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# Study of Biochemical Analysis and Antimicrobial Activity of *Leucas urticifolia* (Vahl) Sm. Medicinal Plant

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**ABSTRACT**

The present study indicates the biochemical analysis and antimicrobial activity of the folk medicinal plant *Leucas urticifolia* (Vahl) Sm. (Family-Lamiaceae). The primary biochemical analysis showed that it is rich in phytochemicals which are extractable in methanol. The qualitative biochemical analysis showed the presence of nine compounds. The quantitative analysis of medicinal plant has revealed the presence of different compounds like reducing sugar, carbohydrate, starch, total amino acids, proteins and chlorophyll pigments. In the given species the concentrations of chlorophyll-a (1.84 mg/g fresh weight) are more than chlorophyll-b (1.32 mg/g of fresh weight). The total amino acids content was estimated to be 19.4 ± 0.32 mg/g and protein content was found to be 7.6 ± 0.32 mg/g in *Leucas urticifolia*. The starch content (10.4 ± 0.16 mg/g) was found to be higher than the reducing sugars (1.84 ± 0.03 mg/g). The carbohydrate content was estimated to be 11.06 ± 0.01 mg/g in *L. urticifolia.* The antimicrobial activity of the plant extract with zone of inhibition against fungi like *Fusarium oxysporum* (0.8 ± 0.1 mm), *Aspergillus niger* (0.9 ± 0.4 mm), *Trichoderma viride* (0.8 ± 0.2 mm) and *Penicillium notatum* (1.0 ± 0.2) for methanolic extracts was found to be higher than the control. The results indicate that the biochemicals are present in higher quantities therefore this medicinal plant can be of pharmacological importance. This study also enlightens the antimicrobial potential of the medicinal plant *L. urticifolia* due to presence of these biochemicals.

***Key words****: Antimicrobial, Biochemical, Karjat, Lamiaceae*, Leucas, *Phytochemicals.*

## Introduction

The family Lamiaceae is acknowledged as a group of plants for many centuries for its pharmaceutical and culinary interest. The essential oil obtained from these plants is used in tooth-pastes, medicine and ointments. The family Lamiaceae consists of 200 genera and 3200 species (Singh, 2001). The studies conducted by Rajput (Rajput, 2020) recorded presence of different useful phytochemicals in the members of family Lamiaceae from Dhar district of Madhya Pradesh, India. The genus *Leucas* is a promising source of natural materials for drug development and discovery because of their phyto-chemistry and pharmacological activity as reported by Sachin Kumar *et al*. (2023). Various plant parts like leaf, flower, fruit and root decoction of *Leucas urticifolia* (Vahl) Sm. is administered against fever,

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cold, cough and to relieve swelling (Katewa and Galav, 2005). According to Fatima *et al.* (2008) *Leucas urticifolia* (Vahl) Sm. entire plant Methanolic extract yielded the organic acids methoxy benzyl benzoate and 4-hydroxyl benzoic acid. The volatile oil obtained from leaves of *L. aspera* shows antimicrobial activity with high sensitivity for microorganisms like *Candida albicans, Haemophilus influenza, Pseudomonas aeruginosa* and *Staphylococcus aureus* and the same fraction revealed no sensitivity for microorganisms like *Aspergillus niger*, *Bacillus subtilis, Neisseria gonorrhea, Proteus vulgaris* and *Trichoderma viride.* These variations may be due to the constituents and composition of the extracts (Gerige *et al*., 2007). This study will focus on biochemical analysis and antimicrobial activity of *L. urticifolia.*

## Materials and Methods

### Study Area

Maharashtra lies in India's central-western region having an area of 3,07,690 sq.kilometers, and it is situated between 22°1' to 16° 4' N Latitude and 72° 6' to 80° 9' E Longitude. It is approximately 800 km east-west and 700 km north-south. Maharashtra has a variety of climatic and edaphic factors and has varied natural ecosystems due to different environmental conditions. Karjat Tehsil lies in Ahmednagar district consisting of 118 villages.

Climate is a major determinant of condition in Karjat tehsil and experiences semi-arid climate. Monsoon rainfall is prime source of this region with approximately 600 mm rainfall during monsoon season. May and June are the hottest months with an average maximum temperature 40 °C. Temperature reduces during December and January with an average minimum temperature of 12 °C. The winter season commences from November up to February whereas rainy season is observed from June to October.

### Survey and Collection

The present work is based on the result of one year (2021 - 2022) of comprehensive study in Karjat tehsil, from Ahmednagar district of Maharashtra state. Efforts were carried out to visit the study area in different seasons to cover all the possible areas of the tehsil. In order to achieve the objectives mentioned above, intensive surveys have been under taken along different topographic and climatic gradient. During the field study a *Leucas urticifolia* was collected and recorded. The photographic evidences of the plant were also gathered from diverse habitats. Most of the photographs of *Leucas urticifolia* have been collected in their flowering and fruiting stages for the correct botanical identification (Figure 1).

### Identification

The identification of collected plant specimen has been done satisfactorily with the help of regional and national floras like 'Flora of Presidency of Bombay' (Cook, 1908, 1958)' Flora of British India '(Hooker, 1885) and from 'Flora of Baramati' (Bhgat *et al*., 2008). The majority of the laboratory work involved accurately identifying the specimens with the help of flora.

### Preliminary screening of phytochemical

The collected *Leucas urticifolia* plant were properly washed and dried. After drying it was used for extract preparation. The plant material weighted was ground in mortar and pestle with equal amount methanol till the formation of fine paste and left for overnight and filtered. This filtrate was used as (100%) crude extract. The freshly prepared extract was used for standard phytochemical analysis to check the presence or absence of compounds like glycosides, terpenoids, alkaloids, tannins, saponins, phenols, flavonoids and steroids (Harborne, 1998).

### Biochemical analysis

The biochemical analysis of different biomolecules was done using standard procedures. The chlorophyll estimation was done as per Arnon method (1949). The proteins were estimated using Lowery's method (1951). The phenol-sulphuric acid method by Dubois *et al*. (1956) was used to determine total carbohydrates. The Moore and Stein (1948) protein method was used to determine total free amino acids. The estimation of starch was done by Holligan *et al.,* (1974) method and reducing sugars by dinitrosalicylic acid method (1966).

### Antimicrobial Screening of *Leucas urticifolia*

#### A. Preparation of extract

*Leucas urticifolia* fresh plant material was thoroughly washed with tap water, distilled water, and dried. Ten grams of dried plant material were obtained, crushed in a mortar and pestle with an equal quan-

tity of methanol, and filtered using Whatman's filter paper. This filtrate was used to assess antimicrobial activity by disc diffusion assay (Bauer *et al*., 1966).

**B. Test**

The effect of *Leucas urticifolia* extract has been studied with four different microbiological strains. The fungal strains –*Aspergillus niger* (NFCCI 3114), *Fusarium oxysporum* (NFCCI 1276), *Penicillium notatum* (NFCCI 1072) and *Trichoderma viride* (NFCCI 1139) were obtained from the National Fungal Culture Collection of India, Agharkar Research Institute Pune, India. They were sub cultured and maintained on Sabouraud's dextrose agar and nutrient agar medium.

**C. Preparation of Muller-Hinton agar media (MHA) and inoculums**

Muller-Hinton agarmedia was prepared, sterilized and poured 20 ml into sterilized petri dishes and allowed for solidification. All fungal strains were inoculated on the given medium, incubated for 48 hr. at 18 -20 ºC by transferring single colony on media and spread on all over using glass spreader.

**D. Preparation of antibiotic solution**

The sterilized distilled water (10 ml) and 1 g antibiotic Nastatin statin was mixed. Two ml of this solution is taken by a sterilized pipette and then added into 98 ml of sterilized distilled water in a flask to make the concentration 10 µg/ml.

**E. Determination of antimicrobial activity by using disc diffusion assay**

The study of antimicrobial activity was undertaken as per the method described by Bauer *et al.* (1966). The disk diffusion assay method is easy and practical and has been well-standardized. As per the National Committee for Clinical Laboratory Standards, the disc diffusion method was used for testing the extract for antimicrobial activity (Santra *et al*., 1999; Veljic *et al*., 2009).

Muller-Hinton Agar medium and Nutrient Agar medium was sterilized at 121°C and distributed in sterilized petri dishes to give a thickness of 4.0 mm ± 0.5 mm (Altuner and Cetin, 2009). The plates were inoculated with test organism suspension of fungal strains of volume 100 µl ($10^6$ CFU/ml spores). The antibiotic Nastatin (10 µg) was used as positive control. The Whatman's No.1 filter paper discs (6 mm in diameter) were sterilized and saturated with 1-3 ml of the crude extract and then placed on inoculated agar plates. The plates were incubated at 30 °C for 48 hr. The inhibition zone around the discs was measured in mm and used to express the antimicrobial activity. The diameter of inhibition zone is found to be directly proportional to the strength of antibiotics as well as sensitivity of fungi. The mean value of zone of inhibition was calculated using replicates.

## Results and Discussion

The primary biochemical analysis of *Leucas urticifolia* indicated that the plant is rich in various phytochemicals. The qualitative biochemical analysis with methanolic extract of *Leucas* showed the active presence of nine compounds except lignin (Table 1). These results are in accordance with the studies conducted by Rajput and Satya (2019), who reported that the phytochemical analysis of leaf extract of *Leucas urticifolia* showed presence of 11 compounds. Similar results were reported from

**Table 1.** Qualitative Phytochemical Analysis of *Leucas urticifolia* (Vahl) R. Br. ex Sm.

| Sr. No. | Class of compound | Leaf extract | |
|---|---|---|---|
| | | Aqueous | Methanol |
| 1. | Alkaloids | + | + |
| 2. | Flavonoids | - | + |
| 3. | Glycosides | - | + |
| 4. | Phenolics | - | + |
| 5. | Tannins | - | + |
| 6. | Lignins | - | - |
| 7. | Steroids | + | + |
| 8. | Carbohydrates | + | + |
| 9. | Amino acids | + | + |
| 10. | Quinone | - | + |

(+ indicates presence in sample & - indicates absence or not detected in sample)

Geethika and Kumar (2018) showing the methanol extracts of *L. stelligera* with the presence of most of the phytochemicals i.e., alkaloids, flavonoids, tannins, phenolic, terpenoids, proteins, carbohydrates and glycosides with the exception of saponins, proteins and total amino acids.

The quantitative analysis of the freshly collected plant samples were tested for the concentration of chlorophyll a, b, and total chlorophyll using Arnon (1949) method. The concentrations of chl-a, was found to be more than chl-b in the given species. The chlorophyll- a content in *Leucas urticifolia* was

**Fig. 1.** *Leucas urticifolia* (Vahl) Sm.

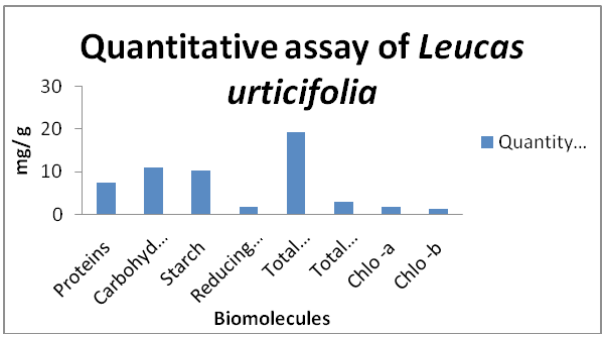


**Fig. 2.** Assay of Bio-compounds of *Leucas urticifolia* (Vahl) Sm.

(1.84 mg/g fresh weight) and chlorophyll- b was (1.32 mg/g fresh weight). The concentration of total chlorophyll in *Leucas urticifolia* was 3.17mg/g fresh weight (Table 2, Figure 2). According to Gayathri and Karthika (2016), the total chlorophyll, chlorophyll-a and chlorophyll -b content were estimated for different medicinal plants. The chlorophyll -a content of *Solanum nigrum* and *Leucas aspera* was estimated to be 0.21± 0.01 and 0.477 ± 0.15 mg respectively and the chlorophyll- b content was estimated to be 0.023± 0.16mg in *L. aspera* compared to 0.076 ± 0.03 mg in *S. nigrum*. The total chlorophyll content was higher in *L. aspera* (0.45 ± 0.04 mg).

The assay of bio-compounds like total amino acids, proteins, carbohydrates, starch and reducing sugars of *Leucas urticifolia* is presented in (Table 3, Figure 2). The total amino acids content was estimated to be 19.4 ± 0.32 mg/g in *L. urticifolia* and the protein content in the given sample was 7.6 ± 0.32 mg/g. The results of Gayathri and Karthika (2016), were similar showing that the protein content in *S. nigrum* was 7.4 ± 3.11 mg and in *L. aspera* was 10.8 ± 1.70 mg.

This study indicates that the levels of reducing sugars (1.84 ± 0.03 mg/g) are lower than starch (10.4 ± 0.16 mg/ g) in *Leucas urticifolia*. The carbohydrate content was estimated to be 11.06 ± 0.01 mg/g in *L. urticifolia*. Similar results were reported from Gayathri and Karthika (2016), showing the carbohydrate content (1.57 ± 0.19 mg) in *S. nigrum* and (1.50 ± 0.20 mg) in *L. aspera*.

The antimicrobial properties of methanolic extract of *L. urticifolia* were examined in comparison

**Table 3.** Assay of Biocompounds of *Leucas urticifolia* (Vahl) R. Br. ex Sm.

| Sr.No. | Contents | Quantity (mg/g sample) |
|---|---|---|
| 1. | Proteins | 7.6 ± 0.32 |
| 2. | Carbohydrates | 11.06 ± 0.01 |
| 3. | Starch | 10.4 ± 0.16 |
| 4. | Reducing sugar | 1.84 ± 0.03 |
| 5. | Amino Acids | 19.4 ± 0.32 |

Values are mean of three replications

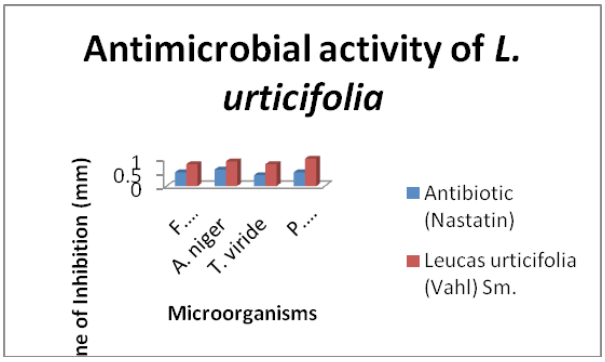


**Fig. 3.** Antimicrobial activity of *Leucas urticifolia* (Vahl) Sm.

**Table 2.** Pigments -Chlorophylls Contents in *Leucas urticifolia* (Vahl) R. Br. ex Sm.

| Sr. No. | Material | Chlo -a | Chlo -b | Total Chlorophyll | Chlorophyll a:b ratio |
|---|---|---|---|---|---|
| 1 | *Leucas urticifolia* (Vahl) R. Br. ex Sm. | 1.84 ± 0.00 | 1.32 ± 0.03 | 3.17 ± 0.04 | 0.71 ± 0.4 |

*Values are mean of three determinations expressed as mg/g fresh tissue (± S.D.).

**Table 4.** Antifungal activity of *Leucas urticifolia* (Vahl) R. Br. ex Sm.

| Sr. No. | Material | Inhibition zone (mm) | | | |
|---|---|---|---|---|---|
| | | *F.oxysporum* | *A. niger* | *T. viridae* | *P. notatum* |
| 1. | *Leucas urticifolia* (Vahl) R. Br. ex Sm. | 0.8 ± 0.1 | 0.9 ± 0.4 | 0.8 ± 0.2 | 1.0 ± 0.2 |
| 2. | Antibiotic (Nastatin) | 0.5 ± 0.1 | 0.6 ± 0.3 | 0.4 ± 0.1 | 0.5 ± 0.2 |

mm- Millimeter, *F-Fusarium*, *A-Aspergillus*, *T-Trichoderma*, *P-Penicillium.*

with common antibiotic, which served as positive control (Table 4, Figure 3). The extract of *L. urticifolia* showed the efficient antimicrobial activity against fungi like *Fusarium oxysporum* (0.8 ± 0.1 mm), *Aspergillus niger* (0.9 ± 0.4 mm), *Trichoderma viride* (0.8 ± 0.2 mm) and *Penicillium notatum* (1.0 ± 0.2). The extract exhibited higher inhibition zones against all studied fungal strains than that of control (Table 4, Figure 3). It means that the plant have antimicrobial potential and can be exploited in pharmacognosy. Similar results were obtained by Sandosskumar *et al.* (2007), where the aqueous extract of *L. aspera* showed an inactive result against fungal strain *Aspergillus flavus.* The reports given by Gerige *et al.* (2007) reported that the oil extracted from the leaves of *L. aspera* showed high sensitivity for microorganisms like *Candida albicans, Haemophilus influenza, Pseudomonas aeruginosa* and *Staphylococcus aureus* while it showed no sensitivity against microorganisms like *Aspergillus niger, Neisseria gonorrhea, Bacillus subtilis, Proteus vulgaris* and *Trichoderma viride*.

It concludes that the plant *Leucas urticifolia* has medicinal properties due to its rich biochemical content. The antimicrobial properties are due to presence of ample amount of constituents in the plant. Thus, the plant can be a source of medicine in Pharmcognosy.

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