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# Antifungal Effects of English Camphor Basil (Ocimum canum) Leaves and Flower Extracts on some Selected Fungi Associated with Skin Infections

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**ABSTRACT**

*Investigations were carried out to ascertain the antifungal activity of English camphor basil (Ocimum canum) leaves and flower extracts on some selected fungi associated with skin infections. Plant samples were collected at Mista Ali, Bassa LGA of Plateau State. Phytochemical analysis was carried out in the Biochemistry Laboratory of National Veterinary Research Institute Vom (NVRI), located in Jos South LGA. Qualitatively, both extract revealed the presence of steroids, cardiac glycosides and flavonoids while tannins were detected in the leaves extract only. There was significant ($p<0.05$) variation in the quantity of inherent phytochemical components of the extracts. Cardiac glycoside was present in the leaves and flower extracts of Ocimum canum. The test organisms, T. mentagrophytes, T. tonsurans, T. rubrum, T. vericosum, M. canis were obtained from the microbial banks of bacteriology and dermatophilosis sections of NVRI, Vom and were standardized with a Nephelometer. T. mentagrophytes was the only fungi specie susceptible to both extracts. The flower extract had significantly higher ($p<0.05$) antifungal activity ($20 \pm 0.93$ mm) against T. mentagrophytes than the leaves extract ($16.7 \pm 1.53$ mm). Both extracts showed MIC of 25 mg/ml against T. mentagrophytes. MFC ranged from 50-100 mg/ml for the sensitive fungal isolate. Fractional Inhibitory Concentration Index (FICI) from the combined extracts varied from 1.89 to 3.97on the fungus and that showed lack of interaction ($FICI<4$). Synergistic activity of O. canum was not effective on fungal isolates tested. However, bioactive constituents of the plant parts can be of pharmacological importance.*

**Keywords:** Antifungal, Leaves, Flower, Extracts, Ocimum canum, English camphor basil.

## INTRODUCTION

From the dawn of civilization, medicinal plants have been part of human society to combat diseases [1]. Herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins, lesser cost and a model for drugs that have made it to the market [2]. Many of today's modern drugs have their origin in traditional plants medicines [3,4]. Natarajan et al. and Iqbal et al. discovered the therapeutic efficacies of many indigenous plants for several disorders described by practitioners of traditional herbal medicines [5,6]. The genus Ocimum involves economically the most important medicinal and aromatic herbs in the world. It belongs to the family Lamiaceae, and comprises more than 30 species distributed in tropical and subtropical regions of Asia, Africa, and Central and South America [1]. Traditionally, the genus Ocimum is widely used for the treatment of various ailments including rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenzae, gonorrhea, mental illness, abdominal pains, colds, coughs and measles and has also antipyretic, anthelmintic and antimalarial effects [3]. It also contains aromatic compounds and essential oils which contains biologically active constituents that possess insecticidal, nematicidal and fungistatic properties [2,4]. *Ocimum canum* is commonly called Ocimum americanum Linn. The main uses of *O. canum* are antimicrobial, antioxidant and antidiabetic. It is also used in the treatment of skin diseases and Genito-urinary problems. It is known as English camphor basil, American basil or hoary basil. Herbal synergism is definitely a line of exploration in the development of new drugs.

## MATERIALS AND METHODS

### Sample collection

The plant materials (leaves and flowers) were collected at Mista-Ali, Bassa Local Government Area of Plateau State. Test organisms; *Trichophyton mentagrophytes, T. tonsurans, T. rubrum, T. vericosum* and *Microsporum canis* were obtained from the microbial banks of Mycological and Bacteriological sections of National Vertinary Research Institute, Vom in Jos South Local Government Area of Plateau State.

### Authentication of selected fungi

Biochemical test such as urea hydrolysis test and lactophenol cotton blue stain was employed for identification of fungi [7].

### Authentication of plant

Authentication of the plant was carried out at Federal College of Forestry located at Bauchi Road in Jos North Local Government Area of Plateau State. Botanical keys were used to obtain specimen (plant) voucher [8].

### Preparation of plant materials

The plant leaves and flowers were separately cleaned with tap water and dried at room temperature (25°C) in a shade for a period of 2-4 weeks. The dried leaves and flowers were then pulverized into fine powder using mortar and pestle and stored in an airtight plastic container.

### Ethanolic extraction

The method of Fatope *et al*. was used for the ethanolic extraction [9]. Two hundred and fifty grams of the powdered flower material was successively extracted separately using cold maceration with 450 ml of ethanol (95%) for 24 hours, and one hundred and eighty grams of powdered leave material was also successively extracted with 180 ml of the same solvent. The extracts were filtered using Whatman No.1 filter paper and allowed to evaporate in a rotary evaporator at 45°C. The dried extracts were preserved at refrigeration temperature.

### Phytochemical screening

Qualitative phytochemical analysis of the extracts of the plant was determined by the methods used by Jigna and Sunitra [10]. The extracts were screened for the presence of alkaloids, saponins, flavonoids, tannins, cardiac glycosides, carbohydrate, steroids and terpenes (Table 1).

**Table 1:** Effect of combined extracts on *Trichophyton mentagrophytes*

| Fungal species | MIC Combined | MIC Leaf | MIC Flower | Fractional Inhibitory Concentration Index (FIC I) | Interaction |
|---|---|---|---|---|---|
| *Trichophyton mentagrophytes* | 23.66[a] | 25.00[b] | 25.00[c] | 1.89[d] | Indifference |

-No activity, ND= Not Determined; Values with the different letters as superscript within the row and column varied significantly at p<0.05.

### Standardization of microbial isolates

Standardization of test organisms was done using a Nephelometer based on McFarland standard.

### Antifungal assay

#### *Well diffusion method*

The agar well diffusion method was employed for the antifungal assay. Sabouraud's Dextrose Agar was used. A sterile cork borer was used to bore holes at equidistance in the plates. Exactly 0.5 ml of plant extracts (of varying concentrations of 25 mg, 50 mg and 100 mg) were introduced aseptically into the holes of the inoculated plates and 10 mg of Itraconazole was used as a control.

The plates were incubated at 25°C for 10-14 days. The zones of inhibition around each hole were measured in mm.

**Combination Assay**

***Well diffusion method***

Varying concentrations of the leaves and flowers extracts were added into wells on prepared plates aseptically.

Flowers to leaves combination were 50:50, 50:25 and 50:12.5. Leaves to flowers combination was 50:50, 50:25 and 50:12.5.

**Determination of Minimum Inhibitory Concentration (MIC)**

This was determined using broth dilution method as described by Junaid [11]. Exactly 4 extract concentrations each of 100, 50, 25 and 12.5, mg/ml of plant materials was used. The 5$^{th}$ and 6$^{th}$ tubes served as positive and negative controls. One (1) ml of the extract was added in each test tube containing Sabouraud Dextrose Agar. The tubes were then inoculated with 0.1 ml of fungal suspensions except for the positive and negative control. The MIC was examined for turbidity (cloudiness) after 5 days of incubation at room temperature (25°C). The MIC was read as the least concentration of extract that showed no growth (clear) after incubation period.

**Determination of Minimum Fungicidal Concentration (MFC)**

MFC was determined by sub-culturing the test dilutions which showed no growth onto fresh drug-free solid media (Sabouraud Dextrose Agar) and were incubated for 5days. The lowest concentration that yielded fungal growth on the medium was taken as the MFC (Table 2).

## DISCUSSION

Both the leaves and flower extracts of *Ocimum canum* showed the presence of pharmacologically active components. The presence of steroids, flavonoids and tannins detected in the extracts of *Ocimum canum* agrees with earlier reports of Rai et al. [12]. In most studies, qualitative phytochemical composition of *Ocimum canum* extracts were reported to contained tannins, saponins, flavonoids, phenolics, terpenoids and alkaloids [13] (Table 3).

In plant based drugs, tannin is one of the major active ingredients reported to exhibiting antimicrobial properties. The mechanism of action is based on the principle that tannins can also be toxic to filamentous fungi and yeasts [14]. The report revealed that cardiac glycosides content confer antimicrobial properties in most plant extracts [15]. Saponins and flavonoids are known for their activity against fungi [16]. Qualitatively, the presence of cardiac glycosides in both the leaves and flower extracts of *Ocimum canum* is contrary to the findings of Aluko et al. and Kosini et al. high level of flavonoids (10.00%) in the leaves were reported[17,18]. A high MIC value indicates low activity and vice versa. In this study, no significant different was observed between the MIC. There was no antifungal activity of both extracts on fungal isolates except for T. mentagrophytes. This could be as a result of absence of some of the bioactive components which would have had effect on the fungal isolates. Results on the effect of combination of leaves with flower extracts showed decrease interaction against test fungi evaluated. Indifference reaction means that the bactericidal rate of a combination is the same as that of the active drug alone (Table 4).

**Table 2:** Minimum fungicidal concentration (MFC) of leaves and flower extracts of *Ocimum canum*

| Fungal Species | MFC (mg/ml) | |
|---|---|---|
| | Leaves | Flower |
| *Trichophyton mentagrophytes* | 100 | 100 |

**Table 3:** Qualitative phytochemical constituents of leaves and flower extracts of *Ocimum canum*

| Constituents | Leaves Extract | Flower Extract |
|---|---|---|
| Tannins | + | – |
| Saponins | - | – |
| Steroids | + | + |
| Cardiac glycosides | + | + |
| Anthraquinone | – | – |
| Flavonoids | + | + |
| Alkaloids | – | – |
| Key: + Presence – Absent | | |

**Table 4:** Antifungal activity of leaves and flower extracts of *Ocimum canum*

| Selected Fungi | Zone of Inhibition (mm)/Extract Concentration (mg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaves Extract | | | Flower Extract | | | | |
| | 100 | 50 | 25 | 100 | 50 | 25 | DMSO | Itraconazole (10 mg) |
| *Trichophyton mentagrophytes* | 18.3 ± 0.15$^{a}$ | 17.0 ± 0.12$^{ab}$ | 15.2 ± 0.15$^{c}$ | 22.0 ± 0.20$^{d}$ | 21.00 ± 0.56$^{e}$ | 17.20 ± 0.12$^{abf}$ | 0.00 ± 0.0$^{g}$ | 18.00 ± 0.00$^{abh}$ |
| *Trichophyton rubrum* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0 .00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 22.00 ± 0.00$^{i}$ |
| *Trichophyton tonsurans* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 21.00 ± 0.70$^{i j}$ |
| *Trichophyton verrucosum* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 22.00 ± 0.30$^{i j k}$ |
| *Microsporum canis* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 30.0 ± 0.00$^{l}$ |

Values are mean ± standard error of duplicate determinations; values with different letters as superscript across the row are significantly different at A p ≤ 0.05
Key: DMSO: Dimethyl Sulfoxide Cipro: Ciprofloxacin

## CONCLUSION

From the analysis of this study, it was concluded that *Ocimum canum* leaves and flower extracts showed antifungal activity on only one of the fungal specie (*T. mentagrophytes*) out the five test fungi used. Therefore the plant contains chemical constituents of pharmacological significance and can be explored for medicinal purpose.

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