Vol. 2 no. 2, pp 120-123 (September 2005)
http://www.ajol.info/journals/jpb

Journal of
PHARMACY AND
BIORESOURCES

Antimicrobial activities of some volatile oils against some pathogenic organisms

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Received 22nd June 2005; Accepted 15th August 2005

Abstract

Antimicrobial activities of volatile oils of *Ocimum gratissimum, Citrus sinensis, C. maxima* and *Eucalyptus globulus* obtained through steam distillation using Clavenger-type apparatus were examined on clinical isolates of some human pathogenic fungi and bacteria using ditch – plate method. The fungal organisms used include *Tinea rubrum, T. megninii* while the bacteria are *Staphylococcus aureus, Escherchia coli, Pseudomonas aeruginosa.* All the organisms were mostly susceptible to the volatile oil of *O. gratissimum* while their susceptibilities to other oils varied.

Keywords: Ocimum gratissimum; Citrus sinensis; Citrus maxima; Eucalyptus globules; Antimicrobial

Introduction

Various synthetic antimicrobial agents of proven potency abound for the chemotherapy of dermatophytes but often they are toxic due to long term use. These classical agents are very costly and sometimes not always available for sustained therapy particularly to those in the rural areas. Much effort is therefore being made to investigate the potential of plant based or natural antimicrobial agents (hitherto of low prominence) in the treatment of dermatophytes and bacterial skin infections.

Some volatile oils obtained from *Cymbopogon citratus, Eugenia uniflora,* and *Ageratum conyzoides* have been reported to possess antimicrobial, larvicidal and insecticidal properties (Onawunmi and Ogunlana, 1988; Adebajo *et al*, 1989; Gbolade *et al.*, 1999; Ayinde and Odigie 2001). There are also some unpublished reports of insect repellent, larvicidal and antibacterial activities of the volatile oils of *Ocimum gratissimum* .The probable effects of volatile oils obtained from waste materials like orange (*Citrus sinensis*) and grape fruit (*Citrus maxima*) peels have received little attention, if any, and there are no literature reports on the effects these volatile oils might have on skin infections due to bacteria or fungi. This investigation therefore, is aimed at examining the antimicrobial activities of volatile oils obtained from *Ocimum gratissimum, Citrus sinensis, Citrus maxima and Eucalyptus globulus* against

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ISSN 0189-8442

dermatophyte organisms of *Trichophyton rubrum, Trichophyton meniginum* as well as the bacterial species of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Experime ıtal

Collection of the plant materials. Orange and grape peels were collected from citrus fruit sellers at a local market in Benin City while *Ocimum gratissimum and Eucalyptus globulus* leaves were collected in August 2004 from cultivated sources in the University of Benin.

Extraction of the volatile oils. The volatile oil of each of the plant materials was extracted by the steam distillation method using Clavenger-type apparatus for 5h. After collection, the volatile oils were kept in a refrigerator maintained at 4°C until needed.

Sources of microorganisms. Trichophyton rubrum, Trichophyton meniginum, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa were collected from the Medical Microbiology laboratory, University of Benin Teaching Hospital. They were re-isolated into pure cultures.

Determination of antimicrobial activity of the volatile oils. The agar diffusion method of ditch – plate technique was employed. The bacterial organisms were tested in sensitest agar while Sabouraud's agar was used for the fungal organisms. Essentially, the ditches were filled with 0.05ml of the respective volatile oil made up to 0.2ml with dimethylsulphoxide (DMSO). The test bacteria were streaked across the isosensitest agar ditches while the fungal organisms were streaked across the Sabouraud's agar ditches. The bacterial and fungal plates were incubated at 37°C and room temperature respectively for 24-72h and zones of inhibition observed. Effect of the undiluted oil on the organisms. Each of the test microorganisms was mixed with each of the volatile oils and allowed to stand. Subcultures were made into appropriate recovery media after 1,2,3, and 7 days contact periods. The plates were incubated and the presence or absence of growth noted and compared.

Results and discussion

The yields of volatile oils from *Citrus sinensis* and *C. maxima* were 1.9% and 0.25% respectively while a yield of 0.5% was recorded for *Ocimum gratissimum* and 0.65% for *Eucalyptus globulus.* At the end of the 24-72h incubation period, *O. gratissimum* and *E. globulus* completely inhibited the growth of both the bacteria and fungi tested while *C. sinensis* had inhibitory effect only on the two fungi used. All the test organisms were unaffected by *C. maxima* volatile oil (Table 1). Sub-culturing the organisms to examine growth recovery after an initial exposure to the volatile oils for 1 day showed the superiority of *O. gratissimum* and *E. globulus* over the two other oils. At the end of 2 and 3 days exposure periods to the volatile oils before subculturing, some of the organisms were inhibited by the volatile oils. However, after 7-day exposure period of the organisms to the oils, the volatile oils of *O. gratissimum and E. globulus* showed fungicidal and bactericidal activities while *C. sinensis* and *C. maxima* oils can be said to be inactive against *T. rubrum* but fungicidal to *T. meniginum* coupled with bactericidal effects (tables 2-5). Methanolic extract of *O. gratissimum* as well as volatile oil of *Cymbopogon citratus* have been reported to possess potent antimicrobial activities (Mbata *et al* 2004; Onawunmi and Ogunlana, 1988) while volatile oils from *Lippia adoensis, C. citratus and Eugenia uniflora* were used to protect stored cowpea seeds from infestation by the cowpea weevil, *Callosobruchus maculatus* (Gbolade and Adebayo, 1993). Also the volatile oil of *O. gratissimum* was reported to show repellent activities against black fly *Simulium damnosum*, the vector of human

onchocerciasis (Aisien *et al.*, 2004). The present study shows that *O. gratissimum* and other volatile oils may be potent fungicidal and bactericidal agents in the order *O. gratissimum* > *E. globulus* > *C. sinensis* > *C. maxima*. The variations observed in the activities of the oils indicate the variations in their constituents. The relative similarity in the activities of both *C. sinensis* and *C. maxima* suggests the similarities in the components of the volatile oils. Also, variations observed in the activities of the oils on the organisms after periods of exposure before sub-culturing suggest physiological differences in the ways various microorganisms respond to the effects of antimicrobial agents.

Table 1: Sensitivities of the test microorganisms to the volatile oils.

Volatile Oils	Micro organisms				
	Trichophyton rubrum	*Trichophyton meniginii*	*Staphlococcus aureus*	*Escherichia coli.*	*Pseudomonas aeruginosa*
Ocimum gratissimum	-	-	-	-	-
E. globulus	-	-	-	-	-
C. sinensis	-	-	+++	+++	+++
C. maxima	+++	+++	+++	+++	+++
Dimethylsulphoxide (DMSO)	+++	+++	+++	+++	+++

- No growth +++ Growth

Table 2: Results of sub-culturing of the test microorganisms after 24-hour exposure to the volatile oils

Volatile Oils	Micro organisms				
	Trichophyton rubrum	*Trichophyton meniginii*	*Staphlococcus aureus*	*Escherichia coli.*	*Pseudomonas aeruginosa*
Ocimum gratissimum	-	-	-	-	-
E. globulus	-	-	-	-	-
C. sinensis	+	-	-	+	+
C. maxima	+	-	+	+	+
Dimethylsulphoxide (DMSO)	+++	+++	+++	+++	+++

- No growth +++ Growth

Table 3: Results of sub-culturing of the test microorganisms after 48-hour exposure to volatile oils

Volatile Oils	Micro organisms				
	Trichophyton rubrum	*Trichophyton meniginii*	*Staphlococcus aureus*	*Escherichia coli.*	*Pseudomonas aeruginosa*
Ocimum gratissimum	-	-	-	-	-
E. globulus	-	-	+	+	-
C. sinensis	+	-	-	-	-
C. maxima	+	-	-	-	-
Dimethylsulphoxide (DMSO)	+++	+++	+++	+++	+++

- No growth +++ Growth

Table 4: Results of sub-culturing of the test microorganisms after 72-hour exposure to volatile oils

Volatile Oils	Micro organisms				
	Trichophyton rubrum	*Trichophyton meniginii*	*Staphlococcus aureus*	*Escherichia coli.*	*Pseudomonas aeruginosa*
Ocimum gratissimum	-	-	-	-	-
E. globulus	-	-	-	+	+
C. sinensis	+	-	+	+	+
C. maxima	-	-	-	+	+
Dimethylsulphoxide (DMSO)	+++	+++	+++	+++	+++

- No growth +++ Growth

Table 5: Results of sub-culturing of the test microorganisms after 7-day exposure to volatile oils.

Volatile Oils	Micro organisms				
	Trichophyton rubrum	*Trichophyton meniginii*	*Staphlococcus aureus*	*Escherichia coli.*	*Pseudomonas aeruginosa*
Ocimum gratissimum	-	-	-	-	-
E. globulus	-	-	-	-	-
C. sinensis	+	-	-	-	-
C. maxima	+	-	-	-	-
Dimethylsulphoxide (DMSO)	+++	+++	+++	+++	+++

- No growth +++ Growth

The volatile oils, particularly those of *O. gratissimum, E. globulus* and *C. sinensis* can be made into appropriate formulations to combat skin infections caused by fungi and bacteria alike.

References

Adebajo A.C., Oloke K.J. and Aladesanmi A.J. (1989); Antimicrobial activities and microbial transformation of volatile oils of *Eugenia uniflora*. *Fitoterapia*, 60, 451 – 455.

Aisien M. S. O., Imasuen A.A., Wagbatsoma V.A and Ayinde B.A. (2004); Preliminary evaluation of the repellent activity of some plant essential oils against *Simulium damnosum* S.L., the vector of human Onchocerciasis. *International Journal of Tropical Insect Science* 24(1), 1-5.

Ayinde B. A. and Odigie F. (2001); Larvicidal properties of the volatile oil of *Ageratum conyzoides* L. (Compositae) against *Culex* species mosquito larvae. *Nigerian Journal of Applied Science* 19; 23-25.

Cowan S.T. and Steel K.J. (1974); Identification of Medical Bacteria (2nd edition) Cambridge University Press, Cambridge.

Gbolade A.A. and Adebayo T.A. (1993); Fumigant effects of some volatile oils on Fecundity and Adult Emergence of *Callosobruchus maculatus* F. *Insect Sci. Applic.* 14(5/6), 631-636

Gbolade A.A., Onayade O.A. and Ayinde. B.A. (1999); Insecticidal Activity of *Ageratum conyzoides* L. volatile oil against *Callosobruchus maculatus* F. in seed treatment and fumigation laboratory tests. *Insect. Sci. Applic.* 19(2/3) 237-240.

Mbata I.T., Orji, M.U. Anukam K. and Ahonkhai. I. (2004); Antimicrobial activities of volatile oil from *Ocimum gratissimum, Ocimum viride* and *Cymbopogon citratus* in selected microganisms. *World Journal of Biotechrology* 5, 847-850.

Onawunmi G.O. and Ogunlana E.O. (1988) .Antimicrobial activities of the oil from lemon grass, *Cymbopogon citratus*, Staff. In: *Drug Production from Natural Products* (Edited by Adesina SK), Medex Publication, Lagos, Nigeria. pp 194 – 206.