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FELIPE TECCHIO BORSOI

EFFECT OF ARAÇÁ-BOI EXTRACT (*Eugenia stipitata* Mac Vaugh - Myrtaceae) ON CANCER PREVENTION: THE ROLE OF POLYPHENOLS IN EPIGENETIC MODULATION

EFEITO DO EXTRATO DE ARAÇÁ-BOI (*Eugenia stipitata* Mac Vaugh - Myrtaceae) NA PREVENÇÃO DO CÂNCER: O PAPEL DOS POLIFENÓIS NA MODULAÇÃO EPIGENÉTICA

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RESUMO

A dieta desempenha um papel essencial na saúde, contribuindo significativamente para a prevenção de doenças crônicas não transmissíveis (DCNTs), como o câncer. Compostos fenólicos, amplamente presentes em frutas e vegetais, destacam-se por suas propriedades bioativas e pela capacidade de modular processos biológicos e epigenéticos relacionadas à carcinogênese. Entre as frutas nativas brasileiras, o araçá-boi (*Eugenia stipitata* Mac Vaugh) destaca-se como uma fonte rica em nutrientes e compostos fenólicos, com potencial terapêutico ainda pouco explorado. Suas atividades biológicas e perfil fenólico sugerem a necessidade de mais estudos, especialmente sobre seu papel em estratégias para o manejo do câncer. Neste contexto, este estudo tem por objetivo avaliar a composição fenólica do extrato da polpa de araçá-boi, compreender sua bioacessibilidade após a digestão gastrointestinal e avaliar seu potencial antioxidante e antitumoral sob uma perspectiva mecanista. Na primeira etapa do estudo, um extrato hidroetanólico da fração comestível do araçá-boi (polpa e casca) foi caracterizado e resultou na identificação de 73 compostos, incluindo ácidos orgânicos, ácidos fenólicos (como ácido gálico, trans-cinâmico, elágico e seus derivados) e flavonoides glicosilados, como kaempferol, miricetina e quercetina. O ácido gálico de forma isolada foi usado para avaliar sua contribuição nas atividades antitumorais. Ambos reduziram significativamente a viabilidade celular nas células tumorais de ovário humano (NCI/ADR-RES). Os ensaios moleculares revelaram a regulação positiva de genes supressores de tumor envolvidos na reparação do DNA, parada do ciclo celular e regulação epigenética, como *BRCA1*, *RASSF1* e *HDAC1* (extrato) e *BRCA1*, *CDKN2A* e *HDAC1* (ácido gálico). Não houve alteração na metilação do promotor do *BRCA1*, sugerindo que os efeitos antitumorais envolvem vias de sinalização e mecanismos epigenéticos diferentes. No segundo estudo foi investigado como a digestão gastrointestinal simulada afeta a recuperação, bioacessibilidade e atividade antioxidante dos fitoquímicos do extrato do araçá-boi. Foram identificados 100 compostos, sendo que apenas 59 foram detectados na fração intestinal. A bioacessibilidade aumentou significativamente para o ácido trans-cinâmico (813%), ácido p-cumárico (232%) e quercetina (106%), indicando que o processo digestivo potencializa a bioacessibilidade de alguns compostos fenólicos. Estudos in sílico mostraram que o ácido trans-cinâmico interage com proteínas NF-κB, IL-1β e PI3K, sugerindo potencial anti-inflamatório. A análise farmacocinética indicou boa solubilidade, absorção e baixa toxicidade reforçando o potencial terapêutico do ácido trans-cinnamico. Por fim, o último estudo investigou os efeitos antitumorais do extrato de araçá-boi e do ácido trans-cinâmico em células de melanoma metastático humano. Foram identificados 11 compostos fenólicos, sendo o ácido trans-cinâmico foi o principal composto, seguido por outros como quercetina-3-O-galactosídeo e ácido siringico. Ambos, o extrato e o ácido trans-cinâmico, reduziram

significativamente a viabilidade celular, migração e estresse oxidativo nas células de melanoma. Eles também influenciaram a expressão de proteínas relacionadas a apoptose (caspase-3) e inflamação (NRLP3). Dessa forma a partir desses estudos buscamos aprofundar a caracterização fitoquímica e quantificação de compostos fenólicos do araçá-boi, uma fruta amazônica pouco explorada, com foco no seu potencial terapêutico no tratamento e prevenção do câncer. Alinha-se aos esforços globais para integrar o conhecimento tradicional e a bioprospecção à medicina moderna, promovendo terapias sustentáveis e destacando a importância da biodiversidade no desenvolvimento de tratamentos inovadores contra o câncer.

Palavras-chave: Compostos fenólicos; Metilação do DNA; Câncer de Ovário; Melanoma; Benefícios à saúde.

ABSTRACT

Diet plays an essential role in health, significantly contributing to the prevention of non-communicable chronic diseases (NCDs), such as cancer. Phenolic compounds, widely present in fruits and vegetables, stand out for their bioactive properties and their ability to modulate biological and epigenetic processes related to carcinogenesis. Among the native Brazilian fruits, araçá-boi (Eugenia stipitata Mac Vaugh) stands out as a rich source of nutrients and phenolic compounds, with its therapeutic potential still underexplored. Its biological activities and phenolic profile suggest the need for further studies, particularly regarding its role in cancer management strategies. In this context, this study aims to evaluate the phenolic composition of the araçá-boi pulp extract, understand its bioaccessibility after gastrointestinal digestion, and assess its antioxidant and antitumor potential from a mechanistic perspective. In the first stage of the study, a hydroethanolic extract of the edible fraction of araçá-boi (pulp and skin) was characterized, resulting in the identification of 73 compounds, including organic acids, phenolic acids (such as gallic, trans-cinnamic, ellagic, and their derivatives), and glycosylated flavonoids like kaempferol, myricetin, and quercetin. Gallic acid, in isolation, was used to assess its contribution to antitumor activities. Both reduced cell viability significantly in human ovarian tumor cells (NCI/ADR-RES). Molecular assays revealed positive regulation of tumor-suppressor genes involved in DNA repair, cell cycle arrest, and epigenetic regulation, such as *BRCA1*, *RASSF1*, and *HDAC1* (extract) and *BRCA1*, *CDKN2A*, and *HDAC1* (gallic acid). No changes in *BRCA1* promoter methylation were observed, suggesting that the antitumor effects involve different signaling pathways and epigenetic mechanisms. In the second study, the impact of simulated gastrointestinal digestion on the recovery, bioaccessibility, and antioxidant activity of the phytochemicals from the araçá-boi extract was investigated. A total of 100 compounds were identified, with 59 detected in the intestinal fraction. Bioaccessibility increased significantly for trans-cinnamic acid (813%), p-coumaric acid (232%), and quercetin (106%), indicating that the digestive process enhances the bioaccessibility of some phenolic compounds. In silico studies showed that trans-cinnamic acid interacts with NF-κB, IL-1β, and PI3K proteins, suggesting anti-inflammatory potential. Pharmacokinetic analysis indicated good solubility, absorption, and low toxicity, reinforcing the therapeutic potential of trans-cinnamic acid. Finally, the last study investigated the antitumor effects of araçá-boi extract and trans-cinnamic acid on human metastatic melanoma cells. Eleven phenolic compounds were identified, with trans-cinnamic acid being the principal compound, followed by others such as quercetin-3-O-galactoside and syringic acid. Both the extract and trans-cinnamic acid significantly reduced cell viability, migration, and oxidative stress in melanoma cells. They also influenced the expression of proteins related to apoptosis (caspase-3) and inflammation (NRLP3). Thus, through these

studies, we aim to deepen the phytochemical characterization and quantification of phenolic compounds in araçá-boi, an underexplored Amazonian fruit, focusing on its therapeutic potential in cancer treatment and prevention. This aligns with global efforts to integrate traditional knowledge and bioprospecting into modern medicine, promoting sustainable therapies and highlighting the importance of biodiversity in the development of innovative cancer treatments.

Keywords: Phenolic compounds; DNA methylation; Ovarian cancer; Melanoma; Health benefits.

CONTENT

GENERAL INTRODUCTION

A balanced diet plays a crucial role in maintaining overall health and preventing chronic non-communicable diseases (NCDs). Among its components, phenolic compounds stand out due to their presence in a variety of foods, including fruits, vegetables, tea, wine, and certain herbs. These bioactive molecules are recognized for their antioxidant, anti-inflammatory, cardioprotective, antidiabetic, and anticancer activities, which contribute significantly to disease prevention and the promotion of well-being (Armas Díaz et al., 2023; Zekrumah et al., 2023).

In 2022, an estimated 4.3 million deaths in females and 5.4 million deaths in males were accounted for by cancer, which is the second leading cause of death globally (GLOBOCAN, 2022). Cancer continues to be one of the biggest global health challenges, with significant difficulties in prevention and treatment due to drug resistance and tumor heterogeneity (Bhat et al., 2024). In this context, phenolic compounds found in plants have gained attention for their ability to interfere with multiple cellular signaling pathways, contributing to both the prevention and treatment of cancer (Foroughi-Gilvaee et al., 2024; Maheshwari & Sharma, 2023). Moreover, these compounds can alter the epigenetic profile of tumor cells, promoting the reactivation of tumor suppressor genes and inhibiting cancer progression (Açar & Akbulut, 2023; Khan et al., 2024).

Brazil is home to an extensive range of native fruits that stand out for their distinctive sensory attributes, significant nutritional value, and potential antitumor effects, largely attributed to their rich content of phenolic compounds (Arruda et al., 2022; Peixoto Araujo et al., 2021). Among these, the Myrtaceae family is particularly prominent, as it represents one of the dominant families in the Atlantic Forest and includes approximately 140 genera and 6,000 species globally (REFLORA, 2024). Within this family, the genus *Eugenia* is particularly relevant and has been widely studied due to its phytochemical composition and its biological potential against several NCDs, including cancer (de Araújo et al., 2019). Among them, the araçá-boi

(*Eugenia stipitata*) stands out as a fruit with remarkable potential, yet it remains underutilized and lacks extensive scientific investigation.

The araçá-boi (*Eugenia stipitata* Mac Vaugh) is an Amazonian fruit tree native to Brazil, Bolivia, Peru, Colombia, and Ecuador. Its fruits are 12 cm in diameter and weigh between 30 and 80 g, with shapes ranging from rounded to slightly flattened (Figure 1). Their yellow peel is thin, and the white pulp is soft, mucilaginous, and highly acidic (pH 2.28). Due to its intense acidity, the fruit is rarely eaten raw and is more commonly used in food products such as juices, ice creams, and jellies (Acosta-Vega et al., 2024). The araçá-boi stands out for its distinctive sensory attributes (such as vibrant coloration, intense taste, and exotic fragrance), rich in nutrients, including dietary fiber, proteins, sugars, vitamin C, and essential minerals like potassium, sodium, calcium, and magnesium. Additionally, the fruit is rich in phenolic compounds and carotenoids. Recent research has highlighted various biological activities of araçá-boi extracts, revealing their antioxidant, antidiabetic, anti-inflammatory, antigenotoxic, and antimutagenic properties (de Araújo et al., 2021; Garzón et al., 2012; Gonçalves et al., 2010; Neri-Numa et al., 2013; Soares et al., 2019). These characteristics indicate that this fruit may have a significant role in the prevention and treatment of various NCDs, emphasizing the importance of exploring its potential in therapeutic strategies.

Figure 1: Fruits of araçá-boi (*Eugenia stipitata* McVaugh). Source: Baldini (2016).

Despite the promising nutritional and bioactive properties of araçá-boi, there is still a significant gap in systematic knowledge regarding its chemical composition and antitumor activities, which limits the understanding of its full therapeutic potential. Thus, further investigation into this fruit is crucial to broaden its applications and uncover additional bioactive compounds with potential for use in cancer prevention and treatment. This will not only increase the attractiveness of araçá-boi but also contribute to its inclusion in dietary practices and its development as a sustainable commercial crop. In this regard, the main objective of this study was to obtain, characterize, and quantify the phenolic compounds from araçá-boi extract and to evaluate the antioxidant and antitumoral potential from a mechanistic perspective. Additionally, the study aimed to understand their bioaccessibility after gastrointestinal digestion.

OBJECTIVES

General Objective

The present research aimed to conduct a comprehensive analysis of the phenolic compound composition in araçá-boi pulp extract (*Eugenia stipitata* Mac Vaugh), to assess its antioxidant and antitumoral potential from a mechanistic perspective, and to investigate the bioaccessibility of these phytochemicals after gastrointestinal digestion.

Specific Objectives

- ✓ To obtain an extract rich in phenolic compounds from the araçá-boi fruit through ultrasound-assisted extraction;
- ✓ To perform a characterization regarding the phytochemicals present in the araçá-boi extract by UHPLC-Q-Orbitrap-MS/MS;
- ✓ To evaluate the antioxidant potential of araçá-boi extract using *in vitro* methods;
- ✓ To evaluate the effect of araçá-boi extract on the inhibition of cell viability and the modulation of tumor suppressor genes in human ovarian tumor cells.
- ✓ To assess the potential of araçá-boi extract as a natural epigenetic modulator on DNA methylation for the prevention of ovarian cancer;
- ✓ To evaluate the bioaccessibility of the araçá-boi extract and to perform a more in-depth characterization and quantification of the phytochemicals present throughout gastrointestinal digestion by UHPLC-Q-Orbitrap-MS/MS and HPLC-DAD;
- ✓ To performed a molecular docking and ADMET analysis using the major compound of araçá-boi after gastrointestinal digestion;
- ✓ To explore the effects of araçá-boi extract on cell viability, migration properties, oxidative stress levels, and protein expression in the human metastatic melanoma cell.

CHAPTER 1

A review concerning how polyphenols can modulate gene expression and modify epigenetic alterations in chronic non-communicable diseases

Dietary polyphenols and their relationship to the modulation of non-communicable chronic diseases and epigenetic mechanisms: A mini-review

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Dietary polyphenols and their relationship to the modulation of non-communicable chronic diseases and epigenetic mechanisms: A mini-review

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ABSTRACT

Chronic Non-Communicable Diseases (NCDs) have been considered a global health problem, characterized as diseases of multiple factors, which are developed throughout life, and regardless of genetics as a risk factor of important relevance, the increase in mortality attributed to the disease to environmental factors and the lifestyle one leads. Although the reactive species (ROS/RNS) are necessary for several physiological processes, their overproduction is directly related to the pathogenesis and aggravation of NCDs. In contrast, dietary polyphenols have been widely associated with minimizing oxidative stress and inflammation. In addition to their antioxidant power, polyphenols have also drawn attention for being able to modulate both gene expression and modify epigenetic alterations, suggesting an essential involvement in the prevention and/or development of some pathologies. Therefore, this review briefly explained the mechanisms in the development of some NCDs, followed by a summary of some evidence related to the interaction of polyphenols in oxidative stress, as well as the modulation of epigenetic mechanisms involved in the management of NCDs.

1. Introduction

Non-communicable diseases (NCDs) are complex conditions associated with cardiovascular diseases, chronic respiratory diseases, diabetes, cancers, and mental illness as a result of a combination of genetic, physiological, behaviors, and environmental factors (https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases). Globally, seven of the top 10 causes of death in 2019 were from NCDs, being

Abbreviations: 8-oxodG, 8-oxo-2́deosyguanosine; *ABCG*, ATP Binding Cassette Subfamily G Member; ADAM10, α-secretase; *ADRB3*, adrenoceptor Beta 3; APP, amyloid-β precursor protein; *ARF*, auxin response factor; ARHGAP24, Rho GTPase Activating Protein 24; *ARH-I*, aplysia ras homology member I; *ATF6*, activating transcription factor 6; *ATP2A3*, ATPase Sarcoplasmic/Endoplasmic Reticulum Ca2+ Transporting 3; *BCL2L14*, apoptosis facilitator Bcl-2-like protein 14; *CDH1*, cadherin-1; *CDKN*, cyclin dependent kinase inhibitor; *CPT*, carnitine palmitoyltransferase; CREBH, cyclic AMP-responsive element-binding protein H; *DANT2*, DXZ4 associated non-noding transcript 2, distal; *DAPK1*, death-associated protein kinase 1; DNMT, DNA methyltransferase; *DOT1L*, disruptor of telomeric silencing 1-like; EWASs, epigenome-wide association studies; *EZH2*, Enhancer of zeste homolog 2; *FAS*, Fas cell Surface Death Receptor; GDNF, glial cell line-derived neurotrophic factor; GFAP, glial fibrillary acid protein; *GSTP1*, Glutathione S-transferases P1; HAT, histone acetylases; HDAC, histone deacetylases; *HSD11B2*, 11 beta-hydroxysteroid dehydrogenase type 2; *IGFBP3*, insulin-like growth factor-binding protein 3; IGT, impaired glucose tolerance; *KCNK3*, potassium two pore domain channel subfamily K Member 3; lncRNA, long non-coding RNA; MBD4, methyl-CpG binding domain 4; *MGMT*, O-6-methylguanine-DNA methyltransferase; NAFLD, Non-alcoholic fatty liver disease; ncRNA, non-coding RNA; oAβ-induced-LTP, oligomeric amyloid-beta induced long term potentiation; *OCT1*, Organic cation transporter 1; OGG1, 8-Oxoguanine DNA Glycosylase; PAI-1, plasminogen activator inhibitor 1; *PHOSPHO1*, Phosphoethanolamine/Phosphocholine Phosphatase 1; *PLIN1*, perilipin 1; *POE3A*, RNA polymerase III; PPAR, peroxisome proliferator-activated receptor; *PPARGC1A*, PPARG coactivator 1 alpha; *PRKCA*, Protein kinase C alpha; *PTEN*, phosphatase and tensin homologue; RASSF1A, Ras association domain family member 1; SAH, S -adenosyl-l-homocysteine; SAM, S-adenosyl-methionine; SD, sleep deprivation; *SOCS3*, suppressor of cytokine signaling 3; *SREBP-1C*, sterol-regulatory element binding protein-1C; *TBX2*, *t*-box transcription factor 2; *TCF7L2*, transcription factor 7 like 2; TET, ten-eleven translocation proteins; *TNNT2*, cardiac muscle troponin T; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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responsible for more than 40 of the 55 million deaths worldwide, representing more than 70 % of all deaths. More than 15 million people die from an NCD between the ages of 30 and 69 years and disproportionately affects low/middle-income countries where occur more than three-quarters of global NCD deaths (https://www.who.int/data/gho/data/indicators/indicator-details/GHO/gho-ghe-ncd-mortality-rate). The percentage of premature deaths from NCDs in the Americas represents about 15 % in countries such as Brazil, Mexico, and the USA. In Europe, in countries like Italy, Spain, and France around 10 % die prematurely from NCDs. On the other hand, countries located in Africa, Southeast Asia, and the eastern Mediterranean account for more than 20 % of premature deaths from NCDs (WHO, 2020). Therefore, NCDs are recognized as a major global challenge and the United Nations has set a goal of reducing premature deaths from NCDs by one-third by 2030 through prevention and treatment (https://sdgs.un.org/goals).

A high-quality diet involving increased consumption of fruits, vegetables, whole grains, and seeds is related to a lower risk of NCDs since these foods are rich in polyphenolic antioxidant compounds. In this sense, polyphenols have been continuously explored for their therapeutic properties (Dominguez et al., 2021). In addition, the epigenetic abnormality is also involved in the pathogenesis of NCDs, and the increasing knowledge of dietary polyphenols as well as their impact on the intestinal microbiota, and their role in epigenetic modulation, make them strong candidates for the treatment and prevention of NCDs (Neri-Numa et al., 2020; Shock et al., 2021). In this context, this review aims to provide an overview of the interplay of polyphenols in oxidative stress, as well as the modulation of epigenetic mechanisms involved in NCDs.

2. A brief overview of the non-communicable diseases (NCDs), reactive species of oxygen and nitrogen (ROS and RNS), and antioxidant modulation

Over the last two decades, several studies have focused on understanding the pathophysiology of oxidative/nitrosative stress related to NCDs (Assi, 2017) as well as searching for effective solutions to mitigate the rising NCDs rates to improve the health span and prevent NCD-driven declines in average lifespan as a strategy to reduce healthcare system costs (United Nations, Department of Economic and Social Affairs, 2019).

Oxidative stress is closely related to the aforementioned diseases once that homeostatic redox imbalance may damage biomacromolecules and modify important proteins, triggering the pathogenesis of NCDs (Neri-Numa et al., 2020; Ruiz et al., 2021). Under physiological conditions, cells require a preferentially reducing environment for the occurrence of several biochemical reactions as well as the creation of the electrochemical gradient necessary for electron flow and energy transfer (Lushchak & Storey, 2021; Ruiz et al., 2021). All these processes comprise redox homeostasis which is determined by the combination of all oxidation–reduction reactions that occur in the cell, involving a regulatory network of molecules that control the rate and amplitude of generation and elimination of reactive species (Ruiz et al., 2021). In other words, the cells have a self-protection system against oxidative and/or nitrosative damages since reactive species of oxygen and nitrogen (ROS and RNS) are inescapable by-products of metabolism being essential for several cellular physiological functions (*e.g.:* cell signaling, proliferation, differentiation, senescence or death) (Al Shahrani et al., 2017; Ruiz et al., 2021). However, when the redox homeostasis is disturbed and there is an overproduction of pro-antioxidants agents, the organisms lose the ability in detoxifying reactive intermediates (Lushchak & Storey, 2021; Neri-Numa et al., 2020).

Although not the main focus of this review, it is important to point out that are differences in the redox status between individuals as well as in different cells or tissues, at the systems level (Meng et al., 2021). So, it does not seem unreasonable to predict that susceptibility to the impacts of oxidative stress will vary amongst those individuals who express polymorphic genes involved in redox signaling and detoxification of reactive species. In a pathophysiological view, the non-detoxified ROS and RNS containing one or more unpaired electrons are more reactive and are involved in cellular dysfunctions, impairment of the intestinal microbiota is trigged beyond several molecular mechanisms which will lead to protein denaturation and enzyme inactivation as well as mutations, genetic instability, and epigenetic modifications (Kumar Saravana et al., 2020; Neri-Numa et al., 2020). For example, redox-active molecules regulate both activities and expression of key enzymes involved in DNA methylation, histone methylation, acetylation, and chromatin remodeling, consequently controlling gene expression and/or enzymatic activity of specific metabolic and redox pathways (Kumar Saravana et al., 2020) (Fig. 1). The redox signaling in human health and disease was nicely illustrated in a recent review (please see: (Zuo et al., 2022).

Despite the oxidative stress producers playing an important role in the pathogenesis of potentially severe conditions, they can be substantially reduced by antioxidant modulation which may act both in the prevention and complementary therapy of NDCs. (Neri-Numa et al., 2020). Antioxidants are the first line of defense against the detrimental effects of ROS and RNS, and they are essential to homeostasis maintenance via different mechanisms of action being categorized as endogenous (enzymatic and non-enzymatic) and exogenous (dietary components, i.e. vitamins C and E, carotenoids, polyphenols, and minerals), working synergistically and together with each other (Azat Aziz et al., 2019). Although this review addresses only exogenous antioxidants (polyphenols, in this case), in a general way, antioxidants can neutralize reactive species as well their by-products by accepting or donating electrons to eliminate the unpaired condition of the radical making them less active, less dangerous, and long-lived substances than those radicals that have been neutralized (Azat Aziz et al., 2019). The rationale behind the exogenous antioxidant protective effects is the autoxidation postponement by inhibiting ROS or RNS generation or by interrupting propagation of the reactive species through chelating metal ions, preventing the formation of peroxides, breaking autoxidation chain reaction, and the elimination of singlet oxygen (Santos-Sánchez et al., 2019).

Polyphenols draw attention due to their chemical and physical properties since they can act as antioxidant and/or pro-oxidant properties, depending on their related structure and/or the cellular redox context, which may include increased levels of oxidant-eliminating proteins or reduced levels of oxidized proteins and lipids; however in this text, we will keep the focus only on the antioxidant properties of these compounds have many phenolic hydroxyl groups (Phenyl-OH or for aromatics, Aryl-OH) which are planar and electron-rich being able to act reducing or inhibiting reactive species (*e.g.:* singlet oxygen quenchers, superoxide radical scavengers and metal chelators over hydroxyl and peroxyl radicals, superoxide anions and peroxynitrites) through hydrogen-atom transfer and/or single-electron transfer (Quideau et al., 2011; Xu et al., 2018).

Although polyphenols are readily ionized (owing to their proneness for electronic delocalization), their bioactivity relies heavily on the position of hydroxyl groups and relative ease of substituent modification. As well, the antioxidant potential of polyphenols depends on the number arrangement of the hydroxyl groups since a large number of methylations will exhibit fewer antioxidants (Quideau et al., 2011; Stockert & Hill, 2018). In general, the antioxidant capacity of natural phenolics is linked with more than one hydroxyl group in the ortho position of the aromatic ring and, what is attributed to the bond dissociation energy (BDE) of O-H which is typically used to evaluate the activity of an antioxidant to neutralize reactive species. It means that the weaker the O-H BDE, the faster the reaction of antioxidants with the free radical (Xu et al., 2018).

It is also important to point out that polyphenols are not restricted in only their antioxidant capacity but also in their interaction with proteins involved in the transcription and expression of genes related to metabolism, proliferation, inflammation, and cell growth (Sharma &

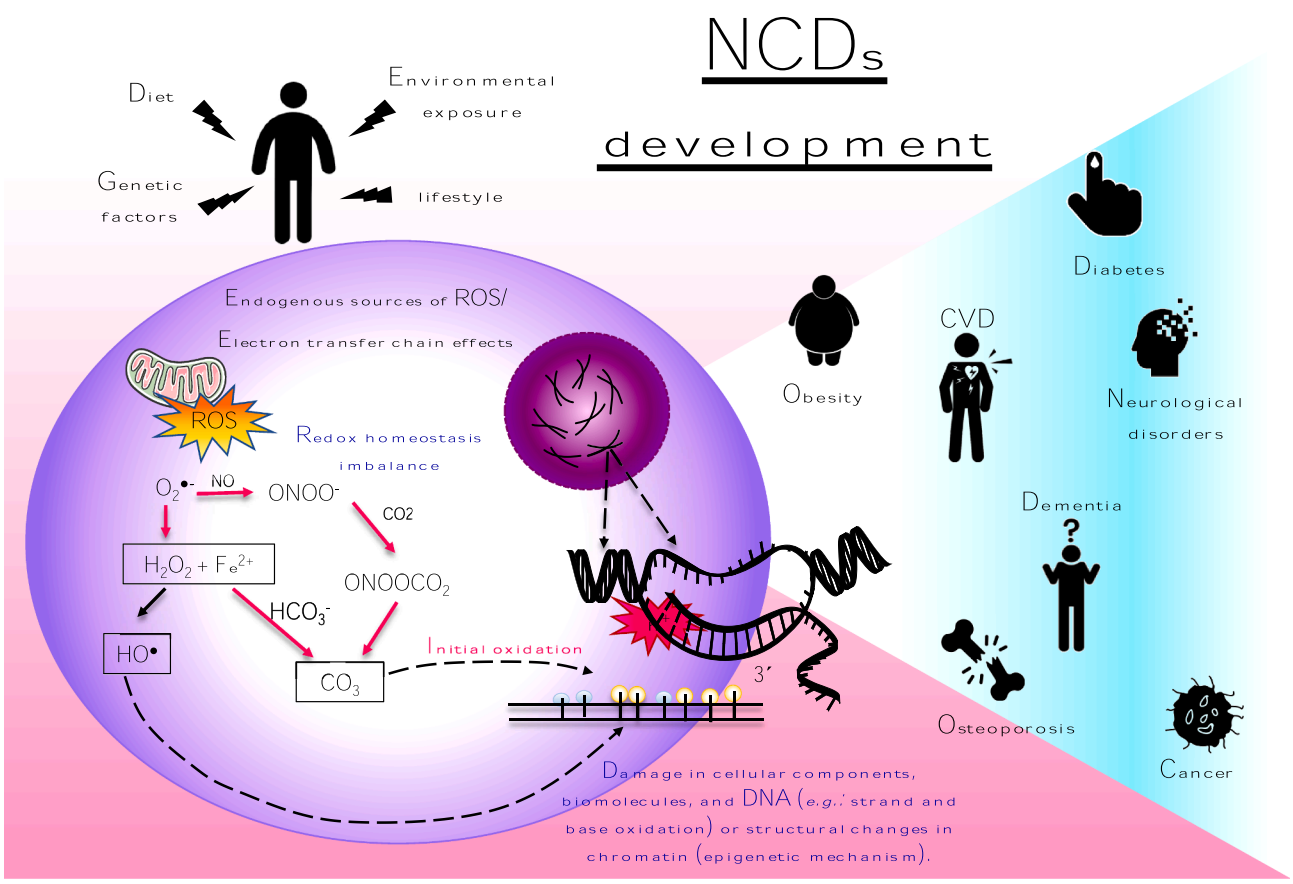

Fig. 1. Schematic representation of redox homeostasis imbalance in chronic non-communicable diseases (NCDs). ROS/RNS generation results from endogenous process of mitochondrial oxidative metabolism and cellular response to external factors plus an inefficient antioxidant mechanism (nonenzymatic and/or enzymatic antioxidants) that can induce inflammation directly by acting on transcription factors (e.g.: nuclear factor κB) and indirectly by modulating other processes such as cellular senescence, mitochondrial dysfunction and microRNA production which in turn have been implicated in the development several NCDs suchs as metabolic sindromes, cardiovascular diseases (CVD), osteoporosis, neurodegenerative diseases and cancer. **ROS,** reactive oxygen species; **RNS,** reactivve nitrogen species; ***O2•-*** *superoxide anion;* ***H2O2,*** *hydrogen peroxide;* ***•OH,*** *hydroxil radical;* ***RO•,*** *alkoxyl radical;* ***ROO•,*** *peroxyl radical;* ***CO3,*** *carbonate radical anion.*

Padwad, 2020). Moreover, all these health-modulating effects are closely linked with bioavailability, intestinal absorption, and metabolism in the gastrointestinal tract (Stockert & Hill, 2018). Thus, considering that there are some preliminary results on the role of some plant-based bioactive compounds in oxidative/nitrosative stress modulation, glucose and lipid metabolism as well as cardiovascular function, in the following sections, this review will provide up-to-date data on intervention studies investigating the modulatory effect of polyphenols in some NCDS from the point of view of redox and epigenetic.

3. The interplay between oxidative stress, NCDs, and epigenetic mechanisms

Pathogenesis of NCDs is closely associated with genetic susceptibility and environmental exposures (*e.g.:* diet, physical activity, environment pollution, stress, and bacterial infections) (Ferrari et al., 2019). This set factor directly affects our genes and can lead to mutations and/or modification of gene expression patterns, dramatically altering cellular metabolism and causing a drop in basal metabolism (Breton et al., 2021; Xiao & Loscalzo, 2020). Additionally, epigenetic mechanisms have been seen as an interesting bidirectional link between oxidative stress and genetic in the consolidation of several chronic diseases (Neri Numa & Pastore, 2020). Firstly, because oxidative stress can impair the proliferative capacity of a cell as well as directly induce DNA damage, and second, genetic changes (*e.g.:* mutations and polymorphisms) may influence gene expression, and thus those functions regulated by the mutated gene (Neri Numa & Pastore, 2020). These contributions to oxidative stress may be partly mediated through changes in epigenetic marks such as DNA methylation, histone modifications, and microRNAs which in turn play an important role gene transcription (Kietzmann et al., 2017). A brief overview of the three major mechanisms of epigenetic regulation is described in Fig. 2.

Basically, oxidative and/or nitrosative stress directly suppress DNA methylation through oxidizing DNA, increasing ten-eleven translocation proteins (TET) mediated hydroxymethylation and altering DNA methyltransferases binding which is responsible for the production of methyl donor S-adenosylmethionine (Ionescu-Tucker & Cotman, 2021). ROS and RNS can also damage DNA by hydroxylating pyrimidines and 5-methylcytosine (5mC), which can interfere with 5-hydroxymethylcytosine (5hmC) epigenetic signals (Ionescu-Tucker & Cotman, 2021; Scaccia et al., 2020). Oxidative stress also may affect post-translational histone modifications, changing chromatin structure, gene expression, gene stability, and replication (Ionescu-Tucker & Cotman, 2021). Frequently, these events are indirect, as ROS impair metabolic efficiency, reducing levels of metabolites such as acetyl-CoA, Fe, NAD+, and ketoglutarate that are essential for histone-modifying enzymes (Ionescu-Tucker & Cotman, 2021; Scaccia et al., 2020).

In the scenario of type 2 diabetes mellitus (T2DM) for example, epigenetic alterations include numerous genes (Scaccia et al., 2020). Retinopathy is a classic complication of diabetes that presents various several epigenetic modifications amongst methylation and histone acetylation; where it is also demonstrated the importance of DNA

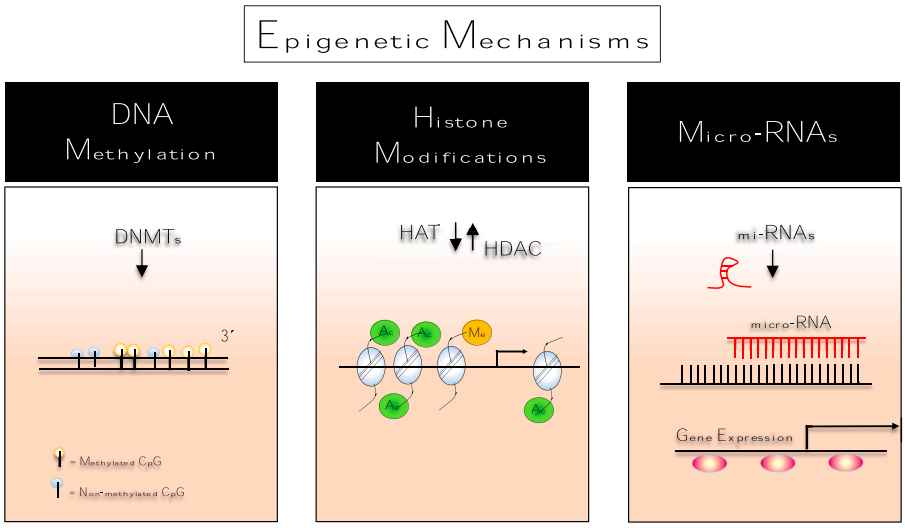

Fig. 2. Schematic mechanism of epigenetic regulation. Epigenetics is the study of changes in gene expression, involving structural changes in chromatin regardless of changes in the DNA sequence. In general, epigenetic mechanisms act to change the accessibility of chromatin for transcriptional regulation by modifications of the DNA and by nucleosome modification or rearrangement from parental to daughter cells. There are three major mechanisms of epigenetic regulation includes (1) DNA cytosine methylation; (2) chromatin remodeling, mostly achieved via histone modification such as methylation, acetylation, phosphorylation, and ubiquitination, or incorporation of histone variants, and (3) small non-coding RNA (miRNAs) regulation. Epigenetic modifications may be reversible and occur naturally in normal development, health as well as in aging or NCDs development once several factors including environmental factors, lifestyle, upbringing, or emotions may interfere with its regulation. Below is a brief overview of the concept of epigenetic mechanisms. **DNA methylation:** is defined as a covalent linkage of methyl groups on the C5 position of cytosine residues in DNA, typically in CpG context, catalyzed by DMNTs enzymes using a SAM donor. **Histone modifications:** refer to covalent post-translational modifications of N-terminal ((including acetylation, phosphorylation, methylation, and ubiquitination) tails of four core histones (H3, H4, H2A, and H2B. Among these alterations, histone acetylation and methylation have received prominence. They are regulated by two groups of enzymes: HAT and HDAC, respectively. The HAT/HDAC system plays a key role in modifying the chromatin structure, which is directly related to the control of the transcriptional process and the gene expression. **miRNA:** induce degradation of targeted mRNAs by sequence-specific base pairing towards their 3́unstranslated regions within the RNA-induced silencing complex (RISC) which further inhibits the translation. ***CpG,*** *cytosine-phosphate-guanine*; ***DNMT,*** *DNA methyltransferase;* ***HAT,*** *histone acetyltransferase;* ***HDAC,*** *histone deacetylase;* ***mi-RNA,*** *micro RNA;* ***SAM,*** *S-adenosyl-methionine.*

methylation/hydroxymethylation machinery in the regulation of Rac1-mediated oxidative stress (Rac Family Small GTPase 1) (Duraisamy et al., 2018; Scaccia et al., 2020). During glucose homeostasis, 8-oxo-2́deosyguanosine (8-oxodG) residues are removed by 8-oxoguanine DNA glycosylase (OGG1) mostly via the short-patch pathway base excision repair (BER) (Banda et al., 2017; Scaccia et al., 2020). However, under glycotoxic conditions, hydroxyl radical (OH) overproduction affects DNA methylation by oxidation of guanosine to 8-oxodG, resulting in the accumulation of 8-oxodG which suppress the methylation of adjacent cytosines (Banda et al., 2017; Davison et al., 2021; Scaccia et al., 2020). This event triggers a cascade of hypomethylation and transcriptional activation responsible for the recruitment of OGG1/TET1 complex proteins to 8-oxodG, facilitating the conversion of 5mC through to 5caC to sites of ROS-induced damage (Davison et al., 2021; Zhou et al., 2016). Another example is cardiovascular disorders, such as atherosclerosis, in which hydrogen peroxide (H_2O_2) is associated with aberrant DNA methylation in atherosclerosis by altering the binding of DNMTs to chromatin. Also, epigenetics silencing of superoxide dismutase (SOD_2) decreases the production of H_2O_2 by selective promoter hypermethylation impaired redox signaling, resulting in vascular smooth muscle cell (SMC) proliferation (Gorabi et al., 2020).

Several studies have proposed a strong connection between the misregulation of ROS (by mitochondrial dysfunction and/or age, or both) and neurodegenerative disorders such as Alzheimeŕs, Parkinsoń s, and Huntingtoń s diseases (Liu et al., 2017; Zuo et al., 2015). In Alzheimeŕs disease (AD), it is believed that the accumulation of reactive species may disrupt the homeostatic balance between anti- and pro-inflammatory cytokines which in turn plays a critical role in the amyloid-beta (Aβ) plaque assembly, resulting in cognitive depletion with impaired speech, vision, behavior, and eventually death (Liu et al., 2017). Epigenetically, ROS alters Aβ-production in the progression of AD by methylation and acetylation. In general, patients with AD not only present a global decrease in DNA methylation and CpG oxidation but also amyloid-β precursor protein (APP)-related mutations, followed by reduced levels of 5hmC and 5mC (Zuo et al., 2015).

Finally, cancer is unquestionably the most studied NCD. Several studies are uncovering the role of ROS on epigenetic modulation and carcinogenesis (García-Guede et al., 2020). Excess of reactive species in addition to triggering a series of changes in the signaling pathways responsible for the control of cell cycle machinery (*e.g.:* MAPK, NF-κB, STAT3, or PPARγ), may trigger an unbalanced expression in both levels of histones (HDAC1, HMT1, and HATI) and epigenetic regulation (DNMT1, DNMT3A, and MBD4) in cancer target cells (Arfin et al., 2021; García-Guede et al., 2020; Mahalingaiah et al., 2017). DNA hypermethylation has been used as a prognosis biomarker in bladder cancers. *ARF, GSTP1, CDH1*, and *CDKN2A* are examples of epigenetically silenced genes by CpG promoter hypermethylation in several molecular pathways contributing to the aggressiveness of urothelial carcinoma once malignant phenotypes present more hypermethylated loci than non-muscle-invasive tumors (Boonla, 2021).

In light of this, the relationship between NCDs and epigenetic changes has attracted much attention in recent years. Therefore, elucidating the physiological/metabolic mechanisms of oxidative stress involved in NCDs, as well as understanding the relationship of polyphenols in the interaction of epigenetic modulation and gut microbiota on these metabolic disorders are essential to advance in the field of epigenetics. Following, this review will address the interplay of polyphenols in the gut microbiota in epigenetic changes and how the relationship between these events could explain the beneficial effects of polyphenols on NCDs.

4. Cross-link of phenolic compounds on the gut microbiota and epigenetic changes

It is increasingly evident the importance of understanding the effects of the consumption of certain bioactive compounds on gut microbiota regulation and its relationship with the health of the host (Farias et al., 2019). Only a small part (5–10 %) of ingested polyphenols are absorbed in the small intestine and, when they reach the colon, they are metabolized by the microorganisms present, leading to the production of short-chain fatty acids (SCFA) and the modulation of the gut microbiota, which in turn play an important role in the prevention/treatment of various NCDs (de Paulo Farias et al., 2021).

Recently, it has been pointed out that there is a mutual relationship

between microbiota/host and how this relationship can regulate gene expression, mainly due to the production of metabolites that are generated after colonic fermentation of bioactive compounds such as polyphenols (Gadecka & Bielak-Zmijewska, 2019). Indeed, the gut microbiota and microbial metabolites may be important mediators of diet-epigenome interactions. Changes in the gut microbiota result in changes in the release, metabolism, bioavailability, and metabolic effects of bioactive compounds, epigenetic mechanisms, and their relationship to health (Gerhauser, 2018). However, it is important to emphasize that there is a reciprocal relationship since the host epigenome also influences the composition of the colonic microbial (Shock et al., 2021).

Some studies have shown that metabolites generated by fermentation of polyphenols and other bioactive compounds can alter the activity of important epigenetic enzymes, including histone acetyltransferases (HATs), histone deacetylases (HDACs), DNA methyltransferases (DNMTs), and DNA demethylases, which are responsible for DNA methylation or histone methylation and/or acetylation (Shock et al., 2021). Likewise, the ingestion of foods such as green tea, soy, fruits, and some vegetables rich in polyphenols that are fermented in the colon can modulate the gut microbiota and alter miRNA expression (Gerhauser, 2018). Some of the major phenolic metabolites resulting from microbial metabolism have been recently reported by Cortés-Martín et al. (2020) (Table 1). Although some stilbenes-derived metabolites such as dihydroresveratrol, dihydropiceid, 3,4′-dihydroxy-trans-stilbene and 3,4′-dihydroxydihydro-stilbene (lunularin) have been reported by Cortés-Martín and colleagues, little information on their epigenetic effects is available in the literature. Likewise, catechol (1,2-dihydroxyphenol), a metabolite derived from hydroxycinnamate (*e.g.:* p-coumaric acid, chlorogenic acid, ferulic acid, sinapic acid, etc.), also has little information in the literature regarding its epigenetic effects. Some effects of polyphenols on the gut microbiota, as well as their metabolites on changes in epigenetic mechanisms, can be seen in Table 1.

Ingestion of yogurt containing phenolic extract of jabuticaba seeds (*Myrciaria jaboticaba*) rich in ellagitannins increased bacterial abundance in male Wistar rats, mainly due to the increase in the *phylum* Bacteroidetes. In addition, the drink containing ellagitannins also reduced the relative abundance of bacteria that are microorganisms considered harmful to the health of the host, such as *Pseudomonas*, *Escherichia*, and *Shigella* (do Carmo et al., 2021). Urolithins are phenolic metabolites formed by the biotransformation of ellagitannins and ellagic acid by intestinal bacteria, mainly *Bifidobacterium*, *Pseudocatenulatum*, *Lactobacilli,* and *Gordonibacter*. These compounds have been extensively studied due to their promising effects in remodeling the epigenome and their beneficial health effects (Selma et al., 2017). A study by Li and collaborators demonstrated that urolithin A, the predominant isoform among the five urolithins in humans, could be a potential therapeutic target to control obesity in relation to thermogenesis activation. According to the authors, this metabolite can control the composition of miRNAs in brown adipocyte tissue, since it was able to increase the expression of miR-124-3p levels, which resulted in increased adipocyte differentiation, in the biogenesis mitochondrial and ATP synthesis (Li et al., 2022). When evaluating the effect of some metabolites on the epigenetic modulation of the mechanisms involved in inflammation, Kiss and colleagues observed that urolithins A and B at a concentration of 5 μM were able to reduce the activity of HATs by more than 50 %, but

Table 1
Effect of phenolic compounds and their metabolites on the gut microbiota and epigenome regulation.

Compound	Effect on gut microbiota	Major phenolic metabolites	Epigenetic effects of metabolites	References
Elagitannins and ellagic acid	↑ *Bifidobacterium* and *Lactobacillus*, and ↓ *B. fragilis*, *Clostridia* and *Enterobacteriaceae in vitro*.	Urolithins (A, B, C, D, M5, M6 and M7)	**Urolithin A**: ↑ the O_2 generation activity, ↑ acetylations of Lys-9 residues of histone H3 within chromatin surrounding the promoter region of gp91-phox gene, regulated protein levels of $p22^{phox}$ and $gp91^{phox}$ in U937 cells.	(Li et al., 2015; Kikuchi et al., 2021)
Isoflavones	Stimulated the growth of *B. bifidum DNG6*, *L. lactisSt66*, *L. plantarum 10.960* and *L. rhamnose* and inhibited pathogenic bacteria (*E. coli* and *S. aureus*) *in vitro*.	*O*-desmethylangolensin and equol	**Equol**: ↓ methylation of the cytosine phosphate guanine (CpG) islands in the *BRCA*1 and *BRCA2* promoters in MCF-7 and MDA-MB-231 cells and ↑ *BRCA1* and BRCA2 proteins expression in nuclei and cytoplasm in cell lines MCF-7, MDA-MB-231 and MCF-10a by immunohistochemistry.	(Chen et al., 2022; Bosviel et al., 2012)
Lignans	↑ Ruminococcaceae and Bacteroidetes (*Bacteroides* and *Rikenellaceae*), ↓ Coriobacteriaceae, especially *Collinsella*, and *Streptococcus* in premenopausal women.	Pinoresinol, secoisolariciresinol, matairesinol, lariciresinol, isolariciresinol, and syringaresinol	**Pinoresinol**: ↑ rate and the expression of collagen type I (Col-I), ALP, osteopontin (OPN), runt-related transcription factor 2 (Runx2) and bone morphogenetic protein-2 (BMP-2), ↑ ALP activity and Alizarin red size, and ↑ cAMP, PKA and phosphorylated cAMP response element-binding protein (CREB) levels.	(Corona et al., 2020; Jiang et al., 2019)
Flavonoids in general, *e.g.* flavonols (quercetin)	↓ Verrocomicrobia and ↑ microbiome diversity and abundance of *Actinobacteria*, *Cyanobacteria* and *Firmicutes*.	Acids and aldehydes phenolic	**Chlorogenic acid**: ↓ proliferation, colony formation, invasion, and metastasis of HepG2 cells both *in vitro* and *in vivo* by down-regulating DNMT1 protein expression, ↑ p53 and p21 activity, ↓ cell proliferation and metastasis, in addition inactivated ERK1/2 and reduced *MMP-2* and *MMP*-9 expression in HepG2 cells.	(Nie et al., 2019; Liu et al., 2020)
Stilbenes, *e.g.* resveratrol	↑ *Bacteroides*, Lachnospiraceae_NK4A136_group, *Blautia*, *Lachnoclostridium*, *Parabacteroides* and *Ruminiclostridium_9*, and ↓ relative abundance of Firmicutes.	Dihydroresveratrol, dihydropiceid, 3,4′-dihydroxy-trans-stilbene and 3,4′-dihydroxydihydro-stilbene (lunularin)	–	(Wang et al., 2019)
Hydroxycinnamates (*p*-coumaric acid, chlorogenic acid, ferulic acid, sinapic acid, etc.)	Reversed dysbiosis of intestinal microbiota, ↓ growth of Desulfovibrionaceae, Ruminococcaceae, Lachnospiraceae, Erysipelotrichaceae and ↑ growth of Bacteroidaceae, Lactobacillaceae.	Catechol (1,2-dihydroxyphenol)	–	(Wang et al., 2019)

did not reduce the activity of HDACs (Kiss et al., 2012).

Likewise, soy flavonoids can also affect the gut microbiota and exert beneficial health effects. When evaluating the modulatory effects of soy milk containing isoflavones on the gut microbiota of male BALB/c mice. Dai and collaborators observed that there was an increase in bacterial taxa such as *Bacteroides*, *Lactobacillus*, *Odoribacter*, and *Alistipes*, while three taxa from the families Parabacteroides and Ruminococcaeae were drastically reduced. In addition, an increase in the levels of SCFAs and isoflavone metabolites, including O-desmethylangolensin and equol, was also observed (Dai et al., 2019).

Importantly, only 30 to 50 % of the intestinal microbiota can metabolize daidzein to equol, while 80 to 90 % of the population produces O-DMA as the main phenolic metabolite. In a study carried out to determine the antioxidant potential of daidzein metabolites, it was observed that O-DMA has the ability to stimulate catalase and total superoxide dismutase (CuZn- and Mn-SOD) activity and mRNA and protein expression in cells HepG2 (Choi & Kim, 2014). Likewise, another study carried out by Dagdemir et al. (2013) reported that equol, produced exclusively by the action of the intestinal microbiota under daidzein, at a concentration of 12.8 μM, has the potential to decrease trimethylated transcriptional repression markers, such as H3K9me3 and H3K27me3, in addition to modulating the expression of EZH2 protein. Finally, the authors concluded such compounds and other flavonoids present in soy tend to modify transcription through demethylation and acetylation of histones in breast cancer cell lines.

Some flavonoids such as catechins and epicatechins also undergo cleavage of the *O*-heterocycle and dehydroxylation by the intestinal microbiota, resulting in the formation of various phenolic acids (Gerhauser, 2018). In this sense, a study carried out to investigate the potential epigenetic mechanisms of some phenolic acids in breast cancer reported that treatment with p-coumaric acid and epigallocatechin-3-gallate (EGCG) significantly reduced the viability of four breast cancer cell lines (BT-20, BT-549, MDA-MB-231, and MDA-MB-436). It has also been reported that these compounds can interact with the MTAse domain of human DNMT1 and compete directly with its intrinsic inhibitor S -adenosyl-l-homocysteine (SAH). In addition, EGCG was also able to partially demethylate the *RASSF1A* promoter region in BT-549 cells (Assumpção et al., 2020). These studies corroborate that polyphenols can be fermented by the intestinal microbiota, being transformed into metabolites capable of modulating the intestinal microbiota and interacting with epigenetic machineries such as DNA methylation and histone modifications. Therefore, understanding the interaction of polyphenols in the gut microbiota and their role in epigenetic modulation is an important step toward new therapeutic opportunities in NCDs.

5. Polyphenols for the management of NCDs: nutritional interactions and actions mechanisms

This section will provide an overview of experimental and clinical studies on dietary polyphenols, focusing on the modulation of epigenetic mechanisms in the main diseases involved in metabolic syndrome, neurodegenerative diseases, and cancer.

5.1. Metabolic syndrome – Obesity/Insulin resistance/diabetes/ cardiovascular disease

Metabolic syndrome (MS) (synonyms: Syndrome X, The Deadly Quartet, and The Insulin Resistance Syndrome) is a global health problem that involves a set of diverse cardiovascular (*e.g.*: heart attack, stroke, and atherosclerosis) and endocrine (*e.g.*: obesity, non-alcoholic fatty liver disease (NAFLD), insulin resistance, and diabetes), and is associated with a high risk of morbidity and mortality (American Heart Association, 2021). Over the years, expert groups have developed clinical criteria for the diagnosis of MS, but there is no consensus. However, the most used protocols are from the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) and the International Diabetes Federation (IDF) which consider a positive diagnosis for MS with three or more factors, in addition to central obesity, differing from within some limits established by the World Health Organization, namely: (i) low HDL cholesterol (1.03 mmol/L; 40 mg/dL; men; 1, 29 mmol/L; 50 mg/dL women); (ii) high blood pressure $\geq$ 130/85 mmHg, (iv) increased fasting blood glucose (5.6 mmol/L; 100 mg/dL) (International Diabetes Federation (IDF), 2005).

MS is increasing worldwide and affects nearly 20–30 % of the general population, with differences between ethnic groups and economic and geographic areas (Oliveira et al., 2020). In Brazil, a recent study, based on the National Health Survey of Brazil and the NCEP-ATP III, reported that the prevalence of MS was 38.4 %, with waist circumference (65.5 %) and HDL cholesterol (49.4 %) the most recurrent components (Oliveira et al., 2020). In addition, the incidence was higher in (i) women (41.8 %), (ii) individuals with low education (47.5 %), and (iii) the elderly (66.1 %) (Oliveira et al., 2020). It is noteworthy that waist circumference is a biomarker of the amount of visceral fat and central obesity (https://www.who.int/health-topics/obesity). In this sense, obesity, which kills 2.8 million people/year worldwide, is a major component of MS, being a direct precursor of (a) dyslipidemia (2.6 million deaths/year, globally); (b) NAFLD ($\leq$1 million deaths/year, global); (c) hypertension (9.4 million deaths/year, global); (d) cardiovascular diseases (17.9 million deaths/year, globally); (e) insulin resistance and type 2 diabetes ($\cong$ 1.5 million deaths/year, global) (Sixty-sixth World Health Assembly, 2013; WHO, 2021; World Health Organization (WHO), 2016; World Health Organization, 2021). MS and its associated comorbidities can be alleviated with increased physical activity, moderate alcohol exposure, and non-smoking (de Oliveira et al., 2021). In addition, dietary changes, such as nutrition rich in polyphenols, can modulate epigenetics to control MS and its associated comorbidities.

MS arises from complex and non-linear interactions between environment, genetics, and epigenetics. Specifically, epigenetic dysregulation has been pointed out as an attractive molecular mechanism, as it plays a significant role in the pathophysiology of metabolic syndromes, involving gene modification via chromatin remodeling, histone modification, noncoding RNA alteration, and methylation (Silva et al., 2019). Epigenome-wide association studies (EWASs) have shown that epigenetic changes in the presence of MS can be global and *locus*-specific (Ramzan et al., 2021). A total of 44 gene *loci* are associated with increased risk of MS, including *TXNTP; TGA; OSRI; KCNK3; PPARGC1A; ARHGAP24; PRDMG; LPL; ADRB3; POE3A; HSD11B2; SREBF1; PHOSPHO1; TBX2/SOCS3; GNAS; CPT1A* and *ABCG1*; the latter two being widely reported in association with MS (Low et al., 2021). For example, EWASs detected a decrease in the methylation of the *CPT1A* gene (regulator of mitochondrial fatty acid oxidation), which is correlated with an increased risk of MS (Chitrala et al., 2020). In other EWASs, increased methylation of the *ABCG1* gene (at *loci*: cg06500161) was reported when in the clinical picture of MS (Nuotio et al., 2020). It is noteworthy that *locus* cg06500161 is an epigenetic link in MS that provides: (i) myocardial infarction and dyslipidemias (Pfeiffer et al., 2015); (ii) insulin resistance (Kriebel et al., 2016); (iii) obesity (Walaszczyk et al., 2018); and (iv) type 2 diabetes (Kulkarni et al., 2015).

In vitro, *in vivo*, clinical and epidemiological studies show that polyphenols have antioxidant and anti-inflammatory properties, with potential preventive and/or therapeutic effects for MS, influencing pathological and physiological processes through multiple mechanisms, namely: scavenging of free radicals, metal chelation, regulation of enzymatic activity, inhibition of cell proliferation and alteration of signaling transduction pathways (De Oliveira et al., 2020). From the point of view of diet-gene interaction, polyphenols can affect gene expression in two ways: (i) direct activation of transcription factors or (ii) indirect modulation of signaling pathways (Silva et al., 2019). As seen in Table 2 (for complementary information, please see Supplementary Table 1, S1), there are a variety of biologically active

Table 2
An overview of the beneficial effects of polyphenols in non-communicable diseases (NCDs).

Polyphenol/ Substrate/ Metabolite	Experimental model/Condition	Epigenetic mechanism	Consequence	Main outcomes	References
Metabolic Syndrome					
Quercetin	Obesity (*In vitro*: PPARγ, C/EBPα and 14–3-3ε, H3K9, LSD1, H3K4 antibodies, and oligonucleotides)	Chromatin remodeling and histone modifications	↓ LSD1 for the 5′ region of the *c/EBPα* and *PPARγ* genes.	↓ Body weight improved dyslipidemia and glucose tolerance. ↓ Hepatic accumulation of lipids.	(Nettore et al., 2019)
Epicatechin	Hyperglycemia (*in vitro:* THP-1 cells pretreated with epicatechin and exposed to 25 mM glucose for a total of 24 h, 5 μM for 4 h)	DNA methylation	↑Acetilação H3K9 e H3K4. ↓ Dimethylation H3K9 ↓HDAC4 levels and TNF-α release.	EC induces increased levels of NF-κB in human monocytes when there is diabetes.	(Cordero-Herrera, Chen, Ramos, & Devaraj, 2017)
Resveratrol/Quercetin	Hypertension (*In vivo*: Male rats aged 6 months, diet with a mixture of 50 mg/kg/day resveratrol + 0.95 mg/kg/day quercetin. Systolic pressure was measured using the tail cuff method)	Histone modification	Modulation of the *SIRT1* and *SIRT3* genes. Regulation of endothelial nitric oxide synthase, nitric oxide, and superoxide dismutase.	↓ Blood pressure in rats resistência à insulina, ↓ Insulin resistance	(Castrejón-Téllez et al., 2020)
Curcumin	Stroke (*In vivo*: Male rats received daily intraperitoneal injections of curcumin (50 mg/kg) for 5 days)	Histone modification	↓ TNF-α and IL-6 levels in the brain. ↑ *SIRT1* expression. ↓ Ac-p53 e Bax expression.	↓ Stroke-induced brain injury. ↓ Inflammation and mitochondrial dysfunction after cerebral ischemia.	(Miao et al., 2016)
Genistein	Diabetes (*In vivo*: 40 female rats were fed a diet containing 1 mg/kg/day of genistein for 8 weeks. After that, pancreas tissue was removed and used for Western blotting and Hematoxylin-Eosin staining)	Histone modification	↓ Nf-κB and IL-1β expression. ↑ *SIRT1* expression	↓ Inflammatory changes in the pancreas. ↓ Pancreatic injury. Improved glucose homeostasis	(Yousefi et al., 2017)
Resveratrol	Diabetes type 2 (*clinical trial:* use of western blot, 192 patients, 40 – 500 mg for 72 h)	Histone acetylation at lysine residue 56 (H3K56ac)	↑ TAS levels, ↓ Percentage of H3K56ac.	SIRT-1 can affect redox homeostasis. ↓ Body fat percentage	(Bo et al., 2018)
Neurodegenerative disorders					
Flavonoid-rich (BBJ) blueberry juice	Exercise-induced protected dopaminergic neurons against MPP + or MPTP-induced toxicity: Fischer 344/Brown (male) Norway hybrid rats aged 3 months, treated with BBJ (20 % solution) for 4 weeks.	NE	↓ 6-OHDA-induced DA cell death, ↓ rotational behavior induced by amphetamine;	BBJ consumption + resulted in a greater reduction in amphetamine-induced rotational behavior and protected against the loss of striatal DA terminals.	(Castro et al., 2022)
Curcumin	Modulation of Synaptic Plasticity Related model: C57BL/6J (male) mice aged 8 weeks, treated with curcumin by intraperitoneal and monitored each 2 h.	DNA methylation, ↓ HAT3, ↓ HAT4.	↓ TREM-1 expression, ↓ p300 activity in the TREM-1 promoter region	Epigenetic modulation inhibits TREM-1 expression in response to lipopolysaccharide.	(Yuan et al., 2012)
Extra virgin olive oil derived (OLE) oleuropein aglycone	Transgenic hemizygous CRND8 male and female mice following 3 age groups of mice treated with OLE-fed mice for 8 experimental weeks	↑ HA3, ↑ HA4 and, ↓HDAC2.	↓ β42 deposits in the brain of young and middle-aged TgCRND8 mice glutaminylcyclase-catalyzed pE3-Aβ generation reduces enzyme expression and interferes both with Aβ42 and pE3-Aβ aggregation.	Improvement of synaptic function in a murine model of Alzheimer's disease.	(Luccarini et al., 2015)
Anthocyanin-rich blueberry (*Vaccinium corymbossum*) extract	Modulation of Synaptic Plasticity Related model: Wistar (male) rats, treated with anthocyanin (2 % w/w) diet treated for 6 weeks.	NE	↑ ERK1, Akt Ser473, mTOR Ser2448 and BDNF protein levels, ↓ α-E-catenin, JNK1, and p38 protein levels.	Activation of ERK-CREB-BDNF pathway; ↑ Spatial and psychomotor performances in aged rats; modulation of key synaptic proteins.	(Vauzour et al., 2021)
Luteolin-7-O-glucoside (LUT-7G)	Neuroprotection PD models:SH-SY5Y cells and C57BL6 (male) mice aged 20 weeks treated with LUT-7G treated for 15 days.	NE	↑ Bcl-2/Bax ratio, ↓ Expression of cleaved caspase 3 ↑ ERα and Erβ expression, ↑ ERK1/2/STAT3/c-Fos activatipn, ↑Muscle strength, ↓ MPTP-induced gliosis in substantia nigra, ↑ TH positive nerve fibers *in striatum.*	Protection of dopaminergic neurons against MPP + or MPTP-induced toxicity, Activation ER-mediated signaling pathway.	(Qin et al., 2019)
Grape juice (*Vitis labrusca*) (GJ)	Randomized human clinical trial - Aquatic exercise + GJ (400 mL/daily) consumption: male/female PD patients, aged over 48 years old, treated for 4 weeks.	↑ HAT4	↑ BDNF levels,	GP + Aquatic exercise resulted in amelioration of functional capacity and mobility in PD patients.	(Oliveira et al., 2020)
Cancer					
Genistein + Sulforophane + sodium butyrate	Breast cancer cell line (MDA-MB-231 and MCF-7) treated with Genistein (5 μM) + Sulforophane (15 μM) + sodium butyrate (2,5 μM) for 72 h	↓ DNMT3A, ↓ DNMT3B, ↓ HDAC1, ↓ HDAC6, ↓ HDAC11	↓ EZH2, ↓ SUV39H1 ↓ KAT2A, ↓ KAT2B, ↓ EP300, ↓ CBP	↑ apoptosis, ↓ cell cycle	(Sharma & Tollefsbol, 2022)
Curcumim	Gastric cancer cell line (HGC-27, MGC-803, MKN-1 and SGC-7901) treated with 30 – 50 μM for 24 h / K19-Wnt1/	↓ DMNT1	↑ *RB1*	↓ cell proliferation, ↑ apoptosis,	(Cao et al., 2020)

(*continued on next page*)

Table 2 (*continued*)

Polyphenol/ Substrate/ Metabolite	Experimental model/Condition	Epigenetic mechanism	Consequence	Main outcomes	References
	C2mE transgenic mice (Gan) Six-week-old treated with curcumin (0.005 % (w/v)) for 3 times a week for 48 weeks			↓ cell cycle, ↓ inflammation	
Quercetin	Acute myeloid leukemia (HL60 and U937) treated with 50 – 75 μM for 72 h /Human xenograft acute myeloid leukemia (AML) models - female mice (NOD.CB17-Prkdcscid/J lineage) 8–11-week-old treated with quercetin (120 mg/kg every 4 days for 20 days	↓ DNMT1, ↓ DNMT3A, ↓ HDACs	↑ *DAPK1*, ↑ *BCL2L1*, ↑ *BAX*, ↑ *APAF1*, ↑ *BNIP3L*	↑ apoptosis	(Alvarez et al., 2018)
Hippophae rhamnoide Polyphenols	Colorectal (HCT116, HT29, and FHC) treated with 40–120 μM for 48 h /tumor xenograft model - BALB/c female nude mice (five weeks old) were treated with *Hippophae rhamnoide* extract (50 mg/kg/day for 21 days)	↑ miR −195-5p, ↑ miR − 497-5p, ↓ miR-1247-3p	↓ *CCND1*, ↓ *CCND2* ↓ *CCND3*, ↓ *CCNE1* ↓ *BCL-2*, ↑*CASP2*	↑ apoptosis, ↓ cell cycle	(Wu et al., 2021)
Apigenin	Breast cancer cell line (MDA-MB-231) treated with 40 μM for 96 h / tumor xenograft model - female athymic nude mice (BALB/c-nu) aged 6 weeks treated with apigenin (25 mg/kg/day for 8 weeks)	↓ HDAC, ↑ HAT	↓ *CDK1*, ↓ *CCNA2*, ↓ *CCNB1*, ↑ *CDKN1A*	↓ cell proliferation, ↓ tumor growth	(Tseng et al., 2016)
Genistein	Head and neck squamous cell carcinoma (HNSCC) - Phase II *clinical trial* – 39 patients (300 mg/day for 3 weeks)	↑ Global Hypermethylation	↓ *LINE-1*	↑ genomic stability	(Rozek et al., 2019)

Symbol: ↔ sample did not affect the parameter; ↓ sample induced a significant reduction; ↑ sample induced significant increase. **NE** not evaluated. **6-OHDA,** 6-hydroxydopamine; **AKT Ser473,** anti-phospho-AKT (ser473) antibody; ***APAF1***, apoptotic protease activating factor-1; **BAX**, BCL2 associated X; ***BCL2***, B-cell leukemia/lymphoma 2 protein; ***BCL2L1***, BCL2 Like 1; **BDNF,** brain-derived neurotrophic factor; **BNIP3L**, BCL2 interacting protein 3 like; **C/EBPα,** CCAAT-enhancer-binding proteins; ***CASP2***, caspase 2; **CBP**, CREB-binding protein; ***CCNA2***, cyclin A2; ***CCNB1***, cyclin B1; ***CCND1***, cyclin D1; ***CCND2***, cyclin D2; ***CCND3***, cyclin D3; ***CCNE1***, cyclin E1; ***CDK1***, cyclin dependent kinase 1; ***CDKN1A***, cyclin dependent kinase inhibitor 1A; ***DAPK1***, death-associated protein kinase 1; **DNMT**, DNA methyltransferase; ***EP300***, E1A binding protein P300; **ERK1**, extracellular signal-related kinase; ***EZH2***, Enhancer of zeste homolog 2; **HAT**, histone acetylases; **HDAC**, histone deacetylases; ***KAT2A***, Lysine acetyltransferase 2A; ***KAT2B***, Lysine Acetyltransferase 2B; ***LINE-1***, long interspersed nuclear element-1; ***LSD1***, lysine demethylase 1A; **NF-κB**, factor nuclear kappa B; **P53**, tumor protein 53; **PPAR**, peroxisome proliferator-activated receptor; ***RB1***, RB Transcriptional Corepressor 1; ***SIRT***, sirtuin; ***SUV39H1***, Histone-lysine N-methyltransferase; **TNF-α**, tumor necrosis dactor alfa; **TREM1**, triggering receptor expressed on myeloid cells.

polyphenols such as anthocyanin, resveratrol, catechin, epicatechin, quercetin, genistein, curcumin, etc. (Bo et al., 2018). For example, a clinical study showed that juçara (*Euterpe edulis* Mart.) supplementation, rich in anthocyanin, modified the serum fatty acid profile, contributing to the reduction of MS, through DNA methylation and histone acetylation (Santamarina et al., 2018). In turn, resveratrol has also been used in different *in vivo* studies for the epigenetic treatment of type 2 diabetes, *NAFLD*, and hypertension through the regulation of genes such as *SIRT-1, ATF6, CREBH, PLIN1*, etc. (see more in Table 2). Quercetin was able to remodel chromatin and modify histones, acting on the expression of *LSD1, CYP2E1*, and *SIRT-1*, attenuating obesity, hypertension, and diabetes in rats (Nettore et al., 2019). Recent *in vivo* models have shown that curcumin can decrease inflammatory cytokines (TNF-α and IL-6) in stroke and obesity by modulating genes such as *BAX, HMGB1*, and *TCF7L2* (Tian et al., 2017). Importantly, complex polyphenols act as probiotics, as they are converted into low-molecular-weight metabolites by the intestinal microbial community (Duttaroy, 2021). In these cases, polyphenols significantly altered the intestinal microbiota, including its metabolic by-products, which, consequently, affect the epigenetics of the host, alleviating MS (Shock et al., 2021). For example, epigallocatechin was able to reduce metabolic disturbances in mice fed a high-fat diet by increasing DNMT1 expression and subsequent hypomethylation of CpG in the colon (Remely et al., 2017). Another study showed that trans-resveratrol, in men with metabolic syndrome, significantly altered the composition of the microbiota (mainly *Akkermansia muciniphila*) and the primary metabolite of dihydroresveratrol, which may have improved insulin sensitivity and reduced fat mass, due to altered expression of the *SIRT* gene (Walker et al., 2018).

These results indicate that polyphenols may have a potential role in innovative MS prevention strategies. However, several challenges in this field of research must be overcome, such as understanding the mechanisms underlying (metabolic, cellular, and molecular) the beneficial effects of polyphenol consumption (Silva et al., 2019). One should also consider the genetic heterogeneity of animals and humans, tissue specificity, and the prominent role of the gut microbiota for a more comprehensive study relating polyphenols, epigenetics, and MS. Therefore, future research is extremely necessary, for more detailed elucidation of the effects of polyphenols on epigenetic dynamics.

5.2. Neurodegenerative disorders

Neurodegenerative diseases (NDDs) comprise a heterogeneous group of disorders (*e.g.:* Alzheimeŕs disease, Parkinson's, and Huntingtońs diseases plus amyotrophic lateral sclerosis) affecting millions of people worldwide. In general, is characterized by loss of function and eventual death of nerve cells in the brain or peripheral nervous system as a result of mitochondrial dysfunction, neurotoxic proteins deposition, and excitotoxicity (Arruda et al., 2020). The most common NDDs are Alzheimer's disease (AD) and Parkinson's disease (PD) (Monzio Compagnoni et al., 2020). In 2021, as many as 6.2 million Americans were living with Alzheimer's disease, which is projected to be nearly 14 million people by 2060 (Alzheimer's Association, 2022). Whereas, approximately 1.2 million people in the United States will be living with Parkinsońs disease by 2030 (Marras et al., 2018).

The pathophysiology of NDDs is complex and not yet completely understood; however, between several downstream degenerative events, prenatal adverse environments, imbalanced redox status, toxicological exposures, and neuroinflammation appear to be the key triggering factors in neurodegeneration, including neurotoxic protein accumulation impairs insulin signals and downregulate neurotrophins expression (Arruda et al., 2020; Kundakovic & Jaric, 2017).

Interestingly, new evidence has addressed the dysregulation of neuroepigenetics related to the same chemical modifications of DNA, and histones but in neurons (Marshall & Bredy, 2016). In the same way, particular attention has been devoted to the gut microbiome, which is deeply involved in nutrient absorption and lipid metabolism,

representing a pillar of the gut microbiome-brain axis besides being activation and upregulation of cellular and molecular pathways in NDDs (Milošević et al., 2021). But how does dysregulation of epigenetic mechanism interface with synapse-specific mechanisms of neural plasticity?

In the brain, DNA methylation, for example, acts by silencing gene expression which is essential in the mediation of memory acquisition and storage (Hwang et al., 2017). In adulthood, it also acts in developing and modifying brain function through DNA methyl-transferases such as DNMT1, DNMT3A, and DNMT3B (Brabson et al., 2021). Some DNA methylation by-products play an important role in the maintenance of genomic stability, and the reasoning behind this is a series of oxidative modifications (*e.g.:* DNA methylation and demethylation processes) in the nervous system (Christopher et al., 2017). For example, DNA demethylation is mediated by Ten-eleven translocation proteins (TETs; family of dioxygenases) by initiating 5-methylcystosine (5-mC) oxidation to generate 5-hydroxymethylcytocine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (Brabson et al., 2021; Christopher et al., 2017).

Alterations in global methylation of histones associated with neuropathogenesis occur mostly at H3 and H4 levels of lysine residues (*e.g.:* H3K4, H3K36, and H3K9), and the main consequence culminates in transcriptional activation (Griñán-Ferré et al., 2018; Wu et al., 2022). While acetylation of histones by HDAC2 is implicated not only in the regulation of synaptic plasticity but also in triggering permanent changes in neurons resulting in a number of learning and memory impairments (Wu et al., 2022). Similarly, deletion of HDAC4 in the brain results in impaired hippocampal-dependent learning, and long-term memory formation (Christopher et al., 2017). Further, histone methylation has been recognized as a pathogenic mechanism in both AD and cognitive deficits where it was observed an increase in both KMT2 and H3K9me2 (Wu et al., 2022). Thus, due to their multifactorial causes, there are still no available treatments for NDDs and it places a huge burden on healthcare systems as they are a common cause of morbidity and cognitive impairment in elderly people and/or neurodegeneration carriers who require increasingly complex and prolonged care (Peplow et al., 2022).

Epidemiological studies have correlated diet, physical activity, and NDDs incidence, indicating that diet is an important modifiable risk factor; since the role of dietary patterns and lifestyle variables in both preventing and slowing down a progression to full-blown diseases is evident (SACN, 2021; WHO/FAO, 2003). Mediterranean diet, for example, is implicated in improving metabolic and lipid profiles in not only NDDs but also in all kinds of chronic diseases (D'Innocenzo et al., 2019; Romanos-Nanclares et al., 2020). The mechanism for these salutary effects resides in the interaction of a plethora of bioactive constituents (*e.g.:* phenolic compounds, sulforaphane, vitamins, MUFA, PUFA, etc.) which may modulate free radical damages and mitigate aging biomarkers in a manner favorable for the longevity and disease prevention (Alasalvar et al., 2020).

Polyphenols, in particular, may exert neuromodulation affects both directly, affecting brain functions and protecting them against oxidative stress and inflammatory injury, or indirectly, modulating the composition of the intestinal microbiota and the metabolites produced since both actions determine the production of neurotransmitters and neuropeptides capable of influencing brain functions (Di Meo et al., 2020; Filosa et al., 2018). Moreover, depending on bioavailability and absorption rates, the polyphenol's active metabolites may directly modulate the synthesis of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) or glial cell line-derived neurotrophic factor (GDNF) (Di Meo et al., 2020). Likewise, emerging evidence points to a number of polyphenols that display the ability to reverse epigenetic regulation involved in NDDs (Russo et al., 2017).

By analyzing a group of 5,209 participants aged 28–62 from the Framingham Heart Study Offspring cohort, it was shown that a higher long-term flavonoid intake is associated with a lower risk of AD and related dementias (ADRD) in US adults (Shishtar et al., 2020). This evidence corroborates another prospective cohort study reporting a positive correlation between a lower risk of mortality among Parkinsońs disease patients and the consumption frequency of flavonoid-rich foods, especially anthocyanins and flavan-3-ols (Zhang et al., 2022). Additionally, we can cite epigallocatechin-3-gallate (EGCG), the major catechin found in green tea (*Camellia sinensis*), and also the resveratrol, a flavonoid commonly found in berries which mitigation of AD and other neurodegenerative processes are not only limited to their antioxidant and anti-inflammatory action but also include disruption of amyloid β protein production, activation of α-secretase (ADAM10), and inhibition of β-secretase (BACE-1) as well as activation of sirtuin 1 (SIRT1) and vitagenes followed by the miRNA upregulation (Folch et al., 2018; Lukiw, 2012). Table 2 shows additional data reported in the literature concerning the biological effects of several plant-based polyphenols and phenolic-rich extracts/fractions on NDDs.

Although polyphenols present a great variety of structures able to modulate several brain functions, there is still a lack of information related to physiological, biochemical, and molecular aspects, in both epidemiological studies as in experimental (*in vitro, in vivo,* and *ex vivo*) models and/or human clinical trials. Another concern is dedicated to the bioavailability of flavonoids and their optimal intake, which should be investigated to elucidate the mechanisms related to the action of these compounds in the modulation of the microbiota, DNA methylation, and their effect on preventing neurodegeneration onset or slowing the progression of the disease. Therefore, scientific research and technological developments still need to advance, aiming to correlate the full potential of polyphenols to design new adjuvant therapies for NDDs.

5.3. Epigenetic targets in cancer modified by polyphenols

Cancer is a generic term for a large group of diseases that can affect any part of the body characterized by unregulated cell growth and spread of abnormal cells (https://www.who.int/cancer/en/). Cancer is the second leading cause of death globally, accounting for an estimated 5.5 million deaths in males and 4.4 million deaths in females in 2020. Lung, colorectal, liver, stomach, pancreas, and prostate cancer are the most common types of cancer in men, while breast, ovarian, lung, colorectal, cervical, and stomach cancer are the most common among women (https://gco.iarc.fr/). Conceptually, all cancers arise as a result of changes in the DNA sequence caused by the accumulation of genetic mutations and epigenetic alterations. Studies have shown that multiple genomic changes contribute to the progression of cancer and it has been incontestable the aberrant pattern in the epigenetic processes leads to altered expression of several genes involved in cell cycle, cell proliferation, cell motility, and apoptosis (Irshad & Husain, 2021).

The main epigenetic mechanisms that occur during cancer progression are DNA methylation, histone modifications, and post-transcriptional gene regulation by miRNAs and lncRNAs. These processes affect transcript stability, DNA folding, nucleosome positioning, chromatin compaction, and complete nuclear organization of the genetic material (Özyalçin & Sanlier, 2020). For instance, ovarian cancer (OC) consists of a complex, heterogeneous group of invasive cancers that originate from different tissues. Tumor suppressor genes such as *BRCA1, RASSF1A, MLH1, CDH1, CDKN2A, CDKN2B, DAPK,* and *APC* have been identified as hypermethylated with associated loss of expression in ovarian cancer (Koukoura et al., 2019). In addition, prostate cancer is considered to be a highly heterogeneous cancer and the hypermethylation of *GSTP, HOXD3, MGMT, CDKN2A, CDH1,* and *APC* genes are the most commonly identified epigenetic alteration (Sugiura et al., 2021). Additionally, histone modifications in chromatin have important effects on gene regulation and carcinogenesis (Özyalçin & Sanlier, 2020). Recent intensive investigations in cancer focus on altering the expression of HATs or HDACs and have found that they contribute to tumorigenesis (M. Sharma & Tollefsbol, 2022). Furthermore, to the activity of DNMTs and histone modifications, ncRNAs such as miRNA

and lncRNAs play important roles in a variety of biological processes, and dysregulation of the expression of these ncRNAs is highly associated with cancer development (Zhang et al., 2020). In breast cancer, the hypermethylation of some suppressor tumor genes such as *APC, RARB, GSTP1, DAPK*, and *SFN* may be related to the dysregulation of ncRNAs (Sher et al., 2020). Therefore, the epigenetic changes mediated mainly by DNMTs, are a promising strategy for developing anticancer agents due to their capacity to reverse hypomethylation in oncogenes and hypermethylation in tumor suppressor genes (Koukoura et al., 2019). This feature is important due to emerging evidence that hypermethylation-induced transcriptional silencing of tumor suppressor genes constitutes a frequent epigenetic defect in many human cancers (Sugiura et al., 2021). Recently, studies have shown that polyphenols play an important role in reversing aberrant epigenetic changes in several cancers polyphenols are known to increase apoptosis and suppress tumor growth in several cancer strains. In addition, polyphenolic compounds have good activity in the reversal of mutant genes and synergy in the effectiveness of conventional cancer treatments (Irshad & Husain, 2021).

Resveratrol is a phytoalexin present in some plants and not only modifies signaling pathways that affect gene expression but also impacts epigenetic mechanisms. Several genes and miRNAs associated with ovarian cancer were modulated by resveratrol by increasing *ARH-I* expression triggering autophagy and inhibiting cell migration in ovarian cancer (Ferraresi et al., 2017). Treatment of breast cancer cell lines with resveratrol demonstrated that DNMT inhibition increases *ATP2A3* gene expression, triggering apoptosis and intracellular Ca^{2+} changes (Izquierdo-Torres et al., 2019). Also, the authors demonstrate that resveratrol decreases HDAC activity, decreases the abundance of nuclear HDAC2 in breast cancer cells, increases HAT activity, and causes enrichment of H3K27Ac. Therefore, the resveratrol-mediated activity of HDAC and HAT enzymes can create a favorable environment for transcription due to the acetylation gain associated with transcriptional activation.

Hesperetin is a common citrus flavanone widely distributed among citrus fruits with great antitumor potential and its ability to reduce histone methylation (H3K79me3) in gastric, breast, lung, liver, and colon cancer cells *in vitro* has been demonstrated (Wang et al., 2021). Furthermore, the authors implemented gastric tumor cells in mice and demonstrated that the reduction of methylation in H3K79 can be explained by the degradation of DOT1L expression through CBP-mediated acetylation and consequently a reduction in cell migration and invasion.

Recently, evidence has shown that polyphenols exert their antiproliferative and pro-apoptotic effects through the regulation of one or more ncRNAs, leading to inhibition of cancer cell growth, induction of apoptosis, or enhancement of conventional cancer therapeutic efficacy (Zhang et al., 2020). Resveratrol acts as a potent regulator of miRNAs decreasing the overexpression of miR-196b/miR-1290 and elevating *IGFBP3* expression in the ALL cell lines triggering apoptosis, antiproliferation, cell cycle arrest, and inhibition of migration (Zhou et al., 2017). Pterostilbene is a stilbenoid chemically related to resveratrol and demonstrated to suppress the cell viability and induced apoptosis of endometrial cancer cells by down-regulation of miR-663b and indirectly increasing the expression of its target, the *BCL2L14* gene (Wang et al., 2017).

Recent reports have demonstrated the contribution of polyphenols as potentiation of several drugs in the treatment of cancer, acting mainly on the gene expression of several signaling pathways and epigenetic mechanisms (Hidetomo Kikuchi et al., 2019). An example is the use of the flavone apigenin to potentiate the anticancer effect of cisplatin in lung cancer by inhibiting HDAC and consequently increasing the degree of histone acetylation and increasing the expression of *CKDN1A* and *BBC3* genes, inducing apoptosis and cell cycle arrest in these cells (Yan et al., 2020).

The polyphenols also act to reverse hypomethylation in oncogenes, such as pterostilbene, that exert anticancer action by remodeling DNA methylation and gene expression. The treatment with PTS decrease the *OCT1* transcription factor with an increase in activity of DNMT3B and was accompanied by *PRKCA* promoter and *TNNT2* and *DANT2* enhancer hypermethylation, and consequently, gene silencing in breast cancer cell line (Beetch et al., 2021).

In addition to the direct contribution of polyphenols to cancer, recent studies demonstrate an indirect influence of polyphenols from the modulation of the composition and metabolism of the intestinal microbiota from epigenetic regulation (Haque et al., 2021). These microorganisms present in the gut are able to transform polyphenols into SCFA such as butyrate, propionate, and acetate that can participate in the modulation of epigenetic processes (Laborda-Illanes et al., 2020). For example, the use of butyrate in combination with sulforaphane and genistein has been shown to increase apoptosis and reduce the viability of breast cancer cells, from epigenetic modulation involved in DNA methylation and histone modifications, such as DNMTs, HDACs, and HATs (Sharma & Tollefsbol, 2022). Table 2 shows additional data reported in the literature concerning the biological effects of several plant-based polyphenols on cancer.

The most recent research covering the antitumor potential of various polyphenols targeting epigenetic regulatory pathways has been shown on this topic. The studies reveal the great potential of polyphenols for cancer prevention and treatment with their individual and/or combined with anticancer drugs to improve anticancer activity. The benefits of polyphenols in epigenetic modulation in tumors are evident, and great progress has been made in experimental models, however, human studies involving the effects of polyphenols in epigenetic modulation in cancer remain insufficient.

6. Concluding remarks and future perspectives

This review highlighted some findings of research developed in the field of polyphenols as antioxidants and their interplay with microbiota followed by epigenetic mechanism modulation, focusing on the management of NCDs. Although many recent data are encouraging, caution is needed when referring to polyphenols as effective therapeutic agents in the treatment of some NCDs, since studies related to the mechanism underlying their protective effects are still incipient. Implementation of technological strategies to overcome the drawbacks of polyphenols extraction and their bioavailability/bioaccessibility and that enable the management of the intestinal microbiota, aiming at obtaining target metabolites, are necessary since few studies address their interaction with other compounds in the diet and/or drug consumption. Likewise, there are still many questions to be answered regarding the role, and mechanism of polyphenols targeting diverse epigenetic landscapes, their parameters as well as signaling pathways, and physiological barriers related to NCDs. Given that, comprehensive studies with age, sex, and ethnicity stratification are also strongly recommended to confirm the main mechanisms of action of polyphenols with regard to regulation of redox status, gene expression, and modulation of epigenetic mechanisms in MS, NDDs, and cancer.

Results presented here emphasize that among the prevention and treatment strategies, the association between nutrition, physical activity, and reduction of sedentary behavior can condition the body to a balance point between health-disease relationship, opening up a range of possibilities in the universe of personalized nutrition. It, consequently, may reduce the impact on public health systems. Thus, we address here only a small slice of all that we can envision of possibilities to identify potential adjuvant therapies (mainly, epigenetic regulators) in a faster, safer, and more economical way. It is now up to future studies to seek ways to overcome the drawbacks of bioavailability and translate them into criteria for the technological design of various innovative diets and food products to promote well-being, as well as to prevent and treat NCDs.

Ethical approval

This article does not contain studies with human participants or animals performed by any of the authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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CHAPTER 2

A review concerning how polyphenols influence ovarian cancer through multi-omics approaches for precision nutrition

A multi-omics approach to understand the influence of polyphenols in ovarian cancer for precision nutrition: a mini-review

Felipe Tecchio Borsoi, Letícia Ferreira Alves, Iramaia Angélica Neri-Numa, Murilo Vieira Geraldo, Glaucia Maria Pastore

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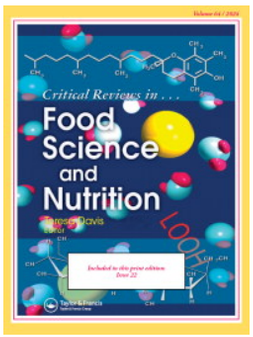

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ReviewA multi-omics approach to understand the influence of polyphenols in ovarian cancer for precision nutrition: a mini-review

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ABSTRACT

The impact of polyphenols in ovarian cancer is widely studied observing gene expression, epigenetic alterations, and molecular mechanisms based on new 'omics' technologies. Therefore, the combination of omics technologies with the use of phenolic compounds may represent a promising approach to precision nutrition in cancer. This article provides an updated review involving the current applications of high-throughput technologies in ovarian cancer, the role of dietary polyphenols and their mechanistic effects in ovarian cancer, and the current status and challenges of precision nutrition and their relationship with big data. High-throughput technologies in different omics science can provide relevant information from different facets for identifying biomarkers for diagnosis, prognosis, and selection of specific therapies for personalized treatment. Furthermore, the field of omics sciences can provide a better understanding of the role of polyphenols and their function as signaling molecules in the prevention and treatment of ovarian cancer. Although we observed an increase in the number of investigations, there are several approaches to data acquisition, analysis, and integration that still need to be improved, and the standardization of these practices still needs to be implemented in clinical trials.

KEYWORDS

Big data; high-throughput technologies; natural compounds; nutrigenetics; nutrigenomics

Introduction

"Cancer" is a generic term for a large group of diseases that can affect any part of the body and are characterized by the uncontrolled growth of cells and invasion of abnormal cells in the body. Cancer is a leading cause of death globally, accounting for an estimated 5.5 million deaths in males and 4.4 million deaths in females in 2020 (GLOBOCAN 2020). Overall, cancer is associated with risk factors such as age, sex, socioeconomic status, family history, genetics, obesity, diet, sedentarism, lifestyle, smoking, alcohol consumption, pollution, radiation, and others. Thus, prevention, early diagnosis, and treatment are essential to reduce the incidence and mortality of this disease (WHO 2022).

Currently, the most commonly found therapies for cancer treatment are surgery, chemotherapy, radiotherapy, and immunotherapy. However, despite advances in this area, these therapies present significant side effects in the vast majority of cases (Abdolmaleki et al. 2020). In this sense, phenolic compounds have shown promise as candidates for the treatment and prevention of cancer (Trisha et al. 2022). Phenolic compounds or polyphenols are chemical substances found mainly in plants and have specific biological activities and can exert a wide variety of beneficial effects on human health (Borsoi et al. 2023). Studies have shown that polyphenols, such as quercetin, kaempferol, curcumin, genistein, resveratrol, catechins, and so on, found in foods, have the ability to inhibit cell proliferation, induce apoptosis, reduce inflammation, inhibit angiogenesis, inhibit cell migration, and inhibit the cell signaling by modulating various molecular pathways in cancer (Braicu et al. 2017; Tavsan and Ayar 2019).

The impact of polyphenols in cancer is widely studied observing gene expression, epigenetic alterations, and molecular mechanisms based on new "omics" technologies (Si et al. 2021). The advancement of omics science around the world and the high-throughput technologies in genomics, epigenomics, transcriptomics, proteomics, and metabolomics allowed for a more comprehensive, precise, and individual analysis of the molecular characteristics of diseases such as cancer. This includes the mechanisms of cancer onset and progression and the identification of biomarkers for diagnosis, prognosis, and selection of specific therapies for each individual (Hasanzad et al. 2022; Karczewski and Snyder 2018). Therefore, mechanistic studies combined with multi-omics approaches may give us insight into the role of polyphenols intake in ovarian cancer from the point of view of prevention of this type of cancer as well as disease management by adding precision nutrition. In light of this, this manuscript provides an updated review involving: (i) current applications of high-throughput technologies in ovarian

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cancer, (ii) the role of dietary polyphenols and their effects on ovarian cancer, and (iii) precision nutrition: definition, current status in cancer research and data management. This knowledge could lead to the development of functional foods rich in polyphenols specifically designed to prevent or treat ovarian cancer.

Trends in omics techniques

The term "omics" refers to a collection of branches of biological sciences dedicated to quantifying, describing, and characterizing distinct groups of biological molecules. Over the last decades, the omic sciences have gained a lot of attention in cancer research and enabled the establishment of molecular profiles of many cancer types in detail. For ovarian carcinoma, the omics approach has contributed to the characterization of its histotypes and the identification of biomarkers, improving the chances for a more suited therapeutic strategy and a potentially better prognosis (Xiao et al. 2022).

From the **genomics** perspective, we can establish two very clearly different eras: the one before and the one after next-generation sequencing (NGS). The pre-NGS era was marked by a lack of scalability and agility in the identification of genetic alterations related to cancer development and progression. With the advent of NGS, a rapid decline in the costs and time for sequencing data generation was observed and a more complete genomic landscape of different diseases, including cancers, was obtained (Mardis 2019). Whole genome sequencing (WGS) is the most known type of NGS and it has enabled the molecular characterization of many cancers at a genomic level. Access to this kind of data revealed the heterogeneity that was behind the tumor diversity but has also helped in establishing the genomic shared features of particular tumor types (Supplitt et al. 2021; Xiao et al. 2022). The dissemination of NGS has made it possible to gather sequencing data of several samples of patients all over the world in databases, such as the Genomic Data Commons Data Portal (GDC Data Portal), which holds data of 74 projects, not only for genomics but also for other omic sciences. Making this data available to the public has greatly contributed to the development of the integrated analysis of multi-omics data (Grossman et al. 2016).

The other two main applications derived from the next-generation technologies are also of great help in research. Whole exome sequencing (WES) is an approach that focuses on the sequencing of exons, mainly used to identify disease-causing mutations (Suwinski et al. 2019). Also derived from NGS, targeted sequencing (Target-seq) focuses on sequencing specific regions of the genome by using custom designs, and its applications are usually related to the deeper coverage that this technique offers (Bewicke-Copley et al. 2019). The advent of highly sensitive techniques comes in handy for the identification of biomarkers on liquid biopsies that for a lot of diseases, the body fluids of patients are enriched with molecules that are a potential source of diagnosis. Even though the field of genomics has allowed the identification of essential genes and mutations for understanding the development and progression of multiple types of cancers, it is important to remember that the genome is far from being enough to define a cell's phenotype and that today it is essential to complement the genomics data with data from different biological levels, represented by the other omics.

Epigenomics, as the name suggests, is the study of the epigenome, the collection of these marks on the genetic material of a cell. The emergence of NGS was also a milestone for the epigenomics field, once combined with other techniques for epigenetic mark investigation, it allowed the comprehensive investigation of the epigenome. Whole genome bisulfite sequencing (WGBS) is one example of the application of NGS for epigenomics. This technique has been used to obtain genome-wide DNA methylation profiles. With high accuracy and lower cost when compared to WGBS, reduced representation bisulfite sequencing (RRBS) is also a high-throughput method based on bisulfite sequencing, but adding a step of digestion with restriction enzymes that enrich the samples for areas with high CpG content, reducing the number of nucleotides to sequence (Wan and Bell 2020). Also for DNA methylation profiling and with lower cost than WGBS, arrays such as the Illumina HumanMethylation450 BeadChip (Illumina 450k) and Illumina HumanMethylationEPIC Bead Chip (Illumina EPIC) have been increasing the coverage of the genome CpGs and are also a frequently used tool on the study of DNA methylation (Fernandez-Jimenez et al. 2019).

Higher-order chromosomal structure makes interactions between spaced regions of the genome possible by bringing together different genes and regulatory elements (Jerković et al. 2020). The chromosome architecture has been explored by a growing field of research with the emergence of highly sophisticated techniques that allow the assessment of nuclear organization in a very high resolution (Jung and Kim 2021). The first exploration of the nuclear organization of genomic *loci* was mainly made by fluorescent in-situ hybridization (FISH). Later the chromosome conformation capture (3C) and its adaptations (4C, 5C, and Hi-C) allowed the large-scale detection of genomic interactions. A variation of Hi-C, known as chromatin interaction analysis by paired-end tag-sequencing (ChIA-PET), provided the possibility to map long-range DNA interactions that are associated with a protein of interest. Together with Hi-C, ChIA-Pet al.lows the establishment of a comprehensive picture of nuclear organization. While chromatin immunoprecipitation sequencing (ChIP-seq) allowed the identification of protein of interest DNA binding sites, the emergence of HiChIP made it easier to investigate the association of proteins with the tridimensional genome structure, producing high confidence contact maps (Liu and Zhao 2021). As the knowledge about chromatin conformation accumulates, tools to manipulate genome interactions are being developed and open up a whole new area to be explored (Hao, Shearwin, and Dodd 2017; Morgan et al. 2017). Single-cell analysis is probably the recent approach that holds the most excitement among cancer researchers. In contrast with in-bulk analysis, the analysis at the single-cell level allows the removal of the noise caused by intercellular differences (Casado-Pelaez, Bueno-Costa, and Esteller 2022). The combination of single-cell methods opens

a promising field to explore the impacts of epigenetic mechanisms on tumor heterogeneity.

Transcriptomics studies the collection of transcripts of a cell and it is known for being much more dynamic than genomics. For that reason, transcriptomics is essential to understand the plastic effects of intra- and extra-cellular conditions in the gene expression profiles of cells. The most used methods for transcriptome profiling include large-scale reverse transcription-quantitative polymerase chain reaction (RT-qPCR), microarrays, and RNA-sequencing (RNA-seq) (Supplitt et al. 2021). RNA-seq represents the most used high-throughput approach to characterize and categorize cancer types, subtypes, and even cell populations (Wang et al. 2019). The databases on cancer data are heavily populated by RNA-seq files, which have made possible several studies exploring the data. The spatial transcriptomics and the single-cell approach have also been recently explored in the field, enabling the determination of specific gene expression profiles for distinct conditions for a given cell (Xiao et al. 2022).

Protein-coding-RNA represents only 2% of the transcribed RNAs, while the remaining portion of the transcriptome is constituted by non-coding-RNAs (ncRNAs). ncRNAs are a large group constituted of several subgroups mostly regulatory and structural RNAs. MicroRNAs (miRNAs) are small single-strand RNAs that act as post-transcriptional regulators of gene expression and are probably the most explored group of ncRNAs. miRNA expression profiles are frequently found as dysregulated in the disease context and normally followed by a dysregulation on the expression levels of their targets (Yi et al. 2019). The first steps in the investigation of the role of these molecules were mainly made by RT-PCR and array-based techniques but recently, high-throughput methods such as miRNA-sequencing have been implemented enabling the development of expression landscapes in a great resolution (Wang et al. 2019). As ncRNAs are known as "promiscuous" molecules, the prediction of their potential interactions by different *in silico* tools has been widely used. Additionally, for the validation of ncRNA interactions, high-throughput approaches such as the sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) or ligation of interacting RNA (LIGR-seq) have been used (Bracken, Scott, and Goodall 2016; Casamassimi et al. 2017).

Proteomics is the study of all proteins produced by an organism or biological system at a specific moment in time. Proteomics identifies the expression level of proteins of interest, post-transcriptional modifications, or particular protein-protein interactions at a specific and precise moment in a biological system (Alharbi 2020). Proteomics, when used in conjunction with other omics techniques such as genomics and transcriptomics, provides a more comprehensive understanding of biological systems and facilitates the validation and complementation of results obtained by each technique (Manzoni et al. 2018). For example, transcriptomics can be used to identify differentially expressed genes in a certain condition, while proteomics can be used to identify the proteins that are produced in response to this condition (Teibo et al. 2022). Several high-throughput techniques in cancer proteomics allow the identification and analysis of a large number of proteins simultaneously, including two-dimensional difference gel electrophoresis (2DE), microarrays, reverse-phase liquid chromatography (LC) coupled with mass spectrometry platforms (MS), including matrix-assisted laser desorption/ionization coupled with time of flight (MALDI-TOF), electrospray ionization (ESI), surface-enhanced laser desorption/ionization (SELDI), isotope-coded affinity tag (ICAT), and isobaric tags for relative and absolute quantification (iTRAQ) (Alharbi 2020; Ghose et al. 2022). These methods ensure high sensitivity, accuracy, and speed to determine and identify thousands of specific proteins associated with cancer and analyze how they interact with other proteins and molecules in the body.

Metabolomics is a science that studies the metabolome during cellular metabolism in a biological system, including cells, tissues, organs, body fluids, and organisms, within a specific time. The metabolome is the consequence of genomic, epigenomic, transcriptomic, and proteomic activities and how they may be associated with certain diseases or physiological conditions (Schmidt et al. 2021). Metabolomics has grown rapidly in recent years, and depending on the goal of metabolomic studies, different types of high-throughput technologies (MS spectrometry combined with LC or GC) can be applied to achieve different resolutions and sensitivities (Cheung et al. 2019). Metabolomics has been widely used in cancer research, as metabolic alterations are common in many types of cancer and can be used to aid in diagnosis and the development of more effective therapies (Schmidt et al. 2021). For instance, the use of nuclear magnetic resonance (NMR) can reveal changes in the metabolomic profile in ovarian cancer cells treated with platinum-based drugs (Ghini et al. 2022). Erben et al. (2021), using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) detected over 950 different metabolites in blood, stool, and urine in patients with colorectal neoplasms and a control group. These techniques allow the analysis of thousands of metabolites in a single sample and provide detailed information about the metabolic composition of cells, tissues, or body fluids. Furthermore, metabolomics has been used to understand how cancer cells adapt to the tumor environment, and how these adaptations can be exploited to develop new therapeutic strategies (Cheung et al. 2019; Schmidt et al. 2021).

Although individually all these techniques have been of great contribution for the purposes cited above, the need to integrate the information to properly establish a profile in a system-wide perspective has been revealed in the last few years (Xuezhu Wang, Zhao, et al. 2021). Network science and the multi-omics approaches through high-throughput technologies have been applied for this purpose and have given us a much more accurate overview of different types of cancer, helping with the molecular characterization that underlies the tumor development and progression (Menyhárt and Győrffy 2021). A more precise and complete landscape of the disease has the potential of improving the chances of a detailed diagnosis and consequently a more tailored therapeutic strategy (Karczewski and Snyder 2018).

Ovarian cancer

Based in GLOBOCAN (2020), ovarian cancer (OC) occupied the eighth place among malignant tumors with more than 310,000 new cases, accounting for 3.4% of the entire cancer-related incidence among women and the seventh place as a cause of death from cancer in women in the world with more than 205,000 deaths, accounting for 4.7% of the entire cancer-related mortality (Sung et al. 2021). In addition, to being one of the most common gynecologic cancers, they have the highest mortality rate between them and occupy the third place in mortality, only after cervical and uterine cancer (Sung et al. 2021). The mortality of this cancer is higher in countries such as China (18%) India (15.5%), the United States (6.9%), Indonesia (4.6%), Russia (3.6%), Germany (2.6%), Japan (2.6%), and Brazil (2.4%) (GLOBOCAN 2020).

The OC consists of a complex, heterogeneous group of invasive cancers that originate from different tissues and are characterized by two distinct subtypes, the most common one is the epithelial OC and only 5% has a non-epithelial origin (stromal cells and germinal cells) (Gaona-Luviano, Medina-Gaona, and Magaña-Pérez 2020). The predominant subtype has five major histologies, namely high-grade serous (the most prevalent, about 70%), endometrioid (10%), clear cell (10%), low-grade serous (<5%), and mucinous (3%), that differ in their origin, pathogenesis, molecular profile, risk factors for their development, and clinical prognosis (Stewart et al. 2019). Moreover, epithelial ovarian malignancies are characterized by type I and type II cells. Type I cells are characterized by a slow-growing phenotype and have a high 5-year survival rate (90%) when diagnosed early. By contrast type II cells, are associated with a more aggressive tumor and with fatal outcomes, because are usually diagnosed later and are often linked to the genetic mutations in *BRCA* (10–15%) and *TP53* (60–80%) genes (Chandra et al. 2019; Stewart et al. 2019). In addition, studies demonstrate that multiple genetic, epigenetic, transcriptomic, proteomic, and metabolomic alterations contribute to the progression of this aggressive cancer (Fang et al. 2018; Khella et al. 2021; Subbannayya et al. 2021; Ye et al. 2021).

The epidemiology of this cancer shows differences between ethnicities and countries due to several risk factors including genetic, reproductive, hormonal, gynecologic, lifestyle, and economic (Gaona-Luviano, Medina-Gaona, and Magaña-Pérez 2020). Despite OC can be treated by removing the tumor through surgery and by chemotherapy, the main consequences of chemotherapy are toxic effects on non-targeted tissues, drug resistance, and recurrence of OC (Lukanović, Kobal, and Černe 2022). Therefore, new tools, therapies, biomarkers, and treatment strategies are being developed, to identify and target these attempts and/or essential signaling pathways for OC.

Current application of omics science in ovarian cancer: an overview

Recently, technological advances in omics sciences in OC have allowed the application of high-throughput techniques to study the tumor in detail. Table 1 provides a summary of the recently published omics studies in OC.

Genomic techniques, such as next-generation sequencing (NGS) allowed the identification of recurrent mutations in genes such as *BRAF*, *KRAS*, *TP53*, *BRCA1/2*, and *PTEN* in OC cells (Govindarajan et al. 2020). An NGS panel consisting of 170 genes was used to analyze the genetic profiles of 132 cases of ovarian carcinoma. The results revealed that over 65% of cases were associated with high-grade serous carcinoma, and novel deletion mutations were identified in the *TP53* gene (Jung, Woo, and Kim 2019). Du et al. (2018) investigated genetic alterations in patients with recurrent OC that were resistant or sensitive to drugs. The NGS indicated that the *TP53* gene was the most frequently mutated in both types of recurrent OC. Furthermore, drug-resistant OC exhibited unique genetic changes in *BRCA1*, *MYC*, *RB1*, and *PIK3CA* compared to drug-sensitive OC. Felicio et al. (2021) utilized WES and bioinformatics analyses to identify potential associations between *RAD54L*, *FAN1*, *DROSHA*, *POLQ*, and *SLC34A2* genes and hereditary breast and/or OC in non-*BRCA1*/*BRCA2* mutation carriers patients.

Epigenomics and transcriptomics techniques are fundamental for the study of OC, as they investigate the chemical modifications in cancer cells that can affect gene expression at different stages of the disease and in different subtypes of tumors (Bisht, Arora, and Sachan 2022; Wang et al. 2019). Two opposing epigenetic phenomena occur in OC: first, an overall global decrease in DNA methylation of heterochromatin, leading to demethylation of several oncogenes (*CLDN4*, *TUBB3*, *HOXA10*, *MAL*, *BORIS*, and *ABCG2*), resulting in the reactivation of these genes, contributing to the development and progression of cancer. The second phenomenon is the hypermethylation of several tumor suppressor genes such as *BRCA1*, *RASSF1A*, *MLH1*, *CDH1*, *CDKN2A/B, DAPK*, and *APC*, which results in the loss of expression and consequently in the deregulation of the cell cycle, in the promotion of uncontrolled cell proliferation and resistance to apoptosis (programmed cell death). Thus, hypermethylation of tumor suppressor genes may contribute to the development and progression of cancer (Bisht, Arora, and Sachan 2022; Borsoi et al. 2023; Reid and Fridley 2020). Improved technology to study DNA methylation has allowed for a more agnostic approach and, with larger sample sets, has begun to unravel how epigenetics contributes to the etiology, response to chemotherapy, and prognosis of OC (Natanzon, Goode, and Cunningham 2018). Hua et al. (2021) utilized reduced representation bisulfite sequencing (RRBS), to detect hyper/hypomethylated CpG sites in *MSH2*, *LINC00261*, *MGRN1*, *ZIC5*, *EVX2*, *CCNL1*, and *DHX40* in primary platinum-resistant tumors, which may play a significant role in treatment response. Li et al. (2022) used whole-genome bisulfite sequencing (WGBS) on tissue and blood samples obtained from benign or malignant ovarian tumors and identified 1272 differentially methylated regions. Based on these regions, a supervised machine learning algorithm was able to correctly predict and classify each blood sample as malignant or nonmalignant with an accuracy of 89.5%. Huo et al. (2020) used chromatin immunoprecipitation sequencing (ChIP-seq) and messenger RNA sequencing

Table 1. Summary of the recently published omics studies on ovarian cancer.

Samples	Omics science/Method	Major Findings	References
• Low-grade serous ovarian carcinoma ($n=78$, age: 21-80 years).	• Genomic (target sequencing).	• 47% of cases with prevalent mutations in *KRAS*, *BRAF*, and *NRAS*. • Mutations in putative novel driver genes including *USP9X* (27%), *MACF1* (11%), *ARID1A* (9%), *NF2* (4%), *DOT1L* (6%), and *ASH1L* (4%).	Cheasley et al. (2021)
• Endometrioid ovarian carcinoma ($n=26$, age: 32-77) and control group.	• Genomic (whole-exome sequencing).	• Endometrioid ovarian carcinoma has a distinct and heterogeneous genomic profile with mutations in *PTEN*, *CTNNB1*, *PIK3CA*, *ARID1A*, and *TP53*. • Mutation in *KMT2D*, *KMT2B*, and *PIK3R1* genes occurred at similar frequencies in endometrial carcinoma.	Pierson et al. (2020)
• Epithelial ovarian carcinoma ($n=25$, age: 32-58 years) and control group: benign ovarian tumors and normal ovarian tissues ($n=25$, age: 39–60 years).	• Epigenomic (pyrosequencing-based DNA methylation analysis).	• The methylation levels at five out of six sites of the *PCDH17* gene promoter were significantly higher in epithelial ovarian cancer compared to the control group.	Elsharkawi et al. (2023)
• High-grade serous ovarian carcinoma ($n=68$, age: 40–79 years) and control group: benign ovarian tumors ($n=53$, age: 32–85 years)	• Epigenomic (methylation-sensitive high-resolution melting). • Transcriptomic (RNA-seq).	• Hypermethylation of *PCDH17*, *CDH13*, *HNF1B*, and *GATA4* genes (100% specificity and sensitivity of 88.5%). • Downregulation of *PCDH17*, *CDH13*, *HNF1B*, and *GATA4* genes compared to the control group.	Baranova et al. (2020)
• High-grade serous ovarian carcinoma ($n=28$, age: 44–75 years)	• Epigenomic (whole genome bisulfite sequencing). • Transcriptomics (RNA-seq).	• Significant methylation pattern in tumors from *BRCA1/2* carriers compared to non-*BRCA1/2* carriers. • Overexpression of genes in *BRCA1/2* carriers compared to non-carriers.	Gull et al. (2022)
• High-grade serous ovarian carcinoma ($n=21$, age: 40–80 years), clear cell carcinoma ($n=6$, age: 50–63 years), and control group: normal ovarian tissues ($n=6$, age: 41–66 years).	• Transcriptomics (RNA sequencing).	• *RXFP1*, *FUT4*, and *CPNE8* are highly expressed genes from high-grade serous ovarian carcinoma. • *BHLHE41*, *NOL4L*, and *PRSS1* are highly expressed genes from clear cell carcinoma.	Nagasawa et al. (2019)
• Epithelial ovarian carcinoma ($n=10$) and control group: normal ovarian tissues ($n=10$).	• Proteomics (iTRAQ and HPLC/MS analysis).	• 408 differentially expressed proteins in epithelial ovarian carcinoma. • CLIC4, STK1, AIMP1, SNX3, FAM49B, FERMET3, TUBB3, and lactotransferrin as potential protein tumor markers.	Peng et al. (2019)
• High-grade serous ovarian carcinoma ($n=10$) and control group: benign ovarian tumors ($n=10$).	• Proteomics (LC-MS/MS analysis).	• Proteome and phosphoproteome analysis reveal differentially regulated signaling processes in ovarian cancer and benign tissues. • Mutations in the *BRCA1* gene are associated with changes in the proteome, affecting areas such as DNA repair, splicing, transcription regulation, and signaling.	Bradbury et al. (2022)
• Epithelial ovarian carcinoma ($n=26$, age: 32–72 years), benign ovarian tumor ($n=25$, age: 26–72 years), and control group: normal ovarian tissues ($n=25$, age: 28–69 years).	• Metabolomic (LC-ESI-MS/MS).	• The level of 21 metabolites differed significantly between the groups. • The pathway analysis revealed 11 metabolic pathways potentially related to ovarian cancer.	Plewa et al. (2019)
• Epithelial ovarian carcinoma ($n=39$, age: 42–51 years) and control group: normal ovarian tissues ($n=31$, age: 40–52 years).	• Metabolomic (UPLC-ESI-MS/MS and GC-MS/MS).	• The levels of 14 metabolites were significantly altered in epithelial ovarian carcinoma. • Methionine, glutamine, asparagine, glutamic acid, and glycolic acid were identified as biomarkers to distinguish ovarian cancer from controls.	Wang, Zhao, et al. (2021) and Wang, Dong, et al. (2021)

Abbreviations: AIMP1: aminoacyl tRNA synthetase complex interacting multifunctional protein 1, *ARID1A:* AT-rich interaction domain 1 A, *ASH1L:* ASH1 like histone lysine methyltransferase, *BHLHE41:* Basic helix-loop-helix family member e41, *BRAF:* B-Raf proto-oncogene, serine/threonine kinase, *BRCA:* Breast cancer gene, *CDH13:* Cadherin 13, CLIC4: Chloride intracellular channel 4, *CPNE8:* Copine 8, *CTNNB1:* Catenin beta 1, *DOT1L:* DOT1 like histone lysine methyltransferase, FAM49B: Family with sequence similarity 49 member B, FERMET3: Fermitin family homolog 3, *FUT4:* Fucosyltransferase 4, *GATA4:* GATA binding protein 4, GC-MS/MS: Gas Chromatography-Tandem Mass Spectrometry, *HNF1B:* HNF1 homeobox B, HPLC/MS: High-Performance Liquid Chromatography/Mass Spectrometry, iTRAQ: Isobaric Tags for Relative and Absolute Quantitation, *KRAS:* Kirsten rat sarcoma viral oncogene homolog, *MACF1:* Microtubule actin crosslinking factor 1, *NF2:* Moesin-ezrin-radixin like (MERLIN) tumor suppressor, *NOL4L:* Nucleolar protein 4 like, *NRAS:* NRAS proto-oncogene, GTPase, *PCDH17:* Protocadherin 17, *PIK3CA:* Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, *PIK3R1:* Phosphoinositide-3-kinase regulatory subunit 1, *PRSS1:* Serine protease 1, *PTEN:* Phosphatase and tensin homolog, *RXFP1:* Relaxin family peptide receptor 1, SNX3: Sorting nexin-3, STK1: Serine-threonine kinase Stk1, *TP53:* Tumor protein p53, TUBB3: Tubulin beta 3 class III, UPLC-ESI-MS/MS: Ultra-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry, *USP9X:* USP9X ubiquitin specific peptidase 9 X-linked.

(mRNA-seq) techniques to explore the function of the *EZH2* gene in OC. The author's findings indicated that the knock-out of *EZH2* activated a key gene involved in steroid biosynthesis (*CYP27B1*) through the methylation of H3K27me3 histones and significantly suppressed cell proliferation, migration, and invasion in OC. The single-cell RNA-seq technique allowed the identification of differential gene expression between OC samples and origins, including genes associated with different tumor grades. Furthermore, the proportion of different cell populations varied between primary and metastatic tumors, suggesting that single-cell RNA-seq plays a key role in providing advanced insights into tumor heterogeneity and detailed insight into gene expression at the single-cell level contributing to advances in understanding and treating cancer (Shih et al. 2018).

Proteomics and metabolomics approaches are also used to investigate the metabolic and signaling pathways involved in OC. The identification of specific proteins that are overexpressed or underexpressed and the discovery of specific metabolites in OC can be used as therapeutic targets

(Tayanloo-Beik et al. 2020). Zhang et al. (2019) employed isobaric tags for relative and absolute quantification (iTRAQ) labeling coupled with liquid chromatography-mass spectrometry (LC-MS) to demonstrate the potential of circulating extracellular vesicles (EVs) as a noninvasive diagnostic tool for OC. The study findings revealed that individuals with epithelial OC showed 200 upregulated and 208 downregulated proteins when compared to the control. Among the 408 significantly differentially expressed proteins, five proteins were chosen as potential candidate markers for the validation of diagnosis evaluation related to EVs, namely EpCAM, C1q, ApoE, Plasminogen (upregulated proteins), and Serpin C1 (downregulated protein), suggesting that circulating EVs may be a potential tool for noninvasive ovarian cancer diagnosis. Due to their ability to carry complex information from donor cells and their ease of collection, EVs are being explored as potential biomarkers in cancer (Zhou et al. 2021). EVs play a crucial role in cell-to-cell communication and have pivotal roles in the coagulation process, implying the inherent mechanism of generation of thrombus which often occurs in OC patients at late stages (Zhang et al. 2019). Additionally, Zhang et al. (2019) using solid phase microextraction (SPME) three orthogonal LC/MS acquisition modes, and multivariate analysis, observed that metabolic and lipid data were able to provide clear discrimination between the five main subtypes of epithelial OC: high-grade serous, low-grade serous, endometrioid, clear cell, and mucinous.

Regarding metabolomics analysis, Hishinuma et al. (2021) conducted a study where they analyzed the plasma metabolome profiles of patients with epithelial OC using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). They found that 77 metabolites were significantly increased and 114 metabolites were significantly decreased in patients with epithelial OC compared to healthy controls, indicating that metabolomics analysis is a promising technique for biomarker discovery.

Recent technological advances in omics sciences, including genomics, epigenomics, transcriptomics, proteomics, and metabolomics, provide new insights into the molecular pathogenesis of OC. These tools provide critical information that can be used to identify specific genes, proteins, and metabolites profile involved in the development and progression of OC. These approaches have the potential to help delineate the molecular mechanisms of OC, significantly impacting therapy, diagnosis, and prognosis of the disease.

Concepts and small observations on nutritional genomics and microbiome related to the intake of dietary polyphenols and their possible effects on ovarian cancer

Our diet consists of a mixture of carcinogenic and anticarcinogenic nutrients which are metabolized by the enzymes of the biotransformation process (Açar and Akbulut 2023). Therefore, understanding the interactions between nutrition, metabolism, and gene expression is crucial to understanding the mechanisms involved as well as establishing diets as therapeutic options and/or adjuvant therapies in cancer (Martínez-Garay and Djouder 2023).

In this context, genomic technologies have led to breakthroughs toward understanding and management of health and disease. Nutritional genomics, for example, keeps the focus on how dietary components (bioactive, macro, and micronutrients) may affect the expression patterns of several genes as well as protein transcription and subsequently, metabolite production (i.e. proteome, transcriptome, and metabolome) (Dall'Asta et al. 2022). Thus, nutrigenomics integrates the so-called nutrigenetics, nutrigenomics, and nutri(epi)genomics (Norheim et al. 2012). It is a tool that has been applied to the understanding of the molecular mechanisms underlying the direct beneficial effects of dietary components such as dietary polyphenols (Dall'Asta et al. 2022). However, due to the fact that there are still errors and conceptual distortions between practitioners and academics, a little contextualization is necessary.

To know both nutrigenetics and nutrigenomics are guided by three important pillars. First, it is understood that the bioavailability and metabolism of dietary components are affected by individual genetic profiles (i.e. by the diversity of genotypes) that are inherited between ethnic groups and individuals. Second, it is known that cultural, economic, and geographic differences have a strong influence on food choices, perception of flavors, and availability of foods and nutrients. Third, it is taken into account that gene expression and genome stability are influenced by malnutrition, either by deficiency or excess; this can lead to mutations in the gene sequence or at the chromosomal level which can cause abnormal gene dosage and gene expression leading to adverse phenotypes during various life stages (Jabeen et al. 2023; Simopoulos 2020).

Given that, nutrigenetics is the science that analyzes how genetic variability (*e.g.,:* single nucleotide polymorphism (SNP) between individuals in relation to nutritional needs, health status, and risk of developing diseases (Balkir, Kemahlioglu, and Yucel 2021; Braconi et al. 2021). Whereas, nutrigenomics focuses on assessing how nutrients and bioactive compounds in food influence the activity of genes, increasing or reducing their protein expression (Jabeen et al. 2023). Unlike genetic variations, which are fixed, epigenetic modifications are temporary and may vary within a generation as an immediate response to the environment or metabolism (Borsoi et al. 2023; Neri Numa and Pastore 2020) (Figure 1).

Whereupon, studies on gene-bioactive compound interaction aim to explain how food intake, at a molecular level, leads to better health outcomes. Polyphenols, for example, are the most abundant group of secondary metabolites in our food, present in fruits, vegetables, and marine organisms. They comprise a wide group of chemical substances (e.g. flavonoids, stilbenes, lignans, and phenolic acids) with different biological activities (Francenia Santos-Sánchez et al. 2019; Tresserra-Rimbau, Lamuela-Raventos, and Moreno 2018). In addition to their antioxidant effects, they are able to act in various biological signaling pathways, including interacting with proteins involved in the transcription and expression of genes related to metabolism, inflammation as

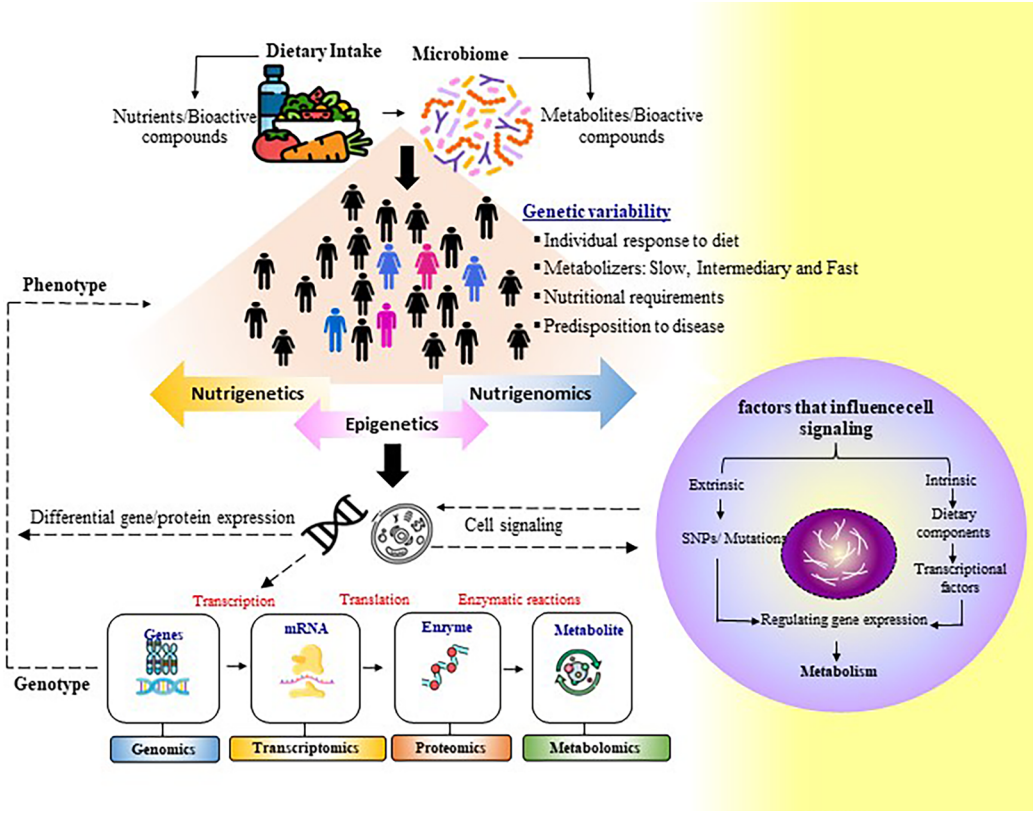

Figure 1. Schematic illustration of nutritional approach showing the typical conditioners related to the dietary polyphenols effects on genes, cell signaling, and metabolic networks involved in a phenotypic characteristic related to the health and/or disease. Foods rich in polyphenols may exert a great impact on health outcomes, mainly because they are able to modulate both genic expression and gut microbiota that are involved in key pathways. However, such response to an ingested polyphenols dose varies between individuals. In this sense, the omics-based biomarkers (transcriptomics, proteomics, metabolomics, and microbiomics) may help us understand how nutrients and bioactive compounds influence: (1) gene expression and consequently the phenotype; (2) which regulatory pathways are involved in the individual's responses; (3) the role of the microbiota in host health and disease, amongst others. To know: the metabolic profile of each individual can be altered by polymorphisms, thus characterizing the following phenotypes: slow metabolizers, intermediate and fast metabolizers. Thus, slow metabolizers, in general, are individuals with a decrease or absence of the metabolizing enzyme, which may result from gene deletion or mRNA instability. Intermediate metabolizers are those with "normal" metabolism, common in the majority of the population. Whereas, the rapid metabolizer phenotype may be due to an increase in the production of the metabolizing enzyme associated with one or multiple duplications of the gene that encodes the enzyme. In addition, each individual has a unique gut microbiota profile which is responsible for performing multiple and specific roles in host nutrient metabolism. Source: (Rowland et al. 2018); Icon credit: https://www.flaticon.com.

well as cell growth and proliferation (Antónia Nunes et al. 2018; Sharma and Padwad 2020).

For example, dietary polyphenols that are able to target a number of key genes in cancer hallmarks leading to carcinogenesis (Cipolletti et al. 2018). In this sense, both meta-analysis and prospective studies have suggested a protective effect of flavonoids, flavonols, and flavanones against OC and a reduced risk of OC (Cassidy et al. 2014; Gates et al. 2007; Hua et al. 2016). Epigallocatechin-3-Gallate (EGCG), curcumin, and resveratrol have been reported as potential chemopreventive and curative agents. Some research on these bioactive compounds has proposed several pathways that interact in cancer management, either interfering or reversing the carcinogenic process. Or even influencing the action of target molecules during intracellular signaling or, in the development and/or maintenance of the disease (Farhan 2023).

Curcumin has been shown to inhibit histone deacetylases (HDACs) and hence control the proliferation of ovarian cancer cells by regulating microRNAs (miRNAs) including miR-125-5p, miR-19a, miR-9, and miR-145 (Farhan 2023; Hassan et al. 2019). Comparatively, resveratrol can modulate the expression of miR-200, miR-122-5p, miR-20, and mIr-663 145 (Farhan 2023). Also, it was suggested that resveratrol-induced apoptosis in cancerous cells is associated with increased levels of miR-424-3p and reduced levels of galectin-3 (El-kott et al. 2019).

In a previous review it was nicely summarized some of the most recent findings regarding the role of quercetin and their possible molecular mechanisms enrolled in ovarian cancer inhibition. One of the results showed that quercetin is able to decrease several anti-apoptotic molecules, including Bcl-2 and Bcl-xL, and increase pro-apoptotic molecules, including caspase-3, caspase-9, Bid, Bax, Bad, and cytochrome c. Likewise, quercetin can induce ER stress, apoptosis, and autophagy through a p-STAT3/Bcl-2 axis (Shafabakhsh and Asemi 2019).

In addition, genistein, a flavonoid commonly found in soy-based products with several health benefits, including its anti-tumor properties, exerts an inhibitory effect in OC cells through estrogen receptor subtype (ERα or ERβ)-mediated molecular mechanisms as well as to show modulate miRNAs

(Chan et al. 2018; Kim, Choi, and Hwang 2015; Parker et al. 2009).

It was also found that a polyphenolic flavonolignan called silibinin, could overcome paclitaxel resistance and decrease the invasiveness of paclitaxel-resistant human ovarian carcinoma cells (Zhou et al. 2008). Similarly, resveratrol reverses ovarian cancer cell resistance to cisplatin by modulating cancer cell proliferation through MAPK inhibition (Lin et al. 2010). While preclinical bioinformatic studies based on molecular docking and molecular dynamics simulation showed that taxifolin (flavonoid commonly found in onion, milk thistle, French maritime pine bark, and Douglas fir bark), might bind molecules of protein kinases B (AKT1) and, thus, inhibit ovarian malignant cells (Ajjarapu et al. 2021). Table 2 shows the current studies of molecular mechanisms of action by polyphenols in ovarian cancer.

It should also be noted that dietary polyphenols (depending on the degree of polymerization of each compound) will only be effective if they are bioaccessible and bioavailable (Cardona et al. 2013). For it, they need to be absorbed and metabolized, and then, biotransformed in the gastrointestinal tract (GIT) by intestinal microbiota (Barreira, Alvarez Arraibi, and Ferreira 2019; Cardona et al. 2013; Stockert and Hill 2018). Thus, the bioavailability of polyphenols happens as the phenolic fractions are released from the food during ingestion; that is, since these are bioaccessible, their metabolic derivatives will be absorbed by the intestinal cells and transported to the tissues, where they will exert their biological functions (de Araújo et al. 2021; Neri-Numa et al. 2020; Sarubbo et al. 2023).

Most polyphenols in order to become bioactive in the human body, undergo complex intraluminal transformation during digestive and absorptive processes as a result of the action of the metabolism of the intestinal microbiota. Since their active metabolites play a fundamental role in the remodeling of the composition and diversity of the intestinal bacterial communities which results in maintaining normal physiology (de Araújo et al. 2021; Frolinger et al. 2019; Neri-Numa et al. 2020). Given that, the overall microbiota diversity is impacted by diet and/or specific dietary compounds which in turn are able to affect not only the gene expression patterns but also the epigenetic mechanisms as well as the production of metabolites and the bacterial composition of the microbiota (Figure 2) (Aruoma et al. 2019). Therefore, in conditions of intestinal microbiota imbalance leading to dysbiosis, that is, increase in the number of pathogenic bacteria to the detriment of commensal bacteria (Neri Numa and Pastore 2020). It, in turn, triggers a series of inflammatory processes, the release of toxins and carcinogenic metabolites which contribute to cancer susceptibility (Chen et al. 2018; Vivarelli et al. 2019).

In the case of OC, both alterations in microbial diversity or the richness of the gut and reproductive tract have been linked to preconditions for the development and/or progression of OC; given that Proteobacteria has been the most dominant taxon in cancer patients as opposed to Firmicutes being the most dominant taxon observed in a normal healthy adult female. Furthermore, stroboloma dysbiosis (Bacteriodete, Firmicutes, Actinobacteria, and Proteobacteria) has an indirect association with OC (Dhingra et al. 2022). Furthermore, healthy diets rich in polyphenols can decrease the risk of developing various types of cancer and reduce the cancer-related mortality rate (Chen et al. 2018). As flavonoids as their microbial metabolites may interact with DNA, RNA, or proteins involved in the activation cascade, changing number, function, and structure. For example, they might be important mediators of the diet-epigenome interaction since there are implicated in reversing adverse epigenetic regulation by altering DNA methylation and histone modification, and they modulate miRNA expression (Neri Numa and Pastore 2020).

However, these modifications translated into phenotypes are still very complex, due to metabolic, physiological, and genetic variability; Roughly speaking, this depends on the complexity of the interaction and variation in the mode of action of this class of bioactive compounds (Norheim et al. 2012; Singh 2023). Thus, microbial contribution to host responses to ingested nutritive molecules has been considered an interdisciplinary subfield in studies of nutritional genomics (Ferguson et al. 2016; Malcomson and Mathers 2023).

For example, some evidence has shown that the estrogen-gut microbiome axis may trigger different gynecological cancers once the production of bioactive circulating levels of estrogen and its metabolites is regulated by the gut microbiome through the estrobolome (AlHilli and Bae-Jump 2020; Hawkins et al. 2022; Walther-António et al. 2016). Also, A well-balanced vaginal microbiome generally contains bacteria from the Firmicutes and Lactobacillus species while gynecological disorders are associated with a decrease in Lactobacillus species and an increase in the prevalence of anaerobic bacteria such as Escherichia and Bacteroides species (AlHilli and Bae-Jump 2020; Walther-António et al. 2016).

A case-control study in two sets of women aged 18–87 years in the Czech Republic, Germany, Italy, Norway, and the UK observed that the presence of ovarian cancer, or factors known to affect risk for the disease (i.e. age and BRCA1 germline mutations), were significantly associated with having a community type O cervicovaginal microbiota (Nené et al. 2019). In the same way, another study demonstrates how gut microbiota dysbiosis promoted epithelial ovarian cancer progression *via* regulating the Hedgehog signaling pathway (Hu et al. 2023). Thus, undoubtedly, both fields of nutrigenomics and microbiome may contribute to understanding the conditions that favor a state of well-being or the emergence of diseases as well as their management.

Another aspect to consider is that the components contained in the food we eat exert a physiological effect on cells, organs, or the entire organism (Balkir, Kemahlioglu, and Yucel 2021; Moore 2019); what also may affect and be affected by the intestinal microbiome and that these microorganisms produce metabolites that act as allosteric regulators and cofactors of epigenetic processes (Dall'Asta et al. 2022; Neri Numa and Pastore 2020). Thus, responses to ingested bioactives are intrinsically related to the unique

Table 2. An overview of the beneficial effects of polyphenols in ovarian cancer.

Polyphenol/Extract	Experimental model/Condition	Molecular Mechanism	Main outcomes	References
Quercetin	Human metastatic ovarian cancer cell line (PA-1) treated with quercetin (0–200 μM) for 24 h.	Quercetin upregulates proapoptotic molecules (caspase-3, caspase-9, Bid, Bad, Bax, and cytochrome c) while downregulates antiapoptotic molecules (Bcl-2 and Bcl-xL) in PA-1 cell line.	↓ cell viability and ↑ apoptosis.	Teekaraman et al. (2019)
Kaempferol	Human ovarian cancer cell lines (CAOV-3, TOV-112D, SKOV-3, and OVCAR-3) were treated with kaempferol (0–50 μM) for 24 h.	Kaempferol upregulates caspase 3, 8, and 9, and Bax and downregulates Bcl-2, Cdc2, and cyclin B1. Kaempferol suppresses MAP2K/ERK and STAT3 signaling pathways concentration-dependently.	↓ cell proliferation, ↑ apoptosis, and ↑ cell cycle arrest at G0/G1.	Yang et al. (2019)
Resveratrol	Human ovarian cancer cell lines (OVCAR-3, OAW42, and KURAMOCHI) were induced by IL-6 and after 5 days treated with resveratrol (0–100 μM).	Resveratrol upregulates ARH-I p21, and p38, downregulates Ki-67, cyclin D, and miR-1305, and suppresses ERK1/2 and STAT3 signaling pathways.	↓ autophagy, ↓ tumor growth, and ↑ cell cycle arrest at G0/G1.	Esposito et al. (2022)
	Human ovarian cancer cell lines (OV-90 and SKOV-3) were treated with resveratrol (0–400 μM) for 24 h.	Resveratrol upregulates miR-34a by targeting and downregulating Bcl-2.	↓ cell viability, ↓ cell invasion, ↓ cell migration, and ↑ apoptosis.	Yao et al. (2021)
Polyphenols extracted from Chinese hickory seed skin *Carya cathayensis* (CHSP)	Human ovarian cancer cell lines (OVCAR-3, A2780/CP70) were treated with CHSP (5–20 μg/mL) for 24 h.	CHSP extract inhibited secretion of VEGF by regulating the HIF-1α-VEGF pathway, upregulates caspase 3/7, Bax, Bad, PARP, Puma, and PTEN, and downregulates NFκB and HIF-1α protein expression.	↑ apoptosis, ↓ angiogenesis, and ↓ cell viability.	He et al. (2020)
Green tea extract + paclitaxel	Human ovarian cancer cell lines (SKOV-3 and OVCAR-3) were treated with a combination of green tea and paclitaxel (20 and 40 μg/mL) for 24 h.	The combination of green tea + paclitaxel inhibited the p-Akt, upregulates caspase-3, caspase-9, Bad, Bax, and cytochrome c, and downregulates Bcl-2.	↓ cell viability and ↑ apoptosis.	Panji et al. (2021)
Epigallocatechin-3-gallate (EGCG)	Human ovarian cancer cell lines (SKOV-3, CAOV-3, and OVCAR-3) were treated with ECGC (0 – 80 μg/mL) for 24, 48, and 72 h. / Xenograft model – Female BALB/c nude mice, aged 4-5 wk ($n = 7$), treated with EGCG (0 – 50 mg/kg twice a week) for 3 wk.	EGCG increased the expression of Bax, caspase-3, and PTEN and decreased the expression of Bcl-2, AKT, and mTOR. EGCG inhibits the PTEN/AKT/mTOR pathway activation	↓ cell proliferation, ↓ cell viability, and ↑ apoptosis	Qin et al. (2020)
Curcumin	Human ovarian cancer cell lines (SKOV-3 and A2780) were treated with curcumin (0–40 μM) for 24, 48, and 72 h/Murine xenograft model – Female BALB/c athymic mice, aged 5 wk ($n = 20$), treated with curcumin (15 mg/kg/every 2 days) for 5 wk.	circ-PLEKHM3 overexpression enhanced curcumin-mediated promotion of c-caspase-3 and Bax protein expression, and reduction of PCNA protein expression. Curcumin regulates the circPLEKHM3/miR-320a/SMG1 axis	↓ cell proliferation and ↑ apoptosis.	Sun and Fang (2021)
Luteolin	Human ovarian cancer cell lines (ES2 and A2780) treated with luteolin (0–30 μM) and luteolin + cisplatin (0–10 μM) for 72 h / patient-derived xenograft models – Female nude mice (NSG and BALB/c) aged 4 wk, treated with luteolin by injections once per week intraperitoneally for 4 wk (control group, 10 mg/kg luteolin, 0.5 mg/kg cisplatin, and 10 mg/kg luteolin plus 0.5 mg/kg cisplatin; $n = 5$/group). Oral administration (0 or 50 ppm/day for 4 wk, $n = 6$/group).	Luteolin downregulates 150 and 54 genes in A2780 and ES2 cells, increased p53 phosphorylation at ser15 and ser46, and decreased MDM2 expression compared to controls by downregulation VRK1, CREB, ERK1/2. Histone H3 phosphorylation was decreased while p53 phosphorylation was increased in tumors in the luteolin-treated group than in the control group in both therapies reducing the tumor growth *in vivo*.	↓ cell proliferation, ↑ apoptosis, ↑ cell cycle arrest at G2/M, and ↓ tumor growth.	Chang et al. (2023)
	Ovarian cancer stem-like cells (OCSLCs) isolated by suspension culture and CD133 + ALDH + cell sorting were employed as OCSCs model and treated with luteolin (30 μM) for 48 h / Cell-derived xenograft model – Female BALB/c nude mice, aged 6-8 wk, treated with luteolin by an intravenous single dose (100 mg/kg/week) for 3 wk.	Luteolin directly binds to KDM4C, blocks KDM4C-induced histone demethylation of the PPP2CA promoter and inhibits PPP2CA transcription and PPP2CA-mediated YAP dephosphorylation, thereby attenuating YAP activity and the stemness of OCSLCs.	↓ stemness and ↓ tumor growth.	Li et al. (2023)

Symbol: ↓ the sample induced a significant reduction; ↑ the sample induced a significant increase.

Abreviations: ARH-I, Aplysia ras homology member I; Bad, BCL2-associated agonist of cell death; Bax, Bcl-2-like protein 4; Bcl-2, B-cell lymphoma 2; Bid, BH3 interacting-domain death agonist; Ccd2, Cyclin-dependent kinase; Cmyc, MYC proto-oncogene; CREB, cAMP response element-binding protein; ERK1/2, Extracellular signal-regulated kinases; HIF-1α, hypoxia-inducible factor-1α; KDM4C, Lysine Demethylase 4C; MAP2K, Mitogen-activated protein kinase kinase; mTOR, Mammalian target of rapamycin; NFκB, Factor nuclear kappa B; P21, cyclin-dependent kinase inhibitor p21; P38, Mitogen-activated protein kinases; P53, Tumor protein p53; p-Akt, Phosphorylated Protein kinase B; PARP, Poly (ADP-ribose) polymerase; PCNA, Proliferating cell nuclear antigen; p-p70S6K, Phosphorylated Ribosomal protein S6 kinase beta-1; PPP2CA, Protein Phosphatase 2 Catalytic Subunit Alpha; PTEN, Phosphatase and tensin homologue; Puma, p53 upregulated modulator of apoptosis; SMG1, Serine/threonine-protein kinase; STAT3, Signal transducers and activators of transcription; VEFG, Vascular endothelial growth factor; VRK1, Vaccinia-related kinase 1; YAP, Yes1 Associated Transcriptional Regulator.

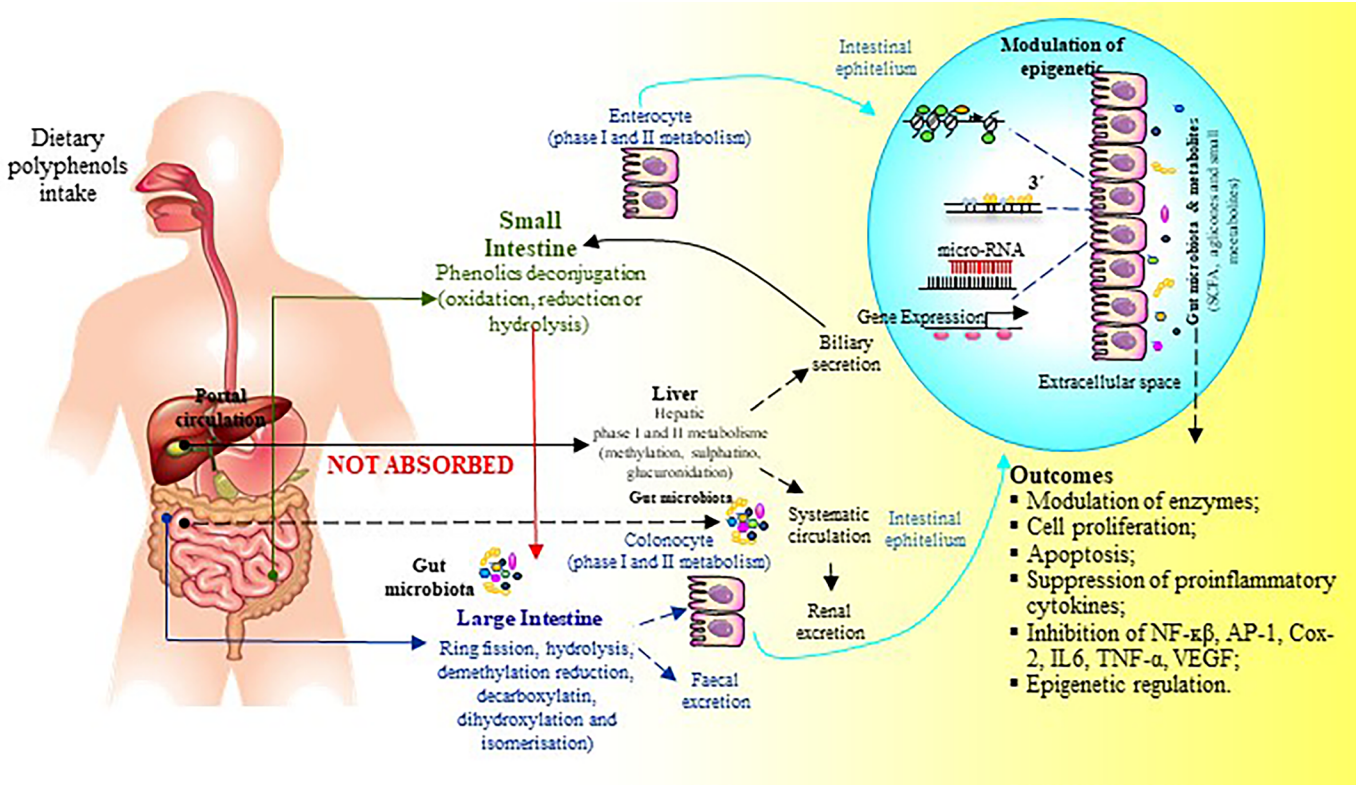

Figure 2. Simplified schematic illustration of bioaccessibility/bioavailability of dietary polyphenols and the impact on human health through interaction with the gut microbiota and epigenetic modulation. For polyphenols exerts their health benefits, first, they need to be released from the food matrix solubilized within the gastrointestinal fluids, be absorbed by the epithelium cells, and reach the target tissues at sufficiently high levels to perform their systemic effects. However, only a small part is absorbed in the small intestine. Then, the compounds that are not absorbed in this first gut portion, reach the colon and undergo important structural modifications where the gut microbiota hydrolyzes glycosides into aglycones and degrades them to active metabolites which in turn are closely related to the biological activity of polyphenols. For example, complex crosstalk occurring between the host and its gut microbiota may affect pathological processes, such as cancer genesis and development, either in a positive or in negative way, depending on its composition. In a positive way, microbiota-derived metabolites act as epigenetic substrates or regulators of chromatin-modifying enzymes, stimulating histone hyperacetylation and the changes in gene expression patterns. These in turn enhance apoptosis and stop cell division with both anti-inflammatory and anticarcinogenic effects. Source: (Borsoi et al. 2023; Neri-Numa et al. 2020; Neri Numa and Pastore 2020). Icon credit: https://www.flaticon.com.

characteristics of each individual concerning metabolic, physiological, and genetic (Norheim et al. 2012; Singh 2023).

In this context, green tea catechins have been demonstrated effectiveness against gynecological cancers due to their capacity to downregulate the expression of proteins involved in inflammation, cell signalization, cell motility, and angiogenesis as well as inhibiting DNA methyltransferases and histone deacetylases in human cervical cancer (Khan et al. 2015; Parish et al. 2023). For example, it was demonstrated that natural epigenetic compounds such as ECGC can regulate the secretion of protumorigenic growth factors, cytokines, extracellular matrix components, and immunoregulatory markers in human ovarian cancer specimens (Kelly et al. 2023).

In light of this, it is possible to combine microbiome and genomic analyses to evaluate polyphenols' effects on the risk of chronic diseases through biomarkers of serum, urine, and feces (Si et al. 2021). Therefore, properly channeled dietary bioactive compounds and nutrigenomic applications can have an extremely positive impact on public health. In parallel, precision nutrition is becoming a newly recognized phenomenon in nutrition research since it combines food and lifestyle, as well as the use of individual biomarkers to create suggestions for healthy menus that are more relevant to the inter-individual variability driven by complex gene-nutrient-environment (Dj Nevena et al. 2022; Rust and Haslberger 2022) as we will discuss in the next section.

Precision nutrition: definition, current status in cancer research and data management

Advances in the fields of oncology and nutrition have served as the basis for establishing personalized nutritional recommendations to improve the quality of life and better survival of cancer patients (Heber 2024). In the case of patients with OC, the nutritional status is closely related to the accuracy of assessments, monitoring, and improvement of nutritional status during clinical follow-up. Therefore, a systematic and comprehensive assessment can provide us with data for an intelligent nutritional diagnosis, as well as laying the foundation for implementing personalized and precise nutritional therapy in order to improve the patient's quality of life and reduce the risk of muscle wasting and the malnutrition (Mu et al. 2022); however, there still lack adequately powered randomized controlled trials evaluating its effectiveness.

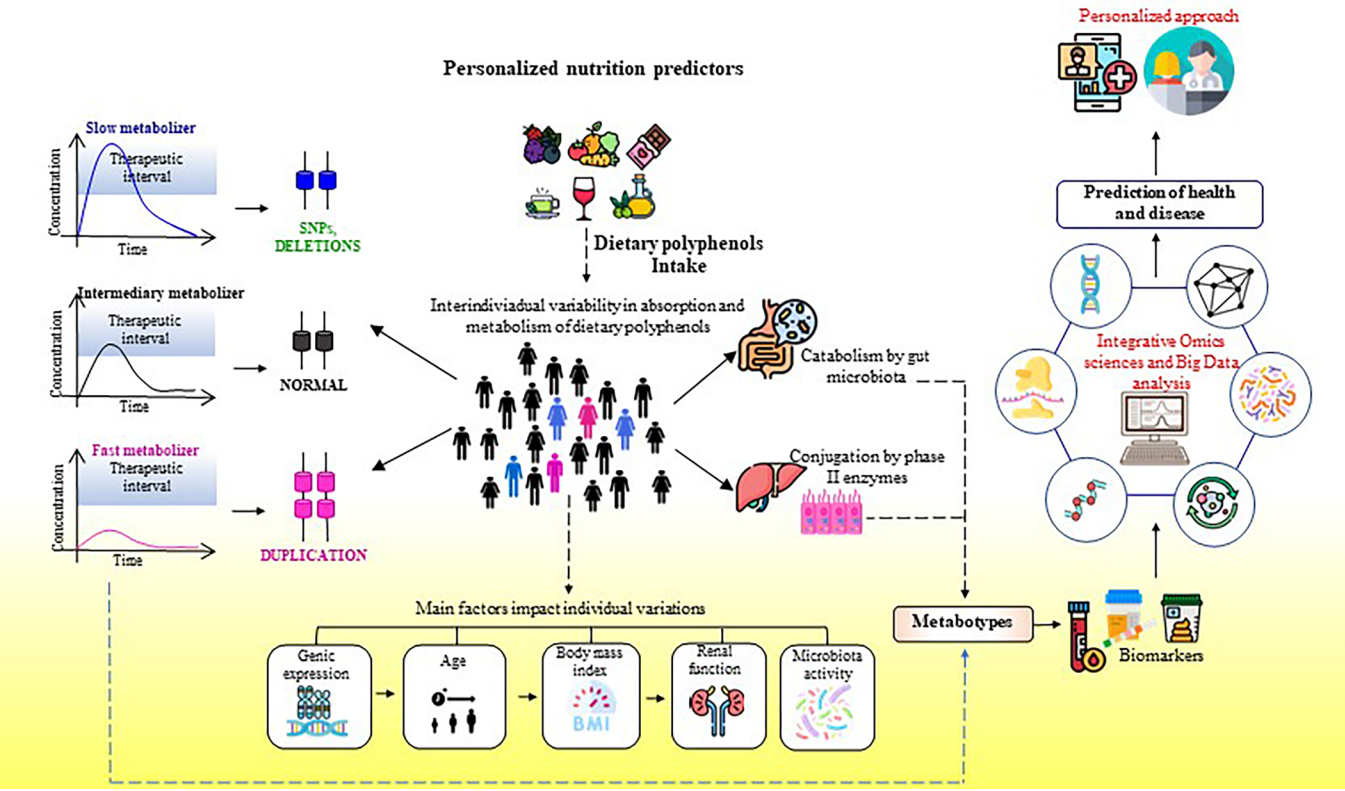

Figure 3. Schematic illustration of the uses of the individual information and responses to the dietary patterns (e.g. polyphenols intake) to predict the prevention and/or management of diseases in a personalized way. Individuals respond differently to the diet, leading to different associations between health and disease. Several factors including genetic background, inherited epigenetics, nutrient/bioactive compounds bioavailability, microbiome-derived metabolites, and lifestyle are closely linked to gene modulation, protein expressions, inflammation, and metabolic phenotypes. Thus, to better understand the influence of polyphenols on the normal biological processes, pathological processes, or pharmacological responses to therapeutic/nutritional interventions and particularly in cancers it is of crucial importance to access biomarkers (genes, proteins, metabolites, glycans, and other molecules). Biomarkers, in turn, coupled with omics-based approaches coupled with and Big data & Analytics may give insights not only for risk prediction and early diagnosis but also for guiding tailored dietary treatments and prognosis schemes. Source: (Antónia Nunes et al. 2018; Ramos López et al. 2022; Singh 2023); Icon credit: https://www.flaticon.com.

The BENITA-study, a pilot randomized controlled trial suggested the effectiveness of the combined exercise and nutritional interventions during and after treatment. This study comprised two phases, where nutritional intervention in phase I aimed to reduce the risk of malnutrition by increasing protein and calorie intake; whereas in phase II, nutritional counseling was carried out based on the Mediterranean diet (known to be rich in dietary flavanones, anthocyanidins, flavones, and flavonoids) which has been proven to reduce malnutrition and cancer risk (Maurer et al. 2022).

Despite there being no consensus on its definition, precision nutrition is a highly interdisciplinary field that combines findings from health history, chronological and biological ages, lifestyle, microbiome composition, and even genome sequencing to guide to increase life expectancy by improving the prevention and management of diseases (Dall'Asta et al. 2022). The main objective is to design dietary interventions according to the phenotypic and genotypic characteristics of individuals in treating and/or preventing diseases in a way more effective (Figure 3) (Chaudhary et al. 2021).

According to Zeisel (2020), precision nutrition is preferred over personalized nutrition, as because in terms of dietetic recommendations it is practically impossible to achieve a unique prescription for each individual. On the contrary, it is perfectly possible to subcategorize people into smaller and smaller groups based on biomarkers aiming to better estimate the different subgroups' dietary requirements.

A substantial body of scientific evidence supports the construction of personalized/accurate nutritional approaches to cancer prevention/treatment. However, unfortunately, most research efforts have been focused on improving dietary intake, combating overweight and obesity, and reducing the risk of cardiometabolic diseases (Malcomson and Mathers 2023). Although there are few studies of individualized nutritional treatment for cancer patients, (Barnett 2023; Malcomson and Mathers 2023) It is expected that in the next 5–10 years, nutrition should become part of the standard of care in treatment plans, with clinical trials supporting the use of specific diets (Barnett 2023; Malcomson and Mathers 2023). However, it is also important to point that there are still great challenges to be overcome in this area. The first concerns the complexities of food matrices and the fact that most chronic diseases are polygenic, that is, diseases consisting of many genes located at different loci, each with small effects on the trait, producing quantitative changes (Pointner and Haslberger 2022). The second point is the requirement of sophisticated research studies and also the high costs of incorporating new technologies entailing restrictions for developing countries.

Likewise, multi-omics analyses applied to precision nutrition generate large, complex, and multidimensional datasets, both in content, structure, and storage format with the use of personal wearable devices (including smartphones) (Dikarlo et al. 2022). These digital biomarkers enable real-time remote monitoring of patients in a comprehensive and personalized non-intrusive manner, which reflects significant variations in functional status, symptom burden, quality of life, and risk of adverse clinical outcomes (Low 2020).

Such huge amounts of data require complex methods for data curation and storage, as well as intensive statistical approaches and programming models to extract relevant information (Bakhtin et al. 2020). Therefore, Big Data techniques have been increasingly necessary to collect and analyze data in an integrated way, in order to enhance investigations into the connections between these organ systems and organisms and to more broadly and accurately assess the intake of nutrients and bioactive compounds as well as the effects of drugs and/or other therapeutic interventions (Alizadeh et al. 2023).

Big Data refers to a set of methods or tools (e.g. data mining, statistics, artificial intelligence (AI), predictive analytics, natural language processing (NLP), etc.) (Bhat and Huang 2021; Sassi, Ouaftouh, and Antr 2019) which has been increasingly become essential in the fields of food science and technology as well as in nutrition. Such tools are able to centralize the collection and analysis of this large set of records. It extracts insights from datasets varied, high volume, and high speed using statistical and processing techniques that allow analysts to identify patterns quickly and predict trends more accurately (see more: https://www.ibm.com/analytics/big-data-analytics). Thus, understanding the application of Data Science may help us in locating certain information and, mainly, applying it assertively in the choice of nutritional interventions and/or disease prevention/management (Kuzminov et al. 2018; Talari et al. 2022).

In general, Big Data is used to defining a large volume of data, structured, semi-structured, and unstructured, generated at high speed and which, due to these characteristics, need specific tools to be analyzed (Gandomi and Haider 2015). The term "Big" usually refers to the three V's which are data that is high in volume, velocity, and variety (3V's), with a fourth V, veracity, also applicable to agriculture and nutrition (Constantiou and Kallinikos 2015; Musker 2019).

According to The World Health Organization (WHO), Big data is defined as "rapidly accumulating, complex, and versatile data that requires unprecedented terabytes (1012 bytes), petabytes (1015 bytes) or zettabytes (1021 bytes) to be stored" (see more: https://www.who.int/europe/news/item/26-05-2021-using-big-data-to-inform-health-care-opportunities-challenges-and-considerations). However, for an environment to be considered Big Data, the large volume of data must be associated with the high speed that needs to be processed for decision-making. The amount, variety, and enormous volume of data bring many possibilities for analysis, information generation, knowledge construction, and increasingly information-based decisions (Belaud et al. 2019; Lutfi et al. 2023).

Definitions of big data volumes are relative and vary by factors such as amount over time, which informs the next component, that is, refers to datasets whose size is beyond the ability of typical database software tools to capture, store, manage, and analyze (Gandomi and Haider 2015; Sonka 2021).

The velocity dimension refers to the capability to acquire, understand, and respond to events as they occur. In nutrition, an often-mentioned benefit of big data is the opportunity for near real-time analysis and decision-making, such as information on nutritional status and other factors, and sending alerts to healthcare professionals (Gandomi and Haider 2015; Musker 2019).

The variety refers to the nature of structured and unstructured data. While the first is an organized intake, such as spreadsheets and CSV or XLSX files, the second consists of data in the most varied formats, such as media content such as audio, images, videos, texts, and the like. In a practical way, it can be used in the assessment of eating habits or sending nutritional monitoring text messages (Musker 2019; Sonka 2021).

Finally, veracity is a fourth dimension of big data required by nutritional fields. It refers to the degree of credibility of the data, and they must present significant reliability to provide value and usefulness to the results generated from them. Good data quality is essential for optimal decision-making, especially when one decision can impact the nutritional status or livelihoods of a large segment of a population (Gandomi and Haider 2015; Musker 2019).

Whereupon, amounts of data may influence day-to-day decisions; since based on our eating habits and exercise routine, it is already possible to have multifactorial and more individualized nutritional recommendations should be developed to recommend healthy menus according to the specific user's needs (lose weight, gain lean mass, lower cholesterol, etc.).

Thus, the importance of having data quality, to compose the fourth V, together with "Volume," "Velocity," and "Variety," we draw attention to the "Veracity" of the data, that is, to the quality of the data (Gandomi and Haider 2015). Recently, Hinojosa-Nogueira et al. (2023), developed and validated the Stance4Health application, aiming to optimize intestinal microbiota activity and long-term consumer engagement by combining healthy parameters, user needs, and new dietary parameters, such as microbiomes or data wearable.

Multibillion-dollar companies such as Nestlé and IBM also jumped on board the Big Data train as it relates to personalized nutrition and healthcare (see: https://www.thecasecentre.org/products/view?id=157889 and https://www.ibm.com/watson-health). Such a dynamic of food innovation has been substantiated by the significant increase in venture capital investments in Big Data Analytics. To have an idea, just in 2016, in the USA, 46% of investors in Big Data spent US$3 billion on digital technology in the agri-food chain (Kuzminov et al. 2018).

In conclusion, it can be said that there is still a long way to precision nutrition in cancer management advance. It is

expected that multidisciplinary efforts and healthcare providers will accelerate the development of both reliable devices and platforms for monitoring specific biomarkers to provide accurate dietary guidelines for effective precision nutrition.

Challenges and future trends

Omics sciences offer great possibilities for advancing knowledge and improving treatments for OC but still face significant challenges that need to be overcome. Inter- and intra-individual variability in gene expression, protein, and metabolic profiles, as well as tumor heterogeneity, can make it difficult to identify reliable therapeutic targets and apply personalized therapies. In addition, omics sciences face the challenge of dealing with the large amount of data generated by genomic, epigenomic, transcriptomic, proteomic, and metabolomic studies, which can be difficult to analyze and interpret. Despite these limitations, there are promising trends in the application of omics in cancer. For example, the integration of multiple omics platforms can increase sensitivity and specificity in biomarker detection. Additionally, omics data analysis can be used for patient stratification and identification of subgroups with a higher likelihood of response to specific treatments (Babu and Snyder 2023). Another promising trend is the use of artificial intelligence and machine learning for omics data analysis and interpretation, allowing for faster and more accurate analysis of large data sets (Srivastava 2023).

On the other hand, polyphenols present challenges in terms of their bioavailability and tumor-targeting specificity. Although they have chemopreventive and therapeutic potential, their low absorption and bioavailability limit their clinical effectiveness. Furthermore, the diversity of polyphenols presents a significant challenge as there are many different types of these compounds, and each may have specific effects on the intestinal microbiota. Understanding the specific interactions between different polyphenols and bacterial species is crucial for understanding their health effects. Another important challenge is the individual variation in the response of the intestinal microbiota to polyphenols. Genetic factors, diet, lifestyle, and other elements can influence how each individual responds to these compounds. It is essential to consider this variability when interpreting study results and personalizing interventions with polyphenols.

Another challenge is the lack of consensus on the optimal dosage of polyphenols, as well as the choice of the most suitable type of polyphenol for OC treatment. Additionally, it is important to consider the possibility of interactions between polyphenols and other drugs used in combination, which can affect therapeutic efficacy. However, trends in polyphenol research for the treatment of different cancers include the development of controlled-release formulations (Abdolmaleki et al. 2020; Imran et al. 2023; Patil and Killedar 2021). Strategies that enhance the release of polyphenols in the colon can increase their effectiveness. This may involve the use of encapsulation technologies or specific release systems that protect the polyphenols during transit through the digestive tract and release them at the desired site to achieve the desired effects (Barboza et al. 2023; Imran et al. 2023).

Concluding remarks

In this review, we discussed the high-throughput technologies represented by omics science (genomics, epigenomics, transcriptomics, proteomics, and metabolomics); they can provide relevant information from different facets for identifying biomarkers for diagnosis, prognosis, and selection of specific therapies or nutrition guidance for personalized treatment. Furthermore, the field of omics sciences can provide a better understanding of the role of polyphenols and their function in the cell cycle, as mediators of gene expression/suppression, epigenetic regulators, a substrate for gut microbiota, etc., being useful in both prevention and management of ovarian cancer. Such data may serve basis for the development of new target antitumor agents, with pharmacologically and biologically active effects superior to current therapies and with fewer or no adverse effects. On the other hand, genomic research, which allows for the biological elucidation of the ability of polyphenols to modulate epigenomic and transcriptomic profiles, and the influence of the expression of proteins and metabolites can be a highly complex task in clinical trials. Although we observed an increase in the number of investigations, there are several approaches to data acquisition, analysis, and integration that still need to be improved, and the standardization of these practices still needs to be implemented in clinical trials. Additionally, future developments in high-throughput technologies as well as the discovery of new relevant biomarkers need to focus on delivering faster and cheaper results on a large scale and the miniaturization of equipment.

Author contributions

Felipe Tecchio Borsoi, Leticia Ferreira Alves, Iramaia Angelica NeriNuma: Contributed equally to Conceptualization, writing - review, and editing; Murilo Vieira Geraldo and Glaucia Maria Pastore: Conceptualization, Supervision, and Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CHAPTER 3

Mechanistic insights into the therapeutic potential of araçá-boi extract and phenolic compounds in ovarian cancer cells

Araçá-boi extract and gallic acid reduce cell viability and modify the expression of tumor suppressor genes and genes involved in epigenetic processes in ovarian cancer

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Araçá-Boi Extract and Gallic Acid Reduce Cell Viability and Modify the Expression of Tumor Suppressor Genes and Genes Involved in Epigenetic Processes in Ovarian Cancer

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Abstract

Natural compounds found in plant species have gained attention for their ability to act as epigenetic modulators promoting the reactivation of tumor suppressor genes and affecting cell proliferation, inflammation, invasion, and metastasis. This study aimed to characterize the phytochemical profile of araçá-boi extract and evaluate its effects on antioxidant activity, cell viability, gene modulation, and DNA methylation in ovarian cancer cells. UHPLC-Q-Orbitrap-MS/MS analysis identified 73 compounds, including organic acids, phenolic acids, and flavonoids. The araçá-boi extract exhibited strong antioxidant activity against synthetic free radicals and reactive oxygen species. Gallic acid was used alone to verify its contribution to antitumor activities. The Chinese hamster ovary (CHO-K1) cell line and human ovarian cancer cell lines (NCI/ADR-RES and OVCAR3) were treated for 24–72 h with araçá-boi extract (0.15–150 μg/mL) and gallic acid (6–48 μg/mL). The araçá-boi extract did not exhibit cytotoxic effects on CHO-K1 cells, whereas gallic acid showed toxicity. Both the araçá-boi extract and gallic acid significantly reduced cell viability in the NCI/ADR-RES cels, mainly after 48 hours, while the OVCAR3 cell line appeared to be more resistant to the treatments. Both treatments modulated genes involved in DNA repair, tumor suppression, and epigenetic regulation. No changes were observed in the methylation status of the *BRCA1* gene promoter region with either araçá-boi extract or gallic acid. These findings highlight the therapeutic potential of araçá-boi extract and its phenolic

compounds in the context of ovarian cancer. Further studies are necessary to comprehensively elucidate these mechanisms and their potential implications.

Keywords: *Eugenia stipitata*; phenolic extract; gene expression; *BRCA1*; DNA methylation.

1. Introduction

Ovarian cancer is a malignant neoplasm that originates in ovarian tissues and is known for being one of the deadliest types of gynecological cancer. It can be classified into two groups: epithelial carcinoma, which is the most common and accounts for about 90% of cases, and non-epithelial origin (germ cells and stromal cells) (Webb & Jordan, 2024). According to GLOBOCAN (2022), ovarian cancer ranks eighth among the most common cancer types in women, with high prevalence and mortality, responsible for over 374,000 new cases and around 240,000 deaths annually. The high mortality rate associated with ovarian cancer is largely due to late-stage diagnosis, as the absence of specific symptoms in early stages and the lack of effective early detection strategies remain significant challenges, highlighting the urgency to adopt new therapeutic strategies focused on tumorigenesis and chemotherapy resistance (Ali et al., 2023).

Recent advancements in omics sciences and big data, such as genomics, epigenomics, transcriptomics, proteomics, metabolomics, and machine learning, have shed light on the molecular pathways involved in ovarian cancer. These tools are essential for identifying metabolites, proteins, and genes involved in the development and progression of cancer, directly impacting diagnosis, prognosis, and therapeutic options (Xiao et al., 2022). In ovarian cancer, Epigenomics plays a crucial role, as alterations like DNA hypermethylation can silence tumor suppressor genes (*e.g.*, *RASSF1A*, *CDKN2A*, *BRCA1*, *MLH1*, and *CDH1*), which not only compromises the regulation of cell growth and vital processes such as apoptosis, DNA repair, and the cell cycle but also disrupts cell adhesion, facilitating metastasis (Borsoi, Alves, et al., 2023; Fu et al., 2024). Additionally, genes involved in epigenetic processes such as *DNMT1* and *HDAC1* play a central role in this context: DNMT1 adds methyl groups

to DNA, perpetuating hypermethylation, while HDAC1 compacts chromatin, inhibiting the transcription of tumor suppressor genes (Chang et al., 2024). Therefore, the use of therapies based on the inhibition of these enzymes is crucial to reverse the silencing of these genes, restoring their expression and potentially reversing tumor processes.

Currently, epigenetic agents, *"epidrugs*, have improved cancer treatment. However, there are still many adverse effects and challenges that limit the effectiveness of these treatments (Castro-Muñoz et al., 2023). In this regard, there is an increasing focus on natural compounds that not only inhibit these epigenetic enzymes but also provide a less toxic alternative, promoting a cellular environment more conducive to apoptosis and cellular repair (Dorna et al., 2023). Natural compounds, such as polyphenols, mainly found in plant species, demonstrate great potential in the treatment of various types of cancer. These compounds are capable of affecting different cancer-related process, such as cell proliferation, invasion, and metastasis (Pavithra et al., 2024; Rathee et al., 2024). Along with all these benefits, their ability to affect epigenetic processes is one of the most important aspects of their impact. Recent studies indicate that polyphenols act as epigenetic modulators by interfering with DNA and histone methylation and acetylation pathways. These alterations promote the reactivation of tumor suppressor genes, helping to control cancer progression (Dorna et al., 2023; Qadir Nanakali et al., 2023). Thus, polyphenols have the potential not only to prevent but also to reverse harmful epigenetic changes, reinforcing their role as promising therapeutic agents in oncology.

Eugenia stipitata Mac Vaugh, widely known as araçá-boi, is a fruit native to the Amazon region and cultivated in various parts of South America (Acosta-Vega et al., 2024). The araçá-boi fruit (peel and pulp) is rich in phenolic compounds (*e.g.*, phenolic acids: trans-cinnamic, ellagic, gallic, and syringic acid, and flavonoids: myricetin, quercetin, and kaempferol derivatives), carotenoids, and vitamins (Acosta-Vega et al., 2024; Borsoi et al., 2024; de Araújo et al., 2021a; Neri-Numa et al., 2013). Phenolic compounds identified in araçá-boi fruit have been recognized for their potential

antioxidant, anticancer, and (Borsoi et al., 2024; Neri-Numa et al., 2013; Soares et al., 2019). However, the phytochemical profile of araçá-boi and its impact on antitumor activity in ovarian cancer, particularly through its influence on epigenetic mechanisms that regulate gene expression and tumor suppression, remain largely unexplored. In light of this, the present study seeks to characterize the phytochemical profile of the araçá-boi extract and investigate its effects on antioxidant activity, cell viability, gene modulation regulation, and DNA methylation in ovarian cancer cells.

2. Material and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, Trolox (6-hydroxy-2578-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,2′-azinobis-(3-ethylbenzothiazo- line-6-sulfonic acid)-diammonium salt (ABTS), TPTZ (2,4,6-tripyridy-s-triazine), 2,2′-azobis(2-methylamidino- propane)-dihydrochloride (AAPH), fluorescein, ethanol, methanol, phenolic compound standards (gallic acid and catechin, grade HPLC, with a purity of ≥96%), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), sodium hypochlorite solution (NaOCl), rhodamine 123 were purchased from Sigma-Aldrich (St. Louis, USA). All chemicals used for cell culture, gene expression, and DNA analysis were obtained from Thermo Fisher Scientific (Grand Island, NY, USA), and Invitrogen Life Technologies (Carlsbad, CA, USA). The primers used for gene expression and DNA methylation analyses were synthesized by Exxtend (São Paulo, Brazil). The other solvents and reagents used in this study were of analytical grade. All solutions were prepared with ultrapure water (18 MΩ cm) obtained from a Milli-Q water purification system.

2.2. *Plant material collection, preparation, and ultrasound-assisted extraction*

Eugenia stipitata (araçá-boi) fruits were harvested during the summer season at 'Kamui Farm' in Ituberá, Bahia, Brazil, located at coordinates 13° 44′ S 39° 9′ W. The botanical identification and voucher specimen (access number 55,875) were deposited at the Herbarium-UEC of the Agronomic Institute of Campinas, São Paulo, Brazil (Baldini et al., 2017). After cleaning with distilled water, the edible parts of the fruit (pulp and peel) were separated from the seeds. These parts were then processed and immediately frozen at -80°C for subsequent lyophilization. The 24-mesh electromagnetic sieve shaker was used to obtain standardized powders.

The phenolic compounds from the edible fraction of araçá-boi were extracted using the method described by Borsoi et al. (2024). Freeze-dried araçá-boi (1 g) was extracted with 15 mL of an ethanol-water mixture (80:20, v/v). This mixture was subjected to ultrasonic treatment using a UNIQUE UCS-2850 model (25 kHz, 120 W, São Paulo, SP, Brazil) for 10 minutes at room temperature. Following ultrasonic extraction, the solution was centrifuged at 4000 × g for 5 minutes at 5 °C. The supernatants were collected after centrifugation, and the residues were re-extracted twice more under the same conditions. The combined supernatants were then evaporated under vacuum at 40 °C and the aqueous phase was concentrated to 50 mL. The resulting araçá-boi extract was stored at −20 °C.

2.3. *Determination of total phenolic content (TPC) and total flavonoid content (TFC)*

The total phenolic content was performed according to the method proposed by Roesler et al. (2007). 30 μL of the extract was added with 150 μL of 10-fold diluted Folin–Ciocalteau reagent, and 120 μL of sodium carbonate solution (NaHCO3 7.5%). After 6 min at 45 °C, absorbance at 760 nm was measured. A gallic acid standard was used for the analytical curve and the results were expressed as mg gallic acid equivalent (GAE)/g dw (dry weight).

Total flavonoid content was determined according to the method proposed by Zhishen et al. (1999) with modifications. 30 μL of the extract was mixed with 110 μL of

ultrapure water and 8 μL of sodium nitrite solution (NaNO2 5%). After 5 min of incubation at room temperature, 8 μL of aluminum chloride solution (AlCl3 10%) was added and incubated for 6 min at room temperature. Finally, 50 μL of sodium hydroxide (NaOH 1 mol/L) and 70 μL of ultrapure water were added and the absorbance at 510 nm was measured. A catechin standard was used for the analytical curve and the total flavonoid content was expressed as mg catechin equivalent (CE)/g dw.

2.4. Phytochemical profile by UHPLC-Q-Orbitrap-MS/MS

A Thermo Ultimate 3000 system (Waltham, MA, USA), coupled with a Q-Exactive mass spectrometer equipped with an electrospray ionization source (ESI), was employed to analyze the phytochemical profile present in araçá-boi extract following the methodology described by Bocker & Silva (2024). The mass spectrometer was configured to operate in negative mode (ESI-), with a 2.5 kV of spray voltage, 51 L/min of desolvation gas flow, 13 L/min of auxiliary gas flow, 3 L/min of sweep gas flow, 266 °C of capillary temperature, 431 °C of auxiliary gas temperature, and RF lens S set to 50. Mass scanning was conducted across a range of 100–1500 Da, using a resolution of 70,000, an AGC target of 3×10^6, and a maximum injection time of 100 ms. The resolution of MS/MS experiments was 17,500 with an AGC target of 1×10^5, a maximum injection time of 50 ms, and fragmentation of the five most intense precursor ions using stepped normalized collision energies (NCE) of 25, 30, and 35 eV within a 3.0 m/z isolation window.

Chromatographic separation was performed on a Poroshell 120 SB-Aq column (100 × 2.1 mm, 2.7 μm, Agilent Technologies) using a gradient program, with a flow rate of 0.45 mL/min and a column temperature of 40 °C. The mobile phase included two eluents: A (formic acid 0.1% in water) and B (acetonitrile 0.1% formic acid). The gradient conditions were: 0–1 min, 95% A; 1–10 min, 95–82% A; 10–13 min, 82–30% A; 13–15 min, 30–0% A; 15–17 min, 0% A; 17–19 min, 0–95% A; and 19–22 min, 95% A (Arruda et al., 2019). Data were acquired and analyzed using Xcalibur 4.3 software,

and the fragmentation profiles of the detected compounds were compared with plant compound databases for identification

2.5. Antioxidant capacity against synthetic free radicals and reactive oxygen species (ROS)

2.5.1. Scavenging of synthetic free radicals DPPH•, ABTS•+, and Ferric-reducing antioxidant power (FRAP)

The DPPH assay was performed according to the method proposed by Roesler et al. (2007) with some modifications. The reaction system consisted of 50 μL of the extract and 250 μL of DPPH• solution (0.004% in ethanol). After a 30-minute incubation at room temperature, the absorbance was measured at 517 nm. The results were expressed as μmol Trolox equivalent (TE)/g dw.

The ABTS•+ assay followed the procedure outlined by Leite et al. (2011). The reaction system consisted of 50 μL of the extract and 250 μL of the ABTS•+ solution (7 mmol/L ABTS with 145 mmol/L potassium persulfate). After a 6-minute incubation at room temperature, the absorbance at 734 nm was recorded. The results were expressed as μmol Trolox equivalent (TE)/g dw.

The FRAP assay followed the procedure proposed by Guerra-Ramírez et al. (2021). The FRAP reagent was prepared by combining 20 mL of acetate buffer (0.3 mol/L, pH 3.6), 2 mL of TPTZ solution (10 mmol/L in 40 mmol/L HCl), and 2 mL of ferric chloride solution (20 mmol/L) in a 10:1:1 ratio. To perform the assay, 20 μL of the extract was mixed with 180 μL of the FRAP reagent and 60 μL of deionized water. After a 30-minute incubation at 37 °C, the absorbance was recorded at 595 nm. The results were expressed as μmol Trolox equivalent (TE)/g dw.

2.5.2. Reactive oxygen species (ROS): peroxyl radicals (ROO•), hydroxyl radical (OH•), superoxide anion (O2•−), and hypochlorous acid (HOCl) scavenging assays

The peroxyl radical (ROO•) scavenging activity was performed according to the method described by Saliba et al. (2023). The reaction took place in a 96-well dark

microplate using phosphate buffer (75 mmol/L, pH 7.4). A mixture of 20 μL of extract, 60 μL of fluorescein solution (508.25 nM), and 110 μL of AAPH solution (76 mM) was incubated at 37 °C, and fluorescence was recorded every 10 minutes for 120 minutes with excitation at 485 nm and emission at 528 nm. The results were expressed as μmol Trolox equivalents (TE) μmol Trolox equivalent (TE)/g dw.

The hydroxyl radical ($OH^{\bullet}$) scavenging activity was performed according to the method described by Andrade et al. (2024). The reaction system was composed of 50 μL of extract, 50 μL of carbonate buffer (0.5 mol/L, pH 10), 50 μL of luminol solution (100 μmol/L) prepared in the same buffer, 50 μL of FeCl2-EDTA solution (125 and 500 μmol/L), and 50 μL of H2O2 solution (17.5 mmol/L). After a 5-minute incubation at 37 °C, luminescence was recorded. Results were expressed as IC_{50} (μg/mL dw).

The superoxide radical ($O2^{\bullet-}$) scavenging activity was evaluated following the method described by Saliba et al. (2023). Briefly, each well of the microplate was added with 100 μL of NADH (166 μM), 50 μL of nitroblue tetrazolium (NBT, 107.5 μM), and 50 μL of phenazine methosulfate (PMS, 2.7 μM), and 100 μL of different concentrations of the extract were dissolved in a 19 mM potassium phosphate buffer solution (pH 7.4) to reach a final volume of 300 μL, and incubated 5 min. The assay was performed at 25 °C and the fluorescence was measured at 560 nm. The results were expressed as IC_{50} (μg/mL dw).

The Hypochlorous acid (HOCl) scavenging activity was performed according to the method described by Saliba et al. (2023). HOCl was prepared by diluting a sodium hypochlorite solution (1% NaOCl) and adjusting its pH to 6.2 with sulfuric acid (10% H_2SO_4). The final concentration was determined using its absorbance at 235 nm (molar absorption coefficient: 100/M/cm) and adjusted to 5 μM in a 100 mM phosphate buffer (pH 7.4). Dihydrorhodamine 123 (DHR) was freshly prepared at 1.25 μM in 100 mM phosphate buffer (pH 7.4). For the assay, 100 μL of the sample (at various concentrations), 100 μL of phosphate buffer, 50 μL of DHR, and 50 μL of the 5 μM HOCl solution were combined in a microplate well. Fluorescence was

immediately measured at 37 °C (excitation: 485 nm; emission: 528 nm). Results were expressed as IC_{50} (μg/mL dw

2.6. Cell lines and culture conditions

The Chinese hamster ovary (CHO-K1) cell line and human ovarian cancer (NCI/ADR-RES) cell line were kindly provided by Prof. Dr. Ana Lúcia Tasca Gois Ruiz (University of Campinas, UNICAMP). The human ovarian cancer (NIH: OVCAR-3) was purchased from the cell bank of Rio de Janeiro (BCRJ, RJ, Brazil). The cells were grown in flasks containing Roswell Park Memorial Institute (RPMI) (Gibco™) 1640 medium with penicillin/streptomycin (1%) supplemented with 10% of fetal bovine serum (RPMI/FBS 10%). The cultures were maintained in 5% carbon dioxide (CO_2) in a humidified incubator at 37 °C (Revco Habitat, Asheville, N.C, USA).

2.7. Cell viability by MTT assay

CHO-K1, NCI/ADR-RES, and OVCAR-3 cells were plated in 96-well plates at a density of 5×10^3 cells per well, using 100 μL of complete medium, and incubated for 24 hours to promote adherence. After that, the cells were exposed to a different concentration of araçá-boi extract (0.15, 1.5, 15, and 150 μg/mL) and gallic acid (6, 12, 24, and 48 μg/mL). The control group cells were treated with only the culture medium. All cells were exposed to the treatments for 24, 48, and 72 hours.

The cell viability by MTT assay was performed according to the method described by Mosmann (1983) with some modifications. The MTT solution was prepared by adding 5 mg of MTT salt in phosphate buffer solution – PBS). After treatment with araçá-boi extract or gallic acid, the culture medium was aspirated and 100 μL of MTT solution was added and incubated at 37 °C for 2 h. Then, the supernatant was removed (85 μL) and 50 μL of DMSO was added. After a 10-minute incubation at 37 °C, the absorbance was measured at 560 nm. The results were expressed as a percentage (%) of cell viability in comparison to control.

2.8. Gene expression analysis

2.8.1. Total RNA isolation

For total RNA extraction, 2 × 10^5 NCI/ADR-RES cells were seeded in a 60 mm cell culture plastic dish with 4 mL of RPMI-1640 medium (Gibco™) with penicillin/streptomycin (1%) supplemented with 10% of fetal bovine serum (RPMI/FBS 10%). The cultures were maintained in 5% carbon dioxide (CO_2) in a humidified incubator at 37 °C. After 24 h, the cells were exposed to a different concentration of araçá-boi extract (1.5 and 15 μg/mL) and gallic acid (24 μg/mL). The control group cells received only the culture medium as treatment. The cells were collected in TRIzol (500 μL) after 48 h. The RNA extraction protocol was performed according to Chomczynski & Sacchi (1987). Chloroform (80 μL) was added to the TRIzol lysate and thoroughly mixed by vortexing. After 3 minutes, the samples were centrifuged at 10,000 × g for 15 minutes at 4 °C, and the upper aqueous phase containing RNA was transferred to a new tube. Then, 200 μL of isopropanol was added, mixed, and kept at room temperature for 10 minutes before being centrifuged at 10,500 × g for 10 minutes at 4 °C. The supernatant was discarded, and the pellet was washed with 200 μL of 75% ethanol solution and centrifuged at 5,200 × g for 5 minutes at 4 °C. The pellet was air-dried, and 30 μL of RNase-free water was added. Finally, the RNA was quantified using a NanoDrop® 2000 spectrophotometer.

2.8.2. cDNA synthesis and primer design

The cDNA synthesis was performed according to *Invitrogen's* M-MLV Reverse Transcriptase (200 U/μL) instructions with 700 ng of total RNA per reaction. The sequences of the internal control gene, *GAPDH*, as well as the genes of interest — *BRCA1*, *RASSF1A*, *CDKN2A*, *DNMT1*, and *HDAC1* — were designed using the UCSC Genome Browser and Primer3Plus software. To ensure the quality and compatibility of the primers, the 'NetPrimers' (Premier Biosoft) and OligoAnalyzer™ Tool software were employed. Table 1 presents a list of the primer names, their sequences, sizes, amplicon length (in bp), and corresponding annealing temperatures.

Table 1: Sequences of primers used for quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Gene names	Forward (5′→3′)	Reverse (5′→3′)	Amplicon (bp)
BRCA1	CTGGACAGAGGACAATGGCT	GTGGGGGATCTGGGGTATCA	139
RASSF1A	ACCCCTCTGCCCTCATTACT	TTCTGTCTGCACCACTCCTG	89
DNMT1	TTCAGCACAACCGTCACCAA	GTCCAGGATGTTGCCGAAGA	147
HDAC1	TTCTTCCCCAACCCCTCAGA	GGCCTTGGTTTCTGTCCCTG	99
CDKN2A	TAAGGGGAATAGGGGAGCGG	ACTGCGAGAACCACATGTCT	149
GAPDH	ACCCACTCCTCCACCTTTGA	CTGTTGCTGTAGCCAAATTCGT	101

bp: base pairs.

2.8.3. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The RT-qPCR was performed in a 20 μL reaction volume, combining 5 μL of cDNA, 10 μL of FastStart Universal SYBR Green Master (ROX), and 5 μL of forward and reverse primers (200 nM). The reactions were carried out using universal cycling conditions on the StepOnePlus™ real-time PCR system. The cycling parameters were as follows: 2 minutes at 50 °C (UDG pretreatment), 10 minutes at 95 °C, followed by 40 cycles of 15 seconds at 95 °C and 60 seconds at 60 °C. A melting curve (15 seconds at 95°C followed by 60–95 °C, increasing by 1.0 °C increments) was generated to verify primer amplification specificity. The relative expression of each gene was represented as the fold change relative to the control and calculated using the comparative $2^{(-\Delta\Delta CT)}$ method. GAPDH was used as the housekeeping gene to normalize gene expression.

2.9. DNA methylation analysis

2.9.1. Bisulfite conversion of DNA

For bisulfite conversion of DNA, 2×10^5 NCI/ADR-RES cells line was seeded into 6-well plates with 2 mL of RPMI-1640 medium (Gibco™) with penicillin/streptomycin (1%) supplemented with 10% of fetal bovine serum (RPMI/FBS 10%). The cultures were maintained in 5% carbon dioxide (CO_2) in a humidified incubator at 37 °C (Revco Habitat, Asheville, N.C, USA) for 24 h to adhere to the plate. Afterward, the cells were exposed to araçá-boi extract (15 μg/mL) and gallic acid (24 μg/mL) for 48 hours. The control group cells received only the culture medium as

treatment. EZ DNA Methylation-Direct™ Kit (Zymo Research Corporation) was used for DNA extraction and direct bisulfite conversion, according to the instructions provided by the manufacturer. The recovered bisulfite-treated DNA was quantified with a NanoDrop® 2000 spectrophotometer.

2.9.2. Primer design and PCR-amplification of the bisulfite-treated DNA

The promoter region of the *BRCA1* gene was obtained from the UCSC Genome Browser and the primers were obtained using the bisulfite primer design tool (Bisulfite Primer Seeker, Zymo Research). Furthermore, to check the quality and compatibility of the primers, the "NetPrimers" (Premier Biosoft) and OligoAnalyzer™ Tool software was used. The *BRCA1* forward primer was 5'-TTTAGTTTTAGGAGTTTGGGGTAAGTAG-3' and reverse 5'-CCTTAAACTTCTCCAAACCCTCTTAATA-3'. PCR was performed on a Mastercycler ep (Eppendorf AG, 22331 Hamburg, Germany) for bisulfite-converted DNA and was conducted in a final volume of 50 µl. The reaction consisted of 5 µL high fidelity PCR buffer (10x), 0.2 µL of 5 U/rxn Platinum® Taq DNA polymerase high fidelity, 1 µL bisulfite-treated genomic DNA (40 ng/uL), 4.5 µL of each primer (10 µM), 1 µL dNTPs (10 mM), 2 µL MgSO4 (2 mM) and autoclaved water to complete the volume. The PCR conditions for the BRCA gene were 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, 54 °C for 30 s, 72 °C for 1 min, and 72 °C for 1 min. PCR amplicons were examined by gel electrophoresis on 1% agarose for the presence of single bands at the expected size.

2.9.3. Purification and Sanger Sequencing

The previously obtained PCR products were purified to remove unincorporated nucleotides and excess primers, using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation), according to the manufacturer's recommendations. After purification of the PCR products, the samples were quantified (ng/µL) with a NanoDrop® 2000 spectrophotometer. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™), with the same

primers used in the PCR, and performed on a 3730xl DNA Analyzer. The runs were made in 36 cm capillaries using the POP7 polymer. The sequences were processed and analyzed using the Genious™ software (version 4.8.5).

2.10. Statistical analysis

The results are expressed as the mean ± standard deviation of at least three independent experiments, analyzed using GraphPad Prism 9. For the cellular assay, statistical analyses were performed using two-way ANOVA, followed by Dunnett's post-hoc multiple comparison test. Gene expression data were analyzed using two-way ANOVA, followed by the Bonferroni test. Significant differences are * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results and discussion

3.1. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity of araçá-boi extract

The results for the total phenolic compounds (TPC), total flavonoid content (TFC), and antioxidant capacity of the araçá-boi extract are shown in Table 2. The TPC and TFC from araçá-boi extract were 25.90 mg GAE/g dw and 6.53 mg CE/g dw, respectively. Following the classification proposed by Rufino et al. (2010), food matrices are categorized based on their dry weight (dw) into low (<10 mg GAE/g), medium (10–50 mg GAE/g), and high (>50 mg GAE/g) TPC. In this context, the araçá-boi extract obtained is considered a valuable source of phenolic compounds, as it falls within the medium range of TPC. These results surpass those reported by de Araújo et al. (2021a) and Llerena et al. (2020), who found for the edible fraction of araçá-boi TPC values of 9.06 and 15.65 GAE/g dw and TFC values of 1.25 and 6.00 mg CE/g dw respectively. Variations in TPC and TFC among studies can be attributed to a combination of methodological, environmental, and agronomic factors, including storage conditions, sample preparation, extraction methods, edaphoclimatic conditions, cultivation practices, and harvest timing, which collectively influence the

stability, recovery, and phytochemical composition of plants (Popescu et al., 2023; Shi et al., 2022).

Table 2: Total phenolic content, total flavonoid content, and antioxidant capacity against synthetic free radicals and reactive oxygen species (ROS) in araçá-boi extract.

Analysis	Parameters	Araçá-boi extract
Phytochemicals	Total phenolics (mg GAE/g dw)	25.90 ± 1.37
	Total flavonoids (mg CE/g dw)	6.53 ± 0.19
Synthetic free radical	DPPH (μmol TE/g dw)	68.78 ± 7.03
	ABTS (μmol TE/g dw)	155.52 ± 8.08
	FRAP (μmol TE/g dw)	161.77 ± 10.21
Reactive oxygen species (ROS)	$ROO^{\bullet}$ (μmol TE/g dw)	366,13 ± 19,39
	$OH^{\bullet}$ (IC_{50} μg/mL dw)	1,91 ± 0,20
	$O_2^{\bullet -}$ (IC_{50} μg/mL dw)	3534,33 ± 111,99
	HOCl (IC_{50} μg/mL dw)	307,66 ± 30,08

CE: catechin equivalents; dw: dry weight; FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; HOCl: hypochlorous acid scavenging activity; IC_{50}: extract concentration that resulted in a 50% reduction in radical concentration compared to the control; $O2^{\bullet -}$: superoxide radical scavenging activity; $OH^{\bullet}$: hydroxyl radical scavenging activity; $ROO^{\bullet}$: peroxyl radical scavenging activity; TE: Trolox equivalents.

The phenolic compounds contain numerous hydroxyl groups, which are electron-rich and planar, allowing them to inhibit or reduce reactive species through single-electron transfer and/or hydrogen-atom transfer (Muflihah et al., 2021). The antioxidant potential of phenolic compounds may contribute to the prevention or mitigation of diseases associated with oxidative stress, such as cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders (Borsoi, Neri-Numa, et al., 2023). Therefore, the antioxidant capacity of araçá-boi extract was assessed in terms of its ability to scavenge synthetic free radicals and reactive oxygen species. As shown in Table 2, the araçá-boi extract demonstrated remarkable peroxyl radical ($ROO^{\bullet}$) scavenging capacity (366.13 μmol TE/g dw). This capacity was followed by similar values obtained in the FRAP (161.77 μmol TE/g dw) and ABTS (155.52 μmol TE/g dw). On the other hand, the DPPH assay was lower, reaching only 66.78 μmol TE/g dw. Borsoi et al. (2024) found a similar trend in araçá-boi extract, with a higher value for peroxyl radical scavenging (583.81 μmol TE/g dw) and lower values for the ABTS and FRAP methods (102.51 and 150.77 μmol TE/g dw). Conversely, Soares et al. (2019)

reported a value of only 32.72 μmol TE/g dw for $ROO^{\bullet}$ in araçá-boi pulp. Similarly, de Araújo et al. (2021b) found values of 8.40, 25.30, and 22.80 μmol TE/g dw for the DPPH, ABTS, and $ROO^{\bullet}$ methods, respectively. Antioxidant methods rely on various mechanisms of action, including single electron transfer (*e.g.*, DPPH and FRAP), hydrogen atom transfer (*e.g.*, $ROO^{\bullet}$), or mixed-mode assays (*e.g.*, ABTS). (Munteanu & Apetrei, 2021; Siddeeg et al., 2021). This suggests that the phenolic compounds in the araçá-boi extract demonstrate antioxidant activity primarily through the hydrogen atom transfer mechanism.

In addition to $ROO^{\bullet}$, other ROS such as hydroxyl radical ($OH^{\bullet}$), superoxide radical ($O2^{\bullet-}$), and hypochlorous acid (HOCl) were also investigated in this study. The results from Table 2 show the highest elimination activity for $OH^{\bullet}$, followed by HOCl and $O2^{\bullet-}$ (1.91, 307.66, and 3534.33 μg/mL dw, respectively). ROS are highly reactive molecules involved in critical biological functions, including maintaining immune defense, signal transduction, and cellular redox balance. However, when ROS levels surpass the antioxidant defense capacity of the organism, oxidative stress arises, causing damage to DNA, RNA, proteins, and cell membranes. This disruption in the body's homeostasis can contribute to the onset of several chronic conditions, such as cardiovascular diseases, diabetes, neurodegenerative disorders, cancer, and inflammation (Gasmi et al., 2022; Joorabloo & Liu, 2024; Rudrapal et al., 2022). It can be inferred that the araçá-boi extract has a relevant and significant antioxidant effect, being particularly effective in neutralizing $OH^{\bullet}$. This radical can react with various biological molecules, leading to damage to proteins, lipids, and membranes. Since there are no enzymatic systems capable of neutralizing $OH^{\bullet}$, it is considered one of the most reactive and harmful species to cells (Y. Sun et al., 2020). Additionally, the araçá-boi extract has significant effects on HOCl and at higher concentrations on $O2^{\bullet-}$. Soares et al. (2019) evaluated different ROS and RNS in the araçá-boi pulp. The authors found an IC_{50} of 758.13, 14.64, and 6.95 μg/mL dw for $O2^{\bullet-}$, HOCl, and nitric oxide ($NO^{\bullet}$), respectively. HOCl generated in excess during the inflammatory response can cause tissue damage, while the $O2^{\bullet-}$ is a precursor to other free radicals and can contribute

to oxidative stress when present at elevated concentrations (Rudrapal et al., 2022; Y. Sun et al., 2020). Thus, the ability of the araçá-boi extract to eliminate different ROS, particularly acting as a strong scavenger of $OH^{\bullet}$, suggests therapeutic potential, especially in conditions associated with oxidative stress, where the increase in these radicals can compromise cellular and molecular integrity. Furthermore, these results highlight the importance of araçá-boi as a potential antioxidant modulator, with the potential to be used in the development of therapies or products that combat the harmful effects of oxidative stress.

3.2. Phytochemical profile by UHPLC-Q-Orbitrap-MS/MS of araçá-boi extract

Studies suggest that the edible part of araçá-boi contains various phytochemicals with antioxidant properties (Acosta-Vega et al., 2024; de Araújo et al., 2021a; Soares et al., 2019). Although recent attempts have been made to identify the phytochemicals in araçá-boi, there is still a lack of extensive research on this topic, and a thorough characterization has yet to be completed. Therefore, the phytochemical profile of the araçá-boi extract was analyzed using UHPLC-Q-Orbitrap-MS/MS (Table 3). The characterization approach was based on exact mass (with a mass accuracy limit of 5 ppm), fragmentation patterns, and comparison with available literature data and existing phytochemical databases, such as MassBank (http://massbank.jp), METLIN Metabolite (http://metlin.scripps.edu), and HMDB (https://hmdb.ca). Xcalibur 4.3 software was employed to process the non-targeted metabolite data.

Table 3: Phytochemicals identified or tentatively annotated in araçá-boi extract using UHPLC-Q-Orbitrap-MS/MS in negative ion mode.

ID	R.T. (min)	Identified/tentatively annotated compound	Molecular Formula	Observed *m/z* value	Theoretical *m/z* value	Error (ppm)	Characteristic MS/MS fragments
	Organic acid and derivatives						
1	0.68	Quinic acid	$C_6H_{12}O_6$	191.0566	191.0556	5.23	191.0568, 173.0456, 127.0408, 93.0347, 85.0294
2	0.74	Malic acid	$C_4H_6O_5$	133.0144	133.0137	5.26	115.0038, 71.0135
3	0.83	Citric acid	$C_6H_8O_7$	191.0201	191.0192	4.71	129.0192, 111.0090
4	0.9	Shikimic acid	$C_7H_{10}O_5$	173.0452	173.045	1.16	155.0004, 111.0087, 93.0350
5	0.9	Succinic acid	$C_4H_6O_4$	117.018	117.0188	-6.84	99.0087, 73.0295
6	0.93	Hydroxyadipic acid	$C_6H_{10}O_5$	161.0457	161.045	4.35	101.0245, 99.0451
7	1.38	Ascorbic acid	$C_6H_8O_6$	175.0251	175.0243	4.57	115.0037, 87.0090, 71.0138
8	1.23	Pantothenic acid (vitamin B5)	$C_9H_{17}NO_5$	218.1033	218.1028	2.29	146.0829, 88.0402
9	4.43	Tuberonic acid hexoside	$C_{18}H_{28}O_9$	387.1662	387.1655	1.81	207.1024, 163.1134
10	10.87	12-hydroxyjasmonoyl-isoleucine	$C_{18}H_{29}NO_5$	338.1984	338.1967	5.03	130.0876
	Phenolic acids and derivatives						
11	0.99	Gallic acid Glucoside	$C_{13}H_{16}O_{10}$	331.0678	331.0665	3.93	271.0489, 211.0272, 169.0155
12	1.14	Gallic acid	$C_7H_6O_5$	169.0146	169.0137	5.33	169.0146, 125.0245, 107.0137, 97.0299, 79.0188
13	1.39	Salicylic acid isomer 1	$C_7H_6O_3$	137.0244	137.0239	3.65	93.0349
14	1.62	Hydroxybenzoic acid hexoside	$C_{13}H_{16}O_8$	299.0775	299.0767	2.67	137.0252
15	1.7	Peduncalagin isomer 1	$C_{34}H_{24}O_{22}$	783.0698	783.0681	2.17	300.9982, 275.0201
16	1.99	Vanillic acid hexoside isomer 1	$C_{14}H_{18}O_9$	329.0886	329.0872	4.25	329.0946, 167.0350, 152.0115, 123.0455, 108.0220
17	2.06	Protocatechuic xyloside	$C_{12}H_{14}O_8$	285.062	285.061	3.51	153.0192, 152.0112, 123.4724, 109.0290, 108.0222
18	2.12	Vanillic acid hexoside isomer 2	$C_{14}H_{18}O_9$	329.0887	329.0872	4.56	329.0908, 167.0358, 123.0451
19	2.19	Salicylic acid isomer 2	$C_7H_6O_3$	137.0243	137.0239	2.92	93.0341
20	2.26	Galloyl shikimic acid	$C_{14}H_{14}O_9$	325.0573	325.056	4.00	169. 0154, 125.0252
21	2.33	Caffeic acid hexoside isomer 1	$C_{15}H_{18}O_9$	341.0885	341.0873	3.52	179.0360, 161.0255, 135.0455
22	2.59	Peduncalagin isomer 2	$C_{34}H_{24}O_{22}$	783.071	783.0681	3.7	300.9995, 275.0200
23	2.62	Coumaric acid isomer 1	$C_9H_8O_3$	163.0403	163.0395	4.91	162.8391, 119.0504
24	2.62	p-coumaric acid hexoside isomer 1	$C_{15}H_{18}O_8$	325.0933	325.0923	3.08	163.0403, 119.0504

26	2.74	Syringic acid hexoside isomer 1	$C_{15}H_{20}O_{10}$	359.0995	359.0978	4.73	197.0839, 138.3060, 123.0091
27	2.78	Caffeic acid hexoside isomer 2	$C_{15}H_{18}O_{9}$	341.0886	341.0873	3.81	179.0361, 135.0459
28	2.95	Vanillic acid hexoside isomer 3	$C_{14}H_{18}O_{9}$	329.0887	329.0872	4.56	167.0352, 123.0456
29	3.1	Coumaric acid isomer 2	$C_{9}H_{8}O_{3}$	163.0403	163.0395	4.91	162.8406, 119.0507
30	3.12	p-coumaric acid hexoside isomer 2	$C_{15}H_{18}O_{8}$	325.0939	325.0923	4.92	163.0410, 145.0301, 119.0498
31	3.22	Caffeic acid hexoside isomer 3	$C_{15}H_{18}O_{9}$	341.0883	341.0873	2.93	179.0356, 135.0453
32	3.28	Coumaric acid isomer 3	$C_{9}H_{8}O_{3}$	163.0402	163.0395	4.29	162.8410, 119.0506
33	3.31	p-coumaric acid hexoside isomer 3	$C_{15}H_{18}O_{8}$	325.0938	325.0923	4.61	163.0408, 145.0292, 119.0506
34	3.72	Vanillic acid hexoside isomer 4	$C_{14}H_{18}O_{9}$	329.0887	329.0872	4.56	167.0357, 123.0456, 108.0218
35	3.81	Digalloyl hexoside isomer 1	$C_{20}H_{20}O_{14}$	483.0797	483.0775	4.55	313.0592, 271.0473, 211.0240, 169.0148
36	4.1	Digalloyl hexoside isomer 2	$C_{20}H_{20}O_{14}$	483.0796	483.0775	4.35	169.0144, 125.0241
37	4.14	p-coumaric acid hexoside isomer 4	$C_{15}H_{18}O_{8}$	325.0939	325.0923	4.92	163.0392, 119.0498
38	4.15	Ferulic acid hexoside	$C_{16}H_{20}O_{9}$	355.1041	355.1029	3.38	193.0498, 175.0413, 134.0370
39	4.15	Di-O-galloyl-rhamnose	$C_{20}H_{20}O_{13}$	467.0806	467.0826	-4.28	315.0174, 169.0143, 125.0251
40	4.16	Ferulic acid	$C_{10}H_{10}O_{4}$	193.0508	193.0501	3.63	134.0376
41	5.11	Coumaric acid isomer 4	$C_{9}H_{8}O_{3}$	163.0402	163.0395	4.29	162.8396, 119.0506
42	5.17	Trans-cinnamic acid	$C_{9}H_{8}O_{2}$	147.0453	147.0446	4.76	147.0457, 103.0549
43	5.88	Syringic acid hexoside isomer 1	$C_{15}H_{20}O_{10}$	359.0964	359.0978	-3.9	197.0831, 153.0923
44	6.1	Caffeoylshikimic acid	$C_{16}H_{16}O_{8}$	335.0784	335.077	4.18	179.0359, 161.0258, 135.0447
45	6.34	Tri-O-galloyl-glucose	$C_{27}H_{24}O_{18}$	635.0926	635.0884	6.61	465.0686, 313.0588, 169.0142, 125.0249
46	7.77	Mirciaphenone B	$C_{21}H_{22}O_{13}$	481.0994	481.0982	2.49	313.0557, 169.0147
47	9.25	Cis-Cinnamic acid	$C_{9}H_{8}O_{2}$	147.0452	147.0446	4.08	147.0459, 103.0549
	Flavonoids and derivatives						
48	3.24	Taxifolin isomer 1	$C_{15}H_{12}O_{7}$	303.0514	303.0505	2.97	285.0428, 217.0512, 175.0395, 125.0245
49	3.44	Taxifolin isomer 2	$C_{15}H_{12}O_{7}$	303.0513	303.0505	2.64	285.0403, 217.0499, 175.0410, 125.0243
50	4.74	(Epi)catechin	$C_{15}H_{14}O_{6}$	289.0723	289.0712	3.81	245.0483, 221.0465, 151.0033, 137.0254, 125.0251
51	5.28	Dihydroquercetin hexoside	$C_{21}H_{22}O_{12}$	465.1045	465.1068	-4.95	285.0390, 151.0038
52	6.99	Taxifolin isomer 3	$C_{15}H_{12}O_{7}$	303.0515	303.0505	3.3	285.0417, 175.0397, 125.0250
53	7.52	Myricetin-3-O-galactoside	$C_{21}H_{20}O_{13}$	479.0848	479.0826	4.59	317.0311, 316.0230
54	8.33	Quercetin-3-O-galloyl hexoside isomer 1	$C_{28}H_{24}O_{16}$	615.1002	615.0986	2.6	463.0884, 301.0350, 300.0306, 169.0145, 151.0046

56	8.68	Quercetin-3-O-galloyl hexoside isomer 2	$C_{28}H_{24}O_{16}$	615.1002	615.0986	2.6	463.0849, 301.0344, 300.0313, 169.0144, 125.2322
57	8.74	Myricetin-3-O-rhamnoside (myricetrin)	$C_{21}H_{20}O_{12}$	463.0895	463.0877	3.89	316.0224, 137.0305
58	8.8	Quercetin maloyl hexoside	$C_{25}H_{24}O_{16}$	579.1018	579.0986	5.53	301.0336, 300.0305
59	8.98	Quercetin-3-O-galactoside	$C_{21}H_{20}O_{12}$	463.0899	463.0877	4.75	301.0342, 300.0311, 179.1588, 151.0037
60	9.08	Quercetin-3-O-glucuronide	$C_{21}H_{18}O_{13}$	477.069	477.0669	4.4	302.0402, 301.0370, 178.9986, 151.0045
61	9.23	Phloretin-C-diglycoside	$C_{27}H_{34}O_{15}$	597.183	597.182	1.67	387.1130, 357.1005, 345.0978, 315.0868, 209.0453
62	9.24	Quercetin-3-O-glucoside	$C_{21}H_{20}O_{12}$	463.09	463.0877	4.97	301.0358, 300.0278, 178.9999, 151.0037
63	9.46	Naringenin	$C_{15}H_{12}O_5$	271.0618	271.0607	4.06	177.0196, 151.0033, 119.0509, 107.0135
64	10.08	Quercetin-3-O-arabinoside	$C_{20}H_{18}O_{11}$	433.0795	433.0771	5.54	301.0359, 300.0279, 271.0620, 151.0031
65	10.16	Kaempferol-3-O-galactoside (trifolin)	$C_{21}H_{20}O_{11}$	447.0938	447.0927	2.46	285.0390, 284.0330, 255.0307,
66	10.18	Phlorizin	$C_{21}H_{24}O_{10}$	435.1302	435.1291	2.53	273.0791, 167.0364
67	10.49	Kaempferol 7-(6'-galloyl glucoside)	$C_{28}H_{24}O_{15}$	599.1061	599.1037	4.01	285.0420, 284.0360, 169.0143
68	10.69	Kaempferol-3-O-glucoside (astragalin)	$C_{21}H_{20}O_{11}$	447.0948	447.0927	4.7	285.0410, 284.0350, 255.0322
69	10.79	Quercetin-3-O-rhamnoside (quercetrin)	$C_{21}H_{20}O_{11}$	447.0948	447.0927	4.7	301.0341, 300.0298, 271.0249, 255.0325, 151.0048
70	11.85	Kaempferol-3-O-rhamnoside (afzelin)	$C_{21}H_{20}O_{10}$	431.0994	431.0978	3.71	286.0453, 285.0408, 284.0348, 255.0320,227.0348
71	12.11	Quercetin deoxyhesoxylhexoside	$C_{27}H_{30}O_{16}$	609.1442	609.1456	-2.3	301.0333, 300.0320
72	12.18	Quercetin-3-O-acetyl rhamnoside	$C_{23}H_{22}O_{12}$	489.1056	489.1033	4.7	301.0337, 300.0306, 271.0245, 255.0322
73	12.18	Quercetin 3-O-hexuronide-7-O-hexoside	$C_{27}H_{28}O_{18}$	639.1212	639.1197	2.35	301.0344, 300.0289, 151.0700
74	12.35	Quercetin	$C_{15}H_{10}O_7$	301.0359	301.0348	3.65	301.0376, 179.0004, 151.0043, 121.0305, 107.0144
75	12.49	Quercetin-3,7-O-dirhamnoside	$C_{27}H_{30}O_{15}$	593.1483	593.1506	-3.88	301.0356, 300.0275, 271.0263, 255.0301, 151.0035

Seventy-three phytochemical compounds were tentatively annotated and characterized, including ten organic acids, thirty-six phenolic acids, and twenty-seven flavonoids (Table 3). As observed, the araçá-boi extract possesses a wide diversity of phenolic acids (e.g., gallic, vanillic, caffeic, coumaric, ellagic acids and/or their derivatives) and flavonoids (mainly as glycosylated forms of myricetin, quercetin, and kaempferol). In addition, it contains a considerable variety of organic acids, such as ascorbic, malic, citric, quinic, shikimic, succinic acid, etc. The presence of organic acids in the araçá-boi extract is important not only for their contribution to the characteristic taste and acidity of the fruit but also for their significant biological roles. Organic acids like malic acid are crucial intermediates in the Krebs cycle, essential for cellular energy production (Igamberdiev & Bykova, 2018). Furthermore, ascorbic acid (vitamin C) is known for its potent antioxidant properties, protecting cells against oxidative stress and contributing to collagen synthesis and immune function (Njus et al., 2020). Other organic acids not only play an important role in nutrient absorption but also contribute to the aroma, taste, and health benefits. These organic acids help improve the bioavailability of other phenolic compounds present in the plant (H. Chen et al., 2023).

Phenolic compounds play a key role in plant defense against both biological and environmental stresses, although they can also be harmful to the plant. To reduce their toxicity, plants attach these compounds to organic molecules, like carbohydrates, via glycosyltransferase enzymes, resulting in less harmful or non-toxic glycosylated forms. These compounds are stored in vacuoles until needed for defense when they are activated by glycosylhydrolase enzymes. Glycosylation also enhances their solubility, stability, and metabolism, allowing better distribution and accumulation in plant cells (Arruda et al., 2023). This may explain why we identified mainly glycosylated phenolic compounds in the araçá-boi extract. Similarly, to our work, de Araújo et al. (2021a) identified a total of 18 compounds in the edible fraction of araçá-boi (pulp and peel) by ESI-LTQ-XL-MS/MS in both positive and negative modes, including only one organic acid (malic acid), phenolic acids (gallic, cinnamic, vanillic,

caffeoyl, and coumaroyl), and flavonoids (mainly glycosylated forms of myricetin, luteolin, kaempferol, and quercetin). On the other hand, Soares et al. (2019) identified a profile mainly composed of hydrolyzable tannins (ellagitannins and their glycosylated derivatives), phenolic acids (ellagic acid, coumaric, vanillic, and their derivatives), and flavonoids (eriodictyol, pinoresinol, epicatechin, quercetin, and their derivatives) through LC–ESI-QTOF-MS analysis. The phenolic acids (*e.g.*, coumaric, gallic, cinnamic, and ellagic acids, and flavonoids (*e.g.*, myricetin, quercetin, and kaempferol), are renowned for their potent against oxidative stress and inflammation (W. Sun & Shahrajabian, 2023). Moreover, phenolic acids and flavonoids can influence the expression of proteins and epigenetic pathways involved in cell cycle regulation, apoptosis, and DNA repair mechanisms. These actions are particularly relevant in the prevention and management of chronic diseases, such as cardiovascular diseases, diabetes, and cancer (Rana et al., 2022; W. Sun & Shahrajabian, 2023). Thus, the araçá-boi extract is rich in a wide variety of phenolic compounds, which may offer several health benefits for humans.

3.3. Cell viability in healthy Chinese hamster ovary cells and human ovarian tumor cells

To evaluate cytotoxicity, an MTT cell viability assay was conducted on Chinese hamster ovary cells (CHO-K1) and two human ovarian tumor cell lines (NCI/ADR-RES and OVCAR-3). The araçá-boi extract was tested at four different concentrations (0.15, 1.5, 15, and 150 μg/mL) and over three distinct exposure times (24, 48, and 72 hours). In parallel, gallic acid, one of the phenolic acids present in the extract, was also tested at concentrations of 6, 12, 24, and 48 μg/mL to assess its isolated effect (Figure 1). The araçá-boi extract did not exhibit cytotoxicity in CHO-K1 cells at any of the tested concentrations and exposure times, suggesting a safety profile for normal cells. In contrast, isolated gallic acid showed a distinct behavior, with a significant cytotoxic effect observed at the highest concentration (48 μg/mL) after 24 and 48 hours of exposure. This effect became evident at all tested concentrations after 72 hours,

indicating a time- and concentration-dependent action for gallic acid that differs from the overall effect of the extract. For the tumor cell lines, the araçá-boi extract did not significantly reduce cell viability at 24 or 72 hours for either line. However, after 48 hours of exposure, a significant reduction in viability was observed for the NCI/ADR-RES line at concentrations of 1.5, 15, and 150 μg/mL, while the OVCAR-3 line showed reduced viability only at the highest concentration (150 μg/mL). In the case of gallic acid, NCI/ADR-RES cells exhibited a significant reduction in cell viability at concentrations of 12, 24, and 48 μg/mL at 24 hours, 24 and 48 μg/mL at 48 hours, and at all concentrations by 72 hours of exposure, indicating a more immediate and potent cell viability reduction effect. On the other hand, for the OVCAR-3 line, a similar behavior to the extract was found, with a significant effect only at the highest concentration (48 μg/mL) after 48 hours, and at 24 and 48 μg/mL after 72 hours.

These results suggest that gallic acid may partially account for the extract's antitumor activity, potentially through a synergistic or antagonistic effect that modulates this reduction in cell viability. The araçá-boi extract is a complex mixture of various phytochemicals, primarily including phenolic acids and glycosylated flavonoids, as noted above. These compounds are known for their antitumor effects. For instance, Neri-Numa et al. (2013) observed that araçá-boi pulp extract is not cytotoxic to green monkey kidney cells. Furthermore, the same authors did not find antiproliferative activity from the extract in nine tumor cell lines, including human ovarian tumor cell lines (NCI/ADR-RES and OVCAR-3). In contrast, Borsoi et al. (2024) demonstrated that araçá-boi extract and trans-cinnamic acid reduce cell viability in melanoma tumor cells (SK-MEL-28).

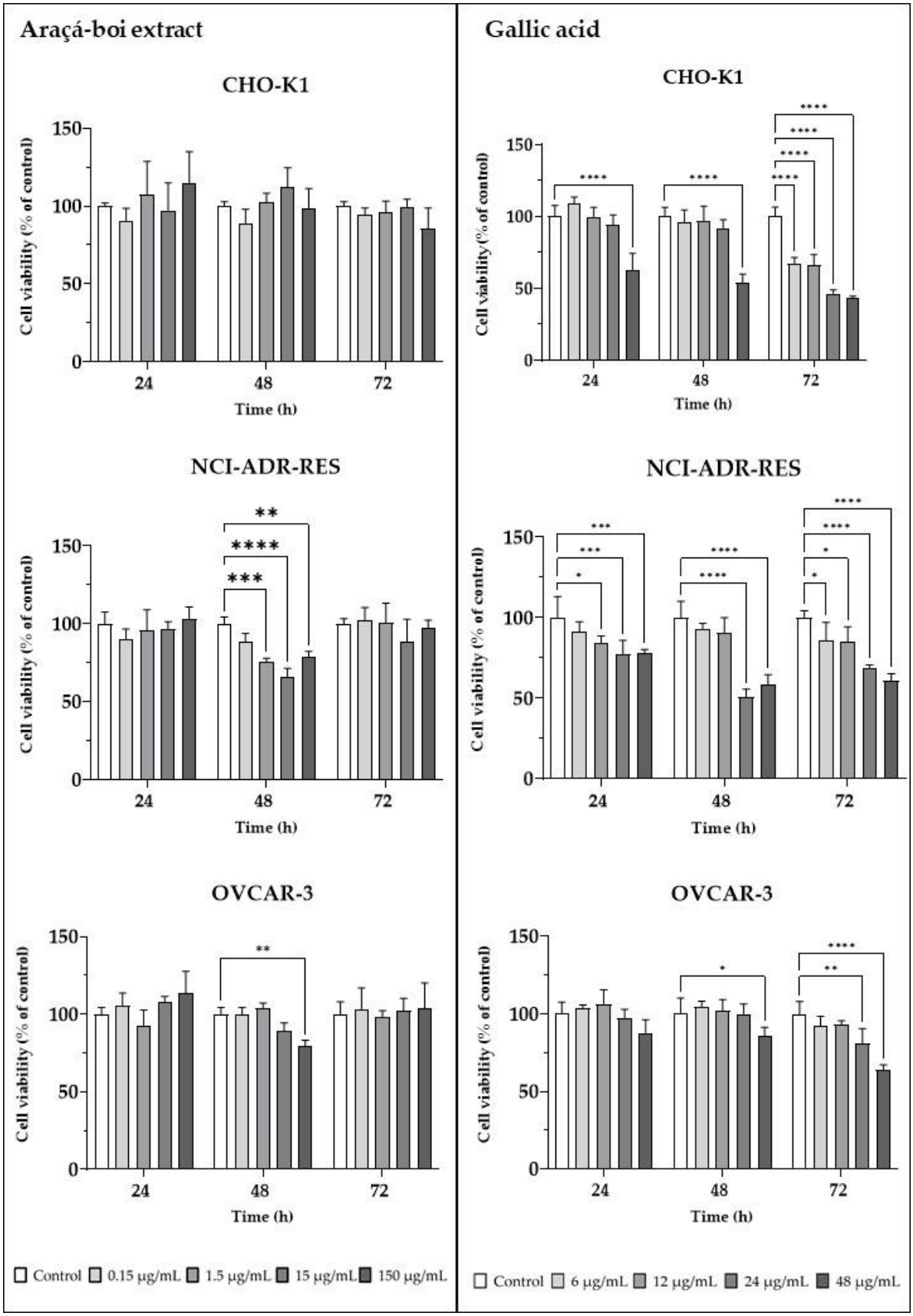

Figure 1. The effect of different concentrations of araçá-boi extract and gallic acid on the viability of normal Chinese hamster ovary (CHO-K1) cell line and human ovarian cancer cell line (NCI-ADR-RES and OVCAR3). Viability was measured by the MTT assay after 24, 48, and 72 h. Values with $p < 0.05$ were considered statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Similar to our findings for gallic acid, Varela-Rodríguez et al. (2020) reported that gallic acid and myricetin exhibited low selectivity, showing cytotoxic activity in both a cell line derived from normal human bronchial epithelial cells (BEAS-2B) and human ovarian tumor cells (OVCAR-3 and SKOV-3), possibly linked to the cellular phenotype. Several mechanisms through which phenolic plant extracts can reduce cell viability include oxidative stress, mitochondrial dysfunction, disruption of cell membranes, DNA damage, induction of apoptosis, cell cycle arrest, and modulation of signaling pathways and gene expression. These mechanisms lead to various cellular consequences, such as damage to cell structures, impaired cellular function, and alterations in the regulation of cell growth and survival (Abotaleb et al., 2020; George et al., 2021; Leri et al., 2020).

The absence of effect of araçá-boi extract on cell viability for the NCI-ADIR-RES tumor cell line after 24 hours can be attributed to an insufficient exposure time, as tumor cells may not have had enough time to accumulate the extract's effects, such as cellular damage or apoptosis induction. By 72 hours, the initial impact may have been attenuated due to cellular resistance mechanisms, reducing the effectiveness of the extract (Russo et al., 2021). On the other hand, the action of isolated gallic acid can be explained by its nature as a single compound, which acts directly on tumor cells without interference from other components present in the extract. Gallic acid, due to its structure and bioactive activity, may induce cellular damage more rapidly compared to the complete extract, which relies on synergistic or antagonistic interactions among its compounds (X. Chen et al., 2022). For OVCAR-3, the more limited response observed for both the araçá-boi extract and gallic acid may be associated with more robust resistance mechanisms against these treatments, which require higher concentrations of the extract or gallic acid to be overcome (Bradbury et al., 2020). Therefore, the results revealed the differential impact of araçá-boi extract and gallic acid on ovarian tumor cells (NCI/ADR-RES), particularly at 48 hours, highlighting the need for further investigation into the molecular mechanisms involved. Additional analyses were performed to assess the expression of genes

essential for cellular damage repair, cell cycle regulation, and epigenetic modulation. These genes play fundamental roles in tumor suppression and cellular response to therapies, providing insights into resistance mechanisms and potential targets for therapeutic interventions.

3.4. Relative gene expression of tumor suppressor genes and epigenetic enzymes

RT-PCR was employed to assess the effects of araçá-boi extract and gallic acid on the expression levels of genes associated with DNA repair (*BRCA1*), tumor suppression (*RASSF1A*), cell cycle regulation (*CDKN2A*), DNA methylation (*DNMT1*), and histone deacetylation (*HDAC1*) on ovarian tumor cells (NCI/ADR-RES) after 48 h (Figure 2). The results showed that treatment with araçá-boi extract significantly increased the expression of tumor-suppressor genes (BRCA1 and RASSF1A) and genes involved in the epigenetic process (HDAC1), especially at 15 μg/mL. Gallic acid (24 μg/mL), a component of the extract, had a more pronounced effect on *BRCA1*, *HDAC1*, and *CDKN2A* gene expression.

The literature features numerous studies on isolated phenolic compounds or plant extracts with the potential to modulate tumor suppressor genes and epigenetic enzymes in cancer (Borsoi, Alves, et al., 2023; Maleki Dana et al., 2022; Rajendran et al., 2022; Vrânceanu et al., 2022). For instance, Homayoun et al. (2020) observed that grape seed extract exerts antitumorigenic effects in chemoresistant human ovarian cancer cells (OVCAR-3), potentially mediated by the modulation of genes involved in signaling pathways (*PTEN*, *AKT*, *mTOR*, *DACT1*, *GSK3B*, and *C-MYC*), cell cycle regulation (*CDK4* and *CCND1*), and apoptosis (*BAX*, *BCL2*, *CASP3*, *CASP8*, and *CASP9*). According to a study by Nowrasteh et al. (2023), a commercial fruit extract, was found to modify the expression of genes related to epigenetic processes in an animal model induced by the carcinogen DMBA (7,12-dimethylbenz(a)anthracene). This alteration may potentially slow down cancer development and tumor progression.

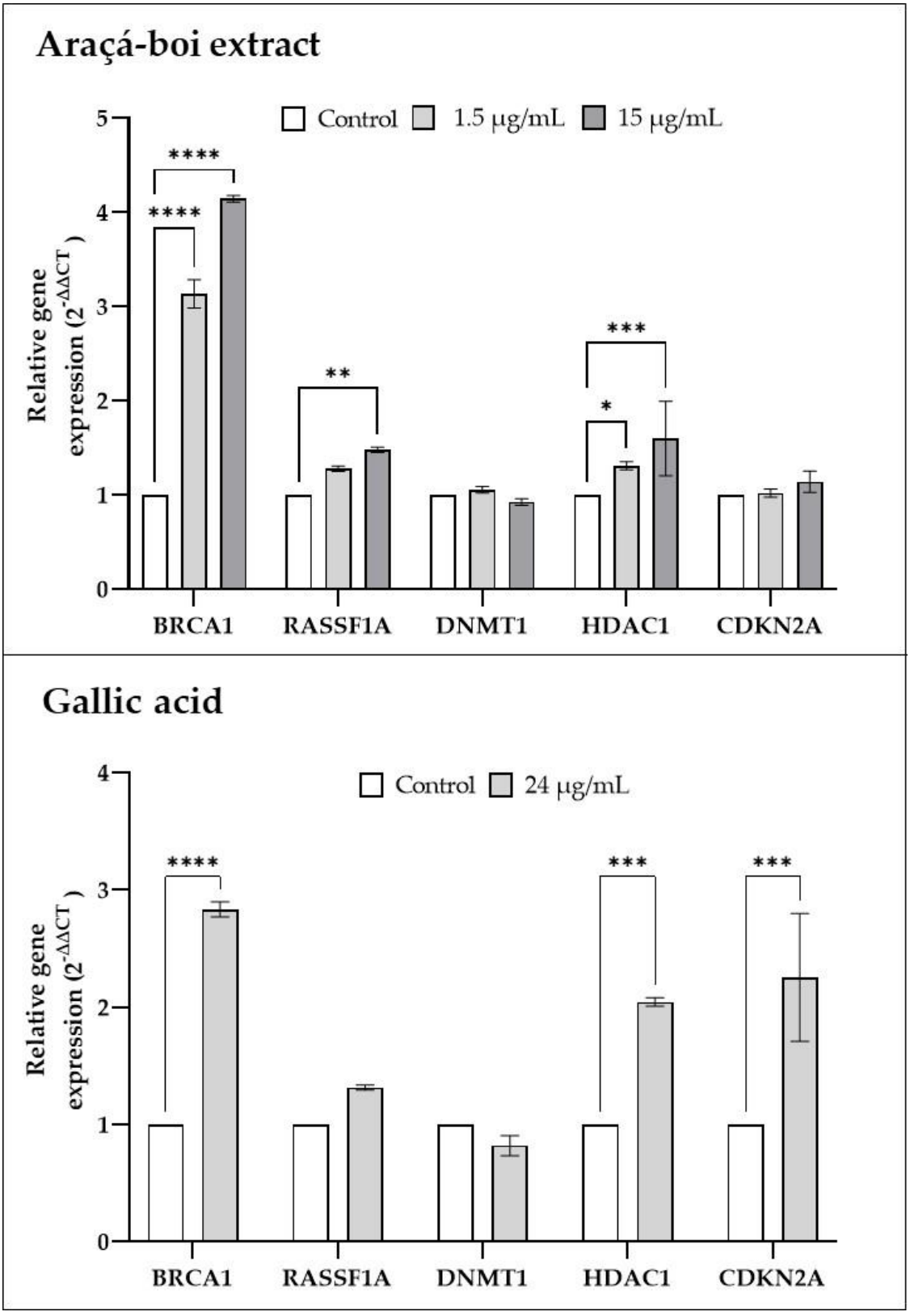

Figure 2. Relative gene expression $2^{(-\Delta\Delta CT)}$ of NCI-ADR-RES cells treated with araçá-boi extract (1.5 and 15 µ/mL) and gallic acid (24 µg/mL) for 48 h. The relative expression of *BRCA1*, *RASSF1A*, *DNMT1*, *HDAC1*, and *CDKN2A* was measured by RT-PCR. GAPDH was used as the housekeeping gene to normalize gene expression. Values with $p < 0.05$ were considered statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

The upregulation of tumor suppressor genes, including *BRCA1*, *RASSF1*, and *CDKN2A*, combined with the downregulation of genes involved in epigenetic processes, such as *DNMT1* and *HDAC1*, has the potential to delay cancer onset and hinder tumor progression (Martinez-Useros et al., 2021). *BRCA1* primarily functions in maintaining genomic integrity by repairing double-strand DNA breaks, which is essential for cellular stability (Al-Yousef et al., 2020). The *RASSF1A* gene is a tumor suppressor that regulates cell cycle, apoptosis, cell migration, cell adhesion, and microtubule stabilization (C. Chen et al., 2024). Thus, the upregulation of *BRCA1* and *RASSF1* by araçá-boi extract suggests a potential activation of DNA repair pathways and an inhibitory function in signaling pathways such as Ras/MAPK, which contributes to cell cycle arrest and the promotion of apoptosis, potentially reducing the cell proliferation typical of cancer. On the other hand, gallic acid has been shown to upregulate the *BRCA1* and *CDKN2A* genes. *CDKN2A* is a tumor suppressor gene that encodes proteins such as p16, which inhibit cyclin-dependent kinase activity, thereby halting cell cycle progression, particularly at the G1 to S phase transition (Z. Chen et al., 2021). The upregulation of *BRCA1* and *CDKN2A* may lead to DNA repair and cell cycle arrest, contributing to a reduction in cell proliferation. Therefore, this suggests that the phytochemicals present in araçá-boi, including gallic acid, may contribute to activating this repair pathway, enhancing cellular defense against mutations that promote tumor development. Additionally, isolated gallic acid influences different molecular pathways compared to araçá-boi extract, highlighting the synergistic and/or antagonistic interactions of the phenolic compounds in the extract.

Regarding the epigenetic enzymes, no changes in *DNMT1* modulation were observed, while upregulation of *HDAC1* was recorded for both the araçá-boi extract and gallic acid. *DNMT* is involved in maintaining DNA methylation, a process that can silence tumor suppressor genes, promoting the proliferation of cancer cells. On the other hand, HDACs, such as *HDAC1*, play a role in removing acetyl groups from histones, resulting in DNA compaction and gene transcription repression. In ovarian

cancer, *DNMT1* and *HDAC1* can facilitate tumor survival by silencing tumor suppressor genes and promoting resistance to apoptosis (Wang et al., 2022). As mentioned above, phenolic compounds derived from plants can downregulate these enzymes, thereby restoring the expression of tumor suppressor genes. Nevertheless, the lack of *DNMT1* modulation observed in our study suggests that, while araçá-boi extract and gallic acid do not inhibit *DNMT1* activity, they also do not promote pro-tumoral epigenetic processes related to DNA methylation. On the other hand, the upregulation of *HDAC1*, although potentially associated with transcriptional repression, may disrupt pro-tumoral pathways or enhance the sensitivity of cells to *HDAC1* inhibitors. This analysis provides a comprehensive perspective on how araçá-boi extract and gallic acid may modulate critical signaling pathways in ovarian tumor cells, identifying promising molecular targets for therapeutic development. The results suggest that the phenolic compounds present in the extract not only have the potential to act specifically on targets related to cell viability and epigenetic regulation but could also be explored in combination therapies. These findings open new avenues to expand the therapeutic spectrum for ovarian cancer treatments, enhancing the effectiveness of current therapeutic approaches.

3.5. DNA methylation profiling of BRCA1 promoter

Genomic DNA was isolated from NCI/ADR-RES ovarian tumor cells and treated with sodium bisulfite using the EZ DNA Methylation-Direct™ Kit (Zymo Research Corporation) to convert all unmethylated cytosines into uracils, leaving methylated cytosines unchanged. In subsequent PCR reactions, unmethylated cytosines are read as Ts (or complementary strand As), while methylated cytosines are read as Cs (or complementary strand Gs). The modified DNA was then used as a template for PCR reactions with primers designed to amplify specific regions in the promoters of the target gene. The PCR products were purified using PCR purification columns and sequenced. In Figure 3, the electropherogram illustrates the methylation status of the *BRCA1* gene promoter region in ovarian tumor NCI/ADR-RES cells

treated for 48 hours with araçá-boi extract or gallic acid. The results showed that, regardless of the treatment, the analyzed CpG islands remained methylated, indicating no demethylation in the evaluated region.

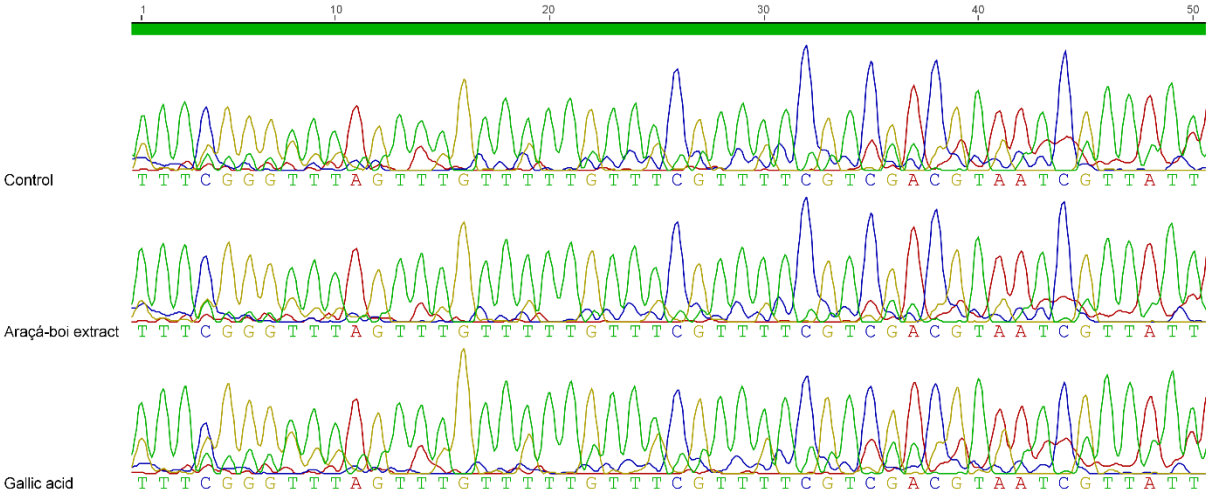

Figure 3. Electropherogram of the methylation status of the *BRCA1* gene promoter region in ovarian tumor cells NCI/ADR-RES treated with araçá-boi extract (15 µg/mL) or gallic acid (24 µg/mL) for 48 hours. Cytosine-C, blue; Thymine-T, green; Guanine-G, yellow; Adenine-A, red.

The BRCA1 gene, known for its critical role in DNA damage repair and tumor suppression, is frequently found methylated in ovarian tumor cells. This anomalous methylation in its promoter region leads to the transcriptional silencing of the gene, not only contributing to pathogenesis but also inducing drug resistance and influencing the prognosis of ovarian cancer (Fu et al., 2024). Phytochemicals such as phenolic compounds have been associated with reversing abnormal methylation status in tumor suppressor genes, including BRCA1, restoring their expression and promoting antitumor effects. This action typically occurs through the inhibition of epigenetic enzymes, such as DNMTs, responsible for the addition and maintenance of methyl groups at CpG dinucleotides, and HDACs, which regulate chromatin compaction levels, directly influencing gene transcription (Fatima et al., 2021; Khan et al., 2022).

The absence of demethylation in the evaluated promoter region of the *BRCA1* gene can be attributed to several factors. Firstly, the concentration and duration of treatment may not have been sufficient to induce significant epigenetic changes.

Previous studies suggest that both the dose and the exposure time to phenolic compounds directly influence their effects on DNA methylation. (Jasek et al., 2019). In our study, the concentration and treatment duration were determined based on the results of the cell viability assay and subsequently on gene expression analysis. Although these conditions effectively reduced cell viability, they may not have been sufficiently intense or prolonged to induce significant epigenetic changes, such as demethylation of the *BRCA1* gene promoter region. Additionally, regional specificity may play a critical role, as different CpG islands within the same promoter region can exhibit distinct responses to epigenetic stimuli (Boettcher et al., 2010). The promoter region of the BRCA1 gene is located approximately 1,000 base pairs upstream of exon 1 and includes both the core promoter and regulatory regions essential for the transcriptional regulation of *BRCA1*. The core promoter spans 326 base pairs and contains 25 CpG islands; however, this study focuses on 6 CpG islands over 50 base pairs, positioned from 103 to 153 within the core promoter. Specific CpG regions in tumor suppressor gene promoters are often preferentially methylated in cancer, regardless of treatment (Gull et al., 2022). This result may indicate that the CpG islands evaluated in the promoter region of the *BRCA1* gene in NCI/ADR-RES ovarian tumor cells may exhibit an epigenetic stability that resists demethylating stimuli, regardless of the applied treatment. Therefore, exploring other regions within the promoter could provide deeper insights into the broader epigenetic dynamics influencing the methylation status of *BRCA1* in ovarian tumor cells.

4. Conclusion

This study characterized the araçá-boi extract and its effects on antioxidant activity, cell viability, and the regulation of genes related to tumor suppression and epigenetic mechanisms in ovarian cancer cells. The extract was found to be rich in phenolic compounds, exhibiting potent antioxidant activity, primarily through hydrogen atom transfer, and efficiently scavenging reactive oxygen species (ROS), suggesting its therapeutic potential in oxidative stress-related conditions. A total of 73

compounds were identified, including ten organic acids, thirty-six phenolic acids, and twenty-seven flavonoids. Gallic acid, a key phenolic compound, was tested independently and contributed to the extract's antitumor effects. The extract had no cytotoxicity on normal CHO-K1 cells, whereas gallic acid showed toxicity at certain concentrations and time points. Both the extract and gallic acid reduced cell viability in the NCI/ADR-RES cell line after 48 hours. Gene expression analysis revealed that the araçá-boi extract upregulated *BRCA1* and *RASSF1A*, suggesting activation of DNA repair pathways and inhibition of cell cycle progression, potentially reducing cancer cell proliferation. Gallic acid also upregulated *BRCA1* and *CDKN2A*, indicating DNA repair and cell cycle arrest through different molecular mechanisms. No changes in BRCA1 promoter methylation were observed, suggesting that the reversal of the tumor phenotype may be driven by oxidative stress, apoptosis, or complex epigenetic effects beyond demethylation, including post-translational modifications and regulation of transcriptional and signaling enzymes. These findings indicate that other phenolic compounds in the araçá-boi extract may also contribute to the observed antitumor effects.

CRediT authorship contribution statement

Felipe Tecchio Borsoi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft, Writing - review & editing. **Henrique Silvano Arruda:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - review & editing. **Amanda Cristina Andrade, Monica Pezenatto, Isabelle Nogueira, Leonardo Augusto Marson, and Ana Sofia Martelli Chaib Saliba:** Data curation, Methodology, Visualization. **Severino Matias de Alencar:** Supervision, Funding acquisition, Resources, Writing - review & editing. **Murilo Vieira Geraldo:** Investigation, Methodology, Supervision Visualization, Funding acquisition, Resources, Writing - review & editing. **Iramaia Angélica Neri Numa:** Methodology, Supervision, Visualization, Writing – review & editing. **Glaucia Maria Pastore:** Project

administration, Supervision, Funding acquisition, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have appeared to influence the work reported in this manuscript.

Data Availability

Data will be made available on request

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Ethical approval

This article does not contain studies with human participants or animals performed by any of the authors.

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CHAPTER 4

Understanding the gastrointestinal behavior of phytochemicals and antioxidants from araçá-boi extract

Understanding the gastrointestinal behavior of phytochemicals and antioxidants from araçá-boi (*Eugenia stipitata* McVaugh) extract: An *in vitro* and *in silico* approach

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Understanding the gastrointestinal behavior of phytochemicals and antioxidants from araçá-boi (*Eugenia stipitata McVaugh*) extract: An *in vitro* and *in silico* approach

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Abstract

Though the araçá-boi fruit's nutritional value and phenolic compounds' functional properties are known, their behavior during gastrointestinal digestion remains unclear. Furthermore, bioinformatics tools have not been used to track the effects of phenolic compounds of araçá-boi extract. Therefore, this study aimed to evaluate the influence of gastrointestinal digestion on the phytochemical profile, phenolic compounds, antioxidant capacity, and sugar content in the araçá-boi extract. Additionally, molecular docking of target proteins and ADMET analysis were performed using the major compound of araçá-boi extract after gastrointestinal digestion. UHPLC-Q-Orbitrap-MS/MS analyses revealed that the araçá-boi extract has a diversity of phytochemical compounds, with 100 compounds identified, and after gastrointestinal digestion, only 59 compounds were identified. Gastrointestinal digestion had a significant impact on phenolic compounds with a highlight on trans-cinnamic acid, whose bioaccessibility increased by an impressive 813%. The extract also contains a variety of mono- and disaccharides, as well as oligosaccharides such as G3 and G4; however, 50 to 65% of the initial content was bioaccessible after digestion. The extract showed an increase in antioxidant capacity in the $ABTS^{\bullet+}$ assay, while it decreased in the DPPH, FRAP, and ORAC assays after gastrointestinal digestion. The *in silico* study showed that trans-cinnamic acid interacts with target proteins such as NF-κB, IL-1β, and PI3K which have a promising pharmacokinetic profile. These findings open new perspectives for future research, emphasizing the importance of

further investigating the effects of the extract in different physiological contexts and its potential applications.

Keywords: *Eugenia stipitata;* phenolic compounds; trans-cinnamic acid; bioaccessibility; molecular docking; ADMET.

1. Introduction

In recent years, there has been an increasing focus on plant-based foods, largely due to their numerous health benefits that extend beyond mere nutritional value. Many biological effects are linked to polyphenols in the food matrix, as they activate antioxidant enzymes, neutralize reactive species, inhibit oxidative enzymes, and donate electrons to free radicals. Despite being present in food only in trace amounts, the antioxidant character of polyphenols gives them the ability to modulate both gene expression and modify epigenetic alterations, consequently reducing the risk of the development of various chronic non-communicable diseases (NCDs), such as diabetes, cancers, cardiovascular diseases, chronic respiratory diseases, and mental illness (Borsoi et al., 2023). However, for their biological effects on the human body, they must be released from the food matrix in the gastrointestinal tract to become available for absorption in the small intestine (bioaccessible fraction), be absorbed, and reach target tissues in effective concentrations (bioavailable fraction) (Dantas et al., 2023).

During the process of gastrointestinal digestion, the stability, bioaccessibility, and bioavailability of phenolic compounds can be affected according to their concentration and the degree of release from the food matrix due to several physical, chemical, and biochemical factors such as changes in intestinal pH, the action of bile salts, temperature, the action of enzymes, degree of polymerization, interaction with fibers, proteins, carbohydrates and minerals, oxidation reactions, etc (Mihaylova et al., 2021). Thus, *in vitro* food digestion systems that simulate the physiological conditions of the human body during gastrointestinal digestion and verify their effect become increasingly popular and predominantly used for preclinical studies. Furthermore, they are fast and simple methods, and without ethical restrictions compared to *in vivo* tests (Ketnawa et al., 2021). Additionally, the application of various bioinformatics

tools has broadened the capabilities and enhanced the effectiveness of phenolic compound screening. For example, methods like molecular docking, along with ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis, are employed to evaluate the physicochemical characteristics, toxicity, and potential biological effects of phenolic compounds (Zhao et al., 2024).

Araçá-boi (*Eugenia stipitata* Mac Vaugh) is a fruit tree native to the Amazon region, which presents peculiar sensorial characteristics and high nutritional and economic potential. The fruit is rich in essential minerals, fibers, vitamins, and phenolic compounds and their derivatives (Acosta-Vega et al., 2024). Although some previous studies have demonstrated the potential of the araçá-boi regarding its nutritional characteristics and its potential functional properties (*e.g.,* antioxidant, anti-inflammatory, antidiabetic, antigenotoxic, and antimutagenic potential (de Araújo et al., 2021a; Garzón et al., 2012; Gonçalves et al., 2010; Neri-Numa et al., 2013; Soares et al., 2019), it is crucial to emphasize that there exists scarce literature remains unclear about the behavior of these compounds throughout the gastrointestinal digestion process. Moreover, bioinformatics tools have not been used to screen the effects of araçá-boi phenolic compounds on the modulation of proteins related to inflammation. Thus, given the scarcity of research in this area, the aim of this study was. To evaluate the influence of gastrointestinal digestion *in vitro* on the phytochemical profile, phenolic compounds, antioxidant capacity, and content of sugars in the araçá-boi extract. Additionally, molecular docking of targeting proteins related to inflammation, and ADMET analysis were performed using the major compound of araçá-boi after gastrointestinal digestion.

2. Materials and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, Trolox (6-hydroxy-2578-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,2′-azobis(2-methylamidino-propane)-dihydrochloride (AAPH), 2,2′-azinobis-(3-ethylbenzothiazo- line-6-sulfonic

acid)-diammonium salt (ABTS), fluorescein, TPTZ (2,4,6- tripyridy-s-triazine), porcine pepsin, pancreatin, and bile solution, methanol and formic acid grade HPLC and all phenolic compound standards (gallic acid, protocatechuic acid, chlorogenic acid, catechin, gentisic acid, epicatechin, caffeic acid, vanillic acid, syringic acid, rutin, *p*-coumaric acid, sinapic acid, ferulic acid, quercetrin, myricetin, benzoic acid, luteolin, quercetin, trans-cinnamic acid, apigenin, naringenin, kaempferol, and hesperetin) with a purity of ≥96% were purchased from Sigma-Aldrich (St. Louis, USA). Sugars (xylitol, mannitol, sorbitol, arabinose, rhamnose, glucose, fructose, and sucrose), and maltooligosaccharides (MOS; maltose to maltoheptaose) were purchased from Sigma-Aldrich (St. Louis, USA), whereas the fructooligosaccharides (FOS; 1-kestose, nystose, and 1F-β-fructofuranosylnystose) were purchased from Wako (Wako Pure Chemicals Industries, Osaka, Japan). The other solvents and reagents used in this study were of analytical grade. All solutions were prepared with ultrapure water (18 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, USA).

2.2. Preparation of plant samples

The harvesting of araçá-boi fruits was conducted in the Ituberá city, Bahia, Brazil (Kamui Farm), with the following specific coordinates: 13º 44' S, 39º 9' W. The species was botanically identified, an exsiccate was registered (access number 55.875) and stored at the Herbarium-UEC of the Agronomic Institute of Campinas in São Paulo, Brazil (Baldini et al., 2017). First, the samples were sanitized, and the seeds were removed from the edible portion, which included both the pulp and peel. The edible portion was processed using a domestic juicer and combined into a single batch. This batch was then rapidly frozen at -80 °C, lyophilized for 52 hours, and subsequently milled using a knife mill. The resulting powder was standardized by particle size using an electromagnetic sieve shaker (24 mesh). The standardized powder was stored at -20 °C.

2.3. *Ultrasound-assisted extraction of phenolic compounds*

The extraction of phenolic compounds followed a slightly modified version of the protocol outlined by de Araújo et al. (2021a). Freeze-dried araçá-boi (10 g) was mixed with an 80:20 v/v ethanol-water solution (150 mL) and subjected to ultrasound-assisted extraction for 10 minutes at 20 °C (UNIQUE, model UCS-2850, 25 kHz, 120 W, Brazil). After this, the samples were centrifuged for 5 min/4000 × g/5 °C. The supernatant was separated, and the remaining solid was extracted two more times under identical conditions. Residual ethanol in the solution was removed under vacuum at 40°C, and the volume was adjusted to 50 mL with deionized water.

2.4. *In vitro gastrointestinal digestion*

The *in vitro* gastrointestinal digestion assay was performed according to the method described by Sancho et al. (2017), with some modifications. To summarize, araçá-boi extract (1 mL) was mixed with 140 mmol/L NaCl + 5 mmol/L KCl saline solution (3.5 mL). The 6 mol/L HCl solution was added until the pH reached 2.0. Next, a porcine pepsin solution was prepared by dissolving 200 mg of pepsin in 5 mL of 0.1 mol/L HCl. Then, 125 μL was added to the sample and incubated in a water bath at 37°C with continuous agitation (130 rpm) for 1 hour. After the gastric process, the pH was adjusted to 6.8 by adding NaHCO3 (1 mol/L). Subsequently, a pancreatin and bile solution was prepared by dissolving 37 mg of pancreatin in 18.7 mL of 0.1 mol/L NaHCO3. Then, 625 μL of the solution was added to the sample and incubated in a water bath at 37°C with continuous agitation (130 rpm) for 2 hours. To eliminate any reagent-related interference, a blank sample with only deionized water was treated under the same digestion conditions. After each digestion step, the samples were cooled using an ice bath. The gastric and intestinal phases were centrifuged (4000 × g/5°C/15 min) and ultrafiltered (Amicon Ultra centrifugal filters, 30 kDa Millipore). The collected samples were subsequently stored at -20°C for future analyses.

2.5. Recovery index (% R) and bioaccessibility index (% B)

During the in vitro digestion process, the release of total phenolics, flavonoids, condensed tannins, and antioxidant activity was monitored. Equations 1 and 2 were applied to calculate the recovery index (% R) and bioaccessibility index (% B), according to Ortega et al. (2011).

$$\%\,R = \frac{A}{C} \times 100 \quad (1)$$

$$\%\,B = \frac{B}{C} \times 100 \quad (2)$$

Where, A represents the content of compounds (phenols, flavonoids, tannins, or antioxidant activity) released during gastric digestion; B corresponds to the content released during intestinal digestion; and C denotes the content present in the extract before digestion.

2.6. Determination of total phenolic content (TPC)

The total phenolic content was performed according to the method proposed by Roesler et al. (2007) with modifications. The araçá-boi extract (30 μL) was added with 10% Folin-Ciocalteau solution (150 μ) and 7.5% $NaHCO_3$ (20 μL). Then, the samples were incubated (45°C/6min), and the absorbances were measured at 760 nm. For the elaboration of the analytical curve, a gallic acid standard was used and the results were expressed as mg gallic acid equivalent (GAE)/g extract dw (dry weight).

2.7. Determination of total flavonoid content (TFC)

Total flavonoid content was determined according to the method proposed by Zhishen et al. (1999). The araçá-boi extract (30 μL) was added to ultrapure water (110 μL), and 5% $NaNO_2$ (8 μL), and incubated (20 °C/5 min). After this period, 8 μL of 10% $AlCl_3$ (8 μL) were added and incubated (20°C/6 min). Finally, 1 mol/L NaOH (50 μL) and ultrapure water (70 μL) were added. Right after, the absorbances were measured at 510 nm. For the elaboration of the analytical curve, a catechin standard was used

and the total flavonoid content was expressed as mg catechin equivalent (CE)/g extract dw.

2.8. Determination of condensed tannin content (CTC)

Condensed tannin content was determined according to the method described by Arruda et al. (2018) with slight modifications. The araçá-boi extract (20 μL), 180 μL of vanillin (4 % w/v) prepared in methanol, and concentered HCl (90 μL) were mixed and incubated (20°C/20 min). The absorbances were recorded at 500 nm. For the elaboration of the analytical curve, a catechin standard was used and the total condensed tannin content was expressed as mg (CE)/g extract dw.

2.9. Antioxidant analysis

2.9.1. DPPH assay

The assay was performed according to the method proposed by Roesler et al. (2007) with some modifications. 50 μL of the extract was mid with 250 μL of $DPPH^{\bullet}$ solution (0.004% in ethanol). After 30 minutes of incubation at room temperature, absorbances were measured at 517 nm. The results were expressed as μmol Trolox equivalents (TE) per gram of dry weight (dw).

2.9.2. $ABTS^{\bullet+}$ scavenging assay

The $ABTS^{\bullet+}$ radical scavenging activity was assessed following the procedure outlined by Leite et al. (2011). Firstly, the $ABTS^{\bullet+}$ radical was generated by reacting 7 mmol ABTS (5 mL) with 140 mmol potassium persulfate (88 μL). This mixture was allowed to stand at room temperature for at least 16 hours. After this, an aliquot of this solution was then diluted with ultrapure water, and the absorbance was adjusted to 0.70 ± 0.02 at 734 nm using a microplate reader. To measure the scavenging activity, 50 μL of the extract was combined with 250 μL of the $ABTS^{\bullet+}$ solution, and the absorbance was recorded at 734 nm. Trolox was used to construct the calibration curve, and the results were expressed as μmol Trolox equivalent (TE)/g extract dw.

2.9.3. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was performed following the method of Guerra-Ramírez et al. (2021), with some modifications. To prepare the FRAP reagent, a solution was made by combining 20 mL of acetate buffer (0.3 mol/L, pH 3.6), 2 mL of TPTZ solution (10 mmol/L) in 40 mmol/L HCl, and 2 mL of ferric chloride solution (20 mmol/L), mixed in a 10:1:1 ratio. Then, 20 μL of the extract was combined with 180 μL of the FRAP solution and 60 μL of deionized water. This mixture was incubated at 37 °C for 30 minutes before measuring the absorbance at 595 nm. A trolox standard was used for the elaboration of the analytical curve and the results were expressed as μmol TE/g extract dw.

2.9.4. Oxygen radical absorbance capacity (ORAC)

Fluorescence-based reactions were carried out using 96-well polystyrene microplates (Corning Co®, NC, USA) according to the method described by Leite et al. (2011), with slight modifications. For this, the samples, standards, and reagents were dissolved in a 75 mmol potassium phosphate buffer (pH 7.4). To each well, 20 μL of Trolox, standard or diluted extracts were added, followed by 120 μL of fluorescein (0.378 μg/mL, pH 7.4) and 60 μL of AAPH [2,2-Azobis-(2-methylamidinopropane)-dihydrochloride] (108 mg/mL). Fluorescence intensity was measured at 37 °C immediately after the addition of AAPH, with readings taken every 60 seconds for 80 cycles. The fluorescence was monitored using excitation and emission filters set at 485 nm and 520 nm, respectively, and the data were analyzed with MARS Data Analysis Software (Version 1.3). A Trolox standard was used for the elaboration of the analytical curve and the results were expressed as μmol TE/g extract dw.

2.10. Determination of phytochemical profile by UHPLC-Q-Orbitrap-MS/MS

The phytochemical profile of the araçá-boi extract, along with its gastric and intestinal fractions, was determined using a Thermo Ultimate 3000 system coupled with a Q-Exactive mass spectrometer. The mass spectrometer was configured to run in negative electrospray ionization (ESI-) mode, following the methodology outlined

by Bocker & Silva (2024). The desolvation gas flow was set to 51 L/min, the auxiliary gas flow to 13 L/min, and the sweep gas flow to 3 L/min. The spray voltage was maintained at 2.5 kV, with the capillary temperature at 266 °C and an RF lens setting of 50. The auxiliary gas was heated to 431 °C. During data acquisition, the instrument scanned a mass range from 100 to 1500 Da with a resolution of 70,000, using an AGC target of 3e6 and a maximum injection time (IT) of 100 ms. For MS/MS analysis, the resolution was reduced to 17,500, with an AGC target of 1e5 and a maximum IT of 50 ms. The five most intense precursor ions were chosen for fragmentation, employing stepped normalized collision energies (NCE) of 25, 30, and 35 eV, and an isolation window of 3.0 m/z.

The sample was chromatographically separated using a Poroshell 120 SB-Aq column (100 × 2.1 mm i.d., 2.7 μm particle size, Agilent Technologies) using a gradient elution program with a flow rate set to 0.45 mL/min. The column temperature was maintained at 40 °C. The mobile phase consisted of two solvents: 0.1% formic acid in water (Eluent A) and acetonitrile with 0.1% formic acid (Eluent B). The gradient conditions were: 0–1 min, 95% A; 1–10 min, 95–82% A; 10–13 min, 82–30% A; 13–15 min, 30–0% A; 15–17 min, 0% A; 17–19 min, 0–95% A; and 19–22 min, 95% A (Arruda et al., 2019). The Xcalibur software (version 4.3) was employed for data acquisition and qualitative analysis. The fragmentation profiles of the identified compounds were compared to phytochemical databases to confirm their identities.

2.11. Phenolic compounds analysis by HPLC-DAD

2.11.1. Preparation of standard solutions

Concentrated solutions of each phenolic compound were prepared in methanol (1 mg/mL). A working solution containing all 23 phenolic compounds (sinapic acid, chlorogenic acid, gallic acid, vanillic acid, benzoic acid, , trans-cinnamic acid, ferulic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, syringic acid, gentisic acid, catechin, epicatechin, quercetin, quercitrin, rutin, luteolin, apigenin, naringenin, myricetin, kaempferol, and hesperetin) was prepared by diluting the concentrated

stock solutions with acidified water (0.1% formic acid). Subsequent serial dilutions were made from this solution at eight distinct concentrations to construct calibration curves. Quality control (QC) solutions were prepared at 1 μg/mL (Level 1), 4 μg/mL (Level 2), and 8 μg/mL (Level 3). All the solutions were stored at -20 °C.

2.11.2. Instruments and analytical conditions

The phenolic compounds were quantified using a Dionex UltiMate 3000 HPLC-DAD system (Thermo Fisher Scientific, Waltham, MA, USA). Separation was carried out on a 250 × 4.6 mm AcclaimTM 120 A C18 column (5 μm particle size, Thermo Fisher Scientific, Waltham, MA, USA). The flow rate was set at 0.5 mL/min, with an injection volume of 20 μL, and the column temperature was maintained at 32 °C. The mobile phases were composed of Eluent A (deionized water with formic acid 0.1%) and Eluent B (100% acetonitrile). The gradient elution was as follows: 0-5 min, 95% Eluent A; 5-27 min, 95-71% Eluent A; 27-33 min, 65% Eluent A; 33-45 min, 50-35% Eluent A; 45-50 min, 5% Eluent A; and 50-60 min, 95% Eluent A. Chromatograms were recorded at four wavelengths (260, 280, 320, and 360 nm). Calibration curves for quantification of phenolic compounds were constructed using standards (0.1 to 10.00 μg/mL) and the identification of individual phenolic compounds was based on their retention times and spectral profiles compared to standard compounds (Table 1). Chromeleon software (version 6.80) was used for data acquisition and analysis. The concentration of each phenolic compound was expressed as μg/g of extract dw.

Table 1. Retention time, optimal absorption wavelength, and detection wavelength of 23 phenolic compounds.

Number	Compound	Retention time (min)	Optimal absorption wavelength (nm)	Detection wavelength (nm)
1	Gallic acid	13.36	270.7	280
2	Protocatechuic acid	20.73	259.2	260
3	Chlorogenic acid	23.98	325.4	320
4	Catechin	24.50	278.8	280
5	Gentisic acid	25.96	327.6	320
6	Epicatechin	26.98	277.5	280
7	Caffeic acid	27.22	323.1	320
8	Vanillic acid	27.53	260.2	260
9	Syringic acid	27.85	275.2	280
10	Rutin	29.90	256.0	260
11	p-Coumaric acid	31.99	309.3	320
12	Sinapic acid	33.15	323.1	320
13	Ferulic acid	33.62	322.0	320
14	Quercetrin	33.91	256.1	260
15	Myricetin	36.05	373.0	360
16	Benzoic acid	37.83	272.7	280
17	Luteolin	40.75	346.5	360
18	Quercetin	41.37	368.2	360
19	Trans-cinnamic acid	45.38	276.2	280
20	Apigenin	45.86	336.1	320
21	Naringenin	46.49	288.4	280
22	Kaempferol	47.04	365.7	360
23	Hesperetin	48.04	287.1	280

2.11.3. Method validation of HPLC-DAD analysis

The validation of the HPLC-DAD method for 23 phenolic compounds was carried out according to the recommendations of the EURACHEM Guidelines (2014). Linearity range, limits of detection (LOD), limits of quantification (LOQ), precision (inter-day and intraday), and accuracy of the method were evaluated.

Linearity was evaluated to confirm that the obtained results show a linear relationship with the concentration of the compound within the studied range. The calibration curves were prepared with a concentration ranging from 0.1 to 10 μg/mL. Eight different calibration levels were injected in triplicate on the same day and used to construct the calibration curves. A linear regression function was fitted to each compound by plotting the peak areas of the phenolic compounds against their concentration. The linearity of the calibration curves was evaluated by the determination of the coefficients of determination (R^2).

The sensitivity of the method was calculated as the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were determined based on the concentration at which the ratio of the analyte peak height (S) to the baseline noise (N) was 3 and 10, respectively.

The precision of the method was evaluated by repeatability (intra-day precision), and reproducibility (inter-day precision). The intraday precision was checked by injecting three independent replicates at three levels (1, 2, and 3) on the same day. Inter-day precision was evaluated in triplicate across three consecutive days, following the same procedure outlined for intraday precision. The acceptance criterion was a relative standard deviation (RSD) of less than 15%.

The accuracy of the method was determined by the recovery experiment that was conducted by analyzing the araçá-boi extract with and without standard added. Araçá-boi extract was spiked with a solution containing all phenolic compounds at three levels (1, 2, and 3). Spiked and unspiked samples were injected into three independent replicates and the recovery (%) for each analyte was calculated according

to Eq. 3. Recovery was considered acceptable when the values varied between 85 and 115%.

$$Recovery\ (\%) = 100 \times \frac{X-Y}{Z} \quad 3$$

where X is the analyte amount in spiked samples, Y is the analyte amount in unspiked samples, and Z is the amount of spiked analyte.

2.12. Chromatographic analysis of sugars

Sugars and oligosaccharides were analyzed using HPAEC-PAD (High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection) on a DIONEX ICS-5000 system (Thermo Fisher Scientific, Waltham, USA), following the methodology outlined by Pereira et al. (2018) with some adjustments. In both separations, the flow rate was set to 1.0 mL/min, the column temperature was maintained at 30 °C, and the injection volume was 25 μL. Sugars (mannitol, sorbitol, xylitol, rhamnose, arabinose, glucose, fructose, and sucrose) were quantified using a CarboPac PA1 column (250 × 4 mm i.d., 10 μm particle size, Thermo Fisher Scientific, Waltham, MA, USA). Separation was performed with an isocratic mobile phase consisting of 0.12 mol/L NaOH. Oligosaccharides (MOS: G2, G3, G4, G5, G6, G7 and FOS: GF2, GF3, GF4) were separated using a CarboPac PA100 column (250 × 4 mm i.d., 8.5 μm particle size, Thermo Fisher Scientific, Waltham, MA, USA). The separation was performed using three mobile phases: Eluent A (0.2 mol/L NaOH), Eluent B (ultrapure water), and Eluent C (0.5 mol/L sodium acetate with 0.2 mol/L NaOH). The elution gradient was programmed as follows: from 0 to 2 minutes, the mobile phase composition was 47% A, 50% B, and 3% C; from 2 to 18 minutes, it was gradually changed to 10% A, 50% B, and 40% C; from 18 to 23 minutes, 100% C was used; and from 23 to 28 minutes, the initial composition (47% A, 50% B, and 3% C) was restored. Identification of sugars and oligosaccharides was done by comparing the retention times of the samples to those of the standards. Calibration curves were created using commercial standards (0.25–12.50 μg/mL) to quantify the compounds, and the results were expressed as mg/g sample.

2.13. Molecular docking analysis

The study was performed by software Open Babel®, PyMOL® verse 1.5.03 Open Source, Autodock ® installed on PC Intel® core i7 8565U 1.80 GHz, RAM 8 GB DDR3 10600.

2.13.1. Data and preparation of ligands and proteins

The trans-cinnamic acid (molecular weight: 148.16 g/mol) was retrieved from the PubChem database (PubChem CID: 444539). The optimized structure was used as initial conformation for the molecular docking analysis.

The crystal structures of NF-kB (PDB ID: 3GUT), TNF-α (PDB ID: 2AZ5), IL-1β (PDB ID: 2NVH), COX-1 (PDB ID: 3N8X), PI3K (PDB ID: 4DK5) and Akt (PDB ID: 6HHF) were retrieved from the Protein Data Bank (http://www.rcsb.org/). Before initiating the docking simulations, all water molecules, ligands, heteroatoms, and undesired chains were removed from the protein, polar hydrogen atoms were added, AD4 atom type was assigned, missing atoms were repaired and Kollman charges were assigned and spread throughout the residue. All target proteins were energy minimized before docking analysis.

2.13.2. Active site prediction

The active sites are typically located in pockets or grooves on the protein's surface, where specific residues are responsible for determining substrate specificity. These residues frequently function as proton donors or acceptors. Active site prediction was developed on the online server PrankWeb (https://prankweb.cz/). Prankweb predicts and ranks the target protein active sites, enabling the visualization of the amino acids involved in each of them. The binding sites considered for each protein were those involved in the binding along the NF-kB, PI3K/Akt, and Arachidonic acid pathways according to the literature. Grid box coordinates (Table 2) were calculated by the medium of the x, y, and z coordinates of each amino acid included in the binding site (data found in .pdb protein files). The grid box was positioned around the approximate center of the binding site, using grid point spacing

of 0.375 Å, and enough dimensioned to fit all protein residue that could be involved in the docking process. The grid maps were calculated by AutoGrid.

Table 2. Grid box coordinates and size.

	COX-1	NF-kB	Akt	TNF-α	IL-1β	PI3K
			Center			
X	-20.59	50,354	3.97	-11,865	40,365	23,30
Y	-50.85	117,189	4.84	63,220	9,463	14,26
Z	1.47	54,361	12.01	18,622	57,721	21,31
			Size			
X	60	50	40	50	40	60
Y	60	40	40	40	40	60
Z	60	50	40	40	40	60

Grid box coordinates were calculated by the medium of the x, y, and z coordinates of each amino acid included in the binding site.

2.13.3. Docking simulation and validation

AutoDock v4.2 was employed to conduct docking simulations, utilizing rigid target proteins and flexible ligands. Therefore, no induced conformational change upon ligand binding in proteins was assumed, while tensional flexibility was permitted for the ligands. The docking protocol chosen to develop the analysis was the Lamarckian genetic algorithm (LGA), a built-in algorithm of Auto Dock, in which the population size was increased to 300. Genetic algorithms typically define an individual and its genetic code concerning the solution space of the problem at hand. In AutoDock, a specific ligand orientation within a protein is considered an 'individual' in the algorithm. Each flexible docking experiment was conducted across 50 trials, resulting in 50 different docked conformations for every ligand. Parameters such as binding energy, inhibitory constant, intermolecular energy, internal energy, and torsional energy are estimated through experimental free energy function calculations performed by AutoDock v4.2. Empirical calculations of binding free energy are made using these energy terms along with a set of coefficient factors. This binding energy is then used to rank the docking positions in order of their effectiveness.

Validation of ligand was conducted as follows: Literature-known inhibitors of each target protein were selected for docking comparisons. Ligands from the docking

process were saved and compared with standard inhibitors for measuring Root Mean Square Deviation (RMSD). If the RMSD difference between the standard inhibitor and docked ligands was similar or smaller than 2, the docking process was validated. Docking analysis were conducted based on interaction between residue and observed ligand along with binding affinity from molecular docking. Standard inhibitors for interleukins and interleukin receptors have not been assayed due to structure unavailability and/or no reports in the literature.

2.13.4. Prediction of absorption, distribution, metabolism, elimination, and toxicity (ADMET) characteristics

The ADMETlab2.0 server (https://admetmesh.scbdd.com/) was used to determine properties related to absorption, distribution, metabolism, and excretion (ADME), while toxicity predictions were predicted using the ProTox 3.0 server (https://tox.charite.de/).

2.14. Statistical analysis

The data were examined using descriptive statistics (mean and standard deviation), one-way analysis of variance (ANOVA), and the Tukey post hoc test ($p \leq 0.05$). Each measurement was repeated three times. Statistical analysis was conducted using Minitab Software (Version 16.1.0, State College, PA, USA).

3. Results and discussion

3.1. Effects of gastrointestinal digestion on total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content (CFC)

The total contents of phenolic, total flavonoids, and condensed tannin before and after the *in vitro* simulated gastrointestinal digestion from araçá-boi extract are presented in Table 3 and the results showed a significant difference ($p \leq 0.05$). As can be seen, the amount of TPC was significantly higher (19.92 mg GAE/g dw) before digestion in comparison to TPC of digested fractions that were significantly decreasing their content throughout the digestive process (8.96 and 5.81 mg GAE/g dw in gastric and intestinal digestion fraction, respectively). The TPC presented a recovery and

bioaccessibility index of 44.95% and 29.15%, respectively. De Paulo Farias et al. (2021) also observed a significant reduction in the TPC after the gastrointestinal process of edible and seed fractions of uvaia fruit (*Eugenia pyriformis*) in 41% and 50%, respectively. Similarly, de Araújo et al. (2021b) evaluated the bioaccessibility of TPC from the araçá-boi fractions and found a decrease in total phenolics for seed fraction after the gastrointestinal process (35.6% and 22%, respectively). On the other hand, there was an increase in TPC for the edible fraction after gastric digestion (123.4%), while after intestinal digestion the content of phenolic compounds decreased and was similar to that of crude extract.

Table 3. Recovery and bioaccessibility of total phenolic content, total flavonoid content, condensed tannin content, and antioxidant activity by DPHH, $ABTS^{\bullet+}$, FRAP, and ORAC methods from araçá-boi extract over the *in vitro* simulated gastrointestinal digestion.

	Fractions			Indexes	
Assay	**Araçá-boi extract**	**Gastric phase**	**Intestinal phase**	**% R**	**% B**
Total phenolic content (mg GAE/g extract dw)	19.92 ± 1.00^{a}	8.96 ± 0.57^{b}	5.81 ± 0.12^{c}	44.95	29.15
Total flavonoid (mg CE/g extract dw)	6.67 ± 0.34^{a}	3.24 ± 0.18^{c}	5.40 ± 0.21^{b}	48.50	80.87
Condensed tannin content (mg CE/g extract dw)	3.27 ± 0.28^{a}	3.08 ± 0.18^{a}	2.17 ± 0.18^{b}	94.42	66.31
DPPH (μmol TE/g extract dw)	66.34 ± 1.91^{a}	11.81 ± 0.20^{b}	6.51 ± 0.38^{c}	17.80	9.82
$ABTS^{\bullet+}$ (μmol TE/g extract dw)	143.12 ± 4.01^{b}	92.80 ± 5.25^{c}	163.08 ± 4.19^{a}	64.84	113.94
FRAP (μmol TE/g extract dw)	122.91 ± 3.07^{a}	98.15 ± 7.47^{b}	42.93 ± 2.28^{c}	79.86	34.93
ORAC (μmol TE/g extract dw)	344.93 ± 9.56^{a}	192.50 ± 16.04^{c}	230.15 ± 4.48^{b}	55.81	66.72

Means ± standard deviation. Different lowercase letters per row indicate significant statistical differences ($p \leq 0.05$). **% R:** recovery. **% B:** bioaccessibility.

Regarding the total flavonoid content (Table 3), it was observed that the araçá-boi extract was superior before the digestive process (6.67 mg CE/g dw) and its content decreased after gastric digestion (3.24 mg CE/g dw). On the other hand, after the intestinal digestion, the TFC of araçá-boi extract increased gradually and significantly, reaching 5.40 mg CE/g dw. Thus, for araçá-boi extract after the gastric phase, the

recovery was 48.50%, and in the intestinal phase a bioaccessibility of 80.87% for TFC. Thomas-Valdés et al. (2019) observed a reduction of TFC by 60.40% and 90.90% after the gastric and intestinal digestion in native Chilean red strawberries *(Fragaria chiloensis ssp. chiloensis f. patagonica)*. Whereas, Liu et al. (2021) found an increase of approximately 25.2% in the gastric phase and 39.1% after intestinal digestion compared with the undigested *Prinsepia utilis* Royle fruits.

The condensed tannin content (CTC) was evaluated and showed the same trend as TPC throughout the gastrointestinal digestion (Table 3). Before digestion, the araçá-boi extract presented a higher value (3.27 mg CE/g dw) and significantly decreased (2.17 mg CE/g dw) at the end of intestinal digestion. Condensed tannins are complex polymers formed by flavonoids, such as catechins and procyanidins, and therefore, they may exhibit good chemical stability during digestive processes (94.42% recovery index), reaching the intestinal phase with a bioavailability of 66.31%. To the best of our knowledge, this is the first study evaluating the effects of gastrointestinal digestion on the content of condensed tannins in araçá-boi extract.

The TPC, TFC, and CTC presented a recovery and bioaccessibility index of 44.95% and 29.15%, 48.50% and 80.87%, and 94.42% and 66.31%, respectively. These data indicate that flavonoids in araçá-boi extract would be more bioavailable than TPC and CTC to exert beneficial effects on health. This behavior is consistent with the findings of Corona-Leo et al. (2021), who observed a lower release of TFC during the gastric stage, followed by an increase in the intestinal stage in apples. These changes in the release and degradation of phenolic compounds throughout the gastrointestinal system can be explained by the various biochemical modifications that occur during the process, such as the action of enzymes, bile salts, pH variations, and temperature (Corona-Leo et al., 2021).

3.2. Effects of gastrointestinal digestion on antioxidant capacity

The antioxidant properties are primarily associated with the phenolic compounds found within the plant structure. These compounds function as donate

hydrogen, chelate transition metals, reducing agents, inhibit enzymes related to oxidative stress, neutralize reactive oxygen and nitrogen species (ROS/RNS), and enhance or safeguard the body's natural defense mechanisms (Arruda et al., 2018). Thus, considering these several methods, the antioxidant capacity of the araçá-boi extracts was determined using DPPH, $ABTS^{\bullet+}$, FRAP, and ORAC, according to Table 3.

As can be seen, the results of antioxidant capacity by DPPH assay gradually decreased significantly after simulated gastrointestinal digestion (66.34 to 6.51 μmol TE/g dw). The same trend can be observed in the FRAP assay, where there was a significant decrease in antioxidant activity throughout the gastrointestinal digestion process of the araçá-boi extract (122.91, 98.15, and 42.93 μmol TE/g dw, respectively).

These findings align with those of Kashyap et al. (2022), who reported a notable decrease in antioxidant capacity by the DPPH and FRAP method for hydroethanolic extracts from Meghalayan cherry pomace extracts submitted to simulated gastrointestinal digestion. By contrast, after the gastrointestinal digestion process, the $ABTS^{\bullet+}$ values significantly improved and surpassed the initial activity of undigested extract (143.12 to 163.08 μmol TE/g dw). This behavior was similar to that observed by de Paulo Farias et al. (2021), who reported a reduction of approximately 45% in the gastric phase and a 72% increase in the intestinal phase, compared to before digestion, in the edible fraction of uvaia.

The ORAC assay was firstly significantly reduced in the gastric phase (344.93 to 192.50 μmol TE/g dw) and right after significantly improved in the intestinal process compared to the gastric phase (192.50 to 230.15 μmol TE/g dw). While the antioxidant capacity of the araçá-boi extract showed an increase during the intestinal phase compared to the gastric phase, it remained lower than the antioxidant capacity of the crude extract. This trend contrasts with the findings reported by Djaoudene et al. (2021) who found a strong increase in the ORAC values after gastric and intestinal digestion compared to the undigested extracts of different Algerian cultivars of date (*Phoenix dactylifera* L.).

In general, the $ABTS^{\bullet+}$ assay presented the highest recovery and bioaccessibility index (64.84% and 113.94%, respectively), meanwhile, the DPPH assay showed the lowest recovery and bioaccessibility index (17.80% and 9.82% respectively). The recovery and bioaccessibility index were 79.86% and 34.93%, respectively, for the FRAP assay, and 55.81% and 66.72%, respectively, for the ORAC assay. The phenolic compounds present in the extract undergo pH variations and interact with digestive enzymes and bile salts present in the simulated gastrointestinal digestion medium, consequently affecting their structure and activity in the methods used (Ketnawa et al., 2021). Antioxidants operate through various mechanisms, mainly by electron transfer (which involves transferring an electron to neutralize compounds like metals, carbonyls, and radicals) and hydrogen atom transfer (which allows the neutralization of free radicals via hydrogen donation). The methods studied here encompass both action mechanisms, electron transfers by DPPH and FRAP and hydrogen atom transfer by $ABTS^{\bullet+}$ and ORAC (Arruda et al., 2018; Lang et al., 2024; Siddeeg et al., 2021). As previously mentioned, the araçá-boi extract, along with its gastric and intestinal phases, exhibited the most significant antioxidant activity when assessed by the ORAC and $ABTS^{\bullet+}$ methods, followed by FRAP and DPPH. This indicates that the phenolic compounds present in the araçá-boi extract and its digested fractions are more effective through hydrogen atom transfer mechanisms. Additionally, the ORAC method provides a more precise measure of a compound's capacity to neutralize peroxyl radicals, simulating the physiological conditions of the human body (Lang et al., 2024). Peroxyl radicals are a type of reactive free radical generated during oxidation processes within the human body. They contribute significantly to oxidative stress, a condition linked to a range of health issues, such as heart disease, cancer, inflammation, and the aging process (Arruda et al., 2018). The ability of the araçá-boi extract and its gastrointestinal phases to scavenge peroxyl radicals demonstrated in this study suggests their potential for preventing and/or managing diseases associated with oxidative stress.

3.3. Determination of phytochemical profile by UHPLC-Q-Orbitrap-MS/MS

Phytochemicals in the araçá-boi extract, before and after *in vitro* simulated gastrointestinal digestion, were identified using UHPL-Q-Orbitrap-MS/MS in negative ion mode. Table 4 lists the fragmentation patterns (main MS/MS fragments), retention times, molecular formulas, precise masses of precursor ions, error (ppm), and tentative identifications for each phytochemical. The analysis was performed using Xcalibur 4.3 software. Characterization was based on precise mass (with a tolerance of 5 ppm), fragmentation patterns, and comparison with data from databases like METLIN Metabolite (http://metlin.scripps.edu), HMDB (https://hmdb.ca), and MassBank (http://massbank.jp).

A total of one hundred compounds from several classes of phytochemicals were tentatively annotated and characterized based on their MS and MS/MS data in the araçá-boi extract. These include eight organic acids, two jasmonates, two nucleotides, three amino acids, five sugars, four stilbenes, forty-five phenolic acids, and thirty-one flavonoids. The extract is predominantly composed of organic acids (*e.g.*, quinic acid, malic acid, ascorbic acid, citric acid, etc.), phenolic acids and their derivatives (*e.g.*, cinnamic acid, gallic acid, *p*-coumaric acid, ferulic acid, hydroxybenzoic acid, etc.), as well as flavonoids, mainly as glycosylated forms of myricetin, quercetin, and kaempferol. Soares et al. (2019), identified seventeen phenolic compounds (including gallic acid, coumaric acid, epicatechin, quercetin, and their derivatives, etc.) in araçá-boi extract through LC–ESI-QTOF-MS analysis. From ESI-LTQ-XL-MS/MS analysis, de Araújo et al. (2021a) identified eighteen compounds in the edible fraction of araçá-boi extract. The authors identified a profile predominantly composed of organic acids (*e.g.*, malic acid), phenolic acids (such as gallic acid, cinnamic acid, and their derivatives), and flavonoids (primarily glycosylated forms of myricetin, luteolin, kaempferol, and quercetin).

Table 4: Identified or tentatively annotated phytochemicals in the araçá-boi extract over the in vitro simulated gastrointestinal digestion by UHPL-Q-Orbitrap-MS/MS under negative ion mode.

No.	R.t. (min)	Identified/Tentatively Annotated Compound	Molecular Formula	Observed *m/z* value	Theoretical *m/z* value	Error (ppm)	Characteristic MS/MS fragments	Araçá-boi extract	Gastric digestion	Intestinal digestion
Organic acids and derivatives										
1	0.68	Quinic acid	$C_7H_{12}O_6$	191.0566	191.0556	5.23	191.0568, 173.0456, 127.0408, 93.0347, 85.0294	+	+	-
2	0.74	Malic acid	$C_4H_6O_5$	133.0144	133.0137	5.26	115.0038, 71.0135	+	+	+
3	0.83	Citric acid	$C_6H_8O_7$	191.0201	191.0192	4.71	129.0192, 111.0090	+	+	+
4	0.9	Shikimic acid	$C_7H_{10}O_5$	173.0452	173.0450	1.16	155.0004, 111.0087, 93.0350	+	+	-
5	0.9	Succinic acid	$C_4H_6O_4$	117.018	117.0188	-6.84	99.0087, 73.0295	+	-	-
6	0.93	Hydroxyadipic acid	$C_6H_{10}O_5$	161.0457	161.0450	4.35	101.0245, 99.0451	+	+	+
7	1.38	Ascorbic acid	$C_6H_8O_6$	175.0251	175.0243	4.57	115.0037, 87.0090, 71.0138	+	+	-
8	1.23	Pantothenic acid (vitamin B5)	$C_9H_{17}NO_5$	218.1033	218.1028	2.29	146.0829, 88.0402	+	+	+
Jasmonates and derivatives										
9	4.43	Tuberonic acid hexoside	$C_{18}H_{28}O_9$	387.1662	387.1655	1.81	207.1024, 163.1134	+	+	+
10	10.87	12-hydroxyjasmonoyl-isoleucine	$C_{18}H_{29}NO_5$	338.1984	338.1967	5.03	130.0876	+	+	-
Nucleotides and derivatives										
11	0.79	Uridine monophosphate	$C_9H_{13}N_2O_9P$	323.0290	323.0280	3.10	211.0010, 150.9799, 111.0194, 96.9691, 78.9591	+	+	+
12	1.03	Guanosine	$C_{10}H_{13}N_5O_5$	282.0845	282.0838	2.48	150.0431	+	+	+
Amino acids										
13	0.67	Glutamic acid	$C_5H_9NO_4$	146.0457	146.0453	2.74	128.0352, 102.0561	+	+	+
14	0.67	Aspartic acid	$C_4H_7NO_4$	132.0303	132.0297	4.54	115.0034, 88.0401	+	-	-
15	1.04	L-Phenylalanine	$C_9H_{11}NO_2$	164.0717	164.0712	3.05	164.0720, 147.0457, 72.0092	+	+	+
Sugars										

16	0.56	Glucoronic or galacturonic acid	$C_6H_{10}O_7$	193.0354	193.0348	3.11	113.0441, 85.0297, 73.0291, 71.0138, 59.0135	+	-	-
17	0.67	Gluconic acid	$C_6H_{12}O_7$	195.0519	195.0505	7.18	177.0399, 159.0301, 99.0089, 87.0088, 77.0085	+	-	-
18	0.69	Arabinose	$C_5H_{10}O_5$	149.0452	149.0450	1.34	130.9996, 89.0247, 75.0087, 71.0137	+	+	+
19	0.69	Glucose or fructose	$C_6H_{12}O_6$	179.0564	179.0556	4.47	113.0249, 101.0247, 89.0244, 71.0136	+	+	+
20	0.69	Sacarose	$C_{12}H_{22}O_{11}$	341.1096	341.1084	3.52	179.0568, 113.0243, 101.0246, 89.0246, 71.0135	+	+	+
	Stilbenes									
21	8.84	Trans-resveratrol hexoside isomer 1	$C_{20}H_{22}O_8$	389.1241	389.1236	1.28	227.073	+	+	+
22	10.1	Trans-resveratrol hexoside isomer 2	$C_{20}H_{22}O_8$	389.1254	389.1236	4.63	227.129	+	-	-
23	11.6	Trans-resveratrol hexoside isomer 3	$C_{20}H_{22}O_8$	389.1228	389.1236	-2.06	227.1307	+	+	+
24	12.05	Trans-resveratrol hexoside isomer 4	$C_{20}H_{22}O_8$	389.1244	389.1236	2.06	227.131	+	+	+
	Phenolic acids and derivatives									
25	0.99	Gallic acid Glucoside	$C_{13}H_{16}O_{10}$	331.0678	331.0665	3.93	271.0489, 211.0272, 169.0155	+	+	+
26	1.14	Gallic acid	$C_7H_6O_5$	169.0146	169.0137	5.33	169.0146, 125.0245, 107.0137, 97.0299, 79.0188	+	+	-
27	1.16	Hydroxybenzoic acid hexoside isomer 1	$C_{13}H_{16}O_8$	299.0775	299.0767	2.67	137.0244	-	-	+
28	1.3	Hydroxybenzoic acid hexoside isomer 2	$C_{13}H_{16}O_8$	299.078	299.0767	4.35	137.0244	-	-	+
29	1.37	Hydroxybenzoic acid isomer 1	$C_7H_6O_3$	137.0244	137.0239	3.65	93.0349	+	-	-
30	1.39	Salicylic acid isomer 1	$C_7H_6O_3$	137.0244	137.0239	3.65	93.0349	+	+	-
31	1.58	Protocatechuic hexoside	$C_{13}H_{16}O_9$	315.0731	315.0716	4.76	153.0198, 109.0294	-	-	+

32	1.62	Hydroxybenzoic acid hexoside isomer 3	$C_{13}H_{16}O_8$	299.0775	299.0767	2.67	137.0252	+	+	+
33	1.7	Peduncalagin isomer 1	$C_{34}H_{24}O_{22}$	783.0698	783.0681	2.17	300.9982, 275.0201	+	-	-
34	1.73	Hydroxybenzoic acid hexoside isomer 4	$C_{13}H_{16}O_8$	299.0777	299.0767	3.34	137.0244	-	-	+
35	1.99	Hydroxybenzoic acid hexoside isomer 5	$C_{13}H_{16}O_8$	299.0776	299.0767	3.01	137.0249	-	-	+
36	1.99	Vanillic acid hexoside isomer 1	$C_{14}H_{18}O_9$	329.0886	329.0872	4.25	329.0946, 167.0350, 152.0115, 123.0455, 108.0220	+	+	+
37	2.06	Protocatechuic xyloside	$C_{12}H_{14}O_8$	285.062	285.061	3.51	153.0192, 152.0112, 123.4724, 109.0290, 108.0222	+	+	+
38	2.12	Vanillic acid hexoside isomer 2	$C_{14}H_{18}O_9$	329.0887	329.0872	4.56	329.0908, 167.0358, 152.3752, 123.0451, 108.6284	+	+	-
39	2.19	Salicylic acid isomer 2	$C_7H_6O_3$	137.0243	137.0239	2.92	93.0341	+	+	+
40	2.2	Hydroxybenzoic acid isomer 2	$C_7H_6O_3$	137.0245	137.0239	4.38	93.0341	+	+	+
41	2.21	p-coumaric acid hexoside isomer 1	$C_{15}H_{18}O_8$	325.0939	325.0923	4.92	163.0405, 145,0290, 119.0505	-	-	+
42	2.26	Galloyl shikimic acid	$C_{14}H_{14}O_9$	325.0573	325.0560	4.00	169. 0154, 125.0252	+	+	-
43	2.33	Caffeic acid hexoside isomer 1	$C_{15}H_{18}O_9$	341.0885	341.0873	3.52	179.0360, 161.0255, 135.0455	+	+	-
44	2.43	Salicylic acid isomer 3	$C_7H_6O_3$	137.0245	137.0239	4.38	93.0341	-	-	+
45	2.43	Hydroxybenzoic acid isomer 3	$C_7H_6O_3$	137.0245	137.0239	4.38	93.0341	-	-	+
46	2.59	Peduncalagin isomer 2	$C_{34}H_{24}O_{22}$	783.071	783.0681	3.70	300.9995, 275.0200	+	-	-
47	2.62	Coumaric acid isomer 1	$C_9H_8O_3$	163.0403	163.0395	4.91	162.8391, 119.0504	+	+	+
48	2.62	p-coumaric acid hexoside isomer 2	$C_{15}H_{18}O_8$	325.0933	325.0923	3.08	163.0403, 119.0504	+	+	+
49	2.74	Syringic acid hexoside isomer 1	$C_{15}H_{20}O_{10}$	359.0995	359.0978	4.73	197.0839, 138.3060, 123.0091	+	+	-
50	2.78	Caffeic acid hexoside isomer 2	$C_{15}H_{18}O_9$	341.0886	341.0873	3.81	179.0361, 135.0459	+	+	+

51	2.86	p-coumaric acid hexoside isomer 3	$C_{15}H_{18}O_8$	325.0933	325.0923	3.08	163.0412, 145.0299, 119.0497	-	-	+
52	2.95	Vanillic acid hexoside isomer 3	$C_{14}H_{18}O_9$	329.0887	329.0872	4.56	167.0352, 152.0893, 123.0456	+	+	-
53	3.1	Coumaric acid isomer 2	$C_9H_8O_3$	163.0403	163.0395	4.91	162.8406, 119.0507	+	+	-
54	3.12	p-coumaric acid hexoside isomer 4	$C_{15}H_{18}O_8$	325.0939	325.0923	4.92	163.0410, 145.0301, 119.0498	+	+	+
55	3.22	Caffeic acid hexoside isomer 3	$C_{15}H_{18}O_9$	341.0883	341.0873	2.93	179.0356, 135.0453	+	+	-
56	3.28	Coumaric acid isomer 3	$C_9H_8O_3$	163.0402	163.0395	4.29	162.8410, 119.0506	+	+	-
57	3.31	p-coumaric acid hexoside isomer 5	$C_{15}H_{18}O_8$	325.0938	325.0923	4.61	163.0408, 145.0292, 119.0506	+	+	+
58	3.6	p-coumaric acid hexoside isomer 6	$C_{15}H_{18}O_8$	325.0929	325.0923	1.85	163.0398, 145.0302, 119.0501	-	-	+
59	3.72	Vanillic acid hexoside isomer 4	$C_{14}H_{18}O_9$	329.0887	329.0872	4.56	167.0357, 123.0456, 108.0218	+	+	-
60	3.79	Ferulic acid hexoside isomer 1	$C_{16}H_{20}O_9$	355.1046	355.1029	4.79	193.0508, 134.0371	-	-	+
61	3.81	Digalloyl hexoside isomer 1	$C_{20}H_{20}O_{14}$	483.0797	483.0775	4.55	313.0592, 271.0473, 211.0240, 169.0148	+	+	-
62	3.86	Mono-HHDP-galloyl-hexoside	$C_{27}H_{22}O_{18}$	633.0754	633.0728	4.11	300.9989, 275.0206	+	+	-
63	4.03	Di-HHDP-galloyl-hexoside isomer 1	$C_{41}H_{28}O_{26}$	935.082	935.0791	3.10	300.9995, 275.0211, 249.0398	+	+	-
64	4.1	Digalloyl hexoside isomer 2	$C_{20}H_{20}O_{14}$	483.0796	483.0775	4.35	169.0144, 125.0241	+	+	-
65	4.14	p-coumaric acid hexoside isomer 7	$C_{15}H_{18}O_8$	325.0939	325.0923	4.92	163.0392, 119.0498	+	+	+
66	4.15	Ferulic acid hexoside isomer 2	$C_{16}H_{20}O_9$	355.1041	355.1029	3.38	193.0498, 175.0413, 134.0370	+	+	+
67	4.15	Di-O-galloyl-rhamnose	$C_{20}H_{20}O_{13}$	467.0806	467.0826	-4.28	315.0174, 169.0143, 125.0251	+	+	-
68	4.16	Ferulic acid	$C_{10}H_{10}O_4$	193.0508	193.0501	3.63	134.0376	+	+	+
69	4.45	Chebulagic acid	$C_{41}H_{30}O_{27}$	953.0920	953.0896	2.52	300.9978, 275.0198	+	+	-

70	4.6	Ferulic acid hexoside isomer 3	$C_{16}H_{20}O_9$	355.1042	355.1029	3.66	193.0507, 175.0409, 134.0382	-	-	+
71	4.64	Di-HHDP-galloyl-hexoside isomer 2	$C_{41}H_{28}O_{26}$	935.0822	935.0791	3.32	300.9989, 275.0205, 249.0431	+	+	-
72	5.11	Coumaric acid isomer 4	$C_9H_8O_3$	163.0402	163.0395	4.29	162.8396, 119.0506	+	+	+
73	5.17	Cinnamic acid isomer 1	$C_9H_8O_2$	147.0453	147.0446	4.76	147.0457, 103.0549	+	+	+
74	5.26	p-coumaric derivative	$C_{30}H_{17}O_4$	441.1105	441.1126	-4.76	163.0402, 145.0299, 119.0509	+	+	-
75	5.88	Syringic acid hexoside isomer 2	$C_{15}H_{20}O_{10}$	359.0964	359.0978	-3.90	197.0831, 153.0923	+	+	-
76	6.1	Caffeoylshikimic acid	$C_{16}H_{16}O_8$	335.0784	335.077	4.18	179.0359, 161.0258, 135.0447	+	+	-
77	6.14	Cinnamic acid isomer 2	$C_9H_8O_2$	147.0453	147.0446	4.76	147.0449, 103.0552	-	-	+
78	6.34	Tri-O-galloyl-glucose	$C_{27}H_{24}O_{18}$	635.0926	635.0884	6.61	465.0686, 313.0588, 169.0142, 125.0249	+	+	-
79	6.38	Trigalloyl-hexoside	$C_{27}H_{24}O_{18}$	635.09	635.0884	2.52	465.0686, 313.0588, 169.0142, 125.0249	+	+	-
80	7.02	Di-HHDP-galloyl-hexoside isomer 3	$C_{41}H_{28}O_{26}$	935.0837	935.0791	4.92	300.9993, 275.0209	+	+	-
81	7.77	Mirciaphenone B	$C_{21}H_{22}O_{13}$	481.0994	481.0982	2.49	313.0557, 169.0147	+	+	-
82	9.25	Cinnamic acid isomer 3	$C_9H_8O_2$	147.0452	147.0446	4.08	147.0459, 103.0549	+	+	+
83	12.32	p-coumaric acid ethyl ester	$C_{11}H_{12}O_3$	191.0714	191.0708	3.14	191.0711, 163.0412, 145.0299, 119,0506	-	-	+
84	13.61	p-coumaric derivative	$C_{22}H_{32}O_4$	359.2238	359.2222	4.45	163.0401, 145.0302, 119.0508	-	-	+
	Flavonoids and derivatives									
85	3.24	Taxifolin isomer 1	$C_{15}H_{12}O_7$	303.0514	303.0505	2.97	285.0428, 217.0512, 175.0395, 125.0245	+	+	-
86	3.44	Taxifolin isomer 2	$C_{15}H_{12}O_7$	303.0513	303.0505	2.64	285.0403, 217.0499, 175.0410, 125.0243	+	+	-
87	4.74	(Epi)catechin	$C_{15}H_{14}O_6$	289.0723	289.0712	3.81	245.0483, 221.0465, 151.0033, 137.0254, 125.0251	+	+	-
88	5.28	Taxifolin hexoside isomer 1	$C_{21}H_{22}O_{12}$	465.1045	465.1068	-4.95	285.0390, 151.0038	+	+	-

89	6.00	Taxifolin hexoside isomer 2	$C_{15}H_{12}O_7$	465.1058	465.1068	-2.15	303.0519, 285.0428, 151.0034, 125.0245	+	+	-
90	6.50	Taxifolin hexoside isomer 3	$C_{15}H_{12}O_7$	465.1055	465.1068	-2.80	303.0548, 285.0419, 151.0046, 125.0248	+	+	+
91	6.99	Taxifolin isomer 3	$C_{15}H_{12}O_7$	303.0515	303.0505	3.30	285.0417, 175.0397, 125.0250	+	+	-
92	7.52	Myricetin-3-O-galactoside	$C_{21}H_{20}O_{13}$	479.0848	479.0826	4.59	317.0311, 316.0230	+	+	-
93	8.33	Quercetin-3-O-galloyl hexoside isomer 1	$C_{28}H_{24}O_{16}$	615.0986	615.1002	-2.60	301.0350, 300.0306, 211.1137, 169.0145, 151.0046	+	+	-
94	8.68	Myricetin-O-galloyl rhamnopyranoside	$C_{28}H_{24}O_{16}$	615.0988	615.1002	-2.28	463.0873, 169.0143, 125.2322	+	+	-
95	8.68	Quercetin-3-O-galloyl hexoside isomer 2	$C_{28}H_{24}O_{16}$	615.0986	615.1002	-2.60	463.0849, 301.0344, 300.0313, 169.0144, 125.2322	+	+	-
96	8.74	Myricetin-3-O-rhamnoside (myricetrin)	$C_{21}H_{20}O_{12}$	463.0895	463.0877	3.89	316.0224, 137.0305	+	+	-
97	8.8	Quercetin maloyl hexoside	$C_{25}H_{24}O_{16}$	579.1018	579.0986	5.53	301.0336, 300.0305	+	+	-
98	8.98	Quercetin-3-O-galactoside	$C_{21}H_{20}O_{12}$	463.0899	463.0877	4.75	301.0342, 300.0311, 179.1588, 151.0037	+	+	+
99	9.08	Quercetin-3-O-glucuronide	$C_{21}H_{18}O_{13}$	477.069	477.0669	4.40	302.0402, 301.0370, 178.9986, 151.0045	+	+	+
100	9.23	Phloretin-C-diglycoside	$C_{27}H_{34}O_{15}$	597.183	597.182	1.67	387.1130, 357.1005, 345.0978, 315.0868, 209.0453	+	+	-
101	9.24	Quercetin-3-O-glucoside	$C_{21}H_{20}O_{12}$	463.09	463.0877	4.97	301.0358, 300.0278, 178.9999, 151.0037	+	+	+
102	9.46	Naringenin	$C_{15}H_{12}O_5$	271.0618	271.0607	4.06	177.0196, 151.0033, 119.0509, 107.0135	+	+	+
103	10.08	Quercetin-3-O-arabinoside	$C_{20}H_{18}O_{11}$	433.0795	433.0771	5.54	301.0359, 300.0279, 271.0620, 151.0031	+	+	+
104	10.16	Kaempferol-3-O-galactoside (trifolin)	$C_{21}H_{20}O_{11}$	447.0938	447.0927	2.46	285.0390, 284.0330, 255.0307,	+	+	+
105	10.18	Phlorizin	$C_{21}H_{24}O_{10}$	435.1302	435.1291	2.53	273.0791, 167.0364	+	+	-

106	10.49	Kaempferol 7-(6'-galloyl glucoside)	$C_{28}H_{24}O_{15}$	599.1061	599.1037	4.01	285.0420, 284.0360, 169.0143	+	+	-
107	10.69	Kaempferol-3-O-glucoside (astragalin)	$C_{21}H_{20}O_{11}$	447.0948	447.0927	4.70	285.0410, 284.0350, 255.0322	+	+	+
108	10.79	Quercetin-3-O-rhamnoside (quercetrin)	$C_{21}H_{20}O_{11}$	447.0948	447.0927	4.70	301.0341, 300.0298, 271.0249, 255.0325, 151.0048	+	+	+
109	11.85	Kaempferol-3-O-rhamnoside (afzelin)	$C_{21}H_{20}O_{10}$	431.0994	431.0978	3.71	286.0453, 285.0408, 284.0348, 255.0320,227.0348	+	+	+
110	12.11	Quercetin deoxyhesoxylhexoside	$C_{27}H_{30}O_{16}$	609.1442	609.1456	-2.30	301.0333, 300.0320	+	+	+
111	12.18	Quercetin-3-O-acetyl rhamnoside	$C_{23}H_{22}O_{12}$	489.1056	489.1033	4.70	301.0337, 300.0306, 271.0245, 255.0322	+	+	-
112	12.18	Quercetin 3-O-hexuronide-7-O-hexoside	$C_{27}H_{28}O_{18}$	639.1212	639.1197	2.35	301.0344, 300.0289, 151.0700	+	+	-
113	12.35	Quercetin	$C_{15}H_{10}O_{7}$	301.0359	301.0348	3.65	301.0376, 179.0004, 151.0043, 121.0305, 107.0144	+	+	-
114	12.47	Genistein	$C_{15}H_{10}O_{5}$	269.0463	269.0450	4.83	269.0487, 225.0549, 181.0668, 159.0446, 133.0298	-	-	+
115	12.49	Quercetin-3,7-O-dirhamnoside	$C_{27}H_{30}O_{15}$	593.1483	593.1506	-3.88	301.0356, 300.0275, 271.0263, 255.0301, 151.0035	+	+	-
116	12.69	Kaempferol-3,7-O-dirhamnoside (Kaempferitrin)	$C_{27}H_{30}O_{14}$	577.1545	577.1557	-2.08	285.0409, 284.0648, 255.0320	+	+	-

R.t. = retention time; Error (ppm): the difference between experimental mass and theoretical mass of the compound.

After gastric digestion, ninety-two of the one hundred compounds initially identified in the araçá-boi extract were tentatively annotated. Only a few compounds, such as succinic acid, aspartic acid, glucuronic/galacturonic acid, gluconic acid, a trans-resveratrol isomer, penducalagin isomers, and hydroxybenzoic acid isomer, were degraded during gastric digestion and were therefore absent from this fraction. The high retention of compounds after gastric digestion can be attributed to the inherent resistance of phytochemicals to the conditions of the stomach. Many of these compounds have chemical structures that confer stability in an acidic environment and against digestive enzymes. This resistance can be explained by the ability of these compounds to form strong covalent bonds and intermolecular interactions, protecting them from degradation (Hu et al., 2023). The observation that over 90% of the compounds identified in the extract remain in the gastric fraction is advantageous, as it increases the likelihood that they will reach the intestines, where absorption and subsequent bioavailability occur.

The araçá-boi extract, after intestinal digestion, exhibited a distinct phytochemical profile compared to the analyses conducted before digestion and during gastric digestion. A total of 59 compounds were characterized, of which four are organic acids, one is a jasmonate, one is a nucleotide, two are amino acids, three are sugars, three are stilbenes, thirty-three are phenolic acids, and twelve are flavonoids. The profile of the araçá-boi extract after gastrointestinal digestion is predominantly composed of certain organic acids (*e.g.*, malic acid and citric acid), phenolic acids (*e.g.*, hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, salicylic acid, ferulic acid, cinnamic acid, and their derivatives), and flavonoids (*e.g.*, glycosylated forms of kaempferol and quercetin). Among the characterized compounds, fifteen new phenolic acids were identified, including derivatives of hydroxybenzoic acid, protocatechuic acid, *p*-coumaric acid, salicylic acid, ferulic acid, and cinnamic acid, as well as one flavonoid (genistein). Conversely, it is possible to observe that organic acids, phenolic acids, and flavonoids were the most affected, and less than 50% of the previously identified organic acids were found. Similarly, only 35% of the phenolic

compounds originally identified in the extract and gastric fraction were detected in the intestinal fraction. The intestinal environment plays a fundamental role in modulating the phytochemical profile after digestion, facilitating not only the emergence of new compounds but also the degradation of others. During gastrointestinal transit, phytochemicals are subjected to the action of digestive enzymes in the alkaline environment of the intestine, which can influence their stability and bioavailability (Hu et al., 2023).

In summary, the araçá-boi extract, after *in vitro* gastrointestinal digestion, revealed a diverse phytochemical profile, with a wide range of phenolic compounds, such as derivatives of cinnamic acid, coumaric acid, hydroxybenzoic acids, ferulic acid, quercetin, and kaempferol. Phenolic compounds possess antioxidant and anti-inflammatory properties, contributing to protection against oxidative stress and inflammation (Sun & Shahrajabian, 2023). Additionally, phenolic acids like cinnamic and p-coumaric acids, along with flavonoids such as quercetin, exhibit anticancer potential by modulating epigenetic pathways (Afnan et al., 2022; Hu et al., 2023; Sun & Shahrajabian, 2023). The presence of dietary polyphenols also suggests prebiotic potential, promoting gut health moreove local and systemic benefits (Dingeo et al., 2020; Neri-Numa & Pastore, 2020). To the best of our knowledge, this is the most detailed study of the phytochemical profile of araçá-boi extract, offering new insights into its stability during *in vitro* gastrointestinal digestion.

3.4. Validation of the analytical method by HPLC-DAD for phenolic compounds

The HPLC-DAD method was validated for linearity, precision (both inter-day and intraday), sensitivity (including limits of detection [LOD] and limits of quantification [LOQ]), and accuracy. Calibration curves were constructed using standard mixture solutions at eight distinct concentrations (0.1, 0.5, 1, 2, 4, 6, 8, and 10 μg/mL). The linearity of the curves was evaluated by determining the correlation coefficients (R^2), with all compounds displaying R^2 values between 0.9960 and 0.9996 (Table 5). Sensitivity was assessed by determining the limits of detection (LODs) and limits of quantification (LOQs),

which ranged from 0.027 to 0.602 μg/mL and from 0.92 to 2.007 μg/mL, respectively, indicating strong sensitivity (Table 5).

Precision was demonstrated through both inter- and intraday variations, with RSD (%) values ranging from 1.16% to 6.45% for repeatability and from 0.93% to 10.25% for reproducibility. These results met the acceptance criterion of RSD (%) < 15% (Table 6). Accuracy was assessed through a recovery study, where the araçá-boi extract was spiked with a mixture of standard solutions at concentrations of 1 μg/mL (Level 1), 4 μg/mL (Level 2), and 8 μg/mL (Level 3) (Table 6). Recovery was considered acceptable when the values fell between 85% and 115%. The method demonstrated good recovery for araçá-boi extract samples, ranging from 86.80% to 111.18%, except for myricetin and gentisic acid at Level 1, which showed recovery values exceeding 115% (Table 6). Overall, the proposed method exhibited a good range of linearity, LOD, and LOQ, with RSD values within the acceptable limit, confirming its precision and accuracy.

Table 5. Linearity, determination coefficient (R^2), limit of detection (LOD), and limit of quantification (LOQ) of HPLC-DAD method for 23 phenolic compounds.

Number	Compound	Wavelength (nm)	Linear range (μg/mL)	Calibration equations	R^2	LOD (μg/mL)	LOQ (μg/mL)
1	Gallic acid	280	0.1-10	y = 1.8542x + 0.0264	0.9990	0.052	0.172
2	Protocatechuic acid	260	0.1-10	y = 2.3589x + 0.0538	0.9990	0.042	0.139
3	Chlorogenic acid	320	0.1-10	y = 1.9901x - 0.0041	0.9990	0.044	0.146
4	Catechin	280	0.1-10	y = 0.4347x + 0.0113	0.9993	0.037	0.123
5	Gentisic acid	320	0.1-10	y = 0.8765x + 0.0109	0.9989	0.059	0.198
6	Epicatechin	280	0.1-10	y = 0.4223x + 0.0247	0.9996	0.078	0.262
7	Caffeic acid	320	0.1-10	y = 3.5356x + 0.0521	0.9989	0.038	0.125
8	Vanillic acid	260	0.1-10	y = 2.4674x + 0.0776	0.9989	0.027	0.092
9	Syringic acid	280	0.1-10	y = 1.8377x + 0.0471	0.9986	0.054	0.179
10	Rutin	260	0.1-10	y = 1.1348x + 0.0254	0.9991	0.042	0.141
11	p-Coumaric acid	320	0.1-10	y = 4.1942x + 0.1273	0.9989	0.038	0.127
12	Sinapic acid	320	0.1-10	y = 3.2729x + 0.0594	0.9989	0.054	0.182
13	Ferulic acid	320	0.1-10	y = 3.3665x + 0.0818	0.9989	0.038	0.126
14	Quercetrin	260	0.1-10	y = 1.8085x + 0.0134	0.9988	0.047	0.155
15	Myricetin	360	0.1-10	y = 1.7682x - 0.3712	0.9960	0.602	2.007
16	Benzoic acid	280	0.1-10	y = 0.2270x + 0.0065	0.9990	0.056	0.188
17	Luteolin	360	0.1-10	y = 1.9861x - 0.1585	0.9984	0.046	0.153
18	Quercetin	360	0.1-10	y = 2.1456x - 0.1543	0.9982	0.125	0.415
19	Trans-cinnamic acid	280	0.1-10	y = 4.9393x + 0.1556	0.9994	0.047	0.157
20	Apigenin	320	0.1-10	y = 2.3977x - 0.1281	0.9978	0.051	0.170
21	Naringenin	280	0.1-10	y = 1.9314x + 0.0367	0.9992	0.043	0.144
22	Kaempferol	360	0.1-10	y = 2.3609x - 0.1719	0.9983	0.098	0.327
23	Hesperetin	280	0.1-10	y = 2.0124x + 0.0342	0.9989	0.041	0.136

R^2: Coefficient of determination**; LOD:** Limit of detection = 3 x standard deviation of the response/slope of the calibration curve; **LOQ:** Limit of quantification = 10 x standard deviation of the response/slope of the calibration curve

Table 6. Precision and recovery of HPLC-DAD method for 23 phenolic compounds.

Number	Compound	Intraday precision (RSD, %)			Interday precision (RSD, %)			Recovery (%)		
		Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
1	Gallic acid	3.06	1.23	2.70	6.07	1.44	1.85	109.80 (0.87)	104.88 (3.68)	103.90 (1.60)
2	Protocatechuic acid	2.92	1.28	2.81	5.95	1.51	2.10	109.88 (2.02)	103.92 (3.82)	102.54 (1.47)
3	Chlorogenic acid	3.19	1.34	2.85	6.38	1.56	2.01	99.18 (1.96)	104.60 (3.91)	99.43 (1.49)
4	Catechin	3.15	1.30	2.47	4.49	1.32	2.12	105.09 (4.72)	91.31 (4.53)	98.30 (1.68)
5	Gentisic acid	2.94	1.44	2.88	6.36	1.70	2.40	116.88 (0.98)	97.59 (6.47)	104.30 (1.40)
6	Epicatechin	2.41	1.81	1.97	3.94	2.09	0.93	104.15 (3.28)	100.70 (4.34)	105.06 (1.56)
7	Caffeic acid	2.94	1.23	2.72	6.07	1.42	1.82	102.72 (1.80)	100.71 (3.84)	100.45 (1.47)
8	Vanillic acid	2.95	1.34	2.82	4.86	1.54	1.95	86.80 (0.29)	105.76 (4.61)	101.06 (1.65)
9	Syringic acid	3.22	1.43	2.97	5.24	1.69	1.96	103.58 (0.30)	107.31 (3.44)	107.21 (1.94)
10	Rutin	2.93	1.55	2.67	5.43	1.66	1.69	105.49 (1.31)	101.06 (3.53)	100.40 (1.47)
11	*p*-Coumaric acid	2.90	1.32	2.73	5.63	1.46	2.04	105.83 (1.46)	103.31 (3.78)	102.38 (1.58)
12	Sinapic acid	2.90	1.45	2.75	5.66	1.63	2.10	100.29 (2.28)	101.44 (4.10)	101.15 (1.76)
13	Ferulic acid	2.87	1.31	2.57	5.15	1.28	2.04	110.64 (1.49)	103.82 (3.89)	102.21 (1.46)
14	Quercetrin	2.95	1.48	2.97	5.05	1.35	2.20	94.03 (2.65)	101.99 (3.12)	101.80 (2.78)
15	Myricetin	6.45	5.43	3.98	10.25	5.11	7.75	119.80 (4.99)	107.65 (4.18)	111.80 (5.22)
16	Benzoic acid	2.66	1.50	2.74	5.89	1.60	2.61	96.74 (0.97)	103.80 (3.42)	100.09 (1.01)
17	Luteolin	3.56	4.65	2.93	2.88	2.56	7.12	107.34 (2.53)	104.02 (3.60)	104.82 (1.57)
18	Quercetin	3.59	3.72	2.95	3.11	2.44	6.65	106.80 (2.13)	108.32 (3.94)	105.32 (1.82)
19	Trans-cinnamic acid	2.66	1.28	2.32	5.47	1.34	1.99	108.59 (0.10)	101.24 (3.27)	99.81 (1.57)
20	Apigenin	3.41	3.36	2.29	4.75	2.45	7.83	107.52 (2.10)	105.98 (3.99)	106.40 (1.63)
21	Naringenin	2.67	1.16	2.42	5.63	1.17	1.68	111.18 (1.44)	103.19 (3.85)	102.32 (1.04)
22	Kaempferol	3.48	3.97	3.10	5.10	2.47	7.59	103.97 (2.44)	105.50 (3.60)	106.86 (1.38)
23	Hesperetin	2.88	1.35	2.79	5.77	1.37	2.10	108.74 (2.21)	104.39 (3.71)	103.50 (1.40)

RSD: Relative standard deviation. RSD (%) are given in parenthesis in case of recovery study; **Intraday precision:** Obtained by analyzing 3 times a mixture of standard solution at concentrations of 1 μg/mL (Level 1), 4 μg/mL (Level 2), and 8 μg/mL (Level 3) on the same day; **Interday precision:** Obtained by analyzing a mixture of standard solution at concentrations of 1 μg/mL (Level 1), 4 μg/mL (Level 2), and 8 μg/mL (Level 3) during 3 consecutive days; **Recovery:** Obtained by spiking the araçá-boi extract with a mixture of standard solution at concentrations of 1 μg/mL (Level 1), 4 μg/mL (Level 2), and 8 μg/mL (Level 3).

3.5. Individual phenolic compound content over gastrointestinal digestion by HPLC-DAD

To examine the content, recovery, and bioaccessibility of individual phenolic compounds over the *in vitro* simulated gastrointestinal digestion, HPLC-DAD analysis was carried out using twenty-three authentic standards. Table 7 shows the effects of gastrointestinal digestion on the phenolic acids and flavonoids of the araçá-boi extract. In Figure 1 (A-H), chromatograms of the standard mix used and the araçá-boi extract and its fractions throughout the gastrointestinal process are represented at two wavelengths (280, 320 nm) that best represent all peaks of the studied phenolic compounds.

Seven phenolic compounds were quantified in the araçá-boi extract such as trans-cinnamic acid (190.60 μg/g), syringic acid (144.03 μg/g), quercitrin (137.54 μg/g), gentisic acid (94.16 μg/g), gallic acid (90.80 μg/g), *p*-coumaric acid (36.03 μg/g) and ferulic acid (4.81 μg/g) (Table 7). Similarly, in a previous study with araçá-boi extract, Borsoi et al. (2024) quantified eleven 155.79 μg/g of trans-cinnamic acid, 151.32 μg/g of quercetin-3-O-galactoside, 116.27 μg/g of quercetin-3-O-rhamnoside, 113.07 μg/g of syringic acid, 73.93 μg/g of gallic acid, and 62.61 μg/g of kaempferol-3-O-glucoside as the major compounds, among others in smaller quantities such as gentisic acid, 4-hydroxybenzoic acid, ferulic acid, p-coumaric acid, and hesperetin. Additionally, another study by Neri-Numa et al. (2013), demonstrated that hydrolyzed *araçá-boi* pulp extract was rich in flavonoids such as kaempferol (37 μg/g), quercetin (51.60 μg/g), and myricetin (170 μg/g). These findings suggest that the presence of these compounds in araçá is linked to sugars, as evidenced by the UHPLC-Q-Orbitrap-MS/MS analysis, which identified several derivatives of these flavonoids.

Table 7. Content, recovery, and bioaccessibility of individual phenolic compounds obtained by HPLC-DAD method in araçá-boi extract over the *in vitro* simulated gastrointestinal digestion.

	Compound	Fractions content (μg/g extract dw)			Index	
		Araçá-boi extract	Grastric phase	Intestinal phase	Recovery (%)	Bioaccessibility (%)
1	Gallic acid	90.80 ± 0.57[a]	83.86 ± 0.56[b]	n.d.	92.36 ± 0.62	n.d.
2	Protocatechuic acid	n.d	n.d	n.d	-	-
3	Chlorogenic acid	n.d	n.d	n.d	-	-
4	Catechin	n.d	n.d	n.d	-	-
5	Gentisic acid	94.16 ± 1.71[b]	103.80 ± 1.02[a]	n.d.	110.24 ± 1.09	n.d.
6	Epicatechin	n.d	n.d	n.d	-	-
7	Caffeic acid	n.d	n.d	1.61 ± 0.11	-	-
8	Vanillic acid	n.d	n.d	n.d	-	-
9	Syringic acid	144.03 ± 0.61[a]	142.53 ± 1.37[a]	n.d	98.96 ± 0.95	n.d.
10	Rutin	n.d	n.d	n.d	-	-
11	p-Coumaric acid	22.77 ± 0.63[c]	24.43 ± 0.25[b]	52.82 ± 0.41[a]	107.30 ± 1.11	231.93 ± 1.78
12	Sinapic acid	n.d	n.d	n.d	-	-
13	Ferulic acid	12.03 ± 0.17[a]	8.21 ± 0.04[b]	3.37 ± 0.07[c]	68.27 ± 0.34	27.98 ± 0.57
14	Quercetrin	137.54 ± 1.22[b]	127.52 ± 1.07[c]	146.25 ± 0.21[a]	92.71 ± 0.78	106.33 ± 0.15
15	Myricetin	n.d	n.d	n.d	-	-
16	Benzoic acid	n.d	n.d	22.18 ± 1.25	-	-
17	Luteolin	n.d	n.d	n.d	-	-
18	Quercetin	n.d	n.d	n.d	-	-
19	Trans-cinnamic acid	190.60 ± 1.11[b]	192.92 ± 1.38[b]	1551.35 ± 40.73[a]	101.22 ± 0.73	813.91 ± 21.37
20	Apigenin	n.d	n.d	n.d	-	-
21	Naringenin	n.d	n.d	n.d	-	-
22	Kaempferol	n.d	n.d	n.d	-	-
23	Hesperetin	n.d	n.d	n.d	-	-

n.d.: not detected.

Likewise, the same compounds found in the extract before digestion were found in the gastric phase with little significant differences in their content, except for p-coumaric and ferulic acid which present a large difference. This is reflected in the recovery of these compounds, where all compounds from the gastric phase remained in the range of 90 to 110% recovery, except ferulic acid, which had a recovery of only 68%, demonstrating good stability of these compounds in the gastric phase (Table 7).

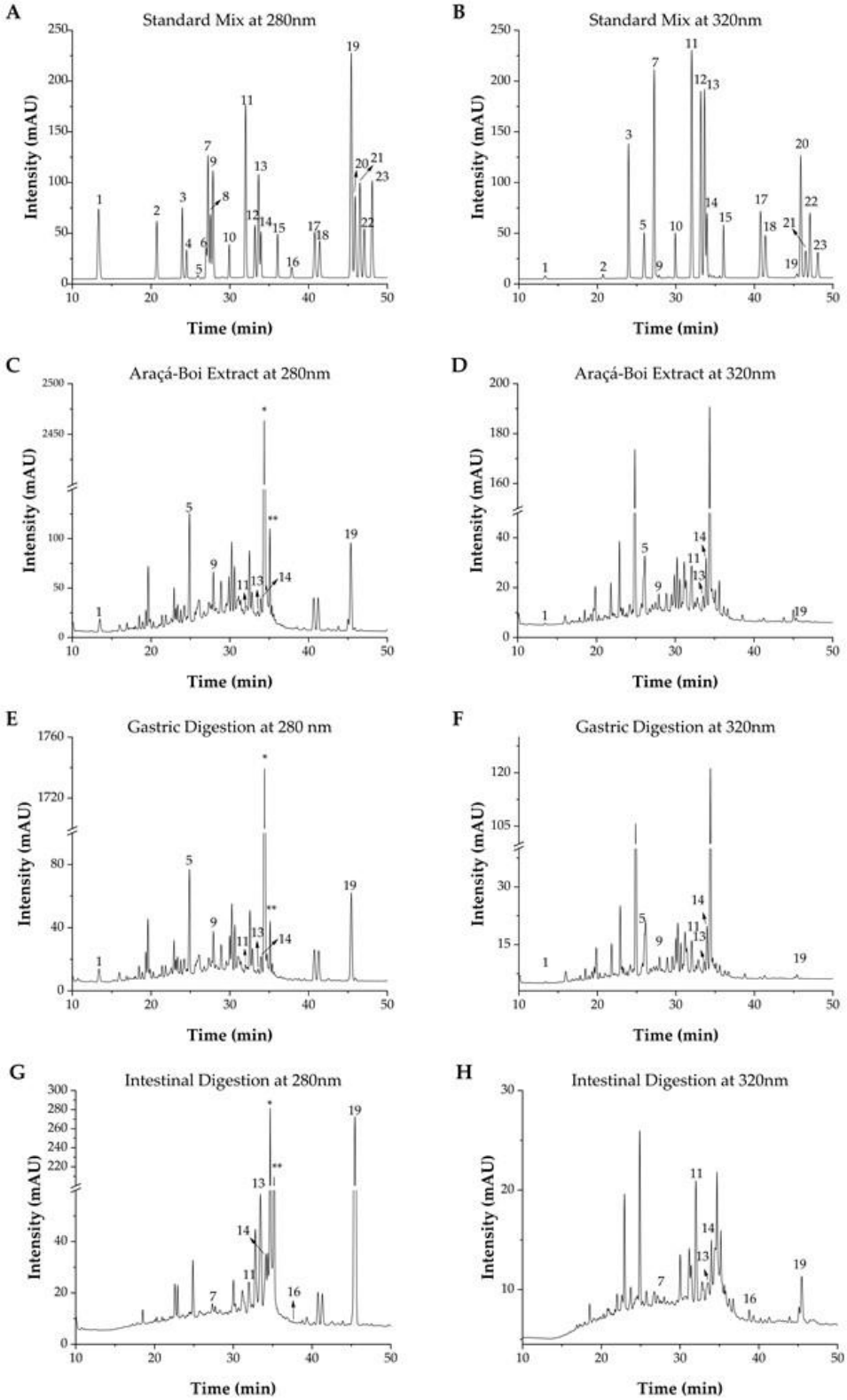

Figure 1. Chromatograms of phenolic compounds obtained by HPLC-DAD method in araçá-boi extract over the *in vitro* simulated gastrointestinal digestion. Peak identification: (1) gallic acid; (2) protocatechuic acid; (3) chlorogenic acid; (4) catechin; (5) gentisic acid; (6) epicatechin; (7) caffeic acid; (8) vanillic acid; (9) syringic acid; (10) rutin; (11) p-coumaric acid; (12) sinapic acid; (13) ferulic acid; (14) quercetrin; (15) myricetin; (16) benzoic acid; (17) luteolin; (18) quercetin; (19) trans-cinnamic acid; (20) apigenin; (21) naringenin; (22) kaempferol; (23) hesperetin. * Unknown compound 1; ** Unknown compound 2. HPLC conditions are described in the text.

The stability of phenolic compounds during the gastric phase can be explained by their interaction with the acidic pH (pH = 2) submitted in this phase and the phenolic substance structure. Under this condition, phenolic compounds become more structurally resistant to protonation and oxidation reactions and maintain their solubility in the medium (Li et al., 2023; Yu et al., 2022). Additionally, phenolic compounds can bind to components present in the extract (i.e., proteins and carbohydrates) through hydrogen bonds, hydrophobic interactions, and covalent bonds, making them more stable throughout the gastric digestion process (Zhu et al., 2021).

Six phenolic compounds were quantified after the intestinal phase, namely: *p*-coumaric acid (52.82 μg/g), ferulic acid (3.37 μg/g), caffeic acid (1.61 μg/g), benzoic acid (22.18 μg/g), quercitrin (146 μg/g), and trans-cinnamic acid (1551 μg/g) (Table 7). Quercitrin, *p*-coumaric acid, and trans-cinnamic acid were the only compounds that showed a significant increase in their bioaccessibility (106, 232, and 813%, respectively). The significant increase in trans-cinnamic acid after intestinal digestion can be explained by several factors: during this phase, alkaline conditions and the action of digestive enzymes can break covalent bonds between phenolic compounds and other components of the extract, such as proteins and polysaccharides, resulting in the release of greater amounts of the compounds in their aglycone form (Pais et al., 2024). Additionally, in the chromatograms obtained for the extract at 280 nm, the presence of an unknown compound 1 can be observed with a peak intensity exceeding 2400 mAU, which is much higher than the intensity of the other peaks. However, throughout the digestion process, its intensity drastically decreases, reaching less than 300 mAU (Figure 1 C, E, and G). The reduction in the intensity of this unknown compound 1 may be associated with an increase in the peak intensities observed for unknown compound 2 and trans-cinnamic acid. In light of this observation, the UV-Vis absorption spectra of trans-cinnamic acid obtained from the standard and the araçá-boi extract were examined and compared with those of unknown compounds 1 and 2 (Figure 2).

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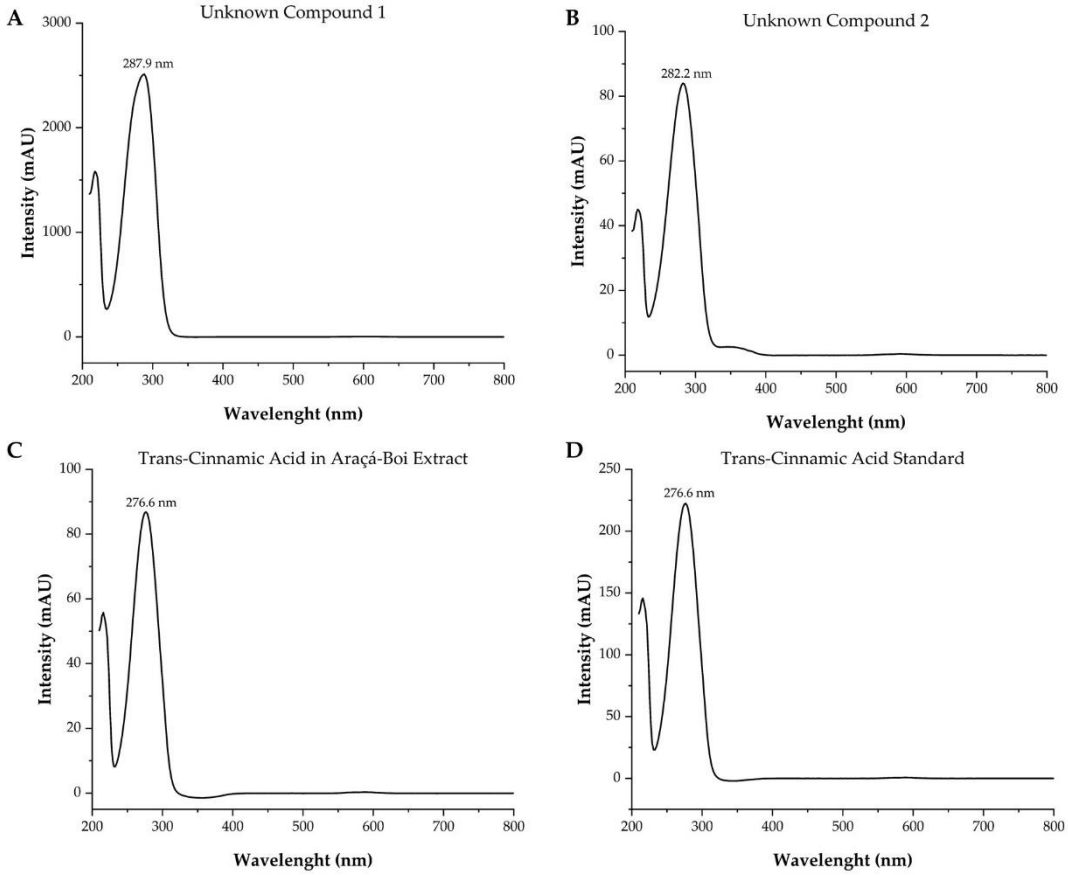

Figure 2: Absorption spectrum of unknown compounds 1 (A) and 2 (B), and of trans-cinnamic acid in araçá-boi extract (C) and in trans-cinnamic acid standard (D).

In this way, these unknown compounds 1 and 2 showed UV-Vis absorption spectra very similar to that observed for trans-cinnamic acid from the standard and araçá-boi. Additionally, we observed an increase in the optimal absorption wavelength from 276.6 nm for trans-cinnamic acid and araçá-boi extract to 287.9 and 282.2 nm for unknown compounds 1 and 2, respectively. This shift in the optimal absorption wavelength may be associated with structural changes, the orientation of functional groups, and steric interactions (Lama-Muñoz & Contreras, 2022), or it could also be due to the presence of covalently saturated groups (e.g., hydroxyls and methoxyls), known as auxochromes, which, when attached to a chromophore, alter both the wavelength and the intensity of the absorption maximum. When this shift in the maximum absorption wavelength increases, it is known as a bathochromic shift (Gürses et al., 2016). Thus, this evidence suggests that the unknown compound 1 is possibly derived from trans-cinnamic acid and, therefore, upon reaching the intestinal

fraction, it might be released as another derivative of trans-cinnamic acid (unknown compound 2) and its aglycone.

As can be seen in Table 7, the intestinal digestion process not only significantly influenced the increase in trans-cinnamic acid but also contributed to the degradation of gallic, gentisic, and syringic acids and the formation of new phenolic acids such as benzoic and caffeic acids. Interestingly, in Figure 3, it can be noticed that gallic and gentisic acids may have been partially transformed into benzoic acid through dehydroxylation. The nearly 30% reduction in the bioavailability of ferulic acid can be explained by its partial conversion into caffeic acid through demethylation (Zahid et al., 2023; Zhang et al., 2023).

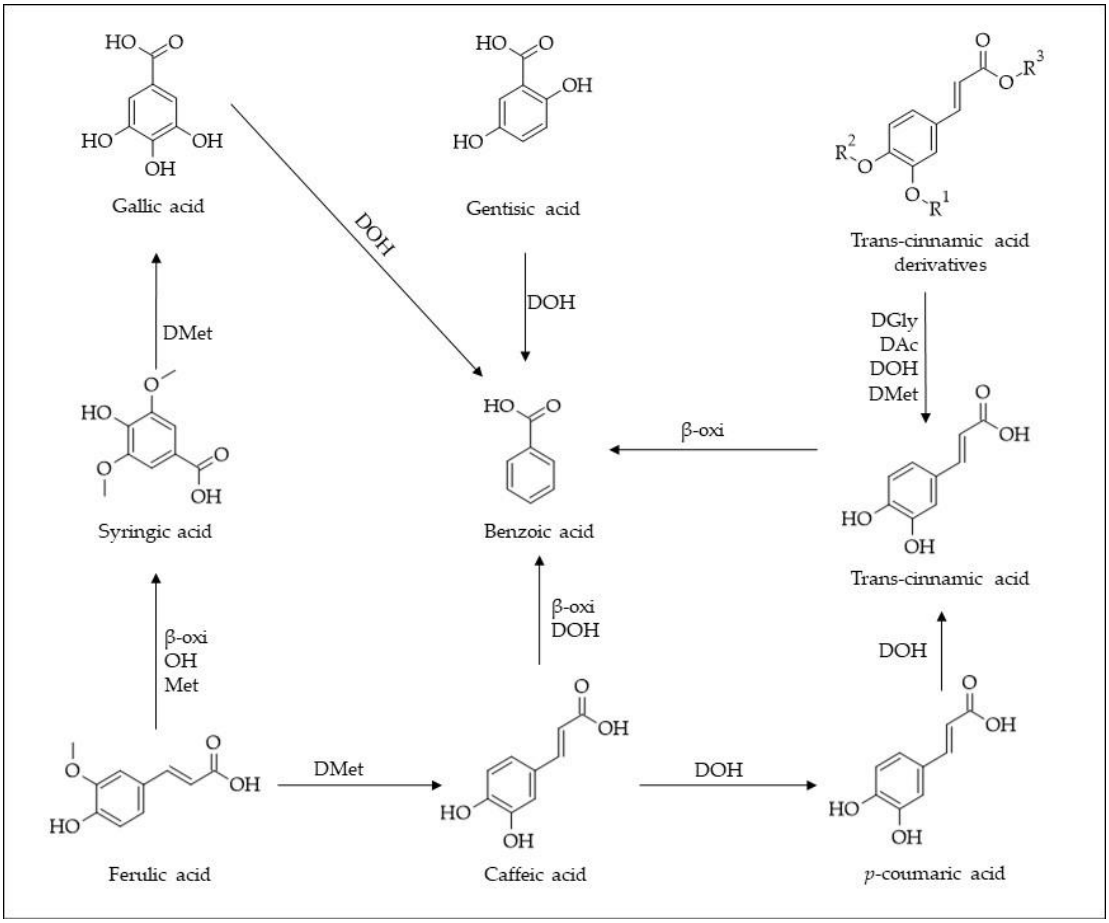

Figure 3. Proposed pathways of major phenolic acids in araçá-boi extract during simulated gastrointestinal digestion. DAc: Deacetylation, DGly: Deglycosylation, DMet: Demethylation, DOH: Dehydroxylation, Met: Methylation, OH: Hydroxylation, β-oxi: β-oxidation, R^1, R^2, and R^3: Hydrogen, methyl, organic acids, or sugars groups.

The total or partial reduction and conversion of the aforementioned phenolic acids into other compounds after the intestinal digestion process can be attributed to the alkaline conditions in the intestinal environment and the action of bile salts and digestive enzymes, which can modify the chemical structures of these compounds, leading to their degradation or even the formation of new compounds (Li et al., 2023). However, more details about the possible pathways of phenolic acids still need to be elucidated.

3.6. Sugar profile and content over gastrointestinal digestion

The individual and total contents of sugars and oligosaccharides found before and after simulated gastrointestinal digestion of araçá-boi extracts were determined using HPAEC-PAD and are presented in Table 8.

Mono and disaccharides such as sorbitol, arabinose, glucose, fructose, sucrose, and maltose were found before and after gastrointestinal digestion, except for mannitol, which was not detected in intestinal digestion. As for oligosaccharides, 1-kestose (GF2), 1-fructofuranosyl nystose (GF4), maltotriose (G3), and maltotetraose (G4) were identified before and after gastrointestinal digestion. Compounds such as xylitol, raffinose, nystose (GF2), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7) were also studied but were not detected. These results contrast with the findings of de Araújo et al. (2021a), where sugars such as glucose, fructose, sucrose, and maltose, as well as oligosaccharides like GF2 and G4, were found in the edible fraction of this fruit.

Table 8: Sugar profile (mg/g) of araçá-boi extract over the *in vitro* simulated gastrointestinal digestion.

Compound	Fractions content (mg/g dw)		
	Araçá-boi extract	Grastric phase	Intestinal phase
Mono- and disaccharides			
Xylitol	n.d.	n.d.	n.d.
Mannitol	1.53 ± 0.07[a]	1.48 ± 0.03[a]	n.d.
Sorbitol	2.40 ± 0.04[c]	2.58 ± 0.0.10[b]	2.86 ± 0.11[a]
Rhamnose	n.d.	n.d.	n.d.
Arabinose	2.17 ± 0.09[a]	1.75 ± 0.07[b]	1.65 ± 0.08[b]
Glucose	80.96 ± 2.17[a]	72.50 ± 7.86[a]	50.82 ± 3.67[b]
Fructose	80.08 ± 2.28[a]	68.69 ± 6.31[b]	45.75 ± 3.32[c]
Sucrose	173.37 ± 4.49[a]	105.74 ± 8.52[b]	86.47 ± 1.17[c]
Maltose (G2)	3.77 ± 0.21[a]	3.14 ± 0.22[b]	2.15 ± 0.30[c]
Total Monossacharides and disscharides	344.27 ± 8.71[a]	255.86 ± 22.73[b]	189.70 ± 6.29[c]
Oligosaccharides			
1-kestose (GF2)	t.r.	t.r.	t.r.
Nistose (GF3)	n.d.	n.d.	n.d.
1-frutofuranosil nistose (GF4)	t.r.	t.r.	t.r.
Maltotriose (G3)	0.39 ± 0.01[a]	0.27 ± 0.01[c]	0.34 ± 0.02[b]
Maltotetraose (G4)	1.60 ± 0.09[a]	1.35 ± 0.09[b]	0.97 ± 0.10[c]
Maltopentose (G5)	n.d.	n.d.	n.d.
Maltohexaose (G6)	n.d.	n.d.	n.d.
Maltoheptaose (G7)	n.d.	n.d.	n.d.
Total Oligosaccharides	1.99 ± 0.10[a]	1.62 ± 0.09[b]	1.31 ± 0.11[c]
Total sugars	346.27 ± 8.70[a]	257.48 ± 22.82[b]	191.01 ± 6.19[c]

t.r.: Traces; n.d: not detected; Values followed by the same lowercase letters in a row are not significantly different ($p > 0.05$.

Sucrose was the sugar that contributed the most to the total sugar content throughout the digestive process (173.37 - 86.47 mg/g dw), followed by glucose (80.96 - 50.82 mg/g dw), fructose (80.08 - 45.75 mg/g dw), maltose (3.77 - 2.15 mg/g dw), arabinose (2.17 - 1.65 mg/g dw), and sorbitol (2.40 - 2.86 mg/g dw). Despite these sugars showing a significant reduction in their content during the digestive process, about 50 to 65% of the initial content reached the intestinal fraction for absorption. Mihaylova et al. (2021) observed a variation in the bioaccessibility of total sugars ranging from 4% to 41% in different juice samples. This can be explained by physiological conditions such as pH, the presence of digestive enzymes, and bile salts, which significantly affect

the bioaccessibility of these compounds. This behavior is reflected in the total sugar value, which was 346.27 mg/g dw before digestion and significantly decreased throughout the digestive process (257.48 and 191.01 mg/g dw in gastric and intestinal digestion, respectively). As shown in Table 8, although the levels of glucose and fructose decrease during digestion, the glucose/fructose ratio remains consistent at 1:1 before and after gastric and intestinal digestion. Fruits and vegetables with a 1:1 or higher glucose/fructose ratio are typically better tolerated, as fructose is fully absorbed. While fructose is absorbed through specific carriers like GLUT5, the presence of glucose in the lumen can enhance fructose absorption via the facultative transporter GLUT2 (Arruda et al., 2017). Therefore, the studied araçá-boi extract has good sugar levels and is well tolerated due to the complete absorption of fructose.

Among the oligosaccharides identified in the araçá-boi extract before and after digestion, trace amounts of 1-kestose (GF2) and 1-fructofuranosyl nystose (GF4) were found, along with quantities ranging from 0.39 to 0.27 mg/g dw of maltotriose (G3) and 1.60 to 0.97 mg/g dw of maltotetraose (G4). These findings demonstrate that although these compounds are degraded during the digestion process, more than 80% of G3 and 60% of G4 reach the intestine for absorption, indicating good chemical stability against the effects of bile salts, digestive enzymes, and pH. Oligosaccharides are recognized for their prebiotic properties. Upon fermentation by intestinal microbiota, they generate short-chain fatty acids (SCFAs), which are linked to various health benefits, including regulation of lipid and glycemic metabolism, enhanced brain function and cognition, and improved mineral bioavailability (Neri-Numa et al., 2020).

3.7. Molecular docking analysis

Molecular docking analysis of the interaction between trans-cinnamic acid (the major compound of araçá-boi extract after gastrointestinal digestion) and target proteins was carried out using Autodock 4.2 tools. Table 9 shows the interaction of trans-cinnamic acid with 6 target proteins: nuclear factor kappa B (NF-κB), interleukin 1β (IL-1β), phosphoinositide 3-kinase (PI3K), cyclooxygenase 1 (COX1), tumor

necrosis factor-alpha (TNF-α), and serine/threonine protein kinase (Akt). Free binding energy, inhibition constant, ligand efficiency, number of hydrogen bonds, bond distances, and involved amino acids were the parameters retrieved from the best docking position, considering the cluster with the highest number of runs, for each ligand versus protein docking analysis. NF-κB, IL-1β, and PI3K should be highlighted as target proteins with better results (-5.79, -5.22, and -5.41 kcal/mol, respectively) considering the lowest free binding energies in the molecular docking analysis compared to COX1, Akt, and TNF-α proteins (-3.64, -4.82, and 3.56 kcal/mol, respectively). In line with previous studies, binding energy below -5.0 kcal/mol is regarded as the threshold for stable ligand-receptor interactions in molecular docking, with lower binding energies indicating increased structural stability (Shahraki et al., 2024). Additionally, the NF-κB, IL-1β, and PI3K proteins showed the lowest inhibition constant values (57.02, 147.98, and 10.57 μM, respectively), indicating a higher affinity of trans-cinnamic acid for these proteins. Therefore, we selected the complexes with binding energy below -5.0 kcal/mol and with the lowest inhibition constant.

Table 9. Summary of molecular docking analysis of the interaction of trans-cinnamic acid and target proteins using Auto Dock Vina.

Protein	Run	FBE (Kcal/mol)	IC (μM)	LE	N° H	DISTANCE	AAs
COX1	31	-3.64	2140.00	-0.53	2	1.814 2.109	HIS164 ARG168
NF-κB	28	**-5.79**	57.02	-0.53	1	1.807	ARG35
Akt	31	-4.82	293.06	-0.44	1	1.975	THR81
TNF-α	31	-3.56	2140.00	-0.33	1	2.234	TYR151
IL-1β	8	**-5.22**	147.98	-0.47	1	1.930	GLU64
PI3K	14	**-5.41**	108.57	-0.52	1	2.201	ALA885

The values in bold represent the lowest free energy of binding (FBE) between the ligand and the proteins; IC: inhibition constant; LE: ligand efficiency; N° H: number of bonds involving hydrogen atoms; AAs: amino acids involved).

The 2-D representation of trans-cinnamic acid interactions with NF-kB, IL-1β, and PI3K is depicted in Figure 4. The interaction between trans-cinnamic acid and NF-κB is primarily stabilized by hydrogen bonds with residues Arg35 and Arg33, while hydrophobic interactions with residues Ala43, Gly44, Met32, Ile46, Phe34, and Ile118 further enhance the binding affinity. IL-1β is primarily stabilized by a hydrogen bond

with the residue GLU64, while hydrophobic interactions with residues Pro87, Tyr90, Tyr68, Asn66, Lys65, Ser43, and Lys63 further contribute to the binding affinity. While, PI3K is primarily stabilized by hydrophobic interactions with residues Glu880, Val882, Ile881, Tyr867, Ile963, Ile879, and Asp964.

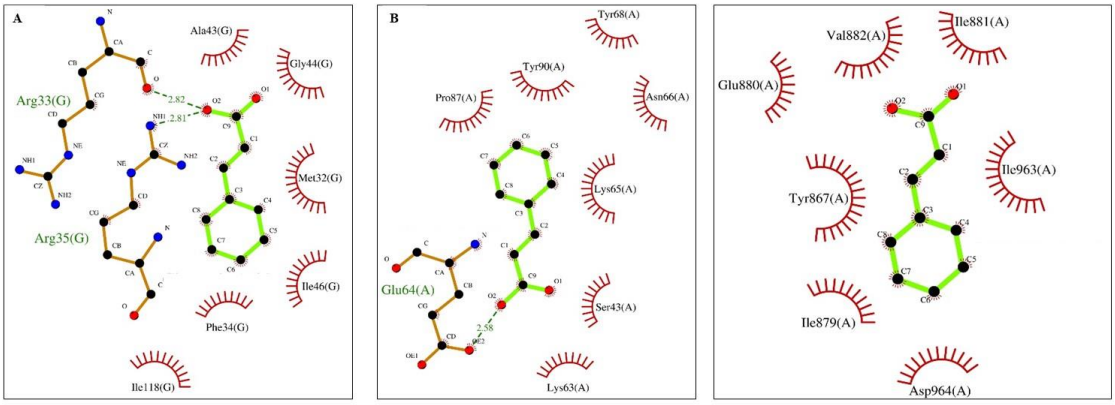

Figure 4. 2-D representations of trans-cinnamic acid interactions with NF-Kb (A), IL-1β (B), and PI3K (C). Trans-cinnamic acid is represented in green; amino acids involved in H bonds are represented in orange; amino acids involved in hydrophobic interactions are represented in red.

The data suggest that trans-cinnamic acid has a high affinity and modulatory potential for the NF-κB, IL-1β, and PI3K proteins due to its stable and strong interactions, indicating a promising therapeutic potential for these targets. Recent studies have shown that trans-cinnamic acid exhibits remarkable biological effects, including anti-inflammatory and antitumor properties. These effects can be attributed to its ability to modulate the activity of key proteins involved in these cellular processes (Borsoi et al., 2024; Onat et al., 2021). Through its interaction with proteins such as NF-κB, IL-1β, and PI3K, trans-cinnamic acid can influence the regulation of inflammatory and tumor growth pathways. For instance, its ability to inhibit NF-κB and IL-1β can reduce the expression of pro-inflammatory genes and create an environment less conducive to tumor development (Afnan et al., 2022; Číž et al., 2020). Additionally, interaction with PI3K may affect cell signaling related to cell survival and proliferation, contributing to the anticancer effects (Afnan et al., 2022). Given this, the interaction profiles of trans-cinnamic acid with NF-κB, IL-1β, and PI3K suggest these proteins are prime candidates for further research into therapeutic applications,

given their favorable binding energies and the robust nature of their amino acid interactions. These findings highlight the potential of araçá-boi extract, rich in trans-cinnamic acid after gastrointestinal digestion, to modulate pathways involving these target proteins effectively.

3.8. ADMET prediction

The ADMET analysis of trans-cinnamic acid provides a detailed overview of its pharmacokinetic and toxicological properties, covering absorption, distribution, metabolism, excretion, and toxicity, which are crucial for understanding its behavior in the body and its therapeutic potential (Table 10). Trans-cinnamic acid demonstrates a promising pharmacokinetic profile, with good solubility (LogS of -2.051 log mol/L), facilitating its absorption in the gastrointestinal tract. Its LogP (2.363) and LogD (2.58) values indicate an appropriate balance between hydrophobicity and hydrophilicity, favoring passage through cell membranes. The intestinal absorption (HIA) of 0.013 and bioavailability (F30%) of 0.059 reinforce its effectiveness in reaching systemic circulation after oral administration. In terms of distribution, a volume of 0.221 L/kg suggests good dispersion throughout body tissues, while the blood-brain barrier (BBB) permeability is moderate (0.614), indicating the compound can partially cross the BBB, with limited effects on the central nervous system. The unbound fraction in plasma (Fu) of 5.90% suggests that a significant portion of the compound is available for interaction with molecular targets. Regarding metabolism, trans-cinnamic acid does not significantly inhibit cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), reducing the risk of drug interactions, which is favorable for therapeutic use. Its elimination is moderate, with a clearance rate of 2.221 mL/min/kg and a half-life of 0.892 hours, suggesting that repeated administration might be needed to maintain adequate therapeutic levels. Trans-cinnamic acid presents mild acute toxicity, with an oral LD50 in rats of 2.5 g/kg, although it shows active hepatotoxicity and nephrotoxicity, indicating potential risks to the liver and kidneys. However, it is inactive for neurotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity,

mutagenicity, and cytotoxicity, minimizing the risk of severe adverse effects. These data provide a solid foundation for the therapeutic potential of trans-cinnamic acid. Its good solubility, absorption, and distribution, combined with the absence of significant cytochrome P450 inhibition and a manageable toxicity profile, make it a promising option that can be further optimized with additional studies.

Table 10. ADMET prediction of trans-cinnamic acid.

Properties	Parameters	Trans-cinnamic acid
Absorption	LogS (log mol/L)	-2.051
	LogP	2.363
	LogD	2.58
	Human intestinal absorption (HIA)	0.013
	$F_{(30\%)}$	0.059
Distribution	Volume distribution (L/kg)	0.221
	Blood-brain barrier permeability (BBB)	0.614
	Fraction Unbound in Plasma (Fu)	5.90%
Metabolism	CYP1A2 inhibitor	Inactive (90.3%)
	CYP2C19 inhibitor	Inactive (96.5%)
	CYP2C9 inhibitor	Inactive (76.3%)
	CYP2D6 inhibitor	Inactive (98.5%)
	CYP13A4 inhibitor	Inactive (99%)
Excretion	Clearance (mL/min/kg)	2.221
	$T_{1/2}$ (h)	0.892
Toxicity	Rat oral LD_{50} (g/kg)	2.5
	Hepatotoxicity	Active (54%)
	Neurotoxicity	Inactive (67%)
	Nephrotoxicity	Active (55%)
	Respiratory toxicity	Inactive (72%)
	Cardiotoxicity	Inactive (53%)
	Carcinogenicity	Inactive (82%)
	Immunotoxicity	Inactive (95%)
	Mutagenicity	Inactive (96%)
	Cytotoxicity	Inactive (83%)

BBB: the ability to cross the blood-brain barrier, a score of 1 indicates a good permeabilibty, while a score closer to 0 indicates poor permeability; Clearance: the rate at which a substance is removed from the body, with values classified as High (> 15 mL/min/kg), Moderate (5-15 mL/min/kg), and Low (< 5 mL/min/kg); F(30%): the probability of the human oral bioavailability less than 30%, its score from 0 to 0.3 represents good; Fu: the proportion that is not bound to plasma proteins, with values categorized as Low (< 5%), Middle (5-20%), and High (> 20%); HIA: the probability of the human intestinal absorption is poorly absorbed, its scores from 0 to 0.3 represent good; LogD: logP at physiological pH 7.4, with a optimal score from 1 to 3; LogP: log of oil-water partition coefficient, its score from 0 to 3 represents good; LogS: log of aqueous solubility, with a score from -4 to 0 representing good solubility; T1/2: A score of 1 indicates a long half-life (> 3 hours), while a score closer to 0 indicates a short half-life (< 3 hours); The Rat oral LD50 test results in LD50 > 15 g/kg, indicating no toxicity; 5 g/kg to 15 g/kg indicates practical non-toxicity; 0.5 g/kg to 5 g/kg indicates mild toxicity; 50 mg/kg to 500 mg/kg indicates moderate toxicity; and < 50 mg/kg indicates high toxicity; Volume distribution: the extent distribution in the body relative to plasma concentration, with scores from 0.04 to 20 L/kg representing optimal distribution.

4. Conclusion

The araçá-boi extract revealed a remarkable diversity of phytochemical compounds, totaling one hundred identifications, including organic acids, phenolic acids, and flavonoids. After gastric digestion, 92 of these compounds demonstrated resistance to acidic conditions, remaining stable. However, the phytochemical profile was altered during intestinal digestion, resulting in the identification of 59 compounds, of which 16 were new phenolic compounds. Gastrointestinal digestion had a significant impact on the phenolic compounds, evidenced by HPLC-DAD, with a highlight on trans-cinnamic acid, whose bioaccessibility increased by an impressive 813%, followed by p-coumaric acid (232%) and quercetin (106%). The extract also contains a variety of mono and disaccharides, as well as oligosaccharides such as G3 and G4, with sucrose making a significant contribution to the total sugar content. Despite the reduction in sugar levels during digestion, approximately 50 to 65% of the initial content reached the intestinal fraction, demonstrating good chemical stability. The analysis of antioxidant capacity revealed significant decreases in activities measured by the DPPH, FRAP, and ORAC assays, while the $ABTS^{\bullet+}$ assay highlighted a notable improvement, exceeding the initial activity of the undigested extract.

Additionally, the *in silico* study of molecular docking and ADMET analysis were conducted for the predominant compound of the araçá-boi extract after gastrointestinal digestion. The analyses showed that trans-cinnamic acid interacts promisingly and significantly with target proteins such as NF-κB, IL-1β, and PI3K, exhibiting substantial free binding energies and low inhibition constants, indicating high affinity. These interactions suggest considerable modulatory potential of trans-cinnamic acid on these proteins. Finally, the ADMET analysis revealed a promising pharmacokinetic profile for trans-cinnamic acid, with good solubility, absorption, and distribution, along with mild toxicity.

The results of this study emphasize the rich diversity of bioactive compounds present in the araçá-boi extract, highlighting the significant increase in the bioaccessibility of trans-cinnamic acid after intestinal digestion. The *in silico* analyses

of molecular docking and ADMET provide a deeper insight into the therapeutic potential of this compound, while the detailed characterization of the phytochemical profile and quantification of individual phenolic compounds reinforce the extract's relevance in promoting health and preventing diseases. These findings open new perspectives for future research, emphasizing the importance of further investigating the effects of the extract in different physiological contexts and its potential applications in the formulation of new functional foods and nutraceuticals.

CRediT authorship contribution statement

Felipe Tecchio Borsoi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft, Writing - review & editing. **Henrique Silvano Arruda:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - review & editing. **Lívia Mateus Reguengo:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, **Iramaia Angélica Neri Numa:** Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Glaucia Maria Pastore:** Project administration, Supervision, Funding acquisition, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have appeared to influence the work reported in this manuscript.

Data Availability

Data will be made available on request

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Ethical approval

This article does not contain studies with human participants or animals performed by any of the authors.

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CHAPTER 5

The therapeutic potential of araçá-boi extract and its phenolic compounds in human metastatic melanoma cells

Extract of araçá-boi and its major phenolic compound, trans-cinnamic acid, reduce viability and inhibit migration of human metastatic melanoma cells

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Extract of Araçá-Boi and Its Major Phenolic Compound, Trans-Cinnamic Acid, Reduce Viability and Inhibit Migration of Human Metastatic Melanoma Cells

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Abstract: Cutaneous melanoma is an aggressive type of skin cancer that is recognized for its high metastatic potential and the challenges it presents in its treatment. There has been increasing interest in plant extracts and their potential applications in melanoma. The present study aimed to investigate the content of individual phenolic compounds in araçá-boi extract, evaluate their antioxidant activity, and explore their effects on cell viability, migration properties, oxidative stress levels, and protein expression in the human metastatic melanoma cell line SK-MEL-28. HPLC-DAD analysis identified 11 phenolic compounds in the araçá-boi extract. Trans-cinnamic acid was the main phenolic compound identified; therefore, it was used alone to verify its contribution to antitumor activities. SK-MEL-28 melanoma cells were treated for 24 h with different concentrations of araçá-boi extract and trans-cinnamic acid (200, 400, 600, 800, and 1600 μg/mL). Both the araçá-boi extract and trans-cinnamic acid reduced cell viability, cell migration, and oxidative stress in melanoma cells. Additionally, they modulate proteins involved in apoptosis and inflammation. These findings suggest the therapeutic potential of araçá-boi extract and its phenolic compounds in the context of melanoma, especially in strategies focused on preventing metastasis. Additional studies, such as the analysis of specific signaling pathways, would be valuable in confirming and expanding these observations.

Keywords: *Eugenia stipitata*; skin cancer; Brazilian fruits; polyphenols; anti-cancer effect; phenolic acids; flavonoids; oxidative stress; caspase-3; inflammasome

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1. Introduction

Cutaneous melanoma is an aggressive type of skin cancer that originates from malignant transformations and uncontrolled proliferation of melanocytes, pigment-producing cells of the skin [1]. It poses significant challenges due to its ability to metastasize, leading to poor prognosis if not detected and treated early [2]. The global incidence of melanoma continues to rise steadily, with 331,722 new cases and 58,667 deaths reported in 2022 alone, underscoring its growing public health concerns [3]. The development of melanoma is multifactorial and arises from the interaction between genetic susceptibility and environmental exposure. However, sixty to seventy percent of melanomas are thought to be caused by ultraviolet (UV) radiation from sunlight, which induces DNA mutations in skin cells [4].

Current treatment options for cutaneous melanoma include surgery, radiotherapy, chemotherapy, immunotherapy, and targeted therapy [5]. Cancer immunotherapies and

targeted therapies have been gaining increasing attention with the development of immune checkpoint blockade strategies targeting programmed death-1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) co-inhibitory receptors and mitogen-activated protein kinases (MAPKs) molecular targeted therapy directed at oncogenic serine/threonine-protein kinase B-Raf (BRAF) and mitogen-activated extracellular signal-regulated kinase (MEK) signaling pathways [4,5]. Although the emergence of immune and targeted therapies has increased the life expectancy of patients with melanoma, some patients relapse or simply do not respond to these regimens. Therefore, the development of new and improved adjuvant therapies or drugs remains a priority for researchers to improve patient survival and health quality [1].

In recent years, there has been growing interest in plant extracts, particularly those rich in phenolic compounds, for their potential applications in cutaneous melanoma [6–10]. Phenolic compounds are a group of naturally occurring organic substances found in the plant kingdom as secondary metabolites and are characterized by the presence of hydroxyl (–OH) groups attached to an aromatic hydrocarbon ring. This structural characteristic allows antioxidant properties and can interact with cellular signaling cascades, regulating the activity of transcription factors and consequently affecting the expression of genes involved in cellular metabolism and cell survival, mainly related to cell growth, apoptosis, and inhibition of metastasis [10–14].

Araçá-boi (*Eugenia stipitata* Mac Vaugh-Myrtaceae) is a fruit tree that grows naturally in the Amazon region and is cultivated in some countries such as Brazil, Peru, Bolivia, Ecuador, and Colombia. It is a fleshy globe-shaped fruit, approximately 12 cm in diameter and weighing around 30–80 g, characterized by a fine, yellow, pubescent epicarp, white soft mucilaginous pulp, very acidic (pH = 2.28), and an attractive flavor [15]. In the literature, data on the characterization and quantification of phenolic compounds do araçá-boi, and their biological effects are still scarce. Some studies reported the presence of quercetin, myricetin, kaempferol, luteolin, gallic acid, vanillic acid, apigenin, catechin, gallocatechin, and their derivatives [15–18]. Additionally, few studies have demonstrated the biological activities of araçá-boi extract, including antioxidant, antimutagenic, antigenotoxic, and anti-inflammatory activities [16,18]. Thus, demonstrating the antitumor activity of araçá-boi could be a tool for managing public health issues, as the inclusion of araçá-boi or its derived products (e.g., juices, extracts, and processed products) in the diet could contribute to the intake of potentially anticarcinogenic phytochemicals, particularly phenolic compounds. This approach may not only play a role in prevention but also serve as a promising therapeutic strategy for the treatment of melanoma. In this context, the present study aims to investigate the content of individual phenolic compounds in araçá-boi extract by high-performance liquid chromatography coupled to a photodiode array detector (HPLC-DAD) and explore their effects on cell viability, migration properties, oxidative stress levels, and protein expression in the human metastatic melanoma cell line SK-MEL-28.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-methyl propionamidine)-dihydrochloride (AAPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein, 2,4,6-tripyridy-*s*-triazine (TPTZ), acetonitrile and formic acid grade HPLC, and all phenolic compound standards (gallic acid, α-resorcylic acid, protocatechuic acid, procyanidin B1, chlorogenic acid, catechin, procyanidin B2, 4-hydroxybenzoic acid, gentisic acid, epicatechin, caffeic acid, vanillic acid, syringic acid, vitexin-2-rhamnoside, rutin, vitexin, quercetin-3-*O*-galactoside, *p*-coumaric acid, procyanidin A2, sinapic acid, kaempferol-3-*O*-glucoside, ferulic acid, quercetrin, apigenin-7-*O*-glucoside, myricetin, benzoic acid, luteolin, quercetin, trans-cinnamic acid, apigenin, naringenin, kaempferol, and hesperetin) with a purity of ≥96% were purchased from Sigma-Aldrich (St. Louis, MO, USA). All materials used for cell culture were obtained from Gibco™ Thermo Fisher Scientific (Grand Island, NY, USA) and

Invitrogen Life Technologies (Carlsbad, CA, USA). antibodies anti-human *Caspase-3* (p17 subunit; catalog no. BS-20363R-A555), and anti-human *NLRP3* (catalog no. MA5-32255) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA).

2.2. Plant Material and Phenolic Extract Obtantion

Mature araçá-boi fruits were collected from the "Kamui Farm" in Ituberá city, Bahia State (Brazil), at 13°44″ S and 39°9″ W. Botanical identification and exsiccate (access number 55.875) were deposited at the Herbarium-UEC of the Agronomic Institute of Campinas, State of São Paulo, Brazil [19]. The edible fractions (pulp and peel) were freeze-dried (Lyophilizer Series LS E, Terroni Scientific Equipment, São Carlos, SP, Brazil) and ground using a knife mill (Marconi, model MA340, Piracicaba, SP, Brazil). The powders obtained were granulometrically standardized using an electromagnetic sieve shaker 24-mesh (Bertel, model AGMAGB, Caieiras, SP, Brazil).

The phenolic extract was obtained according to the method described by de Araújo et al. [15] with slight modifications. A hydroethanolic solution was selected as the extractor solvent because, among the organic solvents suitable for the extraction of phenolic compounds, only ethanol is considered a generally recognized as safe (GRAS) solvent. Furthermore, its combination with water increases the efficiency of phenolic extraction, particularly in the glycosylated fraction. Thus, the use of an ethanol-water mixture (80:20, v/v), along with a non-conventional extraction technique (ultrasound), was employed in the present study to address environmental and safety concerns [20,21].

Freeze-dried samples of araçá-boi (1 g) were extracted with 15 mL of an ethanol-water mixture (80:20, v/v) for 10 min using an ultrasonic bath (UNIQUE, model UCS-2850, 25 kHz, 120 W, São Paulo, SP, Brazil) at room temperature. After extraction, the mixture was centrifuged at 4000× g for 5 min, and the upper layer was collected. The residues were re-extracted two more times under the same conditions. The upper layers were combined, evaporated under a vacuum, and freeze-dried. The obtained powder extract was stored at −20 °C until further analysis.

2.3. Chemical Characterization of Araçá-Boi Extract

2.3.1. Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined using the method described by Pereira et al. [22]. The extract (25 µL), 50% (v/v) Folin-Ciocalteu reagent (25 µL), and 5% (w/v) sodium carbonate (200 µL) were mixed and allowed to react for 20 min in the dark at room temperature. The absorbance was recorded at 760 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany). Gallic acid was used as a standard, and the results were expressed as mg gallic acid equivalents per gram of dried extract (mg GAE/g dw).

2.3.2. Determination of Condensed Tannin Content (CTC)

The condensed tannin content (CTC) was determined according to the method described by Arruda et al. [23] with slight modifications. The extract (20 µL), 4% (w/v) vanillin in methanol (180 µL), and concentrated HCl (90 µL) were mixed and allowed to react for 20 min in the dark at room temperature. The absorbance was recorded at 500 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany). Catechin was used as a standard, and the results were expressed as mg catechin equivalents per gram of dried extract (mg CE/g dw).

2.3.3. Chromatographic Analysis of Phenolic Compounds by HPLC-DAD

The extracts were analyzed following the chromatographic conditions established by Silva et al. [24] using a Dionex UltiMate 3000 (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was carried out on a reverse-phase AcclaimTM 120 A C18 column (250 × 4.6 mm i.d., 5 µm particle size, Thermo Fisher Scientific, Waltham, MA, USA) and thermostated at 32 °C. The solvents used were 0.1% formic acid in deionized

water (Solvent A) and HPLC-grade acetonitrile (Solvent B). The gradient was linear at a flow rate of 0.5 mL/min with 5% solvent B for 5 min, from 5% to 29% solvent B for 22 min, 35% solvent B for 6 min, 35% to 50% solvent B for 12 min, 95% solvent B for 5 min, and 5% solvent B for 10 min. Diode array detection was performed from 200 to 800 nm. The injection volume was 20 µL. Quantification of different phenolic compounds was carried out at different wavelengths depending on the compound (260, 280, 320, or 360 nm). The compounds were identified and quantified based on their retention times and spectral characteristics compared to those of standard compounds. The contents of individual phenolic compounds were expressed as micrograms per gram of dried extract (µg/g dw).

2.3.4. Trolox Equivalent Antioxidant Capacity (TEAC) Assay Using $ABTS^{\bullet+}$ Radical

The TEAC was conducted as described by Arruda et al. [20]. A radical cation $ABTS^{\bullet+}$ solution (7 mmol/L ABTS and 145 mmol/L potassium persulfate) was prepared and incubated in the dark at room temperature overnight. The $ABTS^{\bullet+}$ working solution was diluted with ultrapure water to achieve an absorbance of 0.70 ± 0.02 at 734 nm. The extract (40 µL) was mixed with $ABTS^{\bullet+}$ solution (200 µL) and the absorbance was measured on a microplate reader (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany) after 6 min at 734 nm. A calibration curve with Trolox was used as a reference, and the results were expressed as micromoles of Trolox equivalents per gram of dried extract (µmol TE/g dw).

2.3.5. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed based on the method described by Guerra-Ramírez et al. [25] with some modifications. The FRAP solution was prepared by adding 20 mL acetate buffer (0.3 mol/L) at pH 3.6, 2 mL TPTZ solution (10 mmol/L) in 40 mmol/L HCl, and 2 mL ferric chloride solution (20 mmol/L) (10:1:1). The extract (20 µL), FRAP solution (180 µL), and deionized water (60 µL) were mixed and incubated at 37 °C for 30 min before measuring the absorbance at 595 nm using a microplate reader (SPECTROstar Nano, BMG Labtech, Ortenberg, Germany). A calibration curve with Trolox was utilized as a reference, and results were expressed as micromoles of Trolox equivalents per gram of dried extract (µmol TE/g dw).

2.3.6. Oxygen Radical Absorbance Capacity (ORAC) Assay

The ORAC assay was conducted following the protocol reported by Dávalos et al. [26]. The reaction was performed in phosphate buffer (75 mmol/L, pH 7.4) in a 96-well dark microplate. The extract (20 µL), 1 µmol/L fluorescein (120 µL), and 0.4 mol/L AAPH (60 µL) were mixed and incubated at 37 °C. Fluorescence was measured with excitation at 485 nm and emission at 520 nm every minute for 80 min on a microplate reader (NOVOstar, BMG Labtech, Offenburg, Germany). A calibration curve with Trolox was utilized as a reference, and results were expressed as micromoles of Trolox equivalents per gram of dried extract (µmol TE/g dw).

2.4. Cellular Assays

2.4.1. Cell Culture and Treatments

The human metastatic melanoma cell line (SK-MEL-28, Cell Bank of Rio de Janeiro, Brazil) was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were maintained in an incubator at 37 °C and 5% CO_2. The powdered araçá-boi extract and trans-cinnamic acid were dissolved in 0.4% dimethyl sulfoxide (DMSO) and mixed with culture medium to obtain treatment concentrations of 200, 400, 800, and 1600 µg/mL. The control group (CT) received only a culture medium for treatment.

2.4.2. Cell Viability by Fluorescence Microscopy Assay

The fluorophore acridine orange (AO) was used to stain viable cells due to its ability to permeate the membranes of living cells and interact with DNA, emitting green

fluorescence [27]. Melanoma cells were grown until a monolayer reached a density of 1×10^5 cells/well in 96-well plates and treated with araçá-boi extract and trans-cinnamic acid for 24 h. The cells were then washed twice with PBS and stained with acridine orange (1 µg/mL) for 5 s. The reminiscent staining solution was discarded, and the cells were washed twice with PBS. The readings were taken using an inverted fluorescence microscope (Nikon Eclipse TS2-FL, Tokyo, Japan) with a magnification of 200× at the central point of the wells (Ex = 480 nm, Em = 500 nm). The software ImageJ version 1.54j was used to linearly adjust the images for brightness and contrast. The results are expressed as a percentage (%) of cell viability compared to the control.

2.4.3. Measurement of Mitochondrial Transmembrane Potential (ΔΨm)

The cationic dye tetramethylrhodamine ethyl ester (TMRE) was used to measure the mitochondrial transmembrane potential due to its ability to accumulate in active mitochondria, emitting red fluorescence [28]. Melanoma cells were grown until a monolayer reached a density of 1×10^5 cells/well in 96-well plates and treated with the araçá-boi extract and trans-cinnamic acid for 24 h. Then, the cells were washed twice with PBS buffer and incubated for 30 min with a solution of Hank's balanced salts containing 20 nmol/L TMRE (100 µL). The reminiscent solution was discarded, cells were washed with PBS, and readings were taken under a fluorescence microscope at a magnification of 200× in the center of the wells (Ex = 550 nm, Em = 580 nm). The software ImageJ version 1.54j was used to adjust the images for brightness and contrast linearly. The results are expressed as a percentage (%) of the mitochondrial transmembrane potential compared to the control.

2.4.4. Cell Migration Assay

To verify the influence of the treatments on cell migration, we performed an assay based on the study proposed by Justus et al. [29]. For this, melanoma cells were cultured in a six-well plate at a density of 1×10^6 cells for 24 h. Scratches were made in a confluent monolayer using a sterile tip. Subsequently, the cells were exposed to araçá-boi extract and trans-cinnamic acid for 24 h. The cells were washed with 0.9% NaCl solution to remove nonadherent cell debris. Cells that migrated across the wound area were photographed at 0 and 24 h using optical microscopy at a magnification of 40×. The software ImageJ version 1.54j was used to adjust the images for brightness and contrast linearly. The results are expressed as a percentage (%) of wound healing closure compared to the control.

2.4.5. Detection of Cellular Reactive Oxygen Species (ROS)

To detect cellular reactive oxygen species (ROS) levels in melanoma cells, a cell-permeable fluorescent signal indicator, 2,7-dichlorodihydrofluorescein-diacetate (H_2DCF-DA), was used, according to Wu et al. [30]. Thus, after araçá-boi extract and trans-cinnamic acid treatments, cells were washed with PBS and incubated with 100 mmol/L H_2DCF-DA for 30 min at 37 °C. The final fluorescent product was measured using a fluorescence plate reader (Thermo Scientific™ Varioskan™ LUX, Waltham, MA, USA) (Ex = 488 nm, Em = 525 nm). The results are expressed as a percentage (%) of relative fluorescence compared to the control.

2.4.6. Assessment of Caspase-3 and NLRP3 Expression

To assess caspase-3 and NLRP3 expression in melanoma cells, 1×10^6 cells were collected after 24 h of treatment, washed with PBS, and centrifuged at 3000 rpm for 5 min. Then, the cell pellets were diluted in 100 µL of PBS and mixed with 1 µL of each conjugated antibody (CASP3 or NLRP3). After 30 min, the cells were read in a C6 Plus Personal flow cytometer (Accuri™, BD Accuri, San Jose, CA, USA) every 10,000 events. The results are expressed as a percentage (%) of Caspase-3 and NLRP3 expression compared to the control.

2.5. Statistical Analysis

The results are expressed as the mean ± standard deviation value of at least three independent experiments. Statistical analyses were performed using the GraphPad Prism software version 9. Significant differences are symbolized using p-values of * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, which were acquired using one-way ANOVA, followed by Dunnett's post-hoc multiple comparison test.

3. Results

3.1. Chemical Characterization of Araçá-Boi Extract

3.1.1. Total Phenolic Content, Condensed Tannin Content, and Antioxidant Activity

The total phenolic content, condensed tannin content, and antioxidant activity of the araçá-boi extract are shown in Table 1. Araçá-boi extract showed a total phenolic content of 12.16 ± 0.38 mg GAE/g extract dw. Rufino et al. [31] classified food matrices based on their dry weight (dw) as low (<10 mg GAE/g), medium (10–50 mg GAE/g), and high (>50 mg GAE/g). Therefore, the araçá-boi extract can be considered a good source of phenolic compounds since it exhibits a medium content of these phytochemicals. Our study reported higher total phenolic values than those reported by de Araújo et al. [15] (9.06 mg GAE/g extract dw), who also extracted phenolic compounds from araçá-boi using ultrasound-assisted extraction with ethanol-water mixture (80:20, v/v) solvent system. Variations in total phenolic content across studies can be attributed to factors such as edaphoclimatic conditions of the fruit's region of origin (e.g., temperature, soil composition, light exposure, and harvest timing), along with the plant's physiological and genetic traits, as well as differences in sample preparation and storage conditions [32].

Table 1. Total phenolic content, condensed tannin content, and antioxidant activity by TEAC, FRAP, and ORAC methods from araçá-boi extract.

Assay	Araçá-Boi Extract
TPC (mg GAE/g extract dw)	12.16 ± 0.38
Condensed tannins (mg CE/g extract dw)	5.76 ± 0.07
TEAC (µmol TE/g extract dw)	102.51 ± 1.16
FRAP (µmol TE/g extract dw)	150.77 ± 4.37
ORAC (µmol TE/g extract dw)	583.81 ± 17.67

dw, dried weight; CE, catechin equivalents; GAE, gallic acid equivalents; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content.

The condensed tannin content was estimated in the araçá-boi extract. As shown in Table 1, the amount of extractable condensed tannin content was 5.76 ± 0.07 mg CE/g extract dw. Condensed tannins, also known as proanthocyanidins, are phenolic compounds found in several fruits. They are polymers formed by the condensation of flavan-3-ols, such as catechin and epicatechin [33]. To the best of our knowledge, this is the first study to report the condensed tannin content in araçá-boi extract.

A strong and positive correlation between the total phenolic content/condensed tannin content and the antioxidant activity of different plant matrices has been demonstrated by other researchers [23,34,35]. Antioxidant activity is mainly linked to phenolic compounds present in the plant matrix, acting as reducing agents, hydrogen donors, transition metal chelators, reactive oxygen and/or nitrogen species (ROS/RNS) quenchers, inhibitors of enzymes involved in oxidative stress, and upregulation and/or protection of endogenous defense systems [23]. Thus, considering these mechanisms of action, the antioxidant activity of the araçá-boi extract was determined using TEAC, FRAP, and ORAC assays. As seen in Table 1, araçá-boi extract showed a higher antioxidant activity value in the ORAC assay (583.81 ± 17.67 µmol TE/g extract dw), followed by FRAP assay (150.77 ± 4.37 µmol TE/g extract dw), and TEAC assay (102.51 ± 1.16 µmol TE/g extract dw). Antioxidant methods are based on different mechanisms of action, including hydrogen atom transfer (the ability to quench free radicals by hydrogen donation), single-electron transfer (the

ability to transfer one electron to reduce any compound, such as radicals, metals, and carbonyls), or mixed-mode assays. Therefore, to better understand the antioxidant potential of plant extracts, it is necessary to conduct various antioxidant assays to evaluate their different mechanisms of action [23,36,37]. In the present study, we subjected the araçá-boi extract to antioxidant assays involving each of the aforementioned mechanisms of action, namely hydrogen atoms transfer (ORAC), single electron transfer (FRAP), and mixed-mode (ABTS). As observed above, the araçá-boi extract showed the highest antioxidant activity by the ORAC method, followed by FRAP and TEAC, demonstrating that the phenolic compounds of the araçá-boi extract act more efficiently through hydrogen atom transfer mechanisms. Furthermore, the ORAC method measures a compound's ability to inhibit peroxyl radicals generated by simulating the physiological conditions of the human body more accurately. Peroxyl radicals are a class of highly reactive free radicals formed during oxidation reactions in the human body and play a significant role in oxidative stress, which is associated with various pathological conditions, including cardiovascular diseases, cancer, inflammatory processes, and aging [23]. The effective capacity of the araçá-boi extract as peroxyl radical scavengers found here points to their potential to prevent and/or treat oxidative-related diseases.

3.1.2. Content of Individual Phenolic Compounds by HPLC-DAD Method

To examine the content of individual phenolic compounds in the araçá-boi extract, HPLC-DAD analysis was carried out using thirty-three authentic standards (Table 2). The data were collected according to the retention time and chromatograms were simultaneously recorded at 260, 280, 320, and 360 nm, approaching the optimal wavelength (λmax) for each compound. Eleven phenolic compounds were identified and quantified in the araçá-boi extract, including seven phenolic acids (gentisic acid, 4-hydroxybenzoic acid, ferulic acid, gallic acid, *p*-coumaric acid, syringic acid, and trans-cinnamic acid) and four flavonoids (hesperetin, kaempferol-3-*O*-glucoside, quercetin-3-*O*-galactoside, and quercetin-3-*O*-rhamnoside). A total value of 766.44 ± 4.53 μg/g extract dw of phenolic compounds was quantified in the araçá-boi extract using HPLC-DAD (Table 2). Additionally, about 56% of the total quantified (435.09 ± 2.19 μg/g extract dw) are phenolic acids, and the other 44% were flavonoids (331.35 ± 2.49 μg/g extract dw), demonstrating a diversification of the phenolic classes found. In terms of compounds, trans-cinnamic acid (155.79 ± 0.32 μg/g extract dw) was the major phenolic compound present in the araçá-boi extract, followed by quercetin-3-O-galactoside (151.32 ± 1.86 μg/g extract dw), quercetin-3-*O*-rhamnoside (116.27 ± 0.27 μg/g extract dw), and syringic acid (113.07 ± 0.74 μg/g extract dw), accounting for approximately 70% of the total quantified phenolic compounds. Few studies have been conducted on the identification and quantification of phenolic compounds present in the fractions of the araçá-boi fruit. For example, Cuellar et al. [38] identified and quantified three phenolic acids, such as chlorogenic acid (44.1–515.1 μg/g dw), gallic acid (17.3–64.8 μg/g dw), and caffeic acid (3.0–11.1 μg/g dw) in an acidified methanol solution (80:19:1, *v*/*v*/*v*, methanol-water-HCl) of the epicarp or mesocarp of araçá-boi fruit at different ripening stages. A study conducted by Neri-Numa et al. [18] found three flavonoids, namely myricetin (0.17 μg/g fw), quercetin (0.09 μg/g fw), and kaempferol (0.03 μg/g fw) in an acid-hydrolyzed methanolic extract of araçá-boi pulp.

To the best of our knowledge, the data presented in this study represent the most detailed quantification of the phenolic compounds in the araçá-boi extract. The major phenolic compounds found in araçá-boi extracts have been reported to exert numerous biological effects. For example, trans-cinnamic acid possesses several significant bioactivities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, neuroprotective, hepatoprotective, antiallergic, and wound healing properties [39–41]. Similarly, various studies demonstrate the potential of quercetin and its derivatives in diverse biological activities through several molecular mechanisms, including modulation of oxidative stress pathways, inhibition of inflammatory signaling pathways, and modulation of cancer-related molecular signaling pathways [42–44]. Therefore, these findings suggest that

araçá-boi extract possesses a broad and varied number of phenolic compounds, allowing for a potential antioxidant and a broad spectrum of bioactivities that can contribute to promoting human health and well-being. Moreover, different phenolic compounds can interact synergistically to enhance their overall activity. Trans-cinnamic acid is the major compound in the araçá-boi extract (corresponding to approximately one-fifth of the total phenolic compounds identified and quantified by HPLC-DAD). Therefore, this compound was chosen for comparison in the subsequent analyses.

Table 2. Content of individual phenolic compounds obtained by HPLC-DAD method in araçá-boi extract.

Class	Compound	Araçá-Boi Extract (μg/g Extract dw)
Phenolic acids	2,5-Dihydroxybenzoic acid (gentisic acid)	54.94 ± 0.86
	3,4-Dihydroxybenzoic acid (protocatechuic acid)	n.d.
	3,5-Dihydroxybenzoic acid (α-resorcylic acid)	n.d.
	4-Hydroxybenzoic acid	2.56 ± 0.11
	Benzoic acid	n.d.
	Caffeic acid	n.d.
	Chlorogenic acid	n.d.
	Ferulic acid	6.71 ± 0.19
	Gallic acid	73.93 ± 0.18
	p-Coumaric acid	28.09 ± 0.49
	Sinapic acid	n.d.
	Syringic acid	113.07 ± 0.74
	Trans-cinnamic acid	155.79 ± 0.32
	Vanillic acid	n.d.
	Total phenolic acids	435.09 ± 2.19
Flavonoids	Apigenin	n.d.
	Apigenin-7-*O*-glucoside (apigetrin)	n.d.
	Apigenin-8-*C*-glucoside (vitexin)	n.d.
	Catechin	n.d.
	Epicatechin	n.d.
	Hesperetin	1.15 ± 0.07
	Kaempferol	n.d.
	Kaempferol-3-*O*-glucoside (astragalin)	62.61 ± 0.63
	Luteolin	n.d.
	Myricetin	n.d.
	Naringenin	n.d.
	Procyanidin A2	n.d.
	Procyanidin B1	n.d.
	Procyanidin B2	n.d.
	Quercetin	n.d
	Quercetin-3-*O*-galactoside (hyperoside)	151.32 ± 1.86
	Quercetin-3-*O*-rhamnoside (quercetrin)	116.27 ± 0.27
	Quercetin-3-*O*-rutinoside (rutin)	n.d.
	Vitexin-2″-*O*-rhamnoside	n.d.
	Total flavonoids	331.35 ± 2.49
	Total phenolic compounds	766.44 ± 4.53

dw, dried weight; n.d.: not detected.

3.2. Effects of Araçá-Boi Extract and Trans-Cinnamic Acid on Human Metastatic Melanoma Cells

3.2.1. Cell Viability in Melanoma Cells

The viability of SK-MEL-28 cells was assessed by fluorescence microscopy using an acridine orange fluorophore. After 24 h, melanoma cells stained with acridine orange showed a significant decrease in cell viability at all tested concentrations of araçá-boi extract and trans-cinnamic acid compared to the control group cells ($p < 0.0001$) (Figure 1).

Melanoma Cells

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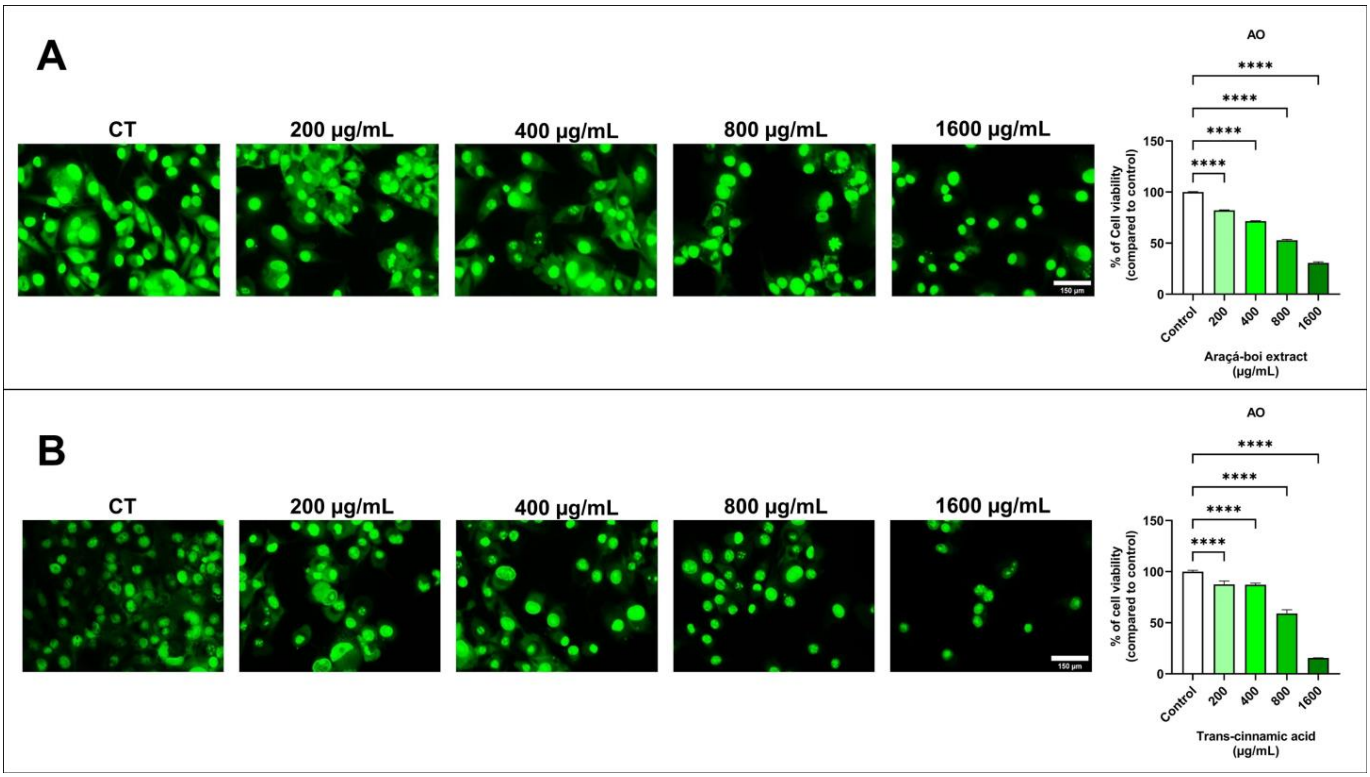

Figure 1. Fluorescence microscopy assay for cell viability using acridine orange (AO) was evaluated in melanoma cells treated with araçá-boi extract (**A**) and trans-cinnamic acid (**B**). CT = control group cells. White scale bar represents 150 µm. Microscope magnification = 200×. All experiments were independently performed three times and in three replicates. Data are presented as the mean ± standard deviation. Statistical analysis: One-way ANOVA followed by post-hoc Dunnett's multiple comparisons test. Statistical significance was set at $p < 0.05$. **** ($p < 0.0001$).

Previous studies have highlighted that phenolic compounds present in plant extracts are primarily responsible for their antitumor activities in various types of cancer, including melanoma [10,45,46]. This antitumor activity may be related to the different stages of cancer progression, such as initiation, promotion, progression, invasion, and metastasis [47]. Therefore, to assess antitumor activity, a cell viability assay was performed using fluorescence microscopy, allowing direct observation of membrane integrity on viable cells through the interaction between acridine orange dye and nucleic acids by emitting green fluorescence [48]. As shown in Figure 1A, araçá-boi extract significantly reduced cell viability as the concentration increased. Similarly, this can be observed for trans-cinnamic acid (Figure 1B), indicating that this phenolic acid may be the main contributor to the reduction in melanoma tumor cell viability. Thus, for the first time, the effect of the araçá-boi extract on reducing cell viability in melanoma cells was evaluated, as well as the actual contribution of its major component, trans-cinnamic acid. Several studies have demonstrated that phenolic plant extracts can inhibit cell viability through different mechanisms, such as inducing apoptosis, cell cycle arrest, oxidative stress induction, modulation of signaling pathways, changes in cell membranes, mitochondrial alterations, and DNA damage, among others [47,49]. Therefore, to understand and elucidate the mechanisms by which the araçá-boi extract and trans-cinnamic acid may reduce cell viability, changes in transmembrane potential, oxidative stress induction, and modulation of proteins related to apoptosis and inflammation were performed and are discussed below.

3.2.2. Transmembrane Potential of Mitochondria of Melanoma Cells (ΔΨm)

The transmembrane potential of the mitochondria in SK-MEL-28 cells was assessed by fluorescence microscopy using TMRE (Figure 2). After 24 h, melanoma cells stained

with TMRE showed a slight reduction in mitochondrial function with the araçá-boi extract at the highest concentration tested (1600 µg/mL) compared with the control group cells (Figure 2A). In contrast, a noticeable reduction in mitochondrial function was observed with trans-cinnamic acid treatment, as indicated by progressively lower fluorescence intensity at concentrations of 200, 400, 800, and 1600 µg/mL (Figure 2B).

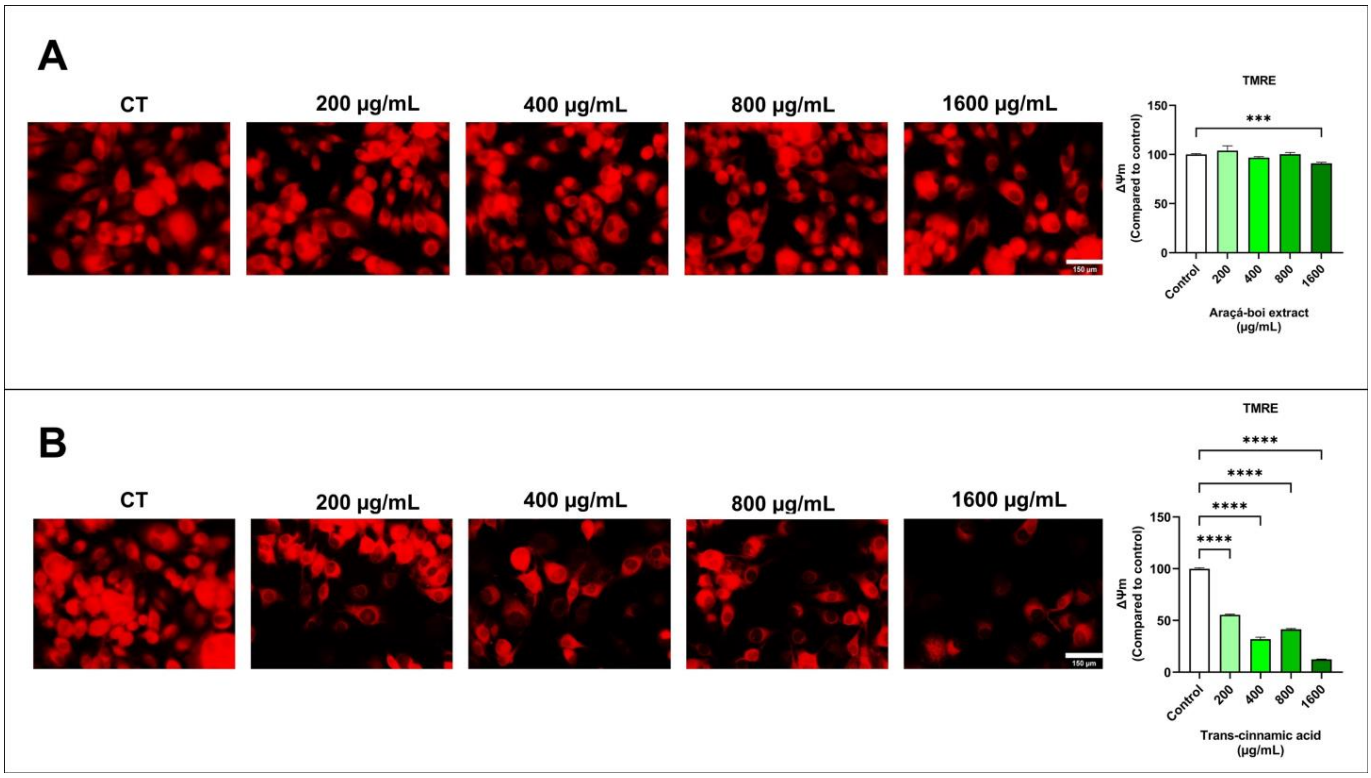

Figure 2. Fluorescence microscopy assay for the transmembrane potential of mitochondria (TMRE) was evaluated in melanoma cells treated with araça-boi extract (**A**) and trans-cinnamic acid (**B**). CT = control group cells. White scale bar represents 150 µm. Microscope magnification = 200×. All experiments were performed independently three times and in three replicates. Data are presented as mean ± standard deviation. Statistical analysis: one-way ANOVA followed by a post hoc Dunnett's multiple comparisons test. Values with $p < 0.05$ were considered statistically significant. *** ($p < 0.001$) and **** ($p < 0.0001$).

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3.2.3. Cell Migration of Melanoma Cells

Figure 3 shows melanoma cell migration (SK-MEL-28) treated for 24 h with araça-boi extract and trans-cinnamic acid. There was a significant decrease in wound closure at all tested concentrations of araça-boi extract and trans-cinnamic acid compared to the control group cells ($p < 0.0001$).

Cutaneous melanoma is an aggressive skin tumor primarily characterized by its high metastatic potential and low survival rate [60]. Metastasis is a multifaceted biological process encompassing invasion, migration, and intravasation into the bloodstream, enabling tumor cells to disseminate and establish colonies in organs distant from the primary tumor site [61]. Recently, several studies have demonstrated that extracts from food matrices rich in phenolic compounds reduce cell migration in melanoma tumor cells [62–64]. The results of the present study demonstrated that araça-boi extract, which is rich in phenolic compounds such as trans-cinnamic acid, induced a significant reduction in wound closure in melanoma cells at all tested concentrations. This finding suggests the potential anti-metastatic activity of the araça-boi extract, as cellular migration is a crucial step in the metastasis process. Additional experiments were performed, to determine the specific contribution of trans-cinnamic acid to the inhibition of cellular migration. When administered alone, trans-cinnamic acid resulted in a significant reduction in cellular migration. These data imply that trans-cinnamic acid may be one of the main contributors to the reduction in melanoma cell migration observed in the araçá-boi extract. The phenolic compounds present in plant extracts can inhibit the migration of melanoma tumor cells by modulating signaling pathways (MAPK/ERK, PI3K/Akt, NF-κB, JAK/STAT, and TGF-β) and enzymes responsible for degrading the extracellular matrix, including matrix

Mitochondria produce adenosine triphosphate (ATP) through oxidative phosphorylation within cells. This metabolic process involves actively transporting positively charged protons across the inner mitochondrial membrane, creating a negative charge known as the mitochondrial transmembrane potential [50]. Dysfunction of the mitochondrial membrane potential in normal cells can induce undesired loss of cell viability and is involved in the onset of several diseases, including chronic respiratory diseases, neurodegenerative diseases, cardiovascular diseases, and cancer [51–54]. However, this effect is desirable for therapies related to the management and treatment of cancer. Recently, phenolic compounds have been studied for their ability to affect mitochondrial function in cancer cells, specifically by reducing the mitochondrial transmembrane potential [55,56]. Phenolic compounds can induce dysfunction in the mitochondrial transmembrane potential of cancer cells through multiple mechanisms, including induction of oxidative stress, inhibition of the electron transport chain, modulation of apoptotic proteins, and disruption of energy metabolism [57–59]. In this study, it was observed that the araça-boi extract caused a slight reduction in mitochondrial transmembrane potential, while trans-cinnamic acid was visibly more aggressive. These findings lead us to infer that the effect of the araçá-boi extract only at higher concentrations is because the extract is a complex mixture of phenolic compounds

metalloproteinases and integrins [65]. Furthermore, dietary polyphenols can act on epigenetic modulation and gene expression related to signaling pathways and key cellular events in cancer [13]. For example, Isacescu et al. [65] presented a comprehensive overview of polyphenol-regulated miRNAs and their modulatory impact on cellular processes involved in melanoma development, including cell growth arrest, apoptosis, epithelial-to-mesenchymal transition, proliferation, invasion, migration, metastasis, and tumor growth. The identification of natural compounds with antimetastatic activity is of great clinical relevance, especially for melanoma, a type of cancer known for its high metastatic rate and resistance to conventional treatments [66]. Thus, araça-boi extract may represent a promising candidate for the development of new antimetastatic therapies. However, additional studies are needed to fully elucidate the mechanisms of action and to evaluate the efficacy and safety of this extract and its compounds in animal studies and clinical trials.

(predominantly phenolic acids and glycosylated flavonoids) and non-phenolic compounds (e.g., sugars and organic acids). Consequently, the concentration of trans-cinnamic acid in the extract is diluted among the other phenolic compounds when compared to isolated trans-cinnamic acid, reducing its impact on the mitochondrial transmembrane potential.

3.2.3. Cell Migration of Melanoma Cells

Figure 3 shows melanoma cell migration (SK-MEL-28) treated for 24 h with araçá-boi extract and trans-cinnamic acid. There was a significant decrease in wound closure at all tested concentrations of araçá-boi extract and trans-cinnamic acid compared to the control group cells ($p < 0.0001$).

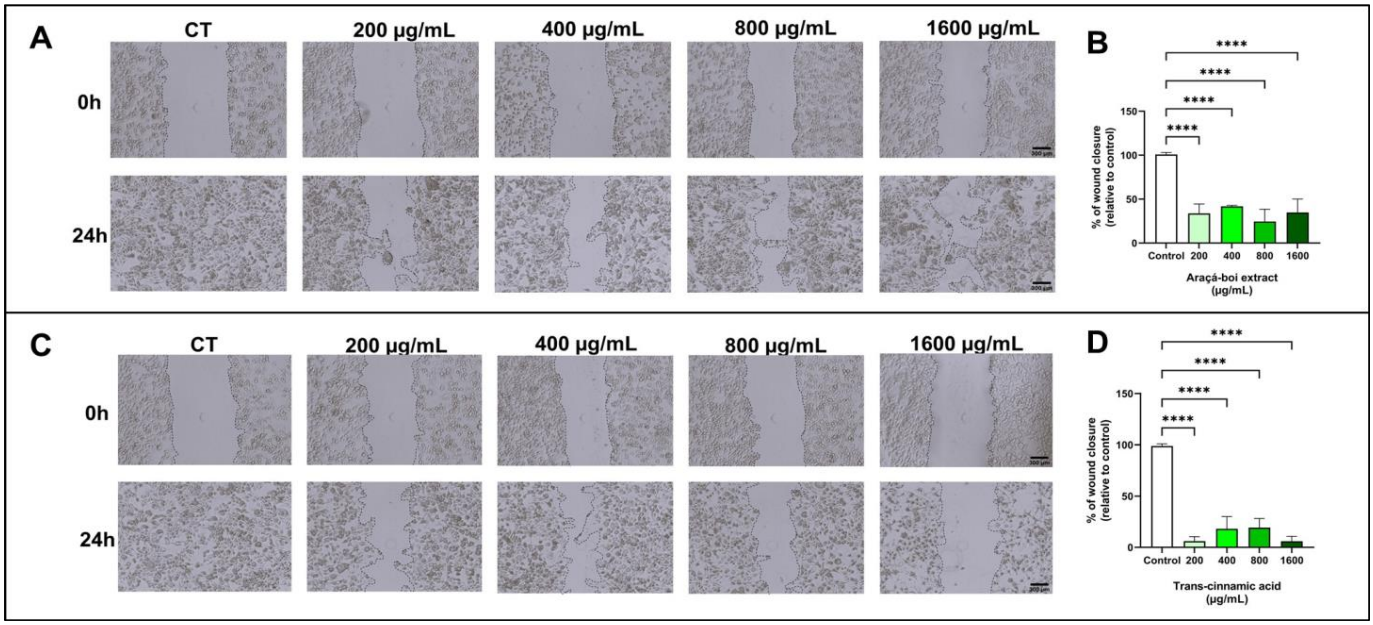

Figure 3. The migration of melanoma cells was evaluated by the wound-healing assay. After cell monolayer formation, a scratch was made, cells were treated with araçá-boi extract (**A**) and trans-cinnamic acid (**C**) for 24 h, and photomicrography was performed. Araçá-boi extract (**B**) and trans-cinnamic acid (**D**) significantly prevented wound closure in the range from 200 µg/mL to 1600 µg/mL. CT = control group cells. Black scale bar represents 300 µm. Microscope magnification = 40×. All experiments were performed independently three times and in triplicate. Data are presented as mean ± standard deviation. Statistical analysis: one-way ANOVA followed by a post hoc Dunnett's multiple comparisons test. Values with $p < 0.05$ were considered statistically significant. **** ($p < 0.0001$).

3.2.4. Detection of Cellular Reactive Oxygen Species (ROS)

The ROS detection assay is widely used to assess the presence of various reactive oxygen species (ROS) within cells, including hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($^{\bullet}O_2^-$), singlet oxygen ($^1O_2$), peroxyl radicals (ROO$^{\bullet}$), and hypochlorite (OCl$^-$). The assay uses a non-fluorescent marker (H$_2$DCF-DA) that is oxidized by ROS, resulting in the formation of DCF (2′,7′-dichlorofluorescein), which is highly fluorescent [67]. Figure 4 shows the ROS levels in cutaneous melanoma cells (SK-MEL-28) treated for 24 h with araçá-boi extract and trans-cinnamic acid. Araçá-boi extract significantly decreased ROS levels only at the highest concentration tested (1600 µg/mL) compared to the control group ($p < 0.01$) (Figure 4A). On the other hand, there was a significant decrease in ROS levels at all tested concentrations of trans-cinnamic acid compared to the control group cells ($p < 0.0001$) (Figure 4B).

Cutaneous melanoma is an aggressive skin tumor primarily characterized by its high metastatic potential and low survival rate [60]. Metastasis is a multifaceted biological process encompassing invasion, migration, and intravasation into the bloodstream, enabling tumor cells to disseminate and establish colonies in organs distant from the primary tumor site [61]. Recently, several studies have demonstrated that extracts from food matrices rich in phenolic compounds reduce cell migration in melanoma tumor cells [62–64]. The results of the present study demonstrated that araçá-boi extract, which is rich in phenolic compounds such as trans-cinnamic acid, induced a significant reduction in wound closure in melanoma cells at all tested concentrations. This finding suggests the potential antimetastatic activity of the araçá-boi extract, as cellular migration is a crucial step in the metastasis process. Additional experiments were performed, to determine the specific contribution of trans-cinnamic acid to the inhibition of cellular migration. When administered alone, trans-cinnamic acid resulted in a significant reduction in cellular migration. These data imply that trans-cinnamic acid may be one of the main contributors to the reduction

in melanoma cell migration observed in the araçá-boi extract. The phenolic compounds present in plant extracts can inhibit the migration of melanoma tumor cells by modulating signaling pathways (MAPK/ERK, PI3K/Akt, NF-κB, JAK/STAT, and TGF-β) and enzymes responsible for degrading the extracellular matrix, including matrix metalloproteinases and integrins [65]. Furthermore, dietary polyphenols can act on epigenetic modulation and gene expression related to signaling pathways and key cellular events in cancer [13]. For example, Isacescu et al. [65] presented a comprehensive overview of polyphenol-regulated miRNAs and their modulatory impact on cellular processes involved in melanoma development, including cell growth arrest, apoptosis, epithelial-to-mesenchymal transition, proliferation, invasion, migration, metastasis, and tumor growth. The identification of natural compounds with antimetastatic activity is of great clinical relevance, especially for melanoma, a type of cancer known for its high metastatic rate and resistance to conventional treatments [66]. Thus, araçá-boi extract may represent a promising candidate for the development of new antimetastatic therapies. However, additional studies are needed to fully elucidate the mechanisms of action and to evaluate the efficacy and safety of this extract and its compounds in animal studies and clinical trials.

3.2.4. Detection of Cellular Reactive Oxygen Species (ROS)

The ROS detection assay is widely used to assess the presence of various reactive oxygen species (ROS) within cells, including hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($^{\bullet}O_2^-$), singlet oxygen ($^1O_2$), peroxyl radicals ($ROO^{\bullet}$), and hypochlorite (OCl^-). The assay uses a non-fluorescent marker (H_2DCF-DA) that is oxidized by ROS, resulting in the formation of DCF (2′,7′-dichlorofluorescein), which is highly fluorescent [67]. Figure 4 shows the ROS levels in tumoral melanoma cells (SK-MEL-28) treated for 24 h with araçá-boi extract and trans-cinnamic acid. Araçá-boi extract significantly decreased ROS levels only at the highest concentration tested (1600 µg/mL) compared to the control group ($p < 0.01$) (Figure 4A). On the other hand, there was a significant decrease in ROS levels at all tested concentrations of trans-cinnamic acid compared to the control group cells ($p < 0.0001$) (Figure 4B).

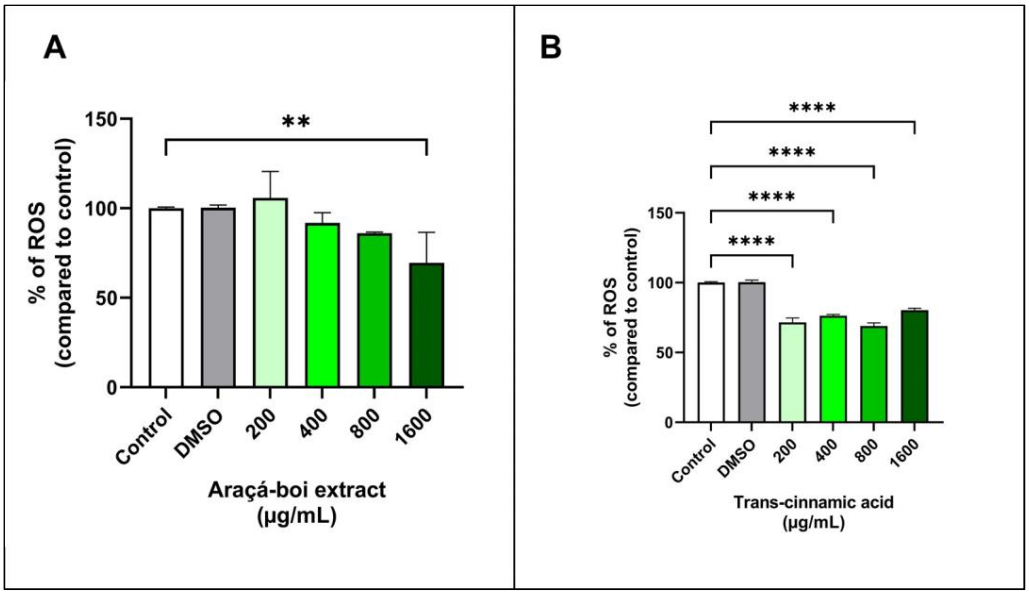

Figure 4. Detection of reactive oxygen species (ROS) levels in tumor melanoma cells treated with araçá-boi extract (**A**) and trans-cinnamic acid (**B**) after 24 h of treatment. CT = control group cells. All experiments were performed independently three times and in three replicates. Data are presented as mean ± standard deviation. Statistical analysis: one-way ANOVA followed by a post hoc Dunnett's multiple comparisons test. Values with $p < 0.05$ were considered statistically significant. ** ($p < 0.01$) and **** ($p < 0.0001$).

ROS, such as hydroxyl radicals, superoxide anions, and singlet oxygen, are inevitable byproducts of cellular metabolism and are essential for various physiological functions such as cell signaling, proliferation, and cell death [68]. Under normal conditions, cells maintain a redox balance in which the generation and elimination of ROS are balanced, thereby preventing oxidative damage. However, tumor cells often exhibit elevated levels

ROS, such as hydroxyl radicals, superoxide anions, and singlet oxygen, are inevitable byproducts of cellular metabolism and are essential for various physiological functions such as cell signaling, proliferation, and cell death [68]. Under normal conditions, cells maintain a redox balance in which the generation and elimination of ROS are balanced, thereby preventing oxidative damage. However, tumor cells often exhibit elevated levels of ROS, leading to an imbalance in redox homeostasis and oxidative stress. Oxidative stress can promote DNA mutations and damage biomacromolecules, contributing to uncontrolled cell growth and resistance to apoptosis [13,69,70]. Phenolic compounds, known for their antioxidant and pro-oxidant properties, play a crucial role in modulating stress [71]. At low concentrations, they act as antioxidants, protecting cells against oxidative damage and inhibiting tumor growth. At higher concentrations, they function as pro-oxidants, increasing the production of ROS, overwhelming the antioxidant defenses of tumor cells, and inducing cell death [13,56,71,72].

Our findings demonstrate that araçá-boi extract reduces ROS levels in melanoma tumor cells at the highest concentration. In contrast, trans-cinnamic acid markedly reduced ROS levels at all concentrations tested. These data suggest that high concentrations of araçá-boi extract are necessary to exert antioxidant effects in melanoma tumor cells, possibly due to the increased levels of trans-cinnamic acid present in the extract. Furthermore, other phenolic acids and flavonoids present in the extract may interact synergistically or antagonistically, influencing ROS levels. Phenolic compounds can reduce free radical levels and decrease oxidative stress via several mechanisms. They act directly as antioxidants and neutralize ROS such as hydrogen peroxide (H_2O_2) and free radicals such as superoxide anions ($^{\bullet}O_2^-$) and hydroxyl radicals ($^{\bullet}OH$) [73]. These phytochemicals can also act as a chelator of transition metals, such as iron and copper, which catalyze free radical formation via the Fenton cycleby binding to these metals, phenolic compounds prevent the formation of hydroxyl radicals and other free radicals [74,75]. Additionally, they can enhance the expression or activity of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which help neutralize free radicals and reduce oxidative stress [76,77]. Phenolic compounds can modulate cellular signaling pathways, contributing to protection against oxidative damage, and consequently, DNA damage, apoptosis control, cell-cycle regulation, senescence, and cell fate [78]. The reduction in ROS levels observed with araçá-boi extract and trans-cinnamic acid demonstrates that they could act as antioxidants in tumor cells and protect them against cellular damage and consequently maintain their viability. On the other hand, the reduction in oxidative stress can indirectly affect signaling pathways and potentially inhibit cell migration, as observed above. Thus, it was not possible to clarify the cellular signaling pathway that explains the exact mechanism by which the araçá-boi extract and trans-cinnamic acid exhibit an antitumor effect on melanoma cells. These results underscore the need for additional studies to fully elucidate the role of phenolic compounds present in the extract, the specific mechanisms involved in modulating ROS levels in melanoma cells, and their impact on tumor cell viability and migration.

3.2.5. Expression of Caspase-3 and NLRP3 in Melanoma Cells

Caspases and inflammasomes play critical roles in regulating apoptosis and the inflammatory response in tumor cells, including caspase-3 and NLRP3 inflammasome [79]. Figure 5 presents the effect of araçá-boi extract and trans-cinnamic acid on caspase-3 and NLRP3 expression in melanoma tumor cells after 24 h of treatment. Araçá-boi extract did not show a significant difference in caspase-3 expression compared to the control group cells ($p > 0.05$) (Figure 5A). However, an increase in NLRP3 expression was observed at the highest concentration tested (1600 µg/mL) compared to the control group ($p < 0.01$) (Figure 5A). Trans-cinnamic acid at concentrations of 200 and 1600 µg/mL showed a significant increase in caspase-3 expression ($p < 0.0001$) (Figure 5B). Conversely, trans-cinnamic acid strongly reduces NLRP3 expression at concentrations of 200, 400, and 1600 µg/mL ($p < 0.05$) (Figure 5B).

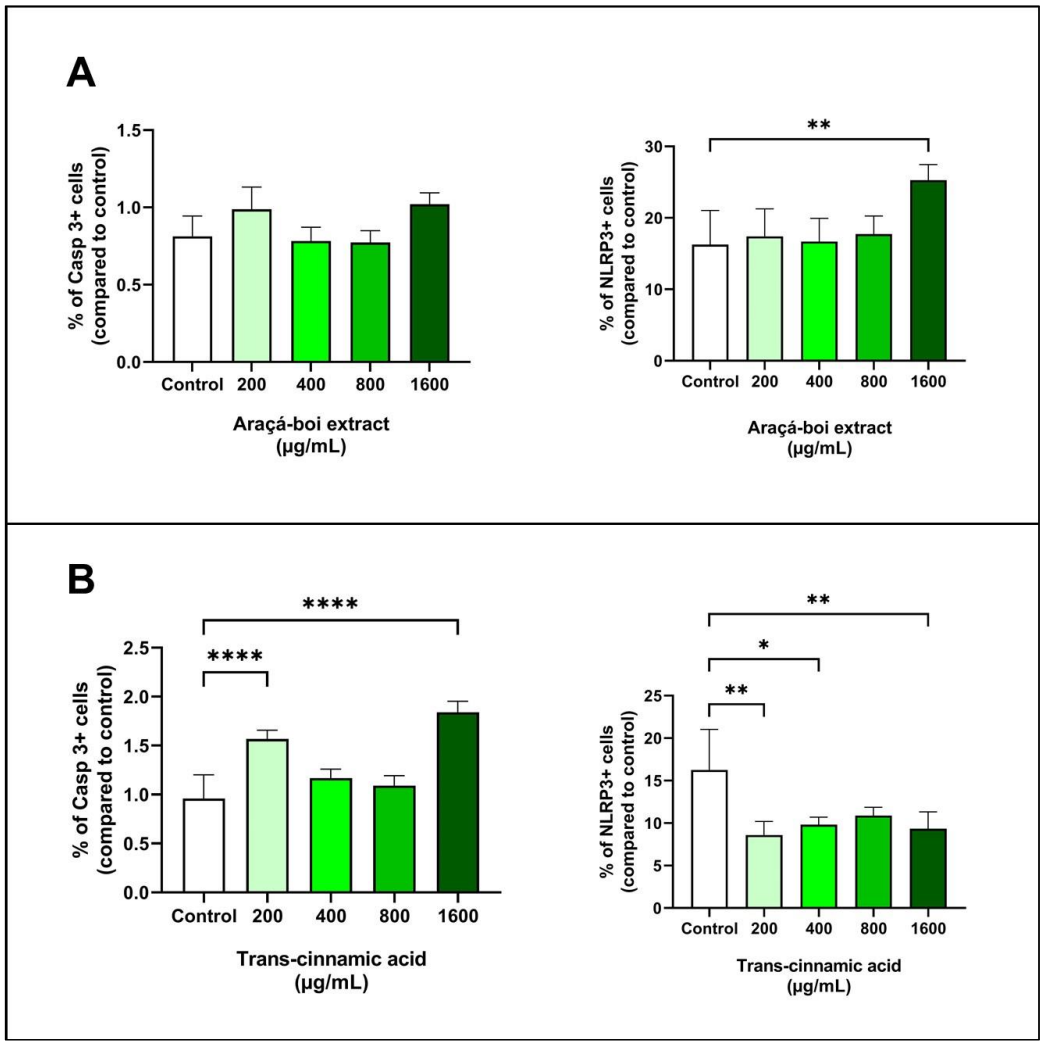

Figure 5. Expression of caspase-3 and NLRP3 in melanoma cells treated with araçá-boi extract (**A**) and trans-cinnamic acid (**B**). CT = control group cells. All experiments were performed independently three times and in three replicates. Data are presented as mean ± standard deviation. Statistical analysis: one-way ANOVA followed by a post hoc Dunnett's multiple comparisons test. Values with $p < 0.05$ were considered statistically significant. * ($p < 0.05$), ** ($p < 0.01$), and **** ($p < 0.0001$).

Caspase-3 is a crucial protease in the execution of apoptosis in tumor cells, and its increased expression is associated with greater activation of apoptotic pathways, which can lead to programmed cell death [79,80]. The activation of caspase-3 is a common endpoint of both the intrinsic and extrinsic apoptotic pathways. The intrinsic pathway is regulated by internal signals from the cell and is often mediated by the release of cytochrome c from the mitochondria and subsequent formation of the apoptosome. The extrinsic pathway is activated by external ligands, such as the FAS and TRAIL ligands, which trigger the activation of executioner caspases-3, leading to cell death [80]. Plant phenolic-rich extracts and isolated phenolic acids, such as rosmarinic acid and curcumin, have been associated with an increase in caspase-3 expression in melanoma tumor cells due to their ability to modulate apoptosis-related cell signaling pathways. These compounds can directly activate apoptotic pathways or influence the expression of pro-apoptotic proteins, leading to the activation of caspase-3 and the subsequent induction of apoptosis [55,56,81]. In our study, we did not observe a significant increase in caspase-3 expression in melanoma tumor cells treated with araçá-boi extract, although this modulation occurred with trans-cinnamic acid at certain concentrations. Even though the extract contains trans-cinnamic acid as a major compound, the presence of other phenolic acids and flavonoids may interfere with the expression. Despite the extract containing trans-cinnamic acid, several other phenolic acids and flavonoids are present, and thus, the extract's activity may be influenced by interactions among these compounds. As mentioned above, phenolic compounds may exhibit

The NLRP3 inflammasome (NOD-like receptor pyrin domain-containing 3) is a protein complex involved in the regulation of inflammation and immune response, particularly through its role in the formation of inflammasomes. These inflammasomes, when activated, can lead to the maturation and release of pro-inflammatory cytokines like IL-1β and IL-18, contributing to innate immunity and an inflammation-related mode of the programmed cell death process called pyroptosis [82]. Additionally, NLRP3 is indirectly associated with apoptotic pathways. Both apoptotic initiator caspases (such as caspase-9) and executioner caspases (such as caspase-3, 6, and 7) are important for the activation of the NLRP3 inflammasome [83]. Because it can be activated by multiple signals, NLRP3 inflammasomes play an important role in the development of a variety of tumors [84,85]. Several phenolic compounds from different natural sources and medicinal plants have been reported to target NLRP3 and exert beneficial effects against NLRP3 inflammasome-related diseases, including cancer [86,87]. In our study, araçá-boi extract increased NLRP3 expression at the highest concentration tested, whereas trans-cinnamic acid reduced its expression. Despite the extract containing trans-cinnamic acid, several other phenolic acids and flavonoids are present, and thus, the extract's activity may be influenced by interactions among these compounds. As mentioned above, phenolic compounds may exhibit

with its ability to induce caspase-3 expression. Moreover, the specific concentrations of these compounds in the extract may not be sufficient to activate the apoptotic pathways responsible for caspase-3 expression. On the other hand, a high concentration of isolated trans-cinnamic acid might favor its interaction with apoptotic pathways, suggesting a potential induction of apoptosis. Thus, the complexity of the extract's composition and the interactions between different phenolic compounds and flavonoids may explain why the araçá-boi extract did not modulate caspase-3 expression compared to the control, while trans-cinnamic acid showed positive effects.

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4. Conclusions

For the first time, we investigated the effects of an extract obtained from araçá-boi fruit on SK-MEL-28 melanoma cells regarding their viability, cell migration, oxidative stress, and expression of proteins related to apoptosis and inflammation. The HPLC-DAD analysis of araçá-boi extract identified and quantified eleven phenolic compounds, comprising seven phenolic acids and four flavonoids. Phenolic acids represented approximately 56%, while flavonoids accounted for 44% of the total phenolic compounds. Trans-cinnamic acid was the main phenolic compound identified in the araça-boi extract and, therefore, was used alone to verify its contribution to biological activities. Both araçá-boi extract and trans-cinnamic acid treatment significantly reduced cell viability and inhibited cell migration. These results are a promising outcome, indicating potential inhibition of the progression, invasion, and metastasis of melanoma cells by the araçá-boi extract and its main phenolic compound. These findings suggest that the action of the araçá-boi extract may be attributable to the presence of trans-cinnamic acid.

The antioxidant activity of araçá-boi extract and trans-cinnamic acid can be attributed to the reduction in ROS levels. Regarding the evaluation of proteins related to apoptosis and inflammation, the extract did not modulate caspase-3 expression but increased NLRP3 expression. In contrast, trans-cinnamic acid increased caspase-3 expression and reduced NLRP3 expression. This indicates that trans-cinnamic acid can suppress melanoma through pro-apoptotic and anti-inflammatory pathways.

This discovery indicates that araçá-boi extract and trans-cinnamic acid possess antiproliferative, anti-migration, and antioxidant activities and the ability to modulate protein expression related to apoptosis and inflammation in melanoma cells. Although trans-cinnamic acid was identified as a key component of the araçá-boi extract, the results indicate that other phenolic compounds and non-phenolics from the araçá-boi extract may also contribute to the observed effects, particularly in the specific modulation of analyzed proteins.

Thus, the ability of trans-cinnamic acid to reduce oxidative stress and stimulate anti-migratory, pro-apoptotic, and anti-inflammatory effects may be a complementary tool or promising agent for the prevention and management of melanoma. Despite some positive effects of araçá-boi extract (e.g., reduced cell viability and anti-migratory activity) in the management of melanoma, the underlying mechanisms still need to be elucidated. Further studies to understand the specific molecular mechanisms and validate these effects in in vivo models are crucial for advancing research in this area.

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GENERAL DISCUSSION

The plant material chosen for the development of this thesis was the edible fraction (pulp and peel) of araçá-boi (*Eugenia stipitata*), a fruit native to the Amazon region and belonging to the Myrtaceae family. It is known for its acidic flavor and characteristic aroma, as well as being rich in phenolic compounds, such as phenolic acids and flavonoids. These compounds provide the fruit with antioxidant properties and potential health benefits. Despite its high nutritional and functional potential, araçá-boi remains underexplored in terms of industrial and scientific applications, representing a valuable opportunity for the development of innovative products and the promotion of Amazonian biodiversity. Our research group had previously investigated this raw material regarding its physicochemical characterization and behavior during the gastrointestinal digestion process. Additionally, our group evaluated the impact of its phenolic compounds on antioxidant and antitumor activities. However, a more in-depth phytochemical characterization and identification of phenolic compounds, as well as an understanding of the potential epigenetic and molecular mechanisms related to the effects of these compounds on tumor cells, had not yet been explored. Therefore, this study aimed to better understand the antioxidant and antitumor effects of araçá-boi and to propose new perspectives for its use in the prevention and treatment of diseases.

In this regard, **Chapter 1** aimed to present the latest research on plant-derived polyphenols as antioxidants and their interaction with the microbiota, leading to the modulation of epigenetic mechanisms, with a focus on managing NCDs, including cancer. Throughout the research, it became clear that while many recent findings are promising, caution is necessary when considering polyphenols as effective therapeutic agents for certain NCDs, as research into the underlying mechanisms of their protective effects is still in its early stages. Additionally, there remain numerous unanswered questions regarding the role of polyphenols in targeting diverse epigenetic landscapes, their associated parameters, as well as the signaling pathways and physiological barriers related to NCDs. Therefore, we have merely scratched the

surface of what is possible to identify potential adjunctive therapies more efficiently, safely, and cost-effectively.

The second literature review presented in **Chapter 2** aimed to explore the current applications of omics science in ovarian cancer research, with a focus on the impact of dietary polyphenols and their mechanisms in the disease. We found that omics technologies offer valuable insights for identifying biomarkers that assist in diagnosis, prognosis, and the selection of targeted therapies for personalized treatments. Furthermore, these methodologies enhance our understanding of how polyphenols influence the cell cycle, act as mediators of gene expression and suppression, serve as epigenetic regulators, and function as substrates for gut microbiota, all of which contribute to the prevention and treatment of ovarian cancer. Thus, based on the two reviews conducted in this thesis, the potential of polyphenols in health is highlighted, especially in non-communicable chronic diseases and ovarian cancer, emphasizing their role as antioxidants, epigenetic regulators, and molecular mediators. However, their mechanisms remain poorly understood, with gaps in molecular signaling, physiological barriers, and epigenetic factors. Further research is crucial to confirm their therapeutic efficacy and safety.

In the first experimental study presented in **Chapter 3,** we evaluated the araçá-boi extract and gallic acid for their antitumor activity against ovarian cancer. From the edible fraction of araçá-boi (pulp and peel), we obtained a phenolic-rich extract characterized by UHPLC-Q-Orbitrap-MS/MS. A total of 73 compounds were identified in this extract, including ten organic acids, thirty-six phenolic acids (such as gallic, vanillic, caffeic, coumaric, and ellagic acids, among others), and twenty-seven flavonoids, primarily glycosylated forms of myricetin, quercetin, and kaempferol. Antioxidant analyses demonstrated a strong antioxidant potential, effectively neutralizing both synthetic radicals and reactive oxygen species. For the cellular and molecular assays, gallic acid, one of the phenolic compounds identified in the extract, was used individually to assess its specific contribution. In cytotoxicity assays, we observed that the extract was non-toxic to normal Chinese hamster ovary

cells (CHO-K1). However, gallic acid exhibited toxicity at certain concentrations and exposure times. For ovarian tumor cells, both the araçá-boi extract and gallic acid significantly reduced cell viability in the NCI/ADR-RES cell line, whereas OVCAR-3 cells showed greater resistance to these treatments. Subsequent analyses were performed to evaluate the expression of tumor suppressor genes and genes involved in epigenetic processes and the DNA methylation status of the *BRCA1* gene promoter in the NCI/ADR-RES cell line. We observed that the araçá-boi extract upregulated genes such as *BRCA1* and *RASSF1*, while gallic acid upregulated *BRCA1* and *CDKN2A*. Furthermore, no changes in the methylation status of the *BRCA1* gene promoter region were detected in NCI/ADR-RES ovarian tumor cells. The impact on gene expression and cell viability, without changes in the *BRCA1* promoter DNA methylation, suggests that the reversal of the tumor phenotype may involve other molecular mechanisms, such as oxidative stress induction or apoptosis, and complex epigenetic effects beyond demethylation. These could include post-translational modifications and regulation of other enzymes involved in transcriptional control and cellular signaling pathways. Furthermore, the results suggest that other phenolic compounds in the extract may synergistically modulate various molecular pathways to decrease cell viability in ovarian cancer cells.

The second study in **Chapter 4** explored the impact of gastrointestinal digestion on the recovery, bioaccessibility of phytochemicals, and antioxidant activity of araçá-boi extract. Furthermore, an *in silico* analysis was performed to evaluate the interactions of the major phenolic compound with inflammatory proteins. Total phenolic compounds were significantly degraded during digestion, as shown by spectrophotometric analyses. Antioxidant activity varied depending on the method: ABTS showed a 113% increase, while DPPH dropped to 9%, highlighting differences in the mechanisms involved. UHPLC-Q-Orbitrap-MS/MS identified 100 compounds in the extract, including organic acids, nucleotides, sugars, phenolic acids, and 31 flavonoids. After gastric digestion, 92 compounds remained, but only 59 were detected in the intestinal fraction, including 15 newly derived phenolic acids

and one flavonoid. This indicates that the intestinal environment plays a crucial role in modulating the phytochemical profile, promoting the degradation of some compounds and the emergence of others. A validated quantification method confirmed significant changes in phenolic compounds during digestion. Trans-cinnamic acid showed an 813% increase in bioaccessibility, followed by p-coumaric acid (232%) and quercetin (106%). Sugar analysis revealed a mix of mono-, disaccharides, and oligosaccharides, with sucrose being predominant. Despite digestion reducing sugar levels, 50–65% of the initial content remained in the intestinal fraction, demonstrating stability. Finally, in silico molecular docking and pharmacokinetic studies of trans-cinnamic acid revealed strong interactions with NF-κB, IL-1β, and PI3K, suggesting anti-inflammatory potential. Pharmacokinetic analysis highlighted favorable solubility, absorption, and distribution, along with low toxicity, reinforcing its therapeutic potential.

Chapter 5 focused on evaluating the phenolic content of araçá-boi and exploring its antitumoral effects in human metastatic melanoma cells. For this, araçá-boi pulp was extracted, and phenolic compounds were identified via HPLC-DAD. A total of eleven phenolic compounds were detected in the araçá-boi extract (trans-cinnamic acid, syringic acid, gallic acid, ferulic acid, p-coumaric acid, 4-hydroxybenzoic acid, gentisic acid, quercetin-3-O-rhamnoside, quercetin-3-O-galactoside, kaempferol-3-O-glucoside, and hesperetin). Melanoma cells were exposed to different concentrations of araçá-boi extract and trans-cinnamic acid for 24 hours. The results showed that both the extract and trans-cinnamic acid significantly reduced cell viability, migration, and oxidative stress. However, while the extract did not affect the expression of apoptosis-related (caspase-3) and inflammation-related (NLRP3) proteins, trans-cinnamic acid modulated these proteins, demonstrating pro-apoptotic and anti-inflammatory effects. These findings indicate that the extract and trans-cinnamic acid reduce cell viability and migration through distinct signaling pathways. Further research is needed to clarify the specific molecular mechanisms and advance this promising field.

GENERAL CONCLUSION

This study investigated the bioactive potential of araçá-boi extract (Eugenia stipitata Mac Vaugh), focusing on the role of its phenolic compounds in cancer prevention and treatment. Through an interdisciplinary approach integrating chemical, biological, and computational analyses, significant progress was made in understanding the antioxidant and antitumor properties of this underexplored Amazonian fruit. The literature reviews presented in **Chapters 1 and 2** explored the potential of polyphenols in managing non-communicable chronic diseases (NCDs), including cancer, offering complementary insights essential for the experimental studies. **Chapter 1** highlighted the antioxidant and epigenetic regulatory roles of polyphenols in NCDs through interactions with the microbiota and epigenetic modulation. **Chapter 2**, in turn, examined the mechanistic effects of polyphenols on ovarian cancer using multi-omics approaches. Together, these studies reinforce the potential of polyphenols as molecular mediators and epigenetic regulators, forming a theoretical foundation for experimental studies.

Thereby, the experimental study in **Chapter 3**, investigates the antitumor potential of araçá-boi extract and gallic acid against ovarian cancer. A phenolic-rich extract revealed 73 compounds, including organic acids, phenolic acids, and flavonoids. The extract exhibited strong antioxidant properties, effectively neutralizing reactive oxygen species. While the extract showed selective cytotoxicity—non-toxic to normal CHO-K1 cells and significantly reducing the viability of NCI/ADR-RES tumor cells—gallic acid exhibited cytotoxicity under certain conditions. Molecular assays revealed upregulation of tumor suppressor genes, such as *BRCA1* and *RASSF1* for the extract, and *BRCA1* and *CDKN2A* for gallic acid. However, no changes in *BRCA1* promoter methylation were observed, suggesting that the antitumor effects involve oxidative stress, apoptosis, and complex epigenetic mechanisms beyond DNA demethylation. These findings highlight the therapeutic promise of araçá-boi, emphasizing the synergistic role of its phenolic compounds in modulating molecular pathways to combat ovarian cancer.

In **Chapter 4**, the study explores how simulated gastrointestinal digestion affects the recovery, bioaccessibility, and antioxidant activity of phytochemicals from araçá-boi extract. Through UHPLC-Q-Orbitrap-MS/MS, 100 compounds were detected in the extract, and after digestion, 92 compounds remained, with 59 detected in the intestinal fraction. Significant increases in bioaccessibility were noted for trans-cinnamic acid (813%), p-coumaric acid (232%), and quercetin (106%), suggesting the digestive process enhances their availability. In silico docking studies showed that trans-cinnamic acid interacts with NF-κB, IL-1β, and PI3K, indicating anti-inflammatory potential. Pharmacokinetic analysis indicated favorable solubility, absorption, and low toxicity, reinforcing its therapeutic potential. This study provides insights into how digestion affects the bioaccessibility and biological activity of phytochemicals, with implications for their therapeutic applications.

Finally, in **Chapter 5**, the study explores the antitumor effects of araçá-boi extract and trans-cinnamic acid on human metastatic melanoma cells. We quantified seven phenolic acids and four flavonoids, with trans-cinnamic acid identified as the major compound, followed by other phenolics such as quercetin-3-O-galactoside and syringic acid. The study demonstrated that both treatments (extract and trans-cinnamic acid) exhibit antitumor activity in melanoma cells by reducing cell viability, metastasis, and oxidative stress. Further studies are necessary to fully understand the molecular mechanisms behind these effects.

The study draws attention to the health benefits of araçá-boi, an underutilized Amazonian fruit, contributing to the growing understanding of biodiversity's role in developing sustainable therapies. The findings not only underscore araçá-boi's promise in cancer treatment but also align with global efforts to integrate traditional knowledge and bioprospecting into modern medicine, fostering sustainable approaches to cancer.

FUTURE PERSPECTIVES

Based on the findings of this research, subsequent investigations may focus on diverse strategies to enhance our understanding of the phytochemicals and health benefits of araçá-boi:

- A study focused on optimizing the extraction process to increase both yield and efficiency;
- Evaluating the effects of the extract on other types of cancer to understand its therapeutic versatility;
- Investigating the potential of the extract as an adjunct in conventional cancer treatments, assessing possible synergies or reduction of side effects;
- Comprehensive research exploring how phenolic compounds influence DNA methylation, histone alterations, and non-coding RNA activity, aiming to clarify their functions within specific epigenetic pathways;
- Expanding experimental models to include in vivo studies, enabling validation of therapeutic efficacy in more complex and relevant systems;
- Assessing the stability of araçá-boi extract and its viability as an ingredient for functional products, such as foods or nutraceuticals;
- Understanding how phenolic compounds interact within the extract and investigating possible synergies that may enhance the observed biological activities;
- Developing studies to evaluate the feasibility of incorporating araçá-boi extract into personalized nutrition approaches.

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APPENDICES

Appendix I – Declaration regarding access to the Brazilian genetic heritage

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A1A1D38

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A1A1D38**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**
Espécie	
Eugenia stipitata	
Título da Atividade:	**EFEITO DO CONSUMO DE ARAÇÁ-BOI (Eugenia stipitata Mac Vaugh - Myrtaceae) NA PREVENÇÃO DO CÂNCER DE OVÁRIO: O PAPEL EPIGENÉTICO DOS POLIFENÓIS ALIMENTARES**

Equipe	
Felipe Tecchio Borsoi	**UNICAMP**
Glaucia Maria Pastore	**UNICAMP**
Iramaia Angélica Neri-Numa	**UNICAMP**
WIlliara Queiroz de Oliveira	**UNICAMP**
Fábio Fernandes de Araújo	**UNICAMP**

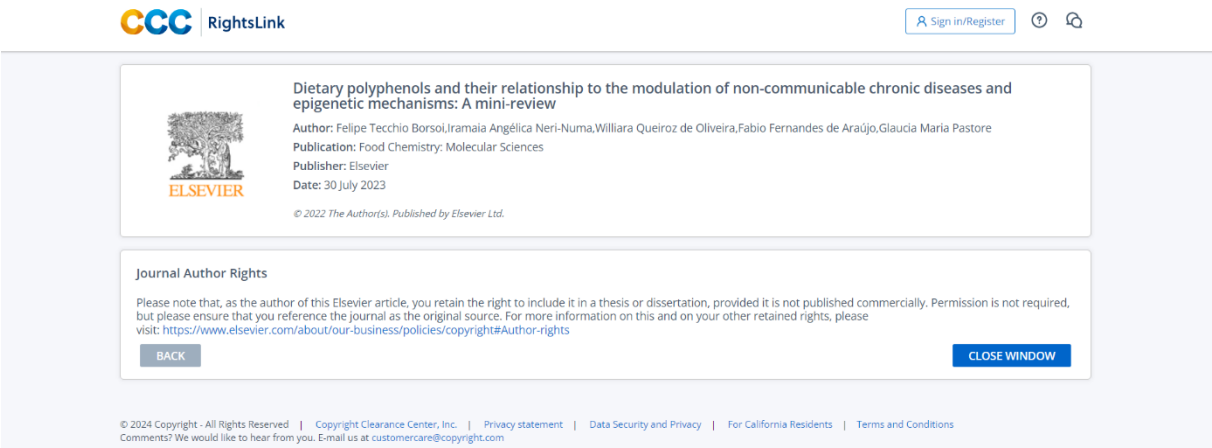
Data do Cadastro:	**19/09/2022 16:43:45**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **16:31** de **28/09/2022**.

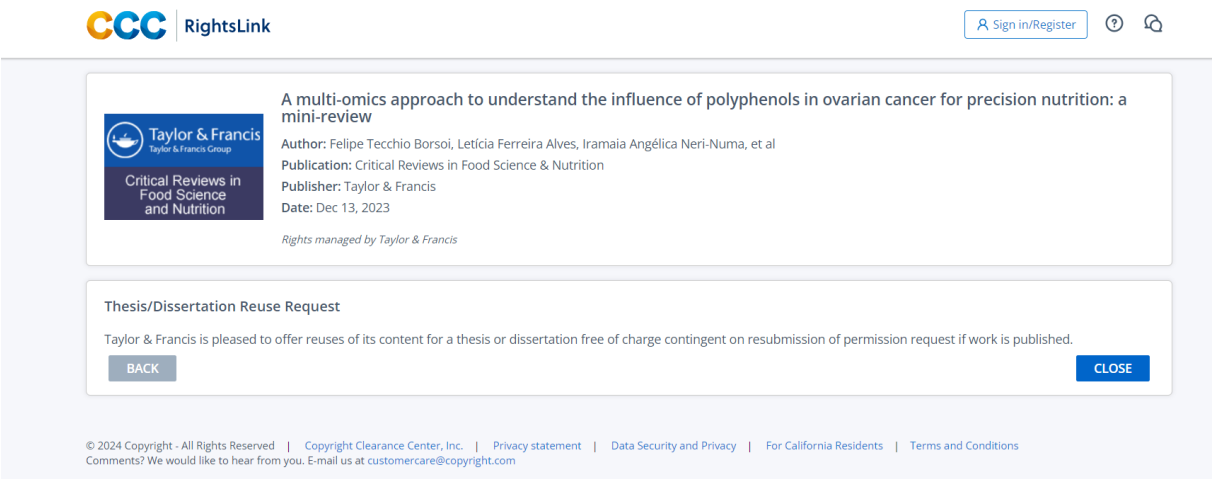
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

Appendix II - Publishers' authorization

✓ Dietary polyphenols and their relationship to the modulation of non-communicable chronic diseases and epigenetic mechanisms: A mini-review



✓ A multi-omics approach to understand the influence of polyphenols in ovarian cancer for precision nutrition: a mini-review



✓ Extract of araçá-boi and its major phenolic compound, trans-cinnamic acid, reduce viability and inhibit migration of human metastatic melanoma cells

Appendix III – Other publications authored during the Ph.D period

- ✓ Health benefits of the alkaloids from lobeira (*solanum lycocarpum* st. hill): a comprehensive review

Open Access Review

Health Benefits of the Alkaloids from Lobeira (*Solanum lycocarpum* St. Hill): A Comprehensive Review

by **Felipe Tecchio Borsoi**, **Glaucia Maria Pastore** and **Henrique Silvano Arruda** *

Departamento de Ciência de Alimentos e Nutrição, Faculdade de Engenharia de Alimentos (FEA), Universidade Estadual de Campinas (UNICAMP), Rua Monteiro Lobato nº 80, Campinas 13083-862, São Paulo, Brazil

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Plants **2024**, *13*(10), 1396; **https://doi.org/10.3390/plants13101396**

Submission received: 23 April 2024 / Revised: 14 May 2024 / Accepted: 15 May 2024 / Published: 17 May 2024

(This article belongs to the Special Issue **Alkaloids: Chemical Structures with Pharmaceutical Potential**)

- ✓ Essential oils from native brazilian plants of the genus *Eugenia* as an innovative and sustainable source of active ingredients for food systems and human health and well-being

Open Access Review

Essential Oils from Native Brazilian Plants of the Genus *Eugenia* as an Innovative and Sustainable Source of Active Ingredients for Food Systems and Human Health and Well-Being

by **Felipe Tecchio Borsoi** [1], **Arícia Possas** [2], **Glaucia Maria Pastore** [1] and **Henrique Silvano Arruda** [1,*]

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Horticulturae **2024**, *10*(7), 768; **https://doi.org/10.3390/horticulturae10070768**

Submission received: 28 May 2024 / Revised: 15 July 2024 / Accepted: 18 July 2024 / Published: 19 July 2024

(This article belongs to the Special Issue **Medicinal and Aromatic Plants: Isolation, Characterization and Biological Activities**)

Appendix IV – Publications performed in partnership during the Ph.D. period

✓ Scientific advances in the last decade on the recovery, characterization, and functionality of bioactive compounds from the araticum fruit (*Annona crassiflora* Mart.)

Open Access Review

Scientific Advances in the Last Decade on the Recovery, Characterization, and Functionality of Bioactive Compounds from the Araticum Fruit (*Annona crassiflora* Mart.)

by **Henrique Silvano Arruda** [1,2,*], **Felipe Tecchio Borsoi** [1], **Amanda Cristina Andrade** [1], **Glaucia Maria Pastore** [1] and **Mario Roberto Marostica Junior** [2,*]

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Plants **2023**, *12*(7), 1536; **https://doi.org/10.3390/plants12071536**

Submission received: 10 March 2023 / Revised: 29 March 2023 / Accepted: 31 March 2023 / Published: 3 April 2023

(This article belongs to the Special Issue **Recovery, Characterization, Functionality and Applications of Bioactive Compounds from Food-Plant Products and Their By-Products**)

✓ Evaluating the galactooligosaccharide stability in chocolate milk beverage submitted to ohmic heating

Food Research International

Volume 188, July 2024, 114429

Evaluating the galactooligosaccharide stability in chocolate milk beverage submitted to ohmic heating

Ramon Silva [a b], Ramon S. Rocha [a e], Marcus Vinicius S. Ferreira [c], Gustavo L.P.A. Ramos [b], Henrique S. Arruda [d], Felipe T. Borsoi [d], Glaucia Maria Pastore [d], Monica Q. Freitas [b], Adriano G. Cruz [a]

✓ Multilayer microparticles for programmed sequential release of phenolic compounds from *Eugenia stipitata*: stability and bioavailability

ELSEVIER

Food Chemistry
Volume 443, 15 June 2024, 138579

Multilayer microparticles for programmed sequential release of phenolic compounds from *Eugenia stipitata*: Stability and bioavailability

Williara Queiroz de Oliveira [a], Iramaia Angélica Neri Numa [a], Izabela D. Alvim [b], Henriette M.C. Azeredo [c], Leticia B. Santos [c d], Felipe T. Borsoi [a], Fábio F. de Araújo [a e], Alexandra C.H.F. Sawaya [e], Gustavo C. do Nascimento [f], Maria Teresa P.S. Clerici [f], Célio K. do Sacramento [g], Glaucia Maria Pastore [a]

✓ *Eugenia calycina* and *Eugenia stigmatosa* as promising sources of antioxidant phenolic compounds

Open Access Article

Eugenia calycina and *Eugenia stigmatosa* as Promising Sources of Antioxidant Phenolic Compounds

by **Juliana Dara Rabêlo Silva** [1,*], **Henrique Silvano Arruda** [1], **Amanda Cristina Andrade** [1], **Patrícia Berilli** [1], **Felipe Tecchio Borsoi** [1], **Yaneth Machaca Monroy** [2], **Marili Villa Nova Rodrigues** [3], **Klicia Araujo Sampaio** [2], **Glaucia Maria Pastore** [1] and **Mario Roberto Marostica Junior** [1,*]

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Plants **2024**, *13*(15), 2039; **https://doi.org/10.3390/plants13152039**

Submission received: 1 July 2024 / Revised: 19 July 2024 / Accepted: 23 July 2024 / Published: 24 July 2024

(This article belongs to the Special Issue **Chemical Characterizations and Biological Activities of Plant Products and By-Products and Their Applications**)

✓ *Passiflora nitida* Kunth fruit: Chemical analysis, antioxidant capacity, and cytotoxicity

Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00258

Passiflora nitida Kunth fruit: Chemical analysis, antioxidant capacity, and cytotoxicity

Zilanir Carvalho PEREIRA[1], Josias Martins dos Anjos CRUZ[1], Débora Raquel Gomes CASTRO[1], Andrezza Silva RAMOS[1], Daniel de Queiroz ROCHA[1], Henrique Silvano ARRUDA[2,3], Felipe Tecchio BORSOI[3], Iramaia Angelica NERI-NUMA[3], Glaucia Maria PASTORE[3], Edgar Aparecido SANCHES[4], Pedro Henrique CAMPELO[5], Jerusa Araújo Quintão Arantes FARIA[6], Lyege Magalhães OLIVEIRA[1], Jaqueline de Araújo BEZERRA[1*]

✓ Optimization of Ultrasonic-Assisted Extraction of Phenolic Compounds and Antioxidant Activity from Araticum Peel Using Response Surface Methodology

Optimization of Ultrasonic-Assisted Extraction of Phenolic Compounds and Antioxidant Activity from Araticum Peel Using Response Surface Methodology

by **Amanda Cristina Andrade** [1], **Felipe Tecchio Borsoi** [1], **Ana Sofia Martelli Chaib Saliba** [2], **Severino Matias de Alencar** [2], **Glaucia Maria Pastore** [1] and **Henrique Silvano Arruda** [1,*]

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Plants **2024**, *13*(18), 2560; **https://doi.org/10.3390/plants13182560**

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✓ Microencapsulation of anthocyanin-rich extract of grumixama fruits (*Eugenia brasiliensis*) using non-conventional wall materials and *in vitro* gastrointestinal digestion

ELSEVIER

Journal of Food Engineering
Volume 389, March 2025, 112393

Microencapsulation of anthocyanin-rich extract of grumixama fruits (*Eugenia brasiliensis*) using non-conventional wall materials and *in vitro* gastrointestinal digestion

Elivaldo Nunes Modesto Junior [a], Rosane Patricia Ferreira Chaves [a], Henrique Silvano Arruda [c d], Felipe Tecchio Borsoi [d], Glaucia Maria Pastore [d], Gustavo Araujo Pereira [a b], Renan Campos Chisté [a e], Rosinelson da Silva Pena [a b]