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IN-VITRO ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM MEDICINAL PLANTS

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Abstract:

The essential oils are of very much interest during recent years because of the need of new therapies against microbes. However, few investigations have compared large numbers of oils and extracts using different methods that are directly comparable. In the present study six plant oils was investigated for activity against Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi, Escherichia coli, Aspergillums flavous, Candida albicans, Microspora gryseus and Aspergillus terus using cup plate method. The selected essential oils showed promising activity against selected pathogenic microbial strains among them the eucalyptus, sandal wood and rose oils showed very good activity against both bacterial as well as fungal strains. From this investigation we can say that the oils and their dilutions can be used as antiseptic and anti-infective agents in pharmaceutical and cosmoceutical formulations.

Keywords: *Essential oils, antifungal, antibacterial, cup plate method.*

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INTRODUCTION:

Essential oils have been traditionally used for treatment of infections and diseases all over the world for centuries [1]. Presently the use of essential oils is a growing market because of its considerable range of applications. The oils are used in the food, beverages, fragrances and cosmetics, besides a broad spectrum of biological activity which has lead to an increased interest among researchers. In recent years there has been extensive investigation to explore and determine the antimicrobial activity of essential oils. All oils tested to date have displayed some antimicrobial activity and some have been shown to be more effective than others. Thymol, carvacrol, linalool and eugenol are main constituents of some essential oils [2, 3], other studies suggest that phenols such as carvacrol and eugenol disturb the cellular membrane and react with active sites of enzymes [4].

The function of essential oils is believed to be largely communicative and a variety of complex interactions have evolved enabling plants to utilize essential oils to influence their environment. The volatile monoterpenes that comprise about half of the oleoresin produced by certain species of conifer act as phytoprotective agents, defending the tree from herbivore and pathogen attack [5]. The use of essential oils as antimicrobial agent has been described qualitatively for many year starting with the phenol coefficient of a number of essential oils published [6].

The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad-spectrum activities [7]. Since ancient times, plants and their derivatives, such as essential oils have been used in folk medicine. In nature essential oils plays an important role in the protection of plants. They also may attract some insects to promote the dispersion of pollens and seeds or keep away other undesirable insects. Thus, essential oils can play a role in mediating the interactions of plants with the environment [8]. The essential oils contain a wide series of secondary metabolites, which can inhibit or slow the growth of bacteria, yeasts and moulds [9-11]. Since essential oils possess complex chemical constituents, which vary depending on the amount of rainfall and daylight to which plants are exposed, and the soil conditions, humidity, elevation, even the time of day at which the plants are harvested [12, 13], resistance among bacteria is not yet detected [14]. A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics and chemotherapeutics which leads to the inefficiency of antimicrobial treatment [15]. The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes [16, 17]. There is a strong necessity for the development of new drugs for the cure of infections provoked by resistant and multi-resistant bacteria species. Essential oils are important both for food preservation control of human diseases that are of microbial origin [18].

Hence, our interest was directed towards antimicrobial activity of some essential oils and possibilities for their application in pharmaceutical field. The aim of the current investigation is to study the antimicrobial activities of six essential oils against some bacteria, fungi and yeast.

MATERIALS AND METHODS:

Essential oils

The essential oils were commercially obtained from different companies. The solvent DMSO was also of analytical grade (SDFCL).

ANTIMICROBIAL SCREENING

Antibacterial activity

Cup plate method

The antibacterial activity of the essential oils was studied systematically against four different strains of bacteria (gram-positive and gram-negative) by the agar diffusion method. All the essential oils were evaluated for antibacterial activity against *Bacillus subtilis* (gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* (gram negative), by using the agar diffusion method of assay [19]. The organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1°C for 24 hr. they were stored in a refrigerator. The flasks were incubated Bacterial inoculums was prepared by transferring a loopful of stock culture to nutrient broth (100 ml) in a clean and at 37±1°C for 18 hr. before the experimentation. Solutions of the test compounds were prepared by dissolving 2 ml each in dimethylformamide (5ml). A reference standard for gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of Amoxicillin respectively in dimethylformamide solution, separately. The nutrient agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch). The Petri-plates, test tubes and flasks containing medium is plugged with cotton were sterilized in hot air-oven at 160°C, for an hour. Into each sterilized pertained plate (10 cm diameter), about 20 ml each of molten nutrient bacteria (6 ml of inoculums to 300 ml of nutrient agar medium) was transferred, aseptically. The plates were left at room temperature to allow for solidification. In each plate, four cups of 6 mm diameter were made with a sterile cork borer. Then, 0.1 ml of the test solution was

added to the cups, aseptically and labeled, accordingly. The plates were kept undisturbed for at least 2 hrs at room temperature to allow diffusion of the solution properly, into nutrient agar medium. After incubation of the plates at 37±1°C for 24 hr. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of dimethylformamide to observe the solvent effects.

Antifungal activity [19]

Preparation of Potato dextrose agar media

Potato - 100 gm
Dextrose - 10 g
Distilled water- 500ml
P^H -5.6

All six essential oils were tested for their antifungal activity. The fungi employed for screening were *Aspergillus flavus*, *Candida albicans*, *Microspora gryseus* and *Aspergillus terus*. The test organisms were sub-cultured using potato dextrose agar medium. The tubes containing sterilized medium were inoculated with test fungi and after 25°C for 48 hr. they were stored at 4° in a refrigerator. The inoculums was prepared by taking a loopful of stock culture to about 100 ml of nutrient broth; in 250 ml clean ad sterilized conical flasks. The flasks were incubated at 25°C for 24 hr. before use the solutions of test substances were prepared by a similar procedure described under the reference standard (0.5 mg and 1 mg/ml conc.) were prepared by dissolving 5 mg and 10 mg of Griseofulvin in 10 ml of dimethylformamide to obtain a solutions of 50 µg/ml and 100 µg/ml concentration.

The potato-dextrose-agar medium was sterilized by autoclave at 121°C (15 lb/sq. inch), for 15 minutes. The Petri-plates, tubes and flasks plugged with cotton were sterilized in autoclave at 121°C, for an hour. Into each sterilized Petri-plate (10 cm diameter), about 30 ml each of molten potato dextrose-agar medium inoculated with respective fungus (6 ml of inoculums to 300 ml of potato-dextrose-agar medium) was transferred, aseptically. After solidification of the medium at room temperature four cups of 6mm diameter were made in each plate with a sterile borer. Accurately 0.1 ml (100 µg/ml conc.) of test solution was transferred to the cups, aseptically and labeled, accordingly. The reference standard 0.1 ml (50µg/ml conc., 100 µg/ml conc.) was also added to the cups in each plate. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion of the solution properly, into potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 hr. The diameter of the zone of inhibition was read with help of an 'antibiotic zone reader'. The experiments were performed in triplicate in order to minimize the errors.

Table-1: Anti-bacterial activity

Sl. No.	Sample code		Zone of inhibition (in mm)			
			P. a.	B.s.	S. t.	E. c.
1	A	a	8	5	2	2
		b	10	3	1	3
		c	0	4	4	3
2	N	a	0	4	4	1
		b	10	6	8	6
		c	12	5	6	5
3	C	a	20	15	22	20
		b	22	18	17	22
		c	23	16	15	23
4	E	a	30	23	21	25
		b	30	22	24	25
		c	30	25	25	25
5	S	a	28	26	24	25
		b	22	22	21	23
		c	20	19	18	21
6	R	a	24	23	24	26
		b	22	22	20	24
		c	21	20	19	22
7	Standard Amoxicillin	S_1	34	32	32	36
		S_2	31	31	30	33
		S_3	31	31	29	31
8	Control	---		---	---	---

A- Almondoil, N- Neem oil, C -Clove oil , E- Eucalyptus oil, S- Sandal wood oil, R-Rose oil

Name of the organisms

P.A- Pseudoaureous aeruginosa
B.S- Bacillus subtilis
S.T- Salmonella typhi
E.C- Escherichia coli

Concentrations

a. 50% (1:1)5ml oil & 5ml DMF solvent (1%)
b. 50% (1:1)5ml oil **(**a**)** & 5ml DMF solvent (0.5%)
c. 50% (1:1)5ml oil **(**b**)** & 5ml DMF solvent (0.25%)

Table-2: Anti-fungal activity

Sl. No.	Sample code		Zone of inhibition (in mm)			
			A. f.	C. a.	M. g.	A. t.
1	A	a	16		--	--
		b	20	--	--	12
		c	22	--	--	14
2	N	a	18	20	30	--
		b	20	23	28	--
		c	23	24	24	--
3	C	a	17	18	--	15
		b	20	23	--	20
		c	22	26	--	14
4	E	a	34	--	--	36
		b	29	--	--	27
		c	20	--	--	23
5	S	a	30	--	25	--
		b	28	--	25	--
		c	22	--	23	--
6	R	a	20	18	22	20
		b	22	16	17	22
		c	23	15	15	23
7	Standard Griseofulvin	S_1	34	32	32	36
		S_2	31	31	30	33
		S_3	31	31	29	31
8	Control	---		---	---	---

Sample Code

- A-Almond oil, N- Neem oil, C -Clove oil , E- Eucalyptus oil, S- Sandal wood oil, R- Rose oil

Name of the organisms

A. f- *Aspergillus flavous*, C. a- *Candida albicans*, M. g- *Microspora gryseus*, A. t- *Aspergillus terus*

Concentrations

a. 50% (1: 1) 5ml oil & 5ml DMF solvent (1%),
b. 50% (1: 1) 5ml oil **(**a**)** & 5ml DMF solvent (0.5%)
c. 50% (1: 1) 5ml oil **(**b**)** & 5ml DMF solvent (0.25%)

DISCUSSION:

As shown in Table-1 and 2, the six essential oils showed antimicrobial activity against eight microorganisms tested: *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Aspergillums flavous*, *Candida albicans*, *Microspora gryseus* and *Aspergillus terus*. The antimicrobial effects may be due to the different constituents which are present in the essential oils. The results of the present study indicate that the presence of Terpene molecules such as geraniol and geranyl acetate, which are found in the essential oils of the aerial parts [20], and are reported to contribute to the antimicrobial activities, were not found in the seed extracts, whereas a number of other molecules, such as the sesquiterpene, that were found in the seed oils have no antimicrobial activity. It has been reported that components of the essential oils obtained from the aerial parts of medicinal plants exhibited antimicrobial activity [20, 21]. When we compared the previously published results with those of the current study, it was apparent that the antimicrobial activity is strongly affected by the essential oil composition. It is well known that the concentration of biologically active constituents varies with the plant parts, which directly reflects this activity.

CONCLUSION:

The essential oils have tremendous medicinal properties that were proved already in many ways and we had lot of supporting data regarding these oils can be used in many therapeutic practices. On the basis of that we have choose six essential oils of the commercially available, more valuable and useful essential oils for against selected pathogenic microbial strains, among them the eucalyptus, sandal wood and rose oils showed very good activity against both bacterial and fungal strains. From our present research investigation we analyse that the oils and their dilution can be used as antiseptic and anti-infective agents in pharmaceutical formulation.

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