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AN EXPERIMENTAL STUDY ON QUALITATIVE PHYTOCHEMICAL TEST & ANTIMICROBIAL ACTIVITY OF *LEUCAS ASPERA* FLOWERS

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Abstract

Objective:

This study set out to explain the phytochemistry and antimicrobial properties of *Leucas aspera* flowers.

Method

Using standard techniques, qualitative analysis was carried out to identify different phytochemical elements. Agar diffusion was used to perform antibacterial properties. Both Gram-positive & Gram-negative bacteria were tested for resistance to the ethyl acetate extract of the *L. aspera* flower. Agar diffusion was also used to measure antifungal activity. Czapek Dox Agar was the agar used to test for antifungal properties.

Results:

Study of the *L. aspera* flower extract found that tannins, flavonoids, glycosides, saponins, alkaloids, reducing sugar, and phenols were present. With the maximum zone of inhibition against *Vibrio cholera* at 24 mm and *Bacillus polymyxa* at 21 mm, *L. aspera* flower extract demonstrated strong antibacterial effect. The *Aspergillus niger* was most inhibited by the *L. aspera* flower's ethyl acetate extract (12 mm zone of inhibition), whereas *Trichoderma viridae* was least inhibited (4 mm zone of inhibition).

Conclusion: The findings indicated that tannins, flavonoids, glycosides, saponins, alkaloids, reducing sugar, and phenols are present in *L. aspera* flower. It possesses broad-spectrum antibacterial action with a zone of inhibition of 24 to 14 mm. The inception and development of

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many traditional herbal medicines have benefited greatly from the therapeutic qualities of many plant species.

Key-Words: Phytochemical, Antimicrobial, *Leucas aspera* flower, Glycosides

INTRODUCTION

Antimicrobial molecules have been shown to be abundant in medicinal plants. Many effective and strong medications are derived from plants, which are utilized in many countries [1]. India has a sizable forest cover that is rich in diverse plant life [2]. Numerous plants have still not been investigated, despite the fact that hundreds of plant species have been tested for their antibacterial characteristics [3]. The most advantageous aspect of plants is that they are the most affordable and efficient source of medications [4]. Numerous therapeutic plants have reportedly been identified as a significant source of bioactive chemicals [5]. In the last 30 years, there has been a significant change in the epidemiology of invasive diseases. The current classification of mycoses & bacterial infections as emerging illnesses reflects this. To combat disease strains that are resistant to antibiotics, it is crucial to find new and potent antimicrobial agents [6].Terpenoids, alkaloids, phenols, unsaturated lactones, phenolic glycosides, saponins, sulfur compounds, cyanogenic glycosides, and glucosinolates are a few examples of bioactive secondary metabolites found in plants with the potential to cure a variety of ailments [7,8]. In order to find new medications for the creation of novel therapeutic agents for the treatment of human diseases including cancer and infectious diseases, numerous pharmacognostical and pharmacological research are being conducted [9]. *Leucas aspera* is extensively used to treat a variety of diseases, suggesting that *L. aspera* has an endless capacity for the development of novel medications. There are 80 species in the *Leucas* genus [10]. L. aspera is an annual plant that branches out and grows to a height of 15 to 60 cm. Its branches and stem are stout and hispid, and its flowers are white, sessile, tiny, and arranged in dense terminal or axillary whorls. [11,12]

The plant is extremely beneficial for treating a variety of medical conditions, including analgesic, antipyretic, antirheumatic, anti-inflammatory, anti-bacterial, anti-fungal, psoriasis, chronic skin eruptions, chronic rheumatism, skin inflammation, painful swellings, stimulant, expectorant, aperients, diaphoretic, and insecticide.[13-16]

MATERIALS & METHODS

Collection of *L. aspera* flowers

Fresh *L. aspera* flowers used in this study were collected from Vindhya Herbals, Bhopal, Madhya Pradesh and was authenticated.

Preparation of plant extracts

Fresh L. aspera flowers were picked, completely cleaned with distilled water three to four times, and then dried in the shade. Electric pulverizers were used to powder the dried flowers. In a thimble

constructed of Whatman No. 1 filter paper, 25 g of shade-dried L. aspera flowers were placed, and for 48 hours, they were progressively extracted with ethyl acetate in a Soxhlet extractor. The solvent was heated to 4°C in an airtight container and concentrated at 40° under decreased pressure in a rotating evaporator.

Test cultures

- Test Bacteria: Bacteria used for this research are *Bacillus megaterium , Bacillus polymyxa* , *Escherichia coli*, *Bacillus pumilus ,* and *Vibrio cholerae* . These were maintained on nutrient agar slants.
- Test fungi: Fungi used for my research are *Aspergillus niger*, *Aspergillus flavus* , *Neurospora crassa* , and *Trichoderma viride*.

Qualitative phytochemical analysis

Qualitative analysis for detection of various phytochemical constituents were performed using standard methods.

Antibacterial activity

Agar diffusion was used to perform antibacterial activity. By inoculating the stock cultures of bacteria in nutrient broth media and growing them for 18 hours at 37°C, the cultures were revived. 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). The diameter of the inhibitory zone was measured in millimeters on each plate after a 24-hour incubation period at 37°C.

Antifungal activity

The agar diffusion technique was used to perform antifungal activity. For the antifungal activity, Czapek Dox Agar medium was utilized. After being reactivated, the stock cultures were cultured at 27 °C for 48 hours while being inoculated in Czapek Dox Agar broth medium. The aforementioned media's agar plates were made.

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. A positive control was fluconazole. The diameter of the inhibitory zone was measured in mm after 96 hours of incubation at 27°C on each plate.

RESULTS

To examine the phytochemicals in the *L. aspera* flower, many phytochemical tests were carried out. **Table 1** provides a summary of the phytoconstituents present. Additionally, this investigation

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established the *L. aspera* floral ethyl acetate extract's capacity to inhibit test microorganisms. **Table 2** provides an overview of the antibacterial activity's results. All the microorganisms were effectively inhibited by *L. aspera* flower extract. With a zone of inhibition of 24 mm in diameter, the extract demonstrated the greatest zone of inhibition against *V. cholera. B. pumilus, B. polmyxa, and E. coli* all showed a 21 mm zone of inhibition. With just a 14 mm zone of inhibition, *L. aspera* flower extract exhibited the least amount of E. coli inhibition.The *L. aspera* flower extract's antifungal activity showed excellent antifungal activity spanning from 12 mm to 4 mm. As demonstrated in **Table 3**, *A. niger* exhibited the maximum zone of inhibition, whereas *Trichoderma viridae* exhibited the lowest.

Table 1. Phytochemical analysis of Leucas aspera flower

S.N.	Phytochemicals	Result
1.	Tannins	+
2.	Phlobatannins	-
3.	Flavonoids	+
4.	Terpenoids	-
5.	Steroids	-
6.	Glycosides	+
7.	Saponins	+
8.	Anthraquinones	-
9.	Carotenoids	-
10.	Alkaloids	+
11.	Reducing Sugar	+
12.	Phenols	+

Table 2 : Antibacterial activity of L. aspera flower ethyl acetate extracts

S.N.	Bacteria	L. aspera flower ethyl acetate extract zone of inhibition in mm (100 µl)
1.	*Bacillus polymyxa*	21±3.71
2.	*Bacillus megaterium*	18±2.0
3.	*Bacillus pumilus*	17±1.63
4.	*Escherichia coli*	14±2.69
5.	*Vibrio cholera*	24±3.62

Table 3: Anti fungal activity of L. aspera flower ethyl acetate extract

S.N.	Fungi	L. aspera flower ethyl acetate extract zone of inhibition in mm (100 µl)
1.	*Aspergillus niger*	12±2.69
2.	*Aspergillus flavus*	5±1.78
3.	*Trichoderma viride*	4±2.0

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4.	*Neurospora crassa*	6±2.45

CONCLUSION

The *L. aspera* flowers used in this study demonstrated the presence of a variety of phytochemicals as well as broad-spectrum antibacterial and antifungal activities. The blossoms are a natural remedy that may be used to cure a number of infectious disorders brought on by fungus and bacteria. The second biggest cause of death is infectious infections. Since antibiotics are often used, bacteria have developed resistance to them. Since antibiotics frequently cause side effects that are harmful to people, it is important to use and research medicinal plants as much as possible to cure infectious illnesses.

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CONFLICT OF INTEREST

We declare we have no conflict of interest.

REFERENCES

1. Srivastava J, Lambert J, Vietmeyer N. Antimicrobial Activity of *Anogeissus latifolia*. Medicinal plants: An Expanding Role in Development. World Bank Technical Paper. No.320. 106. Journal of Ethnopharmacology; 1996. p. 57-61.
2. Parekh J, Chanda S. *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. Afr J Biotechnol 2008;7:4349-53.
3. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985;228:1154-60.
4. van der Watt E, Pretorius JC. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. J Ethnopharmacol 2001;76:87-91.
5. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci 2005;78:431-41.
6. Rapp RP. Changing strategies for the management of invasive fungal infections. Pharmacotherapy 2004;24:4S-28S.
7. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. Curr Med Chem 2001;8:1467- 86.
8. Quiroga EN, Sampietro AR, Vattuone MA. Screening antifungal activities of selected medicinal plants. J Ethnopharmacol 2001;74:89-96.
9. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 2003;66:1022-37.
10. Hedge IC. Labiatae. In: Ali SI, Nasir YJ, editors. Flora of Pakistan. Karachi: University of Karachi Department of Botany; 1990. p. 192.
11. Kirtikar KR, Basu BD. Indian Medicinal Plants. New Delhi: Periodical Experts; 1975. p. 2019-20.

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12. Hooker JD. The Flora of British India. London: The Muston Company; 1984. p. 690
13. Chopra, R.N, Nayar, S.L. & Chopra, I.C. (1996). Glossary of Indian Medicinal Plants. NISC, New Delhi, pp. 153.
14. Gani, A. (1998). Medicinal plants of Bangladesh: Chemical constituents and uses. Pp.467.
15. Kirtikar, K.R. & Basu, B.D. (1991). Indian medicinal plants, Singh B and Singh M.P. Publishers, Vol. 3.
16. Mangathayaru, K., Lakshmikant, J., Shyam, S.N., Swapna, R., Grace, X.F. & Vasantha, J. (2005). Antimicrobial activity of *Leucas aspera* flowers. Fitoterapia, 76, 752–754.