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Phytochemical screening and Antimicrobial activity of the leaves of *Memecylon umbellatum* burm. F.

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ABSTRACT

An ethnomedicinal plant, *Memecylon umbellatum* Burm. f., was investigated for preliminary phytochemical screening and antimicrobial activity. Preliminary phytochemical screening of various extracts of the leaves revealed the presence of various classes of compounds such as amino acids, carbohydrates, flavonoids, gum, oil & resins, proteins, phenolic groups, saponins, steroids, tannins and terpenoids. Bioassay of antimicrobial activity of leaves of petroleum ether, chloroform and ethanol extracts showed significant activity against the human pathogens such as *Streptococcus pneumoniae* causing brain abscesses, pneumonia and septic arthritis, *Proteus vulgaris, Pseudomonas aeruginosa* causing urinary tract infections and the fungus *Candida albicans*. The antimicrobial activity of the petroleum ether, chloroform and ethanolic leaf extract showed concentration-dependent activity against all the tested bacteria with the zone of inhibition at various concentrations. Thus the findings revealed the medicinal potential of *Memecylon umbellatum* against various infectious diseases to develop a drug.

Keywords: Ethnomedicine, *Memecylon umbellatum*, phytochemistry, antimicrobial activity, human pathogens, drug development

INTRODUCTION

WHO (2001) estimated that 80% of world population rely on medicinal plants for their primary health care needs. Out of the 3,50,000 plant species known so far, about 35,000 (some estimate up to 70,000) are used worldwide for medicinal purposes and less than about 0.5% of these have been investigated for their phytochemical and pharmacological potential (Hostettmann and Marston, 2002). This green inheritance thus represents an enormous reservoir of putative lead compounds to be discovered for various diseases. Plants are important sources of medicines and at least 25% of the prescription drugs issued in the USA and Canada contain bioactive compounds that are derived from or modeled after plant natural products (Farnsworth, 1984). Medicinal plants would be the best source to obtain a variety of drugs and therefore such plants should be investigated to understand better about their properties, safety and efficacy (Nascimento et al., 2000). Medicinal plants are major sources of obtaining antimicrobial drugs (Sofowora, 1986). The genus Memecylon L., (family: Melastomataceae) comprises of about 300 species in the world, of which 30 species has been reported from India (Santapau and Henry, 1974; Henry et al., 1989) and 16 species from Tamil Nadu state (Nair and Henry, 1983). The species Memecylon umbellatum Burm.f., is an ethnomedicinal plant used traditionally for treating various diseases. Ethnomedicinally, leaves are used to treat eye troubles, gonorrhea, leucorrhea and wounds (Anonymous, 1998; Dhar et al., 1968; Puratchikodi and Nagalakshmi, 2007), treatment of bone fracture, herpes (Rajakumar and Shivanna, 2009), diabetes (Grover et a., 2002; Ayyanar et al., 2008; Akanksha and Maurya, 2009), skin diseases (Karuppasamy, 2007), snake bite (Kshirsagar and Singh, 2001). Chemical constituents such as umbelactone (4-hydroxymethyl-3-methyl-but-2ane-4,7-olide), amyrin, sitosterol, tartaric acid, malic acid, oleanolic acid, urseolic acid (Asolklar et al., 1956; Ram and Mehrotra, 1993), tannins (Killedar and More, 2010) were reported. Biological activity such as anti-diabetic (Amalraj and Ignacimuthu, 1998; Grover et al., 2002), anti-viral (Dhar et al., 1968; Anonymous, 1998), and wound healing activity (Puratchikodi and Nagalakshmi, 2007) were reported. After scrutiny of published literature, so far no sufficient work has been done regarding the antimicrobial activity on this selected plant. The active principles of many drugs found in plants are secondary metabolites. Hence the basic phytochemical investigation on the extracts for their main phytocompounds is very vital. Hence in the present study the hexane, chloroform and ethanol extracts of the leaves of Memecylon umbellatum Burm.f., were screened for phytochemical constituents and the antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Plant Material and preparation of the Extracts

The leaves of Memecylon umbellatum Burm.f., were collected from Jamunamaruthur, Javadu Hills, Tiruvannamalai District, Tamil Nadu. The collected plant material was botanically identified and confirmed by Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The herbarium specimens were preserved and submitted to Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College, Thanjavur District, Tamil Nadu for further reference (Voucher no. ACT57). The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powders were then subjected to successive extraction with organic solvents such as hexane, chloroform and ethanol by Soxhlet method. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed in vacuo and stored at 4°C. They were used for preliminary phytochemical screening and antimicrobial activity. The graded concentrations (100, 50, 25 and 12.5mg/ml) of different extracts were prepared for the bioassay.

Phytochemical Screening

Phytochemical analysis of the different plant extracts was performed using the methods described (Trease and Evans, 1983; Harbourne, 1998).

Test Organisms

All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh. These microbes include the Gram-negative bacteria such as *Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhi* (MTCC 733), *Vibrio parahaemolyticus* (MTCC 451) and *V. vulnificus* (MTCC 1145); the Gram-positive bacteria such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and *Streptococcus pneumoniae* (MTCC 655)

and fungi such as *Aspergillus flavus* (MTCC 277), *A. fumigatus* (MTCC 343), *A. niger* (MTCC 1344) and *Candida albicans* (MTCC 227) respectively.

Bioassay for antimicrobial activity

Agar well-diffusion method by Perez et al. (1990) was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8h old - broth culture of respective bacteria and fungi. Two wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2h. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for fungal pathogens. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The results of preliminary phytochemical screening were given in the Table 1. Flavonoids, phenolic groups, steroids and terpenoids were present in all the extracts. aminoacids, proteins and saponins were present in petroleum ether extract but absent in chloroform and ethanol extracts. Carbohydrates were present in petroleum ether and chloroform extracts. Anthraquinones, catechins, coumarins, gum, oil and resins, quinones, and tannins were absent in all the three tested extracts.

Table 1 Prelimi	nary phytochemical	screening of	various	extracts	of
the leaves of Men	necylon umbellatum	Burm.f.			

Phytoconstituents	nstituents Leaves extra						
	Petroleum ether	Chloroform	Ethanol				
Alkaloids	-	-	-				
Amino acids	+	-	-				
Anthraquinones	-	-	-				
Carbohydrates	+	+	-				
Catechins	-	-	-				
Coumarins	-	-	-				
Flavonoids	+	+	+				
Gums, oils and resins	-	-	+				
Proteins	+	-	-				

Phenolic groups	+	+	+		
Quinones	-	-	-		
Saponins	+	-	-		
Steroids	+	+	+		
Tannins	-	-	+		
Terpenoids	-	+	+		
+ = present ; - = absent					

The results of antimicrobial activity (Table 2) of the petroleum ether, chloroform and ethanol extract of leaves of Memecylon umbellatum Burm.f., showed concentration-dependent activity against all the tested bacteria with the zone of inhibition ranged from 10-24mm at various concentrations. Only ethanol extract showed antimicrobial activity against the fungi A. flavus and C. albicans with the zone of inhibition ranged from 14-21mm at various concentrations. Petroleum ether extract showed more antimicrobial activity against the gram-negative bacteria than the chloroform extract. The zone of inhibition recorded was ranged from 11-24mm against gram-negative bacteria. The solvents used for extraction were used as control and all the solvent control did not show any activity. Standard antibiotics were also used along with the extracts for comparison as given in the Table 2. Petroleum ether extract showed the maximum zone of inhibition ranged from 19 to 24mm against gram-negative bacteria such as 24mm against P. vulgaris, 22mm each against P. aeruginosa and S. typhi, 19mm against V. vulnificus at 100mg/ml concentration. Chloroform extract showed the maximum zone of inhibition as 20mm each against E. coli and V. parahaemolyticus at 100mg/ml concentration. Ethanol extract showed the maximum zone of inhibition ranged from 19 to 24mm against gram-negative bacteria such as 24mm each against S. typhi and V. parahaemolyticus and 22mm each against E. coli and P. vulgaris 100mg/ml concentration and against the fungi such as 20mm against A. flavus and 21mm against C. albicans at 100mg/ml concentration respectively.

From the results of antimicrobial activity, it was found that the petroleum ether and ethanol extracts exhibited maximum antimicrobial activity against the tested human pathogens. In our study, the maximum zone of inhibition against gram negative bacteria such as E. coli, P. vulgaris, P. aeruginosa, S. typhi and V. parahaemolyticus and against the fungi such as A. flavus and C. albicans might be attributed to the presence of secondary metabolites such as flavonoids, phenolic groups and steroids as suggested by previous reports (Kosalec et al., 2005; Pereira et al., 2007; Lauro Figueroa et al., 2008. The significant activity of the results of ethanol extract against the fungi, A. flavus and Candida albicans provides additional confirmation to the phenolic compounds and steroidal compounds which are more effective in higher concentration inhibited the growth of all fungi (Winkelhausen et al., 2005; Subhisha Subramoniam, 2005; Pereira et al., 2007). Even in hospitals, majority of disinfectants such as phenols, lysol, cresols used are belonging to phenolic groups. Thus recent findings of antimicrobial activity against P. aeruginosa, P. vulgaris, S. typhi, V. parahaemolyticus and V. vulnificus revealed the medicinal potential value of petroleum ether and ethanol extracts against abdominal pain, diarrhea, fever, nausea,

septicaemia, urinary tract infections and vomiting, hospitalacquired wound infections, septicaemia and urinary tract infections by *P. vulgaris* and *P. aeruginosa*, typhoid fever by *S. typhi* and diarrheal infections by *Vibrio* species, skin related diseases by *C. albicans* and Aspergillosis and respiratory tract infections by *A. flavus* respectively.

CONCLUSION

Thus from our findings, it is concluded that the bioactive principles responsible for the antimicrobial activities against these tested microorganisms should be isolated identified and elucidated its structure to develop a new lead of therapeutic interest to cure various human ailments.

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Test Microorganisms	Petroleum ether (mg/ml)		Chloroform (mg/ml)		Efhanol (mg/ml)		5	Standard drug (10 µg/ml)		
)			
	100	50	25	100	50	25	100	50	25	
Gram-positive bacteria										
B. subtilis		-	16	14	11	17	12	12	- 31 (A	0
S. aureus		-	-	-	-	19	13	13	- 30 (A	Ú
S. prieumoniae	13	10	10	15	12	-	17	11	11	31(0)
Gram-negative bacteria										
B. coli –	17	14	12	20	15	-	22	19	12	32(A)
P. aeruginosa	22	16	14	17	15	-	18	16	-	33(A)
P. vulgaris	24	17	-	-	-	-	22	18	13	31(C1)
S. typhi	22	12	-	16	-	-	24	14	10	30 (CE)
V. parahaem olyticus	16	11	-	20	16	-	24	19	12	29(K)
V. vubrificus	19	-	-	-	-	-	17	11	11	32(K)
Fungi										
A flavus	-	-	-	-	-	-	20	17	14	34(P)
A fumigatus		-	-	-	-	-	-	-	-	36(P)
A niger		-	-	-	-	-	-	-	-	31(P)
C. albicans	-		-	-	-	-	21	16	14	33(P)

Table 2 Antimicrobial activity of various extracts of the leaves of Memecylon umbellatum Burn f.

(Measurement indicates the zone of inhibition in mm).

A – Ampir illin; Cl – Clotrima xazole; Cf – Ciprofloxacin; K – Kanamycin; P – Penicillin

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