Full Length

PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *MANGIFERA INDICA* LEAF EXTRACT

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ABSTRACT

Mango (Mangifera indica) is a leading tropical fruit crop that is grown worldwide. Different parts of the mango fruit have traditionally been used for various medicinal applications. The current study explored the phytochemical components of mango leaf extract and examined its antibacterial and antioxidant properties. Phytochemical screening revealed the presence of flavonoids, phenols, steroids, terpenoids, coumarins, glycosides, saponins, and tannins in the extracts. Gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of several bioactive compounds in the extract. The antibacterial activity of the leaf extract showed dose-dependent inhibition against Escherichia coli (11–8 mm), Staphylococcus aureus (13–8 mm), and Salmonella enterica (11–7 mm). The extract also exhibited dose-dependent DPPH radical scavenging activity, reflecting its strong antioxidant potential. Therefore, the ethanolic leaf extract of Mangifera indica has considerable antioxidant and antimicrobial activities, implying its potential use in the food and pharmaceutical industries.

Key words: *Mangifera indica*, GC-MS, antibacterial activity, antioxidant activity

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INTRODUCTION

Mangifera indica (Mango), a member of the *Anacardiaceae* family, is generally regarded as an economically valuable and highly significant tropical fruit crop (Kumar *et al.*, 2021). Although, the mango fruit is considered the major economic product of the tree, most of the other components, especially the leaves are discarded as waste by the mango industry. The leaves of mango plants have been investigated for potential

health benefits derived from a diverse range of phytochemicals, including mangiferin, phenolic acids, benzophenones and various antioxidants such as flavonoids, ascorbic acid, carotenoids and tocopherols.

Phytochemicals are mainly classified as primary and secondary metabolites. The primary metabolites are responsible for the basic development of the plant which includes the sugars, amino acids, proteins, nucleic acids, chlorophyll. Secondary metabolites include flavonoids. tannins, saponins, alkaloids, and steroids.

Mango leaves exhibit a variety of biological, phytochemical and pharmacological properties, including antimicrobial, antioxidant, antidiabetic, antitumor and immunomodulatory properties. (Muralikrishna *et al*., 2014; Samari *et al*., 2018; Kumar *et al*., 2021).

Mango leaves are especially recognized for their abundance of bioactive phytochemicals, such as flavonoids, phenolic compounds, and terpenoids, which contribute to their antioxidant and antimicrobial properties(Akash Dagale*, 2025; Deependra *et al.*, 2022). Antioxidant metabolites are compounds which can reduce or prevent the generation of reactive oxygen species (ROS) in the body during various metabolic processes (Sreelatha, *et al.*, 2025).

The present study, was conducted to analyse the ethanol extract of mango leaves by conventional method and GC/MS analysis and screening of antioxidant, antimicrobial activities were studied against *E. coli, S. aureus*, and *S. enterica.*

MATERIALS AND METHODS

Collection of mango leaf

Fresh mango leaves (*Mangifera indica*) were collected from mango trees located at College of Food and Dairy Technology, Koduvalli, Thiruvallur District in Tamil Nadu, India.

Preparation of mango leaf extract

The mango leaves were washed with distilled water, solar dried for two days and ground to obtain a coarse powder. Thirty gram of mango leaf powder were put into 100 ml of 100% ethanol and stirred for 24 hrs, then sonicated for 30 minutes for extraction of phytochemicals. The extracted phytochemicals were subjected to GC/MS analysis for screening. The extract was concentrated using rotary vacuum evaporator and stored at 4^{o}C for further use.

Phytochemicals screening of mango leaf extract

The ethanol extract of *Mangifera indica* leaves was subjected to phytochemical screening to evaluate the presence of steroid, coumarine, terpenoids, flavonoids, glycosides, porteins, phenol and tannins by the method prescribed by Basavarajappa *et al* 2022.

Gas chromatograph with mass spectrometry analysis of extract

The identification of compounds in the ethanol extract of mango leaves was carried out using a Shimadzu QP2020 GC-MS spectrometer in EI mode. The GC oven temperature was maintained at 300°C at a rate of 10°C /min; the carrier gas with a flow rate of 1 ml/min. The split sampling technique was used to inject the sample into a 30 m column with an internal diameter of 0.53 mm in the ratio of 1:10. Mass-Spectrum was carried out by using the database of the National Institute Standard and Technology (NIST) with more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library.

Antibacterial activity of mango leaf extract

The agar well diffusion method was used to analyze the antibacterial activity of the plant extract against *E. coli, S. aureus*, and *S. enterica.* The concentration of overnight cultures was adjusted to 0.08 to 0.1 OD at 600 nm for the assay. Cultured organisms were swabbed on MHA plates, and 6 mm wells were made in the agar. Different concentrations (50 µL, 40 µL, 30 µL and 10 µL) of the sample were loaded onto each well of MHA plates, with kanamycin (100 µg/mL) as a positive control. Then the plates were incubated at 37°C, and the zone of inhibition was measured and recorded. The experiment was conducted in triplicate, and the average value was calculated for the antibacterial activity.

Antioxidant activity of mango leaf extract

The antioxidant activity of the mango leaf extract in comparison with ascorbic acid was assessed using the free radical DPPH scavenging assay as described by Khorrami *et al.,* (2018) with slight modification. The plant extract, 200 µL, was taken on 1st well of a 96-well plate and serially diluted two-fold up to 10th well. Then, all the wells received 100 µL of DPPH (0.2mM) solution. The UV–vis absorbance of the samples was measured at 517 nm after 30 min of incubation. The experiment was conducted in triplicate, and the average value was calculated for the antibacterial activity. The antioxidant activity was calculated based on the equation as below;

$$\% \text{ Inhibition} = \frac{(\text{OD Control} - \text{OD Test})}{(\text{OD Control})} \times 100$$

RESULTS AND DISCUSSION

Phytochemical characterisation of mango leaf extract

The phytochemical constituents of the ethanol extracts were subjected to biochemical analysis. The test results are presented in Table 1. These tests confirmed the presence of flavonoids, phenols, steroids, terpenoids, coumarins, glycosides, saponins, and tannins whereas carbohydrates and proteins were absent. The similar results were observed in different studies reported by (Ghosh *et al*., 2022; Maharaj *et al.*, 2022; Somkuwar and Kamble, 2013).

Gas chromatography with mass spectrometry analysis of extract

GC-MS analysis identified 22 chemical compounds in the mango leaf extract. The peak, retention time, % area, and retention index of the corresponding compounds are listed in Table 2 and Fig.23.

The highest peak area, with a percentage of 38.79 %, was phenol, followed by 9.43 indicated that 9-Octadecenoic acid, hexyl ester. GC-MS analysis of the mango leaf extract revealed the presence of 22 phytochemical compounds. Acetic acid, phenol, sulfurous acid, butanoic acid, palmitic acid (n-hexadecanoic acid), and phenyl esters exhibit antibacterial activity (Kumar *et al*., 2021). A similar study was conducted on leaf and bark ethanol extracts of *Mangifera indica* using gas chromatography-mass spectrometry analysis, which revealed numerous bioactive compounds, such as phenols, sesquiterpenes, terpenoids, fatty acids, and esters (Karigidi *et al*.,2025).

Antibacterial activity of plant extract

The antibacterial activity of the plant extract were assessed against *E. coli, S. aureus* and *S. enterica*. Various concentrations of the plant extract (50, 40, 30, and 10 µL) were added to each well. The zone of inhibition of plant extract are shown in (Fig 2 and Fig 3).The antibacterial activity of the plant extract showed dose-dependent inhibition against *E. coli* ranging from 11 to 8 mm, *S. aureus* ranging from 13 to 8 mm and *S. enterica* ranging from 11 to 7 mm. In the present study, mango leaf extract had better antibacterial activity against *E. coli, S. aureus*, and *S. enterica*. Polyphenols and phenolic acids found in mango leaf extracts prevented the growth of pathogenic organisms (Ediriweera, *et al.*, 2017). Previous studies have reported that the stem bark of *M. indica* extract inhibited the growth of *Staphylococcus sp.*, *E.coli, Vibrio sp., Penicillium sp*., yeast and mould (Ogidi *et al*., 2021).

Antioxidant activity of plant extract

The antioxidant activity of mango leaf extract was examined based on the DPPH scavenging, the results of the DPPH scavenging assay are shown in Fig 4. The dose dependent inhibition of DPPH was observed in this study. The activity of mango leaves extract was found to be better than the control in all concentrations. The antioxidant activity of mango leaf extract has shown higher free radical scavenging activity when compared with ascorbic acid as a control. The antioxidant activity of mango leaf extract is not only due to phenolic components but also due to mangiferin, glucosides and polyphenols (Bharath *et al*., 2019). Several studies reported that mango leaf extract had antioxidant activity that can be used for various applications (Pan *et al*., 2018; Itoh *et al*., 2020). Ethanolic extract of plant showed highest antioxidant activity reported by (Kshama, *et al.*, 2025) similar results was observed in this study.

CONCLUSION

The present study concluded that the phytochemicals present in the mango leaf extract exhibited significant antibacterial and antioxidant activities. This study revealed that the important phytochemicals present in the extract were flavonoids, phenols, steroids, terpenoids, coumarins, glycosides, saponins, and tannins. Gas chromatography-mass spectrometry analysis revealed the presence of 22 chemical compounds in the mango leaf extract. This study revealed a dose-dependent inhibition of DPPH. Further investigation is required to incorporate the phytochemicals extracted from mango leaves for use as potential antibacterial and antioxidant agents in various applications.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Table.1. Phytochemical constituents of ethanol extract of mango leaf

S. No.	Phytochemical test	Mango leaf extract
1.	Tannin	+
2.	Steroids	+
3.	Flavonoids	+
4.	Phenols	+
5.	Saponin	+
6.	Glycosides	+
7.	Carbohydrate	-
8.	Protein	-
9.	Coumarins	+
10.	Terpenoids	+
11.	Fixed oil	+

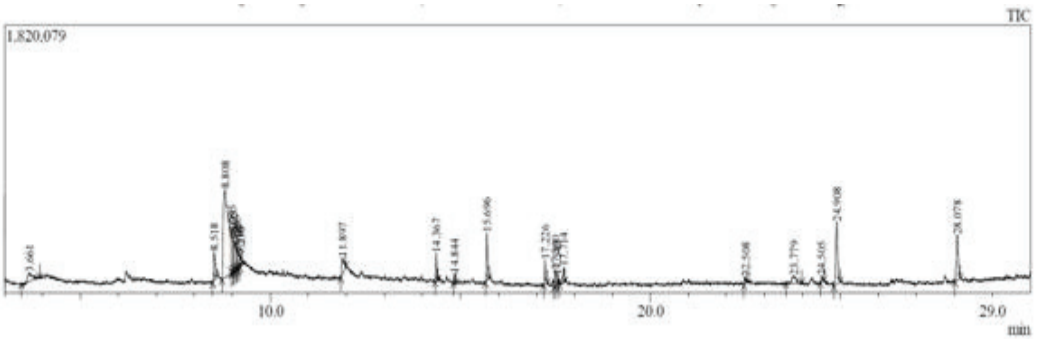

Fig.1. GC-MS chromatogram of ethanol extract of mango leaf

Table.2. Phytochemical profile of mango leaf extract using gas chromatography-mass spectrometry

Peak	R. Time (min)	Area %	Name of the compound	Retention index
1	3.661	2.77	Phenyl ester	1204
2	8.518	3.73	Cyclopentaneundecanoic acid	2011
3	8.808	38.79	Phenol	1547
4	8.995	4.39	2-p-Nitrobenzoyl-1,3,5-tribenzyl-.alpha.-d-ribose	4415
5	9.025	3.07	3-Penten-2-ol	689
6	9.095	3.33	5-Cyclopentylidene-2,2-dimethyl-1,3-dioxane-4,6-dione	1846
7	9.130	1.98	Acetic acid	802
8	9.180	1.82	Hexahydro-1,3-benzodioxol-2-one	1196

9	9.230	0.88	(3-Ethynyl-3-methyloxiran-2-yl)methanol	884
10	11.897	3.23	1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-(2-aminoethyl)-	1793
11	14.367	2.28	Oxirane, dodecyl-	1503
12	14.844	0.86	Hexanesulphonylacetonitrile	1568
13	15.696	5.25	n-Hexadecanoic acid	1773
14	17.226	2.21	Propanoic acid	1253
15	17.500	1.74	1,6-Bis(2-propyn-1-yloxy)hexane	1361
16	17.545	1.25	Butanoic acid	2114
17	17.714	1.81	4-Methyloctanoic acid	1208
18	22.508	0.73	Isobutyl pentyl carbonate	1194
19	23.779	2.46	Thiolane-3,3,4,4-tetracarbonitrile, 2,5-di-tert-butyl	2541
20	24.505	0.75	Sulfurous acid	1674
21	24.908	9.43	9-Octadecenoic acid, hexyl ester, (Z)-	2582
22	28.078	7.23	Oxalic acid	2135

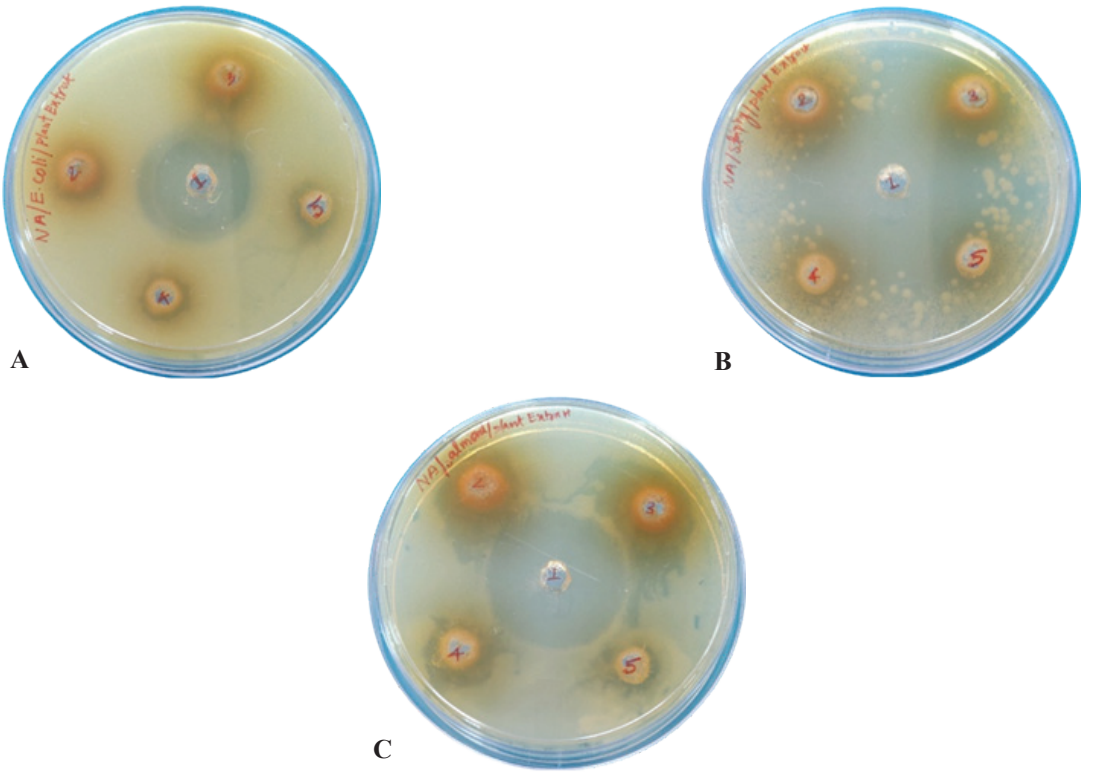

Fig.2. Antibacterial effects of ethanolic mango leaf exract against (A) *E. coli*, (B) *S. aureus* and (C) *S. enterica* on MHA plate after 24 hrs of incubation at 37°C (Well 1: Kanamycin (100 µg/ml), Well 2-5: Plant extract 50, 40, 30, and 10 µL respectively)

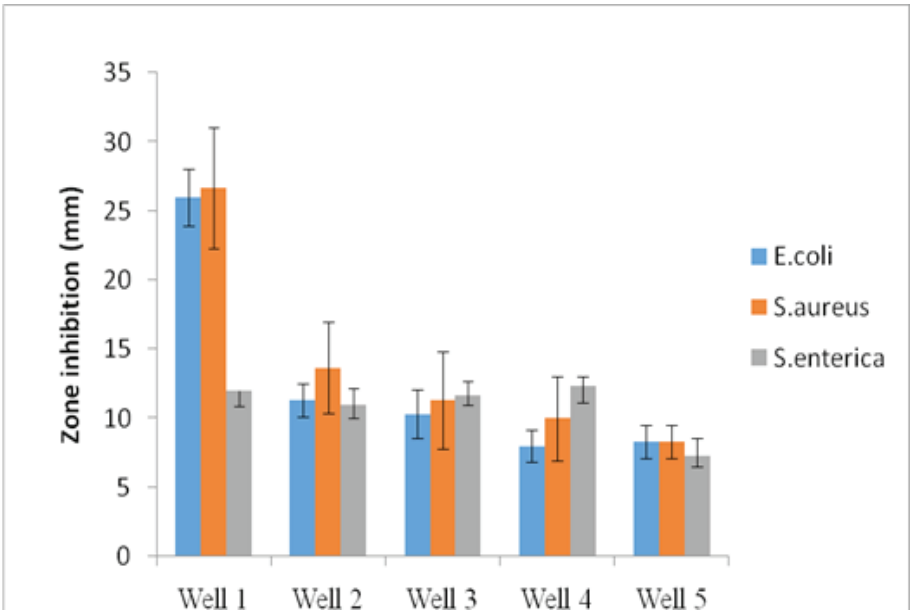

Fig.3. Antibacterial activity of plant extract zone of inhibition (Mean±SE) against *E. coli, S. aureus* and *S. enterica* (Well 1: Kanamycin (100 µg/ml), Well 2-5: Plant extract 50, 40, 30, and 10 µL respectively).

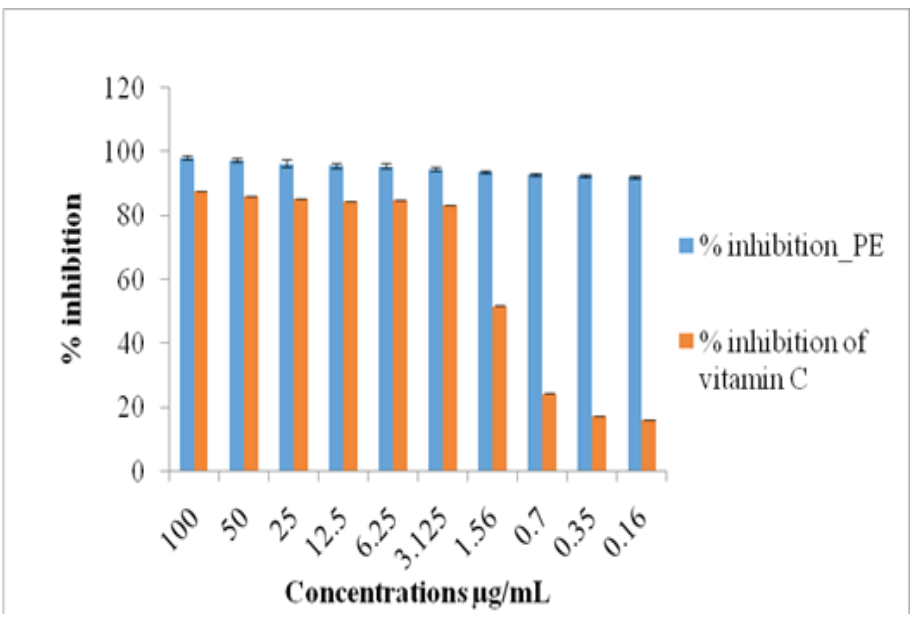

Fig.4. DPPH scavanging activity of mango leaves extract(Mean±SE)

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