

COMPARATIVE INVESTIGATIONS OF BIOACTIVE COMPOUNDS AND NUTRIENT COMPOSITIONS OF CAMELLIA SINENSIS AND ANNONA MURICATA

By

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ABSTRACT

*This study undertook a comprehensive comparative analysis of the bioactive compounds and nutrient composition present in tea leaves (*Camellia sinensis*) and soursop (*Annona muricata*), two plants widely recognized for their medicinal properties. Through a combination of chromatographic and spectroscopic techniques, we identified and quantified a range of phytochemicals in both plants, including flavonoids, alkaloids, phenolic acids, and acetogenins. Notably, our results reveal striking similarities in the bioactive content of tea leaves and soursop, with both plants containing significant amounts of gallic acid, catechins, and annona ceousacetogenins. These compounds are known for their potent antioxidant, anti-inflammatory, and anticancer properties, suggesting potential applications in the prevention and treatment of various diseases. The similarities in bioactive content between tea leaves and soursop also imply that these plants may share common molecular mechanisms underlying their therapeutic effects. This study provides new insights into the phytochemical profiles of tea leaves and soursop, highlighting their potential as valuable resources for the development of natural products and preventive medicine. This study comparatively evaluates the phytochemical analysis, Quantitative analysis, Amino acid Profiling and antioxidant capacity, investigating the potential as alternative sources of bioactive compounds present in the two leave samples. In conclusion, these plants soursop (*Annona muricata*), are important sources of nutrients and bioactive compounds, as well tea leaves (*Camellia sinensis*). Further research is needed to fully elucidate the synergistic effects of these compounds and to explore their implications for human health.*

Keywords: *Annona muricata*, Phytochemical, Antioxidants, DPPH and *C. sinensis*

INTRODUCTION

Worldwide, *Camellia sinensis*, native to Southeast Asia, is one of the most widely consumed beverages, second only to water (Liao *et al.*, 2020). With a history dating over 2,000 years to ancient China, where it was first consumed as a medicinal drink, tea has spread across the world, becoming a key component of cultures in China, India, Japan, and beyond (Ajala *et al.*, 2013). Nowadays, over 18–20 billion cups of tea is consumed on a daily basis globally, which is considered as one of the most popular non-alcoholic beverages (Wang, 2019). Tea can be classified into many types according to the diverse definition methods in different countries. In China, according to the degree of fermentation, tea is divided into six major tea lines: green tea, black tea,

white tea, yellow tea, Oolong tea, and dark tea (Xu *et al.*, 2019). Green tea was the first tea to be discovered, and it is a non-fermented tea. It retains more natural substances in fresh leaves and has less vitamin loss, thus forming the characteristics of green tea as “clear soup with green leaves and strong flavour convergence”. A large number of researchers and analysts have confirmed that tea possesses chemical nutrients that are essential to human health. Tea polyphenols, caffeine, theanine, tea polysaccharides, and other components which are extracted and separated from tea have pharmacological activities such as anti-cancer (Bala *et al.*, 2019), anti-oxidation (Lambert and Elias, 2010), protecting the nervous system (Yoneda *et al.*, 2019), and lowering blood sugar (Amorim *et al.*, 2018). Tea, especially the green one is considered to be suitable for patients with hypertension, hyperlipidemia, coronary heart disease, arteriosclerosis, and diabetes. However, it is important to keep in mind that “natural” does not always mean perfectly safe.

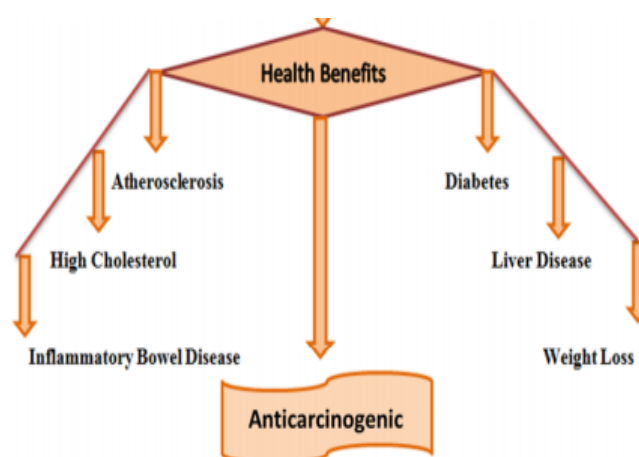


Figure 1: Summary of the health benefits of tea.

Beyond *Camellia sinensis*, many other plants are consumed as herbal infusions and/or remedies, often mimicking the sensory qualities of traditional tea but lacking scientific validation of their nutritional or bioactive potential. *Annona muricata*, commonly known as soursop, is a member of the *Annonaceae* family; a plant widely used in traditional medicine across West Africa, including Nigeria, due to its perceived antimicrobial, anti-inflammatory, and antioxidant properties (Adewole & Ojewole, 2009). Its leaves contain a range of phytochemicals, including flavonoids, phenolic acids, gallotannins, alkaloids, and annonaceous acetogenins (notably annonacin), which exhibit potent bioactivity. Traditional extraction methods, such as maceration and Soxhlet extraction, are often solvent-intensive and may degrade heat-sensitive compounds, prompting interest in modern techniques like ultrasound-assisted extraction (UAE) for improved efficiency (Anuragi *et al.*, 2016).

However, there remains a notable gap in scientific validation regarding *A. muricata*'s phytochemical profile, nutrient composition, and health benefits compared to *C. sinensis*. Given the increasing consumers' shift toward natural and functional beverages, there arises an urgent need for a comparative study. This work is therefore aimed at a comparative evaluation of *A. muricata* and *C. sinensis* which is crucial to assessing the potential of soursop leaves as a viable tea alternative or complementary functional beverage. Specifically, this study addresses the following questions: What differences exist in their bioactive compound profiles? How do their nutrient compositions compare? Which plant demonstrates higher antioxidant activity?

To answer the above questions; the specific objectives of this study are:

- To compare the bioactive compound profiles (polyphenols, flavonoids, alkaloids) of *C. sinensis* and *A. muricata*.

- To assess their nutrient composition, including vitamins, minerals, and amino acids.
- To determine their antioxidant activity using radical scavenging assays.
- To profile major bioactive compounds and analyze UV-visible spectral characteristics of the extracts.

METHODOLOGY

- **Materials**
 - **Plant Samples**

Fresh leaves of *Camellia sinensis* were collected from Mambilla Plateau, Taraba State, Nigeria, while *Annona muricata* leaves were obtained from Abuja Metropolis. These samples served as the experimental plant materials.



Figure 2: *Camellia sinensis* plant



Figure 3: *Annona muricata* plant

- **Chemicals and Reagents**

All chemicals and reagents used were of analytical grade, sourced from Sigma-Aldrich and UK Chemicals, and used without further purification.

- **Methods**

- **Sample Preparation and Extraction**

The leaves were washed, shade-dried, and ground into a fine powder. Each sample (40 g) was soaked in distilled water for 24 hours, filtered through muslin cloth, and the filtrates were concentrated at 50°C to obtain crude aqueous extracts. The percentage yield was calculated for each extract. To mimic the traditional hot-brew tea method, an additional extraction was performed by heating the aqueous extract to 75°C, cooling for 24 hours, and concentrating to obtain clear extracts.

- **Experimental Design**

Both qualitative and quantitative analyses were carried out to characterize the samples:

- **Qualitative:** Phytochemical screening.

- **Quantitative:** Amino acid profiling, UV–visible spectrophotometry, antioxidant analysis, quantitative phytochemical assays, and high-performance liquid chromatography (HPLC).

- **Qualitative Phytochemical Screening**

Qualitative analysis of bioactive constituents was performed using standard procedures described by Vishnu et al. (2019). Tests included:

- **Phenols:** Formation of a bulky white precipitate with 10% lead acetate.

- **Tannins:** Dark green coloration with 5% ferric chloride.

- **Flavonoids:** Yellow precipitate with 10% lead acetate.

- **Alkaloids:** Orange/red precipitate with HCl and Dragendorff's reagent.

- **Glycosides** (Borntrager's test): Pink coloration after chloroform–ammonia treatment.

- **Cardiac glycosides** (Keller–Killiani test): Brown ring at the interface of H₂SO₄ and glacial acetic acid.

- **Saponins:** Persistent froth upon vigorous shaking.

- **Steroids:** Red coloration and yellowish-green fluorescence with chloroform–H₂SO₄.

- **Terpenoids:** Reddish-brown interface in Salkowski test.

- **Anthocyanins:** Pink-red turning blue-violet with HCl and ammonia.

- **Anthraquinones:** Rose-pink coloration with H₂SO₄ and ammonia.

- **Quantitative Phytochemical Analyses**

- **Total Phenolic Content:** Determined using the Folin–Ciocalteu method (Maurya & Singh, 2010). Results expressed as mg gallic acid equivalent (GAE)/g.

- **Total Tannins:** Quantified using Folin–Ciocalteu reagent (Rajeev *et al.*, 2012). Results expressed as mg GAE/100 g.

- **Total Flavonoid Content:** Determined using rutin as standard (Zengnin *et al.*, 2011).
- **Total Alkaloids:** Quantified using the Bromocresol Green (BCG) method (Raheleh *et al.*, 2013). Results expressed as atropine equivalents.
- **Total Sterols:** Measured using Liebermann–Burchard reagent (Attarde *et al.*, 1976). Results expressed as β -sitosterol equivalents.
- **Cardiac Glycosides:** Quantified by Sofowora's method (1995).
- **Total Saponins:** Determined using anisaldehyde and sulfuric acid reagents (Uematsu *et al.*, 2000). Results expressed in digitonin equivalents.
- **Total Glycosides:** Analyzed using Baljet's reagent (Tofighi *et al.*, 2016). Results expressed as digitoxin equivalents.

➤ **Amino Acid Analysis**

Defatting of Samples

Samples were defatted using a 2:1 chloroform/methanol mixture in a Soxhlet extractor for 15 hours (AOAC, 2006).

Nitrogen Determination (Kjeldahl Method)

Nitrogen content was determined to estimate protein composition. Samples were digested with H₂SO₄ and catalysts, distilled into boric acid, and titrated with 0.01 N HCl.

Hydrolysis of Samples

Defatted samples were hydrolyzed in 6 N HCl at 105°C for 22 hours under nitrogen (Peace & Gilani, 2005). Hydrolysates were filtered, evaporated, and reconstituted in acetate buffer (pH 2.0).

Amino Acid Profiling

Amino acids were quantified using an Applied Biosystems PTH Amino Acid Analyzer with norleucine as internal standard. Eighteen amino acids were resolved, including leucine, lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, proline, arginine, tyrosine, histidine, cystine, alanine, glycine, threonine, serine, aspartic acid, and glutamic acid.

➤ **High-Performance Liquid Chromatography (HPLC) Analysis**

HPLC analysis followed the modified method of Adamu *et al.*, (2018) using a Shimadzu UFLC system with UV–DAD detection:

- **Mobile phase:** A (0.1% formic acid in water), B (acetonitrile); isocratic (20% B).
- **Flow rate:** 0.6 ml/min, Temperature: 40°C, Injection volume: 10 μ L.
- **Detection:** 254 nm, Run time: 20 minutes. Bioactive compounds were identified based on retention times and spectral characteristics.

- **Statistical Analysis**

All analyses were conducted in triplicate, and results expressed as mean $\pm$ standard error of mean (SEM). Statistical significance was accepted at $p < 0.05$. IC₅₀ values for antioxidant assays were determined using concentration–response curves in Origin software, while graphs were generated using GraphPad Prism 6.

RESULTS

- **Analysis Of Soursop And Camellia Tea Leaves**

Various analyses were conducted on Soursop and camellia tea leaves to evaluate their potential as a tea product. The results are summarized in a table and in figures below:

- **Antioxidant Analysis**

- **DPPH Assay:** Both Soursop and camellia showed significant antioxidant activity at concentrations of 10mg/ml, 20mg/ml, and 100mg/ml.

- **HRSA Assay:** Camellia and Soursop had similar activity at 20mg/ml, while ascorbic acid showed higher activity at 97mg/ml.

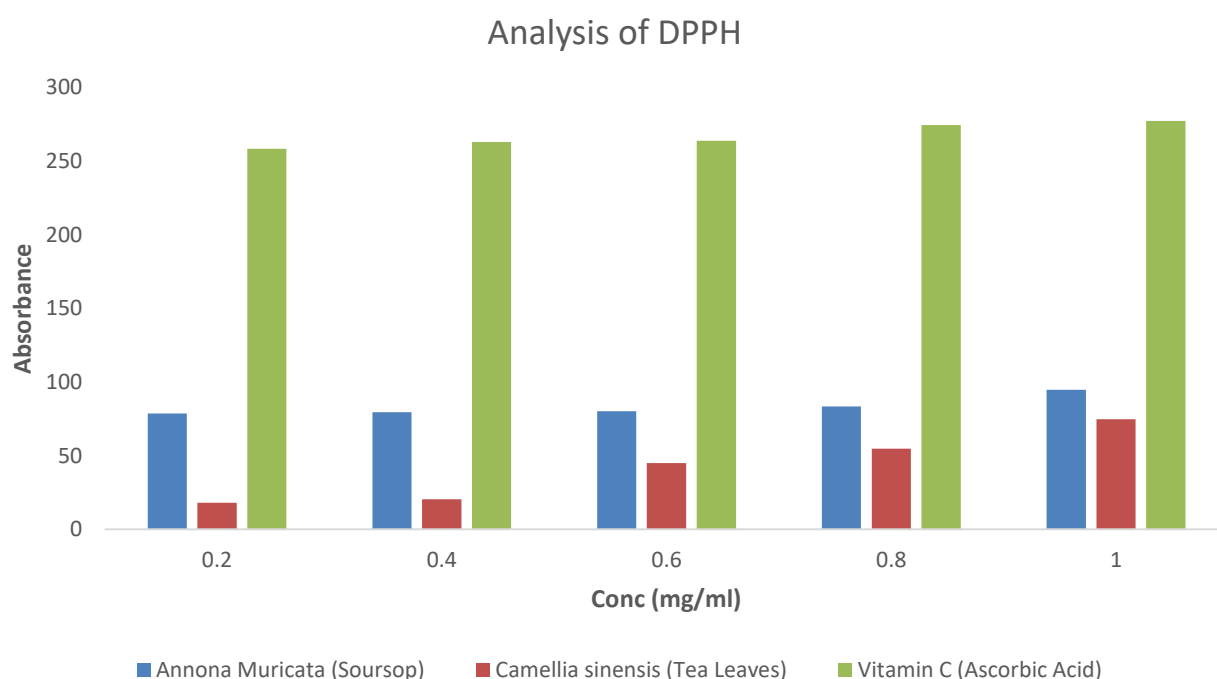


Figure 4: Analysis of DPPH

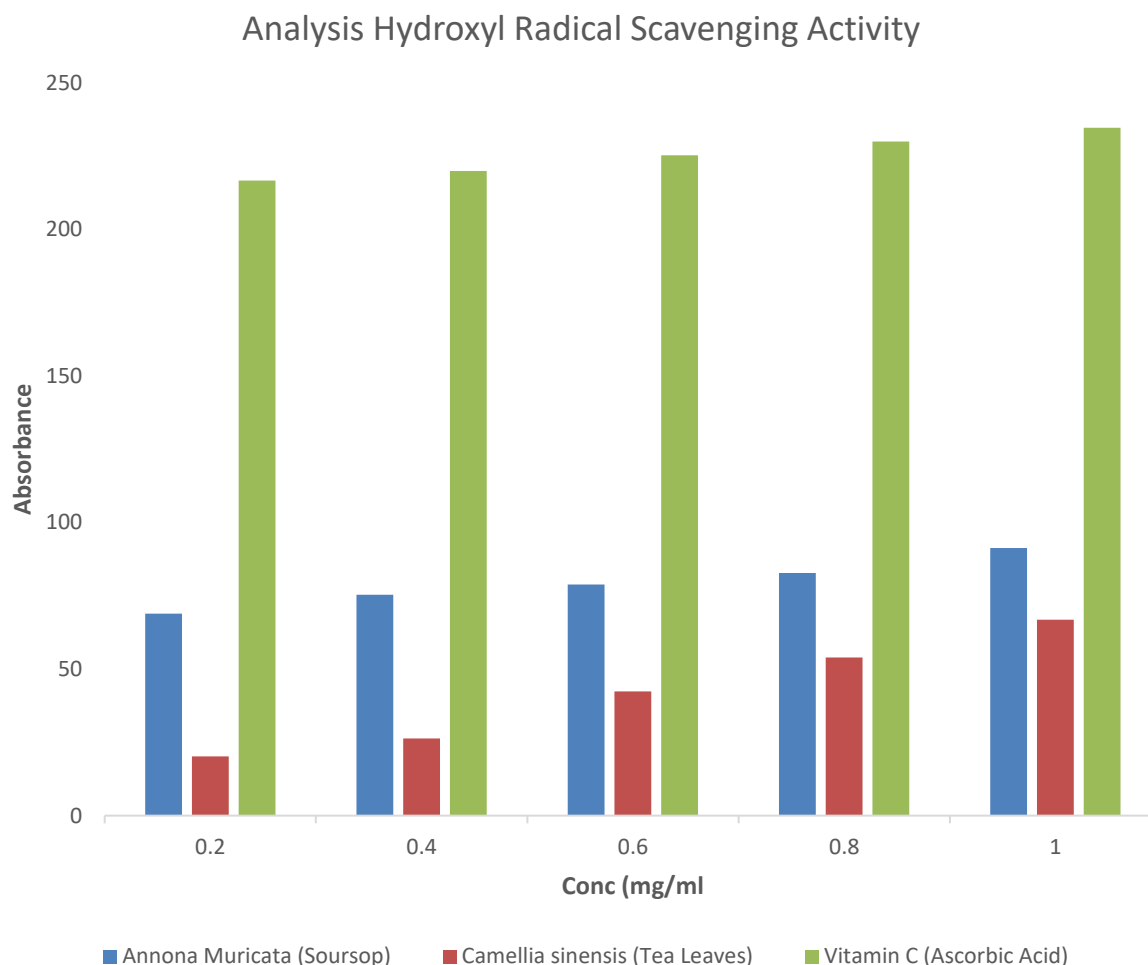


Figure 5: Analysis of Hydroxyl Radical Scavenging Activity

• Phytochemical Analysis

Qualitative Analysis: The qualitative screening revealed the presence of key phytochemicals—phenols, tannins, flavonoids, saponins, and glycosides—in both *A. muricata* and *C. sinensis*, supporting their traditional use in herbal medicine. Alkaloids and cardiac glycosides were detected only in soursop, suggesting potential anticancer, antidiabetic, and cardioprotective effects. Steroids, found uniquely in *C. sinensis*, may contribute to membrane stability and metabolic regulation. The presence of saponins in both plants indicates potential cholesterol-lowering, immune-boosting, and antioxidant properties, though potency may vary.

Quantitative Analysis: The levels of phenols, tannins, flavonoids, saponins, steroids, and glycosides were measured. Soursop had values ranging from 0.01 mg/g to 15.9 mg/g, while camellia had values between 9.78 mg/g and 47.98 mg/g, indicating potentially lower potency in Sour sop

Table 1: Phytochemical Analysis of *Camellia sinensis* and *Annona muricata*

Phytochemical	<i>Annona muricata</i>	<i>Camellia sinensis</i>
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	-
Steroids	-	+
Terpenoids	-	-
Saponins	+	+
Glycosides	+	+
Cardiac glycosides	+	-
Anthraquinones	-	-
Anthocyanins	-	-

Key: + means present; - means absent

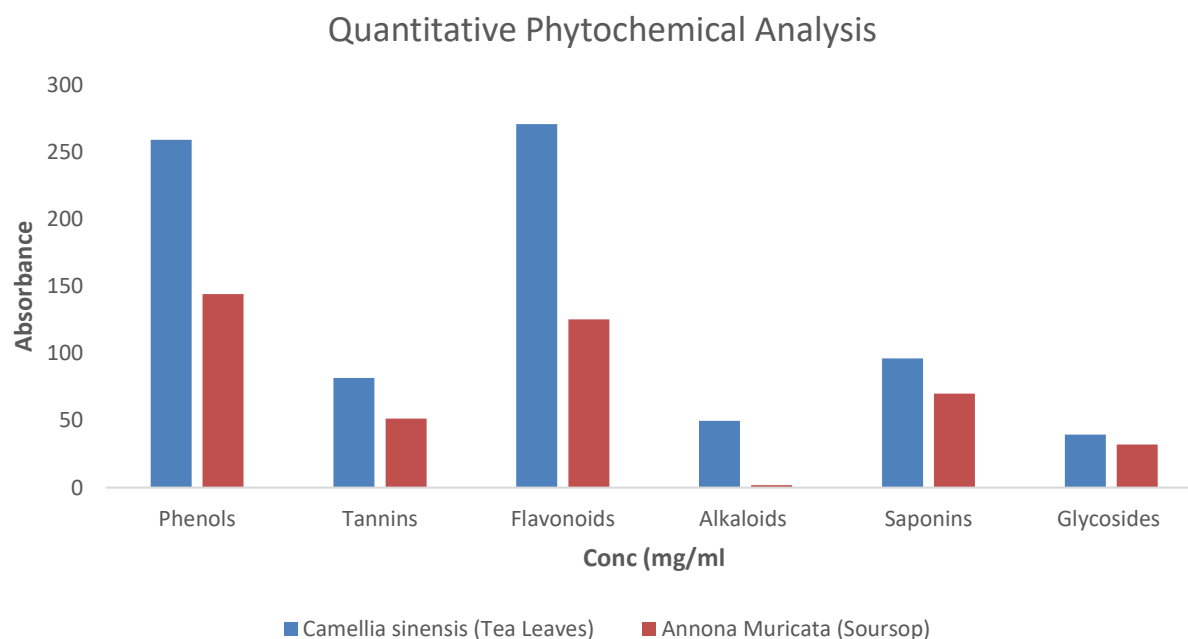


Figure 6: Quantitative Phytochemical Analysis

• Amino Acid Profiling

Both plants showed 19 of 20 common amino acids, including essential ones. Soursop had substantial glutamic acid (up to 18 mg/L), suggesting nutritional relevance. The absence of cysteine, methionine, and tryptophan in soursop may slightly reduce its protein quality, but its diversity supports potential as a functional beverage.

Annona (Soursop) Amino Acid profiling

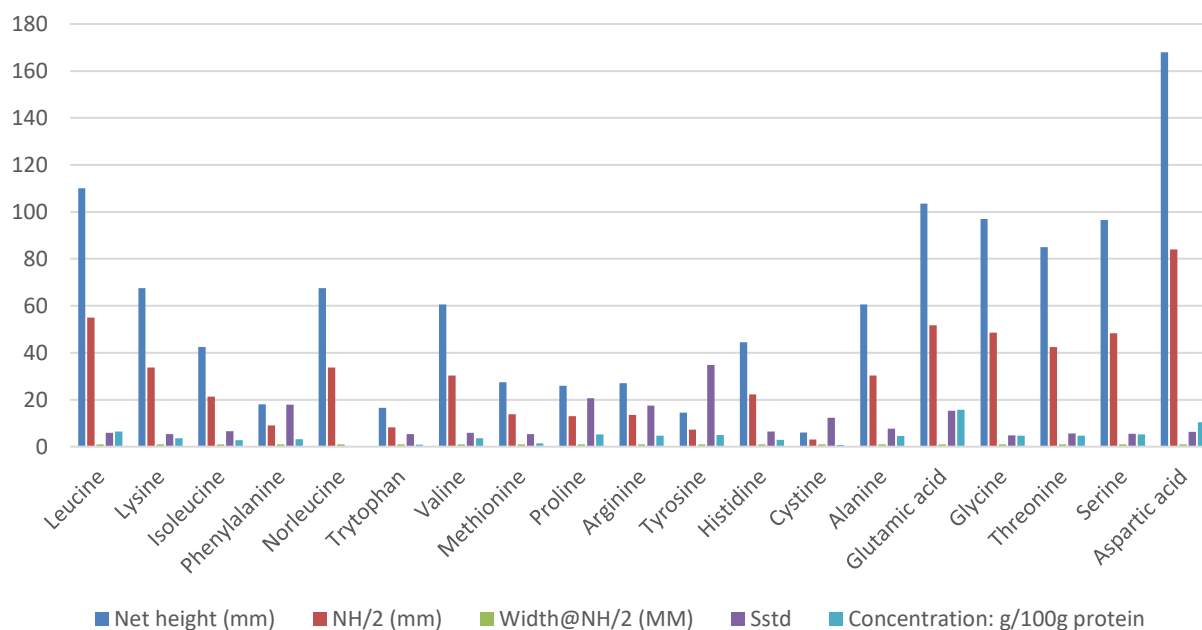


Figure 7: *Annona muricata* (SOUR SOP) Amino Acid Profiling

Camellia sinensis (Tea Leaves) Amino Acid profiling

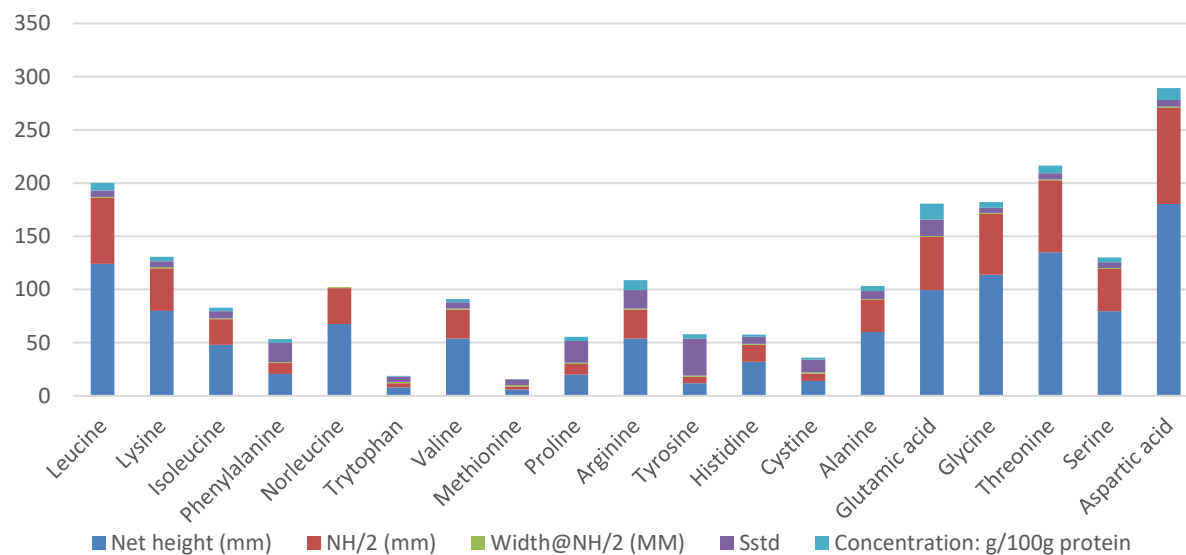


Figure 8: *Camellia sinensis* (Tea Leaves) Amino acid Profiling

- **HPLC Analysis**

Using ascorbic acid as a reference standard, camellia showed a significant presence, while Soursop showed a mere appearance.

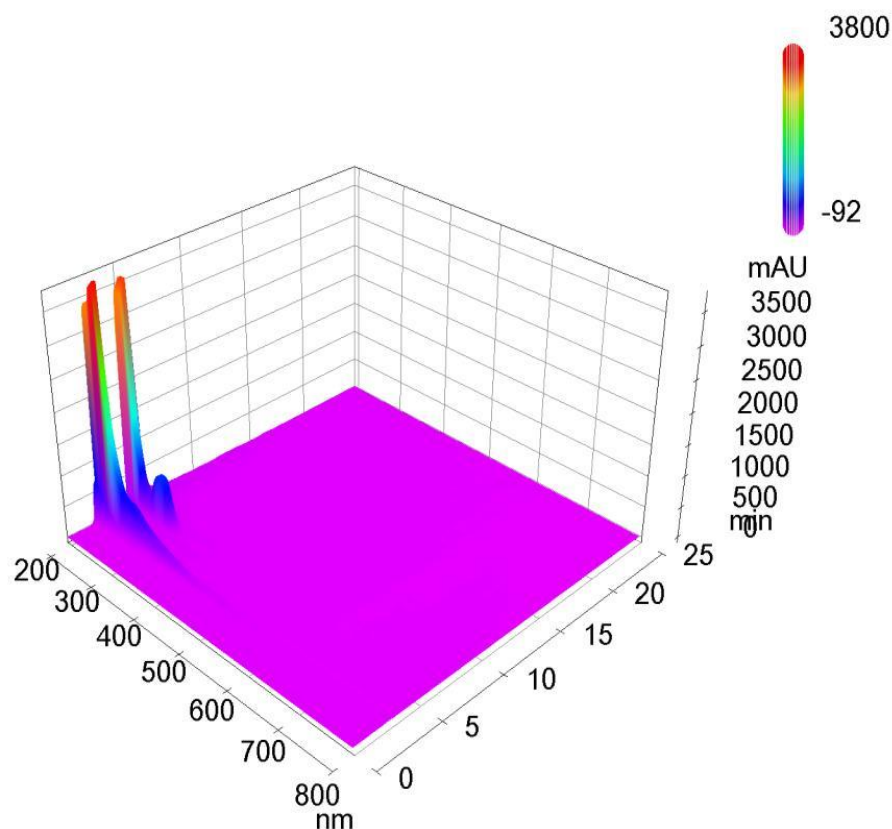


Figure 9: 3D Representation of *Camellia sinensis* HPLC Analysis

<Chromatogram>

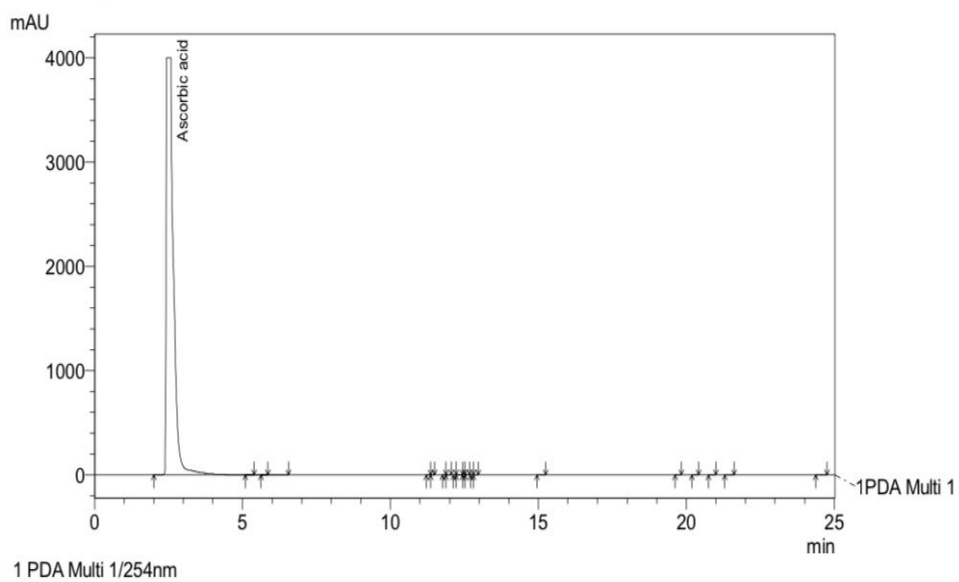
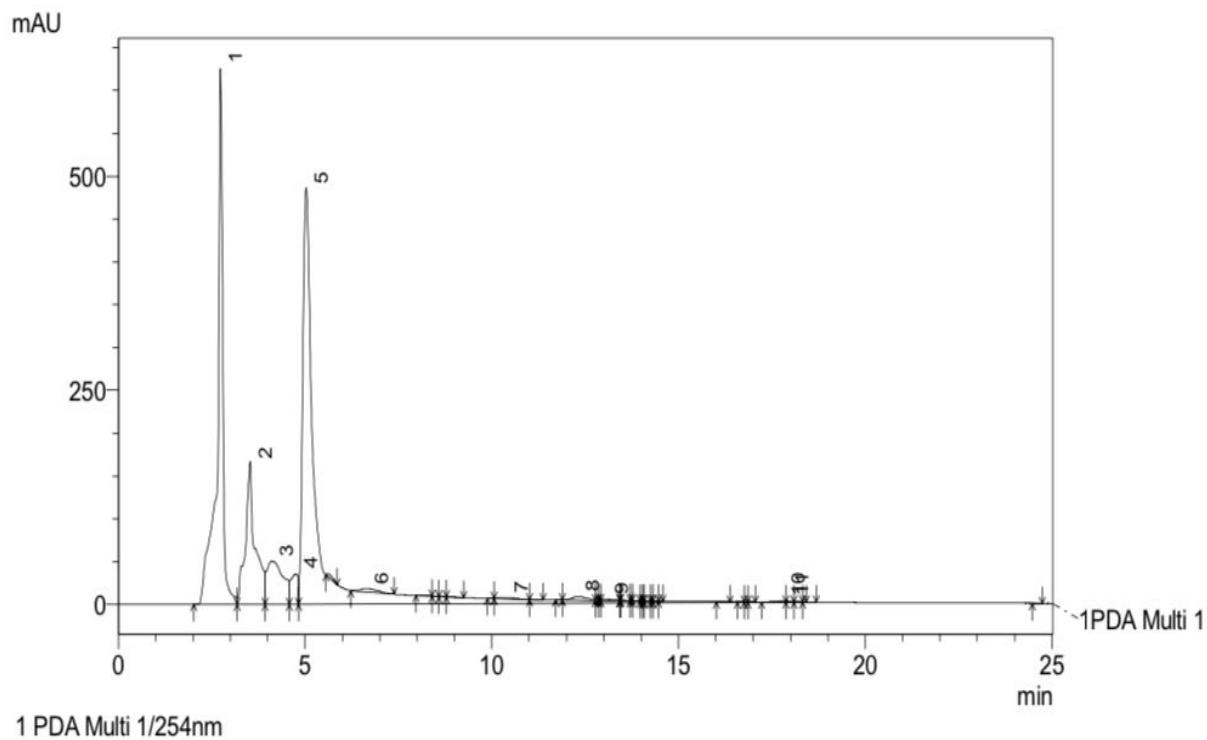


Figure 11: HPLC Chromatogram representation of ascorbic acid a standard used for the analysis having a retention time of 2.571 and area 68582692



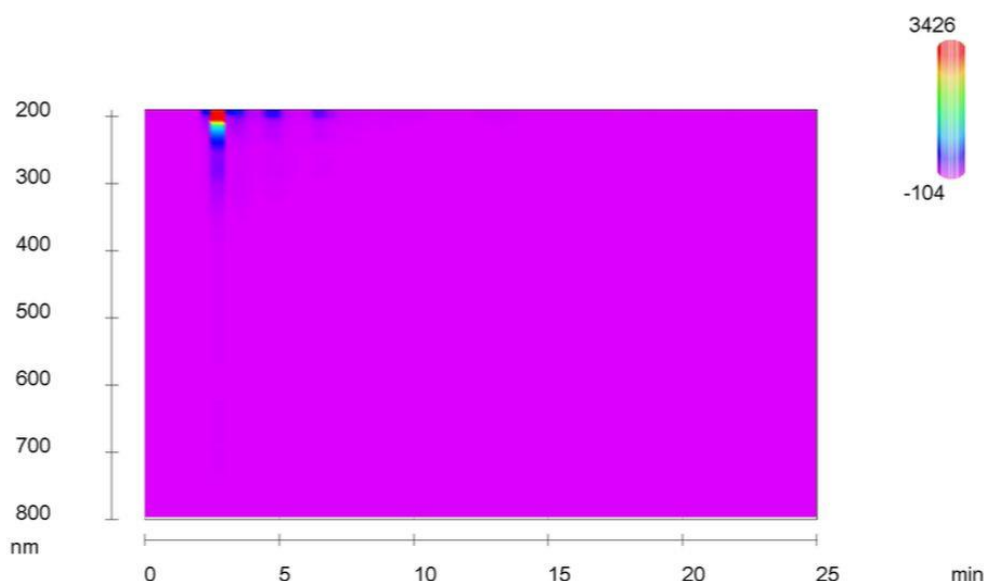


Figure 13: HPLC contour representation of sour soup (*Anona muricata*) a standard

- **Spectrophotometer Analysis**

The highest absorbance was observed at 250nm wavelength for both Soursop (4.196) and camellia (4.361), suggesting this wavelength is optimal for absorbance analysis.

Overall, the results suggest that Soursop has potential as a tea product, but its potency might be lower compared to camellia tea leaves.

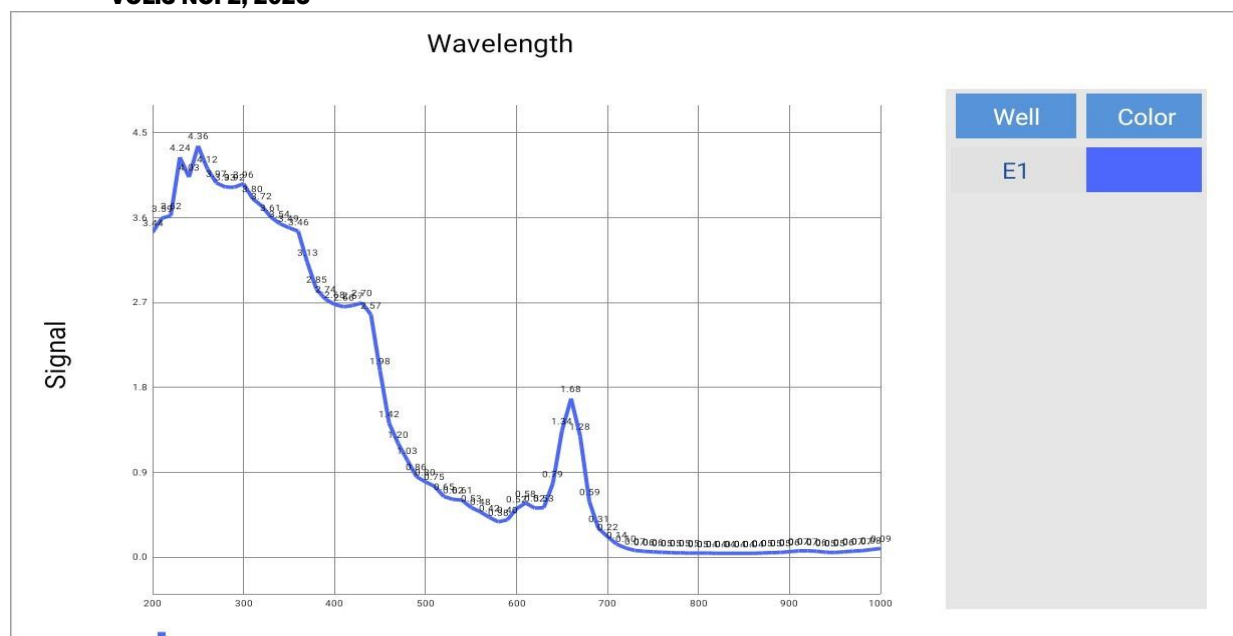


Figure 14: Spectral Studies of *Camellia sinensis*

DISCUSSION

This study evaluated the phytochemical composition, amino-acid profile, antioxidant potential, and chemical characteristics of *Annona muricata* (soursop) leaves in comparison with *Camellia sinensis* (tea) leaves, with the aim of determining the suitability of soursop as an alternative herbal tea. The qualitative and quantitative findings of this research align closely with prior studies reporting that soursop leaves contain key secondary metabolites such as phenols, flavonoids, tannins, and alkaloids, which contribute to their antioxidant and therapeutic potential. Similar profiles have been documented in recent analyses showing that soursop leaf infusions are rich in polyphenols and flavonoids and demonstrate strong radical-scavenging activity, with DPPH inhibition values exceeding 70% in some extracts (Life, 2024). Similarly, other authors have identified major flavonoids such as quercetin and epicatechin derivatives in soursop through HPLC analysis, supporting the diverse chemical profile observed in the present study (Adewole and Ojewole 2015).

In contrast, *Camellia sinensis* leaves exhibited substantially higher concentrations of phenolic and flavonoid compounds, consistent with extensive literature demonstrating the polyphenol-rich nature of tea, especially its high catechin content and strong antioxidant activity (Allassane *et al.*, 2022). These established findings corroborate the higher phytochemical yields, stronger antioxidant responses, and larger HPLC peak intensities observed in tea samples compared to soursop. Studies on tea and herbal infusions similarly emphasize that tea leaves possess

markedly superior free-radical-scavenging properties due to their enriched phenolic pool (Attaguile *et al.*, 2024).

The antioxidant activity measured in this study through DPPH and hydroxyl-radical scavenging assays parallels earlier work showing that both soursop and tea extracts have significant in vitro antioxidant capacity, although soursop generally presents lower potency than tea (Attaguile *et al.*, 2024). Additionally, the detection of alkaloids and cardiac glycosides specifically in soursop is consistent with reports identifying unique bioactive constituents—such as acetogenins and various glycosides—in soursop leaves that may contribute to their documented antimicrobial, anti-inflammatory, and DNA-protective properties (Adewole and Ojewole 2015).

Although few studies have compared amino-acid profiles of herbal leaves directly, the presence of a broad spectrum of amino acids in soursop and tea is consistent with the nutritional potential attributed to many medicinal plants. Broader investigations of herbal blends also support the idea that soursop contributes meaningful nutritional and antioxidative properties when consumed as an infusion, especially when combined with other plants (Adewole and Ojewole 2015). Moreover, research on processing effects indicates that leaf maturity and drying methods significantly influence the phytochemical and antioxidant content of soursop, reinforcing the importance of standardized preparation in comparative studies (Baskar *et al.*, 2007).

Overall, the findings of this study agree with the current scientific consensus that *Camellia sinensis* remains a superior source of antioxidants and polyphenols, while soursop leaves—despite lower phytochemical concentrations—offer valuable bioactive compounds and nutritional constituents that justify their use as a complementary or alternative herbal infusion.

CONCLUSION

This comprehensive analysis suggests that soursop has potential as an alternative to traditional tea leaves, with significant amounts of phytochemical, amino acids, and antioxidants. These results contribute to a growing body of evidence supporting the therapeutic and functional potential of soursop leaf tea, while also highlighting the need for standardized, directly comparative research to further elucidate differences in compositional and health-related properties between soursop and traditional tea leaves. Further studies are necessary to fully explore the potential of soursop as a tea product.

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- **Conflict of Interest**

The authors declare that they have no financial or other relationships that might lead to any conflict.

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