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PHYTOCHEMICAL AND INVIVO ANALGESIC ACTIVITY STUDY OF ETHANOLIC EXTRACT OF THE FLOWER OF MANGIFERA INDICA L.

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ABSTRACT

Mangifera indica L. is medicinally important plant species used to treat different diseases. The aim of the present study was to investigate the phytochemical and in vivo analgesic activity of ethanol extract of flower of Mangifera indica. flowers of Mangifera indica show the presence of alkaloids, carbohydrates, tannins, fixed oils and fats, resins, phenols, flavonoids, and absence of glycosides. Qualitative phytochemical screening was done for Ethanollic extracts by the use of standard methods. For investigation of the analgesic activity, acetic acid induced writhing response model was used in Swiss albino mice. In this case, flower extract were administered and the effects were compared with commercially available analgesic and anti-inflammatory drug, Diclofenac Sodium. The findings of the present study will be helpful for identification of new active principles.

KEYWORDS: Mangifera indica, phytochemical, pharmacological activity, analgesic.

INTRODUCTION

Magnifera indica a plant species is important to make medicine in order to cure many diseases. The object of the study was to look into the phytochemical and pharmacological activity of ethanol leave extract of Magnifera indica. It is revealed from the flowers alkaloids, carbohydrate, tannins, fixed oils, fats, resins, phenols, flavonoids are present; and glycosides and amino acids are absent. To find out the result of the pharmacological activities, acetic acid was used in Swiss albino mice and writhing response mood I was followed. The extract

of the leaves were administered and the effects were compared with analgesic and anti-inflammatory drug, Diclofenac Sodium. The present study will be of great use to the Phytochemists and pharmacologists for the identification of new active principles.

India traditional medicine has a wide range in consulting with its various components like Ayurveda, Siddha and Unani. The traditional systems of medicines are developed for safety, efficacy and equality which will help to secure the traditional heritage and the use of natural products in healthcare.[1] As a result of it many medicinal plants are used to cure various diseases.[2] Most of the people of the world are in the habit of using traditional medicines for primary healthcare.[3] With the help of some chemical active substances the value of medicinal plants are grown that produce a physiological action on the human body [4]. Prolonged studies on different medicinal plants in different countries have proved the medicinal efficiency.[5,8]

An evergreen large, dome shaped tree is Magnifera Indica. It is an Anacordiaceae. It is a tropical plant. It is used for horticulture. The fruits of this plantscontain protein, fat, carbohydrate, minerals, vitamins A, B complex or C and amino acids. We also find a resin containing mangifereue, mangiferic acid, resinol and manifierol and others. Saponins, glycosides, unsaturated sterols, polyphenols, euxanthin acid, mangiferine and tannins etc[9,10], are found in leaves. Antibacterial activity[11] is possessed by flowers, antiulcerogenic action[12], hypoglycemic activity[13], atherogenicity[14], anti-diarrhoeal activity[15] is possessed by seed, effectiveness for dyslipidemia.[16] Immunomodulatory activity[17] is possessed by bark and stem, anti-inflammatory and neuro protective activity.

MATERIALS AND METHODS

Sample collection

The flower of M. indica was collected and identified in March - April, 2014 from West Bengal region, India. The flower were separated, washed thoroughly with tap water, chopped into small pieces, shade dried, homogenized to fine powder and stored in air tight container.

Extraction of Plant Material

150 gm powder was macerated using pet ether, chloroform, ethanol from low to high polar solvent for 7 days for each solvent at room temperature with occasional stirring. After 7 days, ethanol extract was filtered thoroughly with cotton plug and finally with a Whatsman no. 1 filter paper. The extract was concentrated under reduced pressure below 50º C temperature

through rotatory vaccum evaporator. The concentrated extracts were collected in a Petri dish and allowed it to air dry for complete evaporation of ethanol. Finally, 13.8 gm greenish colored concentrated flower extract was obtained (yield 10.87 % w/w) which was kept in desicator.

Phytochemical analysis

Phytochemical examinations were carried out for all the extracts as per the standard methods[18,19]

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 (H_2SO_4) 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 Fehling's 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 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 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. Detection of phenols

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

Pharmacological Activity Analysis

Experimental animals and diets

Swiss albino mice of both sexes weighing between 20 gm and 35 gm were taken from animal house of BCDA COLLEGE OF PHARMACY & TECHNOLOGY laboratory, Barasat-West Bengal with permission from institutional animal ethical committee Registration no. 1682/PO/a/13/CPCSEA. The animals were kept to room temperature (28±5°C) with a relative humidity of 55 ± 5 % in a standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study the animals were supplied with standard diet and water.

Screening of analgesic activity of plant extract

Acetic acid induced writhing test model as described by Koster et al. (1959) [20] was performed to evaluate the analgesic activity of Mangifera indica flower extract.

Acetic acid induced writhing response model

Thirty Swiss Albino mice with average body weight of 20 gm to 35 gm and 3 months of age in both sexes were taken during experiment. They were randomly divided into five groups and each group consisting of 6 animals. The animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. Negative Control group received only distilled

water, positive control group received Acetic acid, Standard received analgesic drug Diclofenac sodium at the dose rate of 10mg/kg body weight and treated group received flower extract at the dose rate of 150mg/kg and 250mg/kg body weight. Ethanollic extract of flower, analgesic drug diclofenac sodium and distilled water administered orally to particular groups, 15 mins prior to acetic acid injection.1 % (v/v) acetic acid solution at the dose rate of 5ml/kg body weight was injected intra-peritoneally in mice and the writhing episodes were recorded for 15 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted. Percentage of inhibition was evaluated using following formula. The results of acetic acid induced writhing method in mice were tabulated in Table-1.

$$\% \text{ Inhibition} = \frac{\text{Mean writhing count (Positive Control group} - \text{Treated group)}}{\text{Mean writhing count of Positive control group}} \times 100$$

Statistical analysis

The results were reported as the Mean ± S.E.M. (Standard Error of Mean) and statistical significance between the groups was determined by means of two-way analysis of variance (ANOVA) followed by paired Student's t-test to determine statistical significance. Probability (P) value of 0.05 or less ($P \leq 0.05$) was considered as significant. Comparison of analgesic activity of Diclofenac sodium and ethanol extract of Mangifera indica flower is shown (Table-1 and Figure-1).

TABLE 1

Compound	%Inhibition
standard	58.2
ex1	36.29
ex2	56.18

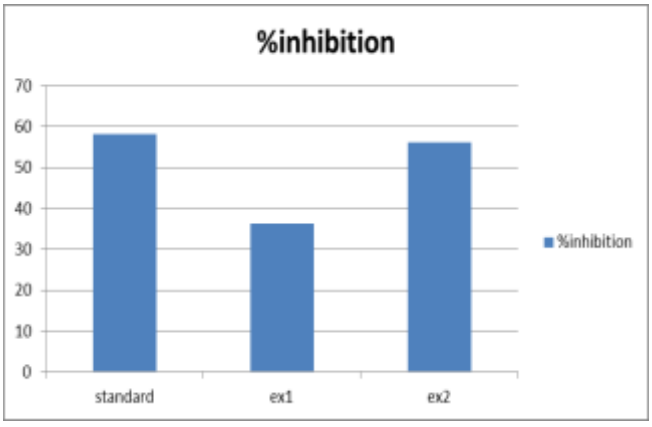

Figure 1:
n=6 Values are Mean±SD in each. p<0.05, p<0.01.

RESULT AND DISCUSSION

A) Phytochemical Parameter

The phytochemical screening and qualitative estimation of Mangifera indica showed that the Flowers are rich in proteins, alkaloids, carbohydrates and flavonoids (Table 2). 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.[21] Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids are have been reported to possess many useful properties, including anti-inflammatory, analgesic, estrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activity.[22] Glycosides are totally absent. Flavonoids and alkaloids have hypoglycemic activities. Tannin compounds are presented. Saponins are present in the flowers of M.indica. 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 the extract of the Mangifera indica. Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. The ethanol extract of Mangifera indica flower at the dose levels of 150 and 250 mg/kg body weight administered orally exhibited significant increase in analgesic activity in a dose-dependent manner (Table-2 and Table-3).The standard drug Diclofenac sodium showed significant increase in analgesic activity when compared with the control group of animals.

Table 2: Phytochemical screening of Mangifera indica L.

Sr. No.	Test	Ethanol Extract of Leaves
A.	**Alkaloids Test**	
1.	Mayer's Test	+
2.	Wagner's Test	+
3.	Hager's Test	+
B.	**Carbohydrates Test**	
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	+

Sr. No.	Test	Ethanol Extract of Leaves
E.	**Proteins & amino acids Test**	
1.	Xanthoproteic Test	+
2.	Ninhydrin Test	-
3.	Biuret Test	+
F.	**Flavonoids Test**	
1.	Alkaline Reagent Test	+
2.	Lead Acetate Test	+
3.	Shinoda Test	+
4.	Zinc hydrochloric acid reduction Test	+
G.	**Tannins Test**	
1.	Gelatin Test	+
H.	**Resins Test**	
1.	Acetone Water Test	+
I.	**Fixed Oils & Fats Test**	
1.	Stain Test	+
J.	**Phenols Test**	
1.	Ferric Chloride Test	+

B) Pharmacological Activity Parameter

Effect of flower extract of Mangifera indica and Paracetamol on acetic acid induced writhing response in Swiss albino mice.

Table 3:

Group	Treatment	Dose	Writhing Count	% Inhibition
Control	Acetic acid	5ml/kg	24.33+/- 1.63	-
Positive Control	Diclofenac Sodium	10mg/kg	10.16+/-1.83	58.2
Treated	Mangifera indica extract	150mg/kg	15.5+/-1.97	36.29
Treated	Mangifera indica extract	250mg/kg	10.66+/_1.37	56.18

n=6 Values are Mean±SD in each. $p<0.05$, $p<0.01$.

CONCLUSION

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. 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 .Preliminary research shows the analgesic activity of Ethanollic extract of flower of Mangifera indica, further studies may helpful to get other therapeutic activities which will be beneficial for human health.

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