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IN-VITRO ANTIBACTERIAL, ANTIFUNGAL AND ANTHELMINTHIC ACTIVITY OF THE BARK OF *MADHUCA INDICA*

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ABSTRACT

The research work was carried on the evaluation of antibacterial, antifungal and anthelminthic activities of the methanolic, petroleum ether and aqueous extracts of *Madhuca indica* bark belonging to family *Sapotaceae*. Disc diffusion technique was used for *in-vitro* antibacterial and antifungal activities using ciprofloxacin (5µg/disc) and clotrimazole (10µg/disc), zone of inhibition was observed against two gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis)*, two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa)*, two fungal species (*Aspergillus Niger, Candida albicans)* at concentration of 50, 100, 150, 200µg/mL). Anthelminthic activity was investigated in *pheretima posthuma* (Indian Earth Worm) at concentration 10, 20, 50mg/mL against standard drug piperazine citrate (10mg/mL). For antibacterial and antifungal activity of all the extracts has shown moderate to good activity against standard drugs. Among all the extracts aqueous extract and methanolic extract have shown good activity when compared to petroleum ether extract which showed moderate activity against standard drugs. For anthelminthic activity of petroleum ether extract has shown good activity against the other extracts.

Keywords: *Madhuca indica bark*, antibacterial, antifungal, anthelminthic activities.

INTRODUCTION

Plant sources as a potential antimicrobial compounds have been widely used by the researchers throughout the world for screening; in which plants have been utilized in the traditional or alternative systems [1]. There is a need to screen medicinal plants for therapeutically bioactive molecules including those with antimicrobial properties has gained an remarkable importance in the recent years and WHO is promoting the development and utilization of indigenous medicinal plants resources in the developing countries so as to extend safe and effective health care to a maximum number of population in those countries [2]. The most widespread infections in humans, is due to helminthic infections, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia [3]. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastrointestinal helminthes become resistant to currently available anthelminthic drugs therefore is a foremost problem in the treatment of helminthes disease [4, 5]. *Madhuca indica* commonly known as mahua belongs

to the family Sapotaceae. Mahua is a large shady, deciduous tree doting much of the central Indian landscape, both wild and cultivated. Mahua seeds are of economic importance as they are good source of edible fats [6]. The distilled juice of the flower is considered as tonic, both nutritional and cooling and also in the treatment of helminthes, acute and chronic tonsillitis as well as bronchitis [7]. The leaves are applied as a poultice to relieve eczema; the aerial parts are used for treatment of inflammation [8]. The stem bark is devoid of tannins, the bark is good remedy for itch swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus, helminthes, acute and chronic tonsillitis, pharyngitis as well as bronchitis previous phytochemical studies of *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides [9,10], it has anti-inflammatory, anti-ulcer and hypoglycemic effect of ethanol & crude alkaloid extract of seed cake on albino rats [11]. 50% alcoholic extract of stem bark of *Madhuca indica* reveal hypotensive activity and devoid of diuretic and anticancer property and LD_{50} was 1000mg/kg in albino mice [12]. In our previous investigation we have reported screening of *Madhuca indica* for anti-diabetic activity in streptozotocin and streptozotocin-nicotinamide induced diabetic rats [13]. The present study is to evaluate invitro antibacterial, antifungal *Madhuca indica* bark, methanolic, petroleum ether and aqueous extract of *Madhuca indica* bark was evaluated for *in-vitro* antibacterial, antifungal and anthelminthic activity [14].

MATERIAL AND METHODS

Plant Material: The bark of *Madhuca indica* was collected and authentified by Dr. Madhav Chetty, Department of Botany, Venkateswara University, Tirupathi, India. The collected plant material was washed thoroughly with water and dried under shade. Dried pieces of bark were powdered in a grinder and the powder was extracted with different solvents such as methanol, petroleum ether and water by soxhelation process. The extracts were evaporated to dryness at a controlled temperature (45^{o}C).

Preparation of Extracts: The methanolic, petroleum ether and aqueous extracts were prepared in 5 successive dilutions namely 50µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL for *in-vitro* antibacterial, antifungal and 10mg/mL, 20mg/mL, 50 mg?mL for anthelminthic activity.

Organisms Used:

Bacteria Gram Positive: *Staphylococcus aureus, Bacillus subtilis.*
Bacteria Gram Negative*:* *Escherichia coli, Pseudomonas aeruginosa.*
Fungi: *Aspergillus Niger, Candida albicans.*

Antibacterial Antifungal activities [15]: The paper disc diffusion method was used to determine the antibacterial and antifungal activities with methanolic, petroleum ether and aqueous extracts of *Madhuca indica*. Muller Hington agar media was prepared, sterilized and used the growth medium for bacterial culture. 20ml of sterilized medium was poured into each sterilized petridish covered and allowed to solidify. The plates were then seeded with test organism (bacterial culture) by sterile cotton swabs. For fungal culture saboraub dextrose agar was prepared and transferred into sterile petri plates and solidified. The medium plates were then swabbed with fungal culture. The sterilized paper discs were soaked in the prepared solution of the extracts with different solvents and were dried at 50^{o}C.The dried paper disc was then placed on both plates (Muller Hington and Saboraub dextrose agar) seeded with test microorganisms. The plates were then incubated for bacterial culture at 37^{0}C for 24 hours and for fungus the plates were incubated at room temperature for 48 hours and the zone of inhibition were measured. The results of antibacterial & antifungal activity of different extracts of Madhuca indica are shown in table 1, 2 &3.

Anthelminthic Activity [16]: The assay was performed on adult Indian Earthworm *pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Five groups of Indian earthworms each containing six earthworms approximately of equal size were used for study. Each group of earthworms were tested with different extracts at concentration (10, 20, 50 mg/ml), distilled water (control) and reference standard piperazine citrate (10mg/ml in distilled water).Observations were made for the time taken for paralysis and death of individual earthworms. Time of paralysis was noted with no movement of any sort could be observed except when the worms were shaken vigorously. Time of death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^{0}C followed with fading of their body color. The results of anthelminthic activity of different extracts of *Madhuca indica* are shown in table 4.

TABLE: 1: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *MADHUCA INDICA* BARK

NAME OF THE ORGANSIM	Methanolic extract (µg/ml) Zone of inhibition in mm				Standard (ciprofloxacin/ Clotrimazole Zone of inhibition in mm)
	50	100	150	200	
Staphylococcus aureus	8.7±0.10^{ns}	11.6±0.18*	12.3±0.21^{ns}	11.9±0.49^{ns}	18.8±0.16**
Bacillus subtilis	10.3±0.12*	13.2±0.07*	14.1±0.11^{ns}	14.4±0.06***	13.1±0.17**
E.coli	10.4±0.35^{ns}	12.3±0.51^{ns}	12.9±0.39*	14.2±0.29*	12.3±0.33*
Pseudomonas aeruginosa	9.5±0.22^{ns}	10.6±0.21*	13.5±0.22*	14.6±0.21^{ns}	15.3±0.33***
Aspergillus Niger	10.5±0.22*	12.3±0.21*	14.4±0.15***	15.1±0.16*	11.5±0.50*
Candida albicans	9.75±0.25*	10.9±0.02^{ns}	11.9±0.02**	13.9±0.02**	12.5±0.50*
DMF(control)	----	----	----	----	----

Values are mean ± SEM (n=3) * P<0.05, **P<0.01, ***P<0.001 were considered significant
-----: No Activity; NS: Not significant
Ciprofloxacin: 5µg/disc
Clotrimazole: 10µg/disc

TABLE: 2: ANTIBACTERIAL ACTIVITY OF PETROLEUM ETHER EXTRACT OF *MADHUCA INDICA* BARK

NAME OF THE ORGANSIM	Petroleum ether extract (µg/ml) Zone of inhibition in mm				Standard (ciprofloxacin /Clotrimazole Zone of inhibition in mm)
	50	100	150	200	
Staphylococcus aureus	7.9±0.08*	9.8±0.10^{ns}	9.5±0.14^{ns}	9.9±0.04**	18.8±0.16**
Bacillus subtilis	10.2±0.11^{ns}	11.9±0.03**	13±0.14*	13.1±0.25*	13.1±0.17**
E.coli	5.5±0.22**	8.1±0.10*	9.2±0.10***	9.2±0.16*	12.3±0.33*
Pseudomonas aeruginosa	7.6±0.21^{ns}	10.2±0.14*	13.1±0.16*	14.0±0.08^{ns}	15.3±0.33***
Aspergillus Niger	13.9±0.02**	15±0.04*	15.9±0.02^{ns}	13.6±0.21^{ns}	11.5±0.50*
Candida albicans	5.1±0.16*	8.2±0.14*	9.16±0.10*	9.3±0.18*	12.5±0.50*
DMF(Control)	----	----	----	----	----

Values are mean ± SEM (n=3) * P<0.05, **P<0.01, ***P<0.001 were considered significant
-----: No Activity; NS: Not Significant
Ciprofloxacin: 5µg/disc
Clotrimazole: 10µg/disc

TABLE: 3: ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *MADHUCA INDICA* BARK

NAME OF THE ORGANSIM	Aqueous extract (µg/ml) Zone of inhibition in mm				Standard (ciprofloxacin/ Clotrimazole Zone of inhibition in mm)
	50	100	150	200	
Staphylococcus aureus	12.33±0.21*	12.81±0.18^{ns}	14.91±0.04*	16.83±0.16*	18.83±0.16**
Bacillus subtilis	8.83±0.16^{ns}	12.33±0.33*	13.33±0.33^{ns}	13.66±0.33^{ns}	13.15±0.17**
E.coli	10.33±0.21*	11.33±0.21**	14±0.25^{ns}	14.5±0.22*	12.33±0.33*
Pseudomonas aeruginosa	11±0.25**	12.66±0.33*	14.33±0.33^{ns}	15.5±0.50*	15.33±0.33***
Aspergillus Niger	8.5±0.34	10.66±0.42*	13±0.25*	13.16±0.60**	11.50±0.50*
Candida albicans	10.5±0.50	12.66±0.66***	13.16±0.65*	15.5±0.34***	12.5±0.50*
DMF(control)	----	----	----	----	----

Values are mean ± SEM (n=3) * P<0.05, **P<0.01, ***P<0.001 were considered significant
-----: No Activity; NS:Not significant
Ciprofloxacin: 5µg/disc
Clotrimazole: 10µg/disc

TABLE: 4: ANTHELMINTIC ACTIVITY OF METHANOLIC, PETROLEUM ETHER AND AQUEOUS EXTRACTS OF *MADHUCA INDICA* BARK

Groups	Concentration (mg/ml)	Pheretima Posthuma	
		Time taken for Paralysis(P) in min	Time taken for death(D) in min
Control(Normal saline)	NA	NA	NA
Methanolic extract	10	33.1±0.74*	69±0.68*
	20	26.6±0.49^{ns}	51.6±1.05^{ns}
	50	16.5±0.34**	34.5±0.34**
Petroleum ether extract	10	28.66±0.66**	64.5±0.50**
	20	22.83±0.65**	62.5±0.50**
	50	14.33±0.21***	36.5±0.34*
Aqueous extract	10	35.5±0.50^{ns}	72.41±0.32^{ns}
	20	30.83±0.65*	66.83±0.54^{ns}
	50	19.66±0.66*	64.5±0.34*
Piperazine citrate (standard)	10	23.3±0.21**	61.1±0.16**

Values are mean ± SEM (n=6) * P<0.05, **P<0.01, ***P<0.001 were considered significant
NA: No activity; NS: Not significant

RESULTS AND DISCUSSION

The bark of *Madhuca indica* is extracted with solvents like methanol, petroleum ether and water. The dried extracts were screened for *in-vitro* antibacterial, antifungal and anthelminthic activities. Preliminary phytochemical studies of the bark of methanolic extract showed the presence of triterpenes, saponins, steroidal saponins, alkaloids, carbohydrates, tannins, glycosides but absence of steroid, whereas petroleum ether extract showed the absence of steroids, triterpenes, whereas aqueous extract showed absence of steroids.

The concentration of extracts for *in-vitro* antibacterial and antifungal activities was 50, 100, 150, 200µg/mL against the standard drugs ciprofloxacin (5µg/mL) for antibacterial activity and clotrimazole (10µg/mL) for antifungal activity. The organisms used for invitro antibacterial activity gram positive bacteria *staphylococcus aureus*, *bacillus subtilis* and gram negative bacteria *Escherichia coli* and *pseudomonas aeruginosa*, whereas for antifungal activity Aspergillus niger, candida albicans were used for testing the activity. Anthelminthic activity was carried out against *pheretima posthuma* at concentration 10, 20, 50 mg/mL against standard drug piperazine citrate at concentration 10 mg/mL.

Tannins may bind to proline rich proteins and interfere with the protein synthesis of bacteria, flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection. The antimicrobial and antifungal activity may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial and fungal cell walls, whereas saponins exert their activity due to its ability to cause leakage of proteins and certain enzymes from the cell.

For antibacterial and antifungal activity all the extracts has shown moderate to good activity against standard drugs. Among all the extracts aqueous extract and methanolic extract have shown good activity when compared to petroleum ether extract which showed moderate activity against standard drugs. All the extracts have shown the activity in a dose dependent manner i.e. at highest concentration the activity of the extract was good. For anthelminthic activity petroleum ether extract has shown good activity against the other extracts, tannins (polyphenolic compounds) interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation. The standard drug for anthelminthic activity i.e. piperazine citrate showed its mechanism of action by increasing the chloride ion conductance of worm muscle membrane, producing hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis, *Madhuca indica* bark extracts may also act in a similar manner.

CONCLUSION

The present study revealed that the extracts of *Madhuca indica* bark have shown potent antibacterial, antifungal and anthelmintic activities against the standard drugs. Extracts showed broad spectrum activities against wide range of bacterial and fungal infections. Results revealed all the extracts showed good activity with increase in the concentration. Further purification and isolation of the active constituents and toxicity studies should be carried out for finding clinically effective compounds, the bark of *Madhuca indica* should be explored for the possible mechanism of action responsible for the activities.

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