Palestinian Medical and Pharmaceutical Journal

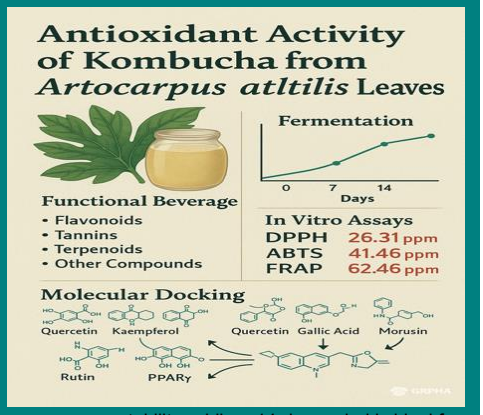
Antioxidant Activities of *Artocarpus altilis* Leaf Kombucha: DPPH, ABTS, FRAP Assays and Potential Phytochemical Targets

Muhammad Andry[1], Tiara Ramadaini[1], Shaum Shiyan[1], Muhammad Amin Nasution[2] & Muhammad Fauzan Lubis[3*]

(Type: Full Article). Received: 11st Aug. 2025. Accepted: 15th Mar. 2026, Published: ××××, DOI: ××××

Accepted Manuscript, In Press

Abstract: **Objective:** This study aimed to investigate the antioxidant properties of kombucha prepared from *Artocarpus altilis* (breadfruit) leaves across various fermentation durations (0, 7, 10, and 14 days) and to elucidate the molecular interactions between its key phytochemicals and essential antioxidant-regulating proteins. **Methodology:** Kombucha was produced under standardized conditions after performing phytochemical screening and pharmacognostic evaluation of the dried leaf powder. The antioxidant capacity was assessed using three complementary in vitro assays: DPPH, ABTS, and FRAP. Additionally, in silico molecular docking analysis was conducted to determine the binding affinities of principal phytochemicals (quercetin, kaempferol, gallic acid, morusin, and rutin) with major antioxidant-regulating targets, including Keap1, Nrf2, and PPARγ. **Key Results:** Antioxidant activity progressively enhanced with fermentation time, with the 14-day sample exhibiting the highest potency (IC_{50} values: 26.31 ppm for DPPH, 41.46 ppm for ABTS, and 62.46 ppm for FRAP), which is comparable to ascorbic acid. Phytochemical profiling identified flavonoids, tannins, and terpenoids as the primary bioactive constituents. Molecular docking results showed strong binding affinities between the identified compounds and Keap1, Nrf2, and PPARγ, supporting a multi-target mechanistic role in mitigating oxidative stress. **Conclusions:** *A. altilis* leaf kombucha serves as a potent multifunctional antioxidant beverage that effectively integrates traditional herbal medicine with modern fermentation science and computational validation. The fermentation process significantly boosts the bioactive potential of the substrate. **Recommendations:** A 7-day fermentation period is recommended for commercial products to balance antioxidant benefits with sensory acceptability, while a 14-day period is ideal for maximum potency. Future research should include in vivo validation and molecular dynamics simulations to further verify these mechanistic insights.



Keywords: *A. altilis*, Antioxidant, Molecular docking, Kombucha.

Introduction

In recent years, the heightened emphasis on preventive healthcare and functional nutrition has significantly increased the global market for natural antioxidant products sourced from traditional medicinal plants. Kombucha, a fermented tea created through the symbiotic interaction of acetic acid bacteria and yeasts (SCOBY), has garnered significant scientific and public interest among numerous functional beverages. Its appeal arises from a wide array of documented health-enhancing attributes, including robust antioxidant, antibacterial, and detoxifying effects. Traditional kombucha is produced using Camellia sinensis (black or green tea), but modern advancements have diversified its formulation to incorporate several herbal bases, hence improving its bioactive properties and broadening its functional capabilities [1-5].

A. altilis (breadfruit) is a notably promising substrate, with its leaves historically employed in ethnomedicine for addressing hypertension, inflammation, and several metabolic diseases. Phytochemical analyses have revealed that A. altilis leaves are rich in bioactive secondary metabolites, such as flavonoids, phenolic acids, tannins, and terpenoids, many of which are acknowledged for their significant antioxidant properties. These natural antioxidants are essential in mitigating oxidative stress, a significant contributor to the initiation and advancement of chronic non-communicable diseases, including cardiovascular disorders, cancer, and neurological problems. Notwithstanding its extensive phytochemical composition and established pharmacological significance, the potential of A. altilis as a fermentation substrate for the creation of functional antioxidant drinks remains significantly underinvestigated, thus offering a noteworthy chance for scientific advancement [5-8].

Oxidative stress, an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses, is a significant contributor to the development of various chronic diseases. Antioxidants originating from plants, especially those included in fermented functional beverages like kombucha, are increasingly acknowledged as safe and efficient natural substitutes for synthetic antioxidant chemicals. These bioactive compounds are essential for neutralizing free radicals, maintaining cellular redox equilibrium, and reducing oxidative damage. Thus, they possess significant promise in mitigating the risk and advancement of disorders associated with oxidative stress, encompassing metabolic, cardiovascular, and neurodegenerative diseases [6-7].

The antioxidant capacity of kombucha can be thoroughly evaluated using many in vitro assays, each representing a

1 Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya, South Sumatra 30662, Indonesia.
2 Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia.
3. Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia.
*Corresponding author: fauzan.lubis@usu.ac.id

unique mechanism of antioxidant activity. Frequently utilized techniques encompass the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, the ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) assay, and the FRAP (ferric reducing antioxidant power) assay. These complimentary methodologies assess hydrogen-donating capacity, electron transfer potential, and ferric ion reduction ability, therefore offering a comprehensive understanding of kombucha's overall antioxidant efficacy throughout fermentation [8-9].

Molecular docking has emerged as a potent in silico method for revealing the interactions between phytochemicals and essential antioxidant-regulating proteins, surpassing traditional experimental experiments. Numerous bioactive chemicals found in A. altilis leaves, including quercetin, kaempferol, gallic acid, morusin, and rutin, exhibit significant binding affinities for essential antioxidant-related targets such as Keap1, Nrf2, NQO1, PPARγ, and TP53. These proteins are essential for sustaining cellular redox equilibrium, regulating inflammation, and influencing cell survival and death pathways, thereby acting as key molecular targets in the prevention and management of illnesses associated with oxidative stress. Molecular docking offers important mechanistic insights; nevertheless, additional in vitro and in vivo validation investigations, including molecular dynamics simulations, are necessary to verify the stability and biological significance of these interactions [10–12].

Notwithstanding the well reported pharmacological potential of A. altilis, a considerable research gap remains in investigations that combine experimental antioxidant profiling of fermented products with in silico mechanistic analysis. Prior research has predominantly concentrated on either the phytochemical characterisation or the molecular docking simulations of single chemicals, neglecting their synergistic interactions within intricate fermentation matrices. Moreover, research integrating multi-assay antioxidant assessment with molecular insights into the relationships between phytochemicals and antioxidant-regulating proteins is limited. Addressing this gap is crucial for clarifying how fermentation dynamically alters bioactive molecule profiles and improves their functional efficacy, hence improving a comprehensive understanding of the biochemical and mechanistic foundations of antioxidant activity in A. altilis kombucha [13,14].

This study aimed to fill the noted research gap by assessing the antioxidant activity of A. altilis leaf kombucha using three complementing in vitro assays: DPPH, ABTS, and FRAP, throughout various fermentation durations (0, 7, 10, and 14 days). A literature-based molecular docking analysis was concurrently conducted to clarify the probable interactions between principal A. altilis phytochemicals and essential antioxidant-regulating proteins. This research integrates experimental antioxidant data with in silico mechanistic insights to develop a comprehensive framework for understanding the impact of fermentation on bioactive chemical behavior and antioxidant performance. The findings seek to strengthen the scientific basis for the development of A. altilis-based kombucha as a validated functional beverage and to offer mechanistic evidence supporting its potential use in formulating plant-derived nutraceuticals for contemporary preventive healthcare [15,16].

Materials and Methods

Materials

Dried leaves of A. altilis (breadfruit) were collected from Medan, North Sumatra, Indonesia, and taxonomically authenticated at the Herbarium Medanese, Department of Biology, Universitas Sumatera Utara (Specimen ID: HM-USU-2025-ALTLIS). The leaves were harvested during the dry season to ensure optimal phytochemical stability and concentration.

All reagents and chemicals used in this study were of analytical grade. For antioxidant assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), and 2,4,6-tripyridyl-s-triazine (TPTZ) were procured from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid was employed as the standard reference antioxidant. Ethanol (96%) was used as the extraction and dilution solvent, while sucrose and distilled water served as the carbon source and aqueous medium, respectively, for kombucha fermentation.

The kombucha starter culture (SCOBY), comprising acetic acid bacteria and yeasts in a symbiotic association, was obtained from a certified Indonesian kombucha supplier. Prior to use, the SCOBY was microbiologically tested to confirm its viability and fermentative activity.

Methods

Preparation of *A. altilis* Leaf Powder

Five kilograms of fresh *A. altilis* leaves were meticulously rinsed under running tap water to eliminate dust and surface contaminants. The cleaned leaves were air-dried in the shade for about 12 hours to remove surface moisture, then oven-dried at 45–50 °C until a steady weight was attained. The desiccated substance was subsequently coarsely crushed and finely pulverized with a mechanical grinder to get a uniform powder. The resultant powder was filtered using a 40-mesh sieve to achieve uniform particle size and subsequently stored in clean, airtight amber glass containers at room temperature, shielded from light and moisture until further utilization [17,18].

Pharmacognostic Evaluation of Simplicia

Pharmacognostic characterization was conducted in accordance with the guidelines established in the Indonesian Herbal Pharmacopoeia and conventional pharmacognostic methods to evaluate the quality and identity of the leaf powder. The assessed parameters were organoleptic features (color, odor, and texture), macroscopic and microscopic characteristics (powder microscopy), moisture content (loss on drying), total ash, acid-insoluble ash, and extractive values (water-soluble and ethanol-soluble).

Procedures were conducted as follows:

1. Organoleptic and macroscopic/microscopic analysis: Color, odor, and texture were documented by professional evaluators. Macroscopic characteristics were recorded (leaf morphology, venation, border, surface texture). Powder microscopy was conducted on cleared and stained slides utilizing a light microscope (×40–400) to identify diagnostic cells, trichomes, stomata, and additional anatomical features. Photomicrographs were obtained for documentation.
2. Loss on drying (moisture content): Ascertained via the gravimetric method. Approximately 2–5 g of powdered material was desiccated in an oven at 105 °C until a consistent weight was achieved; the percentage loss was determined in relation to the initial weight.
3. Total ash: An accurately weighed sample (about 2–3 g) was burned in a muffle furnace at 500–600 °C until white ash was produced, and then weighed; total ash was expressed as a percentage by weight of the original sample.
4. Acid-insoluble ash: The ash obtained was subjected to boiling with 25 mL of 2 N HCl for 5 minutes. The insoluble residue was collected on a Gooch or ashless filter paper,

cleaned, ignited, and subsequently weighed. Acid-insoluble ash was quantified as a percentage by weight (% w/w).

5. Extractive values: A specified mass of 5 g of powder was macerated with 100 mL of solvent for 24 hours, with intermittent agitation. An aliquot of the filtrate was evaporated to dryness and subsequently weighed. The extractive value was determined as a percentage by weight of the dried extract in relation to the original sample [19,20].

Phytochemical Screening

Qualitative Phytochemical Screening

A qualitative phytochemical analysis was conducted to identify the predominant groups of secondary metabolites in the dried A. altilis leaf extract. Standard phytochemical assays were performed in accordance with the methodologies outlined by Tiwari et al. (2011), Rauf et al. (2019), and Kaur et al. (2021), with slight adjustments to suit local laboratory circumstances.

The subsequent assays were employed:

1. Alkaloids: Identified with Dragendorff's and Mayer's reagents. The emergence of an orange-brown (Dragendorff's) or cream (Mayer's) precipitate signified a favorable reaction.
2. Flavonoids: Detected via the Shinoda test. The extract was subjected to magnesium turnings and a few drops of strong hydrochloric acid; the emergence of a pink to crimson hue onfirmed the presence of flavonoids.
3. Tannins: Verified by the ferric chloride ($FeCl_3$) assay. A dark blue, green, or blackish hue signified the presence of phenolic tannins.
4. Saponins: Assessed using the foaming test. The consistent generation of foam after vigorously shaking the aqueous extract for 30 seconds signified the presence of saponins.
5. Steroids and Terpenoids: Identified via the Liebermann and Burchard reaction. The introduction of acetic anhydride, succeeded by concentrated sulfuric acid, yielded a blue-green or reddish-brown hue, so affirming the existence of steroids or terpenoids, respectively.

All qualitative tests were conducted in triplicate to guarantee reproducibility, and the observations were compared with standard reference colorimetric changes documented in the literature [21,22].

Preparation of Kombucha Samples

Ten grams of A. altilis leaf powder were immersed in 1000 mL of hot distilled water (about 90 °C) for 15 minutes to create the herbal tea base. The infusion was filtered using Whatman No. 1 filter paper, and 100 g of sucrose was dissolved in the heated filtrate. The sugared solution was subsequently permitted to cool to 25–27 °C prior to inoculation. Subsequent to cooling, 100 mL of kombucha starter culture was aseptically introduced to the infusion under sterile circumstances. Fermentation was conducted in clean, wide-mouthed glass jars sealed with sterile muslin cloth to facilitate aeration while inhibiting contamination. The jars were stored undisturbed at ambient temperature (28 ± 2 °C) in darkness to reduce photodegradation of bioactive chemicals. Samples were extracted after 7 days (F7), 10 days (F10), and 14 days (F14) of fermentation. A non-fermented control (F0) was created utilizing the identical process, without the incorporation of SCOBY. All fermented and control samples were filtered, transferred to sterile bottles, and kept at 4 °C for subsequent analyses [24].

The SCOBY microbiota utilized in this work primarily comprised acetic acid bacteria, chiefly *Komagataeibacter xylinus*, and yeasts including *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. This symbiotic consortium is renowned for its involvement in sucrose hydrolysis, organic acid synthesis, and the formation of bioactive metabolites during fermentation. The yeast strains commence fermentation by transforming sucrose into glucose and fructose, generating ethanol, which is then oxidized by acetic acid bacteria into acetic acid and other organic acids. Furthermore, both microbial groups engage in the enzymatic biotransformation of polyphenolic glycosides into their more active aglycone derivatives. This transition improves the solubility, stability, and antioxidant efficacy of phenolic compounds, therefore leading to the noted enhancement in antioxidant activity with extended fermentation.

Organoleptic and pH Evaluation

Throughout fermentation, visual criteria such as color, aroma, and turbidity were assessed daily to track the advancement of biochemical alterations. The hue and turbidity of each sample were visually assessed under uniform lighting conditions, while alterations in fragrance were documented descriptively. For pH assessment, 5 mL of each kombucha sample was evaluated using universal pH indicator strips and compared to a defined color reference chart. All measures were conducted in triplicate, and data were presented as mean ± SD [25].

Antioxidant Assays

All antioxidant testing were conducted in triplicate to guarantee repeatability. Before analysis, kombucha samples were filtered using Whatman No. 1 filter paper to eliminate suspended particles and subsequently diluted with ethanol to achieve final concentrations between 100 and 500 ppm. Absorbance measurements were conducted using a UV–Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) using calibrated quartz cuvettes. Blank and standard solutions were produced under uniform circumstances for each experiment [26].

DPPH Radical Scavenging Assay

A 0.2 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was freshly produced in methanol before to utilization. For the assay, 1.0 mL of kombucha sample at different concentrations (100–500 ppm) was combined with 1.0 mL of the DPPH solution in a test tube. The mixture was gently vortexed and incubated in darkness at room temperature (25 ± 2 °C) for 30 minutes to avert photodegradation of DPPH radicals. Following incubation, absorbance was quantified at 517 nm utilizing methanol as a blank with a UV–Vis spectrophotometer [27].

ABTS Radical Cation Decolorization Assay

The ABTS radical cation was produced by combining equal volumes of 7.4 mM 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution and 2.6 mM potassium persulfate, then letting the mixture to incubate in the dark at ambient temperature (25 ± 2 °C) for 12–16 hours. The ABTS stock solution was further diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm.

For the experiment, 1.0 mL of the working ABTS solution was combined with 100 µL of kombucha sample at different concentrations (100–500 ppm). The mixture was gently vortexed and incubated in the dark at room temperature for 6 minutes to ensure reaction completion. Absorbance was measured at 734 nm utilizing a UV–Vis spectrophotometer, employing ethanol as a blank [28].

Ferric Reducing Antioxidant Power (FRAP) Assay

A new FRAP working solution was formulated by combining 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a

10:1:1 (v/v/v) ratio. The combination was freshly made and preheated to 37 °C prior to utilization.

For the test, 100 µL of kombucha sample at several concentrations (100–500 ppm) was combined with 3.0 mL of the FRAP working solution and incubated at 37 °C for 30 minutes in darkness. The conversion of the ferric (Fe^{3+}) TPTZ complex to ferrous (Fe^{2+}) TPTZ resulted in a vivid blue hue, with absorbance quantified at 593 nm by a UV–Vis spectrophotometer.

A calibration curve was established utilizing ascorbic acid as a reference standard at concentrations ranging from 10 to 100 µg/mL. The FRAP values were derived from the standard curve and represented as milligrams of vitamin C equivalent (VCE) per gram of sample (mg VCE/g). All analyses were performed in triplicate, and findings were shown as mean ± SD [29].

Sensory Evaluation

A hedonic sensory evaluation was performed on kombucha samples fermented for 7, 10, and 14 days (F7, F10, and F14). A total of 30 untrained panelists, aged 20 to 35 years, were recruited from the campus community. Each sample was assigned a random three-digit code and presented at 10 °C in a randomized sequence to reduce bias. Panelists were directed to rinse their lips with water between samples to avoid flavor transfer.

Participants assessed three sensory attributes aroma, taste, and color utilizing a 5 point hedonic scale, with 1 denoting “dislike very much” and 5 indicating “like very much.” The sensory data were subjected to statistical analysis via one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test to identify significant differences among samples at a confidence level of $p < 0.05$. Informed consent was secured from all panelists before participation, and assessments were conducted in regulated lighting and odorless environments [30].

Literature-Based Molecular Docking

To clarify the antioxidant processes of A. altilis, molecular docking data were gathered from existing literature. Phytochemicals like quercetin, kaempferol, gallic acid, morusin, rutin, and cyclocommunol were chosen. The protein targets comprised Keap1, Nrf2, TP53, PPARγ, ACLY, and NQO1, all of which are implicated in the oxidative stress response [31]. Docking was previously performed via AutoDock Vina and subsequently analyzed with PyMOL and LigPlot+. Ligand structures were obtained from PubChem (CID) and protein crystal structures from the Protein Data Bank (PDB ID). Parameters including binding energy (kcal/mol), hydrogen bonding, and interaction residues were synthesized and analyzed to yield molecular-level insights that corroborate the experimental antioxidant results [32].

Statistical Analysis

All data were presented as mean ± standard deviation (SD) derived from triple measurements. Statistical analyses were conducted utilizing SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Group differences were assessed using one-way analysis of variance (ANOVA), succeeded by Tukey’s HSD post hoc test to identify significant pairwise differences at a significance threshold of $\alpha = 0.05$. Pearson’s correlation analysis was used to evaluate the linear correlations among the antioxidant assay results (DPPH, ABTS, and FRAP), offering insight into the consistency and interdependence of the antioxidant evaluation methodologies.

Heatmap Visualization of Antioxidant Potential

A heatmap was created to illustrate comparative patterns of antioxidant activity among the kombucha samples. IC_{50} values derived from the DPPH, ABTS, and FRAP experiments used as input variables. The heatmap was produced with a statistical visualization tool, where darker color intensities denoted lower IC_{50} values, signifying greater antioxidant efficacy. This visualization enabled the recognition of trends and clustering patterns related to fermentation duration and antioxidant capacity [33].

Results and Discussion

Pharmacognostic Evaluation of *A. altilis* Leaf Powder

The dried powder of *A. altilis* leaves was evaluated for key pharmacognostic parameters. As summarized in Table 1, all measured values complied with the standards specified in the Indonesian Herbal Pharmacopeia, confirming the acceptable quality and authenticity of the plant material used for kombucha preparation.

Table (1). Pharmacognostic characteristics of *A. altilis* leaf powder

Parameter	Result (%)	Standard (FHI)
Moisture content	3.9	≤ 10
Water-soluble extractive	41.6	≥ 15
Ethanol-soluble extractive	26.6	≥ 8.5
Total ash	0.5	≤ 14.12
Acid-insoluble ash	0.4	≤ 1.0

The low moisture content ensures better shelf stability and minimizes microbial growth, while the high extractive values indicate a rich presence of water and ethanol soluble bioactive constituents, particularly polyphenols and flavonoids, which are known contributors to antioxidant activity [34].

The quality of crude drugs plays a crucial role in determining the efficacy and consistency of herbal formulations. Pharmacognostic evaluation of *A. altilis* leaf powder (Table 1) revealed that all measured parameters conformed to the standards established by the Indonesian Herbal Pharmacopeia (FHI, 2017). The low moisture content (3.9%) ensures long-term stability and minimizes the risk of microbial contamination.

Meanwhile, the high extractive values water-soluble (41.6%) and ethanol-soluble (26.6%) indicate an abundance of polar and semi-polar bioactive constituents, particularly flavonoids and phenolic compounds, which are known to contribute significantly to antioxidant potential [35].

The total ash (0.5%) and acid-insoluble ash (0.4%) contents were well below the permissible limits, indicating minimal inorganic impurities and confirming good sample purity. These results further validate the high quality of the *A altilis* leaves used as substrates for kombucha fermentation [36].

Phytochemical Constituents

Qualitative phytochemical screening confirmed the presence of major secondary metabolites, as summarized in Table 2. These bioactive compounds particularly flavonoids, tannins, terpenoids, and saponins are widely recognized for their antioxidant, anti-inflammatory, and cytoprotective properties, which collectively contribute to the therapeutic potential of *A. altilis* kombucha.

Table (2): Phytochemical screening results.

Phytochemical group	Observation	Result
Alkaloids	Precipitate with Dragendorff & Mayer’s	+
Flavonoids	Reddish color with Mg–HCl test	+
Tannins	Dark color with $FeCl_3$	+
Saponins	Stable foam >2 cm	+
Steroids	Green ring (Liebermann–Burchard)	+
Terpenoids	Brown ring at phase interface	+

These compounds may act synergistically to enhance radical scavenging and reducing effects within the fermented beverage, thereby contributing to its overall antioxidant capacity [37]. Phytochemical screening confirmed the presence of six

major classes of secondary metabolites: alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids (Table 2). The detection of flavonoids and tannins, in particular, supports the expected antioxidant potential, as these compounds are well known for their ability to donate electrons and neutralize reactive oxygen species (ROS) [38]. Saponins and terpenoids may further contribute through cell membrane stabilization and anti-inflammatory mechanisms, while steroids could enhance compound bioavailability and membrane permeability. These findings are consistent with previous studies reporting flavonoid-rich extracts from *A. altilis* leaves with potent free radical scavenging and reducing activities [39].

The high water and ethanol extractive values of *A. altilis* leaf powder indicate a substantial presence of polyphenols and flavonoids. These compounds play a crucial role in antioxidant activity, particularly following fermentation. During the fermentation process, many complex polyphenols undergo enzymatic hydrolysis into aglycone forms, which exhibit higher electron donating and radical scavenging capacities. This biochemical transformation is consistent with the observed decrease in IC_{50} values across all antioxidant assays, indicating enhanced antioxidant potency.

Kombucha pH and Organoleptic Characteristics

Fermentation of *A. altilis* kombucha resulted in a marked decrease in pH (Table 3), indicating progressive organic acid formation during the fermentation process. This acidification not only promotes microbial stability but also plays a vital role in enhancing the antioxidant potential of the beverage by facilitating the release and transformation of phenolic compounds.

Table (3): Changes in pH of *A. altilis* Kombucha at Different Fermentation Periods.

Day	pH (Mean ± SD)	SNI Standard
0	5.62 ± 0.09	2.5 – 4.6
7	4.10 ± 0.05	
10	3.65 ± 0.11	
14	3.02 ± 0.08	

Visual and sensory analyses (Table 4) revealed a characteristic kombucha aroma that intensified with fermentation time, accompanied by a gradual increase in perceived acidity. The color of the beverage remained consistently yellowish throughout the fermentation process, indicating stable pigment composition and minimal browning reactions.

Table (4): Organoleptic characteristics of kombucha

Day	Aroma	Color	Taste
0	Fresh herbal aroma	Light green	Sweet, no acidity
7	Kombucha *A. altilis*	Yellow	Slightly acidic
10	Kombucha *A. altilis*	Yellow	Acidic
14	Kombucha *A. altilis*	Yellow	Strongly acidic

During fermentation, a progressive decrease in pH was observed from day 7 to day 14 (Table 3), reflecting increased production of organic acids, primarily acetic, glucuronic, and gluconic acids. By day 14, the pH reached 3.02 ± 0.08, which falls within the Indonesian National Standard (SNI) for fermented beverages (2.5–4.6), indicating that the product is both safe and microbiologically stable for consumption [40].

Organoleptically, all samples maintained a yellowish color throughout fermentation, while the aroma gradually developed a characteristic kombucha scent. The taste changed from mildly acidic at day 7 (F7) to strongly acidic at day 14 (F14), corresponding to the accumulation of organic acids (Table 4). This increase in acidity not only results from fermentation but also appears to enhance antioxidant activity, as acidic conditions can stabilize polyphenolic compounds [41].

Antioxidant Activity Assays

DPPH Radical Scavenging

Fermentation significantly enhanced the antioxidant activity of *A. altilis* leaf kombucha, as evidenced by decreasing IC_{50} values (Table 5). The lowest IC_{50} was observed on day 14 (26.31 ppm), approaching the activity of vitamin C (9.16 ppm). The DPPH assay measures the ability of antioxidants to donate hydrogen atoms, thereby stabilizing free radicals. Non-fermented tea (F0) exhibited a high IC_{50} value of 116.07 ppm, indicating weak antioxidant activity. With ongoing fermentation, IC_{50} values decreased progressively: 99.21 ppm (F7), 46.31 ppm (F10), and 26.31 ppm (F14). This enhancement suggests that fermentation facilitates the biotransformation of complex phenolic compounds into simpler, more bioavailable forms. The IC_{50} of the F14 sample, nearing that of ascorbic acid, confirms its strong radical-scavenging capacity [42,43].

ABTS Radical Scavenging

A similar trend was observed in the ABTS assay. On day 14, the IC_{50} value reached 41.46 ppm, indicating strong electron-donating potential. The ABTS assay complements the DPPH method by assessing both hydrophilic and lipophilic antioxidant capacities. Fermentation significantly enhanced antioxidant activity, with IC_{50} values decreasing from 118.73 ppm in the non-fermented sample (F0) to 41.46 ppm on day 14 (F14). This pattern closely parallels the DPPH results, confirming that fermentation increases the electron-donating ability of *A. altilis* leaf kombucha, likely through enzymatic degradation of glycosylated polyphenols into simpler, more active forms [44,45].

FRAP Assay

The ferric-reducing capacity of *A. altilis* leaf kombucha improved progressively during fermentation. The lowest IC_{50} value was observed on day 14 (62.46 ppm), which still falls within the “strong antioxidant” classification. This increase in ferric-reducing power suggests that fermentation enhances the electron-donating ability of phenolic compounds, supporting the results obtained from the DPPH and ABTS assays and confirming the overall improvement in antioxidant potential.

Table (5): IC_{50} values of kombucha in antioxidant assays.

Sample	DPPH (ppm)	ABTS (ppm)	FRAP (ppm)
F0 (Control)	116.07	118.73	81.65
F7	99.21	87.85	69.06
F10	46.31	60.18	67.33
F14	26.31	41.46	62.46
Vit C	9.16	29.22	35.96

The FRAP assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The ferric-reducing power of *A. altilis* leaf kombucha increased progressively with fermentation time, as indicated by a decrease in IC_{50} from 81.65 ppm in the non-fermented sample (F0) to 62.46 ppm on day 14 (F14). Although FRAP values were numerically higher than those obtained from DPPH and ABTS assays, this difference is expected because FRAP depends on redox potential, which varies among different polyphenols [46,47].

Overall, all three assays consistently showed that antioxidant activity increased throughout fermentation. Kombucha fermented for 14 days exhibited the lowest IC_{50} values across all methods, indicating that fermentation promotes the biotransformation of active compounds into more bioavailable forms. These findings highlight fermentation duration as a critical factor in enhancing the antioxidant potential of this functional beverage.

Statistical Correlation Analysis

One-way ANOVA results indicated that variations in fermentation duration significantly affected antioxidant activity across all assays ($p < 0.05$). Furthermore, Pearson correlation analysis revealed a very strong positive correlation among the three antioxidant assays ($r > 0.97$, $p < 0.01$), demonstrating high internal consistency and supporting the reliability of the analytical results [48].

Heatmap Visualization of Antioxidant Potential

A heatmap was constructed to visually compare antioxidant strength across samples and assays. Darker cells correspond to lower IC_{50} values, indicating stronger antioxidant activity. Consistently, the F14 samples exhibited the highest potency across all assays, reinforcing the conclusion that prolonged fermentation enhances the bioactivity of *A. altilis* leaf kombucha.

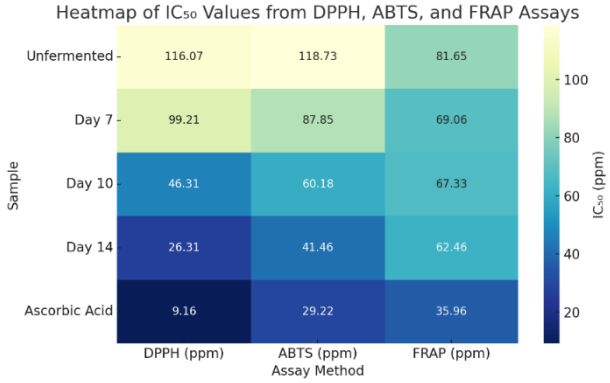


Figure (1) : Comparative Heatmap of Antioxidant Activity in *A. altilis* Leaf Kombucha Across Fermentation Stages Using DPPH, ABTS, and FRAP Assays.

To better illustrate the progressive improvement in antioxidant capacity during fermentation, a heatmap (not shown) was generated using IC_{50} data from all three assays. Darker shades indicate stronger antioxidant activity (i.e., lower IC_{50} values). Across all methods, the F14 sample consistently exhibited the darkest shades, reflecting superior radical-scavenging and ferric-reducing capacities. These findings reinforce that a 14-day fermentation period optimally enhances the antioxidant potential of *A. altilis* leaf kombucha. Importantly, this study provides novel insight by integrating multiple antioxidant assays with fermentation time, highlighting the unique bioactive profile of *A. altilis*, which has been less studied compared to other kombucha substrates [49].

The dark color pattern on the heatmap reflects the progressive increase in antioxidant capacity throughout fermentation. The consistency across the three assays (DPPH, ABTS, and FRAP), all showing peak activity on day 14, demonstrates a stable biological trend and reinforces the reliability of the results. This heatmap provides a clear visual representation that complements the quantitative data, strengthening the comparative assessment of antioxidant enhancement during fermentation.

Sensory Acceptability

The highest hedonic scores for taste and aroma were observed on day 9. However, these scores declined on days 12 and 14, likely due to the increasing acidity as fermentation progressed. In contrast, color scores remained relatively stable throughout the fermentation period, indicating that visual appearance was less affected by acid accumulation.

Table (6): Hedonic test results (n = 30).

Day	Aroma	Color	Taste
7	3.10 ± 1.00	4.27 ± 0.45	3.37 ± 0.88
10	2.57 ± 0.83	4.27 ± 0.45	3.03 ± 0.73
14	2.67 ± 0.93	4.33 ± 0.48	3.13 ± 0.78

Based on consumer perception, a fermentation period of 7 days provides the best balance between functional benefits and palatability. Hedonic testing (Table 6) indicated that samples fermented for 7 days achieved the highest scores for aroma (3.10 ± 1.00), taste (3.37 ± 0.88), and color (4.27 ± 0.45). Sensory acceptability declined after 10 and 14 days, primarily due to increased acidity. Although F14 exhibited the strongest antioxidant capacity, its taste was perceived as excessively sour by most panelists, highlighting a trade-off between functionality and palatability. These findings suggest that a 7–9 day fermentation period represents the optimal compromise for product development, maintaining high antioxidant activity while ensuring consumer acceptability [50,51].

While the highest antioxidant activity was achieved on day 14, sensory test results revealed reduced consumer preference for aroma and taste due to increasing acidity. Therefore, balancing fermentation duration is crucial to produce functional beverages that combine strong antioxidant potential with favorable sensory qualities.

Molecular Docking Overview

Literature based molecular docking analysis revealed strong binding affinities between major phytochemicals from *A. altilis* and key antioxidant-related protein targets (Table 7). For instance, quercetin exhibited binding energies of –9.1 kcal/mol with NQO1 and –7.9 kcal/mol with Keap1, suggesting its potential to modulate the Keap1–Nrf2 signaling pathway. These interactions provide mechanistic insight into how the bioactive compounds in *A. altilis* leaf kombucha may contribute to antioxidant activity beyond direct radical scavenging [52,53].

Tabel (7): Summary of docking energies and target interactions.

Compound	Target	Binding Energy (kcal/mol)	Key Interactions (H-bonds)
Quercetin	Keap1 NQO1	–7.9 –9.1	Arg380, Asn382 Gln104, Tyr128
Kaempferol	Nrf2 PPARγ TP53	–7.8 –11 –7	Glu3, Glu10 Ser342, Ile281 Ser109, Lys74
Gallic acid	Keap1 SESN2	–6.2 –7.5	Arg415, Ser508 Arg390, Glu451
Morusin	TNF-α 3Q05 PPARγ	–7 –7.2 –10.9	Leu74, Leu45 Glu2254, Thr231 His466, Ser342
Rutin	Keap1	–6.47	Ser363, Ser508
Cyclocommunol	6GVZ	–7.9	Asp393, Gly369

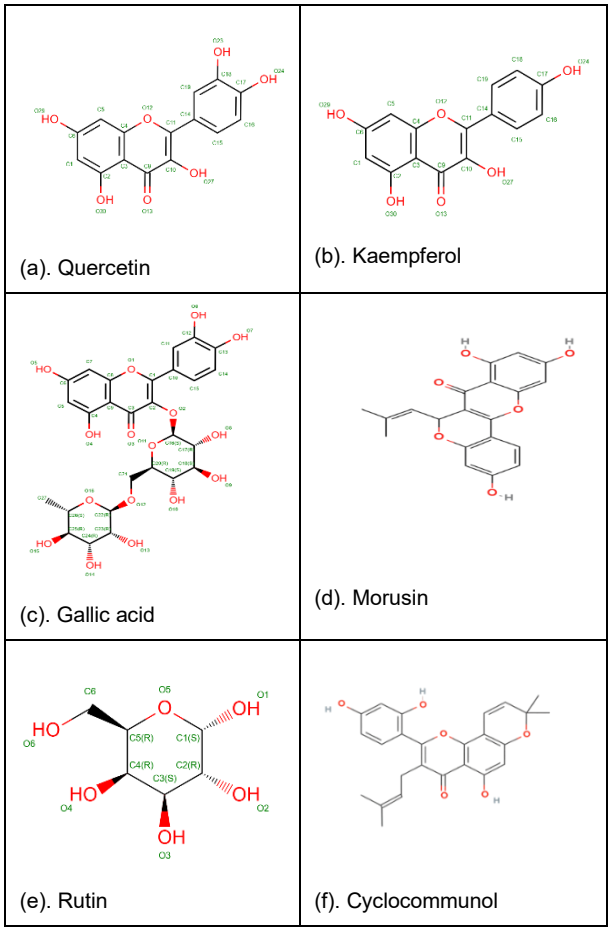


Figure (2): 2D chemical structures of selected phytochemicals discussed in the literature-based docking section (quercetin, gallic acid, kaempferol, morusin, rutin, and cyclocommunol), retrieved from public chemical structure databases.

Previously published molecular docking studies have suggested that major phytochemicals in Artocarpus altilis may modulate key antioxidant response pathways, notably through interactions with Keap1–Nrf2–ARE, TP53, and PPARγ signalling cascades [54]. To explore potential mechanisms of antioxidant action, docking evaluations were performed against key oxidative stress-regulating proteins (Table 7).

Quercetin exhibited strong binding affinity to NQO1 (–9.1 kcal/mol) and Keap1 (–7.9 kcal/mol), suggesting its potential to activate Nrf2, a master regulator of cellular antioxidant defense. Normally, Keap1 binds Nrf2 in the cytoplasm and targets it for ubiquitin–proteasome degradation. Oxidative stress modifies cysteine residues on Keap1, releasing Nrf2, which translocates to the nucleus to bind antioxidant response elements (ARE) and promote transcription of cytoprotective enzymes [55].

Kaempferol showed high affinity to TP53, Nrf2, and PPARγ (–7 to –11 kcal/mol), implying roles in cell cycle regulation, metabolism, and redox balance. Kaempferol has been reported to mitigate pancreatic damage in severe acute pancreatitis by modulating NF-κB and Keap1–Nrf2 signaling, and also exhibits cytoprotective and anticancer effects through regulation of apoptosis and inflammatory mediators [56].

Gallic acid (GA) and rutin interacted with Keap1 (–6.2 and –6.47 kcal/mol, respectively), suggesting competitive inhibition at the Nrf2-binding site. GA can disrupt the Keap1–Nrf2 interaction, facilitating Nrf2 activation and enhancing antioxidant enzyme expression. Similarly, rutin may stabilize Nrf2 and promote nuclear translocation, activating ARE-driven genes such as HO-1 and NQO1 [57,58].

Morusin, a prenylated flavonoid specific to *A. altilis*, showed high affinity toward PPARγ (–10.9 kcal/mol), supporting its anti-inflammatory potential. Morusin exerts both anti-inflammatory and pro-apoptotic effects via multi-target modulation, including binding TNF-α, inhibiting cytokine–receptor interaction, and suppressing NF-κB and MAPK signaling pathways [59].

Cyclocommunol exhibited moderate binding affinity to protein 6GVZ (–7.9 kcal/mol). While its role in oxidative stress regulation is not fully established, this interaction may influence protein–ligand stability and conformational dynamics, suggesting potential allosteric modulation or functional inhibition [60].

It should be noted that molecular docking predictions require experimental validation. Follow-up in vitro or in vivo studies are essential to confirm whether these ligand–receptor interactions occur biologically. Molecular dynamics simulations could further elucidate the stability of the ligand–receptor complexes. Overall, these molecular interactions support the hypothesis that the antioxidant effects of *A. altilis* kombucha involve not only direct free radical scavenging but also modulation of cellular antioxidant defense pathways, particularly Keap1–Nrf2–ARE, PPARγ, and TP53 signaling cascades [61].

Conclusion

This study demonstrates that kombucha derived from *A. altilis* leaves exhibits strong antioxidant activity, which progressively increases with fermentation duration. The 14-day fermented sample consistently showed the lowest IC_{50} values in DPPH, ABTS, and FRAP assays, approaching the antioxidant capacity of ascorbic acid.

Furthermore, literature-based molecular docking analysis revealed that major *A. altilis* phytoconstituents exhibit high binding affinity toward key antioxidant-regulating proteins, including Keap1, Nrf2, PPARγ, and TP53. These interactions suggest that the antioxidant effects of *A. altilis* kombucha may involve not only direct free radical neutralization but also modulation of cellular defense pathways. Collectively, these findings highlight the potential of *A. altilis* leaf kombucha as a functional beverage with multi-target antioxidant mechanisms, integrating traditional herbal knowledge with modern fermentation technology and computational validation.

From a practical perspective, a 7-day fermentation period may offer the most favourable balance between antioxidant benefit and sensory acceptability for market-ready products, whereas a 14-day fermentation period provides the strongest antioxidant activity.

Disclosure Statements

- **Ethics approval and consent to participate:** This study was approved by the Animal Research Ethics Committees (AREC), Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (Approval No. 0671/KEPH-FMIPA/2025) on January 9, 2025. The research involved human participants, and written informed consent was obtained from all subjects prior to participation, in accordance with the principles of the Declaration of Helsinki.
- **Consent for publication:** Not applicable. No individual person's data, images, or videos are included in this published manuscript.
- **Availability of data and materials:** The raw data supporting the findings of this study are presented within the tables and figures of this manuscript. Supplementary datasets produced and/or examined during this work are accessible from the corresponding author upon reasonable request.
- **Author's contribution:** MA devised and formulated the study. TR and SS conducted the experiments and gathered

the results. MAN carried out the data analysis. MFL supervised the research and critically revised the manuscript. All authors reviewed and endorsed the final version of the text.

- **Funding:** This research did not receive any specific support from public, commercial, or non-profit agencies. The research was financed by the author independently.
- **Conflicts of interest:** The authors say that there is no conflict of interest in putting this work out there.
- **Acknowledgements:** The authors express their gratitude to the Faculty of Pharmacy, Universitas Sumatera Utara, for the facilities provided during this research. The authors also thank the sensory evaluation group for their helpful comments and the laboratory staff for their technical support.

Open Access

References

1] Kalogerakou, T., & Antoniadou, M. (2024). The role of dietary antioxidants, food supplements and functional foods for energy enhancement in healthcare professionals. Antioxidants, 13(12), 1508.

2] Chong, A. Q., Chin, N. L., Talib, R. A., & Basha, R. K. (2024). Modelling pH dynamics, SCOBY biomass formation, and acetic acid production of kombucha fermentation using black, green, and oolong teas. Processes, 12(7), 1301.

3] Huda, H. S. A., Majid, N. B. A., Chen, Y., Adnan, M., Ashraf, S. A., Roszko, M., ... & Sasidharan, S. (2024). Exploring the ancient roots and modern global brews of tea and herbal beverages: A comprehensive review of origins, types, health benefits, market dynamics, and future trends. Food Science & Nutrition, 12(10), 6938-6955.

4] Prajapati, K., Prajapati, J., Patel, D., Patel, R., Varshnei, A., Saraf, M., & Goswami, D. (2024). Multidisciplinary advances in kombucha fermentation, health efficacy, and market evolution. Archives of microbiology, 206(9), 366.

5] John, O. D., Surugau, N., Kansedo, J., Panchal, S. K., & Brown, L. (2025). Plant-based functional foods from Borneo. Nutrients, 17(2), 200.

6] Ed-Dahmani, I., El Fadili, M., Kandsi, F., Conte, R., El Atki, Y., Kara, M., ... & Abdellaoui, A. (2024). Phytochemical, antioxidant activity, and toxicity of wild medicinal plant of Melitotus albus extracts, in vitro and in silico approaches. ACS omega, 9(8), 9236-9246.

7] Muscolo, A., Mariateresa, O., Giulio, T., & Mariateresa, R. (2024). Oxidative stress: the role of antioxidant phytochemicals in the prevention and treatment of diseases. International journal of molecular sciences, 25(6), 3264.

8] Andry, M., Ginting, I., Nasution, M. A., & Lubis, M. F. (2025). Impact of Solvent Type on Total Flavonoid Content and Sun Protection Factors in Centella asiatica (L.) Urban Leaves Extract. Malaysian Journal of Fundamental and Applied Sciences, 21(3), 1998-2007.

9] Fitri, K., Andry, M., Khairani, T. N., Nasution, M. A., Bu'ulolo, A. A. C., & Lubis, M. F. (2024). Antioxidant Potential of Ethanol Extracts Leaf and Stem of Nelumbo nucifera Gaertn. with DPPH, ABTS, and Frap Methods. Research Journal of Pharmacy and Technology, 17(12), 6084-6090.

10] Nasution, M. A., Andry, M., Lubis, M. F., Illian, D. N., Rani, Z., & Fauzi, Z. P. A. (2024). Phytochemical Analysis and Nanoparticle Formulations of Extracts Myristica fragrans Houtt Leaves as Antibacterial. Trends in Sciences, 21(10), 8335-8335.

11] Lubis, L. D., Prananda, A. T., Juwita, N. A., Nasution, M. A., Syahputra, R. A., Sumaiyah, S., ... & Atiqah, J. F. (2024). Unveiling antioxidant capacity of standardized chitosan-tripolyphosphate microcapsules containing polyphenol-rich extract of Portulaca oleraceae. Heliyon, 10(8).

12] Mfopa, A. N., Kemzeu, R., Fokom, R., Yamthe, L. R. T., Dize, D., & Boyom, F. F. (2024). Phenolic compounds, antioxidant and antileishmanial activities of kombucha as affected by fermentation time. Heliyon, 10(22).

13] Minarti, M., Ariani, N., Megawati, M., Hidayat, A., Hendra, M., Primahana, G., & Darmawan, A. (2024). Potential antioxidant activity methods DPPH, ABTS, FRAP, total phenol and total flavonoid levels of Macaranga hypoleuca (Reichb. f. & Zoll.) leaves extract and fractions. In E3S Web of Conferences (Vol. 503, p. 07005). EDP Sciences.

14] Balendra, V., & Singh, S. K. (2021). Therapeutic potential of astaxanthin and superoxide dismutase in Alzheimer's disease. Open biology, 11(6), 210013.

15] Liu, L., Huang, Z., & Li, Z. (2025). Potential Natural Plant Polysaccharides for Use as Feed Additives in Aquaculture: Structure–Activity Relationship, Benefits, and Restrictions. Reviews in Aquaculture, 17(2), e70014.

16] Chen, Z., Zhang, S., Sun, X., Meng, D., Lai, C., Zhang, M., ... & Gao, X. (2024). Analysis of the Protective Effects of Rosa roxburghii-Fermented Juice on Lipopolysaccharide-Induced Acute Lung Injury in Mice through Network Pharmacology and Metabolomics. Nutrients, 16(9), 1376.

17] Masiala, A., Vingadassalon, A., Lemoyne, S., Pejcz, E., Wojciechowicz-Budzisz, A., Olędzki, R., ... & Harasym, J. (2024). Sustainable Valorization of Breadfruit (*A. altilis* Leaves) as a Pasta Ingredient. Sustainability, 16(24), 11030.

18] Fitria, F., Sumaiyah, S., & Yuandani, Y. (2025). Formulation and Activity Test of Nanogel from Ethanol Extract of Breadfulness Leaves (*A. altilis*) as A Topical Preparation for Healing Burn Wounds in Male White Rats. Trends in Sciences, 22(6), 9698-9698.

19] Herman, H., Ibrahim, A., Junaidin, J., Arifuddin, M., Hikmawan, B. D., Siska, S., ... & Ahmad, I. (2024). Pharmacognostic profile and antidiabetic activity of Eleutherine bulbosa Mills. bulbs from East Kalimantan, Indonesia. Pharmacognosy Journal, 16(1).

20] Nazaruddin, N., Rosmaidar, R., Siregar, T. N., Wahyuni, S., & Sutriana, A. (2024). Phytocomponents analysis and antioxidant activity of Malacca fruit extract (Phyllanthus emblica) using three different solvents. Biodiversitas Journal of Biological Diversity, 25(5).

21] PM, T., K, A. V., U, N. S., K, N., AP, F. S., & UK, I. (2024). Biosynthesis and Characterization of Silver Nanoparticle by

Using *A. altilis* Fruit Extract for Its Biological Activity. Journal of Pharmaceutical Innovation, 19(2), 15.

22] Mali, S. N., Pandey, A., de Oliveira, M. S., & Jawarkar, R. D. (2024). A decade review of analysis of essential oils from genus Artocarpus: Its phytochemistry and pharmacology. Pharmacological Research-Natural Products, 2, 100016.

23] Zheng, Y., Liu, Y., Han, S., He, Y., Liu, R., & Zhou, P. (2024). Comprehensive evaluation of quality and bioactivity of kombucha from six major tea types in China. International Journal of Gastronomy and Food Science, 36, 100910.

24] Hassler, V., Brand, N., & Wefers, D. (2024). Isolation and characterization of exopolysaccharides from Kombucha samples of different origins. International Journal of Biological Macromolecules, 267, 131377.

25] Sarfraz, M., Shabbir, K., Adnan, Q., Khan, H. M. S., Shirazi, J. H., Sabir, H., ... & Basit, A. (2024). Fabrication, organoleptic evaluation and in vitro characterization of cream loaded with Carica papaya seed extract. Journal of Cosmetic Dermatology, 23(3), 1045-1054.

26] Lang, Y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., ... & Li, B. (2024). Classification and antioxidant assays of polyphenols: A review. Journal of Future Foods, 4(3), 193-204.

27] Kumar, R., Shikha, D., & Sinha, S. K. (2024). DPPH radical scavenging assay: A tool for evaluating antioxidant activity in 3% cobalt–Doped hydroxyapatite for orthopaedic implants. Ceramics International, 50(8), 13967-13973.

28] Tran, M. T., Gomez, S. V., Alenicheva, V., & Remcho, V. T. (2024). A paper-based assay for the determination of total antioxidant capacity in human serum samples. Biosensors, 14(11), 559.

29] Nerlekar, N., Dandge, P., Nandre, V., & Dandge, P. (2025). Bacterial siderophore as a green solvent for the development of FRAP assay as a potential antioxidant test: A comparative study. Talanta Open, 100515.

30] Saito, M. S., dos Santos, W. A., & Mamede, M. E. D. O. (2024). Coffee-Flavoured Kombucha: Development, Physicochemical Characterisation, and Sensory Analysis. Fermentation, 10(7), 334.

31] Gupta, R., Sardana, S., Sethiya, N. K., Bisht, D., & Patil, U. K. (2025). A literature-based overview on chemistry, pharmacology, molecular docking and nanotechnological advancements of capsaicin including potential impact on pharmacological action of other drugs with mechanistic insight. Food Chemistry Advances, 7, 100988.

32] Majumdar, D., Chatterjee, A., Feizi-Dehnayebi, M., Kiran, N. S., Tuzun, B., & Mishra, D. (2024). 8-Aminoquinoline derived two Schiff base platforms: Synthesis, characterization, DFT insights, corrosion inhibitor, molecular docking, and pH-dependent antibacterial study. Heliyon, 10(15).

33] Boonupara, T., Udomkun, P., & Kajitvichyanukul, P. (2024). Quantitative analysis of atrazine impact on UAV-derived multispectral indices and correlated plant pigment alterations: A heatmap approach. Agronomy, 14(4), 814.

34] Masiala, A., Vingadassalon, A., Lemoyne, S., Pejcz, E., Wojciechowicz-Budzisz, A., Olędzki, R., ... & Harasym, J. (2024). Sustainable Valorization of Breadfruit (*A. altilis* Leaves) as a Pasta Ingredient. Sustainability, 16(24), 11030.

35] Shone, S., Siyana, N., & Sahana, S. S. Pharmacognostic Prespective of Artocarpus Heterophyllus Lam Leaf and Its In-Vitro Antirheumatic Activity.

36] Zebua, N. F., Nerdy, N., Septama, A. W., Suryani, D., Suwailim, S., & Bahrianur, R. (2025). Antioxidant Activity Test and Infrared Profile of Hexane, Ethyl Acetate, and Ethanol Extract of Breadfruit Leaves (*A. altilis*). Rasayan Journal of Chemistry, 18(1).

37] sTue, N. M., Quan, N. T., Chi, H. T., & Ly, B. T. K. (2024). An in vitro Study into Antioxidant, Antibacterial, and Toxicity Impacts of *A. altilis* Leaf Extract. Biological, 2307(4), 4637.

38] Narayanaswamy, S., Rangappa, N. B., Rai, B. C., & Mukherjee, D. B. (2025). Biochemical profiling of plant latex Euphorbia hirta and Croton bonplandianum: Unveiling the nuclease and protease activity. South African Journal of Botany, 184, 650-658.

39] Edo, G. I., Ugbune, U., Akpoghelie, P. O., & Owheruo, J. O. (2024). Evaluation of physicochemical, phytochemical, anti-bacterial and antioxidant potential of kola nut (cola acuminata): an approach in food, health and nutritional benefits. Vegetos, 37(6), 2320-2329.

40] Agustina, E., Nafisah, R. F., Fiddaroini, A. F., Tania, C. E., Purnamasari, R., Andiarna, F., & Hidayati, I. (2025). Formulation and Characterization Tests of SCOBY Kombucha Gel. Biology, Medicine, & Natural Product Chemistry, 14(1), 143-151.

41] Selvaraj, S., & Gurumurthy, K. (2024). Metagenomic, organoleptic profiling, and nutritional properties of fermented kombucha tea substituted with recycled substrates. Frontiers in Microbiology, 15, 1367697.

42] Limsuwan, S., Awaeloh, N., Na-Phatthalung, P., Kaewmanee, T., & Chusri, S. (2025). Exploring Antioxidant Properties of Standardized Extracts from Medicinal Plants Approved by the Thai FDA for Dietary Supplementation. Nutrients, 17(5), 898.

43] Aouadi, A., Saoud, D. H., Bouafia, A., Mohammed, H. A., Gamal, H. G., Achouri, A., ... & Al-Lohedan, H. A. (2024). Unveiling the antioxidant power: synthesis and characterization of lemon and orange peel-derived carbon quantum dots with exceptional free radical scavenging activity. Biomass Conversion and Biorefinery, 1-14.

44] Patel, M., Al-Keridis, L. A., Alomrani, S. O., Alshammari, N., Ibrahim, A. M., & Adnan, M. (2024). Antioxidant effects and potential molecular mechanism of action of Diplocyclos Palmatus (L.) C. Jeffrey Fruits based on systematic network pharmacology with experimental validation. Journal of Molecular Structure, 1313, 138638.

45] Li, Y., Chen, M., Shi, Q. S., Xie, X., & Guo, Y. (2024). Biomass fractionation techniques impact on the structure and antioxidant properties of isolated lignins. Separation and Purification Technology, 330, 125499.

46] Nurhayati, R., Shoviantari, F., Munandar, T. E., & Yuwono, M. (2024). Butterfly pea (Clitoria ternatea L.) flower water and ethanol extract: Phytochemical screening, FTIR analysis, and antioxidant activity estimation using comparison of ABTS, DPPH, and FRAP assays. Research Journal of Pharmacy and Technology, 17(5), 1973-1982.

47] Heckmann, M., Stadlbauer, V., Drotarova, I., Gramatte, T., Feichtinger, M., Arnaut, V., ... & Weghuber, J. (2024). Identification of oxidative-stress-reducing plant extracts from a novel extract library—Comparative analysis of cell-free

and cell-based in vitro assays to quantitate antioxidant activity. Antioxidants, 13(3), 297.

48] Liu, C., Chen, J., Che, Y., He, L., Luo, S., Yang, C. S., & Chen, T. (2024). Interactive Effects of Arabinoxylan Oligosaccharides and Green Tea Polyphenols on Obesity Management and Gut Microbiota Modulation in High-Fat Diet-Fed Mice. Journal of Agricultural and Food Chemistry, 72(29), 16237-16249.

49] Boonupara, T., Udomkun, P., & Kajitvichyanukul, P. (2024). Quantitative analysis of atrazine impact on UAV-derived multispectral indices and correlated plant pigment alterations: A heatmap approach. Agronomy, 14(4), 814.

50] Cartwright, J., Smyth, H. E., Netzel, M. E., Sultanbawa, Y. F., & Wright, O. R. (2025). A Hedonic Sensory Trial: Exploring the Relationship Between Sweet-Liker Status, Demographics, and Health Measures on Acceptability in a Beverage System. Journal of Sensory Studies, 40(1), e70008.

51] Akasapu, K., & Uppaluri, R. V. (2024). Role of score-deviation, hedonic and fuzzy logic sensory approaches for the development and identification of optimal vegetable mix soups. International Journal of Gastronomy and Food Science, 37, 100982.

52] Agu PC, Afiukwa CA, Orji OU, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Sci Rep*. 2023;13(1):1-18. doi:10.1038/s41598-023-40160-2

53] Rante H, Alam G, Irwan M. -Glucosidase inhibitory activity of breadfruit leaf extract (*A. altilis* (Parkinson) fosberg). *J Phys Conf Ser*. 2019;1341(7). doi:10.1088/1742-6596/1341/7/072015

54] Rinaldi DH, Kamadjaja DB, Sumarta NPM. The effects of breadfruit leaf (*A. altilis*) extract on fibroblast proliferation in the tooth extraction sockets of Wistar rat. *Dent J*. 2018;51(3):143-146. doi:10.20473/j.djmkg33.v51.i3.p143-146

55] Rosmawaty R, Tehubijuluw H. Screening Of Phytochemicals And Bioactivity Test Of The Leaves Breadfruit (*A. altilis*). *Indones J Chem Res*. 2017;1(1):28-32. doi:10.30598/ijcr.2013.1-ros

56] Devi M, Wibowotomo B, Soekopitojo S, Merawati D. Study of Antioxidant Activity in Sinom Drinks From Breadfruit (*A. altilis*) Leaves. 2019;242(Icovet 2018):7-12. doi:10.2991/icovet-18.2019.3

57] Ghaisani Yumni G, Primitasari R, Nur Afifah N. Various Regions in Central Java. *J Kefarmasian Indones*. 2024;14(2):167-175.

58] P. Agbangnan Dossa C, T. D. Bothon F, E. Kanfon R, et al. Nutritional Quality, Phenolic Compound Content and Radical Scavenging Potential of *A. altilis* of Benin. *Am J Food Sci Technol*. 2018;6(4):195-198. doi:10.12691/ajfst-6-4-9

59] Silva CE da, Narain N. Physicochemical characterization and bioactive compounds in breadfruit (*A. altilis*) and its dried products. *Res Soc Dev*. 2021;10(15):e537101523391. doi:10.33448/rsd-v10i15.23391

60] Riasari H, Sukrasno, Ruslan K. Metabolite Profile of Various Development Bread Fruit Leaves (*A. altilis*. Parkinson. Fosberg) and the Identification of Their Major Componens. *Int J Pharm Sci Res*. 2015;6(5):2170-2177. doi:10.13040/IJPSR.0975-8232.6(5).2170-77

61] Lutfiani F, Wibowotomo B, Devi M. Chemical and Organoleptic Properties Analysis of Breadfruit Leaves (*A. altilis*) Herbal Tea with Cinnamon and Clove Addition. 2020;406(Iconhomecs 2019):208-216. doi:10.2991/assehr.k.200218.033