Research Article **CODEN: IJPRNK** **ISSN: 2277-8713**
Suresh Killedar, IJPRBS, 2014; Volume 3(1): 378-391 **IJPRBS**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF DIFFERENT ROOT EXTRACTS AND FRACTIONS OF *MEMECYLON UMBELLATUM* BURM

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Accepted Date: 16/02/2014; Published Date: 27/02/2014

Abstract: Extraction of air dried root powder with six different solvents was carried out by soxhlet extraction. Extracts were dried under reduced pressure and screened for phytoconsituents. Maximum yield was obtained with acetone root extract (ARE) followed by methanolic extract while that of minimum with petroleum ether extract. Antibacterial activity of roots of *Memecylon umbellatum* has been studied using Cylinder-plate or Cup-plate method. The selected bacterial strains, namely *E. coli, B. subtilis, P. aeruginosa, M. luteus* and *S. aureus* were procured from NCIM, Pune, Maharashtra. The plant extracts were compared with the standard positive control Doxycyclin and Ciprofloxacin. Also fractionation and isolation of compounds from ARE have been carried out and antimicrobial activity was investigated. Also antifungal potential of root extract was determined. The minimum inhibition concentration (MIC) was determined by serial dilution technique. Among the six solvents analyzed ARE of *Memecylon umbellatum* exhibited a maximum resistance against *M. luteus* and possessed a highest inhibitory zone against *P. aeruginosa* when compared to other bacterial strains. Acetone Root Extract Fraction-5 (AREF-5) exhibited a highest antimicrobial activity against *E. coli* amongs the fractions. The total extract has exhibited better activity than fractions confirming synergistic activity due to combined effect of polyphenols and triterpene derivatives. The obtained data was subjected to statistical analysis, using Graph Pad InStat 3.

Keywords: *Memecylon umbellatum* Root, Phytochemical screening, Antimicrobial activity, Cup-plate method, Fractionation.

PAPER-QR CODE

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How to Cite This Article:

Suresh Killedar, IJPRBS, 2014; Volume 3(1): 378-391

INTRODUCTION

For many centuries, plants have been used as a major source in the drug development process because of their vital importance in global health care and human use of plants as medicinal agents [1]. Also the World Health Organization (WHO) has investigated that medicinal plants would be the best source to obtain a variety of drugs [2] and more than 80% of the world's population relies on traditional medicines for their primary health care needs [3]. The recent year's investigation found that medicinal plants contain a large number of physiologically active principles and posses interesting biological activities that could be of interest for all parts of the world [4]. The indiscriminate use of commonly used commercial antimicrobial drugs for the treatment of infectious diseases has lead to development of multiple drug resistance in both human and plant pathogens. The commercial antimicrobial agents have to face the problem of limited life span and hence there is necessity to search new antimicrobial substances to meet the need of today's modern medicine [5]. In traditional medicine various plants are widely used due to their medicinal properties so use of plants will be one of the most promising research avenue in the search of new biologically active compounds.

Memecylon umbellatum Burm. (Family: Melastomataceae) is small evergreen shrub or tree having young terete branches and bears numerous umbellate cymes. In English the plant is known as "Ironwood tree" while in Sanskrit as "Anjani". Geographically plants have been 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 [6,7]. The leaves of the plant have been reported to possess antimicrobial and astringent properties so these are used in the treatment of leucorrhoea and gonorrhoea. Lotion prepared from leaves is used to treat eye troubles [8]. The decoction of the root is used in the treatment of excessive menstrual discharge [9]. Leaves are also reported to possess antiviral activity [10-11]. Bark is used in the treatment of bruises externally as lepa along with coconut kernels [12]. The literature survey reveal that leaves and roots of *Memecylon umbellatum* have been investigated for its hypoglycemic activity using alloxan induced hyperglycemic wistar albino rats [13-14]. Wound healing activity of ethanolic extract of leaves has also been reported [15]. Roots of the plant have been reported for antipyretic, analgesic, anti-inflammatory, hepatoprotective, anthelmintic and antiinsect activities [16-19]. Plant contains wide variety of phytoconstituents such as umbellactone, amyrine, oleanolic acid, ursolic acid, sitosterol and organic acids [20].

The present study was conducted to investigate antimicrobial activity of different solvent extracts of *Memecylon umbellatum* root against various microorganisms.

MATERIALS AND METHODS

Sample was collected in the month of March-April from Gaganbavada hilly region (altitude about 600m) of Kolhapur district. Plant was authenticated by Dr S. R. Yadav, Botany department, Shivaji University Kolhapur and herbarium sheet (Voucher no. SGK-3) was deposited in the department of Pharmacognosy Bharati Vidyapeeth College of Pharmacy Kolhapur. Microbial cultures were procured from NCIM, Pune and maintained at 4^0C. All the solvents, chemicals used were of analytical grade (Loba) and standard Culture media (Hi-media) was used.

Collection, drying and powdering of roots

Roots were collected in the month of March-April and washed thoroughly in running water and dried under shade for 15-20 days. The dried roots were powdered using heavy duty Willey type disintegrator and fine powder was obtained by passing through # 180. Powder sample (1kg) was used for extraction.

Soxhlet extraction

Extraction was carried out by standard procedure [21-23]. Fine powder (1kg) was packed in cotton bag previously washed with polar and non polar solvents and dried. Then pet. ether (60-80^0C) was poured from top and allowed to percolate from the bed of the packed powder in extractor. After pouring sufficient amount (3.5lts) of solvent, the assembly was made air tight using sealing wax and extraction was carried out at low temperature for 18h or appearance of clear solvent in siphon tube. The extract was filtered and powder was dried in hot air oven (Kumar make- Mumbai) at 45^0C for 24h and process was repeated for other solvents with increasing polarity. Finally the dried powder was macerated for 48h with 3.5 lit. of chloroform water IP (0.25% v/v) at room temperature in macerating bottle with frequent shaking. All the filtrates were evaluated for physical characters and evaporated using rotary vacuum film evaporator (Dolphin) and dried using vacuum dryer. The yield of solids was determined by using electronic balance (Shimadzo -AY120).

Table 1: Physical analyses of liquid root extracts and yield of solids

Name of extract	Color of extract	pH	Fluorescence			Sp.gr.	Density	Viscosity	Yield of solids (g)	Nature of solid extract
			D	S	L					
PEER	Y	5.0	G	-	-	0.6125	0.6232	0.6728	0.50	Waxy
ChER	YB	5.4	-	M	-	1.3720	1.4807	0.7791	0.60	Lumpy
EAER	YB	4.2	-	M	-	0.8517	0.9123	0.5862	1.03	Powder
AER	OR	4.5	R	M	DB	0.7826	0.8354	0.5642	14.25	Powder
MER	RB	6.0	R	M	-	0.8235	0.8798	0.9649	12.60	Waxy
AqER	FY	6.0	-	-	-	1.0128	0.8658	0.9251	9.72	Powder

PEER-Petroleum Ether extract Root, ChER-Chloroform Extract Root, EAER Ethyl Acetate Extract Root, AER- Acetone Extract Root, MER-Methanol Extract Root, AqER-Aqueous Extract Root, Y-Yellow, G-Green, YB-Yellowish Brown, M-Milky, OR-Orange Red, RB-Reddish Brown, R-Red, DB-Dark Brown, FY- Faint Yellow.

Phytochemical screening

All the dried extracts were dissolved in small quantity of respective solvents and tested for phytoconstituents using standard reagents [24]. The results are given in table no. 2

Table 2: Phytochemical screening of root extracts

Extract	Sugars		Alk.	Tannins		Glycosides					Steroids		Prot.	Org. acids		
	R	NR		HT	CT	a	c	S	f	co	ST	TT		C	O	T
AqER	+	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+
MER	+	+	-	+	+	-	+	+	+	+	-	+	+	-	+	+
AER	+	+	-	+	-	-	+	+	+	+	+	-	+	-	+	-
EAER	+	+	-	+	+	-	-	-	-	-	+	-	+	-	-	-
ChER	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
PEER	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-

AqER-Aqueous Extract Root, MER-Methanol Extract Root, AER-Acetone Extract Root, EAER-Ethyl Acetate Extract Root, ChER-Chloroform Extract Root, PEEL-Petroleum Ether Extract Root, Alk-Alkaloids, Gly-Glycosides, Org. acids-Organic acids, R-Reducing sugars, NR-Non Reducing sugars, HT-Hydrolysable Tannins, CT-Condensed Tannins, a-anthracene glycosides, c-Cardiac glycosides, s-saponin glycosides, f-flavanoidal glycosides, co-coumarin glycosides, + Positive, - Negative.

Bacterial and fungal strains

Standard bacterial test organisms were sub cultured on freshly prepared nutrient agar and fungal cultures on saboraud's dextrose agar media and organisms were incubated at 37^0C in incubator for 24 h and fungus at 27^0C for 48 h. They were further stored at 40^0C in the refrigerator to maintain stock culture. The organisms with their ATCC code, are given in Table No. 3

Table 3: Microorganisms with their ATCC Codes

Sr. No.	Name of microorganism	ATCC Code
1.	*Escherichia coli*	8739
2.	*Bacillus subtilus*	6633
3.	*Micrococcus luteus*	9341
4.	*Pseudomonas aeruginosa*	27853
5.	*Staphylococcus aureus*	25923
6.	*Aspergillus niger*	16404
7	*Candida albicans*	10231
8.	*Penicillium notatum*	28682

Antibacterial Assay [25-28]

The nutrient agar, test extracts and standard drug solutions were prepared freshly by dissolving in the corresponding solvents under aseptic conditions and used for screening of antimicrobial activity.

Cylinder-plate or cup-plate method

All the sterilized materials were kept in the aseptic area in the Ultra-Violet laminar air flow. Bacterial suspensions (3 ml) were then poured in the plates. As soon as nutrient agar attained 50ºCtemperature, 20 ml of media was poured in to the petri plates containing bacterial suspension and plates were rotated to mix the suspension with media. When the agar got solidified bores were made in the plate with sterile borer of 8 mm diameter. In each plate six bores were made. Out of which one is meant for addition of standard, two for negative control of blank solvents of standard and sample and remaining three bores for addition of same concentrations of sample. 100µl of sample was added in each cylinder. The plates were kept to allow diffusion at room temperature for three hours and then incubated in the upright position in incubator at 37ºC for about 24h for bacterial growth. For fungus, Sabouraud dextrose agar was poured in the petri plates, allowed to solidify. The fungal suspensions were then sprayed uniformly over the surface of agar. All the procedure was same as that of antibacterial activity. Fungi were incubated at room temperature for 48h.The diameter of zone of inhibition was

accurately measured by zone reader in each treated plate as shown and was compared with standard at tested concentrations.

Table 4: Antibacterial Activity of *Memecylon umbellatum* Root Extracts

Part of plant	Sample/ Extract	Conc. used	Zone of inhibition in mm.*				
			E. coli	*P. aeruginosa*	*M. luteus*	*B. subtilus*	*S. aureus*
Control-1	2% acacia suspension	0.2ml	--	--	--	--	--
Control-2	DMSO	0.2ml	--	--	--	--	--
Standard-1	Doxycyclin	01µg	22	18	23	25	23
Standard-2	Ciprofloxacin	01µg	25	21	27	25	26
Root	Pet. ether	3mg	09	--	09	10	10
		6mg	10	--	10	11	11
	Chloroform	3mg	--	--	09	10	10
		6mg	--	--	09	11	11
	Ethyl acetate	0.5mg	22	--	14	21	24
		1.0mg	24	--	16	23	25
	Acetone**	0.5mg	24	--	22	19	18
		1.0mg	26	--	24	20	20
	Methanol	0.5mg	20	--	20	16	14
		1.0mg	25	--	23	17	16
	Aqueous	3mg	--	--	09	11	10
		6mg	--	--	10	12	11

* Average of triplicates

** Statistical significance with standards ($P < 0.05$)

DMSO-Dimethyl Sulphoxide

Fractionation and isolation of compounds from ARE

Different solvents like petroleum ether, diethyl ether, chloroform, ethyl acetate, butanol were used for the fractionation of ARE using column chromatography and each fraction was concentrated. Further each fraction was subjected to TLC and HPTLC studies for confirmation of tannins and finally antibacterial activity of all the fractions was investigated as shown in table no.5.

Table 5: Antimicrobial activity of fractions and isolated compounds

Sample/ extract	Conc.	Zone of inhibition in mm.*						
		E.coli	*M. luteus*	*B.subtilus*	*S. aureus*	*P.notatum*	*C.albicans*	*A.niger*
2% acacia suspension	0.2ml	--	--	--	--		--	--
DMSO	0.2ml	--	--	--	--		--	--
ARE**	0.5mg	24	22	19	18	11	13	15
AREF-3	0.5mg	12	11	10	10	--	--	--
AREF-5	0.5mg	13	12	11	11	--	--	--
AREF-7	0.5mg	12	12	10	10	--	--	--
ARETF	0.5mg	10	11	10	10	10	09	11
ARETFF	0.5mg	12	12	13	11	09	10	10
ARPEF (TF)	0.5mg	--	--	--	--	09	10	09
ARChF (TF)	0.5mg	--	08	09	09	--	--	--
ARSEF (TF)	0.5mg	11	12	11	12	09	09	10
AREAF (TF)	0.5mg	10	11	12	11	09	--	--
ARBuF (TF)	0.5mg	11	10	11	11	09	--	10
ARAqF (TF)	0.5mg	--	09	--	09	--	--	--
TAN-1	0.5mg	08	09	09	09	--	08	--

* Average of triplicates

** Statistical significance with standards ($P < 0.05$)

ARE- Acetone Root Extract, AREF-3- Acetone Root Extract Fraction-3, AREF-5- Acetone Root Extract Fraction-5, AREF-7- Acetone Root Extract Fraction-7, ARETF- Acetone Root Extract Tannin Fraction, ARETFF- Acetone Root Extract Tannin Free Fraction, PEFAR(TFF)- Petroleum ether fraction Acetone Root, DEFAR(TFF)- Diethyl Ether Fraction Acetone Root, CHFAR(TFF)- Chloroform Fraction Acetone Root, EAFAR(TFF)- Ethyl Acetate Fraction Acetone Root, BuFAR(TFF)- Butanol Fraction Acetone Root, AqFAR(TFF)- Aqueous Fraction Acetone Root.

Antifungal Activity

For fungus, SDA (Saboraud Dextrose Agar) was poured in the petri plates, allowed to solidify. The fungal suspensions were then sprayed uniformly over the surface of agar. All the procedure was same as that of for antibacterial activity. Fungi were incubated at room temperature for 48h. Zone of inhibitions of fungal growth were recorded as shown in table no. 6

Table 6: Antifungal Activity of *Memecylon umbellatum* Root Extracts

Part of plant	Sample/ extract	Conc. used	Zone of inhibition in mm.*		
			A. niger	*C. albicans*	*P. notatum*
Control-1	2% acacia suspension	0.2ml	--	--	--
Control-2	DMSO	0.2ml	--	--	--
Standard	Fluconazole	5µg	16	17	17
Root	Pet. ether	3mg	--	--	--
		6mg	--	09	08
	Chloroform	3mg	--	--	--
		6mg	09	09	--
	Ethyl acetate	0.5mg	12	13	11
		1.0mg	13	14	12
	Acetone	0.5mg	12	14	11
		1.0mg	13	15	13
	Methanol	0.5mg	10	09	10
		1.0mg	11	10	11
	Aqueous	3mg	--	--	--
		6mg	09	--	--

* Average of triplicates

Determination of MIC

Promising extracts were tested for MIC using serial broth dilution technique [29]. 10 ml of sterile nutrient broth without agar was taken in a test tube and concentrations of 0.5, 1, 3, 7, 15mg of test samples were added aseptically. 1 ml of inoculums of each test organism in normal saline (105 CFU) was added in each sample. The tubes were incubated at 37°C for 24h (or 48h for the fungi) and then observed for the Minimum Inhibitory Concentration (MIC). The growth of organisms was observed as turbidity determined by a double beam spectrophotometer (Jasco V-530) at 620 nm. Control tubes without the tested extracts were assayed simultaneously. All samples were tested in triplicate and results are as given in table no.7

Table 7: MIC for different extracts of *Memecylon umbellatum* Root and ARE fractions

Sample/Extract	MIC* (conc. in mg)				
	E. coli	*P. aeruginosa*	*M. luteus*	*B. subtilus*	*S. aureus*
Pet. ether	3.0	--	3.0	3.0	3.0
Chloroform	15.0	--	3.0	3.0	3.0
Ethyl acetate	0.5	15	0.5	0.5	0.5
Acetone	0.5	15	0.5	0.5	0.5
Methanol	0.5	15	0.5	0.5	0.5
Aqueous	15.0	32	3.0	3.0	3.0
AREF-3	0.5	NP	0.5	0.5	0.5
AREF-5	0.5	NP	0.5	0.5	0.5
AREF-7	0.5	NP	0.5	0.5	0.5
ARETF	0.5	NP	0.5	0.5	0.5
ARETFF	0.5	NP	0.5	0.5	0.5
ARPEF (TF)	NP	NP	NP	NP	NP
ARChF (TF)	1.0	NP	1.0	1.0	1.0

ARSEF (TF)	0.5	NP	0.5	0.5	0.5
AREAF (TF)	0.5	NP	0.5	0.5	0.5
ARBuF (TF)	0.5	NP	0.5	0.5	0.5
ARAqF (TF)	0.5	NP	0.5	0.5	0.5
TAN-1	0.5	NP	0.5	0.5	0.5

* Average of triplicates

NP- Not Performed

RESULT AND DISCUSSION

Acetone, Ethyl acetate and methanolic extracts have shown better antimicrobial activity as compared to other extracts. All the extracts have been failed to show activity against *P. aeruginosa.* The activity shown by acetone and ethyl acetate was comparable with standards like Doxycyclin and Ciprofloxacin at tested concentrations. Petroleum ether, chloroform and aqueous extracts exhibited antimicrobial activity at higher concentration (3mg) as compared to acetone, ethyl acetate and methanol extracts (0.5mg). The minimum inhibition concentration (MIC) was determined by serial dilution technique. Ethyl acetate, acetone and methanol extracts have shown MIC at 0.5 mg against all the tested organisms except *P. aeruginosa* which shows at 15 mg. All other extracts have shown MIC at higher concentrations (table-7). Among the ARE fractions all fractions have shown MIC at 0.5mg against all the tested organisms except ARChF (TF) at 1mg.

Chloroform and aqueous extracts have been failed to show antimicrobial activity against *E. coli* and *P. aeruginosa*. Along with antimicrobial activity acetone, ethyl acetate and methanolic extracts have also shown good antifungal activity. The ARE preparative TLC fractions ARF-3, ARF-5 and ARF-7 have shown only antibacterial activity and no antifungal activity. Tannin and tannin free fractions of ARE have shown activity against all the tested organisms but comparatively less than that of original acetone root extract. From the tannin free fraction of acetone root, petroleum ether fraction shows only antifungal activity. Solvent ether and butanol fractions have shown activity against all the organisms. Chloroform fraction shows only antibacterial and no antifungal activity while aqueous fraction shows activity only against *M. luteus* and*S. aureus*. Overall activity of fractions was comparatively less than the tannin and tannin free fractions and original extract.

Different extracts showed presence of tannins, glycosides, triterpenes and steroids as important constituents. These constituents may be responsible for antimicrobial activity as per

the reported activities on Camellia sinensis, [30] Rhizophoraapiculata bark, [31] blue and white flowering Silybummarianum, [32] roots of Tecomastans, [33] punicalagin from the peel of Punicagranatum, tannic acid from galls and prodelphinidin oligomers from the bark of Elaeocarpussylvestris var. ellipticus etc [34]. Further study is necessary to isolate the constituents responsible for activity from ethyl acetate and methanol extracts.

Statistical analysis

The obtained data was subjected to statistical analysis, using Graph Pad InStat 3, to determine their significance. One-way Analysis of Variance (ANOVA) was conducted to examine whether there is statistically significant difference in the activity with standards. All results of ARE and its fractions shows statistically significance as $P < 0.05$.

CONCLUSION

From the present investigation it has been revealed that, acetone and ethyl acetate extract has shown maximum antimicrobial activity against *E. coli, M. luteus, B. subtilis* and *S. aureus* and antifungal activity against *A. niger, P. notatum and C. albicans*. So the acetone root extract and its fractions has significant potential antimicrobial activity and to some extent antifungal activity than other extracts to inhibit the growth of pathogenic bacterial and fungal strains. The various compounds identified were needed further study on the toxicological aspects including clinical trials to develop safe drug for the treatment of various human ailments.

ACKNOWLEDGEMENT

Authors are very thankful to Dr. S. R. Yadav, Dept. of Botany Shivaji University, Kolhapur for authentication of plant.

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