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PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACTS OF STEM, LEAVES, FLOWER AND SEED KERNEL OF *MANGIFERA INDICA* L.

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ABSTRACT

Mangifera indica L. is medicinally important plant species used to treat different diseases. The present work is aimed to screen this medicinal plant for phytochemicals. Leaf, stem, flower and seed kernel powder of this plant were extracted in ethanol solvents by soxhlet extraction and screened for secondary metabolites. Leaves of *Mangifera indica* revealed the presence of alkaloids, carbohydrates, phytosterols, tannins, fixed oils and fats, resins, phenols, flavonoids, proteins; and absence of glycosides and amino acids. Stem of *Mangifera indica* showed the presence of alkaloids, carbohydrates, phytosterols, resins, phenols, tannins, flavonoids, proteins; and absence of glycosides, tannins and amino acids. Whereas the flower and seed kernel revealed the presence of alkaloids, carbohydrates, saponins, phytosterols, fixed oils and fats, resins, phenols, tannins, flavonoids, proteins and amino acids but absence of anthranol glycosides. The plant parts showed variation in secondary metabolites. *Mangifera indica* accumulates more number of secondary metabolites. The findings of the present study will be helpful to the phytochemists and pharmacologists for identification of new active principles.

KEYWORDS: **Phytochemicals, Secondary metabolites, *Mangifera indica* etc.**

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INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases [1]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [2]. In India, almost 95% of the prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha [3]. The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products are of plant origin and their sales exceeded US $65 billion in 2003 [4].*Mangifera indica* is a large evergreen tree, with a heavy, dome-shaped crown. It belongs to the family *Anacardiaceae*. It is found all over the tropical regions of the world where it is used as a horticultural and medicinal plant. Fruits contain protein, fat, carbohydrate, minerals, vitamins A, B and C and amino acids. The fruits also yield a resin containing mangiferene, mangiferic acid, resinol and maniferol and others [5, 6]. The leaves have been reported to contain saponins, glycosides, unsaturated sterols, polyphenols, euxanthin acid, mangiferine and tannins etc. The ashes of the leaves are used to treat burns, scalds, sores, cough and diarrhoea in South America and other parts of the world [5, 7]. The use of leaf extracts as antiseptics in the treatment of burns, scalds, sores, wounds, abscesses and other infections in humans and animals has been reported in a number of ethnobotanical surveys [8, 9, 10].Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc. [11]. The present study aimed to find the phytochemical constituents present in the ethanolic extracts of *Mangifera indica* L.

MATERIALS AND METHODS

Plant Materials

The fully matured leaves, stem, flower and seed kernel of *Mangifera indica* were collected from the farm of Akola District, India during December 2010. The plant material were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in an air tight bottle.

Extraction of Plant Material

Twenty grams of shade dried powder of each plant parts was used for extraction with 200ml ethanol for 24 h in Soxhlet apparatus. The solvent was removed in rotary evaporator and the crude extracts were dried at room temperature in steady air-current and stored at 4˚C.

Phytochemical analysis

Phytochemical examinations were carried out for all the extracts as per the standard methods [12, 13].

1. *Detection of alkaloids*

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.
b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide) Formation of brown/reddish brown precipitate indicates the presence of alkaloids.
c) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow colored precipitate indicates the presence of alkaloids.

2. *Detection of carbohydrates*

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of Carbohydrates.
b) Benedict's test: Filtrates were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.
c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehlings A and B solutions. Formation of a red precipitate indicates the presence of reducing sugars.

3*. Detection of glycosides*

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

4. *Detection of saponins*

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
b) Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. *Detection of phytosterols*

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
b) Libermann Burchard's test: Extracts were treated with chloroform and filtered.The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

6. *Detection of fixed oils & fats*

a) Stain Test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

7. *Detection of resins*

a) Acetone-water Test: Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

8. *Detection of phenols*

a) Ferric Chloride Test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

9. *Detection of tannins*

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

10. *Detection of flavonoids*

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.
b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.
c) Shinoda Test: To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

d) Zinc hydrochloric acid reduction Test: To the alcoholic solution of extracts, a pinch of Zinc dust and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

11. *Detection of proteins and amino acids*

a) Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presence of proteins.
b) Ninhydrin test: To the extract, 0.25% ninhydrin reagent was added and boiled for few a minutes. Formation of blue colour indicates the presence of amino acid.
c) Biuret Test: The extracts were treated with 1 ml of 10% sodium hydroxide solution and heated. To this a drop of 0.7% copper sulphate solution was added. Formation of purplish violet colour indicates the presence of proteins.

RESULTS AND DISCUSSION

The phytochemical screening and qualitative estimation of *Mangifera indica* showed that the leaves are rich in proteins, alkaloids, carbohydrates and flavonoids (Table 1). Proteins contributed to the structure and functions of the living cell, they occur as independent units as well as in combination with lipids, nucleic acids, carbohydrates and many other compounds [14]. Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids are have been reported to possess many useful properties, including antiinflammatory, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activity [15]. Glycosides are totally absent in all the plant parts. Flavonoids and alkaloids have hypoglycemic activities. Tannin compounds are present in all plant materials. Tannins isolated from plant species *Solanum trilobatum* Linn exhibited antibacterial activities against *Streptococcus pyrogens, Salmonella typhi, Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* [16]. Saponins are present in seed kernel extracts of the *Mangifera* and absent in other extracts. Vaghasiya and co-workers reported that saponins are absent in methanolic extract of *Mangifera indica* seed kernel[17]. Traditionally saponins have been extensively used as detergents, as pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects. Carbohydrates, reducing sugars and phenols are present in all extracts of the *Mangifera indica.* The results of the phytochemical analysis agree with the findings of other authors: El-Mahmood Muhammad Abubakar revealed the presence of alkaloids, phenols, tannins, saponins and cardiac glycosides in methanolic stem bark extract[18], ethanolic flower extract of *Mangifera indica* showed the presence of alkaloids, phenols, flavonoids and carbohydrates and absence of saponins[19], ethanolic leaves extract revealed the existence of alkaloids, carbohydrates, phytosterols, flavonoids and proteins as mentioned in the study[20].The presence of bioactive compounds indicates the medicinal value of the plants. Antioxidants and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones. Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. Leaves of *Albizzia lebbeck*, *Ocimum americanum*, *Nerium momordica*, *Acalypha ornate*, stem bark of *Pyrus pashim* and leaves, stem, roots and seed kernels of *Jatropha* are rich in secondary metabolites [21, 22, 23, 24, 25, 26].In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic worldwide sales of drugs is based on natural products [27]. Bioactive extracts should be standardized on the basis of phytochemical compounds. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites.

Table 1
Phytochemical screening of Mangifera indica L

Sr. No	Test	Ethanol Extract			
		Stem	Leaves	Flower	Seed kernel
A.	**Alkaloids Test**				
1.	Mayer's Test	+	+	+	+
2.	Wagner's Test	+	+	+	+
3.	Hager's Test	+	+	+	+
B.	**CarbohydratesTest**				
1.	Molisch's Test	+	+	+	+
2.	Benedict's Test	+	+	+	+
3.	Fehling's Test	+	+	+	+
C.	**Glycosides Test**				
1.	Modified Borntrager's /Anthraquinone Test	-	-	-	-
D.	**Saponins Test**				
1.	Froth Test	-	-	-	+
2.	Foam Test	-	-	-	+
E.	**Phytosterols Test**				
1.	Salkowski's Test/Terpenoid test	+	+	+	+
2.	Libermann Burchard's test/Sterols test	+	+	+	+
F.	**Fixed oils & Fats Test**				
1.	Stain Test	-	+	+	+
G.	**Resins Test**				
1.	Acetone Water Test	+	+	+	+
H.	**Phenols Test**				
1.	Ferric chloride Test	+	+	+	+
I.	**Tannins Test**				
1.	Gelatin Test	+	+	+	+
J.	**Flavonoids Test**				
1..	Alkaline Reagent Test	+	+	+	+
2.	Lead Acetate Test	+	+	+	+
3.	Shinoda Test	+	+	+	+
4.	Zinc hydrochloric acid reduction Test	+	+	+	+
K.	**Proteins & amino acids Test**				
1.	Xanthoproteic Test	+	+	+	+
2.	Ninhydrin Test	-	-	+	+
3.	Biuret Test	+	+	+	+

Note: '+' indicates presence '-' indicates absence

CONCLUSION

Mangifera indica L. is widely used in traditional medicine to combat and cure various ailments and found to be rich in secondary metabolites. The presence of alkaloids, carbohydrates, phenol, resins, proteins, amino acids, flavonoids, tannins steroids and terpenoids in plant may be attributed to their curative properties. Exploitation of these pharmacological properties involves further investigation and identification of these active ingredients by implementing techniques like extraction, purification, separation and crystallization.

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