# Analysis of Essential Oils and Antifungal Activity of *Cymbopogon Travancorensis* Bor.

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**Summary**

Essential oils analysis and a rapid assay to determine antifungal activity of lemon grass, *Cymbopogon travancorensis* is described. The composition of the essential oil isolated from the leaves of *C. travancorensis* Bor. collected in Courtallam, Tirunelveli Hills, Tirunelveli District, Tamil Nadu, South India, was determined by capillary GC/MS. Twenty- four compounds were identified corresponding to *ca.* 97.35% of the oil. The major components were Citronellol (36.12%), Citronellal (25.36%), *γ*-Terpinene (5.21%) and *β*-Phellandrene (4.36%). Essential oils of *C. travancorensis* were exhibited a complete arrest of the growth of three fungi, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*.

**Keywords**: *Cymbopogon travancorensis*; essential oils; Citronellol; antifungal activity

## Introduction

Treatment of infectious diseases with antimicrobial agents continues to present problems in contemporary medicine with several studies showing a significant increase in the incidence of side effects and the resistance that pathogenic microorganisms construct against several antibiotics (1-3). However, recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine to treat infections recently (4).

Essential oils are being a major group of active components among the secondary metabolites of higher plants. These compounds comprise terpenes, sesquiterpenes as well as their derivatives. Their volatility, which made them easy to find out in aromatic plant material and at the same time readily obtainable by simple distillation of plant parts, lent to them the term essential oil.

Aromatic plants have great importance for food, cosmetics and pharmaceutical industries. Their use have taken place since ancient times, and despite many of them were substituted by synthetic ones, the demand for natural products is increasing (5). The antimicrobial attributes of essential oils have been recognized long ago but only recently it has been established scientifically (6-7).

The genus *Cymbopogon* belongs to the family Poaceae. Essential oils from several *Cymbopogon* species are known for their antimicrobial activity (8-16). According to literature survey of *C. nardus* and *C. martinii* essential oils had the highest fungistatic activity (17). Effect of *Cymbopogon nardus* essential oil on growth and morphogenesis of *Aspergillus niger* were studied (18). *Cymbopogon travancorensis* Bor. an aromatic grass, is found in thoughtout the growing on an altitude of 500m Southern Western Ghat region, South India. Previously, no studies have been carried out analysis of chemical constituents and antifungal activity of lemon grass of *C. travancorensis.*

## Materials and Methods

### *Plant Materials*

Fresh leaves of *C. travancorensis* Bor. collected in Courtallam, Tirunelveli Hills, Tirunelveli District, Tamil Nadu, South India. The voucher specimen was deposited in the Centre for Biodiversity and Biotechnology, St. Xavier's College, Palayamkottai, South India.

### *Isolation and identification of essential oil*

Fresh leaves of *Cymbopogon travancorensis* were subjected to hydrodistillation in a Clevenger-type apparatus for 6h. The volatile oil was dried over anhydrous sodium sulfate and stored at 4 °C in the dark before analysis. 0.1µl of oil diluted with pentane (1:10,000,v:v) were analyzed on an HP-GCD apparatus equipped with an HP5 (30m x 0.25mm) fused-silica capillary column using helium(1ml/min) as a carrier gas. The injector and detector temperatures were 250 and 280 $^{o}$C, respectively, and the oven conditions were 70 $^{o}$C for 2 min, then rising from 70 to200 $^{o}$C at a rate of 4 $^{o}$C/min and subsequently held at 200 $^{o}$C for 10min. The mass range was recorded from 45 to 450 m/z, with ionization energy of 70eV.

Identification of constituents was based on comparison of the retention times with those of authentic samples, comparing their retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS 95) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (19-24).

***Antifungal activity***

The strain was maintained on malt extract agar at 28°C. Mycelium of the fungus was cultured in a liquid medium containing 10 g/l glucose, 10 g/l malt extract, 3 g/l Yeast extract and mycological peptone. Growth inhibition was determined on Potato Dextrose Agar (PDA).

**Agar medium assay**

MIC determination with 400, 200, 100, and 50µl of pure essential oil were added to 50 mL of liquid PDA medium. Each Petri dish was inoculated at the center with a mycelial disc (5mm diameter) which was taken *A. niger, A. flavus* and *Candida albicans* colony grown on PDA at 28°C for 7days. Control plates without oil were inoculated. MIC was defined as the lowest concentration of essential oil required to prevent fungal growth completely. Fungal colony diameter was recorded seventh day, for each plate. The colony diameter for each oil concentration is the mean of five replicates. The percent inhibition of mycelial growth was calculated using the following formula

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

where C = Mycelial weight in control and T = Mycelial weight in treatment.

## Results and Discussion

The yield of the oil obtained from *Cymbopogon travancorensis* was 0.68%. The *C. travancorensis* oil was examined by GC-MS. The list of compounds identified in the oil of *C. travancorensis* can be seen in Table 1.

Twenty- two compounds were identified, representing 97.35% of the total essential oil, in which the major components were Citronellol (36.12%), Citronellal (25.36%), *γ*-Terpinene (5.21%) and *β*-Phellandrene (4.36%). Fig 1. and 2 other compounds were found in total oils but with some quantitative variation that can be seen in Table 1.

Earlier report indicated that *Cymbopogon nardus* contain Citronellol and Citronellal as the major constituents of their oils (18). The major constituent of limonene, which is found to be *C. densiflorus* (25). The present reported that minor constituent of limonene is present in *Cymbopogon travancorensis*.

In the present study, the antifungal property of the essential oils was screened against pathogenic fungi at 50-400µl concentration using agar medium assay method. The results obtained on the antifungal activity of *Cymbopogon travancorensis* leaves showed that the essential oils possess good antifungal activity of completely inhibition of growth arrested on *Aspergillus flavus*, *A. niger* and *Candida albicans* (Table-2). According to literature survey of *A. flavus* was inhibited by both spices essential oils of *Cuminum cyminum* and *Elettaria cardamomum* (26). Earlier reported that *M. alternifolia* essential oils was fungal toxicity of *Candida albicans* is based on increased permeability of the plasma membranes (7). Essential oil of limonene isolated from the *H. suaveolens* has mild antifungal activity against *C. albicans* (6). Among the reports on isolimonene showed good fungistatic activities against *C. albicans* (27). Similar findings of essential oils of *Foeniculum vulgare* seeds exhibited a marked antifungal activity against *Aspergillus niger* (28). Cardamom oil inhibited the growth of *A. flavus*, and of its components linalool and limonene had the greatest antifungal activity (29).

**Table 1:** Composition of *Cymbopogon travancorensis* essential oil identified by GC/MS

| Sl. No | Active constituents | Composition (%) |
|---|---|---|
| 1 | *α*-Pinene | 0.37 |
| 2 | *β*-Pinene | 0.25 |
| 3 | Myrecene | 1.25 |
| 4 | α-Phellandrene | 4.3 |
| 5 | Limonene | 2.31 |
| 6 | *β*-Elemene | 3.25 |
| 7 | *β*-Phellandrene | 4.36 |
| 8 | *γ*-Terpinene | 5.21 |
| 9 | Terpinolene | 1.32 |
| 10 | Citronellal | 25.36 |

| | | |
|---|---|---|
| 11 | *β*-Caryophyllene | 0.09 |
| 12 | Geraniol | 0.05 |
| 13 | Citronellol | 36.12 |
| 14 | Eugenol | 1.14 |
| 15 | Unkown | 0.26 |
| 16 | Unkown | 1.15 |
| 17 | Linalool | 1.25 |
| 18 | Camphor | 0.45 |
| 19 | Nerol | 2.21 |
| 20 | Geranial | 3.25 |
| 21 | *α*-Terpineol | 1.25 |
| 22 | *γ*-Cadinene | 0.25 |
| 23 | Geranyl acetate | 1.78 |
| 24 | α-Muurolene | 0.12 |
| Total | | 97.35 |

**Table 2:** Antifungal activity of essential oils of *C. travancorensis* leaves

| Tested fungi | % of growth inhibition | | | |
|---|---|---|---|---|
| | 50µl | 100 µl | 200 µl | 400 µl |
| *Aspergillus flavus* | 12.14 ±0.02 | 45.29±0.01 | 58.46±1.23 | 100±0.00 |
| *Aspergillus niger* | 23.78±0.23 | 56.84±0.25 | 78.34±2.40 | 100±0.00 |
| *Candida albicans* | 15.26±0.01 | 51.26±0.23 | 64.87±1.45 | 100±0.00 |

***** All values are triplicates (Mean±S.E)

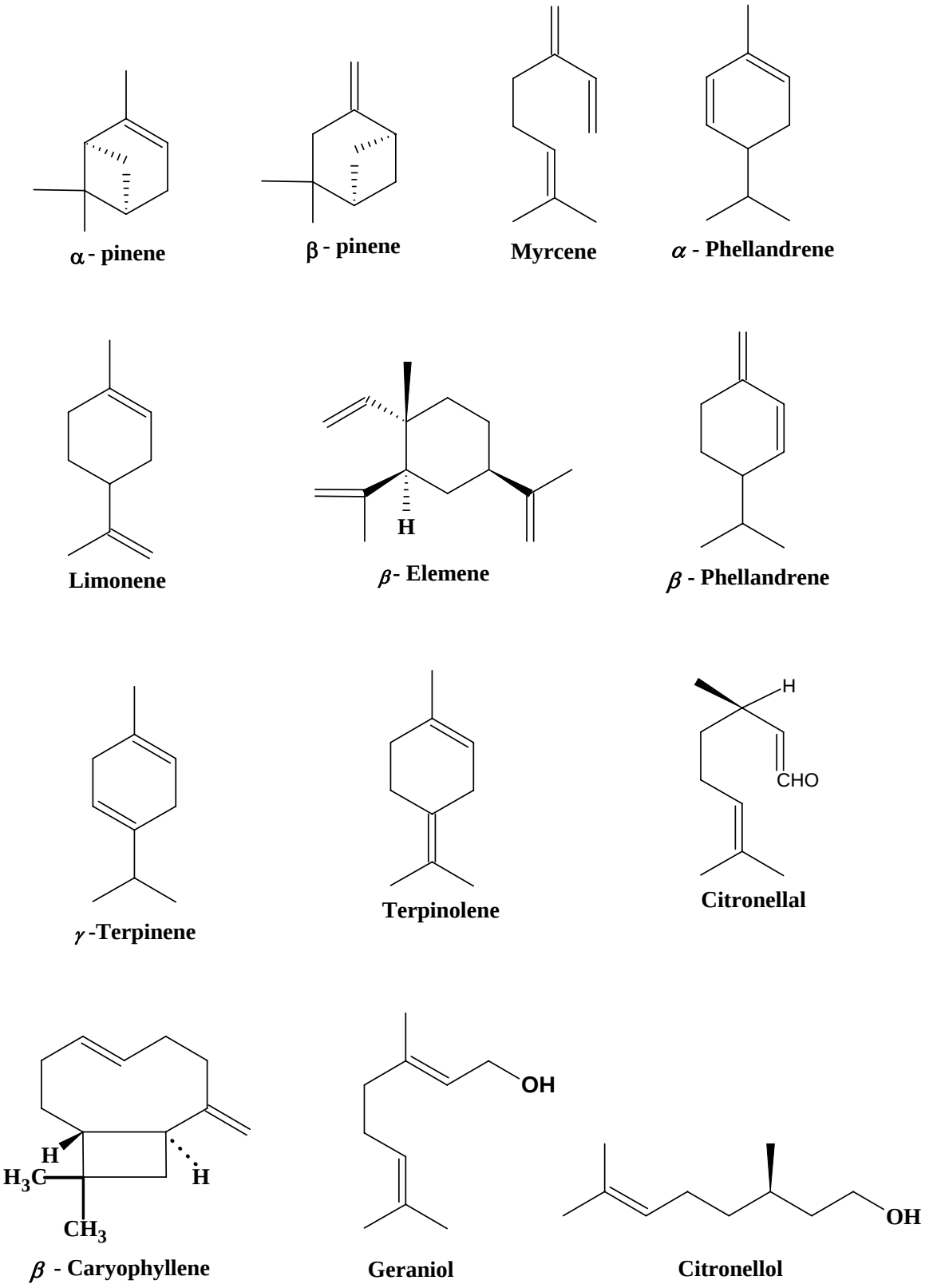


**Fig.1:** Structure of the volatile compounds identified in the oil of *C. travancorensis*

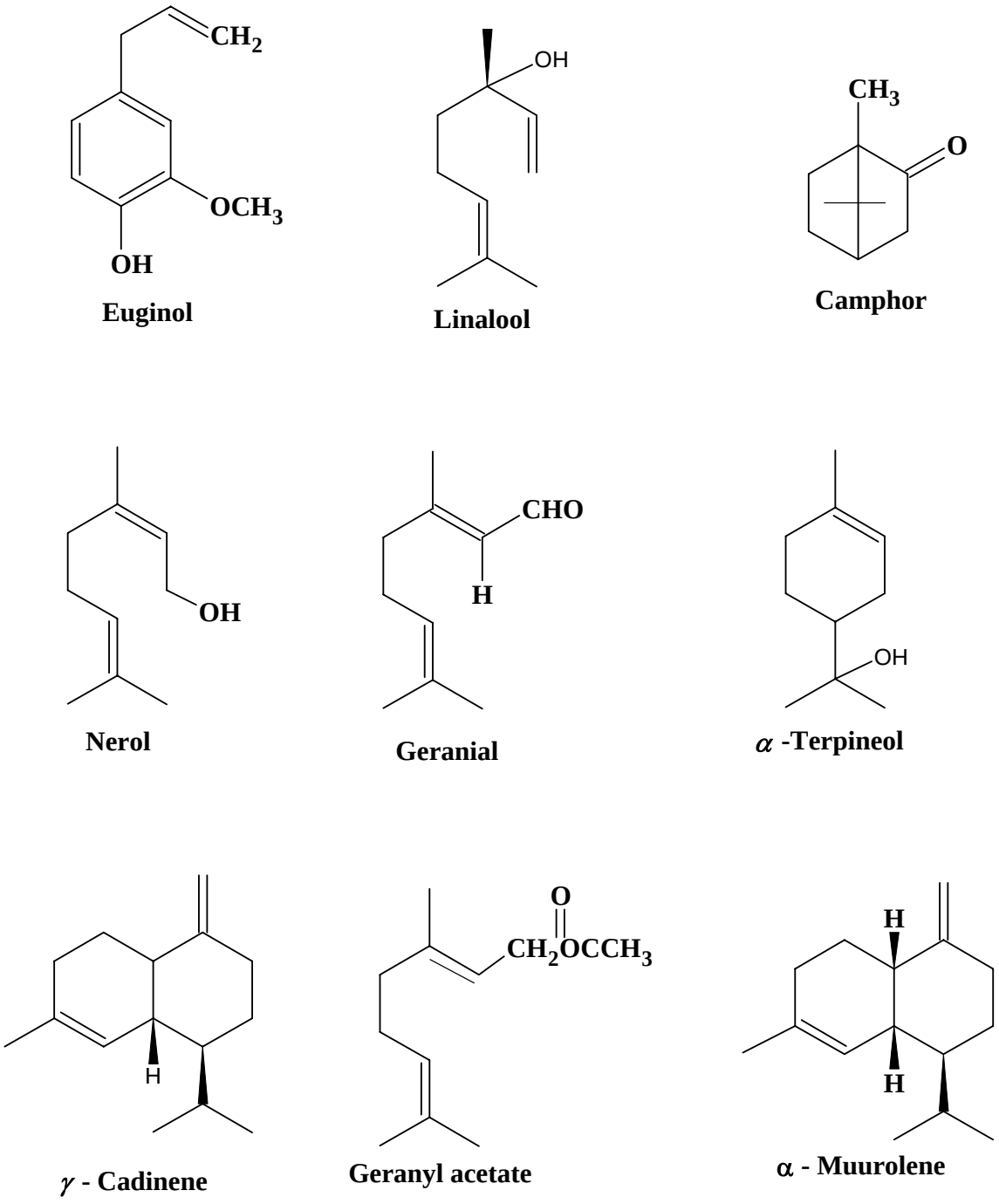


**Fig.2:** Structure of the volatile compounds identified in the oil of *C. travancorensis*

## Conclusion

The result obtained from this study indicate that twenty- four compounds were identified corresponding to *ca.* 97.35% of the oils of *C. travancorensis*. The major components were Citronellol (36.12%), Citronellal (25.36%), *γ*-Terpinene (5.21%) and *β*-Phellandrene (4.36%) present in *C. travancorensis*. Essential oils of *C. travancorensis* were completely growth arrested on three fungi, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*. The conclusion of the results showed that the essential oil of *Cymbopogon travancorensis* were possessed the strong antifungal activity. However, it is important to point out that essential oils such as these need to be further purified through antifungal activity guided fractionation to isolate and identify the compounds responsible for biological activity.

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