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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF VARIOUS EXTRACTSOF LEUCUS ASPERA (WILLD.) LINN. AERIAL PARTS

Tirtha Ghosh[1]*, Swarupa Ghosh[2] and Debabrata Devbhuti[1]

[1]Department of Pharmacy, Jnan Chandra Ghosh Polytechnic, 7 Mayurbhanj Road, Pin.700023, West Bengal, India.
[2]Department of Botany, Alipurduar College, P.O.- Alipurduar Court, Alipurduar, West BengalPin-736123, India.

***Corresponding Author: Tirtha Ghosh**
Department of Pharmacy, Jnan Chandra Ghosh Polytechnic, 7 Mayurbhanj Road, Pin.700023, West Bengal, India.

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ABSTRACT
Various Extracts of *Leucus aspera* (Willd.) Linn. Aerial Parts were investigated for antibacterial and antifungal effects. Ethanolic extract (EA), Ethyl acetate extract (Eac), Petroleum Ether extract (PE) and n-butanol extract (NB) were prepared by successive soxhlation method and estimation of zone of inhibition and Minimum inhibitory concentration(MIC) were carried out. Among the tested strains of micro-organisms, PE extract showed maximum inhibition though all the extracts showed antimicrobial activity and the activity waswas found to be dose dependent. Maximum activity in PE may be attributed to the hydrophobicnature of the extract which might be responsible for breakage of lipid bilayer of cell membrane,leading to permeability dissipation and a subsequent outflow of its cell contents. From these results, it was suggested that PE could effectively act as antimicrobial agent.

KEYWORDS: *Leucus aspera*, *Antibacterial activity*, *Antifungal activity*, Zone of inhibition, Minimum inhibitory concentration.

INTRODUCTION
Presently, there has been an increasing interest in the identification and development of novel antimicrobial agents from different sources to counter microbial resistance. Anti-microbial activity study has a vast range of applications in drug discovery and epidemiology [Balouiri *etal*, 2015].

Leucas aspera (Willd.) Linn. (Family: Lamiaceae) commonly known as 'Thumbai' [Rai *et al*, 2005] is distributed throughout India from the Himalayas down to Ceylon. [Nadkarni, 1976] The traditional use of the plant is as antipyretic and insecticide. Flowers are considered useful as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are usedin chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites. [Rai *et al*, 2005; Shirazi, 1947]

Various phytochemical constituents mainly triterpenoids, oleanolic acid, ursolic acid and b-sitosterol, nicotine, sterols, flavonoids, glucoside, diterpenes, phenolic compounds (4- (24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol) have been reported earlier. As the traditional use of the plant refers to its use in psoriasis and other chronic skin eruptions, it is thought worthwhile to study the antimicrobial activity of various extracts of the aerial parts of *Leucus aspera* (Willd.) Linn in a scientific manner.

MATERIALS AND METHODS
Plant material
The plant was identified by the taxonomists of the Botanical Survey of India, Govt. of India, Howrah. A voucher specimen is kept in our department (JP/PH/TG/03/02) for furtherreference. Fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Salipur, Orissa, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder.

Chemicals used
All the chemicals and reagents used in the study were of analytical grade.

Preparation of extract
The powdered plant material (2 kg) was extracted successively with n-hexane, petroleum ether (60-80^{o}C), ethylacetate and ethanol (95%) in a soxhlet apparatus in increasing order of polarity. The respective solvents were removed under reduced pressure, which produced different extracts. All The dried extract was then mixed

with dimethyl sulfoxide (DMSO) forantimicrobial study.

Drugs used

Ciprofloxacin and Cotrimazole were used as reference standards for the antibacterial and antifungal studies respectively.

Microorganisms used

For the present study, the microorganisms used include *Staphylococcus aureus, Bacillus subtilis, Bacillus polymexia, Streptococcus faecalis, Pseudomonas aerugenosa, Salmonella typhi, Vibrio cholerae, Shigella dysenteriae, Escherichia coli, Penicillum notatum, Aspergillus niger and Candida albicans*. Suitable strains of these microorganisms were procured from the microbiology laboratory of our institute.

Antimicrobial activity determination of zone of inhibition

The zone of inhibition of the extracts were performed by agar disc diffusion method [Cruickshank, 1968] at concentrations of 2, 5 and 10 mg/ml of the extracts in DMSO. Ciprofloxacin (5 µg/ml) and Cotrimazole (25 µg/ml) were used as reference controls for the antibacterial and antifungal studies respectively. Solvent control (only DMSO) was also maintained throughout the experiment.

Determination of Minimum inhibitory concentration (MIC).

Minimum inhibitory concentration (MIC) of the extracts were performed by broth dilution method of Ghosh *et al*, 2006, at concentrations of the extracts ranging from 25 µg/ml to 600 µg/ml in DMSO against all the test microorganisms.

RESULTS AND DISCUSSION

Table 1 depicts the antimicrobial activity of various extracts of *Leucus aspera* aerial parts. Theresult of zone of inhibition study revealed that all the extracts possess antimicrobial activity in a concentration dependent manner against the test organisms and was comparable with the standard drug used in the experiment. The gram-positive bacteria were observed to be more susceptible than gram-negative bacteria and fungi. These observations are possibly due to an outer membrane in gram negative bacteria that acts as a barrier to many environmental stress including antibiotics [Chandrasekaran *et al*, 2005]. Among the tested strains of bacteria, PE extract showed maximum inhibition. It was most effective against *B. polymexia* and least against *P. aeruginosa*, which is naturally resistant to antibacterial agents [Walker and Edward, 1999]. PE extract was most effective against *C. albicans* among the fungal strains.

Table 1: Zone of inhibition (mm) of various extracts of *Leucus aspera* (Willd.) Linn. Aerial Parts.

Microorganisms	Zone of inhibition (mm)[a]												
	EA			Eac			NB			PE			Std[b]
	2	5	10	2	5	10	2	5	10	2	5	10	
Gram-positive bacteria													
Staphylococcus aureus ATCC 25923	9.7	17.3	18.3	10.3	16.7	20.3	6.7	12.3	15.7	12.0	16.7	21.3	27.3
Bacillus subtilis UC 564	8.3	18.0	20.0	9.7	16.3	19.7	6.3	10.7	15.0	10.3	16.7	20.7	25.0
Bacillus polymexia 474	7.7	12.7	16.7	10.7	15.7	19.0	7.0	11.3	15.3	12.7	17.0	22.3	22.3
Streptococcus faecalis ATCC 29212	7.0	10.7	14.7	10.3	12.7	18.3	8.7	11.3	15.7	11.6	14.3	21.3	26.7
Gram-negative bacteria													
Pseudomonas aerugenosa 25619	7.0	11.7	13.7	9.0	12.3	15.3	8.7	9.0	13.3	9.7	12.7	17.0	24.3
Salmonella typhi 57	9.3	16.7	17.3	11.0	18.3	22.3	8.7	14.3	15.0	12.3	17.3	20.0	23.3
Vibrio cholerae 824	8.0	11.3	16.7	10.3	16.3	19.0	8.3	11.7	14.3	11.7	18.0	21.3	24.7
Shigella dysenteriae ATCC C3	8.7	12.7	18.0	9.3	12.7	17.3	8.7	9.7	14.0	10.7	13.7	19.0	26.0
Escherichia coli NCTC 8196	8.0	14.7	15.7	9.3	13.0	17.7	7.7	11.3	12.7	11.0	14.7	18.3	22.3
Fungi													
Penicillum notatum ATCC 11625	8.3	11.7	14.7	9.7	14.3	15.7	7.0	9.7	12.7	12.7	15.7	17.0	21.7
Aspergillus niger AB 41	7.3	12.3	18.3	8.3	13.3	17.7	6.7	10.7	12.3	10.0	14.7	18.3	23.3
Candida albicans ATCC 18804	9.7	14.0	20.0	12.7	18.7	23.3	8.0	13.7	14.7	13.3	20.3	24.7	27.0

[a] Values are mean of three readings

[b] Standards: Antibacterial studies- Ciprofloxacin- 5 µg/ml; Antifungal studies- Cotrimazole- 25 µg/ml

The results of MIC study **(Table 2)** revealed the antimicrobial activity of EA, Eac, NB and PE extracts against the tested strains of microorganisms. MIC of EA ranges between concentration of 50 and 400 µg/ml, while that of Eac and NB are from 25 to 175 µg/ml and 100 to 600 µg/ml respectively. MIC value of PE ranges between 25 to 100 µg/ml. Results from zone of inhibition study and their corresponding MIC values indicate PE extract to be most effective.

Table 2: MIC (µg/ml) of various extracts of *Leucus aspera* (Willd.) Linn. Aerial Parts.

Microorganisms	EA	Eac	NB	PE
Gram-positive bacteria				
Staphylococcus aureus ATCC 25923	50	50	100	25
Bacillus subtilis UC 564	50	25	150	25
Bacillus polymexia 474	50	25	125	25
Streptococcus faecalis ATCC 29212	150	75	300	50
Gram-negative bacteria				
Pseudomonas aerugenosa 25619	400	175	600	100
Salmonella typhi 57	50	25	150	25
Vibrio cholerae 824	150	100	225	50
Shigella dysenteriae ATCC C3	175	75	300	25
Escherichia coli NCTC 8196	250	125	400	50
Fungi				
Penicillum notatum ATCC 11625	275	175	400	50
Aspergillus niger AB 41	200	100	400	75
Candida albicans ATCC 18804	150	75	300	50

Results from previous studies by various researchers indicate different bioactive compounds demonstrate various modes of antimicrobial activity. Maximum activity is shown by PE extract in the present study. This may be attributed to the hydrophobic nature of the extract which might be responsible for breakage of lipid bilayer of cell membrane, leading to permeability dissipation and a subsequent outflow of its cell contents.

Our results from the present study indicate the potential usefulness of *Leucus aspera* aerial parts in the treatment of various pathogenic diseases as mentioned in the Ayurvedic literature. Further study regarding the isolation and characterisation of the active constituents responsible for such activity is currently under progress.

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