ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 2, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *LEUCAS ASPERA* FLOWERS FROM BIHAR, INDIA

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Received: 12 August 2017, Revised and Accepted: 11 November 2017

ABSTRACT

Objective: The aim of this study was to explicate antibacterial, antifungal, and antioxidant activities of *Leucas aspera* flowers.

Methods: Antibacterial activity was done by agar diffusion method. The ethyl acetate extract of *L. aspera* flower was evaluated against both Gram-positive and Gram-negative bacteria. Antifungal activity was also done by agar diffusion method. The agar used for antifungal activity was Czapek Dox Agar. Nitric oxide scavenging assay and free radical scavenging assay were used for the antioxidant activity. Griess reagent was used in nitric oxide scavenging assay. 1,1-diphenyl-2-picryl hydrazyl was used in free radical scavenging assay.

Results: *L. aspera* flower extract showed good antibacterial activity with the highest zone of inhibition against *Vibrio cholera* with 23 mm followed by *Bacillus polymyxa* showing 20 mm zone of inhibition. The ethyl acetate extract of *L. aspera* flower showed quite a good results with the highest inhibitory activity against *Aspergillus niger* with 13 mm zone of inhibition and lowest for *Trichoderma viridae* with 5 mm zone of inhibition. Antioxidant activity of *L. aspera* flower extract was done by free radical scavenging assay and nitric oxide scavenging assay. Nitric oxide scavenging assay showed prominent results almost performed equal to standard compound Butylated hydroxyl anisole (BHA) The values for 10 µl of *L. aspera* extract was 50.27, for the standard (BHA) showed 50.81. *L. aspera* extract values for 50 µl was 69.73 and for BHA, the values was 77.30. For 100 µl, the extract gave 82.70, and for standard BHA, the reading was 89.73.

Conclusion: The results showed that *L. aspera* flower has broad-spectrum antibacterial activity ranging from 23 to 13 mm zone of inhibition. *L. aspera* flower has strong antioxidative power on nitric oxide radicals. The medicinal properties of plant species have made an outstanding contribution to the origin and evolution of many traditional herbal therapies.

Keywords: *Leucas aspera* flower, Antibacterial activity, Antifungal activity, Antioxidant activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i2.21976

INTRODUCTION

Medicinal plants have shown a gilded source of antimicrobial agents. Plants are used in different countries and are a source of abounding potent and powerful drugs [1]. India has an extensive forest cover enriched with plant diversity [2]. Although hundreds of plant species have experimented for antimicrobial properties, there are many plants still not been evaluated [3]. Plants have the large-scale asset of being the most effective and cheaper alternative source of drugs [4]. In 2014, the World Health Organization gave its first report on surveillance of antimicrobial resistance, announcing that it is increasing global threat and scope to treat common nosocomial or community-acquired infection is at risk [5]. A vast variety of medicinal plants have been reported as an essential source of bioactive compounds [6]. The epidemiology of invasive infections has notably changed in the past 30 years. Hence, mycoses and bacterial infections are presently weighed as emerging diseases. This is an important area that needs the discovery of new and effective antimicrobial agents to gear antibiotic-resistant strains of pathogens [7]. Plants contain bioactive secondary metabolites with good potential to treat various diseases, and examples of some these compounds include terpenoids, alkaloids, phenols, unsaturated lactones, phenolic glycosides, saponins, sulfur compounds, cyanogenic glycosides, and glucosinolates [8,9]. Many pharmacognostical and pharmacological investigations are going on to identify new drugs for the development of novel therapeutic agents for the treatment of human diseases such as cancer and infectious diseases [10]. *Leucas aspera* is widely used to cure many diseased conditions, which connote that *L. aspera* has an infinite capacity for the discovery of new drugs. *Leucas* genus embraces 80 species [11]. *L. aspera* is an annual, branched, herb erecting to a height of 15–60 cm with stout and hispid acutely quadrangular stem and branches, and *L. aspera* flowers are white, sessile small, in dense terminal, or axillary whorls [12,13].

METHODS

Collection of *L. aspera* flowers

Fresh *L. aspera* flowers used in this study were collected from Munger, Bihar, with latitude 25.3748° N and 86.4735° E longitude and was authenticated by Dr. S.B. Padal, the Department of Botany, Andhra University. Voucher specimen number-22230 and deposited in Botany Department Herbarium, Andhra University, India.

Preparation of plant extracts

Fresh *L. aspera* flowers were collected washed thoroughly with distilled water 2–3 times and shade dried. Dried flowers were powdered using electric pulverizers. 25 g of shade-dried *L. aspera* flowers was filled in thimble made of Whatman No 1 filter paper and extracted successively with ethyl acetate in Soxhlet extractor for 48 h. The solvent was concentrated under reduced pressure at 40° in a rotary evaporator and stored at 4°C in an airtight bottle until further use.

Test cultures

The cultures were obtained from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

- Test Bacteria: Bacteria used for this research are *B. subtilis* MTCC *736, Bacillus polymyxa* (local isolate), *Escherichia coli* MTCC 723, *B. pumilus* MTCC 2466, and *Vibrio cholerae* MTCC 3906. These were maintained on nutrient agar slants.

- Test fungi: Fungi used for my research are *Aspergillus niger* MTCC 1881, *A. flavus* MTCC 1883, *Neurospora crassa* MTCC 1855, and *Trichoderma viride* MTCC241.

Antibacterial activity

Antibacterial activity was performed by agar diffusion method [14]. The stock cultures of bacteria were revived by inoculating in nutrient broth media and grown at 37°C for 18 h. The agar plates of the above media were prepared. Each plate was inoculated with 18 h, old cultures (100 µl, 10^{-4} colony-forming unit [CFU]), and spread evenly on the plate. After 20 min, the wells of size 6 mm was punctured by sterile cork borer filled with 100 µl of *L. aspera* flower ethyl acetate extract, and ciprofloxacin antibiotic was used as positive control (100 µg). All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone was noted in mm.

Antifungal activity

Antifungal activity was done by agar diffusion method [14]. Czapek Dox Agar media was used for the antifungal activity. The stock cultures were revived by inoculating in Czapek Dox Agar broth media and grown at 27°C for 48 h. The agar plates of the above media were prepared. Each plate was inoculated with 48-h-old cultures (100 µl 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells were punctured by sterile cork borer of 6 mm size and were filled with 100 µl of *L. aspera* flower ethyl acetate extract. Fluconazole was used as a positive control. All the plates were incubated at 27°C for 96 h, and the diameter of inhibition zone was noted in mm.

Antioxidant activity

Assay of free radical scavenging activity

Free radical scavenging activity was done by the method which was expressed by the study of Braca *et al.* [15]. Different concentrations (10 µl, 50 µl, and 100 µl) of *L. aspera* flower extract in dimethyl sulfoxide were taken in a series of test tubes. The volume was adjusted to 500 µl by adding methanol. 5 ml of a 0.1 mM methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) (DPPH; from Sigma-Aldrich, Bengaluru) was added to these tubes and shaken vigorously. A control without the test compound but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 min. The absorbance of the *L. aspera* flower extract was measured at 517 nm. Butylated hydroxyanisole (BHA) was used as reference standard. Free radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{(\text{control OD-sample OD}) \times 100}{\text{Control OD}}$$

Assay of nitric oxide scavenging activity

Nitric oxide scavenging assay was done by the method of Marcocci *et al.* [16]. The nitric oxide scavenging assay is done by Griess reagent. Antioxidant activity of *L. aspera* flower extract was evaluated by nitric oxide scavenging activity. Different concentrations (10 µl, 50 µl, and 100 µl) of *L. aspera* flower extract and BHA were taken in different test tubes and made up to 3 ml with 0.1 M phosphate buffer (potential of hydrogen [pH] 7.2). Sodium nitroprusside (5 mM) prepared in buffered saline (pH 7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at room temperature. A control without the test sample but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the *L. aspera* flower extract was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ NO radical scavenging activity} = \frac{(\text{control optical density-sample OD}) \times 100}{\text{Control optical density}}$$

RESULTS

The ability of *L. aspera* flower ethyl acetate extract to inhibit test bacteria was determined in this study. The result of antibacterial activity is summarized in Table 1. *L. aspera* flower extract showed good inhibitory activity against all the bacteria. The extract gave the highest zone of inhibition against *V. cholera* with 23 mm size of the zone of inhibition. *B. polmyxa* exhibited 20 mm zone of inhibition, followed by *B. pumilus* and *E. coli*. *L. aspera* flower extract showed least inhibitory activity against *E. coli* with only 13 mm zone of inhibition. Antifungal activity of *L. aspera* flower extract revealed good antifungal activity ranging from 13 mm to 5 mm. The highest zone of inhibition was displayed by *A. niger*; whereas the lowest was shown by *Trichoderma viridae* as given in Table 2. *L. aspera* flower extract showed impressive antioxidant activity by nitric oxide scavenging assay, and it showed activity almost equal to standard compound BHA as depicted in Table 3. *L. aspera* flower extracts 10 µl showed the value of 50.27 with the standard of 10 µl showing 50.81. The extract of 50 µl showed 69.73 as standard showing 77.30. The 100 µl of *L. aspera* flower extracts showed 82.70 and standard BHA showing 89.73. *L. aspera* flower showed moderate antioxidant result by free radical scavenging assay as given in Table 4. The 10 µl of the flower extracts gave the reading 15.33, whereas the standard displayed 54.27. The extracts of 50 µl showed 30.69 as standard gave 70.10. The 100 µl extracts showed 57.26, whereas the standard BHA gave 91.82. Diffusion method is broadly used to explore the antimicrobial activity of natural substances and plant extracts. Discs or holes are used in this assay that contains solutions of the sample to be investigated [17]. The discovery of novel antimicrobial metabolites from medicinal plants is an important alternative to

Table 1: Antibacterial activity of *L. aspera* flower EA extracts

Microorganism	*L. aspera* flower (EA) extract zone of inhibition in mm (100 µl)
B. polymyxa	20±3.61
B. megaterium	17±2.0
B. pumilus	16±1.73
E. coli	13±2.65
V. cholera	23±3.61

Values are mean inhibition zone (mm)±SD of three replicates.
E. coli: Escherichia coli, L. aspera: Leucas aspera, B. polymyxa: Bacillus polymyxa, B. megaterium: Bacillus megaterium, B. pumilus: Bacillus pumilus, V. cholera: Vibrio cholera, EA: Ethyl acetate, SD: Standard deviation

Table 2: Antifungal activity of *L. aspera* flower EA extract

Microorganism	*L. aspera* flower (EA) extract zone of inhibition in mm (100 µl)
A. niger	13±2.65
A. flavus	6±1.73
Trichoderma viride	5±2.0
Neurospora crassa	7±2.65

Values are mean inhibition zone (mm)±SD of three replicates.
A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, T. viride: Trichoderma viride, N. crassa: Neurospora crassa, L. aspera: Leucas aspera, SD: Standard deviation, EA: Ethyl acetate

Table 3: % nitric oxide scavenging activity of *L. aspera flower* EA extract

Concentration (µl)	% nitric oxide scavenging activity	
	L. aspera flower (EA) extract	BHA
10	50.27±0.072	50.81±0.062
50	69.73±0.151	77.30±0.458
100	82.70±0.062	89.73±0.139

Values are±SD of three replicates. *L. aspera: Leucas aspera,* BHA: Butylated hydroxy anisole, SD: Standard deviation, EA: Ethyl acetate

Table 4: % free radical scavenging activity of *L. aspera* flower EA extract

Concentration (µl)	% free radical scavenging activity	
	***L. aspera* flower (EA) extract**	**BHA**
10	15.33±0.096	54.27±0.125
50	30.69±0.082	70.10±1.153
100	57.26±0.079	91.82±0.062

Values are ± SD of three replicates. *L. aspera: Leucas aspera*, BHA: Butylated hydroxy anisole, SD: Standard deviation, EA: Ethyl acetate

overcome the increasing levels of drug resistance by human pathogens. Novel antimicrobial substance from medicinal plants is a substitute to overthrown the increasing levels of drug resistance by human pathogens. New antibiotics and chemotherapeutic agents research is increasing in the chemistry of medicinal plants [18].

Medicinal plants are an important source of antioxidants [19]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases. Antioxidant activity of the plasma is increased by natural antioxidants that help in reducing the danger if diseases [20].

DISCUSSION

About 80% of the world's population uses traditional herbal medicine for their primary health care [21]. *L. aspera* plants were evaluated for antimicrobial and antioxidant activitises. This study aimed to regulate the antibacterial activity of five selected bacteria. *V. cholera* showed the highest zone of inhibition with 23 mm. *B. polymyxa* showed 20 mm zone of inhibition. Antibacterial activity of *L. aspera* root, flower, leaf, and stem showed good antibacterial activity against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *S. typhimurium*, *S. choleraesuis*, and *Shigella flexneri* by Chew *et al*. in 2012 [22]. *L. aspera* flower in this study showed satisfying antifungal activity. *A. niger* displayed apical zone of inhibition with 13 mm. *L. aspera* flower showed the lowest zone of inhibition for *T. viridae* with only 5 mm. Tahareen *et al.* in 2016 studied antibacterial activity on *L. aspera* leaves showed that *E. coli* was inhibited at all concentrations, followed by *Klebsiella* and *Pseudomonas* [23]. Gowrish *et al.* in 2016 studied antibacterial activity of *L. marrubioides*, the results depicted that pet ether extract was very much effective against Gram-positive bacteria *B. cereus, S. Pyogenes*, and *B. subtilis* and Gram-negative bacteria *Proteus mirabilis*, *Klebsiella pneumoniae*, and*V. cholerae* [24]. In 1987, Thakur studied antifungal activity of *L. aspera* ethanol and chloroform extracts against *Trichophyton* and *Microsporum*, the plant reported having fungicidal and fungistatic activity. *L. aspera* flower extract showed highly impressive antioxidant activity by ferric radical scavenging activity and nitric oxide scavenging assay. In 2016, Tahareen *et al.* performed antioxidant activity of *L. aspera*, and *L. Aspera* (40%) exhibited maximum DPPH radical scavenging activity which was found to be 80.69±3.68 [23]. In 2007, Rahman *et al.* studied antioxidant activity of ethanolic extracts of *L. aspera* root and exhibited high free radical scavenging assay [25]. Increased bacterial strains to variety of antimicrobial agents have demanded new antibacterial agents effective against pathogenic bacteria [26].

CONCLUSION

In the present study, L. aspera flowers have shown broad-spectrum antibacterial activity and antifungal activity. The flowers can be used as natural medicine in the treatment of various infectious diseases that are caused by fungi and bacteria. The flowers also gave incredible antioxidant activity by nitric oxide scavenging assay. Infectious diseases are the second leading cause of death. Antibiotics are used continuously, due to this microorganism has become resistant. Antibiotics also show a lot of side effects that have an adverse effect on human beings, so the medicinal plant can be used and explored in great amount for treatment of infectious diseases.

ACKNOWLEDGMENT

The authors are thankful to University Grants Commission-Maulana Azad National Fellowship for providing financial support and Department of Microbiology, GITAM University for providing laboratory facilities.

AUTHORS CONTRIBUTION

Gulnaaz sabri performed the experimentation as part of her Ph.D. Gulnaaz sabri and Y.Vimala prepared the manuscript, Y.Vimala supervised the work, evaluated the data and corrected the manuscript for publication. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

We declare we have no conflict of interest.

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