DOI : *10.15613/sijrs/2021/v8i1&2/217892*
ISSN (Print): 2349-8919
ISSN (Online): 2350-0999
***ScieXplore:* International Journal of Research in Science,** *8(1&2), 28-32, January-December 2021*

Antimicrobial Activity of *Leucas aspera* (Willd.) Link on Selected Bacterial and Fungal Species

M. Saravanaraja*, P. S. Arunadevi and M. Aamina

Post Graduate and Research Department of Zoology, Sri Vasavi College, Erode - 638316, Tamil Nadu, India; drmsrsvc333@gmail.com

Abstract

Leucas aspera is a short green and highly branched herb that belongs to the family Labiatae and order tubiflorae. The present study indented to explore the pharmacological potential of the selected plant species *Leucas aspera* through antimicrobial studies and phytochemical studies. *Leucas aspera* plants for the present study are collected from farms of Guruvarediyur Village, Erode District. Extraction of the plant materials is followed by standard methods. The plant extract was tested for antibacterial and antifungal activity by the standard agar well-diffusion method. The antibacterial and antifungal activity of *L. aspera* extracts also supported the results of some previous studies and the study strongly proves the potential antimicrobial activity of the plant.

Keywords: Antibacterial and Antifungal Activity, *Leucas aspera*, Pharmacological Potential

1. Introduction

Leucas aspera is a short green and highly branched herb that belongs to the family Labiatae and order tubiflorae. They have up to 1.5 cm in length, thick, sub-sessile leaves with short petiole and linear narrow oval shapes. Flowers are bright white, sessile and dense in terminal or axillary whorls with modified green leaf bract. Bracts are with cilia and hairs. The varied tubular calyx is around 12 mm long. The flowers are tube curved with contracted just above nutlets, membranous lower half, ribbed upper half, and small mouth with oblique and not villus. Smooth fruit nutlets are around 3 mm long, quadri-lateral, and brown with an angular inner face and round outer face.

The documentation and antimicrobial studies of a plant are worthwhile to know the phytochemical and pharmaceutical importance[1]. Therefore, the present study indented to explore the pharmacological potential of the selected plant species *Lecas aspera* through antimicrobial studies and phytochemical studies.

2. Materials and Methods

Leucas aspera plants for the present study are collected from farms of Guruvarediyur Village, Erode District. Extraction of the plant materials is followed by the standard methods of Chessbrough[2].

Preliminary phytochemical screening was carried out following standard procedures by Harborne, Kokate and Sani *et al.*,[3–5].

The plant extract was tested for antibacterial activity by the standard agar well-diffusion method against pathogenic bacteria[14] (Perez, 1990). After incubation at 37°C for 24 hours, the different levels of the zone of inhibition were measured. For the present antibacterial investigation was conducted against *Staphylococccus aureus, E. coli, Pseudomonas sp.* and *Bacillus sp.*

Well-spread agar medium in a petri dish is used for measuring the antifungal activity of extracts of *Leucas aspera*. By using sterile cork borer, a well with a 10 mm diameter was created in that petri dish. The standard

*Author for correspondence

drug was prepared by dissolving 10 mg of crude extract of *Leucas aspera* in 10 ml of DMSO Solution. 200 µl of the mixture was impregnated in the well. The plates were kept in an incubator at 28 ± 2 °C for 48 hours. Meanwhile, a well with a 10 mm diameter filled with Clotrimazele (200 µg/disc) was kept as a positive control for the present investigation. Fungal species including *Candida tropicalis, Candida albicans, Tricophyton mentagrophytes, Microsprum gypsum, Microsporum nanum, Aspergillus flavus, Epidermophyton floccosum* and *Penicillium sp.* were used for the present antifungal test.

3. Results

The present phytochemical studies include quantitative and qualitative analysis to find out the presence and amount of phytochemical constituents like tannins, flavonoids, protein, saponins, phenols, steroids, alkaloids and carbohydrates.

In the present study, qualitative tests were conducted for water, 50% and 100% extracts of *Leucas aspera*. The results of the study revealed the presence of protein, phenols, alkaloids and carbohydrates in water extracts. However, 50% and 100% of ethanol consist of tannins, flavonoids, protein, saponins, phenols, steroids, alkaloids and carbohydrates.

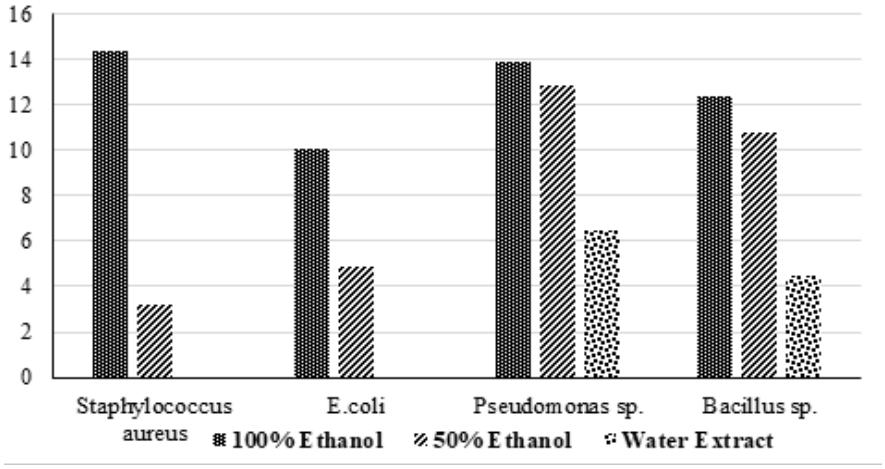
Based on qualitative analysis, extracts were selected for further analysis. In the present quantitative analysis, the ethanol extract of *Leucas aspera* contains 0.3% tannin, 0.4% flavonoids, 6.38% protein, 0.86% saponins, 0.91% steroids, 8.0% alkaloids and 11.92% carbohydrates were quantified.

Qualitative phytochemical tests were carried out for both (aqueous and ethanol) extracts. Flavonoids are excellent phytochemical constituents with great potential to treat diseases like lipid peroxidation. Flavonoids are polyphenolic compounds also recognized as free radical scavenging activity. As antioxidants, these compounds can release hydrogen molecules to free radicals. Results of the present study indicate that the ethanol extract has greater flavonoid content than the ethanol extract but both extracts have high flavonoid content. The presence of flavonoid content in *Leucas aspera* is 100 mg/ml for aqueous extract and 185 mg/ml for ethanol extract.

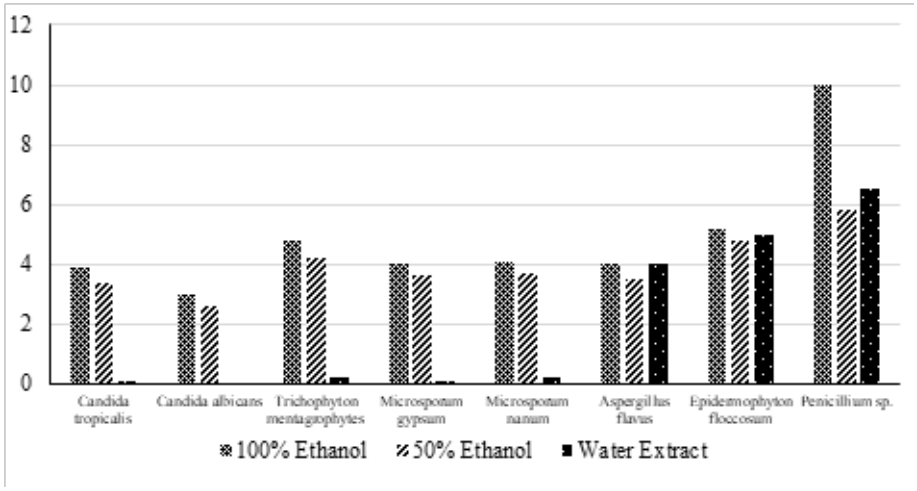
Phenolic compounds are estimated by using the regression equation of the calibration curve at 765 nm. The results are expressed with the unit of Gallic Acid Equivalents (GAE). In the present experiment, total phenolic compounds are 94 mg/GAE/ml and 172 mg/GAE/ml for aqueous and ethanol extracts respectively. Anti-bacterial activity of 100% ethanol, 50% ethanol and water extract of *Leucas aspera* against selected bacterial species like *Staphylococcus aureus, E. coli, Pseudomonas sp.* and *Bacillus sp.* were recorded.

50% ethanol extract showed moderate level of resistance against *Staphylococcus aureus, E. coli,*

Pseudomonas sp. and *Bacillus sp.* with a zone of inhibition 3.2 mm, 4.9 mm, 12.9 mm and 10.8 mm respectively. However, 100% ethanol extract showed a high level of zone inhibition against *Staphylococcus*



Graph 1. Anti-Bacterial Activity of *Leucas aspera.*



Graph 2. Anti-fungal Activity of *Leucas aspera.*

aureus, E. coli, Pseudomonas sp. and *Bacillus sp.* with 14.4 mm, 10.1 mm, 13.9 mm and 12.4 mm respectively.

There was no zone of inhibition against *Staphylococcus aureus* and *E. coli* in water extracts of *Leucas aspera.* However, the water extract showed a minimum level of the zone of inhibition i.e. 6.5 mm and 4.5 mm against *Pseudomonas sp.* and *Bacillus sp.* respectively.

Antifungal activity of water, 50% and 100% ethanol extracts of *Leucas aspera was* recorded. Water extracts of *Leucas aspera* plant showed a minimum level of resistance against selected fungal species including *Candida tropicalis, Candida albicans, Trichophyton mentagrophytes, Microsporum gypsum, Microsporum nanum, Aspergillus flavus, Epidermophyton floccosum* and *Penicillium sp.* were 0.1 mm, 0.2 mm, 0.1 mm, 0.2 mm, 4.0 mm, 5.0 mm and 6.5 mm respectively.

It is evident that all the studied pathogens were found to be susceptible to ethanol extract of *Leucas aspera*. The highest activity was recorded against *Penicillum sp.* by the 100% ethanol extract of *Leucas aspera* (with a zone of inhibition of 10 mm) which is nearer to the control where the control clotrimazele showed an inhibition zone of 10.5 mm. The minimum activity was shown against *Candida albicans* with a 3 mm zone of inhibition. With other fungal species, the extract showed a moderate level of the zone of inhibition within the range from 3.9 mm and 5.2 mm.

The 50% ethanol extract of *Leucas aspera* evaluated for antifungal activity is found to have a higher range of zone of inhibition for *Penicillium sp.*, *Epidermophyton floccosum, Trichophyton mentagrophytes* showed respective highest levels of the zone of inhibition with 5.8 mm, 4.8 mm and 4.2 mm. *Candida albicans*, *Candida tropicalis* and *Aspergillus flavus* showed the least level of zone of inhibition with 2.6 mm, 3.4 mm and 3.5 mm respectively. *Microsporum gypsum* and *Microsporum nanum* showed a moderate level of the zone of inhibition with 3.6 mm and 3.7 respectively.

4. Discussion

In the present experiment, the presence of phytochemicals including alkaloids, flavonoids, proteins, sapnonis, phenols, steroids and carbohydrates. Especially, the presence of alakaloids, tannins and flavonoids is clear evidence for the actual pharmacological potential of the plant *L. aspera* in quantitative estimation itself.

Even though the qualitative analysis showed the presence of these constituents, quantitative estimation of these constituents is essential for the further consideration of pharmacological applications of the plant parts of *Leucas aspera.* Therefore, in the present study, with the assistance of various standard experimental methods, the chemical constituents were estimated.

In the present study, 0.8% of alkaloids are estimated. Karthikeyan *et al.,* reported the presence of alkaloids in the methanol extract of whole plant *L. aspera*[6].

The qualitative and quantitative experiments and various references clearly illustrate the presence of a wide range of phytochemicals in *Leucas aspera.* The potential

of phytochemicals in pharmacology is a well-known fact. In general, various pharmacological applications like treating swelling, arthritis, cough, jaundice, bronchial asthma, digestive impairment and intermittent fever are mentioned in ayurveda articles.

Extracts of *Leucas aspera* showed great antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aerugiousa* and *Bacillus subtillis.* The extracts also showed excellent antibacterial activity against *Salmonella typhimurium*, *Salmonella choleraesis* and *Shigella flexneri*. In addition, the study of Ilango *et al.*,[7] reported about antibacterial activity of methanolic and ethanolic extracts of *L. aspera* against *Staphylococcus epidermids* and *Klebsiella pneumonia.*

However, instead of whole plant extract leaf extract of *L. aspera* showed a very high level of antibacterial activity[8]. But the plant extracts showed a varied range of antibacterial activity against *E. coli* due to differences in the dose. Rahman and Islam reported that gram-positive bacteria are more sensitive than gram-negative bacteria[9].

Experiments by Udayakumar and Begum, Rajakaruna *et al.,* and Valsaraj *et al.,* on antibacterial activity of *L. aspera* extracts also supported the results of present antibacterial activity[10–12].

In the present study, antifungal activity of extracts of *L. aspera* was observed. Pure ethanol extracts of *L. aspera* showed a maximum level of the zone of inhibition against selected fungal species including *Candida tropucalis, Candida albicans, Trichophyton mentagrophytes, Microsporum gypsum, Epidermatophyton flaccsosum and Penillium sp.* The antifungal activity of the plant extract was similar to an experiment conducted by Mangathayaru *et al*[13]. Results of an experiment by Sayedalam *et al.,* and Thakur also supported the results of the present experiment[14,15].

In another study, Akther *et al.,* reported that methanol extracts of *L. aspera* were not showed any antifungal activity, while in the present study ethanol extracts showed a good range of antifungal activity[16].

5. References

1. Kaci Z, Tirchi N, Dahmane T, Berrai H, Sihem RH, Boubekeur, Chebli A, Biche M. First ethnobotanical study relating to usage of medicinal plants in province of Ain Defla Region, South-West of Algeria. Indian Journal of Ecology. 2022; 49(3):655-664. https://doi.org/10.55362/IJE/2022/3576
2. Chessbrough M. Medical laboratory manual for tropical countries. Linacre House, Jordan Hill. Oxford; 2000. p. 260.
3. Harborne JB. Phytochemical methods. Chapman and Hill; 1973.
4. Kokate CK. Practical pharmacognosy. 4th ed. Vallabh Prakasan, Delhi; 1994. p. 107 - 113.
5. Sani AA, Ilyas M, Haruna AK. Phytochemical screening of the leaves of lophiralanceolata (Ochanaceae). Life Science Journal. 2007; 4(4):75-79.
6. Karthikeyan S, Sivakumar A, Anbalagan M, Nalini E, Gothandam KM. Finger printing of alkaloids, steroids and flavonoids using HPTLC of *Leucas aspera* L. whole plant Methanolic extract. J Pharm Sci and Res. 2013; 5:67-71.
7. Ilango K, Ramya S, Gopinath G. Antibacterial activity of *Leucas aspera* spreng. Int J Chem Sci. 2008; 6(2): 526-530.
8. Saritha K, Rajesh A, Manjulatha K, Setty OH, Yenugu S. Mechanism of antibacterial action of the alcoholic extracts of *Hemidesmus indicus* (L.) R. Br. ex Schult, *Leucas aspera* (Wild.), *Plumbago zeylanica* L., and *Tridax procumbens* (L.) R. Br. ex Schult. Front Microbiol. 2015; 6:577. https://doi.org/10.3389/fmicb.2015.00577 PMid:26106379 PMCid:PMC4460426
9. Rahman MA, Islam MS. Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract. Asian Pac J Trop Biomed. 2013; 3:273-279. https://doi.org/10.1016/S2221-1691(13)60062-3. PMid:23620850
10. Udayakumar R, Begum VH. Antimicrobial studies of some selected medicinal plants. Anc Sci Life. 2002; 21:230-239.
11. Rajakaruna N, Harris CS, Towers GHN. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharm Biol. 2002; 40:235-244. https://doi.org/10.1076/phbi.40.3.235.5825
12. Valsaraj R, Pushpagadan P, Smitt UW, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol. 1997; 58:75-83. https://doi.org/10.1016/S0378-8741(97)00085-8 PMID:9406894
13. Mangathayaru K, Thirumurugan D, Patel PS, Pratap DV, David DJ, Karthikeyan J. Isolation and identification of nicotine from *Leucas aspera* (willd) Indian J Pharm

Sci. 2006; 68:88-90. https://doi.org/10.4103/0250-474X.22972

14. Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion methods. Acta Boil Med Exp. 1990; 15:113-115
15. Sayedalam M, Sanjit S, Lee DU. Antibacterial and In vivo cytotoxic activities of the leaves of *Leucas aspera*. J Korean Soc Appl Biol Chem. 2014; 57(2014):551-554. https://doi.org/10.1007/s13765-014-4202-1
16. Thakur DK, Misra SK, Choudhuri PC. *In vitro* trials of plants extracts and chemicals for their antifungal activity. Indian J Animal Health. 1987; 26:31-35.
17. Akther M, Khan MAI, Mushin MDA, Hamid K, Obayed Ullah M., Bulbul IJ, Firoz A., Burkan Uddin M, Urmi KF. Invitro studies on antifungal and cytogenic properties of *Leucas aspera*. B. Mid., 2012; 4:183-187.