PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL POTENTIAL OF *MEMECYLON UMBELLATUM BURN (MELASTOMATACEAE)* AERIAL PARTS

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Summary

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings. In the present study aqueous and ethanol extracts of *Memecylon umbellatum Burn* were investigated for *in vitro* antimicrobial activity by agar well diffusion method against bacterial organisms like *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeuroginosa, Klebsiella pneumoniae* and *Escherichia coli* and fungal strains such as *Aspergillus niger, Aspergillus flavus* and *Candida albicans* at the concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml and 100 µg/ml. The susceptibility of the microorganisms to the extracts was compared with each other and with standard antibiotics Ciprofloxacin and Nystatin. The preliminary phytochemical analysis of the extracts revealed the presence of phytoconstituents like phytosterols, terpenoids, glycosides, tannins and flavonoids. The ethanol extract showed significant antimicrobial activity, whereas aqueous extract showed moderate activity against the tested bacterial and fungal organisms as compared to standard drugs. The results of this study also supports the use of this plant *Memycylon umbellatum Burn* for human and animal disease therapy and reinforce the importance of the ethno botanical approach as a potential source of new bioactive substances.

KEY-WORDS: *Memycylon umbellatum Burn*, Antibacterial activity, Antifungal activity, Agar well diffusion.

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Introduction

Infectious diseases are the most primitive types of diseases which challenge the survival of human beings (1). In treating such infections, which are mainly caused by microorganisms such as bacteria (2), human beings have identified the use of different herbs since ancient times (3). This practice, which evolved through a long process of trial and error, still passes from generation to generation (4, 5). Moreover, over 50% of all modern clinical drugs are of natural product in origin (6). For example, many of the drugs currently used to treat bacterial and other infections were first isolated from natural sources including ethnomedicinal plants (7). Plant metabolites are proved to be the most important group of compounds with wide range of antimicrobial activity (8). According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs (9).

Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance (especially multiple) has increased astronomically (10) in such cases antibiotics are of little help. Moreover in many cases antibiotics produce adverse side effects and allergy. Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry (11). They may provide natural source of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally. Biologically active compounds from natural source have always been of great interest to scientist working on infectious diseases. Numerous useful drugs from higher plants have been discovered by following up ethno-medical uses. The diversity of plants growing in India, along with their known ethno pharmacological uses, offers an enormous possibility of finding novel structures with antimicrobial properties.

Memycylon umbellatum Burn (Family - *Melastomataceae*) commonly known as Iron wood tree (English) Alli and Puvai (Tamil), is a large ornamental shrub or a small tree found mostly in the coastal regions of the Deccan peninsula, the eastern and southern parts of India and in the Andaman islands (12). The leaves have been reported possess astringent properties and are given to treat leucorrhoea and gonorrhoea, a lotion prepared from the leaves is used to treat eye troubles (13). The leaves are also reported to possess antiviral activity (14). The leaves and barks are applied to bruises. It contains wide variety of phytoconstituents which are useful in the treatment of different ailments and include umbelactone (4-hydroxymethyl-3-methyl-but-2-ane-4,7-oide), β-amyrin, sitosterol, its glucoside, tartaric, maleic, oleanolic and ursolic acid (15, 16).

Memecylon umbellatum Burn contains ursolic acid (3-alpha hydroxyl-uro-12-en-oic acid) and isomer of oleanolic acid, is a triterpenoid compound exhibits broad spectrum of pharmacological properties such as hepatoprotective (against carbon

tetrachloride, D-galactosamine induced liver injury and acetaminophen induced cholestasis), analgesia, anti inflammatory, antiviral, antimutagenic, antihyperlipidemic, antiulcer, anticarcinogenic and antiarthritic activity (17). Though the plant has been reported for many biological activities, it has very little report against antimicrobial activity. A survey of literature revealed that no systematic approach has been made to study the antimicrobial activity of this plant. Hence the present study was carried out to evaluate the *in vitro* antimicrobial activity of aqueous and alcoholic extracts of aerial parts of *Memycylon umbellatum Burn* against nine pathogenic microorganisms that cause the most common cases of infectious diseases.

Materials and Methods

Plant material

Fresh aerial parts of *Memycylon umbellatum Burn* was collected from Pacchamalai hills, Trichirapalli district, Tamilnadu, India during the month of March-April 2007. The identity of the leaves has been confirmed by using all official monographic specifications. Voucher samples were prepared and deposited in the Herbarium of the Pharmacognosy Department, Trichy College of Pharmacy, Trichy, India, for reference. Plant parts were dried under shade, pulverized by an electric blender and passed through 40 mesh sieve. It was stored under an airtight container, away from sunlight at room temperature and used for the extraction.

Chemicals used

Ciprofloxacin, Nystatin, Molten Muller Hinton (MH) agar, Nutrient broth and Sabouraud Dextrose Agar (SDA) were obtained from Hi-Media laboratories, Mumbai. All other chemical were of analytical grade and obtained locally.

Preparation of extract

The powdered aerial parts of Memycylon umbellatum burn (500 g) was extracted with ethanol (95%) and double distilled water separately in a soxhlet extractor. The extract was evaporated to dryness at 60ºC under reduced pressure in a rotary evaporator and kept in refrigerator at 4ºC till used. The extracts were dissolved in dimethyl-sulphoxide to make the final concentrations at the time study.

Preliminary phytochemical screening

A small portion of the dry extracts were subjected to preliminary phytochemical screening to detect the presence of various phytoconstituents present in the aerial parts of *Memycylon umbellatum Burn* (18, 19).

Microorganisms used

Clinical strains of six human pathogenic bacteria made up of 3 gram positive (*Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus*) and 3 gram negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were used for the antibacterial assay. While for the antifungal assay, one yeast (*Candida albicans*) and two molds (*Aspergillus niger* and *Aspergillus flavus*) were used for the studies. These organisms were obtained from the department of biotechnology, Mohamed Sathak A. J. College of Pharmacy, Chennai. The purity of the cultures prior to their use was checked by conventional cultural, morphological and biochemical methods. The bacterial and fungal cultures were maintained and stored in nutrient and sabourauds dextrose agar medium at 4ºC.

Preparation of the tested organisms

A) Preparation of standard bacterial suspensions

The average number of viable *Bacillus subtilis*, *Bacillus cereus, Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique (20). About (10^8-10^9) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

B) Preparation of standard fungal suspensions

The average number of viable *Aspergillus Niger, Aspergillus flavus* and *Candida albicans* organisms per ml of the stock suspensions was determined by same method as described above except the media used was Saboraud dextrose broth instead of nutrient broth in case of bacterial organisms and the incubation temperature was 25ºC instead of 37 ºC.

In vitro testing of extracts for antimicrobial activity

Testing for antibacterial activity

The cup-plate agar diffusion method was adapted according to Kavanagh (21), to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (10^8-10^9) colony- forming units per ml was thoroughly mixed with 60 ml of melted and cooled (45-50ºC) sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to

set and in each of these plates 6 cups, 10 mm in diameter, was cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of 1000 µg/ml, 500 µg/ml, 250 µg/ml and 100 µg/ml of each of the extracts were filled in to the wells with the aid of Pasteur pipettes and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Triplicates of tests were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as negative controls. Ciprofloxacin was used as positive standard for antibacterial studies. After incubation the diameters of the results and growth inhibition zones were measured, and the mean ± SD values were tabulated.

Testing for anti-fungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25ºC for two days for the *Candida albicans* and three days for *Aspergillus niger and Aspergillus flavus*. Nystatin was used as positive standard for antifungal studies. All the tests were carried out by triplicate.

Statistical Analysis

All the data's were expressed as mean ± SD (standard deviation) and statistical significance was evaluated by one way ANOVA using SPSS software version 18. *P-value* of <0.05 was considered statistically significant.

Results

The percentage yield for ethanol and aqueous extracts were found to be 27.90% and 17.5%w/w. The highest yield of extractable substances was found in ethanol, whereas the lowest yield of extractable solids was found with aqueous solvent. Phytochemical screening of aqueous and ethanol extracts exhibited different kinds of secondary metabolites.

Table 1 shows the results of phytochemical screening, which indicated the presence of phytosterols, terpenoids, flavonoids, tannins and glycosides with the highest concentration were found in ethanol compared to aqueous extracts. **Table 2** and **Table 3** revealed the restricted growth of bacterial and fungal organisms against aqueous and ethanol extracts of *Memecylon umbellatum Burn* tested at 1000, 500, 250 & 100 µg/ml. It revealed that the ethanol extract has produced a significant antibacterial effect and the aqueous extract has produced a moderate antibacterial effect, whereas both the extracts produced less significant antifungal activity and the observed antimicrobial activity of the ethanol and aqueous extracts of aerial parts of *Memecylon umbellatum Burn* is comparable with the standard antimicrobial agents like Ciprofloxacin and Nystatin.

Table 1. Preliminary phytochemical screening of *Memecylon umbellatum*

S. No	Phytoconstituents	Ethanol extracts	Aqueous extracts
1	Alkaloids	-	-
2	Glycosides	+	+
3	Terpenoids	+++	+
4	Carbohydrates	+	++
5	Proteins	-	-
6	Steroids	+	+
7	Flavonoids	+++	++
8	Phenols	++	+
9	Tannins	++	+
10	Quinones	-	-
11	Saponins	-	++
12	Resins	-	+
13	Fixed oil and fats	-	-
14	Volatile oils	-	-

Note: + ve indicates positive results, whereas – ve indicates negative results.

(-) Absent, (+) slightly present, (++) fairly present and (+++) Abundant

Table 2. Antimicrobial activities of aqueous extracts of *Memecylon umbellatum Burn*

S. No	Concentration (µg/ml)	Zone of restricted growth (mm)					
		Bacterial test organism used					
		S. aureus	***B. Subtilis***	***B. cereus***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
1	**1000**	13.7±0.58	21.0±0.00	20.0±1.00	21.7±0.58	19.0±0.00	15.7±0.58
2	**500**	10.0±1.0	19.0±1.00	17.7±0.58	18.3±1.15	15.3±0.58	12.0±1.00
3	**250**	9.7±0.58	15.0±1.00	14.7±1.15	12.7±1.53	12.3±0.58	12.0±0.00
4	**100**	7.3±0.58	11.0±1.00	12.3±0.58	7.3±1.15	4.7±0.58	-
5	**Ciprofloxacin (50 µg/ml)**	18.3±0.58	20.3±0.58	21.7±0.58	20.7±0.58	18.3±0.58	20.0±1.00
		Fungal test organism used					
		A. niger		***A. flavus***		***C. albicans***	
1	**1000**	15.3±0.58		10.3±0.58		9.7±0.58	
2	**500**	10.7±0.58		8.3±1.15		7.3±1.15	
3	**250**	8.00±1.00		7.3±1.53		-	
4	**100**	-		-		-	
5	**Nystatin (10 µg/ml)**	24.3±0.58		21.7±0.58		21.0±0.00	

Values are expressed as mean ± SD, (-) indicates no inhibition zone

Table 3. Antimicrobial activities of Ethanol extracts of *Memecylon umbellatum Burn*

S. No	Concentration (µg/ml)	Zone of restricted growth (mm)					
		Bacterial test organism used					
		S. aureus	***B. Subtilis***	***B. cereus***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
1	**1000**	21.7±0.58 [b, c]	25.3±0.58 [b, c]	25.0±0.58 [b, c]	22.0±0.00 [a]	20.7±0.58 [c]	15.7±0.58
2	**500**	18.3±0.58 [c]	21.3±1.15	20.7±0.58 [c]	18.0±0.00	15.3±0.58	12.0±1.00
3	**250**	15.0±1.00 [c]	17.0±1.00	17.3±0.58 [d]	14.3±0.58	11.0±1.00	12.0±0.00
4	**100**	12.0±0.00 [c]	13.3±0.58 [d]	12.7±0.58	11.0±1.00 [d]	9.7±0.58 [c]	8.7±0.58 [c]
5	**Ciprofloxacin (50 µg/ml)**	18.3±0.58	20.3±0.58	21.7±0.58	20.7±0.58	18.3±0.58	20.0±1.00
		Fungal test organism used					
		A. niger		***A. flavus***		***C. albicans***	
1	**1000**	18.7±0.58 [c]		18.3±0.58 [c]		16.3±0.58 [c]	
2	**500**	14.0±1.00 [c]		12.0±0.00 [c]		10.7±0.58 [c]	
3	**250**	9.7±0.58		8.0±0.00		-	
4	**100**	5.00±0.00 [c]		3.7±0.58 [c]		-	
5	**Nystatin (10 µg/ml)**	24.3±0.58		21.7±0.58		21.0±0.00	

Values are expressed as mean ± SD, (-) indicates no inhibition zone, [a] indicates $p<0.05$ comparison with standard, [b] indicates $p<0.01$ comparison with standard. [c] indicates $p<0.01$ comparison with Aqueous extracts, [d] indicates $p<0.05$ comparison with Aqueous extracts.

Discussion

Plants serve as vast source for varied phytoconstituents exhibiting varied pharmacological property. Identifying such potential plants is of significance in medicine. In this connection, in the present study the aerial parts of the plant *M. umbellatum Burn* was examined.

Secondary metabolites have proven to be medicinal in nature. They have various protective and therapeutic effects which prevent diseases and maintain a state of well being (22). Phytochemical screening results of aqueous and ethanol extracts revealed that they contain therapeutically essential secondary metabolites, which could account for the antibacterial activity of the plants. The phytochemical analysis of aqueous and alcoholic extracts revealed the presence of phytosterols, terpenoids, flavonoids, tannins and glycosides. These compounds are known to be biologically active. Tannins have been found to form irreversible complexes with proline-rich proteins (23) resulting in the inhibition of the cell protein synthesis. This activity was exhibited against test organisms with the two plant extracts. Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the treatment of inflamed or ulcerated tissues (24). Tannins have important roles such as stable and potent antioxidants (18). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (25), thus exhibiting antimicrobial activity.

It is not surprisingly that there are differences in the antimicrobial activities of the different extracts of tested plant; this could be due to the differences in concentrations of phytoconstituents between them. Among the test microorganisms used, *E. coli, B. cereus* S. *aureus, B. subtilis and K. pneumoniae* were the most susceptible bacteria to all plant extracts. On the contrary, *P. aureginosa* and *C. albicans* were the most resistant microorganisms.

The results from this study demonstrated that the plants have antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis. Staphylococcus aureus* is the causative agent of most skin infection and septicaemia. *Pseudomonas aeruginosa* is known to cause burn wound infection and urinary tract infection (26). *Bacillus subtilis* occasionally produces disease such as meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastro-enteritis in immunocompromised patients (27). Moreover *Staphylococcus aureus* and *Pseudomonas aeruginosa* were found to be the major isolates from ear discharges (28).

Hence from the study it is concluded that the aerial parts of *Memecylon umbellatum Burn* has significant antimicrobial effect and this could lead to the discovery of new antibiotics. The results of this study also support the use of these plants for human and animal disease therapy and reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances. On the basis of results obtained in the present investigation, it is possible to conclude that the ethanol extracts of *Memecylon umbellatum Burn* has significant antimicrobial activity compared to aqueous extracts.

Conclusion

All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these plants were more effective than traditional antibiotics to combat the pathogenic microorganisms studied. The chance to find antimicrobial activity was more apparent in ethanol than water extracts of the same plants.

Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity and to further evaluate the mechanisms of action of extracts on some organisms associated with human diseases. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms.

The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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