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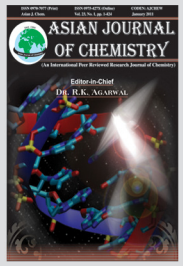

Evaluation of Spermicidal and Antimicrobial Activities of Methanolic Extract of *Leucas aspera* and Structural Elucidation of Separated Active Component

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The methanolic extract of whole plant of *Leucas aspera* was evaluated for its spermicidal and antimicrobial activity. Total four compounds were separated from the extract by column chromatography and the same activities were evaluated for the separated compounds also. The structure of highest active component was elucidated.

Key Words: Spermicidal, Antimicrobial, Structure and *Leucas aspera*.

INTRODUCTION

Leucas aspera is widely found in Tripura and is generally used as expectorant, antibacterial, diuretic, anthelmintic, stimulant, laxative, antipyretic, analgesic, antirheumatic and antiinflammatory and also to relieve burning sensation of eyes, *etc*. It is also effective against blood and urinary disorders[1,2]. As a part of our investigation, we examined methanolic extract of whole plant of *Leucas aspera* growing in Tripura and the results regarding its spermicidal, antimicrobial activity and preliminary phytochemical observations are reported in this communication. Further same activities were tested for separated compounds and the structure of most active component was evaluated.

EXPERIMENTAL

The whole plant of *Leucas aspera* were collected, identified by the expert of Pharmacognosy Department of Institute, cut and shed dried for 7 days, powdered and subjected for extraction in methanol in Soxhlet apparatus. The R_f values of the methanolic extract of *Leucas aspera* was determined by TLC method using various solvent systems *viz*., benzene, butanol:water:dioxane, butanol:acetic acid:water[3-5]. Results are displayed in Table-1. The separation of component from the extract was carried out by column chromatography using ethyl acetate as a mobile phase. Total four numbers of components were separated and they are designated as LA-1, LA-2, LA-3 and LA-4. The solvents were removed and the dried residues were obtained. The dried crude extract and separated components were dissolved in DMF and used for for spermicidal studies and antimicrobial studies. The melting point and structure of most active component was determined. The extract was also subjected for the phytochemical investigation[3,5] by adopting standard procedure.

TABLE-1
R_f VALUE DETERMINATION OF *Leucas aspera*

Solvent system	Spot Number	Distance traveled by components (cm)	Distance traveled by solvent (cm)	R_f Values
BWD	1	0.8	5.4	0.14
	2	1.6		0.29
	3	2.9		0.53
	4	3.6		0.66
	5	4.8		0.88
BAW	1	0.8	5.6	0.14
	2	1.9		0.33
	3	2.9		0.51
	4	3.9		0.69
	5	4.7		0.83
	6	5.2		0.92
B	1	1.2	5.8	0.20
	2	2.0		0.34
	3	3.1		0.53
	4	4.2		0.72
	5	5.2		0.89

BAW = Butanol:acetic acid:water (4:2:1), BWD = Butanol:water: dioxane (4:2:1), B = Benzene.

Spermicidal activity[6]: Spermicidal activity of the extract and separated component were carried out by adopting designed procedure using healthy human sperm (100-150 million sperma-

tozoa/mL, ≥ 80 % motility, 2.1 mL/ejaculate, pH = 7.9). 1 mL of the sperm volume was subjected in the study for each concentration. Sperm volume and extract volume ratio was 10:1 for each case. After the treatment of sperm with crude extracts and separated components the motility of the sperm were recorded after 10, 20 and 30 min. The effect of the vehicle (DMF) was also observed and recorded in Table-2. Total 06 samples of sperm were collected.

TABLE-2
EFFECT OF THE EXTRACT AND SEPERATED COMPONENTS OF *Leucas aspera* ON SPERM

Extract/component (1 mg/mL)	Percentage decrease in motility (average ± SEM)		
	10 min	20 min	30 min
Crude extract	20 ± 0.032	31 ± 0.043	38 ± 0.066
LA-1	0	5 ± 0.005	9 ± 0.008
LA-2	0	0	3 ± 0.009
LA-3	7 ± 0.046	14 ± 0.065	19 ± 0.053
LA-4	10 ± 0.071	18 ± 0.044	22 ± 0.027
LA-3: LA-4 (1:1)	18 ± 0.057	31 ± 0.065	40 ± 0.092

*LA-1 to LA-6 is the separated product of *Leucas aspera* by column chromatography taking ethyl acetate as mobile phase.

Antibacterial activity: The antibacterial activity was determined *in vitro* by filter paper disc method[7] by measuring inhibition zone in cm. Extract was tested against bacterial strains *Staphylococcus aureus*, *Bacillus pertusis* (gram positive) and *Escherichia coli*, *Vibrio cholarae* (gram negative) in a concentration of 200 µg/mL. Nutrient agar media was used as culture medium. All the tests were carried out against standard drug tetracycline. The average results from triplicate observation are given in Table-3.

TABLE-3
ANTIBACTERIAL ACTIVITY OF *Leucas aspera*

Extract/ standard	Average zone of inhibition (cm)			
	Gram positive bacteria		Gram negative bacteria	
	B. pertusis	*S. aureus*	*E. coli*	*V. cholarae*
Leucas aspera	1.1	1.0	1.5	1.1
Tetracycline	2.1	2.5	2.2	2.3

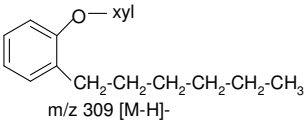
Structure elucidation

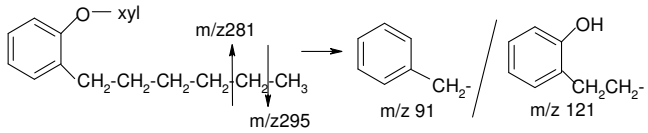
LA-3: The compound LA-3 with molecular formulae $C_{17}H_{26}O_5$ was deduced from its ESI-MS negative mode mass spectrum by exhibiting a pseudo molecular ion $[M-H]^-$ at m/z 309.

In its IR spectrum it exhibits bands at 3479 cm^{-1} for ν(OH) and 1589 and 1641 cm^{-1} for an aromatic ring. Its ^1H NMR spectrum exhibits a triplet at δ 0.49 for a methyl group and multiplets at δ 1.17 and δ 1.25 for methylene protons. In addition to this it exhibits a strong multiplet signal at δ 3.03 indicating the presence of a sugar moiety xylose. Further it shows the presence of aromatic protons at δ 7.59. Taking the above data's into consideration and the mass spectrum the tentative structure of the compound LA-3 may be as below, which also shows supportive ESI-MS fragment.

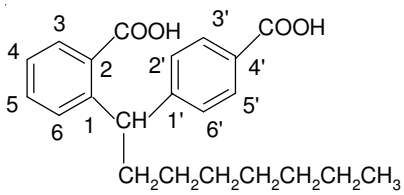
LA-4: IR shows the presence of carboxylic acid by exhibiting characteristic bands at 3400 cm^{-1} ν(-OH) and 1728 cm^{-1} ν(C=O) and aromatic ring system at 1639 and 1589 cm^{-1}. The ^1H NMR spectrum shows the presence of aliphatic chain protons at δ 0.84, 0.87, 0.94 and 1.5 and the aromatic protons at δ 7.7. The ^{13}C NMR spectrum shows the presence of two carboxylic acid groups at δ 167.77 and 160.71. A part from that it shows the presence of 12 other carbon atoms in the aromatic region. From the ^{13}C NMR spectrum the signals for 1'[δ 134.95], (2',6') [δ 123.93], (3',5')[δ 116.01] and 4'[δ 153.95] appear to be that the molecule is symmetrical whereas for the other ring all signals (1: δ 126.08, 2: δ 144.03, 3: δ 124.59, 4: δ 124.36, 5: δ 122.29, 6: δ 121.50) are different and hence an unsymmetrical substitution is provided. The mass spectrum ESI-MS negative mode shows the molecular formulae to be $C_{22}H_{26}O_4$ by exhibiting a pseudo molecular ion $[M-H]^-$ ion at m/z 353. The fragmentation pattern is as follows:

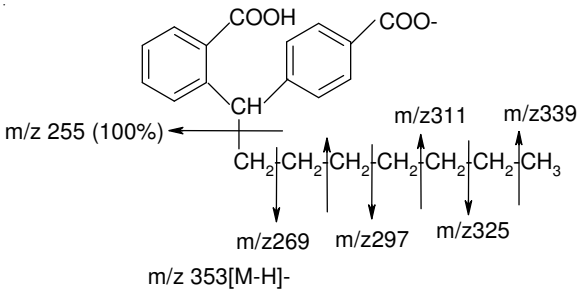
for LA-3





for LA-4





RESULTS AND DISCUSSION

The methanolic extract of *Leucas aspera* was subjected for present investigation as entitled. Five-six spots were observed in TLC study for the extract in different mobile phases as described in Table-1.

Preliminary qualitative chemical tests were showing presence of alkaloids, reducing sugars, steroid. The antibacterial study of the extract showed that the zone of inhibition was not at par to the standard drug tetracycline. The extract showed the highest activity against *E. coli*. as 1.5 cm. The extract also possess a good spermicidal activity. Four components were separated from the extract and these components were also tested for the spermicidal activity. Component No. 3 (LA-3) and component No. 4 (LA-4) in ratio of 1:1 were showing spermicidal activity as equivalent to crude extract, though they were able to show good activity individually. The melting point of LA-3 and LA-4 were found out by open capillary method, not corrected, which were 161 and 128 ºC, respectively. The structures were also elucidated for these two components.

Conclusion

By observing the results it is concluded that the methanolic extract of *Leucas aspera* possess good antimicrobial and spermicidal activity and the most active components were LA-3 and LA-4.

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