Antimicrobial Activity of Essential Oils Isolated from Plants

Zafar S. Khan,* Vishal N. Shinde, Shafikh M. Shaikh,[1] and Sahera Nasreen

Department of Botany, Government Institute of Science, Nipatniranjan nagar, Caves Road, Aurangabad- 431004 (M.S.) India

[1]Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (M.S.) India

**Corresponding Author. E-mail: zfrkhan123@gmail.com*

ABSTRACT

In the present study, essential oils isolated from peppermint leaf (Mentha piperita Linn.), cinnamon bark (Cinnamomum zeylanicum Bl.), flower bud of clove (Syzygium aromaticum Linn.) and leaves of eucalyptus (Eucalyptus globulus Labill.) were tested for their antimicrobial activity against animal and plant pathogenic bacteria and fungi. All the essential oils showed antimicrobial activity against particular target organism. Essential oil of Mentha piperita revealed antimicrobial activity against almost all target organisms except Aspergillus niger. The highest zone of inhibition was observed in essential oil of Mentha piperita and Syzygium aromaticum against Candida albicans 3017 and Proteus vulgaris, respectively (12 mm). Essential oil from Mentha piperita was found to be effective at 200 ppm against Candida albicans 3017.

Keywords: Antimicrobial activity, *Candida albicans* 3017, Essential oils, *Mentha piperita, Cinnamomum zeylanicum*

INTRODUCTION

Due to the infection of bacteria and fungi, lives of millions of people in developing countries of the world are threatened. The present scenario is a consequence of antibiotic resistance developed by pathogens, acute effect of antibiotics on the host and high cost of antibiotics. It is needed to search for new antimicrobials from plant origin as an alternative to currently-available antibiotics which should be effective against resistant pathogens, cheap, safe and affordable by people from any society of the world. Plants are reservoir of biologically-active compounds known as secondary metabolites including essential oils, which play a defensive role in plants. The uses of essential oils or their chemical constituents for the control of various diseases and as preservatives are known to man since ancient time. Eugenol which is the main chemical constituent of clove (*S. aromaticum*) oil has been used for a long time by dentist through intracanal route as a dressing in dentistry for treating minor oral wound, as an analgesic in painful and infective diseases of oral cavity and oropharynx as well as for general oral hygine (Elujoba et al., 2005). Number of essential oil from plants has been tested against various

important pathogens throughout the world (Benkeblia, 2004; Mohanta et al., 2007; Bajpai et al., 2007). In the present investigation, four essential oils isolated from *Cinnamomum zeylanicum* Bl., *Mentha piperita* Linn., *Syzygium aromaticum* Linn. and *Eucalyptus globulus* Labill. were evaluated for antimicrobial activity against animal as well as plant pathogenic bacteria and fungi.

MATERIALS AND METHODS

Dried flower buds of clove and cinnamom bark were obtained from local market of Aurangabad, whereas leaves of peppermint and eucalyptus were obtained from nature around Aurangabad. Pure cultures of *Pseudomonas aeruginosa*, *Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Candida albicans* 3017 were obtained from Department of Microbiology, Government Institute of Science Aurangabad while *Aspergillus niger* was isolated from groundnut (*Arachis hypogaea* L.) seed.

Isolation of essential oils:

Essential oils were isolated by hydrodistillation using a Clavanger apparatus. Oil collected was dried on anhydrous sodium sulphate and preserved at 4°C for further study.

Antimicrobial assay:

Agar disk diffusion method was followed (Bauer et al., 1966) for the determination of antimicrobial activity. Bacterial inoculums were prepared by inoculating a loop-full of target bacteria (24 h old culture) in 5 ml nutrient broth and incubated at 37±2°C for 5-8 h till a moderate turbidity was developed. The turbidity was adjusted to 0.5 which corresponds to 1.5 x 10^8 c.f.u./ml. Surface of the nutrient agar (Hi-media) plates was inoculated with bacterial culture using sterile cotton swab. Sterile paper disks (5 mm) were soaked in essential oils and placed on the surface of nutrient agar using sterile forceps. *Candida albicans* 3017 was grown in Yeast Malt Broth (YMB) and O. D. was adjusted to 1. Standard culture of *Candida albicans* 3017 and *Aspergillus niger* were inoculated in YMA and PDA plates, respectively. All the plates were incubated at 37±2°C for (except *A. niger*) 24 h while *A. niger* was incubated at 28°C for 72 h. Each test was carried out in triplicate and results were recorded after 24 h of inoculation in terms of diameter of the inhibition zone (mm).

Determination of minimum inhibitory concentration (MIC):

MIC of essential oil was determined by well diffusion method. Essential oil was diluted in DMSO into two fold as 200, 400 and 800 ppm. Standard inoculums of bacteria, yeast and fungi were spread on the solidified nutrient agar, YMA and PDA plates, respectively. Wells were made on the plates using sterile cork borer (8 mm diameter). Each sample of essential oil (50 µl) was poured into the well and incubated for overnight at 30°C. The lowest concentration at which zone of inhibition appeared was considered as MIC.

RESULTS

All the essential oils showed antimicrobial activity against specific target organisms (Table 1). Only essential oil from *Mentha piperita* exhibited antimicrobial activity against almost all target organisms except *Aspergillus niger*. Essential oils of *M. piperita* and *Syzygium aromaticum* exhibited greatest zone of inhibition against *Candida albicans* 3017 and *Proteus vulgaris*, respectively (12 mm). Only *Cinnamomum zeylanicum* and *M. piperita* essential oils revealed antifungal activity.

As tabulated in Table 2, essential oils of *M. piperita* and *S. aromaticum* were found to be more effective against *P. aeruginosa, C. albicans* 3017 and *P. vulgaris*, respectively (200 ppm) whereas essential oils of *C. zeylanicum* and *E. globulus* were least effective against target organism at defined concentration. It also showed that essential oil form *C. zeylanicum* was unable to inhibit the growth of *A. niger* up to 800 ppm concentrations.

Table 1. Antimicrobial activity of essential oils from plants.

Essential oil	Zone of inhibition in (mm)*						
	P. a	P. v.	K. p.	E. c.	S. a.	C. a.	A. n.
Cinnamomum zeylanicum	7±0.86	8±1.12	-	-	-	-	11±0.65
Mentha piperita	10±0.91	9±0.19	8±0.71	8±0.44	9±1.12	12±0.97	-
Syzygium aromaticum	-	12±0.86	-	-	-	-	-
Eucalyptus globulus	9±0.44	-	7±1.26	7±0.29	9±0.91	-	-
Streptomycin (100 ppm)	9+0.1	12±0.2	-	8 ±0.2	9 ±0.1	nt	nt
Dithane M-45 (0.2%)	nt	Nt	nt	nt	nt	11±0.2	13±0.1

*Result expressed in mean of triplicates + S.D., - = not detected, nt = not tested, P.a = *Pseudomonas aerugimnosa*, P.v= *Proteus vulgaris*, K.p. = *Klebsiella pneumoniae*, E.c. = *Escherichia coli*, S. a. = *Staphylococcus aureus*, C.a.= *Candida albicans* 3017 and A. s. = *Aspergillus niger*.

DISCUSSION

Antimicrobial activity exhibited by essential oil may attribute to individual compound or synergetic effect of more than one compounds present in them. Antimicrobial activity of essential oil from selected plants was reported against a wide range of pathogens in different regions of the world (Singh et al., 1995; Saeed and Tariq, 2005, 2008). Gupta et al., (2009) found that essential oils from cinnamon, clove, peppermint and eucalyptus possess antimicrobial activity against some bacteria including *Staphylococcus aureus*, which are more or less in compliance with the present results. The difference in results may be due to the geographical region where the plant material is collected, age of plants, different methods of isolation and testing for antimicrobial activity or may be due to the

Table 2. Minimum inhibitory concentration (MIC) of essential oils.

Essential oil	Conc	Zone of inhibition in (mm)*						
	(ppm)	P.a.	P.v.	K.p.	E.c.	S.a.	C.a.	A.n.
Cinnamomum zeylanicum	200	-	-	-	-	-	-	-
	400	-	-	-	-	-	--	-
	800	6 +0.2	8±0.1	-	-	-	--	-
Mentha piperita	200	7+0.3	-	-	-	-	11±0.1	-
	400	7±0.3	-	-	-	-	12±0.1	-
	800	9±0.1	7±0.1	8±0.2	6±0.1	7±0.4	12±0.2	-
Syzygium aromaticum	200	-	7±0.1	-	-	-	-	-
	400	-	8±0.2	-	-	-	-	-
	800	-	10±0.1	-	-	-	-	-
Eucalyptus globulus	200	-	-	-	-	-	-	-
	400	-	-	-	-	-	-	-
	800	7+0.3	-	7+0.2	6±0.2	8±0.1	-	-

*Result expressed in mean of triplicates + S.D., - = not detected, P.a. = *Pseudomonas aerugimnosa*, P.v.= *Proteus vulgaris*, K.p.= *Klebsiella pneumoniae*, E.c. = *Escherichia coli,* S. a. = *Staphylococcus aureus*, C.a.= *Candida albicans* 3017 and A. s. = *Aspergillus niger.*

different strains of bacteria. Overall, paper disk method is not as good as food poisoned method for the determination of antifungal activity against fungi.

Mentha piperita essential oil revealed significant activity against *C. albicans* 3017 which is highly pathogenic to women and causes mycoses. It is also prevalent in AIDS patients. Very few reports are available about the use of *M. piperita* against *C. albicans* in previous literature. Generally, the active antimicrobial compounds of essential oil are terpenes which are phenolic in nature and have enormous potential to strongly inhibit the growth of microbial pathogens. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compound (Cowan, 1999). The present investigation rationalized the use of essential oils in traditional medicinal systems.

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