Evaluation of Mixer of Essential Oils and their Standard Components against Fungi Causing Superficial Mycosis in Human

Neetu Jain

Department of Botany, S.S. Jain Subodh PG (Autonomous) College, Jaipur, Rajasthan, INDIA.

ABSTRACT

Background: Dermatophytoses are responsible for almost one-quarter of all fungal infections globally. When treatable, these challenging fungal infections of the skin, hair, and nails are becoming more resistant to standard antifungal medications and frequently call for lengthy therapeutic regimens. Objectives: Essential oils have been used to cure a range of diseases for ages. In present study combination effect of volatile oils obtained from Trachyspermum ammi, Coriandrum sativum and Cymbopogon martinii were evaluated against four selected dermatophytic fungi namely Microsporum canis, M. fulvum, Trichophyton rubrum and T. mentarophytes. Materials and Methods: Four combinations like T. ammi + Cymbopogon martinii, T. ammi + Coriandrum sativum, Cymbopogon martinii + C. sativum, T. ammi + Cymbopogon martinii + C. sativum were prepared and evaluated through semisolid agar antifungal susceptibility methods. Results: During present investigation T. rubrum was found to be most resistant fungus. Combination of T. ammi with C. sativum showed excellent results as compared to T. ammi and C. martinii. Combination of C. martinii and C. sativum was less effective as compared to other combinations. MIC was ranging from 0.1 μL/mL to 0.4 μL/mL against test fungi. Conclusion: Although essential oils are frequently combined in the use of aromatherapy to cure infectious disorders, there is very little data to back up this practice. This study supports the concurrent usage of blended essential oils in some way.

Keywords: Dermatophytes, Essential oil, Combination, MIC.

INTRODUCTION

Fungal infection in humans and animals is ubiquitous around the world. Fungi were responsible for both external and internal mycoses. A closely related group of keratinophilic fungus called dermatophytes is responsible for most superficial infections.[1] Dermatophytic skin infection has become a major public health issue in socioeconomically poor population of India impacting children, adolescents, and adults. In developing countries, the prevalence of fungal infections has recently increased. This could be due to antibiotic use, environmental factors, immunosuppressive medicines, and a variety of illnesses such as HIV, cancer, and surgery.[1] Recurrence has been documented in up to 25%-40% of instances of dermatophytic infections, despite the availability of novel systemic antifungal treatments.[1] Dermatophytosis has been successfully treated with a variety of synthetic antifungal medications, including griseofulvin, ketoconazole, butenafine and terbinafine imidazoles etc. Due to recrudescence of disease, dermatophytic strains with high resistance, along with various side effect include abdominal pain, itching, nausea, and its toxicity limits its therapeutic application in many times.[4,5]

During last few decades, plants products are found harmless and do not have any side effect, therefore uses of therapeutic plants has been steady resurgence in all over the world. Aromatic plant essences, also known as essential oils, are volatile, fragrant mixtures with an oily viscosity that are typically made with the help of plants. Their unique tints range from pale yellow to emerald green and from blue to dark brownish pink. They can be liquid at room temperature, though some are powerful or resinous.

These are composite blends of various bioactive components like monoterpenes, sesquiterpenes, aldehydes, ketone, esters, phenols and various oxygenated hydrocarbons.[6]

Combinatorial approaches to improve the efficiency of current dermatophytosis treatment regimens and combat fungal resistance involve combining conventional antifungals with...
compounds produced from plants.\cite{7,8} According to some research, the antidermatophytic effect was caused by the interaction of major and minor components rather than by the presence of a single chemical.\cite{9} Synergistic combinations in this situation can have a multi-target fungicide result, boosting the effectiveness of traditional medications and addressing multi-drug resistance in microbes. These relationships also have the benefit of lowering the effective doses of conventional medications, which lessens their toxicity and adverse effects.\cite{10}

Numerous metabolic processes in the microorganisms are typically affected by combinations of essential oil or artificial mixtures of major components that have been purified, which restricts their growth. Application of fundamental disinfectant compounds in blends to suppress food-contaminants microbes has gained popularity in recent years.\cite{11,12} The chemical composition of essential oils, their ability to treat dermatophytes, and their toxicological consequences have all been the subject of numerous investigations.\cite{13,14} However, there aren’t many reports on combination effect of essential oils. Thus, we investigated the combined effect of \textit{Cymbopogon martinii}, \textit{Coriandrum sativum}, and \textit{Trachyspermum ammi} against human pathogenic fungi.

**MATERIALS AND METHODS**

**Oil Extraction Method**

\textit{Trachyspermum ammi} and \textit{Coriandrum sativum} fruits were taken from certified store of medicinal plant, Jaipur, India. While plant parts of \textit{Cymbopogon martinii} were collected from grass land near forest nursery, Nalsandol, Rajasthan. A voucher number RUBL 21181 was obtained upon submission of the plant material in herbarium. For the extraction of essential oil plant materials were macerated with a small quantity of distilled water and the slurry was hydrodistilled with Cleavenger’s apparatus for 7-8 hr, respectively. Anhydrous sodium sulphate was use to dry the essential oil that was gathered in tubes. After that, the moisture-free oil was refrigerated and kept in bottles with an amber vial.

**GC/GC-MS Analysis**

**Gas Chromatography**

Quantitative analysis of the all-selected oils was performed using a Shimadzu GC-2010. GC and GCMS investigation were executed according to calculated procedure applied in our previous research articles.\cite{15} Carrier gas nitrogen was used at 10 psi inlet pressure with FID and Omega SPTm column (30.0 m x 0.25 mm ID, film thickness 0.25 um). The temperatures of the injector and detector were 270°C and 280°C, respectively. The temperature of the column is configured to range from 80°C to 180°C at 4°C/min, 180°C to 230°C at 6°C/min, and 19°C to 6°C with hold times of 6 and 19 min, respectively. The split ratio was 1:80 and the carrier gas flow rate was 1.21 mL/min. Software from GC Solutions was used to process the data for oil composition.

Shimadzu GCMS-QP-2010 plus system with Omega SPTm column (30.0 m x 0.25 mm ID, film thickness 0.25 um) was used for GC-MS analysis. The temperatures of the injector, mass detector, and ion source were 250°C, 280°C, and 270°C, respectively. The temperature of the column is configured to range from 80°C to 180°C at 4°C/min, 180°C to 230°C at 6°C/min, and 19°C to 6°C with hold times of 6 and 19 min, respectively. The split ratio was 1:80 and the carrier gas flow rate was 1.21 mL/min. Helium was used as carrier gas. The mass range and EI source were 40-850 amu and 70 eV, respectively. Utilizing the Willey, NIST, and Perfumery libraries, compounds were identified. By comparing their respective retention indices with values from the literature, the compound identity was ultimately verified.

**Fungal culture**

For current study four selected dermatophytes namely \textit{Trichophyton rubrum} (MTCC 296), \textit{T. mentagrophytes} (MTCC 7687), \textit{Microsporum fulvum} (MTCC2837) and \textit{M. canis} (MTCC2820) were procured from the Imtech Chandigarh. These selected fungi are maintained on Sabouraud’s dextrose agar medium and Potato dextrose agar medium.

**Antidermatophytic activity**

Minimum inhibitory concentration was determined according to Provine and Hadley\cite{16} with slight modification.\cite{15} BHIA was prepared as per maker’s guideline.

For inoculum preparation germ-free gauze into sterile tween 80 were practiced to pick the uncontaminated colony of filamentous fungi from 7-10 day old culture. Inoculated swab was then suspended in 5 mL of sterile normal saline and allowed the heavier particles to settle after being vortexed. The turbidity of the consistent suspension was determined on ~0.5 McFarland standard. For MIC determination test medium tubes with various percentage of test oils as well as oil-free controls were ready in triplicate. One loopful (HiMedia Flexiloop 4) of 0.5 McFarland adjusted culture was inserted deeply into the semisolid agar to inoculate these test tubes. To verify viability and purity, a loopful of the inoculum suspension was also streaked over Sabouraud dextrose agar. As per the NCCLS/CLSI criteria, M27-A and M38-A, the results were computed after 72 hr. Growth was visually examined and rated in relation to the oil-free control group as follows: +4 denotes growth identical to control; +3 denotes a little decline in growth; +2 denotes a severe loss in growth (80% in yeast and 50% in filamentous); +1 denotes a slight increase or few visible fragments of hyphal tissue; 0 denotes no growth.
RESULTS

To confirm the distinct chemotypes, the chemical characterization of the essential oils was examined (Table 1). Although a complete chemical profile for each essential oil was established, just the main components have been mentioned for the sake of conciseness.\textsuperscript{14,17,18}

GC and GC-MS studies revealed that most abundant component of \textit{C. sativum} was linalool, thymol in \textit{T. ammi} and sabinol in \textit{C. martinii} essential oil. Alpha pinene was only component found in all three essential oils. Beta pinene, Myrcene and para cymene and gamma terpinene were found in both \textit{C. sativum} and \textit{T. ammi} essential oils. Limonene and Camphor were the common constituents of \textit{C. sativum} and \textit{Cymbopogon martinii} essential oils. Thymol, Alpha terpinene, Delta -3-Carene and Eugenol were reported only from \textit{T. ammi} essential oil. Citronellal, Fenchone, linalool, trans anethole, geranyl acetate and estragole were present only in \textit{C. sativum} seed essential oil. While Sabinol, Trans p metha-2,8 dienol, Cis p-metha,2,8, dien-1-ol, Durinol, Trans carveol and Cis -carveol were only found in \textit{C. martinii} leave essential oil (Figures 1-3).

Data incorporated in Table 2 represented antidermatophytic properties of three essential oils alone and their combinations against selected fungi. All three essential oils exhibited excellent antidermatophytic activities. More substantial fungitoxic behavior was discovered in \textit{T. ammi} oil (MIC range 0.025±0.000 μL/mL to 0.05±0.033 μL/mL). \textit{T. ammi+ C. sativum} combinations showed superb fungicidal behavior against all fungi. Its maximum effect was observed against \textit{T. rubrum} (0.05±0.003 μL/mL) and \textit{T. mentagrophytes} (0.05±0.003 μL/mL) followed by \textit{M. gypseum} (0.2±0.000 μL/mL). \textit{T. sativum+C. martinii} combination was found less effective as compared to other. Combination of all three oil was found most effective against all selected test fungi. Its maximum activity was seen against \textit{T. rubrum} (0.2±0.000 μL/mL) followed by \textit{T. mentagrophytes} (0.133±0.033 μL/mL), \textit{M. gypseum} and \textit{M. fulvum} (0.2±0.000 μL/mL). Combinations of \textit{T. ammi} and \textit{C. martinii} demonstrated potent antidermatophytic action. \textit{T. mentagrophytes} was the target of its greatest impact (0.2±0.000 μL/mL).

### Table 1: Major chemical constituents of essential oils.

<table>
<thead>
<tr>
<th>Components</th>
<th>\textit{C. sativum}\textsuperscript{17}</th>
<th>\textit{C. martinii}\textsuperscript{14}</th>
<th>\textit{T. ammi}\textsuperscript{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-pinene</td>
<td>3.5581</td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>Beta-pinene</td>
<td>0.3751</td>
<td>-</td>
<td>5.45</td>
</tr>
<tr>
<td>Myrcene</td>
<td>0.2189</td>
<td>-</td>
<td>1.17</td>
</tr>
<tr>
<td>Para-cymene</td>
<td>0.0956</td>
<td>13.19</td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td>0.3058</td>
<td>1.76</td>
<td></td>
</tr>
<tr>
<td>Gamma-terpinene</td>
<td>0.2986</td>
<td>32.32</td>
<td></td>
</tr>
<tr>
<td>Fenchone</td>
<td>1.1733</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>81.3889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camphor</td>
<td>0.0504</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td>Citronellal</td>
<td>0.1879</td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>Alpha terpinene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estragole</td>
<td>1.5351</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans-anethole</td>
<td>1.1864</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>5.5909</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans p metha-2,8 dienol</td>
<td>18.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cis p-metha,2,8,dien-1-ol</td>
<td>11.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durinol</td>
<td>3.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans carveol</td>
<td>7.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cis -carveol</td>
<td>7.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabinol</td>
<td>19.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymol</td>
<td></td>
<td>44.00</td>
<td></td>
</tr>
<tr>
<td>Delta-3-Carene</td>
<td></td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td></td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{14,17,18}
DISCUSSION

The improper use of antibiotics and inadequate infection control have led to the emergence of resistance strains, which pose a major threat to public health and the world economy. Therefore, it has become essential to conduct research and produce a new generation of antimicrobials to slow the spread of antibiotic resistance. To produce the subsequent generation of antibacterial drugs, combinatorial therapies have recently been viewed as a superb platform. Combining different medications has many benefits over using them alone as individual chemical compounds. These benefits include lower dosages of the individual medications, fewer side effects when compared to monotherapy, decreased risk of drug resistance, improved overall

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Mixer of Oils</th>
<th>M. canis (MTCC2820)</th>
<th>M. fulvum (MTCC2837)</th>
<th>T. rubrum (MTCC 296)</th>
<th>T. mentagrophytes (MTCC 7687)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. ammi</td>
<td>0.05±0.033</td>
<td>0.266±0.033</td>
<td>0.025±0.000</td>
<td>0.026±0.002</td>
<td></td>
</tr>
<tr>
<td>Cymbopogon martinii</td>
<td>0.3±0.000</td>
<td>0.4±0.577</td>
<td>0.3±0.000</td>
<td>0.233±0.033</td>
<td></td>
</tr>
<tr>
<td>C. sativum</td>
<td>0.333±0.033</td>
<td>0.233±0.033</td>
<td>0.2±0.000</td>
<td>0.266±0.033</td>
<td></td>
</tr>
<tr>
<td>T. ammi + C. martinii</td>
<td>0.233±0.033</td>
<td>0.5±0.000</td>
<td>0.05±0.003</td>
<td>0.2±0.000</td>
<td></td>
</tr>
<tr>
<td>C. sativum + C. martinii</td>
<td>0.433±0.033</td>
<td>0.333±0.033</td>
<td>0.05±0.003</td>
<td>0.233±0.033</td>
<td></td>
</tr>
<tr>
<td>T. ammi + C. sativum</td>
<td>0.333±0.033</td>
<td>0.133±0.033</td>
<td>0.05±0.003</td>
<td>0.05±0.003</td>
<td></td>
</tr>
<tr>
<td>T. ammi + C. sativum + C. martinii</td>
<td>0.2±0.000</td>
<td>0.2±0.000</td>
<td>0.026±0.002</td>
<td>0.133±0.033</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Antidermatophytic activity of mixer of essential oils (μL/mL).

**Figure 1:** Chemical structure of some major compound of coriander essential oil.
response (synergistic effects), broad-spectrum antibacterial action, and the capacity to simultaneously attack multiple target sites, in many cases. In present investigation combination effect of selected essential oil exhibited excellent fungicidal properties as compared to essential oil alone effect. \textit{T. ammi}+\textit{C. sativum} (1:1) essential oil combination was found more powerful for inhibition of dermatophytes. Jain \textit{et al}.\cite{18} evaluated the \textit{T. ammi} seed essential oil for their chemical compositions, antidermatophytic capabilities, and acute dermal toxicity. MIC was ranging from .025±0.000 μL/mL to 0.5±0.000 μL/mL against dermatophytes.\cite{18} Two albino mice’s skin experienced a mildly irritating impact during toxicological testing at 5% oil content for up to 48 hr of observation. While erythematous effect was seen on all 5 experimental mice upto 72 hr. Similar result was also seen in \textit{Corianderum sativum} seed and \textit{C. martinii} leaf essential oils dermal toxicity testing.\cite{17,19} A 5% concentration was assigned to the insignificant irritating category and a 7% concentration to the mild irritant category. These studies showed that \textit{T. ammi} possesses extraordinary antifungal properties due the presence of thymol but at high concentration also showed some negative impact. Combining \textit{T. ammi} essential oil with other essential oils is a better option for therapeutic use because it may govern a high fungicidal action without causing any negative effects especially for the therapy of superficial fungal diseases.

As a result, using such combinations may be more effective than monotherapy in reducing toxicities and side effects as well as the establishment of resistance strains. These combinatorial techniques may complement conventional therapy by reducing symptoms, promoting healing, and halting the spread of dermatophytosis because of their antidermatophytic properties.

Trifan \textit{et al}.\cite{20} carried out an amalgamation therapy, relating terbinafine and essential oils for the treatment of dermatophytosis. They screened ajowan, coriander, caraway, and anise essential oil for enhancement of antimycotic action of terbinafine against dermatophytes.

There hasn’t been any research done on the combination of \textit{Coriander}, \textit{T. ammi}, and \textit{C. martinii} essential oils and how it affects dermatophytes yet. There have been few studies on the synergistic, additive, and neutral antibacterial properties of other essential oils.

Recently, the potential synergistic effects of combining several oils have been examined in order to increase the efficacy of essential oils. The use of synthetic antifungals in combination with essential oil will likely result in a more effective treatment. Many essential oils only have fungistatic effects, and fungicidal activity requires considerable doses.\cite{21}

According to Nguefack \textit{et al}.\cite{22} mixer of fractions from the same essential oil or from distinct essential oil (\textit{Cymbopogon citratus}, \textit{Ocimum gratissimum}, and \textit{Thymus vulgaris}) had additive, antagonistic, otherwise synergistic effects on fungus. Also, amalgamation of the \textit{C. giganteus} and \textit{C. citratus} oil exercised synergistic, additive and indifferent antimicrobial
effects contingent on the pathogen and amount of the used.

In vitro and in the tomato fruit system (in vivo), Mysore et al. examined the antifungal activity of cinnamaldehyde, eugenol, peppermint, and clove essential oil, as well as their combinations, against 12 species of Aspergillus, Fusarium, Penicillium, and Rhizopus. With the exception of peppermint oil, all examined fungi were totally inhibited by the essential oil at or below the 0.6% level and 80 μL in vitro. They proposed that fractional inhibitory tests, both in vitro and in vivo, demonstrated either an antagonistic or indifferent impact when combining cinnamaldehyde and clove, and either an additive or indifferent effect when combining eugenol and peppermint.

YuJie Fu et al. screened antifungal and antibacterial activities of clove and Rosemary alone and in combination. According to their findings, depending on the microorganism, the combination of clove and rosemary showed antagonistic, additive, and collaborative effects.

Lavandula angustifolia essential oil was tested for antibacterial properties together with 45 other oils by Stephanie de Rapper. The microdilution Minimum Inhibitory Concentration (MIC) method was used to determine the Fractional Inhibitory Concentration (FIC) for each oil combination. When lavender oil was tested in a 1:1 ratio with various oils, interactions were observed that were antagonistic (0.7%), non-interactive (23.7%), synergistic (26.7%), and additive (48.9%).

Research has shown that the cumulative effect of taking drugs in combination is substantially stronger than taking them alone.

**CONCLUSION**

Essential oils have been utilized to cure a variety of illnesses, including fungus infections of the skin, hair, and nails, from antiquity and even in modern popular culture. Although

---

**Figure 3:** Chemical structure of some major compound Cymbopogon essential oil.
essential oils are used all over the world as a supplement to alternative treatment, such as aromatherapy, their application as antimicrobials in conventional medicine has not yet occurred. Due to the complexity of the interactions between the major and minor components of particular oils, our understanding of the mechanism of action of these complex combinations is, at best, basic. Development of new antifungal medicines is necessary since resistance to current antifungals may cause them to lose their effectiveness over time, which emphasises the significance of creating fresh and alternative therapies. Overall, our work evaluated C. sativum, T. ammi and C. martini as a initial point for the creation of novel topical formulations for superficial dermatophyosis. Combinations of essential oil will assist minimise the concentrations of individual essential oil needed to provide the necessary antifungal effect and will lessen their impact on product sensory quality; however, further research is needed to determine their impact on a product’s general acceptability. Development of new antifungal medicines is necessary since resistance to current antifungals may cause them to lose their effectiveness over time, which emphasises the significance of creating fresh and alternative therapies. To provide a more thorough understanding of the mechanism of action of particular essential oils against individual fungi of clinical concern, however, more research will be required.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC: Gas chromatography; GCMS: Gas chromatography and Mass spectrometry; μL: Microliter; mL: Milliliter; MIC: Minimum inhibitory concentration; hr: Hours.

SUMMARY

In present study T. ammi, C. sativum and Cymbopogon martini essential oils extracted through hydrodistillation were evaluated alone and in combination against human pathogenic fungi. Blending of T. ammi with C. sativum showed excellent results as compared to T. ammi and C. martini. Although essential oils are frequently combined in the use of aromatherapy to cure various diseases, there is very little data to back up this practice. This study supports the concurrent usage of blended essential oils in some way. Combinations of essential oil will assist minimise the concentrations of individual essential oil needed to provide the necessary antifungal effect and will lessen their impact on product sensory quality; however, more research is required to find out how they affect a product’s overall acceptability.

AUTHOR’S CONTRIBUTION

Neetu Jain: Conceptualization, experimental studies, data handling and analysis, manuscript preparation and editing, review.

REFERENCES


Cite this article: Jain N. Evaluation of Mixer of Essential Oils and their Standard Components against Fungi Causing Superficial Mycosis in Human. Pharmacog Res. 2024;16(2):339-46.