



ANTIFUNGAL ACTION OF *Trachyspermum ammi* ESSENTIAL OIL AGAINST FUNGI CAUSING SUPERFICIAL SKIN INFECTIONS

ALKA KUSHWAHA^a, SUNIL KUMAR SINGH^b AND PIYUSH MISHRA^{c1}

^{abc}Department of Botany, D.A.V. College Civil Lines, Kanpur, Uttar Pradesh, India

ABSTRACT

Fungi are the very common superficial infectious disease in human being. *Trachyspermum ammi* (ajwain) essential oil has demonstrated potent antifungal activity against dermatophytes and other fungi responsible for superficial skin infections. The oil is rich in bioactive phenolic compounds, particularly thymol, along with carvacrol, *p*-cymene, and γ -terpinene, which contribute to its broad-spectrum antifungal properties. Studies have shown that *T. ammi* essential oil exhibits significant inhibitory effects against common dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Epidermophyton floccosum*, which are the primary causative agents of superficial mycoses. These fungi degrade keratinized material, and found throughout the world especially developing countries in prevailing hot and humid environmental condition. *Trichophyton* and *Microsporum* are main fungal genera responsible for causing various types of superficial infections in human being. The fungal diseases have traditionally been controlled by using synthetic drug like azoles, allylamines etc. These synthetic drugs also cause many harmful side effects on host as well as on environment. Due to its strong antifungal efficacy, natural origin, relatively low likelihood of inducing resistance, high yield and eco-friendly. The *T. ammi* essential oil is considered a promising for the development of topical formulations for the management of superficial fungal infections. However, further in vivo studies and clinical trials are required to establish its safety, efficacy, optimal dosage, and formulation for routine therapeutic use.

KEYWORDS: Essential Oil, *Microsporum*, Superficial Fungi, *Trachyspermum*, *Trichophyton*

Fungal infection of skin nails and hair are worldwide problem and causes various type of diseases. Mycotic diseases are the very common superficial infection in tropical and subtropical countries due to prevailing hot and humid environmental condition and poor hygienic living condition (Weitzman and Summerbell, 1955; Maraki and Tselentis, 1998; Ameen, 2010; Newze, 2010; Jain *et al.*, 2014). Superficial infections are normally referred as ringworm infection due to their ring like appearance (symptoms). In general, it is mainly caused by fungal pathogenic genera like *Trichophyton*, *Epidermophyton* and *Microsporum*. These genera are classified as anthropophilic, zoophilic and geophilic species based on their habitat. These fungi colonize on human and animal tissue as reservoirs like skin, hairs and nails. These reservoirs of dermatophytes communicate to another host in presence of favorable environmental condition (Weitzman and Summerbell, 1995). Dermatophytes are able to invade and decay of human and animal keratinized tissue, result of this lesion appears on skin of all effected body parts. The range of disease severity depends upon many factors including the host reaction to the metabolites produced by fungi, site of infection, infecting strain and environmental factors (Weitzman and Summerbell, 1995; Romani, 2007). Such dermatophytic infection has become severe health

problem affecting children, adolescent and adults. Such types of infections are mostly cutaneous and restricted to the nonliving, cornified layer of skin (Bhatia and Sharma, 2014). But when infection constantly reoccurring, the dermatophytes invade deeper layer and simultaneously causes infection with other organs.

The superficial mycotic infections have traditionally been controlled by using synthetic drug like Azole (clotrimazole, miconazole, econazole, oxiconazole) and Allylamines (Terbinafine and Naftifine) compounds as topical medication (Richard *et al.*, 2009; Gupta and Cooper, 2008) and mild reaction may occur at the site of application (Gupta *et al.*, 2003). Terbinafine and itraconazole have been used as oral drugs (Evans, 2003; Chao and Young, 2000; Baran *et al.*, 2003). When patients displaying large area of infection or in case of persistent or severe infection. Oral administration of synthetic drugs may be causes major side effects including hepatotoxicity, nephrotoxicity, neurotoxicity or hematologic reaction. The Griseofulvin antifungal drugs which had been used for treatment of mycotic diseases for many years. It is still the preferred drug, though fungus has already developed resistance to this drug (Huang *et al.*, 2004). In view of above factors emphasize the urgent need for the development of new alternatives effective treatment. Spices are used as important part of

¹Corresponding author

daily Indian foods for flavoring and having broad spectrum antimicrobial properties including dermatophytes (Mandeel and Al-Laith, 2007).

Our traditional knowledge and modern science prove the antifungal properties of plant derived essential oils (Jain and Sharma, 2003; Shahi *et al.*, 2007; Kalita *et al.*, 2017; Jalila *et al.*, 2017; Jain and Sharma, 2016). *Trachyspermum ammi* L. (ajwain), a member of the Apiaceae family, is an important medicinal and aromatic seed spice native to Egypt and widely cultivated in countries including India, Pakistan, Iran, Iraq, and Afghanistan. In India, it is grown extensively in the states of Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar, and West Bengal. The plant has long been recognized in traditional medicine for its diverse therapeutic applications. The roots exhibit diuretic properties, while the seeds are valued for their aphrodisiac, carminative, antispasmodic, and stomachic effects. Ajwain seeds contain approximately 2–4% essential oil, commonly known as ajwain oil, with thymol as its principal bioactive constituent. Thymol is responsible for many of the oil's pharmacological activities, including potent antifungal, antimicrobial, antiseptic, and antispasmodic effects. Traditionally, ajwain has been used to manage gastrointestinal disorders such as indigestion, flatulence, diarrhea, abdominal pain, and loss of appetite, as well as respiratory ailments including bronchial disorders (Bentley and Trimen, 1999).

The seeds also possess anthelmintic and mild laxative properties and have been used in the treatment of abdominal tumors and piles (Krishnamoorthy and Madalageri, 1999). Owing to its strong antimicrobial (Srivastava, 1988) and fungicidal (Singh and Singh 2000) activities, thymol has found applications in pharmaceutical and oral care products (Joshi, 2000) including toothpaste and antiseptic formulations. These medicinal attributes make *T. ammi* an important natural source for the development of antifungal and antimicrobial therapies.

The least adverse effects improve cure rate, minimum drug interaction and cost effective at available topical agents to combat fungal infections including dermatophytosis. To consider these facts in mind, the new antifungals of plant origin could be an alternative preferred treatment of dermatophytosis for human wellbeing. The above facts promoted us to explore the essential oils for the treatment of dermatophytic infection.

MATERIALS AND METHODS

Maintenance of Human Pathogenic Fungi

The test pathogens, *Epidermophyton floccosum* (Hartz) Langeron et Mitochevitch, *M. canis* Bodin, *M. nanum*, Fuentes, *M. gypseum* (Bodin) Guiart et Grigoraki, *Trichophyton mentagrophytes* (Robin) Blanchard, *T. rubrum* (Castellani) Sabouraud were maintained on Sabouraud dextrose agar (SDA) medium. A seven-day old culture of test pathogens was used for antifungal study.

Trichophyton: Trichophyton is inhabiting either soil (geophilic) or humans (anthrophilic) or animals (zoophilic). This genus is one of the leading causes of skin, hair and nail infection in human being. Most of the Trichophyton species have teleomorphic form. The telomorphic form of Trichophyton is classified in the genus Arthroderma. Many species of these fungal genera have anthrophilic in nature. The most common anthrophilic species are *Trichophyton rubrum*, *T. mentagrophytes*, *T. violaceum*. Among them *T. rubrum* is the most common causative agent of dermatophytosis worldwide (Nenoff *et al.*, 2014). *Trichophyton* is keratinophilic filamentous fungus and possession of several enzymes, such as acid proteinases, keratinases and other proteinases are the major virulence factors of these fungi.

Epidermatophyton floccosum: *Epidermatophyton floccosum* colony on Sabouraud dextrose agar (SDA) medium is moderately fast growing at room temperature, the surface is velvety or felt-like, often with radial furrows and an irregular, folded center, or the surface may be smooth, the colour is pinkish white in beginning turning to dull-white with age. The reverse of colony is yellow-tan.

Microsporum gypseum: Colony are fast growing, producing a flat, spreading, powdery surface that is rich cinnamon buff to brown, occasionally with overtones of violet. The powder consists of masses of macroconidia. The edges of colony are entire to scalloped or ragged. A variety of pigments or none are produced in reverse side of colony.

Trachyspermum ammi (Ajwain): Ajwain is distributed throughout India and mostly cultivated in Gujarat and Rajasthan. Ajwain fruits yield 2% - 4% brownish essential oil. GCMS analysis of oil shows presence of 20 compounds. Thymol was found as a major compound (58.88%) followed by p-cymene (24.02%), γ terpinene (13.77%), β pinene (1.90%) and other compound in very small amount (Jain *et al.*, 2018) The action of ajwain oil against the pathogens like *T. rubrum*, *T. mentagrophytes*,

Microsporum canis, *M. gypseum*, *M. fulvum* and *Epidermatophyton floccosum* shown in following table-

Isolation and Characterization of Essential Oil

The essential oil was extracted from the fruits of *Trachyspermum ammi* by hydrodistillation using Clevenger's apparatus (Clevenger 1928). A colorless or brownish yellow colored oily layer was separated and dried with anhydrous sodium sulfate. The Physiochemical properties of the oil were determined by the technique described by (Langenau Langenau, 1948). Gas liquid chromatography (GLC) analysis of the oil was done.

Efficacy and application of plants secondary metabolite of *Trachyspermum ammi* (Ajwain)

The fruits of *T. ammi* (Ajowan) on hydrodistillation yield 3.0% essential oil. The physiochemical properties of the oil were shown in table 1. The essential oil of *T. ammi* showed four minor and one major components. The percentage purity of the separated fractions was determined using analytical gas chromatography. Several research papers are published each year on the antidermatophytic activity of botanicals from higher plants. Brief discussion on efficacy and application of antimycoti properties essential oil of *Trachyspermum ammi* family Apiaceae. The essential oil fraction is often most inhibitory to growth and survival of superficial fungal infection. Essential oils are highly enriched with terpenoids in plants to inhibit the growth of dermatophytes. The studies show that plants have potential to be effective antidermatophytic agent. More studies are needed on applications of essential oil extracted from seeds of *Trachyspermum ammi* (Ajawain) plants to fully understand how best to optimize their use. The physiochemical properties of the oil of *T. ammi*. Given in table 1.

Table 1: Physiochemical properties of the oil of *T. ammi*.

Properties studied	Observations
Plant height (cm)	158-180
Oil yield (%)	3.0
Colour	Brownish yellow
pH	5.8
Specific gravity at 25 ⁰ C	0.92231
Refractive index at 20 ⁰ C	1.5021
Optical rotation	+3.0 ⁰
Saponification value	12.4
Acid value	3.2
Ester value	9.2
Thymol (%)	60-80
Solubility	90% alcohol

In Vitro Investigation

The nature of antifungal activity (fungistatic/fungicidal) of the oil at MIC was determined by the method of (Garber and Houston, 1959). The inhibited fungal discs (at MIC) were reinoculated upside down on plain SDA medium. Inoculated petri plates wear incubated at 27 ± 1⁰C, observations were recorded on seventh day. Fungal growth on seventh day indicated fungistatic action of the oil while absence of fungal growth denoted fungicidal action. The minimum inhibitory concentrations (MIC) of the oil against test pathogens (dermatophytes) were determined following the poisoned food technique of (Grover and Moore, 1962.) with slight modification (Shahi *et al.*, 1999). The MIC of the oil of *T. ammi* were found to be 0.05 µl ml⁻¹ for *Trichophyton rubrum*, *T. mentagrophytes*, and *M. canis*, 0.1 µl ml⁻¹ for *E. floccosum*, 0.2 µl ml⁻¹ for *M. gypseum*. The minimum fungicidal concentration (MCC) were found 0.1 µl ml⁻¹ for *M. canis*, *T. rubrum*, *T. mentagrophytes*, 0.2 µl ml⁻¹ for *E. floccosum* and 0.3 µl ml⁻¹ for *M. gypseum* (Table 2). The oil inhibited heavy doses (30 mycelial discs, each of 5 mm in diameter) of fungal inoculum at 0.2 µl ml⁻¹ concentration. The activity of the oil did not expire even up to 48 month of storage and persisted up to 100⁰C temperature.

The effects of inoculum potentiality at MIC of the oil were determined by the method of (Shahi *et al.*, 2000) Effect of the temperature, on antifungal activity of the oil was evaluated (Shahi *et al.*, 2000). Five lots of oil were kept in small vials, each containing 5 ml of oil; these were exposed to 40, 60, 80 and 100⁰C in incubator for an hour. Antifungal activity then tested at MIC by the poisoned food technique (Grover and Moore 1962).

To comparative study of the oil with some synthetic antifungals (miconazole, ketaconazole, ciclopirox alamine) was carried out by comparing minimum inhibitory concentrations and minimum fungicidal concentrations (Shahi *et al.*, 2000). Various concentrations 0.01 to 10 µl ml⁻¹, of synthetic antifungal substances were prepared from their stock solutions by incorporating into adequately cooled autoclaved 100 ml SDA medium. In controls, sterilized water (in place of the antifungal substances) was used in the medium in appropriate amount. The MIC was assayed following the poisoned food technique (Grover and Moore 1962). The nature of toxicity at MIC was determined by the method (Garber and Houston, 1959)

Table 2: Antifungal activities MIC ($\mu\text{l ml}^{-1}$) and MFC ($\mu\text{l ml}^{-1}$) of Ajwain oil (*T. ammi*) against dermatophytic fungi

Pathogen	MIC ($\mu\text{l ml}^{-1}$)	MFC ($\mu\text{l ml}^{-1}$)
<i>Trichophyton rubrum</i>	0.05	0.10
<i>T. mentagrophytes</i>	0.05	0.10
<i>Microsporum gypseum</i>	0.20	0.30
<i>M. canis</i>	0.05	0.10
<i>Epidermatophyton floccosum</i>	0.1	0.20

MIC= minimum inhibitory concentration
MFC= minimum fungicidal concentration

RESULTS AND DISCUSSION

The preliminary evaluated of antidermatophytic activity of *Trachyspermum ammi* plants essential oil. This plant essential oil was found to be very effective against various species of the *Trichophyton*, *Epidermatophyton* and *Microsporum* genera.. The MIC of the oil of *T. ammi* were found to be $0.05 \mu\text{l ml}^{-1}$ for *Trichophyton rubrum*, *T. mentagrophytes*, and *M. canis*, $0.1 \mu\text{l ml}^{-1}$ for *E. floccosum*, $0.2 \mu\text{l ml}^{-1}$ for *M. gypseum*. The minimum fungicidal concentration (MCC) were found $0.1 \mu\text{l ml}^{-1}$ for *M. canis*, *T. rubrum*, *T. mentagrophytes*, $0.2 \mu\text{l ml}^{-1}$ for *E. floccosum* and $0.3 \mu\text{l ml}^{-1}$ for *M. gypseum* (Table 2). The oil inhibited heavy doses (30 mycelial discs, each of 5 mm in diameter) of fungal inoculum at $0.2 \mu\text{l ml}^{-1}$ concentration. The activity of the oil did not expire even up to 48 month of storage and persisted up to 100°C temperature.

These dermatophytes are often developing resistant to available synthetic antifungal agents. This open an important frontier in alternative antifungal therapies because some effective essential oils are as antidermatophytic agent with very low MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) and superior to synthetic fungicides. The essential oil is generally nontoxic, biodegradable, renewable source and eco-friendly in nature, while synthetic drugs are toxic, causes various side effects, generally non-biodegradable and nonrenewable. This obviously justifies the use of this plant essential oil in traditional medicine to cure mycotic diseases. Furthermore, work is needed on this effective plant to develop more potent antimycotic drugs.

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