Pesticide Biochemistry and Physiology 98 (2010) 89–93

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Chemical and antimicrobial studies of monoterpene: Citral

Amna A. Saddiq [a,*], Suzan A. Khayyat [b]

[a] Microbiology Department, King Abdul Aziz university, Faculty of Science, Saudi Arabia
[b] Chemistry Department, King Abdul Aziz university, Faculty of Science, Saudi Arabia

ARTICLE INFO

Article history:
Received 3 April 2010
Accepted 17 May 2010
Available online 9 June 2010

Keywords:
6,7-Citral-epoxy derivative
Antifungal and antibacterial studies

ABSTRACT

6,7-Citral-epoxy derivative (a mixture of E and Z isomers with respect to the C2 = C3 double bond) could be react with DNA base producing a major adduct. The mixture of epoxides was condensed with 2 mol of cytosine to give the adduct through condensation between aldehyde and amino groups. Antifungal and antibacterial studies were carried out on citral and citral-epoxide. Studies on the antifungal especially *Penicillium italicum* and *Rhizopus stolonifer* showed that citral and citral-epoxide have good antibacterial action. Antimicrobial studies of *P. italicum* and *R.* stolonifer explained also that citral and citral-epoxide have good antimicrobial activity. Citral epoxide shows high activity against the growth of bacteria methicillin resistant *Staphylococcus aureus* (MRSA) and fungi comparing by citral. The epoxide shows antibacterial activity more than the antibiotics nalidixic acid (NA) and ampicillin (AP) and nitrofurantoin (NI). The results revealed that these complexes are most effective against MRSA.

1. Introduction

Cymbopogon citratus (DC) (*Gramineae*) is an herb worldwide known as lemongrass. The tea made from its leaves is popularly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative [1]. The volatile oil obtained from fresh leaves of this plant is widely used by the perfumes, cosmetics industries and in traditional medicine for various purposes [2].

Citral is the major component of lemongrass oil which was extracted from its leaves, present at levels of, approximately, 65–85%. Citral (3,7-dimethyl-2,6-octadienal) is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral A) and neural (*cis*-citral, citral B) (Fig. 1). In addition to citral, the lemongrass oil consists of small quantities of geranial, geranylacetate and monoterpene olefins, such as myrcene [2].

As a natural acyclic monoterpenes, citral was found in a wide variety of plants [3]. A number of dietary monoterpenes was shown to act effectively in chemoprevention and chemotherapy of different cancers in animal models, at cellular level, and in human clinical trials [4–6]. On the other hand, plant monoterpene citral is subjected to oxidation on exposure to air. Oxidation is enhanced by heat [7] and irradiation [8]. Furthermore, unsaturated trepenes are capable of trapping activated oxygen species in vivo to give intermediate epoxides which can alkylate DNAs, proteins, and other bimolecular [7–12]. In addition, some monoterpenes undergo oxidation by the action of peracid [13]. Hydrogen peroxide consider as oxidative reagent via thermal or photochemical oxidation reactions to give the corresponding epoxy derivatives [14,15,13]. Due to the limitation of currently antifungal drugs, their limited spectrum and the expensive treatment, new drugs and alternative therapies are necessary, including natural product compounds [16]. Taking into account therapeutic importance of monoterpene compounds, it is relevant to examine the citral and oxidative product epoxide as a novel treatment of fungal diseases.

2. Materials and methods

2.1. Lemongrass leaves

Scientific name: *Cymbopogon citrates* (*C. citratus*).

Citral (**1**) was isolated by extraction of *C. citratus*, which was collected from Maddinah city (Saudi Arabia). Citral epoxide can be prepared in last work [13]. The melting points (uncorrected) were determined on a Fisher electric melting point apparatus. The IR spectra were recorded on a Perkin–Elmer 16 FPC FT-IR spectrophotometer from thin films (neat). The NMR spectra were measured from solutions in $CDCl_3$ on a Bruker Advance DPX 400 instrument (400 MHz for 1H). Gas chromatography–mass spectrometry was performed using a Joel JMS 600H mass spectrometer coupled with a Hewlett–Packard HP 6890 Series gas chromatograph (HP-5 capillary column, 30 m × 0.32 mm × 0.25 μm; cross linked 5% dimethylpolysiloxane). A Philips G/5812 SON sodium lamp was used as irradiation source in photoinitiated reactions. Thin-layer chromatography (TLC) and preparative thin-layer chromatography were performed using Polygram SIL G/W 254 silica gel (Mecherey-Nagel).

* Corresponding author.
E-mail address: Kah201017@yahoo.com (A.A. Saddiq).

0048-3575/$ - see front matter Crown Copyright © 2010 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2010.05.004

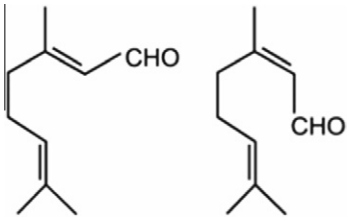


Fig. 1. Chemical structure of the citral.

Solvents were removed from reaction mixtures and extracts using a rotary evaporator (20 °C, 15 mm).

2.2. Test organisms

2.2.1. Fungi pathogenic

Penicillium italicum and *Rhizopus stolonifer* were obtained by the compilation of the Center for microbes (Mircen), Faculty of Agriculture, Ain Shams University – Arab Republic of Egypt. It was cultured on sabaroud dextrous agar media (Oxioid CM 41) at 25 °C.

2.2.2. Bacterial pathogenic

Methicillin resistant *Staphylococcus aureus* (MRSA) from Laboratory of Jeddah king Fahad Hospital in Saudi Arabia. It was cultured on Mueller Hinton media (Oxioid CM 41) at 37 °C.

2.3. Standard antibiotic disc

Nalidixic acid (NA) 30 μg, nitrofurantoin (NI) 300 μg, and ampicillin (AP) 25 μg, Mast Diagnostic Amiens, France.

2.4. The methods

2.4.1. Alkylation of citral epoxide 2a with cytosine[12]

Mixture of citral epoxide 2a & a′ (0.001 gm, 0.37 mol) and (0.22 gm, 0.002 mol) of cytosine was fused at 140 °C for half an hour to give gummy material which was treated with ethyl alcohol (2 ml) to give brown solid material. The residue was subjected to column chromatography on silica gel using petroleum ether (bp 60–80 °C)–diethyl ether (8:2) to isolate compounds **3**. mp. 190 °C.

2.4.2. Citral-cytosine adduct [3]

Colorless semisolid, $C_{18}H_{24}N_6O_3$ (M. wt. 372.00). IR (thin film): ν^{-}: 3413, 3175, 2954, 2857, 1634, 1462, 1149 cm^{-1}. H,H NMR cosy ($CDCl_3$): δ: 0.9 (d, 3H, C^8H_3), 1.3 (br.s, 6H, 2 $C^{9,10}H_3$), 1.70 (Comp. pat., 2H, H-3), 2.3 (Comp. pat., 2H, H-4), 2.03 (Comp. pat., 1H, H-5), 4.23 (Comp. pat., 1H, H-2), 5.36 (d, 1H, H-6), 4.20 (d, 1H, H-7), 7.54 (d, 2H, 2H-5′), 7.71 (d, 1H, H-6'), 7.72 (d, 1H, H-6′), 9.71 (s, 1H, amide proton), 9.73 (s, 1H, amide proton) ppm. MS, m/z, 370 (M^+-2H) (5%), 356 (M^+-CH_4) (36%), 336 ($M^+-C_2H_{12}$) (50%), 278 ($M^+-C_4H_3N_3O$) (3%), 252 ($M^+-C_5H_2N_3O$) (7%), 168 ($C_{10}H_{18}NO$) (3%), 141 ($C_9H_{17}O$) (10%), 114 ($C_7H_{14}O$) (100%).

2.4.3. Biological activity of Lemongrass leaves oil (C. citratus)

Lemongrass oil was tested for in vitro antimicrobial activity by using the agar-well diffusion method [24]. The antibacterial and antifungal activities of lemongrass oil on bacterium (MRSA) and fungi (*P. italicum* and *R. stolonifer*).

2.4.4. Biological activity of citral and citral epoxide

Citral and its epoxide were tested against the fungal species *P. italicum* and *R. stolonifer*, and the bacterial species "*S. aureus*."

2.4.5. Antifungal activities

Diffusion method was used to evaluate the antifungal activities of the tested compounds as follows: 1.0 and 5 ml of the tested compounds dissolved in chloroform ($CHCl_3$) (100.0 μg ml^{-1}) were added to 50 ml of sabaroud dextrous agar media, then poured into sterile Petri dishes (9 cm in diameter) and left to solidify. Mycelia discs measuring 6 mm diameter were taken from the growing margins of cultures of *P. italicum* and *R. stolonifer* (on SDA) and transferred on the surface in the middle of Petri dishes then incubated in 25 ± °C for 6 days in dark. The diameters of the fungal growth were measured after 2, 4 and 6 days [25] (Table 2) .

2.4.6. Antibacterial activities of citral and citral epoxide

About 1.0 and 0.5 ml of the tested compounds dissolved in chloroform (CHCl3) (100.0 μg ml^{-1}) were added into nutrient broth media (Oxioid): the nutrient broth contained then 1 ml from suspension of *S. aureus* (106 CFU/μl) at 37 °C for 24 h was added to it, the growth rate was measured monitoring change in optical density at 650 nm using a spectrophotometer after 24 h Table 3.

The agar disc diffusion method was employed for the determination of antibacterial activities of the citral and its epoxide in ques-

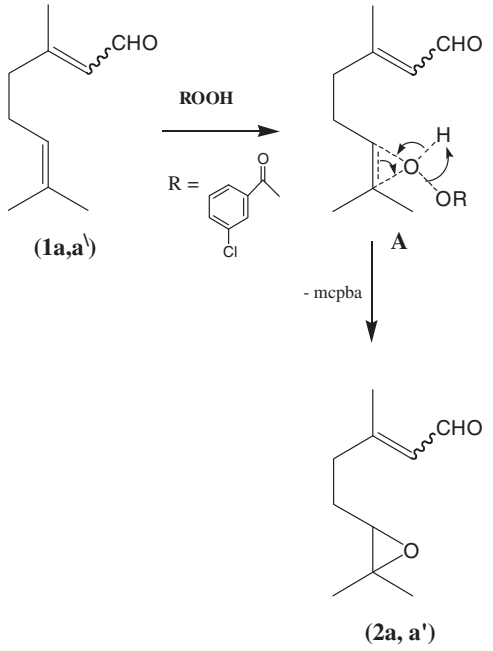
Table 1
Diameter of inhibition zone of the lemongrass oil againt *Staphylococcus aureus*, *Penicillium italicum* and *Rhizopus stolonifer*.

Test strains	Diameter of inhibition zone(mm) lemongrass oil(μl)
Staphylococcus aureus	3.5
Penicillium italicum	2.6
Rhizopus stolonifer	1.8

Table 2
Effect of various concentrations of citral and citral epoxide on the radial growth of *Pencillium italicum* and *Rhizopus stolonifer* grown on solid media (mm/disc; mean of replicates ± SE).

Treatment	Concentration	Fungi pathogenic	D		
			2ays	4	6
Control	0.00	*P. italicum*	7.07 ± 0.23	8.23 ± 0.15	9.00 ± 0.00
		R. stolonifer	4.37 ± 0.19	5.27 ± 0.18	9.00 ± 0.00
Citral	0.5	*P. italicum*	6.70 ± 0.15(ns)	7.77 ± 0.15*	8.03 ± 0.09*
		R. stolonifer	2.27 ± 0.15**	6.17 ± 0.09**	6.67 ± 0.28**
	1.0	*P.italicum*	2.90 ± 0.21**	6.53 ± 0.03**	7.50 ± 0.29**
		R. stolonifer	1.33 ± 0.03**	4.40 ± 0.12**	5.17 ± 0.09**
Citral epoxide	0.5	*P. italicum*	2.23 ± 0.15**	4.60 ± 0.21**	4.93 ± 0.35**
		R. stolonifer	1.93 ± 0.23**	6.37 ± 0.27**	8.80 ± 0.12(ns)
	1.0	*P.italicum*	1.00 ± 0.06**	1.30 ± 0.12**	1.80 ± 0.29**
		R. stolonifer	1.50 ± 0.29**	2.83 ± 0.17**	3.30 ± 0.06**

(ns) Non significant at $P \leqslant 0.05$.
* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.



Scheme 1.

tions [26]. Suspension of the tested microorganisms (10^6 CFU/μl) was spread on Mueller Hinton Agar (Oxioid) for bacteria .Each test solutions was prepared in $CHCl_3$. filter paper discs (6 mm in diameter) were soaked with 20 μl of the stock solutions and placed on the inoculated plates. After keeping at 2 °C for 2 h, they were incubated at 37 °C for 24 h. The diameter of the inhibition zones were measured in millimeters. Some known antibiotics (nalidic acid (NA), ampicillin (AP), nitrofurantoin (NI) and augmantin (Ag) were evaluated for their antibacterial activities and their results compared with citral and its epoxide [27]. The results are in Table 4.

2.5. Data analysis

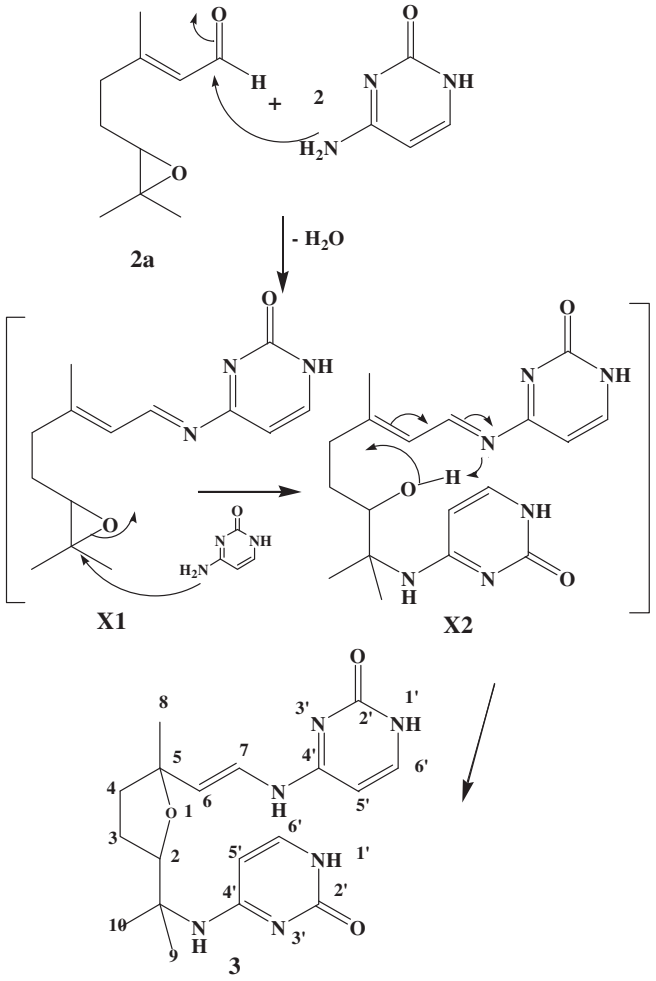
Analysis of data was carried out by student's *t*-test for comparing the means of experimental and control groups [28].

3. Results

Citral, [2-(E), (Z)-3,7-dimethyl-octa-2,6-dienal] (1a,a), is a monoterpene aldehyde which is the major component of lemon grass oil extracted from *C. citratus* belonging to Gramineae [14–16] as a mixture of (2E)-and (2Z)-isomer at a ratio of 3:2, respectively.

Thermal oxidation of citral using m-choroperbenzoic acid (mcpba) in chloroform at room temperature or photochemical oxidation with hydrogen peroxide using a sodium lamp, we obtained a mixture of (E & Z)-epoxides 2a & 2a′ in ca. 60% yield (in the ratio of 60:40 of E:Z configuration). No other products were observed. [13] (Scheme 1).

Epoxides could be reacted with DNA producing a major adduct [12]. Therefore, the mixture of epoxides 2a & a′ was condensed with 2 mol of cytosine to give the adduct 3 through condensation between aldehyde group and amino group in the first mole of cytosine to give intermediate X1, while the other molecule of cytosine was added to amino group on the epoxide ring and open it producing intermediate X2 which cyclized to give adduct 3 (Scheme 2). The ^{1}H-NMR spectrum of 3 contained a doublet at δ 0.90 ppm due to protons of CH_3 group and a singlet at δ 1.30 due to protons of other two CH_3 groups, and three doublets at δ 7.54, 7.71 and 7.72 ppm due to protons of two methylene groups in cytosine ring. Mass spectrum showed the molecular ion peak (M^+-2H) at *m*/*z* 370.



Scheme 2.

Table 3
Effect of various Concentrations of citral and citral epoxide on the growth rates of *Staphylococcus aureus* after 24 h.

Treatment	Concentration	*Staphylococcus aureus*
Control	0.0	185.00 ± 2.89
Citral	0.5	153.33 ± 3.33**
	1.0	110.33 ± 0.88**
Citral epoxide	0.5	123.33 ± 0.88**
	1.0	54.00 ± 3.06**

** Significant at $P \leqslant 0.01$.

Table 4
Antibacterial activity data of citral, citral epoxide and some known antibiotics.

Antibiotic	*Staphylococcus aureus* (MRSA)
Nalidic acid (NA)	++
Ampicillin (AP)	R
Nitrofurantoin (NI)	+++
Augmantin (Ag)	++
Citral	+
Citral epoxide	+++

Fungal and bacterial diseases are among the most common infections, and causative organisms include dermatophytic, yeasts

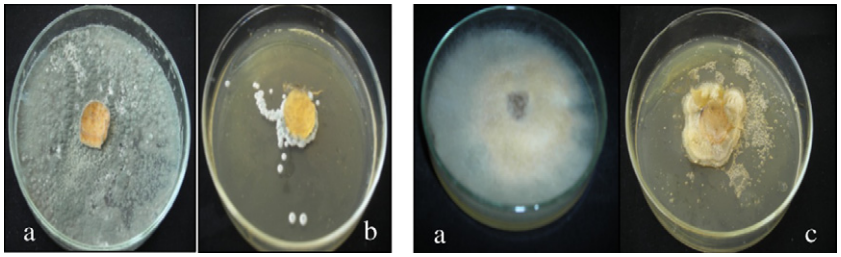


Fig. 2. Effect of citral epoxide on radial growth of fungi grown on the solid media (a) control (b) *Penicillium italicum* and (c) *Rhizopus stolonifer*.

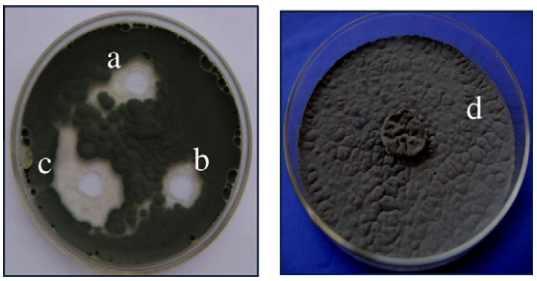


Fig. 3. Effect of lemon grass leaves oil (a) citral (b) citral epoxide (c) on the radial growth of *P. italicum* grown on the solid media and (d) control.

and non-dermatophytic filamentous fungi. The treatment is limited, for many reasons, and new drugs are necessary. In this work, the antimicrobial activity against other *S. aureus* (MRSA), *P. italicum* and *R. stolonifer* was also studied, and the results obtained showed the important antimicrobial activity of lemongrass oil (Table 1). The antifungal result (Table 2) showed that the growth of *P. italicum* and *R. stolonifer* on the solid media was reduced in the presence of citral and its epoxide on fungi. Studies on the antifungal especially *P. italicum* and *R. stolonifer* activity of two essential oil components (citral and menthol) were reported [17].

The data in (Table 3 and 4) revealed that there was a significant decrease in the growth of (MRSA) on liquid media, when (MRSA) were treated by citral epoxide than citral. The same result has been explained in the diffusion method which showed inhibition zones around the antibiotic disc (Fig. 2). If the inhibition zone measures 2 and 3 mm, then the epoxide has a good antibacterial action. If the inhibition zone measures more than 3 mm across, then it are considered very effective, but if there is no inhibition zone then the complex has no activity on the bacterial growth and will not be retained for treatment [18].

Citral epoxide showed more activity than that of the citral against bacteria. The activity of epoxide has been compared with the activity of standard antibiotics nalidixic acid (NA) and ampicillin (AP), but it showed same activity of nitrofurantoin (NI). The results revealed that these complexes are most effective against MRSA.

4. Discussion

From the above result citral epoxide show high activity against the growth of bacteria and fungi comparing by citral, but the inhibition in the case of bacteria more thane that of fungi this may be explained on the basis of bacteria is prokaryotic but fungi are eukaryotic and the sensitivity of prokaryotic is different than that of eukaryotic because the changes in the cell wall and plasma membrane and also the nuclear substances moreover.

Citral and its epoxide can act as fungicidal and bactericidal agents [19]. The comparison between citral and lemongrass oil showed similar effect but citral epoxide showed more inhibitory effect (Fig. 3), this can be explained on the basis that lemongrass oil contains of citral as the major component, small quantities of geranial, geranylacetate and monoterpene olefins, such as myrcene [20] which indicated significant association between their effects and the presence of citral in lemongrass oil [21–23]. The epoxide shows antibacterial activity more than the antibiotics nalidixic acid (NA) and ampicillin (AP) and nitrofurantoin (NI). It is concluded that epoxide have a potent in vitro activity against *P. italicum*, *R. stolonifer* and methicillin resistant *S. aureus* (MRSA).

References

[1] E.A. Carlini, J.D.P. Contar, A.R. Silva-Filho, Pharmacology of lemongrass (*Cymbopogon citratus* Stapf) I. Effects of teas prepared from the leaves on laboratory animals, J. Ethnopharmacol. 17 (1986) 37–64.
[2] M.S.C. Ferreira, M.C. Fonteles, Aspectos etnobotânicos e farmacológicos do *Cymbopogon citratus* Stapf (capim limão), Revista Brasil. Farmácia 70 (1989) 94–97.
[3] D.H. Grayson, Monoterpenoids, Nat. Prod. Rep. 15 (1998) 435–439.
[4] P.L. Crowell, Prevention and therapy of cancer by dietary monoterpenes, J. Nutr. (1999) 129–775.
[5] J.J. Mills, R.S. Chari, I.J. Boyer, M.N. Gould, R.L. Jirtle, Induction of apoptosis in liver tumors by the monoterpene perillyl alcohol, Cancer Res. (1995) 955–979.
[6] S. Carnesecchi, Y. Schneider, J. Ceraline, B. Duranton, F. Gosse, N. Seller, F. Raul, Geraniol, a component of plant essential oils inhibit growth and polyamine synthesis in human colon cancer cells, J. Pharm. Exp. Ther. 197 (2001) 298.
[7] E.M. Elgendy, Epoxidation reactions of natural limonene piperine and piperic acid as intercalative alkylating agents for DNA, Boll. Chim. Farm. 27 (1997) 36–532.
[8] E.M. Elgendy, Photooxygenation of natural limonene, Chim. Pharm J. (1998) 50–225.
[9] A. Méou, M.A. Garcia, P. Brun, Oxygen transfer mechanism in the Mn-salen catalysed epoxidation of olefins, J. Mol. Catal. A Chem. 23 (1999) 138–221.
[10] E.M. Elgendy, Photooxygenation of natural ã-terpinene, Boll. Chim. Farm. (2004) 143–337.
[11] N.W.A. Geraghty, Photochemistry, in: I. Dunkin (Ed.), London: Royal Soc. Chem. 27 (2005) 35–116.
[12] S. Richter, B. Gatto, D. Fabris, K.I. Takao, S. Kobayashi, S.M. Palumbo, Clerocidin alkylates DNA through its epoxide function: evidence for a fine tuned mechanism of action, Nucleic Acids Res. 31 (2003) 5149.
[13] E.M. Elgendy, S.A. Khayyat, Oxidation studies on some natural monoterpenes: citral, pulegone, and camphene, Russ. J. Org. Chem. 44 (2008) 814.
[14] O.I. Yarovaya, D.V. Korchagina, V. Gatilo, V. Yu, V.A. Barkhash, Acid-catalyzed reactions of camphene and α-fenchene epoxides, Russ. J. Org. Chem. 38 (2002) 810.
[15] O.I. Yarovaya, D.V. Korchagina, O.V. Salomatina, M.P. Polovinka, V.A. Barkhash, Synthesis of heterocyclic compounds in acid-catalysed reactions of citral epoxides., Mendeleev Commun. (2003) 27.
[16] B.S. Cristiane, S. Sílvia, V.W. Guterres, E.S. Elfrides, Antifungal activity of the lemongrass oil citral against Candida spp., Braz. J. Infect. Dis. 27 (2008).
[17] M. Venkataramana, N. Pattisapu, Discipline of fruit and vegetable technology, Central Food Technological Research Institute, Mysore 570 013 India, J. Sci. Food Agric. 39 (2006) 239.
[18] D. Baudoux, Antiviral and Antimicrobial properties of essential oils, Available from: http://www.aromabar.com/ articles/ baud55.htm on Science Direct (2005).
[19] D. Cristiane, S.G. Silva, W. Vanessa, E.S.S. Elfrides, Antifungal activity of the lemongrass oil and citral against Candida spp, Braz. J. Infect. Dis. 12 (2008) 63.
[20] M.S.C. Ferreira, M.C.Fonteles, Aspectos etnobotanicos e farmacologicos do Cymbopogon citrates Stapf (capim limao), Revista Brasileira de Farmacia,70 (1989) 94.
[21] G.O. Onawunmi, Evaluation of the antimicrobial activity of citral, Lett. Appl. Microbiol. 9 (1989) 105.
[22] B. Wannissorn, S. Jarikasem, T. Soontorntanasart, Antifungal activity of lemon grass and lemon grass oil cream, Phytotherapy Res. 10 (1996) 551.

[23] G.O. Onawunmi, W.A.B. Yisak, E.O. Ogunlana, Antibacterial constituents in the essential oil of Cymbopogon citrates (DC.) Stapff, Ethnopharmacology 12 (1984) 279.
[24] C.H. Collins, P.M. Lyne, J.M. Grange, Microbiol. Methods 6 (1989) 410.
[25] G.L. Bollen, A comparison of the vitro antifungal spectra of thiophanates and benomyl, Neth. J. Plant Pathol. 78 (1972) 55–64.
[26] U. Hasenekoğlu, Laboratory techniques for micro fungi, Atatürk University, Erzurum, Turkey (1990) 66.
[27] F.J. Baker and M.R.Breach, Medical Microbiological Techniques, Butterworths, 1980, 450p.
[28] H .B. Hill, Principles of Medical Statistic, ninth ed. Oxford Univ., 1971.