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Research Article

ANTIBACTERIAL ACTIVITIES OF LEAVES EXTRACTS OF INDIAN BUTTER TREE (*MADHUCA INDICA)*

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ABSTRACT

Leaves of *Madhuca indica* L. were collected, air dried and powdered. Aqueous and ethanol and extracts were prepared and their antibacterial activity on four human pathogenic bacteria; *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa* was observed by paper disc method. The significant results were obtained by Aqueous and methanol extracts of leaves.

Key words: *Madhuca indica* antibacterial activity, human pathogic Bacterias.

INTRODUCTION

Now days, medicinal and aromatic plants are important to the global economy in the world. Medicinal plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavor and aroma industries. These secondary metabolites of plant are commercially important and find use in a number of phytochemicals compounds.

These secondary metabolites substances have been used as food, medicine etc. Amongst them, the metabolites having medicinal value have been extensively used for treating various disease conditions. Medicinal plants being easily available to human beings have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, fruits leaves, seeds bark, exudates etc. are used as per medicinal properties. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious human diseases. These diseases represent a critical problem to health and they are one of the major courses of morbidity worldwide[1]. The modern pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants and secondary metabolites of which at least 12,000 have been isolated, however, a number estimated to be less than 10% of the total[2].

Laboratories of the world have found literally thousands of phytochemicals showing inhibitory effects on all types of microorganisms *in vitro* Bacterial resistance to currently used antibiotics is becoming a concern to public Health[3]. The development of bacterial super resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents[4].

Mahua (*Madhuca indica* J.F. Gmel. Is a medium sized tree and belongs to the family Sapotaceae. The medicinal properties of *Madhuca indica* such as nutritive and medicinal values of the have been clearly established with the research outcome that was completed in different places in India. Mahua flowers are well known for their high reducing sugar and nutrient content. They are edible and used as a sweetener in preparation of many local dishes like halwa, kheer, puri and burfi[5]. Mahua is very useful and vital information will be emerged out of the research activities initiated on this wonder tree. In the light of the above facts, the present investigation have been carried out on aqueous and methanol leaves, extracts of *M. indica* to show the antibacterial activity on four human pathogenic bacteria *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of Mahua (*Madhuca indica* J.F. Gmel. were collected from Botanical garden R.B.S.College Agra (U.P.), India. The leaves were washed under running tap water and shade dried for three weeks. The dried leaves were then homogenized by using a grinder to make fine powder and stored in air tight bottles.

Preparation of aqueous extract

The 15gm. of dried powder was taken in 250 ml distilled water in separate conical flasks, air tight with cork and then kept on a shaker for 8 hours .After it the extract were filtered by using a vacuum filtration system and stored at 4^{o}C degree in airtight containers.

Preparation of solvent extract

The plant samples were air dried for 48 hours and ground into uniform powder using a grinder. 15gm. of dried powder was taken in 250 ml of organic solvent according to their polarity (methanol) in separate conical flasks, air tight with cork and then kept on a shaker for 8 hours. After it the extract were filtered by using a vacuum filtration system. Now solvent was evaporated to make the final volume one – forth of the original volume and stored at 4^{o}C degree in airtight containers until for further use.

Microorganism and culture condition

Present investigations were carried out on four human pathogenic bacteria viz. *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa.* Bacteria cultured were maintained on Muller Hinton (MH) medium .The antibacterial activities were examined for aqueous and methanol leaf extracts of *Madhuca indica.*

Antibacterial Screening

Screening of antibacterial activity was carried out by paper disc method (Gould and Bowie [6]. High media sterile disc were used for activity, saturated disc with the extract (0.04ml) and known quantity of standard reference antibiotic separately were air dried at room temperature. The molten Muller Hinton (hi media) was inoculated with the 100 ml of the inoculums and poured into sterile Petri plates (borosil) .The disc with test compound placed on the upper surface of sterilized Muller Hinton plate that had been inoculated with the test organism (using a sterile swab) and air dried to remove the surface moisture . The thickness of MH medium was kept equal in all Petri plates and the standard disc (tetracycline) was used in each plate as control. The plates were inoculated 24 hours at 37 degree ^{0}C in incubator. After 24 hours growth of bacteria was measured for its zone of inhibition. The results were obtained by measuring the zone diameter. The experiment was conducted in replicates of 3 and the mean value is presented. The results were compared with the contro chloramphenicol.

Table 1. Antibacterial activity of aqueous leaf extract of *Madhuca indica against* five human pathogens as tested by disc diffusion assay

Species of Bacteria	Zone of Inhibition (mm)		
	Extract	Antibiotic	Control
Escherichia coli	25.50	20.00	0
Staphylococcus aureus	24.25	20.00	0
Salmonella typhimurium	24.50	20.00	0
Pseudomonas aeroginosa	23.50	20.00	0

Antibiotic – Chloramphenicol (1mg/ml)
Control- Distilled Water

Table 2. Antibacterial activity of methanol leaf extract of *Madhuca indica against* five human pathogens as tested by disc diffusion assay

Species of Bacteria	**Zone of Inhibition (mm)**		
	Extract	Antibiotic	Control
Escherichia coli	27.00	22.00	0
Staphylococcus aureus	26.00	24.00	0
Salmonella typhimurium	25.75	26.00	0
Pseudomonas aeroginosa	24.00	20.50	0

Antibiotic – Chloramphenicol (1mg/ml)
Control- Distilled Water

RESULTS AND DISCUSSION

The present investigation, on antibacterial activities of aqueous as well as methanol leaf extracts of *Madhuca indica* against four human pathogenic bacteria viz. *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Pseudomonas aeruginosa* are summarized in Table1 and Table 2. The present study reveals that the leaf extracts of *Madhuca* (aqueous and methanol) exhibited significant antibacterial activity against all the four tested bacteria. The aqueous extract performed strongest antibacterial activity in *Escherichia coli* with (25.50 mm) zone of inhibition and least antibacterial activity was observed in *Pseudomonas aeroginosa* with (23.50 mm) zone of inhibition. On the other hand, methanol leaf extract of *M.indica* also displayed potential antibacterial activity against all the tested bacteria. The strongest activity was recorded in *Escherichia coli* with (27.00 mm) zone of inhibition while, lowest antibacterial activity was observed in *Pseudomonas aeroginosa* with (24.00 mm) zone of inhibition. The moderate antibacterial activities were recorded by *E.coli,* and *Proteus vulgaris* with moderated inhibition zone of growth of bacteria. The above results show that the activity of aqueous and methanol extracts of *Madhuca indica* shows significant antibacterial. The present study also shows the presence of different phytochemical compounds with biological activity. The ethanolic extract of bark was tested for antibiotic activity using the well method on 16 clinical bacterial isolates seeded in Mueller Hinton Agar in *Tephrosia purpurea* (Fabaceae) and *Mimusops elengi* (Sapotaceae)[7]. The antibiotic activity were tested for their Minimum Inhibitory Concentrations (MICs) using the MIC agar dilution method. The ethanolic bark extract shows significant activity against three Staphylococcus isolates including *Staphylococcus aureus.*

The compounds have been shown to posses' antimicrobial activities against a number of human pathogenic bacteria. Antifungal activity of different crude extracts of leaves of *Madhuca indica*[8] the methanol extract According to them *M. indica* leaves showed the highest antifungal activity may be due to the presence of phytochemicals, alkaloids, tannins, saponins, proteins and flavonoids strongly than the other extracts. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Active compounds may be present in insufficient quantities in the crude extracts to show antibacterial activity with the dose levels employed[9].

The solvents used in the extraction procedure were found to have pronounced effect on the solubility of the antibacterial compounds[10]. Therefore, it may suggest that aquous and ethanol are the effective solvents for the extraction of antibacterial compound from leaves of bark of *Madhuca indica.* Further research has to be carried out on these extracts in order to purify and identify active components with the view of their use for pharmaceutical studies

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