



Comparative Phytochemical Screening and In vitro Antioxidant Activity of *Artocarpus heterophyllus* leaf extract versus *Camellia sinensis* (Green Tea): Implications for a tea-like herbal infusion

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Abstract	Article History
In this study we compared the phytochemical screening and in-vitro antioxidant activities of <i>Artocarpus heterophyllus</i> leaf extract against those of <i>Camellia sinensis</i> (Green Tea) to see the implications or potentials or tea-like herbal infusion of <i>A. heterophyllus</i> Extracts were obtained from the dried leaves of the two teas/samples/ and the extracts were evaluated by qualitative phytochemical screening; in vitro antioxidant activity using DPPH and hydrogen peroxide scavenging assays; amino acid profiling of leaf hydrolysates; and HPLC-DAD fingerprinting, samples tested positive for phenols, tannins, flavonoids and saponins in qualitative screening. In the antioxidant assays (0.2–1.0 mg/mL), <i>A. heterophyllus</i> exhibited higher DPPH and H₂O₂ scavenging than <i>C. sinensis</i> at the tested concentrations, while ascorbic acid showed the highest activity. Because neither plant extract reached 50% inhibition in the tested range, IC₅₀ values could not be reliably estimated (IC₅₀ > 1.0 mg/mL). Amino acid profiling showed a mixture of essential and non-essential amino acids in both leaf hydrolysates. However, this analysis does not directly reflect infusion chemistry. Jackfruit leaves contain UV-active constituents and show measurable in vitro antioxidant activity, supporting further study as a tea-like herbal infusion. Nevertheless, claims that <i>A. heterophyllus</i> is an alternative to <i>C. sinensis</i> should be limited until tea-defining markers (Epigallocatechin gallate (EGCG), Epigallocatechin (EGC), Epicatechin galleate (ECG), caffeine and theanine) are quantified using validated chromatographic methods (like., ISO 14502-2).	Received: 24/08/2025 Accepted: 29/10/2025 Published: 31/12/2025
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1.0 Introduction

Tea prepared from *Camellia sinensis* is among the most consumed beverages worldwide and its bioactivity is commonly attributed to catechins (e.g., EGCG, EGC, ECG), methylxanthines (notably caffeine), and free amino acids (especially theanine). The chemistry of tea is well studied and standardized analytical approaches exist for targeted quantification of catechins and caffeine (e.g.,

international organization for Standard (ISO) 14502-2) (Vishnu *et al.*, (2019). In all of these compounds, a catechin compound was found to possess antioxidant activity due to the large number of hydroxyl groups in its chemical structure (Green *et al.*, 2022). ECG is the most effective free radical scavenger among tea polyphenols, then EGCG, EGC and ECG Chao *et al.* (2000) found that EGCG has the ability to reduce the high level of reactive oxygen

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species ROS in diabetic oocytes and improved their quality (Xie *et al.*, 2020). More so, Xie *et al.* (2004) found that EGCG cleared ROS through nuclear Factor signaling, thus inhibiting apoptosis and ferroptosis in human intestinal epithelial cells and improving intestinal injury induced by radiation. EGCG also protect human skin fibroblasts from UV-induced photoaging through its antioxidant activity and strength of regulating related gene expression (Jia, *et al.*, 2023). Tea polyphenols were demonstrated to enhance the antioxidant capacity and induce heat shock proteins (HSPs) to protect cardiomyocytes when subjected to heat stress in vivo and in vitro (Yinlddy *et al.*, 2021). EGCG ameliorated the carbon tetrachloride CCl₄-driven liver damage by reducing oxidant activity, causing a decrease in malon dialdehyde and an increase in Reduce Glutathione (GSH).

In many regions, other plant leaves are consumed as tea-like infusions for sensory or perceived health benefits. Jackfruit (*Artocarpus heterophyllus*) is widely available in tropical regions, and its leaves have been reported in the broader literature to contain phenolic compounds with antioxidant activity. However, demonstrating that a plant is a true alternative to tea requires more than showing general antioxidant activity (Xie *et al.* 2004). It requires beverage-relevant preparation (infusion), standardized extraction for compositional assays, and measurement of tea-defining markers as well as robust statistics (Xie *et al.* 2004).

This is necessary to meet the increasing demand for healthy food, the industrial application of tea is expanding (Yan *et al.*, 2023). The development of tea-based functional products with cardiovascular protection, prevention of neurological disorders, and antidiabetic and antiobesity properties and as well antioxidant holds promising prospects. In addition, tea components find applications as food additives. Research showed that tea polyphenols has potential to reduce the digestion rate of starch in bread, increase the strength of gluten, and most importantly improve the texture of noodles (Kan *et al.*, 2020).

If this is properly achieved then the ever increasing demand for tea will be significantly reduced as more research tailored toward tea and *Artocarpus species* will be explored.

This study provides a preliminary comparative assessment of *A. heterophyllus* leaves and *C. sinensis* leaves using qualitative phytochemical screening, in vitro antioxidant assays, amino acid profiling, and HPLC-DAD fingerprinting, and highlights methodological requirements for substantiating a 'tea-like' claim.

2.0 Materials and Methods

2.1 Plant Material and Authentication

Fresh leaves of *C. sinensis* were obtained from Mambilla Plateau Gembu Taraba State, Nigeria, and leaves of *A. heterophyllus* were obtained from Jabi Daki biyu, Abuja, Nigeria. The leaves were washed, shade-dried to constant mass, pulverized, and stored in airtight containers until analysis.

Metadata on Jackfruit

Number of Independent Plant: 4 plants. Number of Batches: 3 batches. Collection Date and Season: 30th Oct, 2024 during raining season. Coordinates: 9.060104 to 7.429997, Jabi Abuja 900108 and was identified at Baze University Herbarium with voucher number as BUHOOOO127 while **Metadata on *Camellia sinensis*** number of Independent Plant: Numerous. Number of Batches: Significant batches. Collection Date and Season: 31st Oct, 2024 during raining season. Coordinates: 6.720631 to 11.254934, Gembu Taraba 663002 and was identified at Baze University Herbarium with voucher number as BUHOOOO128.

2.2 Extraction and Extract Yield

Powdered leaf material (40 g) was macerated in 500 ml distilled water for 24 h at room temperature with intermittent agitation. Extracts were filtered through muslin cloth and concentrated at 50 °C to obtain crude extracts. Extraction yields 16.77% of *C. sinensis* and 26.15% of *A. heterophyllus*.

2.3 Chemicals and Reagents

All reagents were of analytical grade. Gallic acid (for total phenolics), rutin (for total flavonoids), digitonin/digitoxin (for saponins/glycosides), and ascorbic acid (positive control for antioxidant assays) were used as standards as appropriate. These were purchase from GHTECH with identification number of GB/T: 15347 from Sigma Aldrich, Germany Chemicals

2.4 Qualitative Phytochemical Screening

Qualitative phytochemical tests for phenols, tannins, flavonoids, saponins and other secondary metabolites were carried out using standard colorimetric reactions as described in established phytochemical screening protocols. All tests were performed in at least triplicate.

The qualitative phytochemical screening was carried out according to the method by Vishnu *et al.*, (2019). **Test for Phenol** To 5 ml of extract 3ml of 10%lead acetate solution was added and mixed gently. The production of bulky white precipitate indicted positive for phenols (Vishnu *et al.*, 2019). **Test for Tannins** To 5ml of extract, few drops of 5% ferric

chloride solution was added. The production of dark green colour indicates the presence of tannins (Vishnu *et al.*, 2019). **Test for Flavonoids** 1ml of extract was taken and 10% of lead acetate was added. The yellow precipitate indicated positive inference for the flavonoids (Vishnu *et al.*, 2019). **Test for Alkaloids** To 5ml of extract, 2ml of HCl was added and 1ml of Dragendorff's reagent was added. An orange or red precipitate shows a positive result for alkaloids (Vishnu *et al.*, 2019).

While the Test for Glycosides (Borntrager's test)

To 2ml of the extract, 3ml of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added. The pink colour indicated the presence of glycosides (Vishnu *et al.*, 2019). **Test for Cardiac Glycosides (Keller-Killani test)** 5ml of the extract was mixed with 2ml of glacial acetic acid and a drop of ferric chloride solution was added followed by the addition of 1ml of conc. H₂SO₄. A brown ring in the interface indicated the presence of deoxy sugars of cardenoloides (Vishnu *et al.*, 2019). **Test for Saponins** 0.5 mg of extract will be vigorously shaken with few ml of distilled water. The formation of frothing indicated positive for saponins (Vishnu *et al.*, 2019). **Test for Steroids** To 2ml of extract, 2ml of chloroform and 2ml of concentrated H₂SO₄ were added. The appearance of red colour and yellowish-green fluorescence indicated the presence of steroids (Vishnu *et al.*, 2019).

And last batch were Test for Terpenoids (Salkowski test)

3ml of the extract was taken and 1ml of chloroform and 1.5 ml of concentrated H₂SO₄ were added along the sides of the tube. The reddish-brown colour in the interface was considered positive for the presence of terpenoids (Vishnu *et al.*, 2019). **Anthocyanins** 2ml of the extract was taken to 2ml of 2N HCl and it was followed by the addition of ammonia, the conversion of pink-red turns blue-violet indicated the presence of anthocyanins (Vishnu *et al.*, 2019).

Anthraquinones To 5ml of extract, few ml of conc. H₂SO₄ was added and 1ml of diluted ammonia was added to it. The appearance of rose pink confirms the presence of anthraquinones (Vishnu *et al.*, 2019).

2.5 Quantitative Phytochemical Assays: The quantitative phytochemical screening look at the following parameters:

Total Phenolic Content: The total phenolic content was determined according to the method described by

Maurya and Singh (2010). This is basically a colour reaction that produces a blue colour which is measured spectrophotometrically. Folin Ciocalteu Reagent was used to evaluate the amount of total phenolic content and garlic acid was used as standard expressed as mg GAE/g. The concentration of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml were prepared in methanol. The concentration of 1 mg/ml of plant extract was prepared in methanol then placed in a test tube, and it was mixed with 2.5ml of 10-fold diluted Folin Ciocalteu Reagent. The mixture was added to 2ml of 7.5% of sodium carbonate. The tubes were allowed to stand for 30 minutes covered with parafilm at room temperature before taking the absorbance at 760 nm. Reading was taken in triplicates.

Total Flavonoid Content: The total flavonoid content was determined according to the method described by Zengninet *al.*, (2011). A standard solution (10-50 µg/ml) of rutin were added to 75 µl of 5% sodium nitrite (NaNO₂) solution and mixed properly. After 5-6 minutes, 0.5 ml of aluminium chloride (AlCl₃, 100g/l) was added. 0.5 ml of 4% sodium hydroxide (NaOH) was added after 5 minutes. The final volume was kept at 2.5ml with distilled water and thoroughly mixed. The absorbance of the mixture was determined at 510 nm against the same mixture without the sample as blank. Total flavonoid content was expressed as mg RE/g dry weight through the calibration curve of rutin. All samples were analyzed in three replicates.

Total Alkaloid Content: The total alkaloid content was determined according to the method described by Rahelehet *al.*, (2013). 100ppm solution of atropine expressed as mg AE/ml (1 mg in 10 ml of distilled water) was used as standard. From this stock solution, exactly 0.5, 1.0, 1.5, 2.0 and 2.5 ml of atropine solutions were transferred to five different separating funnels. To each separating funnel, 5 ml of phosphate buffer (pH 4.7) and 5 ml Bromocresol green (BCG) solution were added and mix vigorously. The formed complex mixture was extracted with chloroform (5ml). The chloroform fraction was collected in a 10 ml volumetric flask and make up the volume with chloroform. Absorption at a wavelength of 470 nm of each flask was measured, and a calibration graph was drawn.

For the preparation of sample, the plant extract (1 mg/ml) was dissolved in 2N HCl and then filtered. The pH of the extract was adjusted to neutral with 0.1N NaOH. 1 ml of this solution was transferred to separating funnel and to the mixture of 5 ml Bromocresol green solution along with 5 ml of phosphate buffer were added and mixed properly.

The formed mixture was extracted further with 5 ml of chloroform and transferred to 10 ml volumetric flask and make up the volume with chloroform. The absorbances of the complex in chloroform were measured at 470 nm.

Estimation of Total Sterol: Estimation of total sterols in the extract was done by the method of (Attardeet *al.*, 2006). The Liebermann–Burchard (LB) reagent was employed for the quantitative estimation of sterol. It was prepared by adding 0.5 ml of concentrated sulfuric acid in 10 ml of acetic acid anhydride. To 1 ml of each of the extract (1 mg/ml), chloroform was added to make the volume to 5 ml in a test tube. A volume of 2 ml of LB reagent was added and mixed well. These tubes were then covered with foil paper and kept in the dark for 15 min to avoid any exposure to light. The reaction mixture turn green, which was measured spectrophotometrically by using UV-vis spectrophotometer at 640 nm against the blank. Beta-Sitosterol was used as the standard to prepare a calibration curve. All the tests were carried out in triplicates and the results were expressed as Beta-Sitosterol equivalent ($\mu\text{g BS/mg extract}$).

Determination of Cardiac Glycosides: The procedure described by Sofowora (1995) was used. 10ml of the extract was pipetted into a 250 ml conical flask. 50ml chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100ml conical flask. 10ml of pyridine and 2ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10 min. 3ml of 20% NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin) was used at concentration range from 0 – 50mg/ml from stock solution and the absorbance was read at 510nm.

Determination of Total Saponins: The total saponins content was determined by spectrophotometry as described previously (Uematsuet *al.*, 2000). To 2 mL of extract, 1 ml of reagent A (p-anisaldehyde 0.5 in ethyl acetate 99.5) and 1 ml of reagent B (H_2SO_4 50% in Ethyl acetate 50%) were mixed and homogenized in a vortex. The methanol extract and standard (digitonin) were dissolved in 2 ml ethyl acetate. Then, 1 ml of reagent A and 1 ml of reagent B were added. The mixture was stirred and incubated at 600°C for 10 min in a water bath. The solutions were cooled at room temperature for 10 min and the absorbencies of the colour-developed solution were recorded at 430 nm. Ethyl acetate was used as a control for the measurement of absorbance. Solutions containing 75-175 μg standards (digitonin) in 2

ml ethyl acetate were used to obtain a calibration curve. The total saponins was determined from the calibration curve and expressed as milligrams of digitonin equivalent per gram of extracts (mgDE/g).

Determination of Total Glycosides Content (TGC): Balet's reagent spectrophotometer method was used for the determination of the total glycosides content of the extracts as previously reported with slight modification by Tofighiet *al.*, (2016). The extract (1 mg/ml) was dissolved with 6 ml of distilled water and 1 ml of 12.5% (w/v) lead acetate solution was added. The mixture was made up to 10ml with distilled water and filtered. The filtrate (5 ml) was transferred to a volumetric flask, 1 ml of 4.77% (w/v) Na_2HPO_4 solution was added, and made up to 10ml with distilled water and filtered. Baljet's reagent (10 ml) was added to 1 ml of the clear filtrate and the mixture was allowed to stand for 1h and diluted with 20 ml of distilled water. The absorbance of the mixture was read against the blank at 495 nm with a UV/Visible spectrophotometer. The total glycosides content was determined from the calibration curve and expressed as milligram of digitoxin equivalent per gram of extracts (mgDE/g).

2.6 HPLC-DAD Analysis (fingerprinting) and requirements for targeted Tea Markers

HPLC analysis was performed using a Shimadzu HPLC system with UV-DAD detection and a C18 column ($150 \times 4.6 \text{ mm}$, $5 \mu\text{m}$). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B) under isocratic conditions (20% B), flow rate 0.6 mL/min, column temperature 40 °C, and injection volume 10 μL . Detection was performed at 254 nm and run time was 20 min. Under these conditions, chromatograms serve as fingerprints of UV-absorbing compounds.

2.7 Antioxidant Activity Assays

DPPH radical scavenging activity was assessed by monitoring the decrease in absorbance at 517 nm after reaction with DPPH in methanol. Hydrogen peroxide scavenging activity was assessed spectrophotometrically at 430 nm using a hydrogen peroxide-based assay. For each assay, percent inhibition was calculated as: % scavenging = $(1 - \text{As/Ab}) \times 100$, where As is sample absorbance and Ab is blank/control absorbance. IC50 values should be calculated using non-linear regression of dose-response curves (e.g., four-parameter logistic model) and reported with confidence intervals.

2.8 Amino acid profiling

Amino acid profiles were determined using an amino acid analyzer after hydrolysis of dried leaf samples. Because tea sensory quality is influenced mainly by free amino acids extracted into infusions (not total amino acids after hydrolysis), this measurement is reported for nutritional context and should not be over-interpreted as evidence of ‘tea-likeness’.

2.9 Statistical Analysis

Experiments were performed with clear biological replication (independent plant batches) in addition to technical replication. Data reported as mean $\pm$ SD (or SEM) with the number of replicates (n) stated. Differences among groups tested using ANOVA with appropriate post hoc tests at $\alpha = 0.05$.

3. Results

3.1 Qualitative Phytochemical Screening

Table 1: Qualitative Phytochemical Screening Results (+ present; – not detected under the test conditions).

Phytochemical	<i>A. heterophyllum</i>	<i>C. sinensis</i>
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Alkaloids	–	–
Steroids	–	+
Terpenoids	–	–
Glycosides	–	+
Cardiac glycosides	–	–
Anthraquinones	–	–
Anthocyanins	–	–

3.2 Antioxidant activity

DPPH and hydrogen peroxide scavenging activities are shown in Figures 1 and 2. Ascorbic acid showed the highest scavenging activity across the tested concentration range. The plant

extracts did not reach 50% inhibition at concentrations up to 1.0 mg/mL. Thus, IC₅₀ values for both extracts are reported as > 1.0 mg/mL under these experimental conditions.

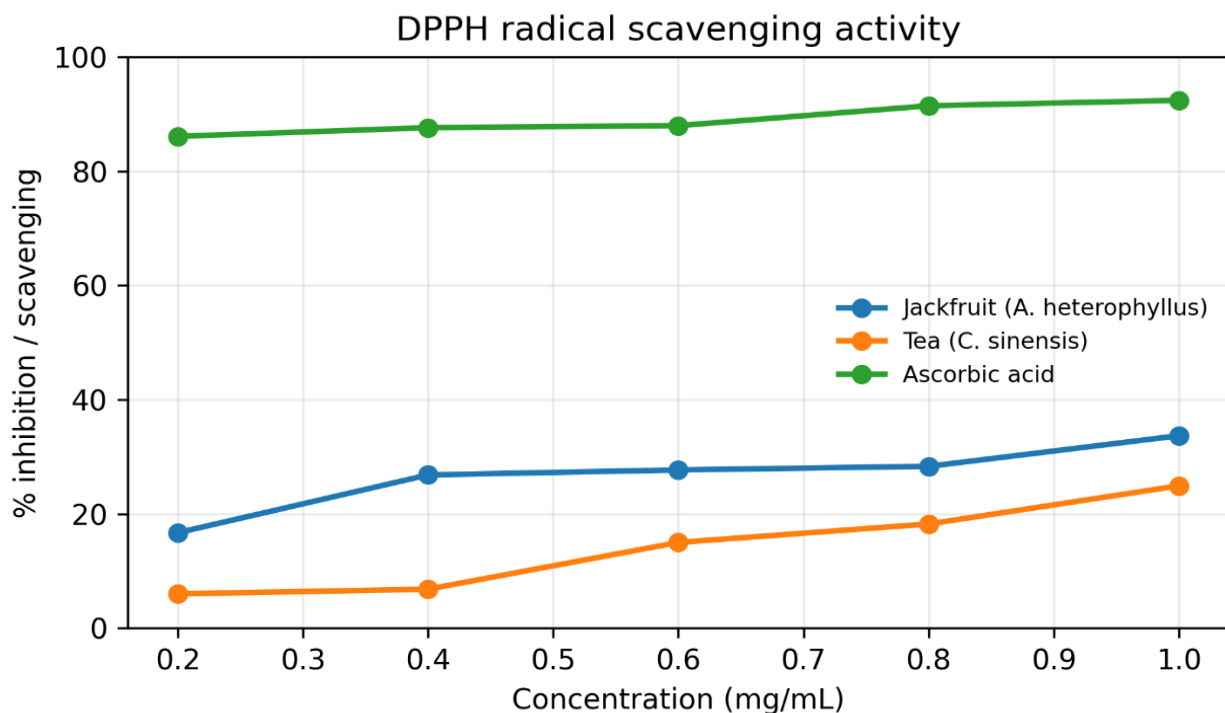


Figure 1. DPPH radical scavenging activity (% inhibition) of *A. heterophyllus*, *C. sinensis*, and ascorbic acid (0.2–1.0 mg/mL) with Values are mean $\pm$ SD

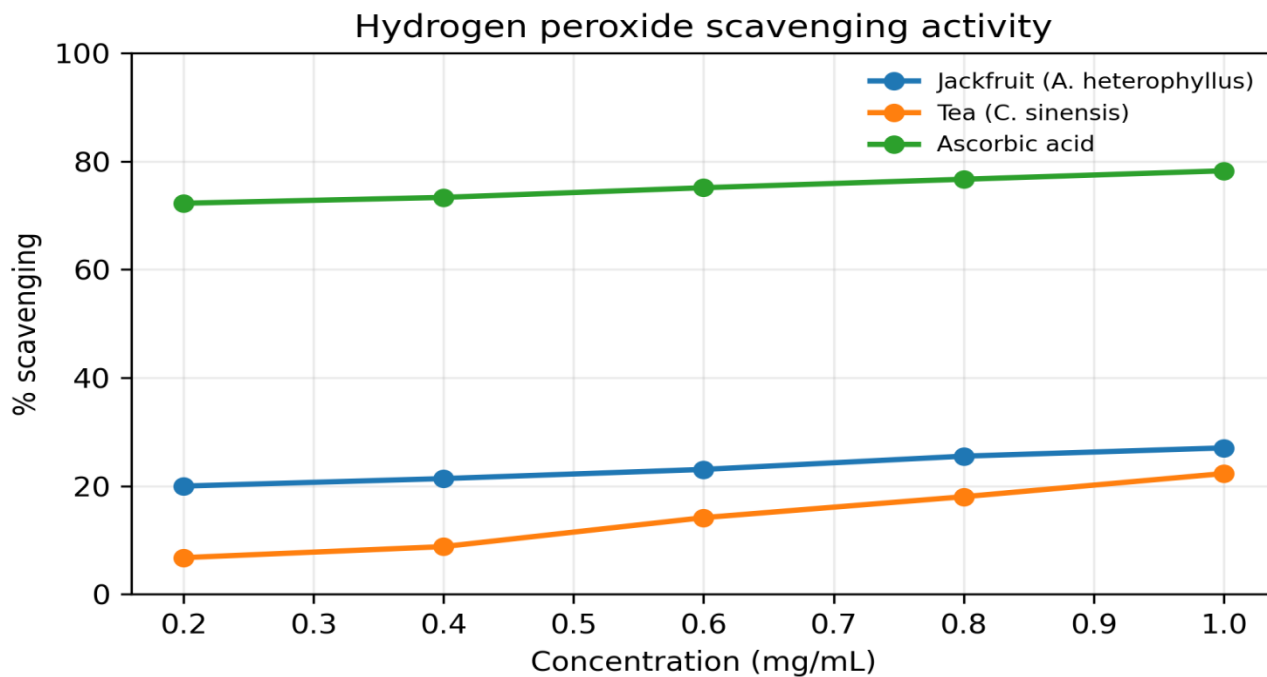


Figure 2. Hydrogen peroxide scavenging activity (% scavenging) of *A. heterophyllus*, *C. sinensis*, and ascorbic acid (0.2–1.0 mg/mL) Values are mean $\pm$ SD3.3

Amino acid profile (leaf hydrolysates)

Figure 3 summarizes the amino acid profiles of the leaf hydrolysates. Values are reported in the

units provided by the analyzer output (mg/L). This profile describes total amino acids after hydrolysis and may differ substantially from free amino acids in a tea infusion.

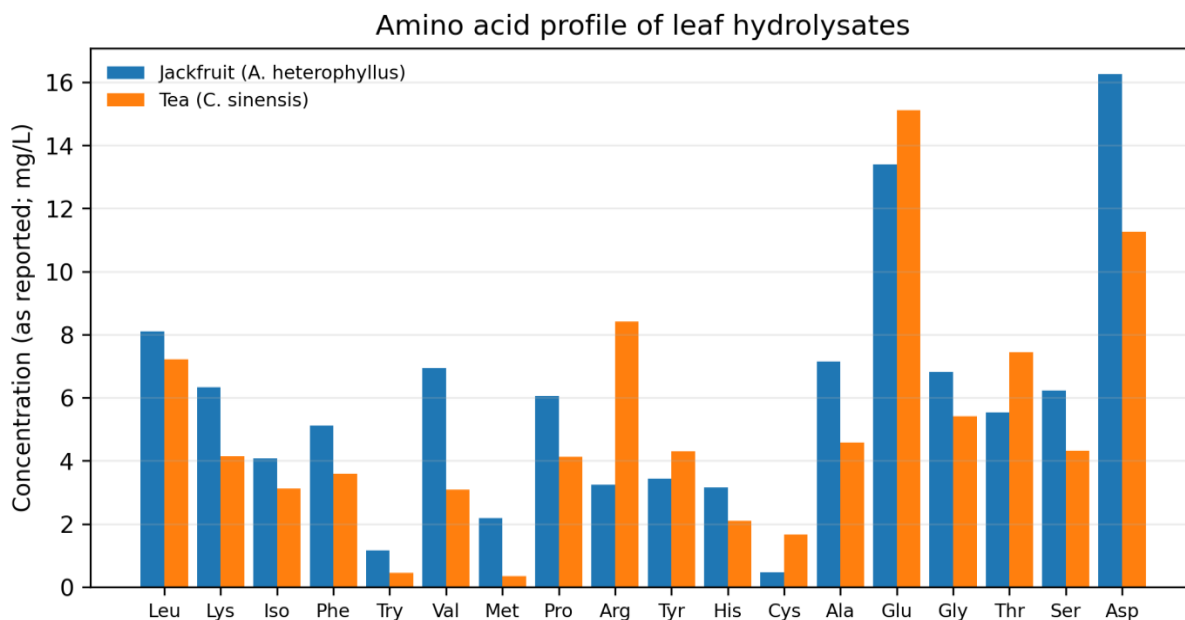


Figure 3: Amino acid profile of *A. heterophyllum* and *C. sinensis* leaf hydrolysates.

3.4 HPLC-DAD fingerprints

HPLC-DAD fingerprints (254 nm, isocratic 20% acetonitrile) indicated multiple UV-absorbing constituents in both samples. Since authentic standards and confirmatory evidence (UV

spectra/peak purity and/or MS) were not applied, under the current conditions, peaks were not assigned to catechins or other tea markers.

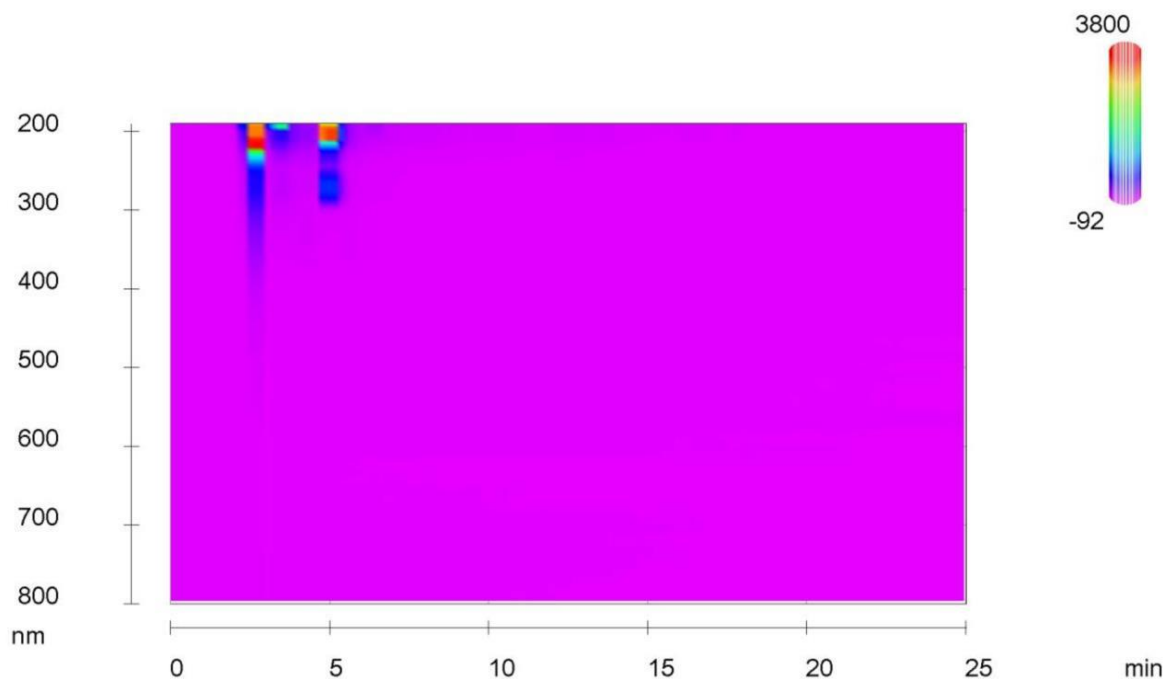


Figure 4: HPLC contour representation for *C. sinensis* sample.

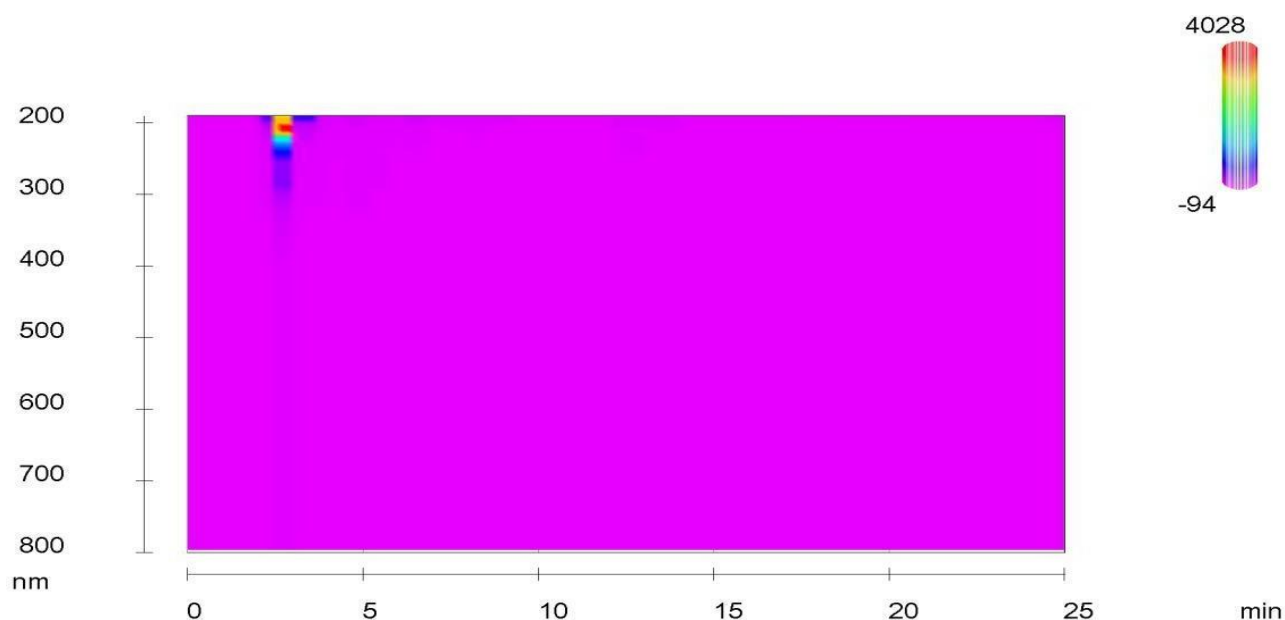


Figure 5: HPLC contour representation for *A. heterophyllus* sample.

4. Discussion

The research project basically work on Jackfruit (*Artocarpus heterophyllus*) and Tea leaves camellia popularly known Tea, the analysis try to justify the former as an alternative to tea by various analytical

methods, The qualitative part of chemical analysis showed, phenols, tanins, flavonoids as positive in both samples as such for treating, cancer treatment of free radical these is also in line with a research of (Matthew, *et al.*, 2022). Phenol, tannins, flavonoid, saponins,

steroids and glycoside of which indicate *camellia sinensis* and jackfruit fruit which significant value between 5mg/ml and 10mg/ml for jackfruit and 25mg/ml and 65mg/ml for *camellia spp*, the jackfruit having 10 mg/ml is a significant. This is seen in respect to phenols, flavonoid and steroid while tannis, saponin and glycosides are less than 10 this indicates the it can be consider as a potential alternative for tea which is standard of *camellia sinensis*, similar report was captured in a work of (Del Rio *et al.*, 2013)

Both essential and non-essential amino acids were significant with a good significant value of *Artocarpus heterophyllus* in all cases having a value or a concentration in milligram up to 16 mg per liter almost all the amino acid few of their minor acids having value less than 2 mg which can be seen in tryptophan and methionine while others indicate amino acid profiling in the case that the fruit is a good source of protein having known that amino acid are building blocks of protein and can be used or consider as a source of tea we can say significant amount of the essential amino acids in the *camellia spp* and jackfruit this indicate that the jackfruit drink can be an alternative source having range of consumptions (Kan *et al.*, 2020).

HPLC-DAD fingerprints (254 nm, isocratic 20% acetonitrile) indicated multiple UV-absorbing constituents in both samples. Since authentic standards and confirmatory evidence (UV spectra/peak purity and/or MS) were not applied, under the current conditions, peaks were not assigned to catechins or other tea markers signifying active presence which uses ascorbic acid as standard.

Though good for consumption, DPPH and hydrogen peroxide scavenging activities as seen above proved with minimal activity, Ascorbic acid showed the highest scavenging activity across the tested concentration range. The plant extracts did not reach 50% inhibition at concentrations up to 1.0 mg/mL. Thus, IC50 values for both extracts are reported as > 1.0 mg/mL under these experimental conditions (Marrero *et al.*, 2012)

5. Conclusion

Artocarpus heterophyllus leaves contained detectable phytochemical classes and demonstrated measurable in vitro antioxidant activity in DPPH and hydrogen peroxide scavenging assays. However, based on the current analytical scope and the absence of targeted quantification of tea-defining markers, *A. heterophyllus* may be concluded to be an alternative to *Camellia sinensis* in a compositional sense. Future work should standardize sampling and extraction,

report extraction yields and full calibration/validation parameters for quantitative assays, quantify catechins, caffeine and theanine using validated chromatographic methods, and evaluate beverage-relevant infusions with appropriate sensory and chemical benchmarks.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

All data are presented in the report

Competing interests

All authors declare no competing interests.

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