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PHYTOCHEMICAL PROFILING AND ANTIOXIDANT POTENTIAL OF ETHANOL EXTRACT OF *MANGIFERA INDICA* LEAVES

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ABSTRACT

Mangifera indica, an Indian traditional medicinal plant has been used for various diseases. The present study was carried out to characterize the bioactive constituents present in ethanolic extract of *Mangifera indica*. The extraction process was performed using the maceration method. The present study was aimed to reveal the phytochemicals and Antioxidant activity of Ethanolic extract of *Mangifera indica*. The phenolic content of ethanol extract of *Mangifera indica* was 82 µg/100mg was expressed as gallic acid equivalent. The flavonoid content of ethanol extract of *Mangifera indica* was 196 µg/100mg was expressed as catechin equivalents. GCMS analysis of ethanol extract of *Mangifera indica* leaves revealed the existence of 1,2,3-Benzenetriol (11.77), 2-(1-piperidyl)propan-2-ol (12.73), Isoniazid (14.85), 3-Methylene-7,11-dimethyl-1-dodecene (22.03), n-Hexadecanoic acid (25.19), 2,5-Dimethylcyclohexanol (29.03), Cyclooctene, 3-ethenyl (29.55). The Antioxidant activity was investigated using DPPH radical scavenging assay. Ethanol extract of *Mangifera indica* showed the highest antioxidant activity (63.06µg/ml). The phytochemical constituents and antioxidant activity may be responsible for the pharmacological effects of *Mangifera indica* in various diseases.

INTRODUCTION

Herbal remedies from medicinal plants to cure and prevent several ailments differ between communities [1]. For many years, medicine depended exclusively on flowers, barks, and the leaves of plants [2]. India has a great wealth of medicinal and aromatic plants due to its rich plant diversity [3]. Phytotherapy, a branch of medicine, deals with the application of plants and their products in the prevention and treatment of several diseases. Besides, as per the World Health Organization (WHO), about 2500 plants are being used for the treatment of various diseases [4]. The phytochemical components have been utilized as therapeutic agents [5]. Plants are naturally containing several phytochemical constituents which are responsible for their medicinal properties [6]. Plant- based medicines are drawing significant interest due to their lower toxicity and minimal side effects [7].

Mangifera indica, commonly known as mango, is an evergreen species of flowering plant in the family Anacardiaceae. It is a large fruit tree, capable of growing to a height of 30 metres (100 feet). There are two distinct genetic populations in modern mangoes – the "Indian type" and the "Southeast Asian type". *Mangifera indica*, commonly used herb in ayurvedic medicine. Mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone, it has strong antioxidant, anti-lipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities [8].

Antioxidants play a crucial role in neutralizing harmful free radicals and reducing oxidative stress in living organisms [9]. They aid in the battle against reactive oxygen species, which cause cell damage in the body and are linked to several heart- related issues as well as the pathogenesis of many diseases [10].

The current investigation focused on examining phytochemicals in the ethanol extract of *Mangifera indica*. In this study, we aim to evaluate the phytochemicals and antioxidant activity of the ethanol extract of *Mangifera indica* using DPPH radical scavenging assay.

Materials and methods:

Plant collection

Mangifera indica leaves were collected from Thovalai, India. After being collected, the leaves was found to be healthy and alive. The leaves was cleansed with running water to get rid of dirt, then rinsed again with distilled water. The leaves were allowing to shade dried for 15-20 days. Dried leaves were chopped and grounded into a fine powder. It was stored in air tight container for further use.

Preparation of extracts

Extraction was performed via maceration. The extracts of samples were prepared by soaking 25 grams of dried powder in 250ml of ethanol for 72hrs. Later, the mixture was filtered through Whatman filter paper no. 42. And leave it for evaporation. After evaporation the remaining extracts were used for next tests [11].

Phytochemical quantitative analysis

Quantitative estimation of phenolic compounds

The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, different concentrations of the 1 ml of the extract were mixed with 0.4 ml FCR (diluted 1:10v\v). After 5 min 4 ml of 7% sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using Gallic acid solutions as standard (20 to 200μg\μl).

Quantitative estimation of flavonoids

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1 ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5% sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin\g dried extract) [12].

GCMS Analysis

GCMS investigation of the volatile compounds from *Mangifera indica* has been executed using an Agilent 8890 system comprising an AOC-20i auto-sampler and a gas chromatograph connected to a Mass spectrometer (GCMS) fitted out with an Elite-5MS (5% diphenyl /95% dimethyl polysiloxane) glued a capillary column (30 × 0.25μm ID × 0.25µm df). For GCMS finding, an electron ionization system was worked in electron stimulus mode and with ionization energy of 70 eV. Helium gas (99.99%) was applied as a carrier gas at an unceasing flow rate of 1.2 ml/min, and an injection volume of 1µl was engaged (a split ratio of 15:1). The injector temperature was preserved at 250°C, the ion source temperature was 230° C, the oven temperature was planned from 350°C, with an escalation of 5°c/min to 300°C/5 min. Mass spectra were booked at 70 eV; a scan intermission of 0.5 sec. and fragments from 45 to 450 Da. The solvent delay was 3 min, and the total GC/MS running time was 53.5 min. The comparative proportion quantity of each constituent was calculated by equating its average peak area to the total areas. The mass detector applied in this examination was Turbo-Mass Gold-Perkin-Elmer, and the software implemented to knob mass spectra and chromatograms was a Turbo-Mass ver-5.2.28-29 [13].

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of ethanol extract of *Mangifera indica* (10-160µg\ml) were evaluated through a free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. 2ml of DPPH solution were added to both standard and test tubes. 100µl of test sample and standard solution of various concentration (10,20,40,80, and 160µg\ml) and were added to respective tubes. Then stand for 30 mins incubation. After incubation, observe the colour change and measured the absorbance of each tubes at 517nm. Calculate the % of inhibition of each concentration with the formula.

$$\% \text{ of inhibition} = (1 - As/Ac) \times 100$$

Where Ac is the absorbance of the control and As is the absorbance in the presence of the sample extract or standard [14].

Results and discussion:

Phytochemical quantitative analysis

Total flavonoid and phenol content of ethanol extract of *Mangifera indica*

The flavonoid content of ethanol extract of *Mangifera indica* was 196μg/mg and was expressed as catechin equivalents. The phenolic content of ethanol extract of *Mangifera indica* was 82μg/mg and was expressed as gallic acid equivalents (Table 1).

Table: 1 Total flavonoid and phenol content of ethanol extract of *Mangifera indica*

Test	Result
Flavonoid	196μg/100mg
Phenol	82μg/100mg

GCMS Analysis

Seven compounds were identified in ethanol extract of *Mangifera indica* leaves by GCMS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in table 2. The compounds are 1,2,3- Benzenetriol (11.77), 2-(1-piperidyl)propan2-ol (2.73), Isoniazid (14.85), 3-Methylene-7,11-dimethyl-1-dodecene (22.03), n-hexadecanoic acid (25.19), 2,5-Dimethylcyclohexanol (29.03), Cyclooctene, 3-ethenyl (29.55). In the present study seven chemical constituents have been identified from ethanol extract of the plant of *Mangifera indica* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis.

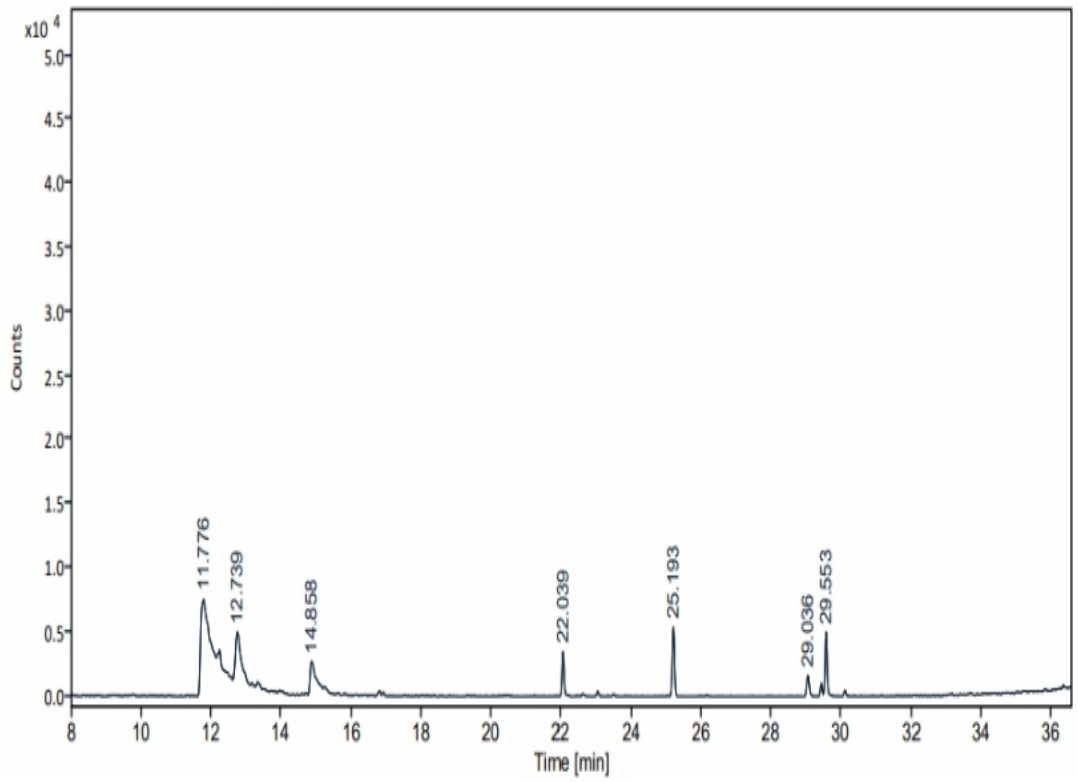


Fig 1 : GCMS analysis of ethanol extract *Mangifera indica*

Table 2: GCMS analysis of ethanol extract *Mangifera indica*

S.No	Retention Time (min)	Compound Name	Molecular formula	Molecular weight	Peak %	Compound Nature	Biological activity
1	11.776	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	31.64	benzenetriol	Antiseptic,Antioxidant,Antidermatitic,Fungicide,Antimicrobial [15]
2	12.739	2-(1-Piperidyl)propan-2-ol	$C_8H_{17}NO$	143.23	16.81	-	NAF
3	14.858	Isoniazid	$C_6H_7N_3O$	137.14	10.11	hydrazide compound and an isonicotinic acid derivative	Antibacterial activity [16]
4	22.039	3-Methylene-7,11-dimethyl-1-dodecene	$C_{15}H_{28}$	208.38	9.04	-	NAF
5	25.193	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	15.02	Fattyacid	Anti-inflammatory,antibacterial,and Antioxidant [17]
6	29.036	2,5-Dimethylcyclohexanol	$C_8H_{16}O$	128.21	5.08	Fattyacid	Anti-inflammatory,antibacterial,and Antioxidant [18]
7	29.553	Cyclooctene, 3-ethenyl-	$C_{10}H_{16}$	136.23	12.30	Alkene	NAF

NAF – No activity found

Antioxidant activity

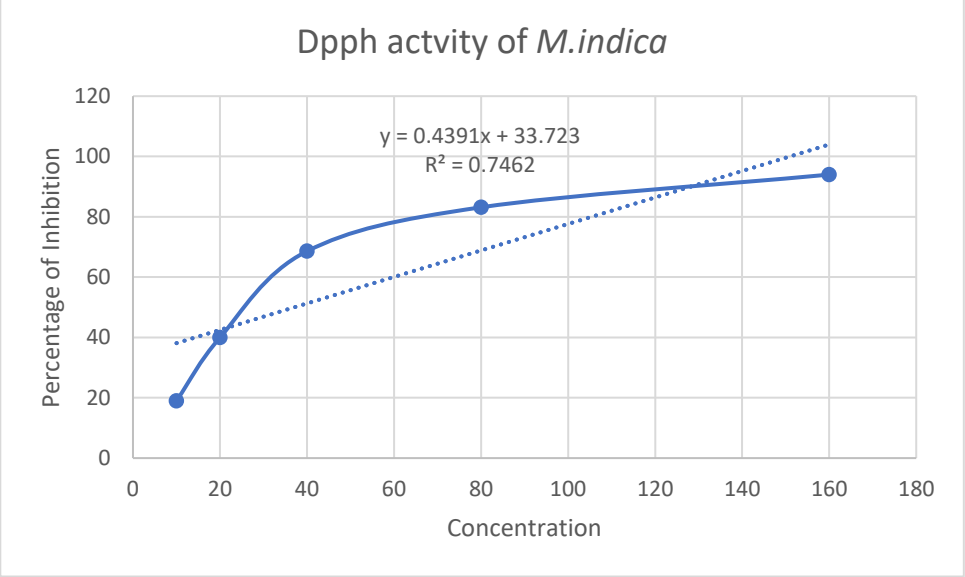
Percentage of inhibition of *Mangifera indica*

In this present study, the antioxidant activity of ethanol extracts of *Mangifera indica* were investigated by using DPPH radical scavenging assay. Ethanol extract of *Mangifera indica* was found to have the most potent antioxidant property with an IC50 value of 63.06μg/ml. The DPPH, a stable free radical, to decolourize in the presence of antioxidants. The antioxidants present in the *Mangifera indica* can acts as radical scavengers may protect the cells against various diseases such as cancer, neurodegenerative disorders.

Table 3: Antioxidant activity of ethanol extract of *Mangifera indica*

Concentration of the sample	OD value at 517nm	Percentage of inhibition
10mg/ml	0.856	11.57
20mg/ml	0.713	26.34
40mg/ml	0.526	45.66
80mg/ml	0.226	76.65
160mg/ml	0.124	87.19
IC50	63.06	

Fig 2: Graphical representation of DPPH scavenging activity of *Mangifera indica*



CONCLUSION

The dried powder of the leaves of *Mangifera indica* was extracted with ethanol. In the present study seven chemical constituents have been identified from the ethanolic extract of *Mangifera indica* by Gas Chromatogram / Mass spectrometry (GC/MS) analysis. The presence of these bioactive compounds in *Mangifera indica* lends credence to its use by the human community. It could be concluded that *Mangifera indica* contains various bioactive compounds. The results of the present investigation indicate that *Mangifera indica* ethanol extracts exhibited the highest antioxidant activity. So, it is recommended as a plant of phytopharmaceutical importance.

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