World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Phytochemical analysis of *Citrus aurantifolia* (LIME) fruit

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World Journal of Advanced Research and Reviews, 2025, 26(02), 4158–4163

Publication history: Received on 20 April 2025; revised on 28 May 2025; accepted on 31 May 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.2.2113

Abstract

Phytochemicals are naturally occurring plant-derived compounds known for their numerous health benefits, particularly their antimicrobial, antioxidant, and therapeutic properties. This study investigates the qualitative and quantitative phytochemical composition of lime fruit (*Citrus aurantifolia*) using ethanol, water, and n-hexane as solvents. The qualitative screening revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, coumarins, and quinones, with varying solubility across different solvents. Cardiac glycosides and coumarins were notably abundant in ethanol and water extracts, while alkaloids were consistently present in all solvents. Quantitative analysis confirmed the presence of alkaloids (0.881 %), saponins (0.881 %), tannins (4.4 mg/L), and glycosides (2.54 mg/ml), while flavonoids and phenols were not detected. Comparisons with previous studies revealed some inconsistencies, particularly in the absence of flavonoids and terpenoids, which are typically present in citrus species. These variations may be attributed to differences in plant part analyzed, solvent polarity, extraction methods, or geographic origin. The findings highlight the therapeutic potential of lime fruit and emphasize the need for improved detection methods in future research.

Keywords: *Citrus aurantifolia*; Phytochemicals; Alkaloids; Cardiac Glycosides; Tannins; Saponins; Flavonoids; Lime Fruit; Secondary Metabolites

1. Introduction

Medicinal plants have long been considered valuable resources for maintaining health and treating diseases. They hold a significant place in traditional healing systems, reflecting the accumulated knowledge of ancient civilizations. In recent years, interest in plant-derived compounds has increased due to their diverse applications in health and industry. Medicinal plants are recognized as rich sources of bioactive compounds used in traditional and modern medicine, dietary supplements, and pharmaceuticals (1).

Phytochemicals are naturally occurring chemical substances in plants that provide health benefits beyond basic nutrition. These compounds play protective roles in plants, shielding them from environmental stressors such as UV radiation, pathogens, and pollution (2,3). When consumed in adequate amounts, phytochemicals can support human health by reducing the risk of chronic diseases such as cancer, diabetes, cardiovascular disorders, and more (4).

Plant nutrients, including proteins, fats, carbohydrates, vitamins, and minerals, contribute significantly to human and animal development. Phytochemicals are divided into primary and secondary metabolites. Primary metabolites, such as amino acids and nucleotides, are essential for plant growth, while secondary metabolites, including flavonoids, alkaloids, tannins, and terpenoids, aid in plant defense and ecological functions like pollinator attraction (4). Many

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secondary metabolites have commercial value and are used in food, pharmaceutical, fragrance, dye, and pesticide industries. Recent research highlights their role in disease prevention and health promotion in humans (4).

Citrus fruits, which belong to the Rutaceae family, are globally cultivated and widely appreciated for their nutritional and therapeutic values. According to Okwu and Emenike (5), five primary species include Citrus sinensis (sweet orange), *Citrus aurantifolia* (lime), Citrus reticulata (tangerine), Citrus limonum (lemon), and Citrus vitis (grapefruit).

The use of plants in traditional medicine continues to grow, with many plants proven effective against infections, while others may pose toxicity risks. Lime fruit, often used in folk medicine, remains under-researched in terms of its chemical composition. This gap in knowledge prevents proper understanding and safe usage of the plant. This study aims to address this issue by identifying the specific phytochemicals present in lime fruit, thus broadening its potential uses. The research focuses on the phytochemical analysis of lime fruit, examining various components of the plant to determine its chemical profile.

2. Method of analysis

Sample Collection and Preparation: Fresh lime fruits (*Citrus aurantifolia*) were procured from a local vendor at Abakpa Market in Enugu State, Nigeria, specifically for phytochemical analysis. The samples were immediately transported to Pymotech Research Centre and Laboratories, Abakpa, Enugu, for processing. Upon arrival, the fruits were thoroughly washed under running tap water to eliminate dirt and potential contaminants. The fruits were then peeled, and the seeds carefully removed. The pulp was homogenized using an electric blender to obtain a uniform extract. This crude extract was stored in air-tight, stoppered glass containers to preserve its integrity prior to analysis. For solvent extraction, aliquots of the extract were placed in test tubes and treated separately with ethanol, distilled water, and n-hexane. Each mixture was thoroughly agitated and then filtered using Whatman filter paper. The resulting filtrates were collected and used for subsequent qualitative and quantitative phytochemical screening.

2.1. Qualitative Phytochemical Analysis

Test for alkaloids (Wagner's Test): To a 1 ml of the filtrate, 1 ml of Wagner's reagent was added by the side of the test tube. A slightly reddish precipitate indicates the test is positive. 0.5 ml of the extract was added to 0.5 ml of HCl. To this acidic medium, 0.5 ml of Dragendroff's reagent was added. A reddish-brown precipitate produced immediately indicates the presence of alkaloids.

- Test for Tannins: 0.5 ml of FeCl3 was added to 1 ml of extract in the test tube. A greenish coloration indicates the presence of tannins.
- Test for Saponin: 2 ml of the sample extract was mixed with 8 ml of distilled water and shaken vigorously for a stable/persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.
- Test for Flavonoids: 2 drops of 10 % NaOH solution was added to 1ml of the extract. The addition was followed by 2 drops of $AlCl_3$ and few drops of concentrated H_2SO_4. A yellow coloration observed in extract indicates the presence of flavonoids. The yellow coloration disappeared on standing.
- Test for Terpenoids (Salkowski test): 2 ml of the extract was mixed in 1 ml of chloroform and 1 ml of concentrated H_2SO_4. A reddish-brown coloration of the interface shows positive results for the presence of terpenoids.
- Test for Cardiac glycosides (Keller-Killani test): 2 ml of extract was treated with 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.
- Quinone Test: 1ml of the extract was treated with 1 ml of 0.1 M acidified potassium iodide. A blue-black coloration indicates the presence of quinone.
- Phenol Test: 1ml of the extract was added to 1ml of distilled water, followed by few drops of 5% sodium hydroxide. The color changes from yellow to bright orange and this indicates the presence of phenol.
- Test for Steroids: To 1 ml of the extract, 5 drops of concentrated sulphuric acid was added. A red coloration of the two mixtures indicates the presence of steroids.

2.2. Quantitative Phytochemical Analysis

2.2.1. Determination of Alkaloid

Alkaloid determined by the method of Harborne (1973). 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

$$\% \text{ Alkaloid} = \frac{W2 - W1}{W3} \times \frac{100}{1}$$

Where
W1 = Wt of empty Paper
W2 = Wt of alkaloid and filter Paper
W3 = Wt of Sample

- Determination of Saponins (Modified Getsetner Method): 20 g of sample was suspended in 100 ml of 20 % absolute ethanol in 100 ml of distilled water. The solution was stirred and placed on a water bath at 56^{o}C (which was filtered after heating). The residue was re-extracted with 20 % ethanol and filtered again.
- The combined extract was subjected to the water bath where it was reduced to 40 ml and was allowed to cool. 20 ml of diethyl ether was added to remove impurities and pigments and was separated using 250 ml separating funnel. The pH of the solution was adjusted to 4.5 and 90 ml of N-butanol was added (which was separated using the separating funnel). The combine butanol extract was washed twice using 10 ml of 50 % sodium chloride and evaporate to dryness. This was repeated using distilled water.

$$\% \text{ saponification} = \frac{W2 - W1 \times}{W3} \frac{100}{1}$$

Where:
W1 = wt of empty beaker
W2 – wt of beaker and sample after drying
W3 = wt of sample used

- Determination of Tannin: 0.5 g of the sample was weighed and added to 50 ml of distilled water in 250 ml beaker. The beaker was placed on magnetic stirrer and allowed to stand for one hour. The solution was filtered and was made up to the mark. 5 ml of the filtrate was transferred to a test-tube with the addition of 2 ml of 0.1M of $FeCL_3$ in 0.1 M HCL and 0.008 M potassium ferrocyaninde. The absorbace was read at 320 nm after 10 mins.
- Determination of Glycosides: 5 g of the sample was weighed into a beaker and 100 ml of distilled water was added and was allowed to stand for 3 hrs. The solution was then filtered and 1 ml of the filtrate was collected with the addition of 2 ml of 3,5-DNS and the solution was boiled for 10-15 minutes. The test-tube was cooled and 10 ml of distilled water was added and read with absorbance at 540 nm. The blank was also measured using 2 ml of DNS.

3. Results

Table 1 Results of the qualitative phytochemical analysis of lime fruit

Test	Ethanol	Water	N-Hexane
Alkaloid	++	++	++
Phenol	_	_	_
Tannins	+	_	_
Saponin	_	+	+
Flavonoids	_	_	_
Quinone	_	+	+
Steriod	_	_	_
Terpenoids	_	_	_
Cardiac Glycosides	+++	+++	+
Coumarins	+++	+	+

Key: _ Not detected; + Present; ++ Moderately Present; +++ Abundantly Present

Table 2 Results of the quantitative phytochemical analysis of lime fruit

Test	Lime
Alkaloids	0.881 %
Tannin	4.4 mg/L
Saponin	0.881 %
Flavonoids	_
Glycoside	2.54 mg/ml
Phenol	_

4. Discussion

The phytochemical analysis of lime (*Citrus aurantifolia*) fruit using different solvents ethanol, water, and n-hexane revealed a varying degree of compound presence, reflecting the complex chemical nature of the fruit and the influence of solvent polarity on extraction efficiency. The qualitative analysis showed that alkaloids were consistently present across all solvents (++), suggesting a relatively high and broad-spectrum solubility. Tannins were only detectable in the ethanol extract (+), which aligns with their moderate polarity. Saponins, on the other hand, were absent in ethanol but detected in water and hexane, hinting at an amphiphilic behavior or potential for emulsification-based extractability in these solvents. Surprisingly, flavonoids and phenols were undetected across all solvents a result that diverges from the known rich flavonoid content of citrus fruits. Quinones, cardiac glycosides, and coumarins showed the most selective solubility and abundant presence. Notably, cardiac glycosides were highly abundant (+++) in both ethanol and water, with mild presence in hexane, while coumarins followed a similar trend. Quantitative analysis further confirmed the qualitative presence of alkaloids (0.881 %), saponins (0.881 %), tannins (4.4 mg/L), and glycosides (2.54 mg/mL). However, flavonoids and phenols were absent, again raising questions regarding the methodological sensitivity, sample preparation, or varietal specificity.

When compared with the findings of Rahmiati *et al*. (6), a study that employed TLC and UV-visible spectroscopy for phytochemical screening of *Citrus aurantifolia* leaves, notable discrepancies emerge. Rahmiati *et al*. reported the presence of flavonoids, saponins, terpenoids, and steroids contradictory to this present analysis, which found no trace of flavonoids, terpenoids, or steroids. The difference could be due to the plant part analyzed (leaves versus juice), variations in environmental growing conditions, or the superior sensitivity of chromatographic methods over basic

colorimetric assays. Similarly, Ezinne & Uzoamaka (7) identified a broader array of phytochemicals, including flavonoids, saponins, terpenoids, and cardiac glycosides in citrus juices and peels. Their detection of flavonoids and terpenoids absent in the current study reinforces the hypothesis that either the analytical method or the fruit sample used (ripeness, freshness, storage) may have limited the current findings. Both studies agree, however, on the strong presence of saponins and cardiac glycosides, affirming their role in the therapeutic potential of lime as an antioxidant and cardioprotective agent.

Further comparison with Oikeh *et al*. (8), who focused on Citrus sinensis (orange) peel extracts, indicates consistent detection of tannins, saponins, and phenolic compounds, alongside flavonoids. This partial agreement supports the universality of certain phytochemicals like tannins and saponins in citrus species. The absence of phenols and flavonoids in the present work may be explained by the difference in fruit part analyzed juice versus peel since peels generally contain more polyphenols. Anmol & Veena (9), who worked with ethanolic and aqueous extracts of citrus fruits, also found flavonoids, tannins, and saponins again challenging the current study's negative results for flavonoids. A similar trend is observed in Aveen (10), who identified flavonoids, phenols, and steroids in Citrus medica. Although differences in citrus species might account for some variation, the consistent detection of flavonoids and phenols in other studies highlights a significant limitation in this present analysis either due to compound degradation or insensitivity of reagents used. Gupta *et al*. (11) and Peter *et al*. (12) likewise confirmed the presence of flavonoids, terpenoids, tannins, and saponins in various citrus parts and extracts, offering strong evidence that these compounds are typically found in Citrus spp. Thus, the exclusion of flavonoids and phenols in the present study may require a reconsideration of assay techniques, extraction conditions, and possibly integrating more sensitive instrumentation like HPLC or LC-MS.

The present phytochemical analysis of lime fruit offers partial alignment with established literature, confirming the presence of bioactive compounds such as alkaloids, saponins, cardiac glycosides, tannins, coumarins, and quinones. These results justify the traditional use of lime fruit in managing various ailments, especially for its anti-inflammatory, antimicrobial, and cardioprotective potentials. However, the absence of key phytochemicals like flavonoids, phenols, terpenoids, and steroids stands in stark contrast to the broad body of citrus literature. This discrepancy calls for more comprehensive analysis using advanced analytical methods, standardized sample preparation, and broader solvent systems. Additionally, since many previous studies utilized leaves, peels, or whole fruit powders, future work on lime fruit should compare these parts directly under uniform experimental conditions. Ultimately, while this study contributes meaningful data on the phytochemistry of *Citrus aurantifolia* juice, it also emphasizes the critical role of methodology in phytochemical research and the importance of cross-validation with more sensitive and robust analytical platforms.

5. Conclusion

This study confirms that lime fruit (*Citrus aurantifolia*) is a rich source of several key phytochemicals, including alkaloids, cardiac glycosides, tannins, coumarins, and saponins. These compounds are known for their therapeutic applications and contribute to the fruit's traditional medicinal use. The absence of flavonoids and phenols in this study, despite being reported in other works, suggests a possible limitation in detection methods or sample characteristics. Solvent polarity played a critical role in phytochemical extraction, with ethanol and water yielding more compounds than non-polar hexane. Overall, the results support the potential use of lime in nutraceutical and pharmaceutical applications, particularly as an antimicrobial, antioxidant, and cardioprotective agent.

Future perspective

Future studies should aim to employ more advanced analytical tools such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), or Liquid Chromatography-Mass Spectrometry (LC-MS) to enhance the accuracy and sensitivity of phytochemical detection. There is also a need to explore seasonal, geographical, and varietal influences on the phytochemical composition of lime fruit. Furthermore, the study can be extended to evaluate the biological activities such as antioxidant, antibacterial, and anti-inflammatory properties of isolated compounds. A comparative study involving other citrus species and plant parts (e.g., peel, seeds, leaves) under standardized conditions will provide a broader insight into the therapeutic value of *Citrus aurantifolia*.

Compliance with ethical standards

Acknowledgments

The authors express their heartfelt gratitude to the management and staff of Pymotech Research Centre and Laboratories, Abakpa, Enugu State, Nigeria, for their technical assistance, laboratory access, and support during this

study. Special thanks to the local vendors at Abakpa Market for their cooperation during the sample collection phase. We also acknowledge the contributions of all colleagues and mentors who provided valuable insights and encouragement throughout the research process.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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