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Isolation, Identification and Functional Group Analysis of Averrhoa Carambola L. (Star Fruit) Leaves using FTIR Spectroscopy

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KEYWORDS

Averrhoa carambola, Flavonoids, Alkaloids, Antioxidant

ABSTRACT:

Introduction: In this research, the phytochemical content and bioactivity of Averrhoa carambola leaves, a medicinal plant species with therapeutic interest in traditional medicine are studied. The plant is rich in varied phytochemicals like terpenoids, flavonoids, alkaloids, and phenolic acids and has a broad spectrum of pharmacological activities. The work targets the identification and characterization of the bioactive constituents of Averrhoa carambola leaves by Fourier Transform Infrared (FTIR) spectroscopy. The research supports its traditional medicinal applications.

Methods: Leaves of Averrhoa carambola were collected, dried, and subjected to extraction using the Soxhlet process with petroleum ether and ethanol as solvents in turn. Exhaustive phytochemical screening was performed to determine different classes of bioactive molecules. FTIR spectroscopy was utilized to characterize the functional groups of the ethanolic extracts. Biological activities, such as antioxidant and anti-inflammatory, were also screened by conducting a battery of in vitro tests.

Results: Phytochemical screening validated the occurrence of alkaloids, flavonoids, phenolic compounds, terpenoids, steroids, and carbohydrates. FTIR spectroscopy revealed major functional groups such as hydroxyl, carbonyl, and aromatic rings, signifying the occurrence of bioactive compounds. The plant extract showed strong antioxidant activity, with comparable results to standard antioxidants, and anti-inflammatory assays indicated a significant decrease in inflammatory markers.

Conclusion: This research confirms the potential antioxidant and anti-inflammatory activities of Averrhoa carambola leaves, supporting their traditional medicinal uses. The results highlight the merits of phytochemical investigation and sophisticated spectroscopic methods in authenticating and characterizing new natural medicines. The plant is richly endowed for the construction of science-based herbal drugs.

1. Introduction

Herbal medicine is the earliest known type of healthcare, with herbs having had a long history of use as medicines to treat numerous human conditions. A lot of pharmaceutical drugs today have been developed from plant compounds[1]. According to WHO, it is estimated that almost 80% of the population in developing nations is now dependent on traditional medicine for healthcare[2]. According to WHO, over 21,000 plant species have been found to be used medicinally worldwide. There are over 100 plant genera in India's traditional systems of medicine, covering nearly 2,500 species[3]. India is the second-largest producer and exporter of medicinal plants, both in volume and value[4]. Additionally, India is among the 12 mega biodiversity hotspots in the world, with 16 different agro-climatic zones[5,6].

Averrhoa carambola, a member of the Oxalidaceae family, has traditionally been used to stimulate appetite, act as a diuretic, and provide anti-inflammatory, antidiarrheal, and fever-reducing effects[7,8].

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Antioxidant supplements maintain normal cell function and are important for overall health[9]. Gallic acid, the most prevalent phenolic compound in plant extracts, and ellagic acid have been reported to exhibit anticancer[10], antiviral[11], antimicrobial[12], and anti-inflammatory[13] activities. Release of reactive oxygen species (ROS), reduction of intrinsic cardiac antioxidants, and the ensuing oxidative stress are all causally linked with myocardial infarction[11].

For identification of the bioactive potential of medicinal plants, a thorough investigation of their phytochemicals is necessary. Among the high-end analytical techniques, Fourier Transform Infrared (FTIR) spectroscopy is one of the strongest tools both for qualitative as well as quantitative estimation of bioactive compounds[12]. FTIR spectroscopy is especially useful for the investigation of plant materials since it aids in determining molecular composition, recognizing functional groups, and examining the structural features of compounds[13]. Through the analysis of infrared radiation absorption by chemical bonds in a sample, FTIR spectroscopy generates distinctive spectra that are useful in understanding molecular vibrations and structural features[14].

The research is intended to investigate the phytochemical constitution and bioactivities of Averrhoa carambola leaves using comprehensive FTIR spectroscopic analysis[15]. The study seeks to isolate and characterize leaf extracts, analyze their phytochemical constituents, and determine their bioactivities[16]. FTIR analysis will facilitate the determination of major functional groups and bioactive molecules and provide useful insights into the molecular bases of the plant's pharmacological activities[17]. Moreover, the research aims to maximize extraction methods to produce higher yields of bioactive compounds, thus maximizing the medicinal properties of Averrhoa carambola.

The results of this study will greatly add to the current knowledge of Averrhoa carambola, validating its traditional medicinal uses and highlighting its potential for the formulation of evidence-based herbal products. Through the integration of traditional medicinal knowledge with scientific verification, this research underscores the value of phytochemical analysis and sophisticated spectroscopic methods in the discovery and development of new natural products for a range of therapeutic uses.

2. Methods

Collection and authentication of the plant:

The leaves of Averrhoa carambola, a member of the Oxalidaceae family, were collected from A Block in Baksa, Assam. The plant was identified and authenticated by Dr. Souravjyoti Borah, Assistant Professor in the Department of Botany at Gauhati University, Assam.

Plant extraction:

Averrhoa carambola leaves were air-dried in the laboratory and pulverized to a coarse powder. The plant was then extracted continuously under the hot Soxhlet apparatus[18]. A 500 ml petroleum ether was initially employed as solvent and the process of extraction took 48 hours. The resultant extract was concentrated through solvent evaporation in a rotary vacuum evaporator at 40°C. After petroleum ether extraction, marc was subjected to another extraction using 500 ml of 95% ethanol. This second extraction also lasted for 48 hours, and after this, the ethanol extract was removed and concentrated via evaporation at 40°C using the rotary vacuum evaporator.

Preliminary Phytochemical Screening:

The crude extracts of Averrhoa carambola underwent qualitative phytochemical screening to identify various secondary metabolites, such as alkaloids, glycosides, tannins, phytosteroids, terpenoids, carbohydrates, proteins, amino acids, and other bioactive compounds[19].

Fourier Transform Infrared (FTIR) Spectroscopy:

FTIR spectroscopy was employed to analyze the functional groups and molecular composition of the Averrhoa carambola leaf extract.

Principle:

FTIR spectroscopy detects the absorption of infrared radiation by the sample, distinguishing chemical bonds based on their characteristic absorption bands. This technique enables the identification of functional groups present in the extract.

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Procedure:

Sample Preparation: The extract was mixed with potassium bromide (KBr) and pelletized for analysis.

Spectral Recording: FTIR spectra were recorded on an FTIR spectrometer (Model: FTIR-8400, Shimadzu).

Calibration and Analysis: The spectrometer was first calibrated with a standard KBr pellet, and the sample was scanned over the wavelength range 4000-400 cm^{-1}. Absorption peaks were compared with reference spectra and analyzed to detect functional groups.

Biological Activity Assessment:

The biological activities of Averrhoa carambola leaf extract were tested by in vitro assays, considering its antioxidant and anti-inflammatory activities. Antioxidant activity was quantified using the lipid peroxidase inhibition assay, which determines the capacity of the extract to scavenge free radicals. Anti-inflammatory activity was studied using appropriate in vitro models to determine the effectiveness of the extract in inhibiting inflammation-related markers.

Lipid peroxidase inhibition assay:

A modified thiobarbituric acid-reactive substance (TBARS) assay will be performed to quantify lipid peroxidation, employing egg yolk homogenates as a lipid-rich medium. Malondialdehyde (MDA), a secondary oxidation product of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) to form a pinkish-red chromogen[20]. For the assay, 500 µL of 10% (v/v) egg yolk homogenate in phosphate-buffered saline (pH 7.4) will be combined with 300 µL of the test sample (1 mg/mL in the corresponding organic solvents) in a test tube and the volume will be made up to 1.0 mL by using distilled water. For inducing lipid peroxidation, 50 µL of $FeSO_4$ (0.075 M) and 20 µL of L-ascorbic acid (0.1 M) will be added and the sample will be incubated at 37°C for 1 hour. 0.2 mL of EDTA (0.1 M) and 1.5 mL of TBA reagent (3 g TBA, 120 g TCA, and 10.4 mL of 70% $HClO_4$ in 800 mL distilled water) will be added to each sample after incubation and then heated at 100°C for 15 minutes. After cooling, the samples will be centrifuged at 3000 rpm for 10 minutes, and the supernatant will be measured for absorbance at 532 nm. The percentage inhibition of lipid peroxidation will then be calculated by using the following equation:

% Inhibition = [(Control OD- Sample OD)/Control OD] × 100

3. Results

Phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, flavonoids, saponins, carbohydrates, steroids, proteins, and amino acids in the leaves of Averrhoa carambola. The results are summarized in Table 1.

Table 1. Preliminary phytochemical analysis of petroleum ether and ethanolic extract

Sl No	Test	Petroleum ether	Ethanol
1	Test for alkaloids 1. Dragendorff's test 2. Hager's test 3. Wagner's test 4. Mayer's test 5. Tannic acid test	 - ve - ve - ve - ve - ve	 +ve +ve +ve +ve +ve
2	Test for glycosides 1. Borntrager trest 2. Legal's test 3. Keller-Killani test	 - ve - ve - ve	 +ve +ve +ve
3	Test for steroid 1. Salkowski test	 - ve	 +ve
4	Test for tannins 1. Ferric chloride test 2. Lead acetate test 3. Potassium permanganate test	 - ve - ve - ve	 +ve +ve +ve
5	Test for flavonoids 1. Shinoda test 2. Lead acetate test & NaOH test	 - ve - ve	 +ve +ve
6	Test for Fats & Oils 1. Saponification test	 +ve	 -ve
7	Test for proteins & amino acid 1. Nitric acid test 2. Ninhydrin test	 - ve - ve	 +ve +ve
8	Test for carbohydrates 1. Molisch's test 2. Fehling's test 3. Benedict's test	 - ve - ve -ve	 +ve -ve +ve

Fourier Transform Infrared (FTIR) Spectroscopy Analysis:

The FTIR analysis gave a good indication of the chemical composition of the bioactive compounds found in Averrhoa carambola leaves. The FTIR spectrum revealed some major functional groups responsible for the plant's bioactivity. The spectra of the extracts (Figure 1) exhibited clear-cut absorption peaks at 3500 cm^{-1} (-OH), 2927 cm^{-1} (CH_2 stretching), 2868 cm^{-1} (CH_3 stretching, side chain), 1461 cm^{-1} (phenyl group), and 1378 cm^{-1} (CH_3 bending).

Hydroxyl groups (-OH):

Found at about 3500 cm^{-1}, these groups are generally found in phenolic compounds and flavonoids and are very important in the antioxidant activity of the extract. Their presence is responsible for enhancing radical

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scavenging activities, which are important in preventing oxidative stress.

Carbonyl Groups (C=O):

Peaks seen around 1620 cm^{-1} indicate the existence of carbonyl groups, which are typical for flavonoids and terpenoids. Such functional groups are crucial for cell enzyme interactions and are responsible for the anti-inflammatory and antimicrobial activities of phytochemicals.

Aromatic Rings:

Bands of absorption in the vicinity of 1461 cm^{-1} suggest the existence of aromatic rings, which are a ubiquitous structural component among phenolic compounds and flavonoids. This confirms the occurrence of these bioactive constituents and suggests their relevance to increasing the plant's curative potential.

Functional Group Confirmation of *Averrhoa carambola* leaves Using FTIR Analysis

Table 2: This table provides the FTIR analysis results of *Averrhoa carambola* leaves, highlighting the specific absorption bands, corresponding functional groups, and their respective stretching modes.

Compound	Absorption band (cm^{-1})	Functional group and stretching
Averrhoa carambola leaves extract	3500 cm^{-1}	Alcohol, OH stretch
	2927 cm^{-1}	Alkanes, C-H stretch
	2868 cm^{-1}	Methyl group, CH_3 stretch side chain
	1461 cm^{-1}	Phenyl group, C=C stretch
	1378 cm^{-1}	Methyl group bending, CH_3 bend

Table 2: This table provides the FTIR analysis results of *Averrhoa carambola* leaves, highlighting the specific absorption bands, corresponding functional groups, and their respective stretching modes.

The analysis confirms the presence of the fundamental bioactive groups such as alcohols, phenols, carboxylic acids, aromatics, and alkyl groups that significantly contribute to the plant's pharmacological properties such as antioxidant and anti-inflammatory activity. The FTIR results correspond with the results of the screening of phytochemicals and confirm the presence of functional groups responsible for supporting the plant's multiplicity in pharmacological activity.

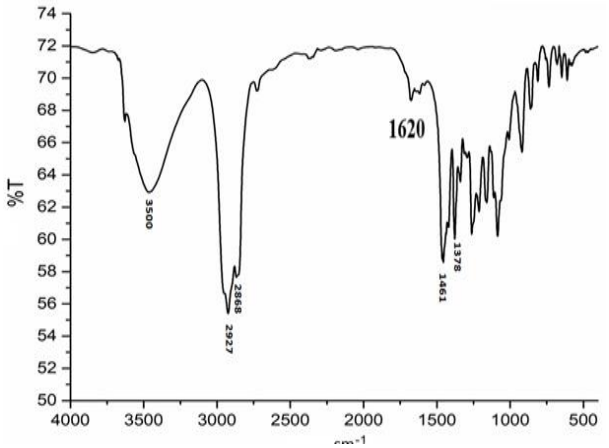

Figure 1: FTIR spectra of Averrhoa carambola leaves extract

Biological Activity Assessment:

The assessment of the biological activity of Averrhoa carambola extract proved its potential as a natural therapeutic agent with significant antioxidant and anti-inflammatory activity. The extract showed considerable antioxidant activity, as determined by the lipid peroxidase inhibition assay, with an IC_{50} value that was similar to that of quercetin, a reference antioxidant. This high antioxidant activity is mainly due to the richness of phenolic and flavonoid molecules, which are efficient in neutralizing free radicals, pointing to its potential for the prevention of oxidative stress-induced diseases like cardiovascular diseases, cancer, and neurodegenerative disorders. Additionally, the anti-inflammatory activity of the extract was validated using in vitro models, with a remarkable decrease in inflammatory markers when compared to controls. The terpenoids and flavonoids present, which inhibit the major inflammatory pathways and inhibit pro-inflammatory cytokines, further supports its historical use in the treatment of inflammatory diseases such as arthritis and skin inflammations.

Results of Lipid peroxidase inhibition assay:

The effect of the ethanolic extract of Averrhoa carambola leaves and the commercial antioxidant quercetin on in vitro inhibition of lipid peroxidation is shown in Table 5. The ethanolic extract showed inhibitory activity against lipid peroxidation caused by Fe^{2+}-ascorbate in egg yolk homogenate. Although inhibition occurred, its efficiency was not as high as that of quercetin. The inhibition percentage, as measured by the TBARS assay, was

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36.27% to 78.15% for the leaf extract, while quercetin showed inhibition of 46.55% to 81.44% at the lowest and highest concentrations, respectively. The IC_{50} value of the leaf extract was 80.35 µg/mL, while that of quercetin was 33.45 µg/mL. The greater IC_{50} value of the A. carambola leaf extract in the TBARS assay suggests that it is less active than quercetin in inhibiting lipid peroxidation.

Table 3: IC_{50} and % inhibition of Plant extract and Quercetin for Lipid peroxidase inhibition assay

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Averrhoa carambola	25	36.27	80.35
	50	42.72	
	100	56.22	
	200	78.15	
Quercetin	25	46.55	33.45
	50	54.33	
	100	64.65	
	200	81.44	
Absorbance of the control: 0.827. Each test was carried out triplicate (n=3)			

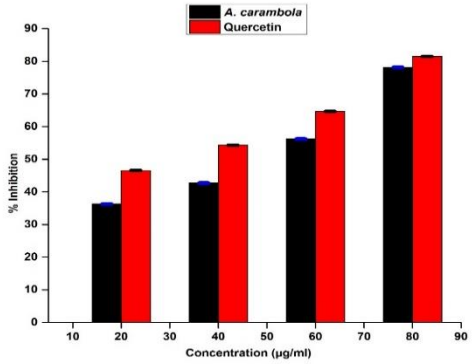

Fig 2: Lipid peroxidase inhibition activity of quercetin and ethanol plant extract of *A. carambola*

4. Discussion

The research presents the significant antioxidant and anti-inflammatory activity of Averrhoa carambola extract, reaffirming its use as a natural drug agent. These findings are in accord with prior investigations on the analogous Averrhoa species, highlighting the richness of the plant in phytochemical composition and bioactive nature.

The lipid peroxidase inhibition assay proved that Averrhoa carambola extract contains high levels of antioxidant activity, as evidenced by the IC_{50} value compared to quercetin. This high level of activity is contributed by the high content of phenolic and flavonoid compounds, which are highly known for their antioxidant activity.

Anti-inflammatory activity of Averrhoa carambola extract was evidenced through an in vitro attenuation of inflammatory markers. The inclusion of terpenoids and flavonoids in the extract most probably contributes to these effects through the suppression of pro-inflammatory cytokines and maintenance of critical inflammatory pathways. This finding is in agreement with reports by Pathak BJ et al. (2025), who stated that ethanol extracts of Averrhoa species efficiently inhibit inflammation through the downregulation of cytokine production, such as TNF-α, IL-1, and IL-6.

The general conclusion of this research is consistent with other studies on Averrhoa species, further supporting the concept that Averrhoa carambola is a good source of bioactive compounds that have significant therapeutic value. These compounds are responsible for the plant's multifaceted health benefits, ranging from antimicrobial, antioxidant, to anti-inflammatory.

Conclusion:

This research highlights the exceptional therapeutic potential of Averrhoa carambola leaf extract with its powerful antioxidant and anti-inflammatory activity. Its high antioxidant activity, similar to that of reference antioxidants such as quercetin, illustrates its efficacy in neutralizing free radicals and thus providing protection against oxidative stress-related diseases such as cardiovascular disorders, cancer, and neurodegenerative disorders. Moreover, the in vitro observed anti-inflammatory activities justify its long-standing use in the treatment of inflammatory diseases, which is presumably due to the bioactive contents of flavonoids and terpenoids.

These results concur with previous studies on other Averrhoa species and highlight the importance of phytochemical constituents such as phenolic acids, flavonoids, and terpenoids in the mediation of the plant's biological activities. Averrhoa carambola stands out as a potential herbal choice for the creation of scientifically

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proven herbal products, merging traditional medicinal practices with scientific investigations. Potential areas of investigation in the future will include the isolation of active compounds, the investigation of their mode of action, and the determination of their safety and efficacy in clinical use. These kinds of studies would greatly boost the knowledge of Averrhoa carambola as a potent natural therapeutic molecule possessing multiple health benefits.

Competing Interests

The authors confirm that they have no conflicting interests.

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