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STUDY OF ANTIBACTERIAL ACTIVITY OF LEUCAS ASPERA SPRENG

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ABSTRACT

In this study, the whole plant of Leucasaspera was first defatted with hexane and discarded.Then the remaining marc was successively extracted with ethyl acetate and methanol and both the extract were concentrated under vacuum to yield corresponding ethyl acetate extract (EAE) and methanolic extract (ME). Extractive value was found to be 5.68% w/w and 9.73% w/w, respectively. Preliminary phytochemical screening reveals the presence of alkaloids, glycosides, terpenoids and sterols in both the extracts. Both EAE and ME were screened for its antibacterial activity against four gram positive and six gram negative bacteria at different concentrations of 50, 100, 200, 300 and 400 µg/disc by agar diffusion method. The activities of both the extracts were compared with standard antibiotics, by measuring the dimension of the zone of microbial growth (zone of inhibition) around the disc. Both the extracts exhibited a significant antibacterial activity against all the screened microorganisms.

Keywords: Leucasaspera, Antibacterial activity

I. INTRODUCTION

Leucasaspera S. (Labiate) has been reported to possess antipyretic and insecticidal properties1. Leaves of the juice is used as an external application for psoriasis, chronic skin eruption, and painful swelling2,3. An alcoholic extract of the leaves shows anti-bacterial activity against Micrococcus pyogenes and Escherichia coli 4.Its anti-inflammatory activity has been shown in rats through prostaglandin inhibition. The entire plant is also used as an insecticide and indicated in traditional medicine for coughs, colds, painful swelling and chronic skin eruption. Apart from this, the plant possesses wound healing property and is used in cobra venom poisoning. Leaves of *L. aspera* are useful in chronic rheumatism, psoriasis, scabies, chronic skin eruptions and their juice used as antibacterial agent. Its chloroform and ether extracts possess antifungal activity. Compounds isolated from the plant include long chain aliphatic compound, triterpenes, sterols and novel phenolic compounds.

II. EXPERIMENTAL

2.1 Plant Material

The whole plant of Leucasaspera S. (Labiate) was collected from botanicalgarden.The sample was authenticated at our campus The plants were segregated into four parts consisting of the leaf, stem, root and flower. Then, the segregated parts were cut into small size and sun dried for 1 week.

2.2 Preparation of Extract

All the plant parts (root, flower, leaf and stem) were ground into powdered form with a grinder. All the plant parts (100 g) were then soaked in 500 mL methanol (80% v/v) separately in a beaker and let to soak for 4 days at room temperature [(26–28) °C]. Removal of dry plant parts was done by filtration through cheesecloth and Whatman filter paper. The filtrate was then further concentrated using rotary evaporator. The extracts were all placed in glass Petri dishes. The dried plant extracts were then redissolved in 80% (v/v) methanol in order to obtain a solution containing 2.0 mg/mL of extract, respectively which were then used for assays.Air dried and powdered, whole plant of Leucasaspera was defatted with hexane by

maceration process. The defatted material was successively extracted with ethyl acetate and methanol. The extractive value was found to be 5.68% and 9.73% w/w, respectively. Preliminary phytochemical screening 5,6 reveals the presence of alkaloids, glycosides, terpenoids and steriods (Table 1).

Table 1.Preliminery phytochemical screening of extracts of Leucasaspera

Constituents	EAE	ME
Alkaloids	+++	++
Anthraquinones	_	_
Coumarins	_	_
Fatty acids	++	_
Steriods	+++	++
Terpenes	+++	++
Glycosides	++	_
Flavanoids	_	_

2.3 Activity

Ethyl acetate extract (EAE) and methanolic extract (ME) was studied for its antibacterial activity using different clinically important strains at different concentrations of 50, 100, 200, 300 and 400 µg/disc by agar diffusion method 7,8 against Bacillus cereusBacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,Klebsiellapneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, and Salmonella typhi. The activity of EAE and ME were compared with the standard antibiotics, mentioned in the Table 2. The plates were incubated at 37oC for 48 hrs. The zone of inhibition was calculated by measuring the dimension of the zone of no microbial growth around the disc. For each value, averages of three determinations were recorded

(Table 2).

Table 2. Antibectarial activity of extracts of Leucasasperaspreng

Microorganisms		EAE					ME					
		IZ at mg/disc					IZ at mg/disc					Standards
		50	100	200	300	400	50	100	200	300	400	
Bacillus cereus	G+			8	8.5	9						24(Am)
Bacillus subtilis	G+	8	10	12	13	13.5						33(Nv)
Staphylococcus aureus	G+	15	7	8	9	9.5	9	11	12	12.5	13	31(Nv)
Staphylococcus epidermidis	G+	17	19	21	22	24	10	12	14	15	16	36(Tr)
Escherichia coli	G-	10	12	13	14	16	20	22	24	25	27	28(Ch)
Klebsiellapneumoniae	G-	17	18	19	20	21	15	16	18	20	21.5	32(Nv)
Proteus mirabilis	G-	7	8	9	10	11.5	10	12	13	14	16	13(Cip)
Proteus vulgaris	G-				8	8.5				9	10	22 (Te)
Pseudomonas aeruginosa	G-		9	10	12	13			8	9	10	26(Ce)
Salmonella typhi	G-		10	11	12	13		12	13	15	17	23(Ce)

a values (mean of three replicates) are; IZ, inhibition zone (mm) ; -, no inhibition.
bCe, Ceftriaxone (30 µg/disc); Ch, Chloramphenicol (30 µg/disc); Er, Erythromycin (15 µg/disc); Nv, Novobiocin (30 µg/disc);
Tr, Trimethoprim (5 µg/disc); Te, Tetracycline (10 µg/disc); Ci, Ciprofloxacin (10 µg/disc); Am, Ampicillin (10 µg/disc).

III. RESULTS AND DISCUSSION

Both EAE and ME of Leucasaspera exhibited moderate to significant and concentration dependent antibacterial activity against all the tested microorganisms at the concentrations of 50, 100, 200, 300 and 400 µg/disc and comparable to the various antibiotics used for individual microorganism. This study also reveals that EAE was found to be highly active against Staphylococcus epidermidis and Klebsiellapneumoniae, where as ME was highly active against Escherichia coli. Our results indicate the potential usefulness of Leucasaspera, in the

treatment of various bacterial infections. Furthephytochemical studies are needed to identify the active principle responsible for the observed antibacterial activity.

The above results indicate that both the extracts possessed some antimicrobial activity against all the tested organisms. So it can be concluded that EAE was found to be much more active than ME. The results may support the uses of this plant in traditional medicines.

Plant has long been a very important source of drug and many plants have been screened if they contain compounds with therapeutic activity[17]. Therefore, it is vital to evaluate the antimicrobial activity of *L. aspera*. In this study, the antibacterial activity of the different parts of *L. aspera* was evaluated by using disk diffusion method. The microorganisms chosen to be studied were Gram positive, *S. aureus* and Gram negative *E. coli*, *P. aeruginosa*, *S. typhimurium*, *S. choleraesuis* and *S. flexneri*. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance as antibiotic use increases.

In a research conducted by using the entire plant of *L. aspera*, Srinivasan *et al*[20] indicated that the aqueous extract of the whole plant exhibited a higher range of zone of inhibition against *S. aureus*, *E. coli* and *P. aeruginosa*. However, the aqueous extract did not exhibit any activity against *S. typhimurium*. The difference in the zone of inhibition is probably due to the different solvents used for extraction and also the fact that the extracts in the previous study were from the entire plant whereas in this study the plant parts were segregated and tested individually. Moreover, growth area also affects the chemical components of the plants and leads to the activity difference. Apart from that, research done by Mangathayaru *et al*[21] indicated that the methanol extract of *L. aspera* (flower) was effective against *S. aureus*, *E. coli* and *P. aeruginosa* which were similar to the results from this study.

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