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Antioxidant, Antiproliferative, Antibacterial, and Antimalarial Effects of Phenolic-Rich Green Tea Kombucha

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Abstract: Green tea kombucha, produced using a green tea (*Camellia sinensis*) grown in Brazil, was characterized and its in vitro bioactive properties were evaluated. Overall, 92 phenolic compounds were identified (70.7% flavonoids, 25% phenolic acids, 2.2% lignans, and 1.1% other polyphenols), contributing to the observed high antioxidant capacity. The major phenolics identified were gallocatechin, catechin 5-O-gallate, and epicatechin. Green tea kombucha exhibited antibacterial activity against all tested bacteria, being more effective against *Salmonella* spp. In addition, green tea kombucha demonstrated antimalarial activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*, and antiproliferative activity against cancer cell lines A549, HCT8, HepG2, and HUVEC. Additionally, it presented antioxidant properties by effectively reducing the generation of reactive oxygen species (ROS) and provided protection to erythrocytes against AAPH-induced oxidative stress. Thus, green tea kombucha is abundant in antioxidants and possesses intriguing bioactive properties that can be investigated by both the food and pharmaceutical sectors.

Keywords: green tea; kombucha; bioactive compounds; biological activity; phenolic profile; UPLC-MS^E

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1. Introduction

Kombucha is a beverage obtained from the fermentation of green or black tea from the *Camellia sinensis* plant, in which a symbiotic consortium of bacteria and yeast, known as SCOBY, is inoculated [1,2]. According to [3], an annual growth in kombucha consumption of over 15% is expected between 2022 and 2030; it is estimated that the kombucha market

will reach $4.26 billion in 2028. This market growth is associated with an increased public concern for healthier lifestyles and a search for food and beverages with bioactive properties, especially post-COVID-19, as seen in the case of kombucha [4]. In Brazil, kombucha consumption is also increasing, leading to the creation of Normative Instruction 41 in 2019, which establishes the identity and quality standards for kombucha produced in the country [5].

The bioactive characteristics of kombucha stem from its rich chemical and microbiological composition, which varies depending on factors such as the type of tea used, fermentation conditions (e.g., time and temperature), and microorganisms present in the SCOBY [1,6]. Kombucha contains organic acids such as acetic, lactic, gluconic, and glucuronic acids, various vitamins, and phenolic compounds, known for their antioxidant potential and free radical scavenging abilities [1,7–9]. Phenolic compounds found in green tea kombucha include catechins, epigallocatechin, various isomers, quercetin, and phenolic acids, among others, derived from tea and the fermentation process, which undergo numerous biotransformations, leading to a diversified phenolic profile compared to the original tea [7,8].

Moreover, there are studies indicating the bioactive properties of kombucha, due to its composition rich in phenolics, such as anticancer and antimalarial properties, an improvement in glucose metabolism, oxidative stress reduction, the potential to reduce dyslipidemia, the modulation of intestinal microbiota, etc., [7,10–12]. The antiproliferative potential of green tea kombucha has been evaluated against lung and colorectal cancers, yielding satisfactory results [7]. The black tea kombucha demonstrated antimalarial activity against strains of both chloroquine-sensitive and chloroquine-resistant Plasmodium falciparum at various stages of *Plasmodium* development [8].

While research on kombucha has seen a significant increase in recent years, there are no studies evaluating the antibacterial activity and bioactive potential, specifically antimalarial and antioxidant properties, of green tea kombucha using a Brazilian green tea. Moreover, the use of green tea produced in Brazil may be an alternative with which to reduce production costs and promote its cultivation in Brazil, which will motivate the kombucha industry, since imported teas are more expensive. The hypothesis posits that green tea kombucha contains a significant amount and diversity of phenolic compounds, which are expected to positively influence the biological activities assessed. Thus, the aim of this study was to characterize and to evaluate the antibacterial and bioactive activities of a green tea kombucha.

2. Materials and Methods

2.1. Kombucha Production

Kombucha was produced from green tea (*Camellia sinensis*) leaves produced in Brazil according to the methodology described by [7], with some modifications. Green tea was obtained from the Amaya brand, grown in the city of Registro, São Paulo, Brazil. Preliminary tests were carried out to define the infusion time and temperature binomial, according to recommendations of the manufacturer, 70 °C for 1 min. Before initiating fermentation, a starter tea from a previous batch of kombucha was added, ensuring that the initial pH remained in the range of 4.4 to 4.2.

Fermentation

Kombucha fermentation was carried out in three batches at 25 °C for five days. The kombucha was stored in plastic containers (fermenters) with a capacity of 20 L and kept in an incubator (BOD). At the end of fermentation, aliquots were collected through a tap located at the bottom of the fermenter and the SCOBY was removed. Kombucha samples

were tested for microbiological counts, pH, and total acidity. Aliquots were also collected and transferred to tubes, centrifuged at 10,000 rpm for 10 min, and stored at −18 °C until analyses (total phenolics, volatile acidity, antioxidant capacity, sugars, and ethanol and acetic acid). Some of the kombucha was freeze-dried, and then used to evaluate its in vitro bioactive properties and phenolic profile.

2.2. Kombucha Characterization

2.2.1. Total Acidity, Volatile Acidity, and pH

Total acidity was determined by titration with standardized 0.01 N NaOH and phenolphthalein as indicator, and the result was expressed as % (*w*/*v*) acetic acid [13]. The pH was determined by a previously calibrated pH meter. To determine volatile acidity, the methodology of the Adolfo Lutz Institute [13] was followed.

2.2.2. Determination of Sugars, Acetic Acid, and Ethanol

The quantification of sucrose, fructose and glucose, ethanol, and acetic acids were carried out using a High-Performance Liquid Chromatograph (HPLC), SHIMADZU brand. For this purpose, the methodology of [7] was followed, with some modifications. The HPX87H column (BIO-RAD) and pre-column of the same phase were used, with oven temperature of 32 °C and flow rate of 0.6 mL·min^{-1}, and, for the mobile phase, H_2SO_4 was used at 5 mM. The obtained results were expressed in g/L.

2.2.3. Microbiological Counting

The kombucha samples were serially diluted and plated on a standard medium of glucose, yeast extract, and calcium carbonate (GYC) agar (Merck, Darmstadt, Germany) for acetic acid bacteria counting. Lactic acid bacteria counting was performed on MRS agar (Merck, Germany) supplemented with bromocresol indicator (0.004%), with colonies identified as lactic acid bacteria being those that were yellow (acid producers), catalase-negative, and Gram-positive. PDA agar (potato dextrose agar, Merck, Germany) was used for yeast counting. Plates were incubated at 30 °C for 3 days under aerobic conditions. Lactic acid bacteria, on the other hand, were incubated under microaerophilic conditions. All results were expressed in CFU/mL.

2.2.4. Antibacterial Activity

The antibacterial activity of the kombucha was tested against the following pathogenic bacteria: *Salmonella enterica* subsp. *enterica* (ATCC 13076), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), and *Listeria monocytogenes* (ATCC 49594). The antibacterial activity was determined by calculating the percentage of inhibition using the broth microdilution method with a 96-well microtiter plate [14], with some modifications. Initially, the cultures were activated in BHI broth at 35 °C/24 h, in two steps. After activation, the inoculum was standardized to approximately 1.0×10^8 CFU/mL using the McFarland scale of 0.5. Serial dilutions were prepared (250 µL/mL to 0.9765 µL/mL) by adding 100 µL of kombucha samples to 100 µL of quadruple-concentrated Mueller–Hinton broth. To assess the effect of phenolic compounds and eliminate the influence of low pH on microbial inhibition, kombucha neutralization was performed. Thus, 1 M NaOH was used in the original kombucha until reaching a pH of 7, measured with a pH meter. All wells were inoculated with 100 µL of each standardized bacterial culture, except for negative control wells (containing only 200 µL of Mueller–Hinton broth). The final concentration of bacteria in each well was approximately 5.0×10^5 CFU/mL. As a positive control, 100 µL of double-concentrated Mueller–Hinton broth and 100 µL of the respective standardized bacterial culture were added to the wells. To assess the action of the original kombucha and the neutralized kombucha (both with a final concentration of 40% relative to the volume of

kombucha aliquot added), 50 microliters of seven-times-concentrated Mueller–Hinton and 200 microliters of kombucha were added. Subsequently, 150 microliters of this mixture (kombucha and culture medium) were discarded, and 100 microliters of each standardized culture were added. The plates were incubated at 35 °C/24 h, and the percentage of microbial inhibition was calculated based on optical density, measured at 625 nm using a spectrophotometer [14]. Here is the formula for calculating the percentage of inhibition:

$$\%inhibition = \left(\frac{(P_c - N_c) - (K_{40\%} - N_c)}{(P_c - N_c)} \right) * 100$$

P_c represents the OD_{625}nm value of the positive control, N_c represents the OD_{625}nm value of the negative control, and $K_{40\%}$ represents the OD_{625nm} value that was treated by 40% kombucha.

2.2.5. Total Phenolics

The concentration of total phenolics was assessed using the Folin–Ciocalteu colorimetric method, with gallic acid serving as the standard [15]. Absorbance readings were taken at 760 nm. The results were reported as milligrams of gallic acid equivalent per milliliter of kombucha (mg GAE/mL).

2.2.6. Antioxidant Capacity

The antioxidant capacity was determined by its ability to inhibit the ABTS+ radical, (2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonate), according to the methodology of [16]. Trolox was used as standard and results were expressed as µmol of Trolox equivalent per milliliter of kombucha (µmol TE/mL).

2.2.7. Phenolic Profiling Using UPLC-MS^E

The phenolic profile was performed in an ultra-performance liquid chromatography system coupled to mass spectrometry using an electrospray source and multiplex acquisition method (UPLC-MS^E) following [7] with some modifications. Green tea kombucha was lyophilized and reconstituted in 3 mL of 2% methanol (LC-MS grade), 5% acetonitrile (LC-MS grade), and 93% Milli-Q water. The reconstituted extracts were filtered through a 0.22 µm hydrophilic PTFE filter (Analytical) and stored in vials. A mixture of analytical standards (Sigma Aldrich, St. Louis, MO, USA) of phenolic compounds was prepared at a final concentration of 10 ppm. This solution was injected in triplicate before sample injection, using the same parameters described to ensure instrument reproducibility and to be used as confirmation of the identified phenolic compounds in the samples. Five µL of each sample were injected into the UPLC Acquity system (Waters Co., Milford, MA, USA) coupled to the Xevo G2S Q-Tof (Waters Co., Wilmslow, UK) equipped with electrospray ionization (ESI) source and quadrupole time-of-flight (QTof) mass analyzer. Data were collected in MS^E mode utilizing argon as the collision gas, with a collision energy ramp ranging from 25 to 55 V. The acquisitions were conducted in negative and centroid mode, covering the m/z range of 50 to 1000. The ionization parameters included a cone voltage of 30 V and a capillary voltage of 3.0 kV, with a desolvation gas flow of nitrogen (N_2) at 1200 L/h and a temperature of 600 °C; the cone gas flow was set at 50 L/h, and the source temperature was maintained at 150 °C. All acquisitions utilized leucine enkephalin (Leu-Enk, m/z 554.2615, [M-H]-) for lock mass calibration. The annotated compounds were categorized into phenolic classes before semi-quantification, based on representative standards of phenolic compounds analyzed under identical experimental conditions. Processed data were exported to XLSTAT software (Addinsoft, Paris, France, https://www.xlstat.com/en/download/customer/xlstat (accessed on 11 November 2024)),

where abundance values from the ion mass spectra were employed for relative quantification and statistical analysis of the data (one-way ANOVA, Tukey post-test, $p < 0.05$).

2.3. In Vitro Bioactive Potential

2.3.1. Cell Lines

The lung adenocarcinoma epithelial cells (A549), human colon carcinoma cells (HCT8), human liver cancer cells (HepG2), human umbilical vein endothelial cells (HUVEC), and normal human lung fibroblast (IMR90) cell lines were obtained from the Rio de Janeiro cell bank (Rio de Janeiro, Brazil) and maintained in Dulbecco's Modified Eagles' Medium/Nutrient Mixture F-12 Ham (DMEM) supplemented with a heat-inactivated fetal bovine serum to final concentration of 20% (IMR90) and 10% (A549, HCT8, Hep-G2, and HUVEC). The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

2.3.2. Cytotoxicity Profiling of Green Tea Kombucha

The assays for cytotoxicity and proliferation of kombucha phenolic compounds were determined by the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). The cells are seeded into 96-well plates at 5×10^3 cells/well (A549), 6×10^3 cells/well (IMR90), 1×10^4 cells/well (HCT8, HUVEC), 2×10^4 cells/well (HepG2), and 100 µL/well, for 24 h. After 24 h, the cells were treated with the freeze-dried kombucha at different concentrations, based on total phenolic content (0.5, 5, 50, 100, 200, and 500 µg GAE/mL) diluted in culture medium for 48 h. Then, 10 µL of MTT (0.5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The metabolically active cells reduced the MTT to blue formazan crystals, which are dissolved in 100 µL of DMSO/well. The absorbance (570 nm) was measured using a microplate reader (SynergyTM H1, Biotek, Waltham, MA, USA), and the dose response was determined by nonlinear regression (curve fitting) using GraphPad Prism® software 10 (GraphPad Software, Inc., San Diego, CA, USA). According to the method described by [17], the IC 50 (50% cell viability inhibition), GI 50 (50% growth inhibition), and LC 50 (50% cell death) parameters were obtained. The kombucha selective index (SI), which indicates the selectivity of the sample to the cell lines tested, was calculated by the ratio IC 50 (normal cell line)/IC 50 (cancer cell lines). Any sample that has an SI value higher than 3 will be considered to have high selectivity [18].

2.3.3. Intracellular Reactive Oxygen Species (ROS) Activity

We assessed the intracellular ROS using the DCFH-DA assay [18] with modifications. First, the cells (HUVEC and HCT8) were seen in a 96-well plate at 6×10^4 cells/well with culture medium. After 24 h to adhesion, the cells were treated with different concentrations of freeze-dried kombucha at different concentrations, based on total phenolic content (10, 50, and 100 µg GAE/mL), and H_2O_2 (15 µmol/L) for the positive control, and incubated for 1 h at 37 °C. After the treatment, the cells were carefully washed with PBS and received HANKS solution containing H_2O_2 (15 µmol/L) as a post-treatment. The fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 538 nm, with a microplate reader (SynergyTM H1, Biotek). The data were expressed as a percentage of fluorescence intensity relative to the untreated group (negative control) and were analyzed using the one-way ANOVA test followed by the Tukey test using GraphPad Prism® software (GraphPad Software, Inc., San Diego, CA, USA).

2.3.4. Erythrocyte Cellular Antioxidant Activity and Protection

The oxidative stress of erythrocytes was induced by AAPH, according to [19] with adjustments. Red blood cells (RBC, hematocrit 20% in PBS; 100 µL) were mixed with 100 µL PBS (negative control) or quercetin (25–50 µg/mL dissolved and diluted in PBS), or freeze-dried kombucha extracts (50 to 150 µg GAE/mL dissolved and diluted in PBS) and

incubated for 20 min (37 °C, 150 rpm). Then, 200 µL of AAPH (200 mM, in PBS) was added and the microtubes were incubated at 37 °C for 2 h. Then, the mixture was centrifuged at 1200× *g* for 3 min, to obtain the supernatant (SN) and the precipitate (PT). To assess the effect of Kombucha in RBC hemolysis and hemoglobin oxidation, an aliquot of SN or PBS as a blank (100 µL) was added to a 96-well plate and mixed with 200 µL of PBS. The hemolysis was recorded at 523 nm and the hemoglobin oxidation rate (%) was calculated by the ratio between the 630 nm and 540 nm absorbance. To evaluate the ROS generation, PT was washed with 400 µL of PBS and centrifuged (1200× *g*, 3 min) before adding 400 µL of DCFH-DA solution at 10 µmol/L. Then, 300 µL was transferred to a 96-well microplate and incubated at 37 °C for 20 min in the dark. The fluorescence intensity was measured using a microplate fluorometer (Synergy™ H1, Biotek, Waltham, MA, USA) at 485 and 520 nm for excitation and emission, respectively. Quercetin was used as the standard. The results are expressed as a percentage.

2.3.5. Antimalarial Properties

The anti-plasmodial effect of kombucha phenolic compounds was performed according to [20], using chloroquine-resistant (W2) and sensible (3D7) strains. *Plasmodium* strains were cultivated in RPMI culture medium, with 10% albumax II and 4% hematocrit, incubated at 37 °C using the candle jar method. The parasites were diluted and incubated in 96-well plates with freeze-dried kombucha at different concentrations, based on total phenolic content (5, 50, 100, and 200 µg GAE/mL) or culture medium as a positive control. After 48 h, the supernatant was removed, and we subsequently added 100 µL of lysis buffer solution (20 mM, pH 7.5), EDTA (5 mM), saponin (0.008%; *w*/*v*), and Trition X-100 (0.08%; *v*/*v*), in addition to 0.2 µL/mL Sybr Safe. The microplates were incubated in the dark for 30 min and the reading was carried out in a microplate reader (Synergy™ H1, Biotek, Waltham, MA, USA) with excitation at 485 nm and emission at 535 nm.

2.4. Target Fishing Analysis

Compounds with relative abundance above 10^6 were selected for SEA webserver [21], predictions, covering the twelve most abundant compounds present in the kombucha sample. The molecular structures were retrieved from PubChem database in the SMILE format and each compound was submitted to the SEA webserver. The predicted human/plasmodium targets were examined. In this sense, this server predicts based on the Tanimoto similarity between the compound and datasets tested against different targets (please see the literature for more information on how the predictions work) [21]. This target prediction strategy was based on previous reported works in the literature [8,22–26] as method for suggesting potential mechanism of action of compounds and mixtures (e.g., extracts).

2.5. Statistical Analysis

The results were expressed as mean ± standard deviation. The differences between the means were analyzed using Student's *t* test, with $p < 0.05$ level. All statistical analyses were performed using the Rstudio program, version 4.3.1.

3. Results and Discussion

3.1. Total Acidity, Volatile Acidity, and pH

The green tea kombucha had a pH of 3.41 ± 0.09 and total acidity of 0.20% ± 0.02 (*w*/*v* acetic acid). The increase in acidity over time, leading to a subsequent reduction in pH, is caused by the production of organic acids throughout the fermentation process, with an emphasis on acetic acids [7,27]. Some studies suggest that green tea kombucha has a

higher acidity when compared to black tea kombucha under the same fermentation time and temperature conditions [7]. This fact can be explained by the difference in terms of the microbiological composition present in green tea and black tea kombuchas. Bacteria of the *Acetobacter* genus, for example, were found in greater quantities in green tea kombuchas and are closely related to the generation of acetic acid, which could explain the lower pH and higher acidity [12].

In our study, the kombucha exhibited a volatile acidity of 35.71 mEq/L, which is also an important parameter related to the quality of the beverage. According to Normative Instruction 41 from the Ministry of Agriculture, Livestock, and Supply in Brazil, the volatile acidity of kombucha should be in the range of 30 to 130 mEq/L of acetic acid [5]. In another study, green tea kombucha also produced in Brazil and fermented at 25 °C for four days reached a volatile acidity of 126.7 mEq/L, and, from the fifth day onwards, it was already outside Brazilian standards [5,28]. Standardizing the initial pH, along with the microbiological composition of the SCOBY, may have contributed to keeping the final pH of the beverage and volatile acidity within the limits recommended by regulations.

3.2. Sugars, Acetic Acid, and Ethanol

The green tea kombucha had 22.24 g/L of sucrose. In the preparation of green tea kombucha, 50 g/L of sucrose was added, meaning that approximately 55.5% of the sucrose, equivalent to 27.56 g/L, was consumed by the microorganisms during the five-day fermentation period. At the beginning of fermentation, sucrose is hydrolyzed by invertase produced by yeast into its constituent monomers, glucose, and fructose, for metabolism. Yeasts, in turn, can metabolize both glucose and fructose, producing ethanol through alcoholic fermentation [29]. On the other hand, acetic acid bacteria can use both ethanol or sugars to generate acetic acid [30].

The residual glucose and fructose concentrations were 11.49 g/L and 12.57 g/L, respectively. Similar to sucrose, this remaining amount contributes to the sensory aspects of the beverage. In a previous study, green tea kombucha was prepared using 50 g/L of sucrose, and, after 10 days of fermentation at 25 °C, lower values were obtained for the concentration of glucose and fructose [7]. One possible explanation for this difference in concentration is the longer fermentation time (10 days), allowing microorganisms to consume these sugars to maintain their activities.

The acetic acid content in this study was consistent with the findings of [7], and higher than that found by [31]. It is worth noting that these differences are expected due to the combination of the fermentation time and temperature, microorganisms present in the SCOBY, and type of tea, among other factors [9,32]. The ethanol content was 4.7 g/L. Thus, our green tea kombucha can be classified as a non-alcoholic beverage according to the Brazilian Normative Instruction, as the ethanol content is below 5.0 g/L [5]. Kombuchas from different brands were evaluated, revealing discrepancies in residual sugar levels, and alcohol content, as well as organic acids. All these factors impact the sensory characteristics and potential bioactive properties that these commercial kombuchas may exhibit [33]. Therefore, standardizing fermentation conditions, and sugar content, among other factors, can be a key piece in developing a beverage that caters to the market niche unwilling to consume alcoholic beverages.

3.3. Microbiological Counting

For the microbiological counting of green tea kombucha, lactic acid bacteria, acetic acid bacteria, and yeast were assessed. Their populations were estimated at 7.29 log CFU/mL, 7.02 log CFU/mL, and 7.17 log CFU/mL, respectively. Similar results were found in a study on kombucha obtained from fractions of sweetened green and black tea (100 g/L),

fermented for 10 days, with populations also around 10^7 CFU/mL [34]. It is evident that, despite having twice the added sugar and fermentation time compared to the present study, this factor did not impact the microbial count. Even with a shorter fermentation time of 5 days, a higher microbial growth was obtained compared to other studies where these populations ranged between 10^5 to 10^6 CFU/mL [7].

3.4. Phenolic Compounds and Antioxidant Capacity

The kombucha had a total phenolic content of 0.32 ± 0.007 mg GAE/mL, about half of that found in green tea kombucha produced using imported green tea, and a longer fermentation time [7]. Additionally, it is worth noting that the infusion time and water temperature (75 °C for 2 min) during the tea preparation for our study were lower (70 °C for 1 min), which may have impacted the reduction in phenolic compound extraction.

The antioxidant capacity of green tea kombucha was 3.24 ± 0.43 µmol TE/mL. The antioxidant capacity is correlated with the content of phenolic compounds (a major contributor); however, other metabolites present in kombucha also contribute to this antioxidant capacity, such as vitamin C [35]. The antioxidant activity of each phenolic compound depends on its chemical characteristics, degree of polarity, and stability in the environment where it will react, as they can easily participate in redox reactions [9,35].

The green tea kombucha phenolic content was evaluated using UPLC-MS^E after 5 days of fermentation, revealing the presence of 92 phenolic compounds (Table S1). The majority (70.7%) belonged to the class of flavonoids, followed by phenolic acids (25%), lignans (2.2%), and other polyphenols (1.1%). Among the 92 compounds, the ten most abundant are presented (Figure 1).

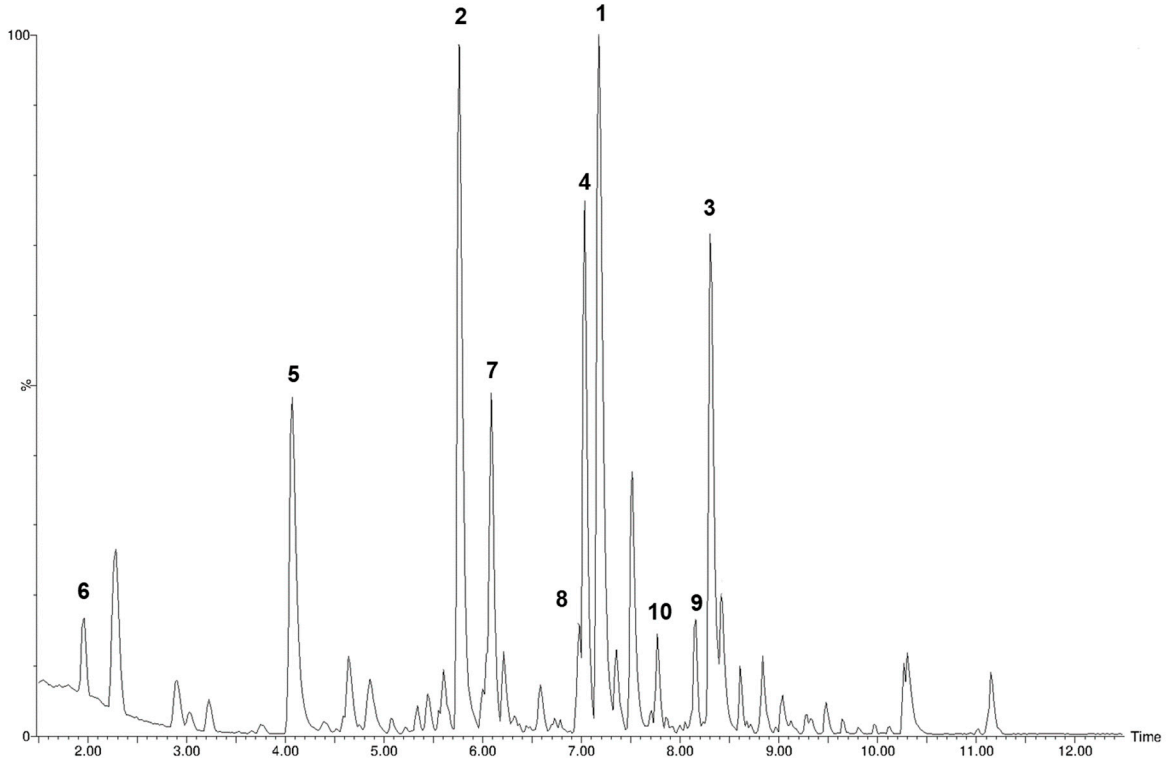

Figure 1. Raw chromatogram of the ten most abundant phenolic compounds found in green tea kombucha. **1** (+)-gallocatechin 3-O-gallate/(-)-epigallocatechin 3-O-gallate; **2** (+)-gallocatechin; **3** Catechin 5-O-gallate; **4** (-)-epicatechin; **5** (-)-epigallocatechin; **6** 5-O-Galloylquinic acid; **7** catechin; **8** 5-p-coumaroylquinic acid; **9** quercetin 3-O-rutinoside; and **10** myricetin 3-O-glucoside.

In other studies that assessed the phenolic compound profiles of green and black tea kombuchas, the flavonoid class also emerged as the predominant one, followed by phenolic acids [7,8]. Comparing the phenolic profile obtained here with other studies on green tea kombuchas, it is evident that there was some similarity in terms of the most abundant phenolics [7,36]. As shown in (Figure 1), epigallocatechin 3-O-gallate was the most abundant phenolic compound found, which also occurred in both green tea and black tea kombuchas according to [7]. The presence of catechin, epicatechin, epicatechin gallate, and epigallocatechin is even reported for kombucha analogs using oak [37].

Generally, green tea kombucha exhibits a lower diversity and abundance of phenolic compounds compared to black tea kombucha [7]. However, more than half of these compounds were found in both beverages [7]. Fermentation is a process that promotes the diversification of phenolic compounds, as various biotransformations can occur due to the presence of microbial enzymes [8]. Among them is the hydrolysis of high-molecular-weight flavonoids into lower-weight ones, contributing to an increase in phenolic acids over time [1,8]. Approximately one-third of the tea mass (dry basis) is composed of phenolic compounds, and optimizing this content through the fermentation process is valuable, given the well-known health benefits of these compounds [38].

Gallocatechin gallate consumption improves the cognitive function of elderly rats and inhibits advanced glycation end products, helping combat diabetic complications [39,40]. Green tea catechins, especially epicatechin-3-gallate (ECG), epigallocatechin-3-gallate (EGCG), and gallic acid-3-gallate (GCG), controlled B16F10 melanoma cells [40]. Meanwhile, quercetin-3-O-rutinoside can prevent gastrointestinal injuries, oxidative stress, and inflammation [41,42]. The rich phenolic composition of kombucha contributes to its bioactive potential.

3.5. Antibacterial Properties

The green tea kombucha was inoculated at 40% after mixing the aliquot of kombucha with the culture medium and adding the tested microorganisms. The beverage inhibited the four tested pathogenic microorganisms (Table 1).

Table 1. Antimicrobial inhibition percentage of neutralized and non-neutralized green tea kombucha.

Amostra	*S. aureus*	*L. monocytogenes*	*Salmonella* sp.	*E. coli*
Non-neutralized	56.82 ± 14.96 [a]	54.24 ± 19.43 [a]	72.82 ± 17.34 [a]	41.98 ± 1.13 [a]
Neutralized	34.37 ± 13.77 [a]	48.95 ± 15.82 [a]	66.23 ± 0.17 [a]	47.91 ± 17.66 [a]

Equal letters indicate that there was no significant difference by the *t*-test ($p > 0.05$) between the original and the neutralized kombucha.

Among the analyzed microorganisms, kombucha was able to act more satisfactorily against *Salmonella* and, to a lesser extent, against *E. coli*, Gram-negative bacteria. The neutralization of kombucha did not result in a significant decrease ($p > 0.05$) in its antimicrobial potential compared to kombucha at its normal pH, around 3.3. These results suggest that the antimicrobial activity of the beverage is not solely attributed to its low pH (the presence of organic acids). One possible explanation for the antimicrobial mechanism of action is that, during the fermentation process, microorganisms can generate compounds such as bacteriocins, proteins, and enzymes, among others, capable of creating selective pressure in the environment and inhibiting the development of certain microorganisms [43–45]. Additionally, compounds such as isorhamnetin and catechin have been previously identified as capable of increasing the permeability of microorganisms' membranes and consequently causing cellular dysfunction [45,46]. In another study, kombucha prepared from lemon balm tea (*Melissa officinalis* L.), also neutralized with NaOH solution, demonstrated antimicrobial activity against Gram-negative bacteria *E. coli* and *Salmonella* sp., but was not effective against *L. monocytogenes* [47].

3.6. In Vitro Bioactive Properties of Green Tea Kombucha

3.6.1. Antiproliferative Properties of Green Tea Kombucha

Cancer emerges as a primary contributor to global public health issues, causing the demise of countless individuals. It is estimated that one in every five individuals will be affected by this disease at some point in their lives [48]. Lung cancer holds the second position among the most prevalent cancers, followed by colorectal cancer in third place. In Brazil, it is projected that more than 700 thousand new cancer cases will arise between 2023–2025 [49]. Due to the potential for numerous side effects associated with cancer treatment, there is a growing body of research dedicated to evaluating the therapeutic efficacy of natural products in combating this disease [50].

Green tea kombucha was tested against various cell types, including lung adenocarcinoma epithelial cells (A549), human colon carcinoma cells (HCT8), human liver cancer cells (HepG2), human umbilical vein endothelial cells (HUVEC), and normal human lung fibroblasts (IMR90). The results of these tests are presented in Figure 2.

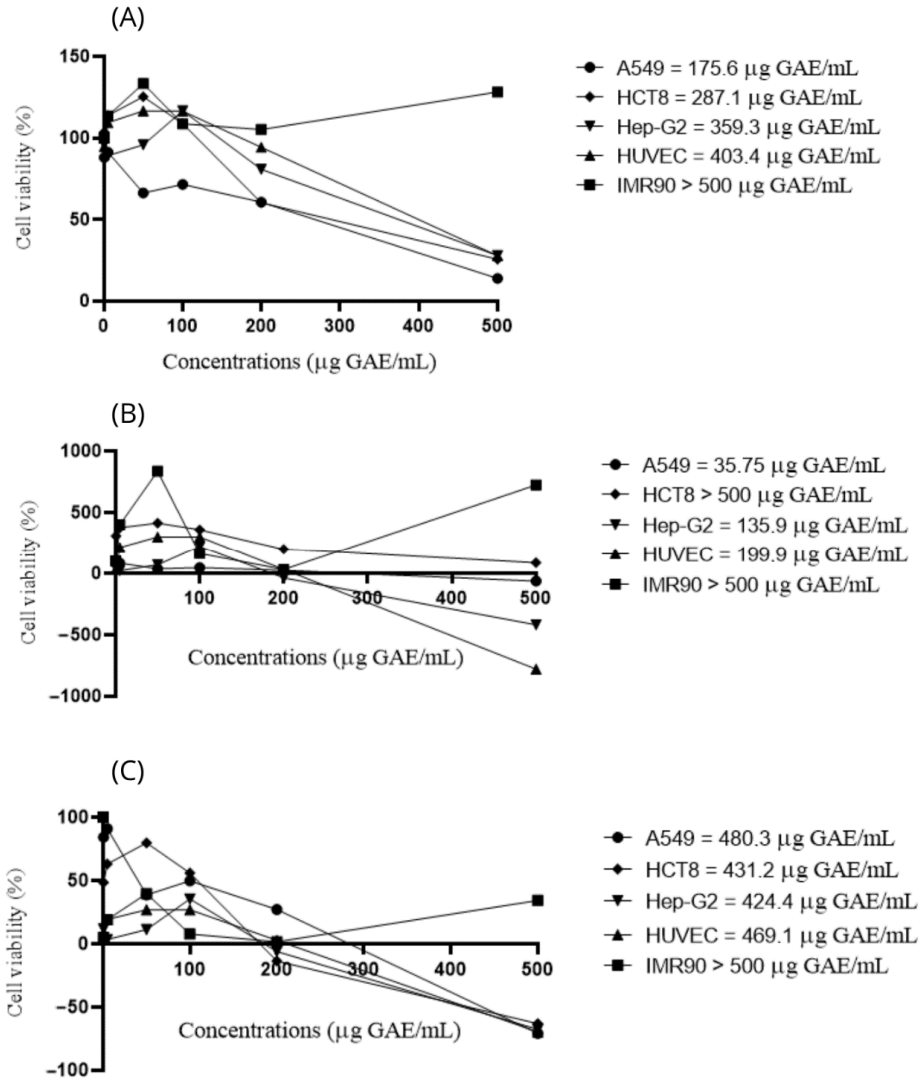

Figure 2. (**A**) IC_{50} (50% cell viability inhibition); (**B**) GI_{50} (50% growth inhibition); and (**C**) LC_{50} (50% cell death) of green tea kombucha.

The green tea kombucha showed a cytotoxic profile in all tested cancer cell lines, evidenced by growth inhibition (GI50), the decreased cell viability (IC50), and the lethal concentration (LC50). The A549 cell line appeared to be more sensitive to the effects of

the extract. In line with this, we observed that the extract presented a selective index (A549 = 2.84; HCT8 = 1.74; HepG2 = 1.4, and HUVEC = 1.23) concerning the normal cell lineage, as IMR90 was not affected by treatment (IC50, IG50, and LC50 > 500 μg GAE/mL). When analyzed separately, the LC50 showed better effects for the Hep-G2 cell line at 424.4 μg/mL, followed by HCT8 at 431.2 μg/mL, while A549 required the highest kombucha concentration, 480.3 μg/mL, and IMR90 > 500 μg/mL to cause a lethal effect on the cells. Ref. [7] assessed the antiproliferative and cytotoxic potential of green and black tea kombuchas against HCT8 (ileocecal colorectal adenocarcinoma) and CACO-2 (colorectal epithelial adenocarcinoma), using IMR90 cells as a control for healthy cells. It was found that, for all these, green tea kombucha was more efficient than black tea kombucha. This higher potential of green tea kombucha was attributed to the action of its phenolic compounds, which had previously been reported in the literature for their anticancer activities [7].

In our study, the cytotoxic potential obtained for IMR90 cells (IC50, GI50, and LC50 > 500 μg GAE/mL) was lower than that reported by Cardoso et al. (2020) [7]. According to the classification of the National Cancer Institute, a compound is considered non-toxic when it presents IC50 > 500 [51]. Thus, it is possible to infer that green tea kombucha acted satisfactorily on cancer cells but without affecting healthy cells, confirming non-toxicity in the latter and an antiproliferative effect on the target cells (A549, HCT8, and HepG2). It is worth noting that the toxic effect of a compound on the cell, whether healthy or not, depends on the exposure time and the characteristics of that compound [52]. Green, black, and oolong tea kombuchas were tested for their antiproliferative potential in colorectal cancer cells (CACO-2). Of these, only green tea kombucha and black tea kombucha showed a high toxicity to CACO-2 cells and a low toxicity to healthy cells [53].

Some findings suggest that kombucha could act by inhibiting certain functions of cancer cells, such as IL-8, VEGF, COX-2, HIF-1α, MMP-2, and MMP-9, leading to apoptosis [54]. Among the phenolic compounds present in green tea kombucha, flavonoids were the most abundant class, comprising more than 70%. Therefore, understanding how they act as anticancer agents is crucial. Dietary flavonoids act pleiotropically in the tumor environment by controlling various pathways such as PI3K/Akt, NF-κB, JNK/STAT, p38/MAPK, and VEGF, thus preventing the development of tumor cells and the chance of metastasis [55]. In general, the pro-oxidant action of bioactive compounds, especially phenolic compounds, is important to assist in cellular apoptosis [56]. The structure of the phenolic compound also affects the efficiency of cellular apoptosis; for example, the structure of pyrogallol in the B-ring is a key factor in its occurrence [57]. Although epigallocatechin gallate has the highest anticancer potential, it is known that the presence of others, such as epicatechin gallate, epigallocatechin, and epicatechin, can potentiate the bioactive effect, as their bioavailability in the body is increased [56]. A noteworthy point is the need for studies that create strategies to increase the bioavailability of these compounds through the use of nanotechnology and liposomes, among other emerging technologies, to improve their overall health effects [58]. Since kombucha contains phenolic compounds from tea, it is believed that the mechanisms by which tea acts can also be applied to a possible action of kombucha.

Target Fishing Analysis: Human Targets Related to Cancer Treatment

Most of the compounds (83.3%) were predicted to interact with ELAV-like protein 3 as a target. This target is a member of the ELAV-like protein family, playing an important role post-transcriptional process. The four isoforms of this family predominantly work positively in regulating the gene expression, and their dysregulation is related to different diseases, such as inflammation and cancer [59]. Additionally, 6-phosphogluconate (6PGD)

dehydrogenase was predicted as a target for 58.3% of the compounds. This enzyme is capable of mediating biological functions, for example, redox homeostasis, metastasis, and the proliferation of cells. The 6PGD enzyme is involved in the oxidative pentose phosphate pathway, and its overexpression is described in multiple cancers. The upregulation of this enzyme is already described in colorectal tumors, breast carcinoma, hepatocellular cancer, lung carcinoma, and others [60].

Abnormal protein glycosylation is associated with malignant transformation in cells [61,62], and, following this mechanism, two target groups were predicted by the target fishing approaches: the alpha-(1,3)-fucosyltransferase (FUT) family and sialyltransferases. The FUT family are enzymes responsible for synthesizing fucosylated oligosaccharides involved in interactions between cells [63]. Both FUT4 and FUT7 were predicted by 58.3% of the compounds, and these two promote neoplastic cell proliferation and hepatocellular carcinoma cell growth [63]. The same frequency was found for the CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase (SIAT6), an enzyme from the sialyltransferases family. This protein is expressed in different cancers [64,65]; nevertheless, it is not a well-established molecular target for small-molecule drug design. Another frequent target (58.3%) was the phosphoglycerate mutase 1 (PGAM1), an enzyme involved in the glycolysis pathway catalyzing the conversion of 2-phosphoglycerate into 3-phosphoglycerate. PGAM1 is involved in the upregulation of the pentose phosphate pathway and is overexpressed in different cancers [66]. Carbonic anhydrase (CA) protein family members were also predicted as targets for the compounds (-)-epicatechin, (+)-catechin, and quercetin 3-O-glucoside. They play important roles in facilitating the transport of carbon dioxide and protons in the intracellular space, across biological membranes, and in the unstirred layers of the extracellular space [67], thus controlling the pH in the tumor microenvironment [68] (Mboge et al., 2018). From the twelve CA, the active isoforms CA9 and CA12 are considered molecular targets for anticancer therapy [68]. The (-)-epicatechin (+)-catechin, and quercetin 3-O-glucoside displayed a Tanimoto coefficient equal to 1 against CA9 and CA12 isoforms, and they already have experimentally validated activity against these targets [69,70]. CA enzymes were already predicted to be potential targets of flavonoids and phenolic acids from sorghum flours with anticancer properties [25]. The metabolites present in the kombucha display potential activity for different targets related to cytotoxicity activity, and some compounds have been previously described, highlighting the complexity of the sample composition. Furthermore, the minority compounds were not considered along with synergistic effects between the components. The complete description of the predicted targets is described in Table S2.

3.6.2. Measurement of Intracellular ROS

Reactive oxygen species (ROS) are generated by aerobic metabolism, and they are highly reactive against various biological targets. These molecules are associated with oxidative stress but can also serve as indicators of biological processes [71]. In recent years, there has been increasing concern about oxidative stress and ways to mitigate it. In this context, phenolic compounds are molecules with high antioxidant potential due to their chemical structure containing aromatic rings. Consequently, they can transfer electrons to free radicals, inhibit the formation of ROS, and remain stable due to the resonance chemical process [9,72,73].

As kombucha is a beverage rich in phenolic compounds, it was evaluated for its ability to inhibit ROS generation. The kombucha phenolic extract demonstrated a protective and antioxidant effect against ROS induced by H_2O_2, reducing the levels of oxidation under basal conditions in both tested cell lines (Figure 3).

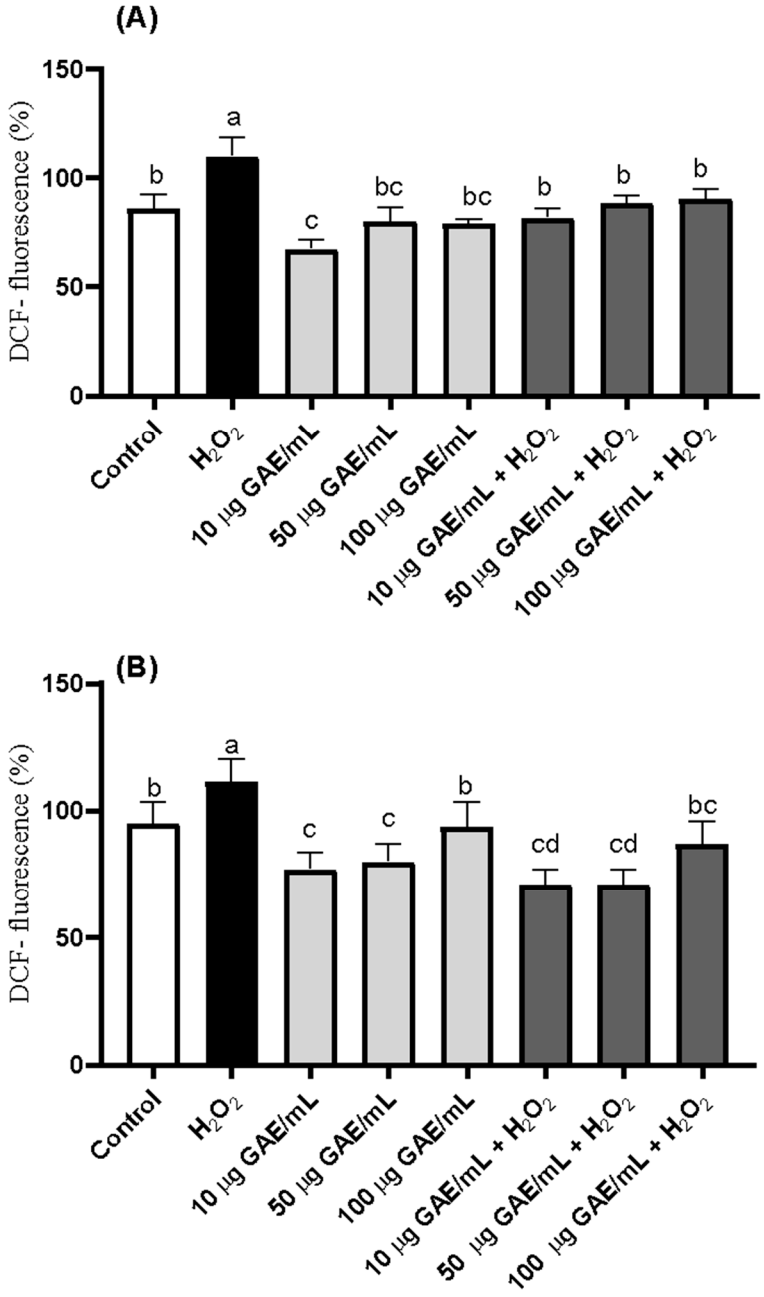

Figure 3. Intracellular ROS measurement in HCT8 (**A**) and HUVEC (**B**) cells by spectrofluorimetry that received treatment with kombucha extract at 10, 50, and 100 µg/mL. Quantitative data are the mean ± standard deviation (n = 4). Different letters represent statistically significant differences ($p \leq 0.05$).

It can be observed that both HCT8 and HUVEC cells, when treated with 10 µg GAE/mL of green tea kombucha without the addition of hydrogen peroxide, showed protection against the generation of reactive oxygen species (ROS), as the treated cells exhibited a lower concentration compared to cells under normal conditions. HCT8 cells treated with kombucha concentrations between 10 and 100 µg GAE/mL, despite the induction of ROS formation due to the presence of hydrogen peroxide, did not show a significant difference compared to the negative control. In contrast, in HUVEC cells, only at the highest concentration, in this case, 100 µg GAE/mL, was there no significant difference compared to the negative control. This demonstrates that kombucha was effective in combating the generation of reactive oxygen species in both cell types.

Bovine mammary cells treated with green tea showed a reduction in hydrogen-peroxide-induced oxidative damage, attributed to the activation of pathways such as NFE2L2, HMOX1, and NQO1. This activation led to an increase in endogenous antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), resulting in a decrease in the levels of malondialdehyde and reactive oxygen species [24]. The NFE2L2 pathway is linked to the activation of antioxidant enzymes, playing a fundamental role in combating potential oxidative damage [74]. Thus, catechin can act directly as an antioxidant or, concurrently, by activating or increasing the activity of antioxidant enzymes [75]. Additionally, the presence of multiple compounds with antioxidant characteristics, such as phenolic compounds and some vitamins, enhances the effect on the elimination of ROS [73]. The mechanism of ROS generation and combat is multifactorial, and green tea kombucha has demonstrated its ability to control ROS generation.

3.6.3. Effects of Kombucha in the Protection of RBC Against AAPH-Induced Stress

The 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) is a highly unstable molecule capable of generating various free radicals. When these radicals interact with lipids present in the erythrocyte plasma membrane, they can lead to the failure of this membrane, causing hemolysis. Erythrocytes, in turn, are highly sensitive to oxidative damage and, at the same time, have a metabolism that is easily understood [19,76]. As green tea kombucha contains phenolic compounds in its composition, it was tested for its ability to protect erythrocytes against potential damage caused by oxidative stress induced by AAPH. For all the parameters, an antioxidant effect of green tea kombucha extract was observed, as the levels of hemolysis and ROS generation were reduced in a dose-dependent manner, and hemoglobin oxidation was decreased in all concentrations tested, conferring RBC protection (Figure 4). Quercetin was used as a standard to compare the protective effects of kombucha extracts against the effects of quercetin, a potent flavonoid with strong antioxidant properties.

It can be observed that, at a concentration of 150 μg GAE/mL, kombucha was able to inhibit the formation of ROS at values equivalent to the negative control, completely neutralizing the effects of AAPH on the cells. Green tea kombucha also acted preventively against hemolysis at lower concentrations (50 μg GAE/mL) without a significant difference compared to the negative control, demonstrating that, even with the stress induced by AAPH, the erythrocyte was able to reach values similar to a cell that was not exposed to this harmful molecule. In another study, at the same concentration of 50 μg/mL, both the green tea extract and quince leaf extract were able to prevent hemolysis to the extent of matching the negative control [77]. Flavonoids, by interacting with the erythrocyte membrane, make it more organized, preventing hemolysis. Additionally, the site of interaction between flavonoids and the membrane is crucial for their action to be greater or lesser [78,79]. In a simplified manner, dietary antioxidants can convert the peroxyl radical into another form that is non-reactive [80].

Regarding hemoglobin oxidation, the increase in green tea kombucha concentration did not cause a significant improvement in preventing this phenomenon (Figure 4). Ref. [81] pointed out that catechin can act directly on the heme group of hemoglobin, potentially oxidizing Fe (II) to Fe (III), exhibiting a pro-oxidant action at higher doses. This fact may help explain why the increase in kombucha concentration did not improve the hemoglobin oxidation. Similarly, the polyphenols from green and black tea showed a protective effect on red blood cells at concentrations of up to 10 μg/mL, and, beyond this point, no significant observation was made [82]. This protective effect on erythrocytes mediated by the action of green tea kombucha helps to explain the antimalarial activity that was verified for both sensitive (3D7) and non-sensitive (W2) strains to the chloroquine drug.

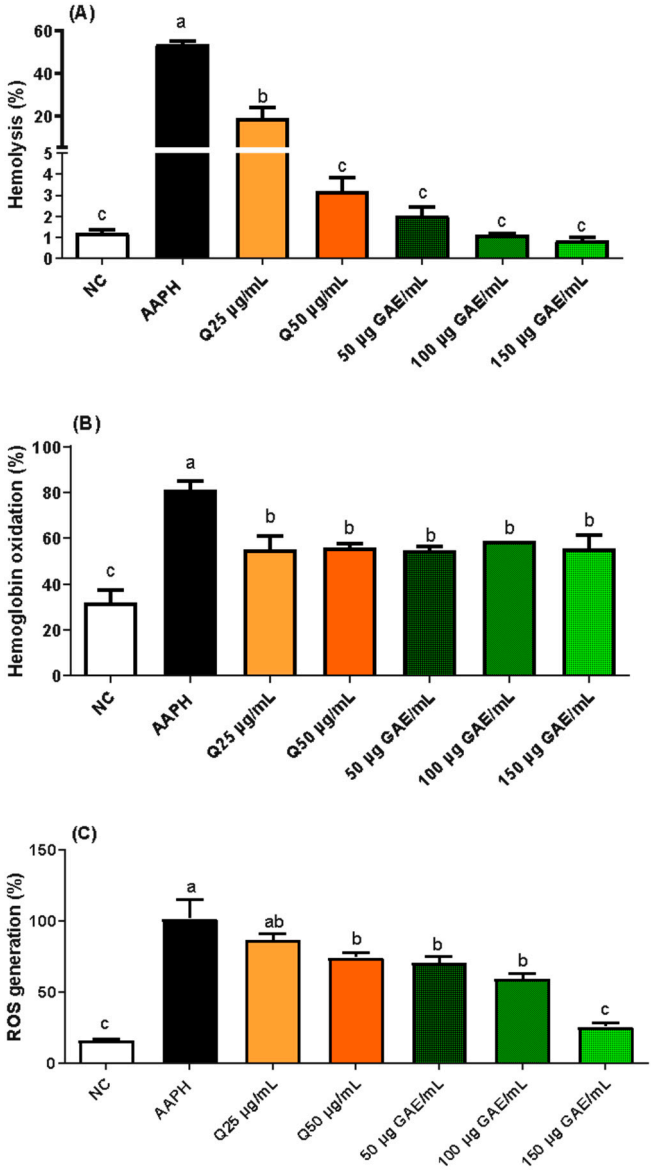

Figure 4. (**A**) Effect of kombucha extract on blood hemolysis induced by 2′-azobis(2-amidinopropane) dihydrochloride (AAPH); (**B**) effect of kombucha extract on hemoglobin oxidation induced by AAPH; and (**C**) effect of kombucha extract on intracellular reactive oxygen species (ROS) induced by AAPH. Note: NC: negative control; AAPH: positive control; Q: quercetin. Quantitative data are the mean ± standard deviation (n = 4). Different letters represent statistically significant differences ($p \leq 0.05$).

3.6.4. Antimalarial Activity

Malaria is a disease that affects millions of people worldwide, caused by *Plasmodium falciparum*, *P. vivax*, and *P. malariae*, which can infect the Anopheles mosquito [83–85]. The life cycle of *Plasmodium* encompasses phases in the Anopheles mosquito and the human host. In the intraerythrocytic cycle of *Plasmodium*, the invasion, growth, and replication of erythrocytes occur. In the ring stage, *Plasmodium* is in its youngest form, which is associated with drug resistance, as these parasites can undergo a temporary growth arrest. Among commonly used medications, most target the parasite in later blood stages, not addressing the ring stage [86]. In Brazil, malaria is endemic in the Amazon region, and the resistance of some strains of *Plasmodium* to commonly used drugs is alarming [84]. Consequently, studies have emerged to evaluate the efficacy of alternative plant-based treatments against malaria [87].

Kombucha green tea demonstrated toxicity against the W2 and 3D7 strains of *P. falciparum*, with the 3D7 strain showing greater sensitivity (IC50 = 4.2 µg GAE/mL) to treatment than W2 (IC50 = 26.7 µg GAE/mL), as illustrated in (Figure 5).

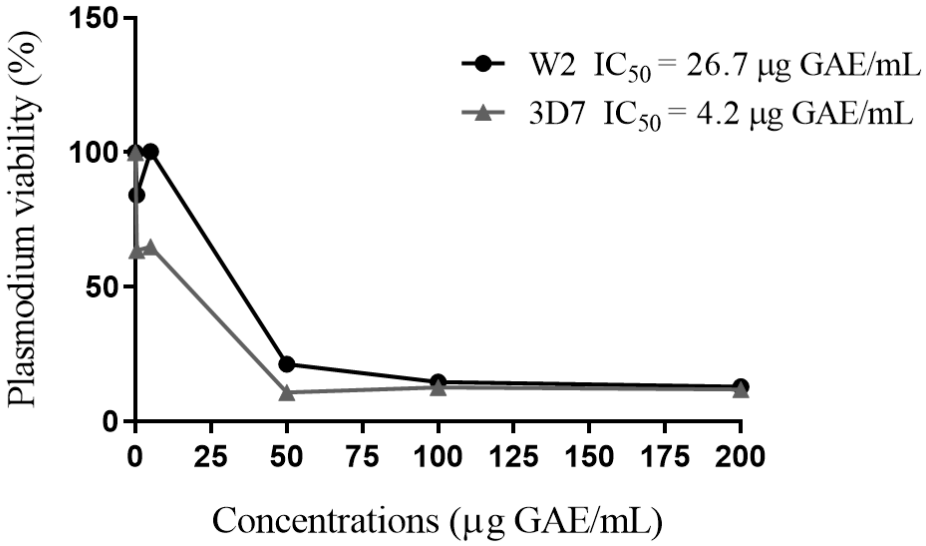

Figure 5. Antiplasmodial activity and cytotoxicity of kombucha phenolic extract, against chloroquine-resistant strain (W2) and chloroquine-sensitive strain (3D7).

The results obtained were encouraging and better than those found in the study by [8], as the IC50 values obtained were lower. This suggests that a smaller amount of kombucha is needed to act against *P. falciparum*, depending on the IC50 values. Flavonoids can be classified from highly active to inactive based on the IC50 values [83]. Following this classification, green tea kombucha for the W2 strain is highly active (IC50 < 10), and, for 3D7, it is moderately active (20 < IC50 < 50).

The presence of phenolic compounds in black tea kombucha, notably, catechin, quercetin, and epigallocatechin gallate, has been attributed to its ability to combat the spread of *P. falciparum* [8]. Furthermore, an increase in their presence is directly proportional to a decrease in the concentration required to cause a 50% reduction in parasitemia (IC50) [20]. It is believed that the action of black tea kombucha on *P. falciparum* involves the rupture of its membrane, affecting fatty acid synthesis, and impairing the action of proteases [8]. Plasmodial lipids play a crucial role in signaling, subcellular transport, and even protein anchoring in the membrane, as changes in the lipid structure can alter the substrate hydrophobicity and prevent the binding of proteins essential for *Plasmodium* survival [88]. Among the mechanisms by which drugs act on *Plasmodium* are the blockade of pyrimidine biosynthesis, the reduction in mitochondrial membrane function, or even the neutralization of the heme group [86].

It is believed that the mechanism of action of catechin against *P. falciparum* is due to the generation of oxidative stress, leading to a redox imbalance and inhibiting its growth [89]. Among flavonoids, for example, epigallocatechin gallate was able to reduce the ability of *Plasmodium* to adhere to the ICAM-1 receptor, essential for the development of cerebral malaria with a high mortality rate [90]. A noteworthy point is that the catechins EGCG and ECG, when administered together with artemisinin, one of the main drugs used in malaria treatment, intensify the drug's antiplasmodial action [73]. Since green tea kombucha contains several phenolic compounds, it is believed that the antimalarial effect is caused by the synergy between different bioactive compounds through various pathways.

Target Fishing Analysis: Antimalarial Targets

Most compounds were predicted to interfere in the fatty acid biosynthesis of *Plasmodium* sp. as inhibitors of 3-oxoacyl-acyl-carrier protein reductase (83.3%), beta-hydroxyacyl-ACP dehydratase (83.3%), 3-oxoacyl-[acyl-carrier-protein] reductase (58.3%), and enoyl-acyl-carrier protein reductase (58.3%). Specifically, (-)-epigallocatechin and (+)-gallocatechin displayed a Tanimoto coefficient equal to 1 against the enoyl-acyl-carrier protein reductase, suggesting that both compounds have already been reported in the literature with experimental activities against those targets. The first one is already described as an inhibitor of the plasmodial enoyl-acyl-carrier protein reductase along with other flavonoids [84,91]. Nevertheless, biological data for (+)-gallocatechin were not found; this could be related to the fingerprint used to measure the Tanimoto similarity not being sensitive enough to distinguish between (+)-gallocatechin and their optical isomers. Previously reported works also called attention to flavonoids present in kombucha samples and their correlation to the antimalarial activity. These findings reinforce the importance of flavonoids targeting the Plasmodium falciparum fatty acid biosynthesis to the antimalarial activity [8]. Enoyl reductase enzymes from Plasmodium were already predicted to be potential targets of flavonoids and phenolic acids from sorghum flours with anticancer properties [25] as well as other Kombucha samples [8]. Moreover, (-)-epicatechin and (+)-catechin displayed the maximum Tanimoto coefficient value (Tc = 1) for the M18 aspartyl aminopeptidase (+)-gallocatechin inhibitors, and these compounds were already tested against this target [92]. This enzyme is an exopeptidase, involved in the host's hemoglobin degradation [93,94]. In this sense, the in vitro activity observed in the kombucha sample could be related to the parasite membrane biosynthesis disruption, and, in in vivo assays, the sample could have additional mechanisms of action. We also do not discard the influence of other compounds with minor concentrations that were not predicted by the SEA webserver as well as possible synergistic effects within the kombucha constituents. The complete description of the predicted targets is described in Table S2.

4. Conclusions

Ninety-two phenolic compounds were found in green tea kombucha, produced using a Brazilian green tea, with 70.7% being flavonoids and 25% phenolic acids. This rich phenolic profile contributes to the beverage's excellent antioxidant capacity. Green tea kombucha exhibited antibacterial activity against all tested strains. The phenolic compounds were largely responsible for the bioactive properties of the kombucha, which showed antimalarial activity against sensitive strains of *P. falciparum* (3D7) and strains not sensitive to chloroquine (W2). The beverage provided dose-dependent protection to erythrocytes, preventing their hemolysis even when subjected to 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), and reduced intracellular ROS formation. Additionally, the kombucha demonstrated antiproliferative activities against epithelial cells of adenocarcinoma (A549), human colon carcinoma cells (HCT8), and human liver cancer cells (HepG2), and showed low toxicity to human umbilical vein endothelial cells (HUVEC) and normal human lung fibroblasts (IMR90). Along with these bioactive properties, the green tea kombucha met all parameters of the Brazilian regulation that outlines the identity and quality criteria that kombucha produced in Brazil must meet.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages11010007/s1, Table S1: Phenolic compounds identified in green tea kombucha by UPLC-MS^E, Table S2: Targets for prediction of compounds present in higher abundance in the tested kombucha sample.

Author Contributions: U.V.L.: Formal Analysis, Investigation, Methodology, Writing—Original Draft. C.V.P.d.C.: Formal Analysis, Investigation. R.R.C.: Investigation, Methodology, Resources, Writing—Review & Editing. C.T.d.S.D.: Formal Analysis, Methodology. M.A.V.d.C.: Formal Analysis, Methodology. A.d.S.L.: Formal Analysis, Methodology. L.d.S.C.: Formal Analysis, Methodology. A.B.d.S.: Formal Analysis, Methodology. P.O.F.: Formal Analysis, Methodology. V.G.M.: Formal Analysis, Methodology, Writing—Review & Editing. M.S.L.F.: Formal Analysis, Methodology, Writing—Review & Editing. L.A.: Formal Analysis, Methodology, Writing—Review & Editing, Supervision, Resources. M.R.E.: Formal Analysis, Methodology, Writing—Review & Editing, Supervision, Resources. V.C.: Writing—Review & Editing, Funding Acquisition. A.G.: Writing—Review & Editing, Funding Acquisition. F.A.R.d.B.: Funding Acquisition, Project Administration, Resources, Supervision, Writing—Review & Editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Jayabalan, R.; Marimuthu, S.; Swaminathan, K. Changes in Content of Organic Acids and Tea Polyphenols during Kombucha Tea Fermentation. *Food Chem.* **2007**, *102*, 392–398. [CrossRef]
2. Tran, T.; Grandvalet, C.; Verdier, F.; Martin, A.; Alexandre, H.; Tourdot-Maréchal, R. Microbial Dynamics between Yeasts and Acetic Acid Bacteria in Kombucha: Impacts on the Chemical Composition of the Beverage. *Foods* **2020**, *9*, 963. [CrossRef] [PubMed]
3. Kombucha Market Size | Mordor Intelligence. Market Research Company—Mordor Intelligence™. Available online: http://www.mordorintelligence.com/pt/industry-reports/kombucha-market (accessed on 2 November 2024).
4. Chong, A.Q.; Lau, S.W.; Chin, N.L.; Talib, R.A.; Basha, R.K. Fermented Beverage Benefits: A Comprehensive Review and Comparison of Kombucha and Kefir Microbiome. *Microorganisms* **2023**, *11*, 1344. [CrossRef] [PubMed]
5. Brasil. Instrução Normativa no 41 de 17 de Setembro de 2019. Estabelece o Padrão de Identidade e Qualidade da Kombucha em Todo o Território Nacional, na Forma Desta Instrução Normativa e do seu Anexo. 2019. Available online: https://abkom.org.br/wp-content/uploads/2021/05/INSTRUCAO-NORMATIVA-No-41-DE-17-DE-SETEMBRO-DE-2019-INSTRUCAO-NORMATIVA-No-41-DE-17-DE-SETEMBRO-DE-2019-DOU-Imprensa-Nacional.pdf (accessed on 13 February 2024).
6. Villarreal-Soto, S.A.; Beaufort, S.; Bouajila, J.; Souchard, J.; Taillandier, P. Understanding Kombucha Tea Fermentation: A Review. *J. Food Sci.* **2018**, *83*, 580–588. [CrossRef]
7. Cardoso, R.R.; Neto, R.O.; dos Santos D'Almeida, C.T.; do Nascimento, T.P.; Pressete, C.G.; Azevedo, L.; Martino, H.S.D.; Cameron, L.C.; Ferreira, M.S.L.; de Barros, F.A.R. Kombuchas from Green and Black Teas Have Different Phenolic Profile, Which Impacts Their Antioxidant Capacities, Antibacterial and Antiproliferative Activities. *Food Res. Int.* **2020**, *128*, 108782. [CrossRef]
8. de Noronha, M.C.; Cardoso, R.R.; dos Santos D'Almeida, C.T.; do Carmo, M.A.V.; Azevedo, L.; Maltarollo, V.G.; Júnior, J.I.R.; Eller, M.R.; Cameron, L.C.; Ferreira, M.S.L.; et al. Black Tea Kombucha: Physicochemical, Microbiological and Comprehensive Phenolic Profile Changes during Fermentation, and Antimalarial Activity. *Food Chem.* **2022**, *384*, 132515. [CrossRef] [PubMed]
9. Jayabalan, R.; Subathradevi, P.; Marimuthu, S.; Sathishkumar, M.; Swaminathan, K. Changes in Free-Radical Scavenging Ability of Kombucha Tea during Fermentation. *Food Chem.* **2008**, *109*, 227–234. [CrossRef]
10. Cardoso, R.R.; Moreira, L.D.P.D.; De Campos Costa, M.A.; Toledo, R.C.L.; Grancieri, M.; Nascimento, T.P.D.; Ferreira, M.S.L.; Da Matta, S.L.P.; Eller, M.R.; Duarte Martino, H.S.; et al. Kombuchas from Green and Black Teas Reduce Oxidative Stress, Liver Steatosis and Inflammation, and Improve Glucose Metabolism in Wistar Rats Fed a High-Fat High-Fructose Diet. *Food Funct.* **2021**, *12*, 10813–10827. [CrossRef] [PubMed]

11. de Costa, M.A.C.; de Vilela, D.L.S.; Fraiz, G.M.; Lopes, I.L.; Coelho, A.I.M.; Castro, L.C.V.; Martin, J.G.P. Effect of Kombucha Intake on the Gut Microbiota and Obesity-Related Comorbidities: A Systematic Review. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 3851–3866. [CrossRef] [PubMed]
12. Costa, M.A.; Dias Moreira, L.D.; Duarte, V.D.; Cardoso, R.R.; São José, V.P.; Silva, B.P.; Grancieri, M.; Corich, V.; Giacomini, A.; Bressan, J.; et al. Kombuchas from Green and Black Tea Modulate the Gut Microbiota and Improve the Intestinal Health of Wistar Rats Fed a High-Fat High-Fructose Diet. *Nutrients* **2022**, *14*, 5234. [CrossRef] [PubMed]
13. IAL-Instituto Adolfo Lutz (São Paulo, Brasil). *Métodos Físico-Químicos Para Análise de Alimentos*, 4 edição (1a edição online); IAL: São Paulo, Brazil, 2008.
14. Cockerill, F.R.; Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard*, 9th ed.; CLSI document; v. 32, no. 2; M07-A9 = v. 32, no. 2; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012.
15. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144. [CrossRef]
16. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
17. Escher, G.B.; Santos, J.S.; Rosso, N.D.; Marques, M.B.; Azevedo, L.; do Carmo, M.A.V.; Daguer, H.; Molognoni, L.; do Prado-Silva, L.; Sant'Ana, A.S.; et al. Chemical Study, Antioxidant, Anti-Hypertensive, and Cytotoxic/Cytoprotective Activities of *Centaurea cyanus* L. Petals Aqueous Extract. *Food Chem. Toxicol.* **2018**, *118*, 439–453. [CrossRef] [PubMed]
18. Boechat, N.; Ferreira, M.D.L.G.; Pinheiro, L.C.S.; Jesus, A.M.L.; Leite, M.M.M.; Júnior, C.C.S.; Aguiar, A.C.C.; De Andrade, I.M.; Krettli, A.U. New Compounds Hybrids 1 *H*-1,2,3-Triazole-Quinoline Against *Plasmodium falciparum*. *Chem. Biol. Drug Des.* **2014**, *84*, 325–332. [CrossRef] [PubMed]
19. Liao, W.; Ning, Z.; Chen, L.; Wei, Q.; Yuan, E.; Yang, J.; Ren, J. Intracellular Antioxidant Detoxifying Effects of Diosmetin on 2,2-Azobis(2-Amidinopropane) Dihydrochloride (AAPH)-Induced Oxidative Stress through Inhibition of Reactive Oxygen Species Generation. *J. Agric. Food Chem.* **2014**, *62*, 8648–8654. [CrossRef]
20. do Carmo, M.A.V.; Fidelis, M.; Sanchez, C.A.; Castro, A.P.; Camps, I.; Colombo, F.A.; Marques, M.J.; Myoda, T.; Granato, D.; Azevedo, L. Camu-Camu (*Myrciaria dubia*) Seeds as a Novel Source of Bioactive Compounds with Promising Antimalarial and Antischistosomicidal Properties. *Food Res. Int.* **2020**, *136*, 109334. [CrossRef]
21. Keiser, M.J.; Roth, B.L.; Armbruster, B.N.; Ernsberger, P.; Irwin, J.J.; Shoichet, B.K. Relating Protein Pharmacology by Ligand Chemistry. *Nat. Biotechnol.* **2007**, *25*, 197–206. [CrossRef]
22. Adnan, M.; Jeon, B.-B.; Chowdhury, M.H.; Oh, K.-K.; Das, T.; Chy, M.N.; Cho, D.-H. Network Pharmacology Study to Reveal the Potentiality of a Methanol Extract of *Caesalpinia sappan* L. Wood against Type-2 Diabetes Mellitus. *Life* **2022**, *12*, 277. [CrossRef] [PubMed]
23. Azevedo, L.; Serafim, M.S.M.; Maltarollo, V.G.; Grabrucker, A.M.; Granato, D. Atherosclerosis Fate in the Era of Tailored Functional Foods: Evidence-Based Guidelines Elicited from Structure- and Ligand-Based Approaches. *Trends Food Sci. Technol.* **2022**, *128*, 75–89. [CrossRef]
24. Ma, Y.; Ma, X.; An, Y.; Sun, Y.; Dou, W.; Li, M.; Bao, H.; Zhang, C. Green Tea Polyphenols Alleviate Hydrogen Peroxide-Induced Oxidative Stress, Inflammation, and Apoptosis in Bovine Mammary Epithelial Cells by Activating ERK1/2–NFE2L2–HMOX1 Pathways. *Front. Vet. Sci.* **2022**, *8*, 804241. [CrossRef]
25. Paes, L.T.; dos D'Almeida, C.T.S.; do Carmo, M.A.V.; da Silva Cruz, L.; de Souza, A.B.; Viana, L.M.; Gonçalves Maltarollo, V.; Martino, H.S.D.; Domingues de Almeida Lima, G.; Larraz Ferreira, M.S.; et al. Phenolic-Rich Extracts from Toasted White and Tannin Sorghum Flours Have Distinct Profiles Influencing Their Antioxidant, Antiproliferative, Anti-Adhesive, Anti-Invasive, and Antimalarial Activities. *Food Res. Int.* **2024**, *176*, 113739. [CrossRef] [PubMed]
26. Serafim, M.S.M.; Lavorato, S.N.; Kronenberger, T.; Sousa, Y.V.; Oliveira, G.P.; Dos Santos, S.G.; Kroon, E.G.; Maltarollo, V.G.; Alves, R.J.; Mota, B.E.F. Antibacterial Activity of Synthetic 1,3-bis(Aryloxy)Propan-2-amines against Gram-positive Bacteria. *MicrobiologyOpen* **2019**, *8*, e814. [CrossRef] [PubMed]
27. Jayabalan, R.; Malbaša, R.V.; Lončar, E.S.; Vitas, J.S.; Sathishkumar, M. A Review on Kombucha Tea—Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Comp. Rev. Food Sci. Food Saf.* **2014**, *13*, 538–550. [CrossRef]
28. Dartora, B.; Hickert, L.R.; Fabricio, M.F.; Ayub, M.A.Z.; Furlan, J.M.; Wagner, R.; Perez, K.J.; Sant'Anna, V. Understanding the Effect of Fermentation Time on Physicochemical Characteristics, Sensory Attributes, and Volatile Compounds in Green Tea Kombucha. *Food Res. Int.* **2023**, *174*, 113569. [CrossRef]
29. Wang, B.; Rutherfurd-Markwick, K.; Zhang, X.-X.; Mutukumira, A.N. Kombucha: Production and Microbiological Research. *Foods* **2022**, *11*, 3456. [CrossRef]
30. Dufresne, C.; Farnworth, E. Tea, Kombucha, and Health: A Review. *Food Res. Int.* **2000**, *33*, 409–421. [CrossRef]
31. Ivanišová, E.; Meňhartová, K.; Terentjeva, M.; Harangozo, Ľ.; Kántor, A.; Kačániová, M. The Evaluation of Chemical, Antioxidant, Antimicrobial and Sensory Properties of Kombucha Tea Beverage. *J. Food Sci. Technol.* **2020**, *57*, 1840–1846. [CrossRef] [PubMed]

32. Barros Santos, M.C.; da Silva Lima, L.R.; Ramos Nascimento, F.; do Nascimento, T.P.; Cameron, L.C.; Simões Larraz Ferreira, M. Metabolomic Approach for Characterization of Phenolic Compounds in Different Wheat Genotypes during Grain Development. *Food Res. Int.* **2019**, *124*, 118–128. [CrossRef] [PubMed]
33. Andreson, M.; Kazantseva, J.; Kuldjärv, R.; Malv, E.; Vaikma, H.; Kaleda, A.; Kütt, M.-L.; Vilu, R. Characterisation of Chemical, Microbial and Sensory Profiles of Commercial Kombuchas. *Int. J. Food Microbiol.* **2022**, *373*, 109715. [CrossRef] [PubMed]
34. Neffe-Skocińska, K.; Sionek, B.; Ścibisz, I.; Kołożyn-Krajewska, D. Acid Contents and the Effect of Fermentation Condition of Kombucha Tea Beverages on Physicochemical, Microbiological and Sensory Properties. *CyTA—J. Food* **2017**, *15*, 601–607. [CrossRef]
35. Antolak, H.; Piechota, D.; Kucharska, A. Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). *Antioxidants* **2021**, *10*, 1541. [CrossRef]
36. Mizuta, A.G.; de Menezes, J.L.; Dutra, T.V.; Ferreira, T.V.; Castro, J.C.; da Silva, C.A.J.; Pilau, E.J.; Machinski Junior, M.; de Abreu Filho, B.A. Evaluation of Antimicrobial Activity of Green Tea Kombucha at Two Fermentation Time Points against *Alicyclobacillus* spp. *LWT* **2020**, *130*, 109641. [CrossRef]
37. Vázquez-Cabral, B.D.; Larrosa-Pérez, M.; Gallegos-Infante, J.A.; Moreno-Jiménez, M.R.; González-Laredo, R.F.; Rutiaga-Quiñones, J.G.; Gamboa-Gómez, C.I.; Rocha-Guzmán, N.E. Oak Kombucha Protects against Oxidative Stress and Inflammatory Processes. *Chem.-Biol. Interact.* **2017**, *272*, 1–9. [CrossRef] [PubMed]
38. Dinh, T.C.; Thi Phuong, T.N.; Minh, L.B.; Minh Thuc, V.T.; Bac, N.D.; Van Tien, N.; Pham, V.H.; Show, P.L.; Tao, Y.; Nhu Ngoc, V.T.; et al. The Effects of Green Tea on Lipid Metabolism and Its Potential Applications for Obesity and Related Metabolic Disorders—An Existing Update. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2019**, *13*, 1667–1673. [CrossRef]
39. Ahn, J.-W.; Kim, S.; Ko, S.; Kim, Y.-H.; Jeong, J.-H.; Chung, S. Modified (−)-Gallocatechin Gallate-Enriched Green Tea Extract Rescues Age-Related Cognitive Deficits by Restoring Hippocampal Synaptic Plasticity. *Biochem. Biophys. Rep.* **2022**, *29*, 101201. [CrossRef] [PubMed]
40. Wu, X.; Zhang, G.; Hu, M.; Pan, J.; Li, A.; Zhang, Y. Molecular Characteristics of Gallocatechin Gallate Affecting Protein Glycation. *Food Hydrocoll.* **2020**, *105*, 105782. [CrossRef]
41. Dutta, A.; Dahiya, A. Quercetin 3-O Rutinoside Prevents Gastrointestinal Injury through Regulation of Apoptosis in 7.5 Gy Total Body Irradiated Mice. *Phytomedicine* **2023**, *112*, 154692. [CrossRef] [PubMed]
42. Sharma, S.; Dahiya, A.; Kumar, S.; Verma, Y.K.; Dutta, A. Quercetin 3-O-Rutinoside Prevents Radiation Induced Oxidative Damage and Inflammation by Coordinated Regulation of Nrf2/NF-κB/NLRP3-Inflammasome Signaling in Gastrointestine. *Phytomed. Plus* **2023**, *3*, 100385. [CrossRef]
43. Al-Mohammadi, A.-R.; Ismaiel, A.A.; Ibrahim, R.A.; Moustafa, A.H.; Abou Zeid, A.; Enan, G. Chemical Constitution and Antimicrobial Activity of Kombucha Fermented Beverage. *Molecules* **2021**, *26*, 5026. [CrossRef]
44. Barbosa, C.D.; Trovatti Uetanabaro, A.P.; Rodrigues Santos, W.C.; Caetano, R.G.; Albano, H.; Kato, R.; Cosenza, G.P.; Azeredo, A.; Góes-Neto, A.; Rosa, C.A.; et al. Microbial–Physicochemical Integrated Analysis of Kombucha Fermentation. *LWT* **2021**, *148*, 111788. [CrossRef]
45. Bhattacharya, D.; Bhattacharya, S.; Patra, M.M.; Chakravorty, S.; Sarkar, S.; Chakraborty, W.; Koley, H.; Gachhui, R. Antibacterial Activity of Polyphenolic Fraction of Kombucha Against Enteric Bacterial Pathogens. *Curr. Microbiol.* **2016**, *73*, 885–896. [CrossRef] [PubMed]
46. Bhattacharya, D.; Ghosh, D.; Bhattacharya, S.; Sarkar, S.; Karmakar, P.; Koley, H.; Gachhui, R. Antibacterial Activity of Polyphenolic Fraction of Kombucha against Vibrio Cholerae: Targeting Cell Membrane. *Lett. Appl. Microbiol.* **2018**, *66*, 145–152. [CrossRef] [PubMed]
47. Velićanski, A.S.; Cvetković, D.D.; Markov, S.L.; Šaponjac, V.T.; Vulić, J.J. Antioxidant and Antibacterial Activity of the Beverage Obtained by Fermentation of Sweetened Lemon Balm (*Melissa officinalis* L.) Tea with Symbiotic Consortium of Bacteria and Yeasts. *Food. Technol. Biotechnol.* **2014**, *52*, 420–429. [CrossRef] [PubMed]
48. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
49. Instituto Nacional do Câncer (Brasil). *Estimativa 2023: Incidência de Câncer no Brasil*; Instituto Nacional do Câncer: Rio de Janeiro, Brazil, 2023.
50. Yang, H.; Wang, M.; Sun, H.; Zhu, S.; Jin, J. Synergetic Effect of EP_1 Receptor Antagonist and (-)-Epigallocatechin-3-Gallate in Hepatocellular Carcinoma. *Pharmacology* **2019**, *104*, 267–275. [CrossRef] [PubMed]
51. Geran, R.I.; Greenberg, N.H.; MacDonald, M.M.; Schumacher, A.M.; Abbot, B.J. Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and Other Biological Systems. *Cancer Chemother* **1972**, *3*, 51–61.
52. Di Nunzio, M.; Valli, V.; Tomás-Cobos, L.; Tomás-Chisbert, T.; Murgui-Bosch, L.; Danesi, F.; Bordoni, A. Is Cytotoxicity a Determinant of the Different in Vitro and in Vivo Effects of Bioactives? *BMC Complement. Altern. Med.* **2017**, *17*, 453. [CrossRef]

53. Kaewkod, T.; Bovonsombut, S.; Tragoolpua, Y. Efficacy of Kombucha Obtained from Green, Oolong, and Black Teas on Inhibition of Pathogenic Bacteria, Antioxidation, and Toxicity on Colorectal Cancer Cell Line. *Microorganisms* **2019**, *7*, 700. [CrossRef] [PubMed]
54. Srihari, T.; Arunkumar, R.; Arunakaran, J.; Satyanarayana, U. Downregulation of Signalling Molecules Involved in Angiogenesis of Prostate Cancer Cell Line (PC-3) by Kombucha (Lyophilized). *Biomed. Prev. Nutr.* **2013**, *3*, 53–58. [CrossRef]
55. Duan, N.; Hu, X.; Zhou, R.; Li, Y.; Wu, W.; Liu, N. A Review on Dietary Flavonoids as Modulators of the Tumor Microenvironment. *Mol. Nutr. Food Res.* **2023**, *67*, 2200435. [CrossRef] [PubMed]
56. Cheng, Z.; Zhang, Z.; Han, Y.; Wang, J.; Wang, Y.; Chen, X.; Shao, Y.; Cheng, Y.; Zhou, W.; Lu, X.; et al. A Review on Anti-Cancer Effect of Green Tea Catechins. *J. Funct. Foods* **2020**, *74*, 104172. [CrossRef]
57. Saeki, K.; Hayakawa, S.; Isemura, M.; Miyase, T. Importance of a Pyrogallol-Type Structure in Catechin Compounds for Apoptosis-Inducing Activity. *Phytochemistry* **2000**, *53*, 391–394. [CrossRef] [PubMed]
58. Almatroodi, S.A.; Almatroudi, A.; Khan, A.A.; Alhumaydhi, F.A.; Alsahli, M.A.; Rahmani, A.H. Potential Therapeutic Targets of Epigallocatechin Gallate (EGCG), the Most Abundant Catechin in Green Tea, and Its Role in the Therapy of Various Types of Cancer. *Molecules* **2020**, *25*, 3146. [CrossRef] [PubMed]
59. Nasti, R.; Rossi, D.; Amadio, M.; Pascale, A.; Unver, M.Y.; Hirsch, A.K.H.; Collina, S. Compounds Interfering with Embryonic Lethal Abnormal Vision (ELAV) Protein–RNA Complexes: An Avenue for Discovering New Drugs. *J. Med. Chem.* **2017**, *60*, 8257–8267. [CrossRef] [PubMed]
60. Sarfraz, I.; Rasul, A.; Hussain, G.; Shah, M.A.; Zahoor, A.F.; Asrar, M.; Selamoglu, Z.; Ji, X.; Adem, Ş.; Sarker, S.D. 6-Phosphogluconate Dehydrogenase Fuels Multiple Aspects of Cancer Cells: From Cancer Initiation to Metastasis and Chemoresistance. *BioFactors* **2020**, *46*, 550–562. [CrossRef]
61. Costa, A.F.; Campos, D.; Reis, C.A.; Gomes, C. Targeting Glycosylation: A New Road for Cancer Drug Discovery. *Trends Cancer* **2020**, *6*, 757–766. [CrossRef] [PubMed]
62. Wang, P.H. Altered glycosylation in cancer: Sialic acids and sialyltransferases. *J. Cancer Mol.* **2005**, *1*, 73–81.
63. Cheng, L.; Luo, S.; Jin, C.; Ma, H.; Zhou, H.; Jia, L. FUT Family Mediates the Multidrug Resistance of Human Hepatocellular Carcinoma via the PI3K/Akt Signaling Pathway. *Cell Death Dis.* **2013**, *4*, e923. [CrossRef]
64. Sjöstedt, E.; Zhong, W.; Fagerberg, L.; Karlsson, M.; Mitsios, N.; Adori, C.; Oksvold, P.; Edfors, F.; Limiszewska, A.; Hikmet, F.; et al. An Atlas of the Protein-Coding Genes in the Human, Pig, and Mouse Brain. *Science* **2020**, *367*, eaay5947. [CrossRef]
65. ST3GAL3 Protein Expression Summary—The Human Protein Atlas. Available online: https://www.proteinatlas.org/ENSG00000126091-ST3GAL (accessed on 2 October 2024).
66. Yang, G.-J.; Tao, F.; Zhong, H.-J.; Yang, C.; Chen, J. Targeting PGAM1 in Cancer: An Emerging Therapeutic Opportunity. *Eur. J. Med. Chem.* **2022**, *244*, 114798. [CrossRef] [PubMed]
67. Potter, C.P.S.; Harris, A.L. Diagnostic, Prognostic and Therapeutic Implications of Carbonic Anhydrases in Cancer. *Br. J. Cancer* **2003**, *89*, 2–7. [CrossRef]
68. Mboge, M.Y.; Mahon, B.P.; McKenna, R.; Frost, S.C. Carbonic Anhydrases: Role in pH Control and Cancer. *Metabolites* **2018**, *8*, 19. [CrossRef]
69. Karioti, A.; Ceruso, M.; Carta, F.; Bilia, A.-R.; Supuran, C.T. New Natural Product Carbonic Anhydrase Inhibitors Incorporating Phenol Moieties. *Bioorganic Med. Chem.* **2015**, *23*, 7219–7225. [CrossRef] [PubMed]
70. Karioti, A.; Carta, F.; Supuran, C.T. Phenols and Polyphenols as Carbonic Anhydrase Inhibitors. *Molecules* **2016**, *21*, 1649. [CrossRef] [PubMed]
71. Schieber, M.; Chandel, N.S. ROS Function in Redox Signaling and Oxidative Stress. *Curr. Biol.* **2014**, *24*, R453–R462. [CrossRef]
72. Heleno, S.A.; Martins, A.; Queiroz, M.J.R.P.; Ferreira, I.C.F.R. Bioactivity of Phenolic Acids: Metabolites versus Parent Compounds: A Review. *Food Chem.* **2015**, *173*, 501–513. [CrossRef] [PubMed]
73. Queiroz, M.; Oppolzer, D.; Gouvinhas, I.; Silva, A.M.; Barros, A.I.R.N.A.; Domínguez-Perles, R. New Grape Stems' Isolated Phenolic Compounds Modulate Reactive Oxygen Species, Glutathione, and Lipid Peroxidation in Vitro: Combined Formulations with Vitamins C and E. *Fitoterapia* **2017**, *120*, 146–157. [CrossRef] [PubMed]
74. Merry, T.L.; Ristow, M. Nuclear Factor Erythroid-derived 2-like 2 (NFE2L2, Nrf2) Mediates Exercise-induced Mitochondrial Biogenesis and the Anti-oxidant Response in Mice. *J. Physiol.* **2016**, *594*, 5195–5207. [CrossRef] [PubMed]
75. Nobari, H.; Saedmocheshi, S.; Chung, L.H.; Suzuki, K.; Maynar-Mariño, M.; Pérez-Gómez, J. An Overview on How Exercise with Green Tea Consumption Can Prevent the Production of Reactive Oxygen Species and Improve Sports Performance. *Int. J. Environ. Res. Public Health* **2022**, *19*, 218. [CrossRef]
76. Halliwell, B. Free Radicals and Antioxidants: Updating a Personal View. *Nutr. Rev.* **2012**, *70*, 257–265. [CrossRef] [PubMed]
77. Costa, R.M.; Magalhães, A.S.; Pereira, J.A.; Andrade, P.B.; Valentão, P.; Carvalho, M.; Silva, B.M. Evaluation of Free Radical-Scavenging and Antihemolytic Activities of Quince (*Cydonia oblonga*) Leaf: A Comparative Study with Green Tea (*Camellia sinensis*). *Food Chem. Toxicol.* **2009**, *47*, 860–865. [CrossRef] [PubMed]

78. Chaudhuri, S.; Banerjee, A.; Basu, K.; Sengupta, B.; Sengupta, P.K. Interaction of Flavonoids with Red Blood Cell Membrane Lipids and Proteins: Antioxidant and Antihemolytic Effects. *Int. J. Biol. Macromol.* **2007**, *41*, 42–48. [CrossRef] [PubMed]
79. Zheng, Q.; Tan, W.; Feng, X.; Feng, K.; Zhong, W.; Liao, C.; Liu, Y.; Li, S.; Hu, W. Protective Effect of Flavonoids from Mulberry Leaf on AAPH-Induced Oxidative Damage in Sheep Erythrocytes. *Molecules* **2022**, *27*, 7625. [CrossRef]
80. Banerjee, A.; Kunwar, A.; Mishra, B.; Priyadarsini, K.I. Concentration Dependent Antioxidant/pro-Oxidant Activity of Curcumin: Studies from AAPH Induced Hemolysis of RBCs. *Chem.-Biol. Interact.* **2008**, *174*, 134–139. [CrossRef] [PubMed]
81. Das, D.; Sen, S.; Sen, K. Caffeine and Catechin towards Prevention of Drug Induced Oxidation of Hemoglobin: A Spectroscopic Study. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2020**, *232*, 118167. [CrossRef] [PubMed]
82. Grinberg, L.N.; Newmark, H.; Kitrossky, N.; Rahamim, E.; Chevion, M.; Rachmilewitz, E.A. Protective Effects of Tea Polyphenols against Oxidative Damage to Red Blood Cells. *Biochem. Pharmacol.* **1997**, *54*, 973–978. [CrossRef] [PubMed]
83. Boniface, P.K.; Ferreira, E.I. Flavonoids as Efficient Scaffolds: Recent Trends for Malaria, Leishmaniasis, Chagas Disease, and Dengue. *Phytother. Res.* **2019**, *33*, 2473–2517. [CrossRef] [PubMed]
84. Lima, R.B.S.; Rocha E Silva, L.F.; Melo, M.R.S.; Costa, J.S.; Picanço, N.S.; Lima, E.S.; Vasconcellos, M.C.; Boleti, A.P.A.; Santos, J.M.P.; Amorim, R.C.N.; et al. In Vitro and in Vivo Anti-Malarial Activity of Plants from the Brazilian Amazon. *Malar. J.* **2015**, *14*, 508. [CrossRef] [PubMed]
85. Puttappa, N.; Kumar, R.S.; Yamjala, K. Artesunate-Quercetin/Luteolin Dual Drug Nanofacilitated Synergistic Treatment for Malaria: A Plausible Approach to Overcome Artemisinin Combination Therapy Resistance. *Med. Hypotheses* **2017**, *109*, 176–180. [CrossRef] [PubMed]
86. Platon, L.; Ménard, D. Plasmodium Falciparum Ring-Stage Plasticity and Drug Resistance. *Trends Parasitol.* **2024**, *40*, 118–130. [CrossRef] [PubMed]
87. Devi, G.; Gorki, V.; Walter, N.S.; Sivangula, S.; Sobhia, M.E.; Jachak, S.; Puri, R.; Kaur, S. Exploring the Efficacy of Ethnomedicinal Plants of Himalayan Region against the Malaria Parasite. *J. Ethnopharmacol.* **2024**, *321*, 117394. [CrossRef] [PubMed]
88. Counihan, N.A.; Chernih, H.C.; de Koning-Ward, T.F. Post-Translational Lipid Modifications in Plasmodium Parasites. *Curr. Opin. Microbiol.* **2022**, *69*, 102196. [CrossRef] [PubMed]
89. Abdulah, R.; Suradji, E.; Subarnas, A.; Supratman, U.; Sugijanto, M.; Diantini, A.; Lestari, K.; Barliana, M.; Kawazu, S.; Koyama, H. Catechin Isolated from Garcinia Celebica Leaves Inhibit Plasmodium Falciparum Growth through the Induction of Oxidative Stress. *Phcog. Mag.* **2017**, *13*, 301. [CrossRef] [PubMed]
90. Patil, P.R.; Gemma, S.; Campiani, G.; Craig, A.G. Broad Inhibition of Plasmodium Falciparum Cytoadherence by (+)-Epigallocatechin Gallate. *Malar. J.* **2011**, *10*, 348. [CrossRef] [PubMed]
91. Wang, X.; Tian, W. Green Tea Epigallocatechin Gallate: A Natural Inhibitor of Fatty-Acid Synthase. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1200–1206. [CrossRef] [PubMed]
92. Wang, Y.; Xiao, J.; Suzek, T.O.; Zhang, J.; Wang, J.; Zhou, Z.; Han, L.; Karapetyan, K.; Dracheva, S.; Shoemaker, B.A.; et al. PubChem's BioAssay Database. *Nucleic Acids Res.* **2012**, *40*, D400–D412. [CrossRef] [PubMed]
93. Rout, S.; Mahapatra, R.K. Plasmodium falciparum: Multidrug resistance. *Chem. Biol. Drug Des.* **2019**, *93*, 737–759. [CrossRef] [PubMed]
94. Sivaraman, K.K.; Oellig, C.A.; Huynh, K.; Atkinson, S.C.; Poreba, M.; Perugini, M.A.; Trenholme, K.R.; Gardiner, D.L.; Salvesen, G.; Drag, M.; et al. X-Ray Crystal Structure and Specificity of the Plasmodium Falciparum Malaria Aminopeptidase PfM18AAP. *J. Mol. Biol.* **2012**, *422*, 495–507. [CrossRef] [PubMed]