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# Antifungal Activity and Qualitative Phytochemical Analysis of Some Medicinal Plants in Jaffna (Sri Lanka)

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## Abstract

Many members of the Labiatae family are used in traditional and folk medicine and also used as culinary and ornamental plants. Leaves are the most used plants parts of this family. Ethanolic extract of the leaves, stem, seeds of *Leucas zeylanica*, *Ocimum canum*, *Ocimum sanctum* and leaves of *Mentha arvensis*, *Ocimum basilicum* were subjected to phytochemical screening and antifungal assays against *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Mucor* sp., *Rhizopus* sp. was determined by using the agar streaking assay method after 48 and 72 hours of incubation. All parts of the plants were found to contain flavonoid/s and alkaloid/s except for the absence of alkaloids in seeds of *L. zeylanica* and stem of *O. sanctum* respectively. Tannins were present in all parts of plants such as *L. zeylanica*, *O. canum*, *M. arvensis* and absent in *O. sanctum* and *O. basilicum*. Phlobatannins were only present in leaves of *L. zeylanica* and saponins were present only in leaves of *O. basilicum*. The leaves of *L. zeylanica*, *O. basilicum*, *M. arvensis*, *O. sanctum* and seeds of *O. sanctum* and *O. canum* showed the presence of steroids. Terpenoids were present in all parts of *O. sanctum* and *O. canum* than the other plants. The cardiac glycosides were present in all parts of *O. sanctum* than the other plants tested. Leaves of *O. sanctum* and *M. arvensis* exhibited strong positive antifungal activity against *Aspergillus* sp. Leaves of *O. canum*, *O. basilicum* and *M. arvensis* and stem of *O. canum* showed strong positive activity against *Mucor* sp. *L. zeylanica* only exhibited the antifungal activity against *Mucor* sp. *Penicillium* sp. was inhibited by the leaves and seeds extracts of *O. sanctum*. Degree of activity was low in *L. zeylanica* compared with other plant extracts. Most of these plant parts did not show any activity against *Trichoderma* sp. and *Rhizopus* sp. This study revealed that the antifungal activity of leaves of these plants was high than other plant parts against tested fungi.

**Keywords**

*Leucas zeylanica*, *Ocimum canum*, *Ocimum sanctum*, *Mentha arvensis*, *Ocimum basilicum*, Antifungal Activity

## 1. Introduction

Many medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse type of plants grow in different parts of the country [1]. The Labiatae family (Lamiaceae) is one of the largest and most distinctive families of flowering plants. In Sri Lanka, there are 63 species belonging to 12 genera of these 51 are indigenous and 12 being endemic [2]. *Leucas zeylanica* (muditumpai-T; geta-thumba-S), *Ocimum canum* (ganjankorai-T; heentala-S), *Ocimum sanctum* (karunthulasi-T; madurutala-S), *Ocimum basilicum* (tirnutpachi-T; suwanda-tala-S) and *Mentha arvensis* (pudina-T; odutalan-S), are used in herbal medicine in Sri Lanka and other countries. Bioactive compounds with antimicrobial activity play an important role in herbal medicine. Objective of this study is the extraction of different plant parts in ethanol, identifying the phytochemicals qualitatively and screening for their antifungal activity.

## 2. Materials and Methods

The whole plant of *L. zeylanica*, *O. canum*, and *O. sanctum* were collected from uncultivated farmlands located at meesalai (chavakachcheri), Jaffna in the Northern Province of Sri Lanka. *O. basilicum* and *M. arvensis* were collected from farmlands at Thirunelvely (Jaffna) in the Northern Province of Sri Lanka. The taxonomic identities of these plants were confirmed by using herbarium samples preserved in the department of Botany, University of Jaffna.

### 2.1. Method of Extraction of Plant Materials

The plant materials were air dried and these samples were ground into uniform powder with electric blender. 100 g of each ground samples were taken into a container separately and adequate amount of ethanol was added to an each container with occasional shacking. Each extract was filtered after 24 hours period. This procedure was repeated and both filtrates were combined. The solvent was evaporated by using rotatory evaporator and the weights were recorded.

### 2.2. Phytochemical Screening Tests for Ethanol Extract

The phytochemical tests have carried out on the crude of the ethanol extract (0.2 g) using standard phytochemical procedures [3]. The compounds interests are alkaloids, saponins, terpenoids, steroids, flavanoids, tannins, phlobatannins, and cardiac glycosides.

Test for alkaloids: A few drops of Wagner's reagent were added to the crude

(0.2 g) of the sample along the wall of the test tube.

Test for tannins: 0.2 g of crude of the sample was boiled with 20 ml of distilled water and it was filtered. Then few drops of 0.1% $FeCl_3$ were added.

Test for phlobatannins: 0.2 g of crude of the sample was boiled with 1% aqueous hydrochloric acid.

Test for saponins: 0.2 g of crude of the sample was boiled with 20 ml of distilled water in a water bath and it was filtered. Then filtrate was mixed with 5 ml of distilled water and it was shaken vigorously for a stable persistent froth.

Test for flavonoids: 0.2 g of crude of the sample was heated with 10 ml of ethylacetate over a steam bath for 3 minutes. The filtrate was shacked with 1 ml of dil $NH_3$ solution.

Test for steroids: 2 ml of acetic anhydride to 0.2 g of crude sample with 2 ml of con $H_2SO_4$ were added.

Test for terpenoids: 3 ml of con $H_2SO_4$ was added carefully to the mixture of 5 ml of extract and 2 ml of $CHCl_3$.

Test for cardiac glycosides: Treat 0.2 g of crude with 2 ml of glacial acetic acid which contains one drop of $FeCl_3$ solution and this underplay with 1 ml of con $H_2SO_4$.

### 2.3. Anti Fungal Activity Assay for the Ethanol Extracts

All plants extracts were tested for their antifungal activity against different fungi such as *Pencillium* sp., *Aspergillus* sp., *Rhizopus* sp., *Mucor* sp. and *Trichoderma* sp. Antifungal activity of the extracts was determined by using the agar streaking assay method [4]. The extracts of *L. zeylanica* (seeds, stem and leaves) were streaked on Potato Dextrose Agar (PDA) in the Petridish at equal distance among them. A fungal disc (5 mm) (*Penicillium* sp.) was taken with the help of sterile cork borer and it was placed on the centre of above PDA medium. The antifungal activity was determined in terms of inhibition/antagonism (strong positive, weak positive and no activity) after 48 hours, 72 hours of incubation periods. All these test plates were compared with control plate. The above procedure was repeated to fungi such as *Aspergillus* sp., *Mucor* sp., *Trichoderma* sp. and *Rhizopus* sp. and other plants.

## 3. Results and Discussion

The phytochemical analysis of five medicinal plants is summarized in **Table 1**. Phytochemical screening tests of the plants such as *L. zeylanica*, *O. canum*, *O. sanctum*, *O. basilicum* and *M. arvensis* showed that the flavanoids were the common constituents. *L. zeylanica* and *O. canum* contained tannins in the stem and leaves except seeds. Alkaloids were found in leaves, stem of *L. zeylanica* and absent in seeds. Similarly alkaloids were transparently seen in all plant parts of *O. canum*. But the leaves of *L. zeylanica*, *O. canum*, *O. sanctum*, *O. basilicum* and *m. arvensis* also showed the presence of alkaloids. Saponins and phlobatannins were only found in leaves of the *O. basilicum*, *L. zeylanica* respectively and

absence in other parts of these plants. The extract from leaves of all plants exhibited the presence of steroids except *O. canum*. All plant parts of *O. sanctum*, leaves and seeds of *O. canum* and leaves of *O. basilicum* and *L. zeylanica* showed the presence of cardiac glycosides. Flavanoids and terpenoids were found in leaves, stem and seeds of the *O. sanctum* and *O. canum*. The presence of alkaloids in *O. sanctum* has been reported by the researchers and this plant widely used in herbal medicine [5].

The crude extracts from seeds and leaves of *O. sanctum* exhibited significant antifungal activity on most of the tested fungi. The degree of inhibition varied among fungi. Only seeds and stem of *L. zeylanica* showed positive activity against *Mucor* sp. after 48 hours and weak positive after 72 hours. This plant didn't show any activity against *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp. after 48 and 72 hours (Table 2).

**Table 1.** Phytochemical analysis of different plants parts.

| Plants | *L. zeylanica* | | | *O. canum* | | | *O. sanctum* | | | *O. basilicum* | *M. arvensis* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Seeds | Leaves | Stem | Seeds | Leaves | Stem | Seeds | Leaves | Stem | Leaves | Leaves |
| Tannins | − | + | + | + | + | + | − | − | − | + | − |
| Phlobatannins | − | + | − | − | − | − | − | − | − | − | − |
| Alkaloids | − | + | + | + | + | + | + | + | − | + | + |
| Saponins | − | − | − | − | − | − | − | − | − | + | − |
| Flavonoids | + | + | + | + | + | + | + | + | + | + | + |
| Steroids | − | + | − | + | − | − | + | + | − | + | + |
| Terpenoids | − | + | − | + | + | + | + | + | + | − | − |
| Cardiac glycosides | − | + | − | − | + | + | + | + | + | + | − |

Note: +presence of constituent; −absence of constituent.

**Table 2.** Effects of crude of plant extracts on the growth of fungi after 48 and 72 hours.

| Micro organisms | *L. zeylanica* | | | | | | *O. canum* | | | | | | *O. sanctum* | | | | | | *O. basilicum* | | *M. arvensis* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Seeds (1) | | Stem (2) | | Leaves (3) | | Seeds (4) | | Stem (5) | | Leaves (6) | | Seeds (7) | | Leaves (8) | | Stem (9) | | Leaves (9) | | Leaves (9) | |
| | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 | 48 | 72 |
| *Aspergillus* sp. | − | − | − | − | − | − | − | − | − | − | ++ | + | +++ | ++ | +++ | ++ | − | − | ++ | + | +++ | ++ |
| *Mucor* sp. | ++ | + | ++ | + | − | − | + | − | +++ | + | +++ | ++ | ++ | + | − | − | ++ | − | +++ | + | +++ | + |
| *Penicillium* sp. | − | − | − | − | − | − | − | − | − | − | − | − | +++ | ++ | +++ | ++ | + | − | +++ | ++ | +++ | ++ |
| *Trichoderma* sp. | − | − | − | − | − | − | − | − | − | − | + | − | + | − | ++ | − | − | − | − | − | + | − |
| *Rhizopus* sp. | − | − | − | − | − | − | − | − | − | − | ++ | + | − | − | ++ | + | − | − | − | − | − | − |

+++strong positive activity, ++positive activity, +weak positive activity and −no activity.

Leaves of *O. canum* showed strong positive activity against *Mucor* sp. after 48 hours and positive activity after 72 hours. Stem and seeds extracts of this plant did not show any activity against other fungi. Seeds of *O. canum* exhibited less activity compared with leaves and stem. *O. sanctum* showed the highest inhibition and strong positive activity against *Aspergillus* sp., *Penicillium* sp. at 48, 72 hours except stem of *O. sanctum. A*t the same time leaves of *O. sanctum* showed positive activity against *Trichoderma* sp. and *Aspergillus* sp.

Stem of *L. zeylanica*, *O. canum* and *O. sanctum* commonly showed antifungal activity against *Mucor* sp. Similarly, leaves of *O. canum*, *O. sanctum*, *O. basilicum* and *M. arvensis* commonly inhibited *Aspergillus* sp. except leaves of *L. zeylanica*. Leaves of *M. arvensis*, *O. basilicum* and *O. canum* showed strong positive activity against *Mucor* sp. Leaves, stem, seeds of *L. zeylanica* didn't show activity against *Trichoderma* sp. This was due to inheritance of fungi, type and the amount of antifungal compounds present in the plant extract to exhibit inhibition. Previous studies indicated that these plants leaves had antibacterial as well as antifungal activity especially against dermatophytic fungi [6] [7] [8] [9]. But the present study showed that not only leaves but other plant parts exhibited antifungal activity.

This study revealed that the extract of *O. basilicum* and *O. sanctum* and *M. arvensis* contained significant amount of antifungal compounds and most of these compounds were present in leaves than other parts of these plants.

## 4. Conclusion

The present study of medicinal plants in family Labiatae showed that distribution of phytochemicals such as alkaloids, terpenoids, steroids, flavanoids, tannins and cardiacglycosides were rich compared to the distribution of phlobatannins, and saponins. Degree of antifungal activity varied among plants as well as among plant parts. Growth of *Aspergillus* sp., *Mucor* sp. and *Penicilium* sp. were strongly inhibited by *O. sanctum*, *O. basilicum* and *M. arvensis* extracts while *Trichoderma* sp. and *Rhizopus* sp. growth was only inhibited by *O. canum*, *O. sanctum*. Antifungal compounds were rich in leaves than the other parts except *L. zeylanica.* Leaves of Labiatae family members except *L. zeylanica* could be used to treat fungal diseases in herbal medicine. Further studies could be carried out to purify these bioactive compounds.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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