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PHYTOCHEMICAL ANALYSIS AND ANTITUBERCULAR ACTIVITY OF FLOWERS EXTRACT OF *MANGIFERA INDICA*

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ABSTRACT: About 5 extract (hexane, acetone, ethanol, methanol, aqueous extraction) from the flowers of *Mangifera indica* was extracted. Ethanol extract was characterized by Gas Chromatography-Mass Spectroscopy. Seven constituents from 7 peaks were identified. Icosanedioic acid monomethyl ester, nonadec-16-enyl-benzene, 1, 9 diphenyl nonane, icosane, octadecane, dodecanoic acid butyl ester, tetracosyl- benzene were identified. The antimicrobial activity of different extract was tested against human and plant pathogenic bacteria. Ethanol extract showed significant role on inhibiting almost all tested pathogenic organisms and antitubercular activities at various concentration.

INTRODUCTION: India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. Antimicrobials of plant origin have enormous therapeutic potential[1]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body [2].

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Mango *(Mangifera indica L.*) is one of the choicest fruit of tropical and sub-tropical region of the world, especially in Asia. Its population and importance can easily be realized by the fact that it is often referred as "King of Fruits in the Tropical World". Mango is popular due to its excellent flavour, delicious taste, delicate fragrance, attractive colour and nutritive value which make at rank among the best fruits of world [3].

Many phenolic compounds have been detected in mango peels [4], mango bark [5], mango puree concentrate [6], mango pulps and seed kernels [7]. Carotenoids from mango [8], alkaloids, carbohydrate, phytosterols, resins, phenol, tannins, flavonoids and amino acid, triterpene [9], alkaloids [10] isolated from leaves Several pharmacological activities of mango extracts have been reported including anti-inflammatory [11], antioxidant [12], antiallergic and anthelmintic [13], antiamoebic [14], antitumor [15], antidiabetic [16], antibone resorption [17], antiviral[18], antibacterial [19], antifungal [19], antiparasitic [20] and lipolytic activity [21]. In the present study certain works such as phytochemical characterization,

antimicrobial activity of extract and antituberculosis activity.

MATERIALS AND METHODS:

Plant Material: The flowers of *Mangifera indica* was collected from tropical area of Madhya Pradesh (**Fig.1, 2**)

FIG.1: *MANGIFERA INDICA* TREE

FIG. 2: MANGO FLOWERS

Flower extract:

An amount of 5kg of fresh flowers was weighed and shade dried, cleaned, and then extracted serially hexane, acetone, ethanol, methanol and aqueous extraction for 95-126 hours each in a soxhlet extractor. Solvent was removed by rotary film evaporator and concentrated extracts were preserved in refrigerator for further use.

Analysis of ethanol extract:

Mass spectrometry analysis was performed on Shimadzu GCMS-QP-2010 SE model using Direct Injection Probe technique.

Antimicrobial activity:

The different flower extracts were subjected to the antimicrobial assay followed by agar well diffusion method [22]. 38 gm of Muller Hinton Agar was suspended in 1000ml of distilled water and heated up to boiling point for complete mixing. To sterilize, it was autoclaved at 15 lbs pressure at 121^0C for 15 minutes. 100 mg of each extract was suspended in 5ml of 10% DMSO. Approximately 25 ml of sterilized selective medium was poured in to each Petridis and solidified at room temperature. Using a sterile cotton sweb, the bacterial culture was swabbed on the surface of pre-poured nutrient agar plates.

The plates were allowed to dry for 15minutes, before use in the test. A well of 10mm diameter, punched off at previously marked Petri plates in to agar medium with sterile cup before then it was filled with 100 ul of extract every time. Plates were places for 30 minutes in refrigerator for diffusion of extracts and then incubated at 37^0C for 24 hours. Zone of inhibition (excluding well diameter) formed was measured as a property of antibacterial and antifungal activity.

Antitubercular activity:

The different flower extracts were screened for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}R_V$ strain using Lowenstein–Jensen medium method [23]. Ten mg of each extract was dissolved in 10 ml of DMSO to get a concentration of 1000 ug/l. Further dilutions were made with DMSO to get different concentrations such as 100, 10, and 1 ug/ml. 0.8 ml of each concentration was used for the study. To this, 7.2 ml of Lowenstein–Jensen medium was added.

RESULT AND DISCUSSION: In the present study, an amount of 5kg of *Mangifera indica* flowers and solvents such as hexane, acetone, ethanol, methanol and water were used for the extraction. From each sample 10 ml extracts were collected for screening microbiology.

GC-MS analysis of ethanol extract: GC-MS analysis indicated that the ethanol extract contained about 7 peaks. The composition of ethanol extract and its retention time are given in **Table 1.** Icosanedioic acid monomethyl ester, nonadec-16-enyl-benzene, 1, 9 diphenyl nonane, icosane, octadecane, dodecanoic acid butyl ester, tetracosyl-benzene were identified.

TABLE 1: COMPOSITION OF *MANGIFERA INDICA* ETHANOL EXTRACT

Number of Peaks	Retention Time (minutes)	Compounds
1	3.305	Icosanedioic acid monomethyl ester
2	4.105	nonadec-16-enyl-benzene
3	6.990	1, 9 diphenyl nonane
4	24.295	icosane,
5	26.750	octadecane
6	41.705	dodecanoic acid butyl ester
7	51.125	tetracosyl- benzene

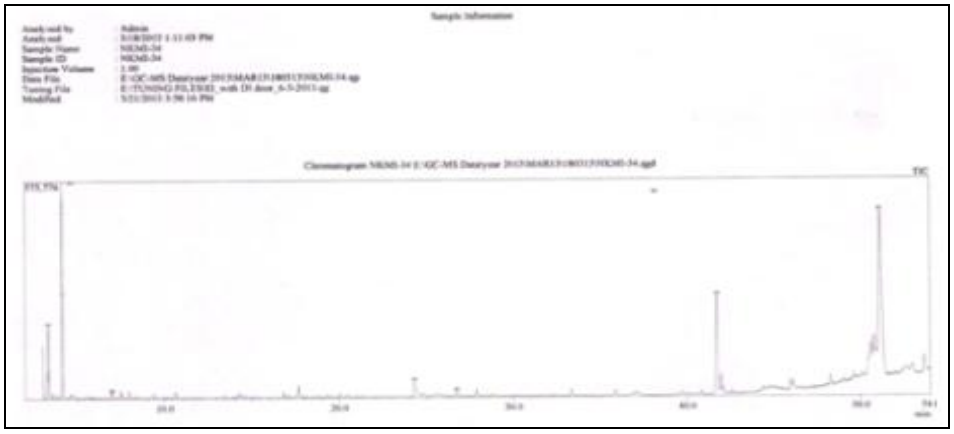

FIG.3: CHROMATOGRAM OF *MANGIFERA INDICA* ETHANOL EXTRACT

Antimicrobial activity of flowers extract of *Mangifera indica*:

In the present study, the antimicrobial activity of different extract of *Mangifera indica* was tested against nine bacteria (*Staphylococcus albus, Staphylococcus aureus, Staphylococcus heamolyticus, Vibrio cholera, Pseudomonas aerusinosa, Klebisella aerogenes, Escherichia coli, Pseudomonas pyocyneaus, Diplococcus peunoniae*). It was clear from the present result, that ethanol extract exhibited pronounced activity against all the bacteria. The presence of phytoconstituents in the flower extracts may be responcible for the antibacterial activity of plant. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents. The result was represented in **Table 2.**

TABLE 2: ANTIMICROBIAL ACTIVITY OF *MANGIFERA INDICA* FLOWER EXTRACT AGAINST 9 BACTERIAL STRAIN BY AGAR WELL DIFFUSION METHOD

Microorganisms	Zone of inhibition in 100µl of 20mg/ml(mm)				
	Hexane extract	Acetone extract	Ethanol extract	Methanol extract	Aqueous extract
Staphylococcusalbus	9.0	9.0	11.0	6.0	5.0
Staphylococcusaureus	8.0	6.0	10.0	6.0	4.0
Staphylococcusheamolyticus	9.0	7.0	12.0	9.0	9.0
Vibrio cholerae	6.0	9.0	6.0	17.0	4.0
Pseudomonasaerusinosa	6.0	10.0	17.0	6.0	6.0
Klebisellaaerogenes	7.0	6.0	9.0	6.0	9.0
Escherichiacoli	9.0	9.0	16.0	6.0	10.0
Pseudomonaspyocyneaus	7.0	6.0	13.0	9.0	6.0
Diplococcuspeunoniae	2.0	3.0	5.0	3.0	4.0

Antitubercular activity:

Pyrazinamide was used as the standard drug. The dilution of Pyrazinamide was made with DMSO to get different concentrations of 100, 10, and 1µg/ml. 0.8 ml of each concentration was used for the study. A sweep from the *Mycobacterium tuberculosis* $H_{37}R_V$ culture was discharged with the help of nichrome wire loop with a 3 mm external diameter, into a sterile distilled bijou bottle containing 6 mm glass beads and 4 ml of sterile distilled water. The bottle was shaken with the help of a mechanical shaker for 2 min, and then using

nichrome wire loop, 3 mm external diameter, a loopful of suspension was inoculated on the surface of each of Lowenstein–Jensen medium containing the test compounds. Lowenstein–Jensen medium containing pyrazinamide as well as control were inoculated with *Mycobacterium tuberculosis* $H_{37}R_V$ strain. The inoculated medium was incubated at 37^0C for 4 weeks. At the end of 4 weeks, readings were taken and recorded in **Table 3.**

TABLE 3: ANTITUBERCULAR ACTIVITY OF DIFFERENT FLOWER EXTRACT OF *MANGIFERA INDICA*

Compound	*Mycobacterium tuberculosis* concentration in *µg/mL*		
	100	10	1
Control	+++	+++	+++
Hexane extract	-ve	-ve	-ve
Acetone extract	-ve	-ve	-ve
Ethanol extract	-ve	-ve	-ve
Methanol extract	-ve	-ve	-ve
Aqueous extract	-ve	-ve	-ve

+++ indicates intensive growth of *M. tuberculosis* -ve indicates complete inhibition of $H_{37}R_V$

FIG.4: ANTITUBERCULAR ACTIVITY

CONCLUSION: from this study it can be concluded that the ethanol extract of *Mangifera indica* exhibited pronounced activity against all the tested bacteria.

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