

Anti-Microbial Activities of *Michelia champaca* L. Essential Oil

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ABSTRACT

The essential oils contain chemical components, which impart the anti microbial activity. Champa oil or Champaca oil extracted from the flowers of *Michelia champaca* L. is highly esteemed in preparation of attars and perfumed hair oils. The flower oil of *Michelia champaca* L. is used as an application in cephalagia, ophthalmia, gout and rheumatism and fruits and seeds are considered useful for healing cracks in feet. Many essential oils have been explored but the essential oils in *Michelia champaca* L. have not been fully explored and researched in India and thus a very basic and practical study is required. The essential oil from *Michelia champaca* L. flowers was obtained using n-Hexane and the assay was carried out using freshly prepared *M. champaca* L. essential oil extract. The hexane extract of Champaca essential oil was prepared and screened for its anti-microbial activity. A slight inhibition was found against *Staphylococcus aureus*, a Gram-positive test organism by the susceptibility disc method. 25% inhibition was observed against *Staphylococcus aureus* by the Durham's diffusion tube assay indicating anti-microbial principles of lower potency in the champaca essential oil extract.

Ker Words: *Michelia champaca* , essential oil, n-Hexane, anti-microbial, *Staphylococcus aureus*.

INTRODUCTION

Most of the flower essential oils in addition to their antioxidant property also possess anti-bacterial and anti-fungal activity. *Artemesia annua* contains compounds such as camphor (41%), germacrene (16%), trans-pinocarveol (11%) which were found to possess inhibitory activity against Gram positive bacteria and

some Fungi (Juteau et al., 2002). Many plants have been exploited as a source of biologically active compounds. Over 25% of the biological preparations contain at least one component origination from plant sources. Plants possess anti microbial and anti oxidant components which are sometimes used to preserve food reserves in the world. Several research groups have tried to isolate and identify individual constituents present in the plant and to relate the chemical composition to the observed biological activity. The greatest antimicrobial activity of plants lies within 'Volatile oils' (Williams, 1996)

The essential oils of three *Micromeria* species such as *M. dalmatica*, *M. albanica* and *M. thymifolia* were analysed and the essential oils showed strong fungitoxicity. The essential oils also exerted anti bacterial effect against bacteria at low concentrations (Marinkovic et al., 2003).

The essential oils contain chemical components, which impart the anti microbial activity. Compounds such as methyl pyrimidine, β -glucopyranosyl triene isolated from flowers of *Alangium* showed antibacterial activity against many gram positive and gram-negative organisms (Arjun et al, 2002).

Thymol is simple phenol, which is present in the essential oil several plants and has been used for its antiseptic activity. A major component of essential oils Cineol (Eucalyptol) which is found in many essential oils showed in vitro antimicrobial activity at an inhibitory concentration of 0.28 to 2.25 mg/ml. Camphor and its derivatives borneol, terpine 4-ol also can be considered to have anti microbial activity (Daferera, et al, 2003).

Geraniol and Eugenol are the chemical components found in many essential oils. The anti fungal activity of these compounds has been investigated by the well agar diffusion method and the compounds show considerable anti fungal activity. Thus the various activities of essential oils can be the property given them by their chemical components.

Michelia genus consists of trees and shrubs belonging to the family Magnoliaceae. *Michelia* genus consists of many species with flower and essential oil bearing plants. The different *Michelia* species have been exploited and considerable research has been done on the various parts of the plant such as the leaves, flowers, fruits, stem and the bark. *Michelia champaca* is a tall evergreen tree cultivated throughout India in gardens and near temples for its fragrant flowers and handsome foliage. Champa oil or Champaca oil extracted from the flowers of *M. champaca* is highly esteemed in preparation of attars and perfumed hair oils.

The flower oil of *Michelia champaca* is used as an application in cephalagia, ophthalmia, gout and rheumatism and fruits and seeds are considered useful for healing cracks in feet (Khan et al, 2002).

There is great scope for the use of essential oils in various purposes. The review of literature reveals that many essential oils have been explored but the essential oils in *Michelia champaca* have not been fully explored and researched in India and thus a very basic and practical study is required. The present studies are aimed at:

The qualitative analysis on the anti-microbial studies of the extracted essential oil by two methods:

1. Disc susceptibility method
- Durham's fusion tube method (for volatile compounds).

MATERIAL AND METHODS

- The fresh Champaca flowers were collected in the months of September, October and November. The flowers were then air dried.
- The essential oils were extracted using the solvent extraction method (Guenther, 1972).
- For antimicrobial assays, Nutrient Agar Media was used for maintaining standard test microorganisms and sub-cultures.

- Mueller Hinton Agar was used for the actual disc susceptibility test.
- Nutrient broth and Trypticase Soya Broth (TSB) were used for preparation of the inoculum densities.
- All the medias were obtained from Hi Media Laboratories.
- Four test organisms were used for the anti microbial assays of the essential oil. The four test organisms included two Gram positive and two Gram negative strains. The standard cultures and their culture conditions have been listed in Table:

Table 1: Micro organisms used for present study and their culture conditions:

Microorganisms	Media	Type	Incubation Temp.
<i>Escherichia coli</i> ATC10148	Nutrient agar	Standard Isolate	37°C
<i>Pseudomonas aeruginosa</i>	Nutrient agar	Clinical Isolate	37°C
<i>Staphylococcus aureus</i> ATCC 25923	Nutrient agar	Standard Isolate	37°C
<i>Staphylococcus epidermis</i>	Nutrient agar	Nutrient agar	37°C

Note: the standard cultures were obtained from C.B. Patel Research Centre, Vile Parle (W).the cultures were subcultured to maintain their purity and viability.

Anti microbial assay:

Susceptibility Disc method: The anti microbial assay was carried out using susceptibility disc method (Jacques, 1980).

- Collection and maintenance of cultures: The standard cultures obtained from C.B.Patel Research Centre have been listed in table and were preserved at 4°C in refrigerators after 24hr inoculation. The cultures were sub-cultured every week to maintain their purity and viability.
- Preparation of inoculum: Few of colonies were selected from the initial (Master) plates/slants

and suspended in a small volume of saline (about 5 ml). the inoculums can also be prepared in TSB(Trypticase Soya Broth) or BIH (Brain Heart Infusion) medium.

The inoculum was incubated for 1-2 hours and turbidity of culture was matched with that of '1' standard of Mc Farland opacity tubes. The turbidity was adjusted with the help of saline, TSB or BHI.

Note- Mc Farland turbidity standard of '1' is equal to inoculum of 3x10⁸ cells/ml

- **Inoculation of Nutrient Agar Plate:** the entire surface of the plate was to be inoculated and therefore 0.1 ml of the adjusted culture was added to the surface of the plates with the help of sterile pipettes. A sterile cotton swab was rotated several times in several directions to dry the added inoculum on the plate and thus ensure even distribution and diffusion of the culture.

- **Preparation of Anti-microbial Discs:** In accordance to the FDA standards, sterile plain filter paper discs (Sterile Discs SD067) were purchased from HI Media Laboratories Pvt. Ltd. 20 µl of the Champaca essential oil extract was carefully added on the discs with the help of a sterile pipette. The discs were stored at -20°C for 30 minutes and then used for inoculation. The same procedure was repeated for n-Hexane to check for its anti microbial activity.

- **Application of Discs:** 10 to 15 minutes after inoculation of plates the Champaca essential oil and n-Hexane discs were placed in center of each inoculated plate. The results were observed 18-24 hrs after the inoculation and incubation.

Durham's Diffusion Method:

The bioassay for volatile essential oils was carried out by Durham's diffusion method . The aromatic substances (essential oils) of natural origin are used medicinally in Ayurveda and can have diverse biodynamic actions. A novel method

to study the anti-microbial properties of volatile components of aromatic oils such as *Michelia champaca* is being used here. The existing methods might not be adequate an oil on to study an exclusive effect of the volatile components of an oil on microbes due to lack of lateral diffusion and evaporation from surface (Agnihotri and Vaidya, 1996).

- **Collection and Maintenance of Standard Test Cultures:** The same standard cultures as tabulated earlier were used for the anti-microbial assay Durham's diffusion assay.
- **Preparation of inoculums:** the same inoculums as used in the Disc Susceptibility Test i.e. Mc Farland's standard of '1' were used which corresponds to inoculum of 3×10^8 cells/ml.
- **Inoculation of Nutrient Agar Slants:** Nutrient agar slants were inoculated by streaking a loopful of the prepared inoculum and incubating at 37°C for 24 hrs.
- **Preparation of Durham's Tubes:** Autoclaved Durham's fusion tubes (2mm diameter) were picked up by sterile forceps and 0.1 ml of the Champaca essential oil extract was added to it. Control tubes with same quantity of n-Hexane are also filled and were used as a positive control. These tubes were then introduced in pre inoculated culture tubes (slants) with the help of sterile forceps. The tubes were then incubated in a tilted position (30° angle) o let

the emerging vapours from the essential oil extract and hexane act on the inoculated slants. The results were read after 24 hrs and the percentage of inhibition was recorded.

- **Interpretation of the Results:** The inhibition of bacterial growth was expressed in terms of the total slant expressed. Thus bacterial growth restricted only to the proximal end of the fusion tube and approximately covering 25% of the slant area was designated as single (+), similarly 50% inhibition of bacterial growth was represented by (++), 75% of the area under inhibition by (+++) and when there was 100% inhibition of bacterial growth it was shown by (+++). The results were recorded after incubation for 24 hours and changes in the growth pattern were noted down.

RESULTS AND DISCUSSION

The essential oil from *Michelia champaca* flowers was obtained using n-Hexane. The assay was carried out using freshly prepared *M. champaca* essential oil extract. The hexane extract of Champaca essential oil was prepared and screened for its anti microbial activity by Durham's diffusion method. The activity of the oil was tested against four standard organisms and the results were recorded after 24 hours. The results obtained from the primary qualitative screening of the essential oil are tabulated in table 2:

Table 2: Effect of Champaca essential oil extract and n-Hexane on the test organisms (Durham's diffusion method)

Microorganisms	Essential oil tube (24 hrs)	n-Hexane Control Tube (24 hrs)
<i>Escherichia coli</i> ATC10148	-	-
<i>Pseudomonas aeruginosa</i>	+	-
<i>Staphylococcus aureus</i> ATCC 25923	-	-
<i>Staphylococcus epidermis</i>	-	-

(+): 25% inhibition of organisms from proximal to distal end of the tube

(-): No inhibition of organism from proximal to distal end of tube.

24 hrs: Results read after 24 hours of inhibition.

Table 3: Effect of Champaca essential oil extract Disc and n-Hexane Discs on the test organisms - Disc diffusion method (Inhibition Zones in mm)

Test organisms	Essential oil Disc Diameter (mm) D ₁	n-Hexane Control Disc Diameter (mm) D ₂
<i>Escherichia coli</i> ATC10148	NI	NI
<i>Pseudomonas aeruginosa</i>	11	NI
<i>Staphylococcus aureus</i> ATCC 25923	NI	NI
<i>Staphylococcus epidermis</i>	9	NI

NI: No Inhibition ;
D1: Diameter of zone of inhibition of essential oil disc.
D2: Diameter of zone of inhibition of hexane control disc.

From the results presented in the table, it is clear that 25% inhibition was observed in the case of *Staphylococcus aureus*. It was observed that 25% of growth on the slant from the mouth of Durham's tube was inhibited by the essential oil extract. No such inhibition was seen for the essential oil extract in case of the other three organisms. It is also observed that n-Hexane, which was used as a control, did not show any inhibition to any of the test organisms. The growth was present on the whole slant in case of the hexane tube. After 24 hours it was seen that the essential oil extract and the hexane had completely volatilized.

Michelia champaca essential oil extract was used to screen the anti microbial activity of the essential oil. This time the standard disc diffusion method was performed against four test organisms. The results of the test were obtained after 24 hours and the results are tabulated in table 3.

It can be seen that essential oil extract was found to be slightly active against the Gram positive test organisms. The activity of the essential oil was not found against Gram negative test organisms. The n-Hexane discs which were put as controls, did not show any inhibition against all the test organisms as can be seen from the table. The solvent thus does not have any antibacterial activity.

The activity of the essential oil extract was seen against standard strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* as can be noted from table. It is also observed from the table that the essential oil showed an inhibitory zone diameter of 11 mm against *Staphylococcus aureus*. The essential oil extract also showed a very low activity against *Staphylococcus epidermidis*, zone of 9 mm diameter. No activity was observed against the other organisms.

CONCLUSION

The bioassay of the essential oil of *Michelia champaca* did not show encouraging results. However, a slight inhibition was found against *Staphylococcus aureus*, a Gram-positive test organism by the susceptibility disc method. A 25% inhibition was observed against *Staphylococcus aureus* by the Durham's diffusion tube assay indicating anti-microbial principles of lower potency in the champaca essential oil extract. The compound Farnesol seems to be the major bioactive principle as evident from the present study. In contrast, anti-microbial compounds of broad spectrum activity were observed in the alcohol extract of leaves, bark and stem of *Michelia champaca* and this activity may be related to the presence of alkaloids. The alkaloid Liriodenine has been reported having anti microbial activity (Khan et al, 2002). It

appears that the bioactive alkaloids are absent in the flowers and thus none of the other components possess effective or potent antimicrobial activity.

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