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IJPBS |Volume 3| Issue 2 |APR-JUN |2013|373-378

Research Article
Biological Sciences

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF METHANOLIC SEED EXTRACT OF *MEMECYLON UMBELLATUM BURM*

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ABSTRACT

The present study was carried out to evaluate the antibacterial activity of methanolic extract of Memecylon umbellatum seeds using cup plate method against five strains of bacterial species, viz., Escherichia coli, Salmonella typhi, Bacillus subtilis, Klebsiella pneumonia and Staphylococcus aureus. The methanolic extract of plant seeds showed minimum to moderate activity against both Gram positive and Gram negative bacteria indicating a broad spectrum of activity. A preliminary phytochemical screening was conducted on plant extract using standard qualitative procedures that revealed the presence of two (tannins, phenolic compounds and fats and oils) secondary metabolites. The study concludes that the methanolic seed extract of plant possesses potent antibacterial property and justifies the need for further investigations and characterization of the bioactive compounds present in it.

KEY WORDS

Antibacterial activity, Cup-plate method, Memecylon umbellatum, Secondary Metabolites.

INTRODUCTION

Infectious diseases are the most primitive types of diseases which challenge the survival of human beings. In treating such infections, which are mainly caused by microorganisms such as bacteria, human beings have identified the use of different herbs since ancient times. This practice, which evolved through a long process of trial and error, still passes from generation to generation. Moreover, over 50% of all modern clinical drugs are of natural product in origin. For example, many of the drugs currently used to treat bacterial and other infections were first isolated from natural sources including ethnomedicinal plants. Plant metabolites are proved to be the most important group of compounds with wide range of antimicrobial activity. According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [1].

For the past five decades, the need for new antibiotics has been met largely by screening of natural product scaffolds discovered in the middle of the 20th century. More recently, however, advances in technology have sparked resurgence in the discovery of herbal antibiotics [2]. Antibiotics are used to cure bacterial illnesses as well as infections. Since the discovery of antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious diseases [3].

The use of plant extracts and phytochemicals both, with known antimicrobial properties, can be of great significance in therapeutics. In the last few years, a number of studies have been conducted in different countries to prove such efficiencies. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known as their active substances, for example, the phenolic

compounds which are part of the essential oils, as well as tannins [4].

Memecylon umbellatum Burm (Family: *Melastomataceae*) is small evergreen shrub or tree having young teeter branches and bears numerous umbellate cymes. The plant is known as *"Anjani"* in Sanskrit and *"Ironwood tree"* in English. Plants are distributed mostly in coastal regions of the Deccan peninsula, the eastern and southern part of India all along Western Ghats and in the Andaman islands. The leaves have been reported to possess astringent properties and are given to treat leucorrhoea and gonorrhea. Lotion prepared from leaves is used to treat eye troubles. The decoction of the root is used in the treatment of excessive menstrual discharge [5, 6].

In Ayurveda, the leaves are used as a cooling astringent; used in conjunctivitis as a lotion; and given internally in leucorrhoea and gonorrhea. A lotion prepared from leaves is used to treat eye troubles. The leaves are reported to possess antiviral activity. Traditionally the leaves and barks are applied to bruises. The leaves yield a yellow dye, which gives beautiful light shades, used in dyeing industry. The leaves were also reported to be used in snakebite [7]. The fruits are pulpy, astringent and edible. The paste of leaves is also used in the treatment of herpes. The decoction of roots is used for the treatment of menorrhagia and other genital dysfunction. The leaf powder is even used for the treatment of diabetes. The seeds are used to cure cough and found to have sedative effect [8]. The literature survey revealed that, no reports or papers are available on antibacterial activity of seeds of *Memecylon umbellatum Burm.,* hence the present research work seems to be of enormous contribution.

Phytoconstituents:

Memecylon umbellatum Burm. plant contain wide variety of phytoconstituents which are useful in the treatment of different ailments, which include umbelactone (4-hydroxymethyl-3-methyl-but-2-ane-4,7-oide), β-amyrin, sitosterol, its glucoside, tartaric, maleic, oleanolic and ursolic acid [1].

Pharmacological Actions [8]**:**

1) Hypoglycemic Activity-

The leaves and the roots of *Memecylon umbellatum* were screened for hypoglycemic activity. Oral administration of an alcoholic extract of the leaves of plant showed a significant lowering of serum glucose levels in normal and alloxan induced diabetic mice.

2) Antimicrobial Activity-

There are reports regarding the antibacterial activity of leaves extracts of the plant. The plant was also found to be active against ranikhet virus. The antimicrobial activity of another species *M. malabaricum* was also reported.

3) Wound Healing Activity-

The plant was selected and successfully evaluated for its wound healing potential using alcoholic leaves extracts in the form of ointment.

4) Anthelmentic and Anti-insect Activities-

Different extracts of *Memecylon umbellatum* root were screened for anthelmentic and anti-insect activities using Indian earthworms (*Pheretima posthuma*) and red flour beetle (*Tribolium castaneum*) respectively.

5) Antispasmodic Activity-

The antispasmodic activity of the ethanol extract of the plant roots were assessed in vitro using rat ileum.

6) Anti-inflammatory Activity-

The ethanolic extract of the roots of plant was evaluated using acute rat model by carrageenan induced rat paw oedema and a sub-acute rat model by cotton pellect induced granuloma.

7) Nephroprotective Activity-

The ethanolic extract of plant roots was investigated for nephroprotective activity against cisplatin induced acute renal damage in rats.

8) Anti-pyretic Activity-

The ethanolic extract of plant roots was subjected for anti-pyretic activity in yeast induced pyrexia model in rats. The results obtained, showed a significant and dose dependent reduction in yeast induced hyperpyrexia in rats when compared to the standard drug paracetamol.

9) Hepatoprotective Activity-

The leaves of the plant were also screened for hepatoprotective activity, and found to possess significant hepatoprotective activity too.

MATERIALS AND METHODS

1) Collection of Plant Material:

The seeds were collected from fully ripped, fresh fruits of *Memecylon umbellatum* from nearby area of

Satara, Maharashtra during the month of September and authenticated at Department of Botany, Y. C. Institute of Science, Satara, (MS) India. The obtained seeds were sundried and powdered using electric blender so as to obtain fine powder; which was subjected for extraction process.

2) Extraction of Plant Material:

The well dried, fine powder of *Memecylon umbellatum* seeds was extracted successively using methanol (at about 60-80^{0}C temperature) in a 'Soxhlet Extraction Assembly'. After completion of extraction the solvent was evaporated so as to obtain crude residue of methanolic extract; that was then subjected to preliminary phytochemical screening.

3) Preliminary Phytochemical Screening [9]:

All the dried extract residues were dissolved in small quantities of respective solvents as mentioned in standard qualitative tests and screened for phytoconsituents using standard reagents and indicators. The results obtained for preliminary phytochemical screening are given in **Table No. 2** in results and discussion.

4) Dilution of Extract:

The extract of seed was then diluted using dimethyl sulfoxide (DMSO) solution. Different dilutions of concentrations such as 10 mg/ml, 15 mg/ml, 20 mg/ml and 25 mg/ml were prepared as test solutions. Ciprofloxacin (15 mg/ml) was used as standard and DMSO was used as control.

5) Antibacterial Activity Assay [10, 11]:

The potency and activity of antibacterial agents is usually determined by zone of inhibition they produce when they act upon bacteria grown on agar plates. Antibacterial activity of seed extract was determined by cup plate method using nutrient agar medium plates prepared aseptically by following procedure as per formula given in **Table No. 1** [12].

Table No. 1: Formula for Nutrient Agar Medium.

Name of ingredient	Quantity
Peptone	1 gm
NaCl	0.5 gm
Beef extract	0.3 gm
Agar-agar	2.5 gm
Distilled water	100 ml
pH	7-7.5

Procedure-

1) All ingredients were accurately weighed as per given proportion.

2) Ingredients were then dissolved in distilled water except agar-agar by gentle heating and constant stirring.

3) Desired pH was then adjusted by adding dilute acid or alkali and lastly agar-agar was added and dissolved.

4) The conical flask was plugged and sterilized in an autoclave.

5) After sterilization, about 20 ml of medium was aseptically poured in each Petri plate, and kept at room temperature for cooling.

6) Preparation of Saline Solution:

The 100 ml of saline was prepared by dissolving 0.9 gm of sodium chloride in 100 ml distilled water. Prepared saline solution was sterilized before preparation of microbial suspensions at 121 ^{0}C for 15 min. in an autoclave.

7) Preparation of Bacterial Suspension [5]: Microbial suspensions were prepared by transferring one loop full of stock culture of respective microorganisms to 10 ml of normal saline solution. All this procedure was conducted in the laminar air flow.

8) Sterilization of Equipments and Media [5]:

Dry Heat Sterilization: All the glass wares previously washed were sterilized in hot air oven. Petri-dishes, pipettes, test tubes were wrapped separately in the paper and kept in the hot air oven for sterilization at 180^{0}C for 1 h.

Moist Heat Sterilization: Normal saline solution and nutrient media were sterilized in autoclave at 121 ^{0}C for 15 min.

9) Spreading of Bacterial Suspension:

For determination of antibacterial activity many gram-positive and gram-negative organisms were used. With the help of sterile glass spreader the suspension was spread on the plates uniformly under aseptic conditions.

10) Preparation of Wells:

Wells were prepared with the help of sterile cork borer having a size of 8 mm diameter.

11) Addition of Solution in Wells [11]:

Different concentrations of extract were added in respective wells, generally 0.1 ml of sample was added in each well and plates were kept for diffusion in refrigerator for 30 min. Later plates were kept for

incubation in an incubator at 37 ^{0}C for 24 h. After incubation antibacterial activity was determined by measuring the zone of inhibition around each well.

RESULT AND DISCUSSION

The secondary metabolites present in the test plant *Memecylon umbellatum* are tannins and phenolic compounds, fats and oils. By virtue of vast literature review and phytochemical screening it is concluded that the presence of one or more of these secondary metabolites is accountable for the antibacterial activity of methanolic seed extracts.

The antibacterial efficacy of methanolic extract of seeds of plant showed varied level of inhibition against human pathogenic bacteria (such as *Escherichia coli, Salmonella typhi, Bacillus subtilis, Klebsiella pneumonia and Staphylococcus aureus*) as shown in **Figure No.1**. The activity of extract of seeds was compared with standard drug Ciprofloxacin. The results of preliminary phytochemical analysis and antibacterial activity of methanolic seed extract are given in **Table No. 2 and Table No. 3** respectively.

Table No. 2: Phytochemical Screening of *Memecylon umbellatum* Seed Extract.

Sr. No.	Secondary Metabolite	Inference**
1	Alkaloids	-
2	Glycosides	-
3	Tannins and Phenolic Compounds	+
4	Fats and Oil	+
5	Flavonoids	-

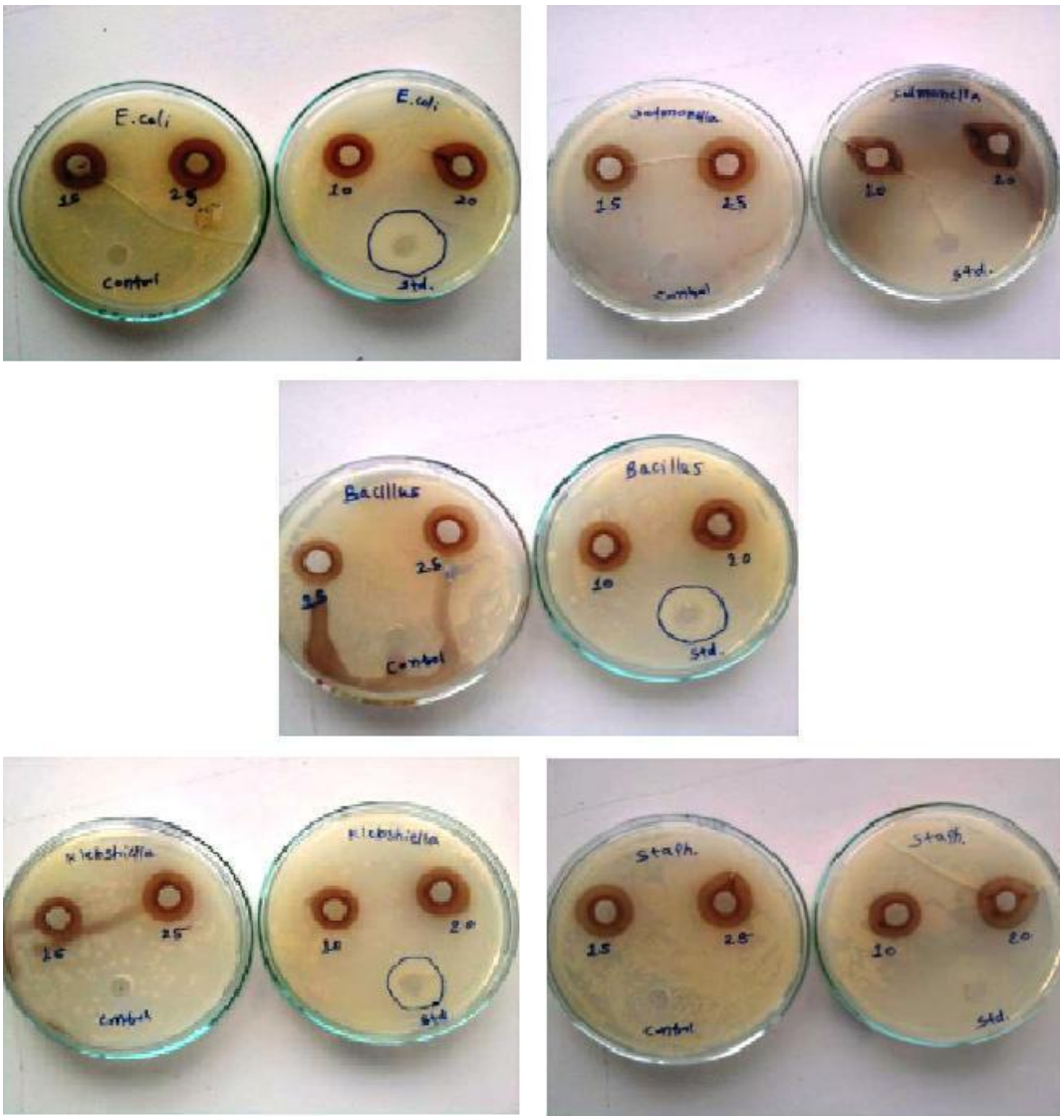
** **(+) Present, (-) Not detected**

Table No. 3: Effects of *Memecylon umbellatum* Seed Extract on Test Bacteria at Different Concentrations.

Sr. No.	Concentration	Zone of Inhibition (mm)				
		Bacterial Test Organism Used				
		Escherichia coli	*Salmonella typhi*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
1	10 mg/ml	19	15	19	17	18
2	15 mg/ml	20	19	17	17	20
3	20 mg/ml	19	18	20	20	21
4	25 mg/ml	21	22	20	21	20
5	Std. (Ciprofloxacin) 15 mg/ml	21	-	23	21	-
6	Control (DMSO)	-	-	-	-	-

'- Denotes no zone of inhibition'

Figure No. 1: Zone of Inhibition of *Memecylon umbellatum* Seed Extract on Different Test Bacteria.



CONCLUSION

The potential for developing antimicrobials from higher plants appears rewarding nowadays as it leads to the development of new drugs which is need of hour. The results obtained from this research study not only support the use of *Memecylon umbellatum* plant since from ancient era but also exposed a way for developing novel anti-infective phyto-formulations. As present work is the first report about the antibacterial activity of methanolic seed extract, further research is needed to find the active components within test plant along with their full spectrum of efficacy. However, the present study of in vitro antibacterial activity forms primary platform for further phytochemical and pharmacological studies.

ACKNOWLEDGEMENTS

We express our sincere thanks to Prof. (Dr.) S. P. Gawade, Dr. A. S. Kulkarni and Dr. N. H. Aloorkar of Satara College of Pharmacy, Satara for their support and guidance throughout the research work. Authors also pay a profound gratitude to Mr. R. Khutale for timely help and laboratory attendants of Satara College of Pharmacy for their unstinted co-operation.

REFERENCES

1) Basha et al. *Preliminary phytochemical screening and evaluation of antimicrobial potential of Memecylon*

umbellatum Burm (Melastomataceae) aerial parts. Pharmacologyonline, *1*: 174-184, (2011).

2) Clardy J., Fischbach M. A., Walsh C. T. *New antibiotics from bacterial natural products.* Nature Biotechnology, *24* (12): 1541, (2006).
3) Khan R., Islam B., Akram M., Shakil S., Ahmad A., Manazir S., Siddiqui M., Khan A. *Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin.* Molecules, *14*: 586-597, (2009).
4) Gislene G. F. Nascimento et al. *Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria.* Brazilian Journal of Microbiology, *31*: 247-256, (2000).
5) Killedar S. and More H. *Screening of antimicrobial potential and phytoconsituents for different extracts of Memecylon umbellatum Burm inflorescences.* Asian J. Pharm. Res, *1* (4): 114-118, (2011).
6) Kirtikar K. R. and Basu B.D. Indian Medicinal Plants. Popular Prakashan, New Delhi: 1063-66, (1996).
7) Joshi H. et al. *Analgesic potential of the roots of Memecylon umbellatum (Burm).* International Research Journal of Pharmacy, *1* (1): 395-400, (2010).
8) Joshi H., Gururaja M. P. *Memecylon umbellatum (Melastomataceae): A review.* International Journal of Pharmaceutical Sciences Review and Research, *11* (2): 54-58, (2011).
9) Khandelwal K. R. Practical Pharmacognosy - Techniques and Experiments. 29th edn, Nirali Prakashan, Pune: 25.1-25.6, (2010).
10) Jayalakshmi B., Raveesha K. A. and Amruthesh K. N. *Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria.* Journal of Applied Pharmaceutical Science, *1* (5): 124-128, (2011).
11) Suryawanshi J. S., Belhekar S. N., Pachhade A. P. and Dias R. J. *Antibacterial potential of Canavalia virosa seed extracts.* Indian Journal of Natural Products, *27* (2): 14-15, (2011).
12) Kokare C. R. Pharmaceutical Microbiology. 2nd edn, Career publication: 58-65, (2008).

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