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ORIGINAL ARTICLE **OPEN ACCESS**

Antifungal Activity of Essential Oils against Fungi causing Otomycosis

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ABSTRACT

Otomycosis is a chronic fungal infection of the external ear canal which may also occur in the middle ear and tympanic membrane. Essential oils are the mixtures of aromatic compounds which are used as herbal medicines for the treatment of various types of infectious diseases. The objective of this study is to evaluate the antifungal action of various essential oils i.e. Lemongrass oil (Cymbopogon citratus), Eucalyptus oil (Eucalyptus globulus), Tea tree oil (Melaleuca alternifolia), Cinnamon oil (Cinnamomum verum), Peppermint oil (Mentha piperita), Fennel seed oil (Foeniculum vulgare), Basil oil (Ocimum basilicum), and Oregano oil (Origanum vulgare) against four pathogenic fungi Aspergillus flavus, Aspergillus niger, Candida and Aspergillus tamarii. To examine the antifungal activity of essential oils by using disc diffusion method. The spore suspension was inoculated in Sabouraud's dextrose agar and placed sterilized essential oil-loaded filter paper disc. After incubation period, the diameter of the zone of inhibition was measured in millimeters. The highest value of the diameter of inhibition zone by Cinnamon oil in Aspergillus flavus (36 mm), Aspergillus niger (32.3 mm), Candida (37 mm), and Aspergillus tamarii (31 mm) and Fennel seed had the lowest inhibitory effect on Aspergillus flavus (12 mm), Aspergillus niger (14 mm), Candida (19 mm) and Aspergillus tamarii (17 mm). In the present study, Cinnamon oil shows strong antifungal activity as compared to the other essential oils. This study reveals that aromatic essential oils showed strong antifungal activity against Otomycotic pathogens. It can be used in the advancement of new natural antifungal drugs.

Keywords: *Essential oil, Aromatic, Otomycosis, Antifungal, Herbal medicines*

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INTRODUCTION

Aromatic medicinal plants are used traditionally as pure compounds to treat various types of infectious diseases such as Otomycosis [1]. It is a common medical problem all over the world. The major fungal agents of Otomycosis are *Aspergillus* and *Candida* species [2]. Several medicinal plants are used to produce essential oils and more than 300 different types of essential oils are available on market [3]. It is also used in agriculture, food, cosmetics, aromatherapy, and in the field of pharmacology. Essential oils are aromatic volatile oils that are made from organic plant matter. The formation of essential oil is depending on the plant parts (root or aerial part) and season [4]. Essential oil is an intricate mixture of aldehydes, ketones, ethers, esters, hydrocarbons, phenols, flavonoids, quinones, terpenes, lactones, and other chemical compounds [5]. All these components are important in the biological properties of aromatic essential oils and fragrance. The aromatic organic compounds have a low molecular weight. The scientific studies on essential oils showed that antibacterial, antifungal, anti-inflammatory, and anxiolytic effects [3]. In recent years the prevalence of fungal infections in humans is increasing and there are limited remedial strategies. The natural antifungal property of essential oils produces a toxic effect on cell membrane structure and function which inhibits the growth of fungal pathogens [6]. The available antifungals drugs and their side effects fascinate the researchers towards the development of new cost effective natural antifungal drugs. In the present study, the antifungal activity of eight essential oils is tested against fungi causing Otomycosis. Essential oils are natural substances that contain powerful antifungal properties which are more effective in the treatment of diseases.

MATERIAL AND METHODS

Fungal strains:

In the present study, samples were collected at the ENT hospitals Ujjain (M.P.) India. All samples were collected aseptically using sterile cotton swabs from the external auditory canal [7]. The samples were inoculated on Sabouraud's dextrose agar (SDA) with chloramphenicol and incubated at 28°C ± 1°C for

seven days. After incubation, cultural and morphological identification of fungal isolates was followed by standard monographs [8,9]. The four isolated test fungi i.e. *Aspergillus flavus, Aspergillus niger*, *Candida sp*, and *Aspergillus tamarii* are used in this study.

Essential Oils:

The eight distinct types of essential oils i.e. Lemongrass oil (*Cymbopogon citratus*), Eucalyptus oil (*Eucalyptus globulus*), Tea tree oil (*Melaleuca alternifolia*), Cinnamon oil (*Cinnamomum verum*), Peppermint oil (*Mentha piperita*), Fennel seed oil (*Foeniculum vulgare*), Basil oil (*Ocimum basilicum*), Oregano oil (*Origanum vulgare*) were purchased from the local herbal shop in Ujjain (M.P.) India.

Inoculum preparation:

The antifungal activity of essential oils was performed by the disc diffusion method [10,11,12]. The fungal spore suspension was prepared from 5 days old cultures of all test fungi and spread on the surface of Sabouraud's dextrose agar. The sterilized filter paper disc (Whatman's filter paper no. 1) was immersed in 25µl of essential oil and placed in the center of the agar surface. All Petri plates were incubated at 28°C ± 1°C for 5 days. Each set was performed in triplicates. The diameter of the zone of inhibition was measured in millimeters with the help of an antibiotic zone scale (HiMedia Laboratories Pvt. Ltd.).

RESULTS

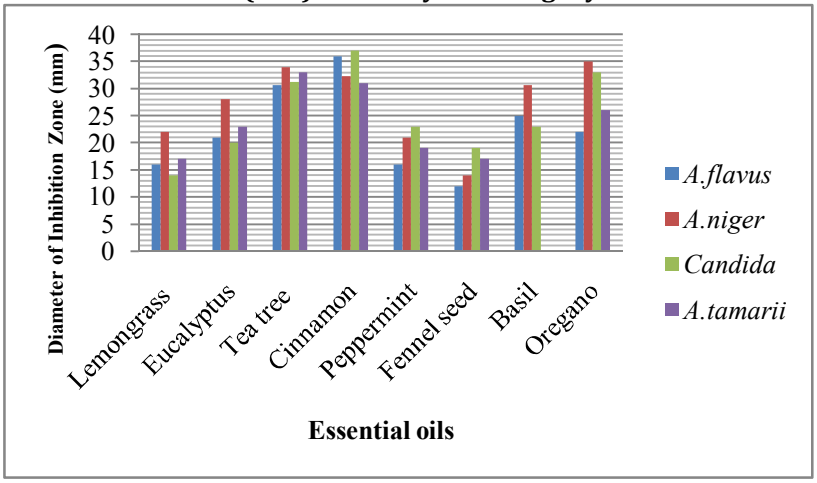
The results of the antifungal activity of essential oils were tested against pathogenic fungi are shown in Table 1 and Figure 1. The highest antifungal activity of essential oils such as Cinnamon oil followed by Tea tree oil, and Oregano oil showed the inhibitory effect on mycelium growth of all test fungi. The highest value of the inhibition zone of Cinnamon oil is showed *Aspergillus flavus* (36 mm), *Aspergillus niger* (32.3 mm), *Candida* (37 mm), and *Aspergillus tamarii* (31 mm), respectively. Cinnamon oil is extremely sensitive to all test fungi. The inhibition of Tea tree oil showed *Aspergillus flavus* (30.6 mm), *Aspergillus niger* (34 mm) *Candida* (31.3 mm), and *Aspergillus tamarii* (33 mm), respectively. Oregano oil showed an inhibitory effect on *Aspergillus flavus* (22 mm), *Aspergillus niger* (35 mm), *Candida* (33 mm), and *Aspergillus tamarii* (26 mm) respectively. Fennel seed showed minimum inhibitory effect on *Aspergillus flavus* (12 mm), *Aspergillus niger* (14 mm), *Candida* (19 mm), and *Aspergillus tamarii* (17 mm), respectively. All test fungi sensitive towards various essential oils, except *Aspergillus tamarii* resistant towards the Basil oil and shows no zone of inhibition.

Table 1: Antifungal effect of essential oils on Otomycotic fungi

Essential oils	Diameter of Inhibition Zone (mm)			
	A.flavus	*A.niger*	*Candida*	*A.tamarii*
Lemongrass	16±0.93	22±0.93	14±0.46	17±0.81
Eucalyptus	21±0.81	28±0.81	20±0.81	23±0.81
Tea tree	30.6±0.71	34±0.81	31.3±0.81	33±0.46
Cinnamon	36±0.46	32.3±0.71	37±0.46	31±0.93
Peppermint	16±0.81	21±0.93	23±0.64	19±0.38
Fennel seed	12±0.94	14±0.46	19±0.38	17±0.81
Basil	25±0.93	30.6±0.71	23±0.81	NI
Oregano	22±0.93	35±0.46	33±0.46	26±0.46

Each value is mean of triplicates ± Standard error; NI = No Inhibition Zone

Figure 1: Inhibition zone (mm) on Otomycotic fungi by various essential oils



DISSCUSION

In the present study, the antifungal effect of eight essential oils on the growth of *Aspergillus flavus, Aspergillus niger*, *Candida sp*, and *Aspergillus tamarii*. Aromatic essential oils contain strong antifungal activity against various fungal pathogens [13]. The data in the current study showed that the highest antifungal activity of Cinnamon oil is effective against all test fungi. Cinnamon is primarily rich in essential oils and other active components such as cinnamaldehyde, cinnamate, and cinnamic acid [14]. The antifungal properties of Cinnamon oil are present in their bark and leaves [5]. Several studies suggested that the compounds present in Cinnamon oil offer various health benefits [15]. Tea tree oil consists of various active ingredients which are used against various types of cutaneous infections. The mechanism of antifungal action of Tea tree oil changes their fungal membrane inhibits the growth of dermatophytes and various filamentous fungi [16]. Oregano oil possesses extensive antimicrobial properties. It inhibits the growth of fungus which causes systemic and superficial infections [17]. In our present study, the effect of Oregano essential oil inhibits the mycelium growth of test fungi.

In several studies, the Eucalyptus essential oil shows antibacterial, antifungal, antioxidant, anti-inflammatory activity on pathogens [18]. In vitro studies of Eucalyptus essential oil showed effectiveness against various pathogenic fungi. Peppermint essential oil is well known effective antimicrobial agent against various fungal pathogens [19]. Peppermint oil and its leaves are widely used as herbal medicinal products to treat various etiological agents of dermatophytosis [20]. Basil essential oil showed a natural antifungal effect against the number of fungal pathogens [21]. Some researchers reported that estragole, eugenol, linalool, and methyl cinnamate are the important antimicrobial and antifungal components which are present in Basil essential oil [22]. In various studies suggested that the mechanism of inhibitory action of essential oil on fungal membrane ATPases, mitochondria, and cytokine interaction [23]. The inhibitory action of volatile oil blocks an enzyme synthesis, nutrient uptake and membrane structure [11]. Several researchers recorded the antifungal activities of different essential oils were carried out by disc diffusion method which is a rapid method to evaluate the susceptibility of fungal pathogens to different essential oils [10,24].

In the present study, numerous essential oils showed antifungal action against various Otomycotic fungi. Lemongrass essential oil consists of monoterpenes, geraniol, and geranyl acetate compounds. Several studies reported the antibacterial and antifungal activity of lemongrass oil. Some studies reported that Lemongrass oil shows an inhibitory effect on fungal pathogens of cutaneous candidiasis and dermatomycosis [25]. Fennel seed and bark extract show the antifungal action against various fungal strains and dermatophytes [26].

CONCLUSION

Essential oils are used since ancient times as a strong anti fungicidal agent. It contains natural antifungal active compounds which are used to treat distinct types of fungal infections. The present study focuses on the efficacy of all essential oils shows inhibitory effects on Otomycotic pathogens. Essential oil treatment is a new therapeutic remedy that is safe and efficient as compared to the use of synthetic chemical fungicides. This study can help design new antifungals drugs against fungus with proper clinical examination.

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