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EVALUATION OF ANTIBACTERIAL POTENTIAL AND PHYTOCHEMICAL STUDIES OF *MANGIFERA INDICA L.* LEAVES

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ABSTRACT

The in vitro antibacterial activity of leaf extracts of *Mangifera indica* has been evaluated using disc diffusion method. Aqueous and ethanolic leaf extracts of *Mangifera indica* leaves were evaluated against pathogenic strains of four Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebseilla pneumoniae* and *Salmonella typhi* and two Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Ethanolic extract of *Mangifera indica* was found to be effective against *Salmonella typhi*, *Staphylococcus aureus*, *Klebseilla pneumoniae* and *Pseudomonas aeruginosa*, while its aqueous extract did not showed any activity against all the bacteria under study. Least effect of ethanolic extract was found on *Bacillus subtilis* and *Escherichia coli*. Thus, the ethanolic leaf extract of *Mangifera indica* was found to possess more antibacterial potential than its aqueous extract. Phytohemical tests showed the presence of alkaloids, carbohydrate, phenol, tannins, flavonoids and amino acid in the ethanolic portion of *Mangifera indica* leaves. These findings may open up the possibility of finding of new antimicrobial compound.

KEYWORDS: Antibacterial activity, *Mangifera indica*, pathogenic strains, phytochemical studies.

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. 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 (Saranraj and Sivasakthi, 2014).

Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. (Ahmad et al., 1998; Bhavnani and Ballow, 2000).

Mangifera indica (Anacardiaceae) is one of the important tropical fruits in the world and India contributes major part of the world production (Sahrawat et al., 2013). Mango is national fruit of India. There are many traditional medicinal uses for the different parts of *M. indica* throughout the globe. It is used as astringent, ophthalmia, eruptions, haemorrhages, menorrhagia, dysentery, antiscorbutic, laxative in traditional medicine (Ghani, 1998).

With this view point, the objective of this research was to evaluate the antibacterial activity of leaf extract of *Mangifera indica* leaves against some pathogenic bacteria and also to determine the phytoconstituents that may be present.

MATERIALS AND METHODS

Plant and preparation of extracts

The green leaves of *Mangifera indica* collected from Gondia (M.S.) region India in September 2015 and taxonomic identification of leaves was carried out by the Botanist at the Botany Department of the present institution.

After washing with tap water, plant leaves were initially allowed to air-dry in the laboratory for a week and then finally ground to a fine powder, using a mortar and pestel and stored for further use.

25 g of the powdered leaves were extracted in Erlenmeyer flasks with 100 ml of deionised distilled water (aqueous extraction) and 100ml of ethanol (ethanolic extraction) separately. The flasks were plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5 days with occasional agitation using a sterile glass rod at intervals of 24 hours. The extracts were separately filtered using Whatman filter paper no. 1.

The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness (Oluduro, 2012).

Test Microorganisms

The test organisms used were pure culture of Staphylococcus aureus (NCIM 2672), Bacillus subtilis (NCIM 2063), Escherichia coli (NCIM 5010), Klebsiella pneumoniae (NCIM 2707), Salmonella typhi (NCIM 2501) and Pseudomonas aeruginosa (NCIM 5029) which were purchased from the National Chemical Laboratory (NCL), Pune. NCIM stands for National collection of industrial microorganisms.

Chemicals and media

All the chemicals, media and medium ingredients used in the present study were purchased from Himedia (Mumbai, M.S. India).

Phytochemical Screening

The phytochemical analysis of ehanoloc extract of *Mangifera indica* leaves was carried out using the method previously described (Brain and Turner, 1975; Evans, 1996). The phytoconstituents assayed were alkaloids, carbohydrate, glycosides, saponins, phytosterols, resins, phenol, tannins, flavonoids and amino acid.

Antimicrobial Disc Preparation

Antimicrobial activity of the aqueous and ethanolic extracts of the leaves was assayed using the paper disc diffusion method of Oluma et al (Oluma et al., 2004; Doughari et al., 2007). Discs of about 6mm diameter were made from Whaman filter paper no. 1 using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 121^0C for 15 minutes. The concentrated leaf extracts were redissolved in 5% dimethyl sulfoxide (DMSO) and sterile discs were impregnated with 30 micro L of 30mg/ml of each extract. The discs were carefully and firmly placed on the Mueller Hinton Agar (MHA) plates lawned previously with the test organism.

Preparation of Inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4^0C and subcultured into nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002).

Evaluation of Antibacterial Potential

Disc agar diffusion technique described by Bauer et al (1966) was employed for antibacterial activity. Paper discs were impregnated with 30 microL of a solution of 30 mg/ml and the standard antibiotic Gentamicin was used as a control for comparison. Filter paper discs dipped into sterile distilled water, solvents blank and the standard antibiotic were used as control. The plates were then incubated at 37^0C for 24 hours. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc. Inhibition zones were calculated as the difference between the disc diameter (6 mm) and the diameters of zone of inhibition (Sainath et al., 2009).

RESULTS AND DISCUSSION

Phytochemical analysis of *Mangifera indica* leaves revealed the presence of different classes of compounds including alkaloids, carbohydrate, phytosterols, resins, phenol, tannins, flavonoids and amino acid shown in Table 1.

Marjorie, 1999 reported Triterpenes and alkaloids possessed antibacterial activity. This suggests that the antibacterial activity of *Mangifera indica* leaves might be due to different classes of these phytocontituents. Alqasim et al., 2014 reported the absence of glycosides and saponins in the ethanolic leaf extract of *Mangifera indica* which is in accordance with the present investigation.

The results of the antibacterial assay of aqueous and ethanolic leaf extracts of *Mangifera indica* indicated that the plant exhibited antibacterial activity against the tested microorganisms at concentrations of 30mg/ml. The potential sensitivity of the ethanolic extract was obtained against all the microorganisms tested and the zone of inhibition was recorded and presented in the table shown below (Table 2). No antibacterial activity was found in aqueous extract of *Mangifera indica.*

Salmonella typhi and *Staphylococcus aureus* and *Klebseilla pneumoniae* were found to be most sensitive to ethanolic extract of *Mangifera indica* leaves followed by *Pseudomonas aeruginosa. E. Coli* and *Bacillus subtilis* were least affected by the treatment of ethanolic leaf extract of *Mangifera indica.* These findings show that the ethanolic leaf extract of *Mangifera indica* was more potent as compared to its aqueous extract.

Zhu, et al., 1993 studied the in vitro effect of mangiferin was studied against Herpes simplex virus type 2; mangiferin does not directly inactivate HSV-2 but inhibits the late event in HSV-2 replication. Ethanol extract showed no inhibitory effect on *Staphylococcus aureus* and weak inhibitory effects against *E. coli* in the work by Mustapha et al., 2014. This effect is not in agreement with the present work.

Antibacterial activities of leaf extract of *Mangifera indica* were studied against bacteria such as *Proteus vulgaris, Pseudomonas fluorescens, Shigella flexneri, Klebsiella pneumoniae, Salmonella typhi* by Sahrawat et al., 2013. Their findings shows the ethanol extracts of *Mangifera indica* leaves showed maximum growth inhibition against Pseudomonas fluorescens and also showed minimum growth inhibition against *Klebsiella pneumoniae*. But in the present work ethanolic extracts of *Mangifera indica* leaves showed adequate activity against *Klebsiella pneumoniae.* Begum P. et al 2015 evaluated the antimicrobial activity of some traditional medicinal plants of Bangladesh.

No antibacterial activity was found in the aqueous extract of the leaves of *M. Indica* but it was reported to possess hypoglycaemic activity (Aderibigbe, et al., 2001).

Table-1. Phytochemical constituents of *Mangifera indica* leaves.

Phytoconstituents	**Ethanolic extract of *Mangifera indica* leaves**
alkaloids	+
carbohydrate	+
glycosides	-
saponins	-
phytosterols	+
resins	+
phenol	+
tannins	+
flavonoids	+
amino acids	+

"+" indicates presence and "–" indicates absence.

Table-2. Bactericidal activity of *Mangifera indica* by disc diffusion method.

Test organism	**Antibacterial activity in terms of Zone of inhibition in mm**			
	Aqueous extract (30mg/ml)	**Ethanolic Extract (30mg/ml)**	**Ethanol (30 micro litre)**	**Gentamicin (30mg/ml)**
E. coli	NZ	10	10	13
Pseudomonas aeruginosa	NZ	12	10	10
Klebseilla pneumoniae	NZ	14	10	12

Salmonella typhi	NZ	14	10	10
Bacillus subtilis	NZ	10	10	12
Staphylococcus aureus	NZ	14	NZ	12

NZ indicates No Zone of inhibition

CONCLUSION

The present results showed that ethanolic leaf extract of *Mangifera indica* had antibacterial activity and can be used as a source for developing broad spectrum antimicrobials. This could validate its use in traditional herbal medicine to treat a variety of caused by bacteria. No bactericidal activity was observed in the aqueous extract of *Mangifera indica* leaves. Presence of phytochemicals indicates possible preventive and curative properties of *Mangifera indica* leaves. However, there is need to carry out more pharmacological studies to support the use of *Mangifera indica* as a medicinal plant.

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