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Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic *Aspergillus fumigatus* and *A. niger*

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Abstract: Oils extracted from fifteen medicinal plants were screened for their activity against *A. fumigatus* and *A. niger* by disc diffusion method. Minimum inhibitory concentrations (MICs) of oils (%v/v) against *Aspergillus fumigatus* and *Aspergillus niger* done by agar dilution method and minimum inhibitory concentration (MIC) and minimum cidal concentration (MCCs) data (%v/v) obtained by the broth micro dilution method. The results showed that the maximum antimycotic activity was demonstrated by oils of *Cymbopogon martini, Eucalyptus globulus* and *Cinnamomum zylenicum* as compared to control, followed by *Cymbopogon citratus* which showed activity similar to control (miconazole nitrate). The oils of *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The oils of *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculum vulgare* and *Elettaria cardamomum* demonstrated comparatively low activity against *A. niger* and *A. fumigatus* as compared to control. Mixed oils showed maximum activity as compared to standard. These results support the plant oils can be used to cure mycotic infections and plant oils may have role as pharmaceutical and preservatives.

Key words: Essential oil % Antifungal activity % Minimum inhibitory concentration % *Aspergillus*

INTRODUCTION

Infectious diseases accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created [1]. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal plants [2]. There are alarming reports of opportunistic fungal infections [3]. The infections caused by opportunistic fungi are included under new spectrum of fungal pathogens. Such fungi were earlier reported from various plants as pathogens. But now they are known to cause disease in human beings. There is an increasing awareness amongst clinicians and microbiologists pertaining to importance of infection caused by opportunistic fungi [4]. Aspergillosis is caused due to inhalation of Aspergillus fumigatus spores. *Aspergillus fumigatus* is an opportunistic pathogen which usually affects cavities that have formed in the lungs from preexisting lung diseases. In the lungs, *Aspergillus fumigatus* forms tangled mass of fungus fibers, blood clots. The fungus mass gradually enlarges, destroying lung tissue in the process, but usually does not spread to other areas.

There are 2600 plant species of which more than 700 are noted for their uses as medicinal herbs [5]. In folk medicine, medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases. Today, a great number of different medicinal tea and other plant products are available in market (including cosmetics and pharmaceuticals), which contains biologically active substances. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have been reported to be safe and without side-effects.

A survey of literature reveals that there are many essential oils which possesses antifungal activity [6-13]. In tuberculosis, treatment contains high doses of antibiotic due to resistance and side effects of this antibiotic, patients take more time for cure Therefore, we

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Table 1: Oils of Indian medicinal plants including the botanical name, Local name, family and traditional use

Botanical name	Local name	Family	Plant part used	Medicinal use
Allium sativum	Garlic oil	Liliaceae	Bulb	expectorant, antibacterial, antifungal
Azadirachta indica	Neem oil	Meliaceae	leaf and seeds	Antiviral activity, antifungal activity
Cuminum cyminum	Jira oil	Umbelliferae	Dried ripe fruit	Stimulant, carmative, antifungal
Cymbopogon martini	Tikhadi oil	Poaceae	Leaves	Rheumatism and skin diseases
Cymbopogon citratus	Lemon grass oil	Poaceae	Leaves	Antimicrobial, Vitamin A
Cinnnamomum zylanicum	Dalchini oil	Lauraceae	Bark and leaves	Carminative and antimicrobial
Eucalyptus globulus	Nilgri oil	Myrtaceae	Leaves	Antiseptic and antimicrobial
Eugenia caryophyllata	Clove oil	Myrtaceae	Dry flowers	Antimicrobial
Elettaria cardamomum	Cardamom	Zingiberaceae	Dried fruit	Carminative, antimicrobial
Foeniculum vulgare	Fennel	Umbelliferae	Dried fruit	Carminative, antimicrobial, expectorant
Mentha spicata	Mint oil	Lamiaceae	Leaves	Antimicrobial
Ocimum sanctum	Tulsi oil	Lamiaceae	leaves	Antibacterial, antifungal
Trachyspermum captivum	Ajwain	Umbelliferae	Dried fruits	Antispasmodic throat infection, bronchitis, antibacterial, antifungal
Withania sominifera	Ashwagandha	Solanaceae	Roots and stem	Tonic, antimicrobial
Zingiber officinale	Zinger oil	Zingiberaceae	Rhizome	Antimicrobial, carminative

need to search plant derived antifungal drugs which are safe and without side-effects. Hence, it is of interest to determine the scientific basis for the traditional use of medicinal plants.

The aim of this study was to assess the antifungal activity of 15 essential oils against *Aspergillus niger* and *Aspergillus fumigatus.* In the present study, 15 different medicinal plants have been chosen for the investigation of *in vitro* antifungal activity which is acts as expectorant and not having toxic properties (Table 1). The purpose of this was to create directly comparable, quantitative, antimicrobial data and to generate data for oils for which little data exist and which are used in to developed antituberculosis and antifungal drugs.

MATERIALS AND METHODS

Medicinal Plants Oils: Leaves of *Cymbopogon citratus* Stapf., *C. martinii* Roxb., *Eucalyptus globulus* Labill., *Azadirachta indica* Linn. and *Ocimum sanctum* Linn., were collected from wild fields of Amravati university, Amravati district of Maharashtra India. *Allium sativum* Linn., *Mentha spicata* Linn., *Withania sominfera* Linn., *Zingiber officinale* Roscoe., *Cinnamomum zeylanicum* Nees. collected from PKV Akola,Maharashtra India, Seeds of *Cuminum cyminum* Linn., *Trachyspermum copticum* Linn., *Elettaria cardamomum* Maton., *Foeniculum vulgare* Miller. and *Eugenia caryophyllata* Thunb., were purchased from the plant medicinal shop of Amravati maharastra india. The seeds and roots of above plants were dried at room temperature and powdered and leaves of above plants were cut into pieces for extraction.

Extraction Procedure: The seeds, roots and leaves for oil were dried and ground to semi-powdered state. The air-dried aerial parts (50 g) were hydro distilled in a clevenger apparatus (sigma chemical company) for 5 h. in accordance with the British pharmacopoeia. The yield was 0.62% dry weight.The aqueous phase was extracted with dichloromethane (Qualigens) (3 x 50 mL). The organic phase was dried with sodium sulphate (Bio-RAD), filtered and the solvent evaporated until dryness by air-dry. The fractions obtained were combined into calibrated flasks, evaporated to dryness and weighted in order to determine the extraction's efficiency. The oils were solublized in DMSO (Bio-RAD) to a final concentration 5 mg/mL [14]. The oils were stored in a sealed glass vial (bijoux bottle) in a refrigerator at 4 ºC until required. These all oils of above plants were screened for their antimycotic activity.

Fungus Selection and Growth Condition: In our survey in different hospitals and primary health center of Washim District (Central India) under the Revised National Tuberculosis Control Programmed (RNTCP) of WHO as a senior tuberculosis laboratory supervisor (STLS), we found 1357 patients of tuberculosis were recorded during June 2002 to September 2003 by sputum examination in ten PHC (primary health center) laboratories of tuberculosis in Washim District. These patients were recorded in (tuberculosis unit register) main hospital of washim. Out of 1357 patients, highly chronic pulmonary tuberculosis patients were found to be 500, which on CAT-I and CAT-II category of tuberculosis (DOTS) treatment. We found, out of 500 (CAT-I and CAT-II) patients 200 patients were suffering from mycotic infection and out of 50 patients of symptomatic TB patients' only 23 patients suffering from mycotic infection

rest of negative. The percentage of mycotic infection in pulmonary tuberculosis patients was 46.0%. Mainly for types of fungi, viz. *Aspergillus niger, A. fumigatus, Histoplasma capsulatum* and *Cryptococcus neoforman* were recovered. Culture characteristic of defferent fungi analysis were done using sputum sample of chronic tuberculosis patients and symptomatic patients (Non tuberculosis). Selection of *Aspergillus fumigatus* and *Aspergillus niger* on the bases of higher percentage in pulmonary tuberculosis patients.

A.fumigatus and *A. niger* were isolated from the sputum samples of pulmonary tuberculosis patients under the programme DOTS from the civil hospital of Washim, Washim District, maharashtra state, India. Deep sputum samples (2-4ml) tuberculosis patients were obtained under sterile conditions. Organisms were maintained on Czapek's dox agar (diffco) (Sodium nitrate: 02 g; Sucrose: 30 g; Dipotassium phosphate: 1g; Magnesium Sulphate: 0.5 g; Potassium Chloride: 0.5g; Ferrous Sulphate: 0.01g; Agar-Agar:15.0g; Distilled Water:1000 ml; pH:7.3 ± 0.2) in the dark at 28ºC for 5-10 days. Culture confirmed by microscopy and PCR. Inocula were prepared by diluting overnight cultures in saline to approximately 10^5 CFU ml^{-1}. These suspensions were further diluted with saline as required.

Antimycotic Assay by Disc Diffusion Technique: Oils were screened for their antifungal activity against *A. fumigatus* and *A. niger* by disc diffusion method. Different concentration of each oil and mixed oils (equal ratio of each oil), i.e., 100 µg, 50 µg, 25 µg and 12.5 µg/disc were used for assay. The fungal cultures were grown on czapex dox broth (diffco). The mycelial mat of *A. fumigatus* and *A. niger* of 7-day old culture was washed, suspended in normal saline solution and then filtered through glass wool aseptically. The colony forming units (CFU/ml) of suspension of the test fungus was determined and test inoculum was adjusted 1-5 X 10^5 ml. These conidia were used for antifungal assay tests. Inocula (0.1ml) were applied on the surface of the Czapek's dox agar (Diffco) plate and spread by using sterile glass spreader. The sterile discs (5mm diameter, Whatmans filter paper No.42) were soaked in added concentrations (100µg / disc, 50µg /disc, 25µg /disc and 12.5µg / disc) of essential oils. The test was performed in triplicate. These dishes were incubated for 48 h at 28ºC. Zone of inhibition in mm were determined after 48 h. Standard antibiotics miconazole (Sigma) and intraconazole (Sigma) were used in order to control the sensitivity of the tested fungus. Miconazole was used as positive control because miconazole and meconazole those that followed such as ketconazole, fluconazole and intraconazole proved to be important drugs for combating human fungal infections. The clinical efficiency and safety of flucanozole in particular has resulted in wide sprade use. The resultant emergence of resistant to azoles has intensified the search for new compounds that are active against resistant organisms [15-18]. DMSO (Sigma) as negative control and filter paper disc which is soaked with distilled water no oil is used as negative control. The zones of different oil were measured. the data of all the parameters were statistically analyzed.

Agar Dilution Method: The agar dilution method followed that approved by the NCCLS with the following modification: a final concentration of 0.5% (v/v) Tween-20 (Sigma) was incorporated into the agar after autoclaving to enhance oil solubility. Briefly, a series of twofold dilutions of each oil, ranging from 2% (v/v) to 0.03% (v/v), was prepared in sabouraud dextrose agar (SDA) (Himedia) with 0.5% (v/v) Tween-20. Plates were dried at 35 °C for 30 min prior to inoculation with 1–2 ml spots containing approximately 10^5 CFU of each organism. SDA, with 0.5% (v/v) Tween-20 but no oil, was used as a positive growth control. Inoculated plates were incubated at 35°C for 48 h. Minimum inhibitory concentrations (MICs) were determined after 48 h for *Aspergillus fumigatus* and *Aspergillus niger*. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded.

Broth Micro Dilution Method: The broth micro dilution assay was performed as described previously [19] with the following modifications: Sabouraud dextrose agar (SDA) (Himedia) was used, in tests with *Aspergillus fumigatus* and *A. niger*, sub-cultures were performed after 48 h incubation. For most oils, the highest concentration tested was 4.0% (v/v), although for some this was 8.0% (v/v). The lowest concentration tested was 0.008% (v/v).

Statistical Analysis: Minimum inhibitory concentrations of the essential oils were compared using the Wilcoxon signed rank test (Graph Prism software for Win-dows, GraphPad Software, San Diego, USA).

RESULTS

The antifungal activities of fifteen plant oils obtained by the disc diffusion method are shown in Table 2. All the oils tested exhibited different degrees of antifungal activity against *Aspergillus fumigatus* and *A. niger*

Table 2: Antifungal activity of the fifteen oils of medicinal plants against *Aspergillus fumigatus* and *Aspergillus niger* growth *in vitro*

S. No.	Plant oils	Aspergillus fumigatus100 50 25 12.5µg /disc /Zone ofinhibition (mm)				Aspergillus niger100 50 25 12.5µg /disc / Zone ofinhibition (mm)			
1	Allium sativum (Garlic oil)	15±0.5	13±0.5	12±0.1	10±0.1	14±0.5	12±0.5	10±1.1	8±0.5
2	Azadirachta indica(Neemoil)	16±0.5	13±0.5	12±1.1	10±1.1	14±0.5	13±0.5	11±1.1	8±0.5
3	Cuminum cyminum(Jira Oil)	13±0.5	12±0.5	10.5±0.5	10±0.5	12±0.5	11±0.1	10±0.5	9±0.5
4	Cymbopogon martinii(Tikhadi oil)	24±0.5	22±0.5	21±0.5	19±0.5	22±0.5	20±0.5	19±0.5	15±0.5
5	Cymbopogon citratus (Lemon- Grass Oil)	22±0.5	20±0.5	19±0.5	18±0.5	21±0.5	20±0.5	18±0.5	18±0.5
6	Cinnnamomum zylanicum (Kalmi-Dalchini oil)	19±0.5	17±0.5	16±0.5	12±0.5	19±0.5	17±0.5	16±0.5	12±0.5
7	Eucalyptus globulus (Nilgri oil)	22±0.0	20±0.5	19±0.5	18±0.5	21±1.0	20±1.0	19±0.5	18±0.5
8	Eugenia caryophyllata(Clove oil)	15±0.5	13±0.5	12±1.1	10±1.1	14±0.5	12±0.5	10±0.5	8±0.1
9	Elettaria cardamomum (Cardamomseeds)	13±0.5	12±0.5	10.5±0.5	10±0.5	12±0.5	11±0.5	10±0.5	9±0.5
10	Foeniculum vulgare(Fennel fruits oil)	13±0.5	12±0.5	10.5±0.5	10±0.5	12±0.5	11±0.5	10±0.5	9±0.5
11	Mentha spicata (Mint oil)	16±0.5	13±0.5	12±0.5	10±0.5	14±0.5	13±0.5	11±0.5	8±0.5
12	Ocimum sanctum (Tulsi oil)	13±0.5	12.5±0.5	11±0.5	9±0.5	12±0.5	10±0.1	9±0.5	8±0.5
13	Trachyspermum copticum(Ajowan)	13±0.5	12±0.5	10.5±0.5	10±0.5	12±0.5	11±0.5	10±0.5	9±0.5
14	Withania sominifera (Ashwagandha oil)	15±0.5	13±0.5	11±1.1	10±1.1	14±0.5	12±0.5	10±1.1	8±0.0
15	Zingiber officinale(Zinger oil)	15±0.5	13±0.5	12±1.1	10±0.0	14±0.5	12±0.5	10±1.1	8±0.0
16	Mixed oils C. martinii, C. citratus, E. globulus and C. zylanicum)	22±0.5	19±0.5	18±0.5	16±0.5	24±0.5	21±0.5	19±0.5	16±0.5
17	DMSO negative control	No Zone				No Zone			
18	Miconazole (positive control)	24±0.5	20±0.5	19±0.5	18±0.5	25±0.5	22±0.5	20±0.5	18±0.5
19	intraconazole	23±0.5	18±0.5	16±0.5	12±0.5	21±0.5	15±0.5	12±0.5	10±0.5

Table 3: Minimum inhibitory concentrations (MICs) of selected essential oils (%v/v) against *Aspergillus fumigatus* and *Aspergillus Niger* using agar diffusion method

Plant oils	*Aspergillus fumigatus*	*Aspergillus niger*
Allium sativum (Garlic oil)	>2	>2
Azadirachta indica (Neemoil)	0.25	0.5
Cuminum cyminum (Jira Oil)	>2	>2
Cymbopogon martini (Palmarosa)	0.06	0.06
Cymbopogon citratus (Lemon- Grass Oil)	0.06	0.06
Cinnnamomum zylanicum (Kalmi-Dalchini oil)	0.12	0.12
Eucalyptus globulus (Nilgri oil)	0.12	0.12
Eugenia caryophyllata(Clove oil)	0.12	0.12
Elettaria cardamomum (Cardamomseeds)	>2	>2
Foeniculum vulgare (Fennel fruits oil)	0.5	0.5
Mentha spicata (Mint oil)	0.5	0.25
Ocimum sanctum (Tulsi oil)	0.5	0.5
Trachyspermum copticum(Ajowan)	0.5	0.5
Withania sominifera (Ashwagandha oil)	0.5	0.5
Zingiber officinale (Zinger oil)	>2	>2
Mixed oils (C. martinii, C. citratus, E. globules and C. zylanicum)	0.03	0.03

isolated from pulmonary tuberculosis patients. The maximum antimycotic activity was shown by *C. martinii* followed by *C. citratus*, *Eucalyptus globulus* and *Cinnamomum zylenicum*. The oils of *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity and the oils of *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum capticum, Foeniculum vulgare* and *Elettaria cardamomum* showed comparativiely low activity against *A. niger* and *A. fumigatus.*

The MICs of 15 plant oils obtained by the agar dilution method are shown in Table 3. *Cymbopogon martini* (Palmarosa), *Cymbopogon citratus* (Lemon-Grass Oil), *Eucalyptus globulus* (Nilgri oil) *Eugenia caryophyllata* (Clove oil) and *Cinnnamomum zylenicum* (Kalmi-Dalchini oil) inhibited *Aspergillus fumigatus* and *Aspergillus niger* at = 0.25% (v/v). *Azadirachta indica (Neemoil), Foeniculum vulgare(feenl oil),Mentha spicata (Mint oil), Ocimum sanctum* (Tulsi oil) and *Withania sominifera* (Ashwagandha oil) inhibited *Aspergillus* and *Aspergillus niger* at = 2.0% (v/v). Five oils (*Allium sativum (Garlic oil)*, *Cuminum cyminum (Jira Oil), Elettaria cardamomum (Cardamomseed)*, *Trachyspermum copticum* (Ajowan) and *Zingiber officinale* (Zinger oil) failed to inhibit any organisms at the highest concentration, which was 2.0% (v/v).

Table 4 shows MICs and minium cidal concentrations (MCCs) of 15 plant oils and mixed oils (equal ratio of *C. martini, C. citratus Eucalyptus globulus* and *Cinnnamomum zylenicum*) obtained by the broth micro dilution method. *Cymbopogon martini* and *Cymbopogon citrates* had lowest MICs of (0.03%v/v) against *Aspergillus fumigatus* and *Aspergillus niger*.

Table 4: Minimum inhibitory concentration and minimum cidal concentration data (% v/v) of *A. fumigatus* and *A. niger* obtained by the broth micro dilution method

	Aspergillus fumigatus		Aspergillus niger	
Plant oils	MIC	MCC	MIC	MCC
Allium sativum (Garlic oil)	>4.0	.>4.0	>4.0	>4.0
Azadirachta indica (Neemoil)	0.12	0.12	0.25	0.25
Cuminum cyminum (Jira Oil)	>4	4	>4	>4
Cymbopogon martini (Palmarosa)	0.03	0.03	0.03	0.03
Cymbopogon citratus (Lemon- Grass Oil)	0.03	0.03	0.03	0.03
Cinnnamomum zylanicum (Kalmi-Dalchini oil)	0.12	0.12	0.12	0.12
Eucalyptus globulus (Nilgri oil)	0.06	0.06	0.06	0.06
Eugenia caryophyllata(Clove oil)	0.06	0.12	0.12	0.06
Elettaria cardamomum (Cardamomseeds)	>8.0	>8.0	>8.0	>8.0
Foeniculum vulgare (Fennel fruits oil)	0.5	1.0	0.5	0.5
Mentha spicata (Mint oil)	0.12	0.12	0.12	0.12
Ocimum sanctum (Tulsi oil)	0.5	1.0	0.5	1.0
Trachyspermum copticum (Ajowan)	>8	>8	1.0	1.0
Withania sominifera (Ashwagandha oil)	1.0	0.5	1.0	0.5
Zingiber officinale (Zinger oil)	>4	>4	>4	>4
Mixed oils (C. martinii, C. citratus, E. globulusand C. zylanicum)	0.008	0.008	0.06	0.06

Mixed oils had lowest MICs of 0.008% (v/v) against *Aspergillus fumigatus* 0.06% (v/v) against *Aspergillus niger*. Comparison of MICs obtained by agar and broth methods showed that differences exceeding two serial dilutions were seen with *Mentha spicata, Allium sativum Azadirachta indica (Neemoil), Cuminum cyminum Eucalyptus globulus* and *Eugenia caryophyllata.* The greatest difference was for *Aspergillus fumigatus* against with *Trachyspermum copticum,* where the MIC obtained by agar dilution was 0.5 %(v/v) compared with the MIC by micro dilution >8.0 %(v/v).The lowest minimum inhibitory concentrations were 0.008 % (v/v) of mixed oils against *Aspergillus fumigatus.*

DISCUSSION

The traditional use of plants as medicines provide the basis for indicating which essential oils and plant oils may be useful for specific medical conditions. Historically, many plant oils and extracts, such as tea tree, myrrh and clove, have been used as topical antiseptics, or have been reported to have antimicrobial properties [20,21]. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [22]. Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel [23-27]. All the oils tested exhibited different degrees of antifungal activity against *Aspergillus fumigatus* and *A. niger*. The maximum antimycotic activity was shown by *C. martinii* followed by *C. citratus*, *Eucalyptus globulus* and *Cinnamomum zylenicum*. Aggarwal, *et al*. [28] reported antimycotic activity of *C. martinii* against *A. niger*. The oil of *C. citratus* was effective against fungal pathogens causing diseases in plants and humanbeings [29]. Quale, *et al*. [30] treated infections caused by *Candida* in AIDS patients with a drug based on Cinnamon. In our study we also found that essential oil extracted from *Cinnamon zeylanicum* demonstrated strong antifungal activity on both the species of *Aspergillus*. The antimycotic activity of cinnamon bark due to presence of cinnamaldehyde is well known [31]. Similarly, *in vitro* antimicrobial activity of *Cinnamon zelyanicum* (bark) against human pathogenic fungi and commensally bacteria was studied by Chaumont *et al*. [32] and Matan *et al.* [33]. The oils of *Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The essential oil of mint was found to have strong antimycotic activity against *Candida albicans* Kishore *et al.* [34] also reported that essential oil of mint showed high antimycotic activity against dermatophytes. The oils of *Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum capticum, Foeniculum vulgare* and *Elettaria cardamomum* showed comparativiely low activity against *A. niger and A. fumigatus* as compared to control. Nigam and Rao [35] reported that oil of *C. cyminum* was toxic to *Aspergillus.* Similarly, Singh N, [3] reported high antifungal activity of *C. cyminum.* Oil of *Allium sativum* showed significant growth inhibition of fungi including dermatophytes [36]. Singh, [37] found antimycotic activity in oil of *Ocimum sanctum* against pathogenic fungi. Plant oils are important source of fungitoxic compounds and they may provide a renewable source of useful fungicides that can be utilized in antimycotics drugs against *A. fumigatus* and *A. niger* infection in patients suffering from pulmonary tuberculosis. Among the plant oils tested, *C. martini*, *C. citrates, E. globulus* and *C. zeylanicum* showed high antimycotic activity. Mixed oils were inhibited the *Aspergillus fumigatus* and *Aspergillus niger* at lowest MICs. These results support the notion that plant essential oils have a role as pharmaceuticals and preservatives.

When comparing data obtained in different studies, most publications provide generalizations about whether or not plant oil possesses activity against fungi. However, not all provide details about the extent or spectrum of this activity. Some publications also show the relative activity of plant oils and extracts by comparing results from different oils tested against the same organism(s). Comparison of the data obtained in this study with previously published results is problematic. First, the composition of plant oils and extracts is known to vary according to local climatic and environmental conditions [38,39]. Furthermore, some oils with the same common name may be derived from different plant species [40]. Secondly, the method used to assess antimicrobial activity and the choice of test organism(s), varies between publications [38]. A method frequently used to screen plant extracts for antimicrobial activity is the agar disc diffusion technique [23,41]. The usefulness of this method is limited to the generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium [38,24]. Agar and broth dilution methods are also commonly used. The results obtained by each of these methods may differ as many factors vary between assays [28,26]. These include differences in microbial growth, exposure of micro-organisms to plant oil, the solubility of oil or oil components and the use and quantity of an emulsifier. These and other elements may account for the large differences in MICs obtained by the agar and broth dilution methods in this study. *In vivo* studies may be required to confirm the validity of some of the results obtained. The need for a standard, reproducible method for assessing oils has been stressed by several authors [42,43]. In view of this, many methods have been developed specifically for determining the antimicrobial activity of essential oils [42,45]. The benefits of basing new methods on preexisting, conventional assays such as the NCCLS methods are that these assays tend to be more readily accepted by regulatory bodies [42,45]. Also, these methods have been designed specifically for assessing the activity of antimicrobial compounds and factors affecting reproducibility have been sufficiently investigated. Although NCCLS methods have been developed for assessing conventional antimicrobial agents such as antibiotics, with minor modifications these methods can be made suitable for the testing of essential oils and plant extracts [42]. For some plant oils, such as wintergreen, eucalyptus, clove and sage, there has been much research and reporting of toxic and irritant properties [21,40]. In spite of this, most of these oils are available for purchase as whole oils or as part of pharmaceutical or cosmetic products, indicating that toxic properties do not prohibit their use. However, the on-going investigation of toxic or irritant properties is imperative, especially when considering any new products for human use, be they medicinal or otherwise. In summary, this study confirms that many essential oils possess *in vitro* antifungal activity. However, if plant oils are to be used for preservation or medicinal purposes, issues of safety and toxicity will need to be addressed. This obviously justifies the use of above mixed oils in traditional medicine to cure mycotic infections in tuberculosis patients.

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