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In-vitro evaluation of Antibacterial and Antioxidant activity of ethanolic extract of *Leucas aspera* roots and leaves

Sourabh Jain*, Akhilesh Gupta and Karunakar Shukla

College of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore (M.P.) - India

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Abstract

There is a currently worldwide upsurge in the use of herbal preparations and active ingredients of medicinal plant in health care. This is particularly true in the rural areas of Asian countries where herbal medicines are the only choice for treating human ailments. Present study reveals the data of *in-vitro* antibacterial and antioxidant activity of ethanolic and chloroform extracts of roots and leaves of *Leucas aspera* against the *selected bacterial strains*. Among all the extracts, ethanolic extract showed greater activity with a zone of inhibition (by disc diffusion assay) ranging from 6.5mm to 19mm. Ethanol extract of *Leucas aspera* exhibited better antibacterial activity with IC_{50} value range 17.95-50mg/ml when compared with chloroform extract. The antioxidant activity stable 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH assay) of ethanolic extract of *Leucas aspera* showed the highest antioxidant properties among all.

Keywords: DPPH Assay, *Leucas aspera*, Antibacterial, Solvent extracts, Disc diffusion.

Introduction

Plants are widely used as indispensable sources of food, medicine, dyes, flavoring agents and timber worldwide. Plants have been considered as an integral part of ethnomedicine and people from several parts of the world rely on plant based neutraceuticals and pharmaceuticals for primary healthcare needs. Plants are key ingredients in some medicinal systems such as Ayurveda, Traditional Chinese Medicine, Siddha and Unani.[1-7] The genus Leucas belonging to the family Labiatae includes shrubs or weeds. *Leucas aspera* commonly known as 'Thumbai' is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities.

An antioxidant could be a molecule capable of prevent the oxidation of other molecules. Oxidation could be a reaction that transfers electrons or hydrogen from a substance to an oxidant[8]. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a very cell, it can cause damage or death to the cell. When the chain reaction occurs in a much purified monomer, it produces a polymer resin, like a plastic, an artificial fiber, or a paint film.

***Corresponding Author**
E.mail: drsourabhjain@aku.ac.in

Antioxidants terminate these chain reactions by removing atom intermediates, and inhibit other oxidation reactions. They are doing this by being oxidized themselves, so antioxidants are often reducing agents like thiols, water-soluble vitamin, or polyphenols[9-12].

Material and Methods

Collection and identification of plant material

The plant *Leucas aspera* was collected from the "Satpura reserve forest" area near Amarkantak, MP in the month of December, 2018. Identification of the voucher specimen was done by available literature. The herbarium of plant was deposited in the Department of Pharmacognosy, College of Pharmacy, Dr. APJ Abdul Kalam University, Indore, MP. The specimen voucher no. received is AKUCOP/COG/SV/19-114. The areal parts of plant was collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

Processing of plant material and preparation of extract

The collected areal parts was shade dried and ground to form coarse powder and had been successively extracted[13-15] with the solvent per. ether, ethyl acetate and ethanol by Soxhlet apparatus and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

Evaluation of the extracts for antibacterial activity

The *in-vitro* antibacterial screening was carried out against selected bacterial pathogens. The bacterial pathogens were *Bacillus cereus* (MTCC1305), *Streptococcus aureus* (MTCC287), *E. coli* (MTCC-1424) and *Pseudomonas aureginosa* (MTCC-1401). These species were procured from Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. These organisms were identified by standard microbial methods [18]. The antibacterial screening of the extracts was carried out by determining the zone of inhibition using agar well diffusion method for bacteria. Levofloxacin was taken as reference antibiotic[16-17].

Standard drugs used and preparation of doses for antibacterial assay

Levofloxacin was used as Reference Antibiotics (RA). The stock solutions of RA were prepared in 10 % dimethylsulphoxide (DMSO) to give a concentration of 0.5 mg/ml for RA.

Agar well diffusion assay

Agar well diffusion method was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, Hi Media Laboratories Ltd., Mumbai). Plates were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria. Wells (8 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37 °C for 24 hrs for bacterial pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and statistical analysis was carried out.

Antioxidant Test

Antioxidant potential of the extract was estimated on the basis of the extract's scavenging activity of stable DPPH radical. First 25 mg of extract was dissolved to 50 ml of ethanol to prepare stock solution. Then 250, 200, 150, 100 and 50 µg / ml solution was prepared by diluting the stock solution. Then 1.5 ml of solution from the above was added to 1.5 ml of DPPH. This was kept in dark room for 20 min for allowing reaction. After that, absorbance or OD was measured by using UV Spectrophotometer at 517 nm against blank prepared. A blank was prepared without adding the extract. 10 mg of dry ascorbic acid was dissolved in 10 ml of methanol to prepare 5 different concentrations viz. 10, 20, 40, 60, 80 and 100 µg / ml of ascorbic acid. That was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical

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scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged} = A_{Control} - A_{Text} \times 100$$

Results and Discussion

Antibacterial Screening

The pet.ether extract (PELA), ethylacetate extract (EALA) & ethanolic extract (ETHLA) of plant *Leucas aspera* subjected to antibacterial screening against *Bacillus subtilis*, *Streptococcus aureus*, *E. coli* and *Staphylococcus aureus*. The results indicated that ethanol extract of *Leucas aspera* (ETHLA) exhibited zone of inhibition (in mm) at 250mg/ml against *Bacillus cereus* (15.75±0.957), *Streptococcus aureus* (11.50±0.577), *E. coli* (20.50±0.577) and *Pseudomonas aureginosa* (17.50±0.577) also ethyl acetate extract (EALA) showed *Bacillus cereus* (13.75±0.500), *Streptococcus aureus* (15.75±0.957), *E. coli* (18.75±0.500) and *Pseudomonas aureginosa* (18.50±0.577). Standard drug Levofloxacin (10 µg/ml) shows the zone of inhibition against *Bacillus cereus* (22.75±0.500), *Streptococcus aureus* (23.50±0.577), *E. coli* (24.50±0.577) and *Pseudomonas aureginosa* (21.75±0.817).

Table 1: Antibacterial activity (Zone of Inhibition) of *Leucas aspera* extracts against various organisms

Organisms	Extract	Zone of Inhibition (mm)				
		100 mg/ml	150 mg/ml	200 mg/ml	250 mg/ml	Standard (Levofloxacin) 10mcg/ml
Bacillus cereus	**PELA**	9.75±0.577	12.25±0.957	12.75±0.500	13.75±0.500	22.75±0.500
	EALA	8.50±0.577	10.50±0.577	12.25±0.500	13.75±0.500	
	ETHLA	11.50±0.577	12.00±0.000	13.50±0.577	15.75±0.957	
Streptococcus aureus	**PELA**	8.25±0.957	11.00±0.817	12.00±0.817	13.50±0.577	23.50±0.577
	EALA	10.00±1.141	11.75±1.258	13.75±0.957	15.75±0.957	
	ETHLA	9.75±0.500	10.50±0.577	11.00±0.816	11.50±0.577	
E. coli	**PELA**	10.00±0.817	11.50±0.577	12.50±0.577	15.75±0.957	24.50±0.577
	EALA	12.50±0.577	13.75±0.577	15.50±0.577	18.75±0.500	
	ETHLA	12.75±0.957	15.25±0.957	18.00±0.817	20.50±0.577	
Pseudomonas Aureginosa	**PELA**	9.50±0.577	10.75±0.500	11.50±0.577	12.50±0.577	21.75±0.817
	EALA	12.00±1.155	14.50±0.577	18.50±0.577	18.50±0.577	
	ETHLA	11.75±0.500	13.50±0.577	15.50±0.577	17.50±0.577	

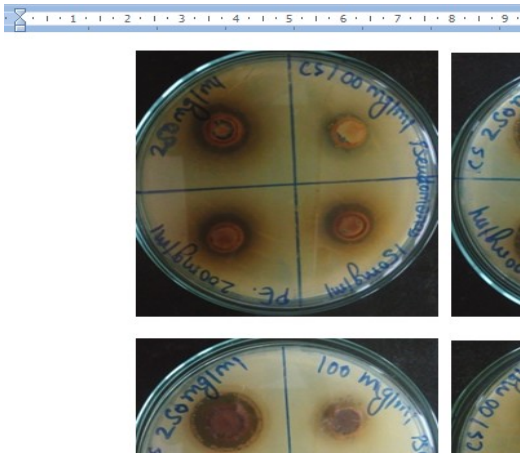

Fig. 1: Antibacterial activity of *Leucas aspera* extracts

Fig. 2: Antibacterial activity of standard drug (Levofloxacin) 10mcg/ml

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Antioxidant activity

DPPH radical scavenging activity of *Leucas aspera*

All the extracts exhibited scavenging activity against DPPH. The observations are given in Table.

Table 2: DPPH radical scavenging activity of *Leucas aspera*

Concentration (µg/ml)	% Inhibition of DPPH*			
	PELA	EALA	ETHLA	AA
10	22.24±0.204	42.16±0.245	45.32±0.425	45.89±0.624
20	25.69±0.444	46.80±0.539	47.80±0.478	51.60±0.178
40	28.81±0.539	48.15±0.353	52.57±1.164	58.30±0.272
60	32.39±0.204	50.26±0.467	59.77±0.390	67.42±0.178
80	37.80±0.444	53.09±0.176	62.97±0.664	78.76±0.642
100	41.09±0.636	56.97±0.176	65.27±0.941	89.05±0.332

* Mean±SD values are represented for n=3

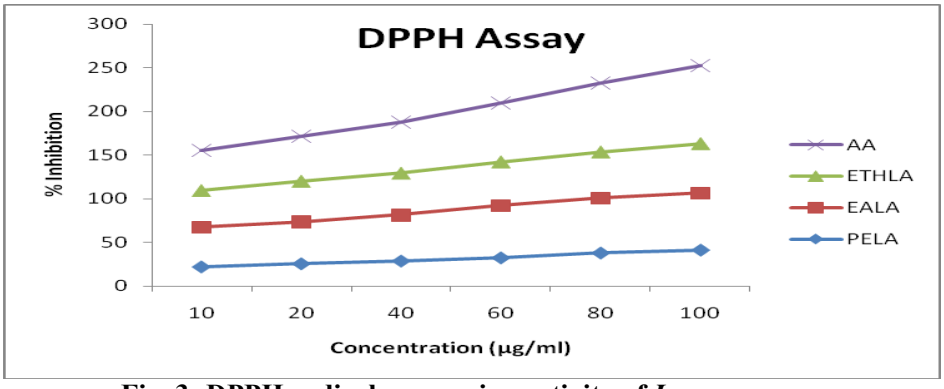

Fig. 3: DPPH radical scavenging activity of *Leucas aspera*

Conclusion

From the above observation we conclude that the ethanolic extract of the plant (ETHLA) have the antibacterial activity against bacteria collected from the soil. So we want to convey more attention to be told the precise mechanism of action and actual chemical constituents responsible for the antibacterial activity. Above observations also showed that the ethanolic extract of the plant (ETHLA) have the antioxidant activity so, we want to administer more mechanism of action and actual chemical constituents answerable for the antioxidant activity.

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