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Eugenol and linalool: Comparison of their antibacterial and antifungal activities

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Antimicrobial resistance is one of the greatest threats to human health. Alternatives to antimicrobials are needed to combat the rise of bacterial resistance. Essential oils (EOs) and their components are potential sources of new antimicrobials. The present study was conducted to evaluate the antibacterial and antifungal activities of two components of EOs. The antimicrobial mechanisms of eugenol and linalool were investigated against five bacterial strains and four Candida strains. Broth macrodilution method was used to compare the antibacterial and anticandidal activities of the two compounds. They exhibited antimicrobial activity against all tested strains. Germ tube formation by *Candida albicans* was investigated and it was found that it was completely inhibited at sub-MICs of eugenol while linalool showed minor activity compared to eugenol. Time kill kinetic studies indicated that eugenol was highly toxic to all bacterial and fungal strains within 2.5 h of exposure. Absorbance of intracellular constituents was measured at 260 nm. Only eugenol was highly effective toward lysis and cellular content leakage compared to control drugs. In addition, scanning electron microscopy (SEM) was used to charactize the effect of the two components on cell morphology and showed that both compounds induced cellular deformity of nearly all tested cells. Also, it was found that only eugenol inhibited the Beta-lactamase production and urease activity and it diminished bacterial motility of all tested bacterial strains. These results indicate that eugenol and linalool are effective antimicrobial agents and both antibacterial and antifungal activities of linalool were much weaker than that of eugenol.

Key words: Antimicrobial resistance, antimicrobials, germ tube formation, time kill kinetic, cellular deformity.

INTRODUCTION

During the past few decades, the incidence of both community-acquired and nosocomial bacterial and fungal infections has significantly raised, increasing the number of patients who are at risk especially those with impaired immunity. There has been a worldwide rapid increase in resistance to antimicrobial agents in almost all bacterial and fungal genera and to all drug classes. The most important factor influencing the spread of antimicrobial resistance is the excessive microbial exposure to antimicrobials that results in selection pressure in microbial population, allowing only the fittest genotype to thrive (Canton and Morosini, 2011; WHO, 2012).

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Currently, high rates of antimicrobial agents use in the community, hospital and agriculture have contributed to fuel this crisis. The cross resistance to two or more antimicrobial agents is often mediated by a single resistance mechanism (Leclercq, 2002). This might be arisen due to plasmids (Tenover, 2006) or emerging mutation of chromosomal DNA (Sanders and Sanders, 1992). The cost of treatment is increased due to antimicrobial resistance. It may result in prolonged hospital stays, higher mortality rates, and creates broader infection control problems (Neu, 1992).

Natural products, either as pure compounds or as plant extracts provide unlimited opportunities for new drugs due to an increasing demand for chemical diversity (Cos et al., 2006). Essential oils (EOs) extracted from plants have been used primarily for flavoring and perfumery (Ben Arfa et al., 2006). It has long been recognized that EOs have antimicrobial properties, and recent studies have demonstrated that these activities are mainly due to the presence of numerous substituted aromatic molecules. Examples of these molecules include eugenol, cinnamaldehyde, and carvacrol (Moleyar and Narasimham, 1992). Currently, there is a trend in food processing to avoid the application of chemical preservatives such as sodium chloride and nitrates. Thus, the use of bioactive compounds derived from EOs as alternative antimicrobial agents is garnering great interest (Gill and Holley, 2004; Tsukiyama et al., 2002). Although, EOs have been empirically used as antimicrobial agents, their spectrum of activity and mechanisms of action remain unknown for most of them.

Linalool (3,7-dimethylocta-1,6-dien-3-ol) is a terpene alcohol that has broad spectrum antimicrobial activity (Alviano et al., 2005). Eugenol (4-allyl-2-methoxyphenol) is the main component of clove oil (phenolic compounds). It is used primarily as a flavoring agent in food and cosmetic products. Eugenol and linalool possesses various biological abilities, including antimicrobial, antioxidant, anti-inflammatory, anticarminative, anti-spasmodic, and antiparasitic activities. They are also effective as antiseptic agents in dentistry. Many studies most focused on the possibility of using clove oil as a replacement for some chemical additives in the preservation of main food categories such as meat and fish (Burt, 2004; Oussalah et al., 2007).

Combinational therapy is essential in the treatment of serious infection and to reduce the risk of resistant microbes (Kamatou et al., 2012). When linalool or menthol is combined with eugenol it showed the highest synergy, indicating that a monoterpenoid phenol combined with a monoterpenoid alcohol is an effective combination (Bassolé et al., 2010). Eugenol shows excellent synergistic activities and decreases MICs of conventional antibiotics as vancomycin, gentamicin and Beta-lactams (Moon et al., 2011). This synergistic effect can be linked to eugenol's ability to damage the membrane of Gram-negative bacteria. Combination between eugenol and cinnamate, cinnamaldehyde, thymol or carvacrol leads to greater antimicrobial effect (Pei et al., 2009; Rico-Molina et al., 2012). Certain combinations of eugenol and thymol show a synergistic effect thus potentiate their inhibition of *C. albicans* colonization and infectivity (Braga et al., 2007).

The present study evaluates the antibacterial and antifungal activity of eugenol and linalool and investigates the antimicrobial mechanisms of action against some Gram positive, Gram negative and *Candida* species.

MATERIALS AND METHODS

Test organisms

In the current study, five standard strains; *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 7839), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 10145) and *Candida albicans* (ATCC 10231) were used. They were obtained from MIRCIN culture collection of the Faculty of Agriculture, Ain Shams University. Clinical strains of *Proteus mirabilis* and Candida (*Candida albicans, Candida glabrata* and *Candida krusei*) were obtained from the Department of Microbiology, Faculty of Pharmacy, Minia University. All cultures were maintained in their appropriate agar slants at 4°C and used as stock cultures.

Antimicrobial agents

Two compounds; Eugenol and linalool (Sigma-Aldrich, Germany) with 98% purity were used in this study.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) or fungicidal (MFC) concentrations

A broth macrodilution method was used to determine the MIC and MBC or MFC according to the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts (2012, 2002). The Mueller Hinton broth (MHB) (Merck) or RPMI-1640 [Roswell Park Memorial Institute medium (with glutamine, without bicarbonate, and with phenol red as a pH indicator) was 1640, buffered to pH 7.0 with MOPS (morpholine propane sulfonic acid) at 0.165 M] (Sigma-Aldrich) was supplemented with dimethylsulfoxyde (DMSO) (Merck) at a 2% concentration in order to enhance sample solubility. Two fold serial dilutions of eugenol and linalool were prepared. The inocula of the microbial strains were prepared from overnight broth cultures and suspensions then adjusted to the turbidity of a 0.5 McFarland standard. Standardized suspension of the test organism was transferred into each tube. Controls without the test compound were prepared.

To determine MBC or MFC, 100 µL of bacterial inoculum was taken aseptically from tubes that had not presented visible turbidity and inoculated in Mueller Hinton agar (MHA) for 20 h at 35°C for bacteria or in MHA supplemented with 2% glucose and 0.5 mg/mL methylene blue for 24 h at 35°C for fungal strains. The MBC/MFC is defined as the lowest concentration of the essential oil at which 99.9% of the initial inoculum was killed. The experiments were repeated three times. To determine the nature of antibacterial or antifungal effect of these compounds, the MBC/MIC or MFC/MIC ratio was used; when the ratio was lower than 4, the compound was

considered as a bactericidal or fungicidal and when the ratio was higher than 4, it was considered as a bacteriostatic or fungistatic (Levison, 2004).

Germ tube inhibition assay

C. albicans (ATCC 10231) and clinical *C. albicans* strain were cultivated overnight on SDA at 37°C. Cell suspensions were prepared in NYP medium [N-acetylglucosamine (Sigma; 10^{-3} mol/L), yeast nitrogen base (Difco; 3.35 g/L), proline (Fluka; 10^{-3} mol/L) and NaCl (4.5 g/L), pH 6.7±0.1] and adjusted to 0.2×10^{6} c.f.u. ml^{-1}. Eugenol and linalool were diluted in DMSO and 10 µl volumes were added to 990 µl of the Candida suspensions (final DMSO concentration of 1%, v/v) to obtain 1/2, 1/4 and 1/8 of the MIC values. Untreated eugenol and linalool control suspensions were used. After 3 h incubation with gentle shaking at 37°C, cell suspensions were examined for the presence of germ tubes using a light microscope. Germ tubes were considered positive when they were at least as long as the blastospore (Pinto et al., 2009).

Time kill assay

Four concentrations (0.5, 1, 2 and 4 MIC) of each compound were tested against each microbial strain. The time-kill assay was performed with a final inoculum of approximately 5×10^{5} CFU/ml in a final volume of 30 ml. The final inoculum was adjusted to match the 0.5 McFarland standards. The tubes were continuously shaken on an orbital shaker and incubated at 35°C. 10 µl samples were withdrawn from each tube at 30, 60, 90, 150 and 24 h and streaked on MHA plate. Ten fold dilutions were prepared when necessary and plated onto each MHA plate. Plates were kept in triplicates for each compound and they were incubated for 24 h at 35°C, and colony counts were determined. Time-kill curves were plotted as $\log_{10}$ CFU/ml versus time functions. The compound concentration and the 3 log10-fold reductions in the bacterial count compared with the growth control were determined (Devi et al., 2010).

Estimation of the yeast cells or bacterial cytosol release

Bacteria and Candida cells were treated with eugenol or linalool then the release of cytosolic material absorbing at 260 nm from these cells was detected (Bennis et al., 2004b). Aliquots of 1.5 ml of cells suspension for 1 h in phosphate buffer saline (PBS) containing various concentrations of test compound ranging from 1.5 to 12 mM were used. Eugenol, linalool, levofloxacin (Sedico, Egypt) against bacterial cells and ketoconazole (Amriya, Egypt) with fungal cells were tested. Microbial cells suspended in PBS were used as control. Correction was made for the absorption of the suspending liquids containing the same concentration of compound after two minutes contact with bacteria or Candida cells at 260 nm in Beckman UV spectrophotometer.

Scanning electron microscopy (SEM)

Microbial suspensions treated with the MBC or MFC concentrations of eugenol or linalool for 1 h. Treated and untreated (negative control) cells were prefixed in 2.5% glutaraldehyde for 1 h at 4°C. After each fixation, the cells were rinsed twice with PBS. The cells were then dried through an ethanol series. The samples were gold coated by cathodic spraying (Edwards S 150 B). Finally, scanning electron microscopy examinations were done on a JSM-840 SEM (JEOL Ltd., Tokyo, Japan) (Benyahya et al., 1992).

Detection of β-lactamases production

All the bacterial strains were screened for β-lactamase production using two methods:

1. Iodometric method.
2. Acidometric method.

Iodometric method

Beta-lactamase production was tested by an iodometric method (Catlin, 1975) as follows: Sterile potassium or sodium penicillin G powder (6 lac units) was dissolved in potassium phosphate buffer pH 6.0, 0.05 M at a concentration of 6000 µg/ml (freshly prepared). Starch solution was prepared by adding 1 g of soluble starch to 100 ml distilled water and slowly heat to boiling to obtain a clear solution. Iodine solution was prepared by dissolving 2.03 g of iodine and 53.2 g of potassium iodide in 100 ml distilled water. One hundred microliters of the penicillin solution was dispensed. Several colonies of the microorganism were suspended in 0.9% saline solution to get dense suspension. Two drops of starch were added and then the mixture was kept at room temperature for 30 to 60 min. One drop of iodine was added which turn the solution blue. Disappearance of the blue colour in 10 minutes indicates that the organism is β-lactamase producer. Penicillin alone without any culture suspension was used as negative control.

Acidometric method

Acidimetric method used to assay enzyme β-lactamase using benzylpenicillin as substrate (Koneman, 2006). A single colony was resuspended and mixed with the indicator solution. One hundred µl of 1% phenol red solution was added to 1 ml of sterile distilled water for preparation of indicator solution. After mixing, this solution was added to a vial of one million units of sodium benzylpenicillin (Crystapen, Glaxo). Since this solution was at an acidic pH due to citrate buffer in the penicillin, a solution of 1 N sodium hydroxide was added drop-wise until the development of violet colour (pH 8.5). To get a dense suspension, several colonies were suspended 0.9% Sodium Chloride solution then added to 150 µl of penicillin phenol red solution. The color development observed within 1 h. The solution turned yellow if β-lactamase enzyme was produced.

Effect of sub-MIC and MIC levels of eugenol and lialool on bacterial urease activity

Urease activity was determined according to the method described by Derakhshan et al. (2008). Urea broth containing sub-MIC and MIC levels of eugenol and linalool was used for cultivation of bacterial strains overnight. Untreated cultures were used as negative control. All tubes were incubated at 37°C for 24 h. Suspensions were centrifuged at 5000×g for 3 min and the supernatants were recovered. Color intensity was measured at 560 nm using a universal microplate reader (Biotek, China). The samples were performed in triplicate.

Motility test

The motilities of the bacterial cells were determined according to Wojnicz and Tichaczek-Goska (2013). Bacterial strains were incubated for 24, 48 and 72 h at 37°C with and without eugenol and linalool. Suspensions were centrifuged at 2500 rpm for 2 min. Separated bacterial cells were washed three times with PBS then inoculated into motility agar tubes. Controls without the test

Table 1. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) values against tested strains*.

Strain	Eugenol			Linalool		
	MIC (mM)	MBC or MFC	MBC/MIC or MFC/MIC	MIC (mM)	MBC or MFC	MBC/MIC or MFC/MIC
S. aureus (ATCC 6538)	6.25	25	4	100	100	1
E. coli (ATCC 7839)	25	25	1	50	50	1
*K. pneumonia*e (ATCC 10031)	12.5	50	4	12.5	50	4
P. mirabilis	12.5	25	2	25	50	2
P. aureginosa (ATCC 10145)	50	100	2	50	100	2
C. albicans (ATCC 10231)	25	50	2	25	25	1
C. albicans	25	25	1	25	50	2
C. glabrata	12.5	12.5	1	25	50	2
C. krusei	12.5	12.5	1	25	25	1

*Tests were performed in triplicate and modal values were represented.

compounds were used.

RESULTS AND DISCUSSION

Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of eugenol and linalool

In order to achieve precisely the antimicrobial properties of eugenol and linalool compounds, determination of MICs and MBCs were necessarily performed. The assayed compounds showed effectiveness in inhibiting all tested strains. Eugenol showed the highest effect against *S. aureus* (the lowest MIC) compared to the other Gram-negative bacterial strains. Generally, Gram-negative bacteria are more resistant to EOs and other natural extracts with antimicrobial activity than Gram-positive bacteria (Trombetta et al., 2005). This resistance could be attributed to the structure of the cell walls of Gram-negative bacteria, mainly with regard to the presence of lipoproteins and lipopolysaccharides that form a barrier to restrict entry of hydrophobic compounds (Russell, 1995).

Eugenol and linalool had nearly the same effect against all other tested microorganisms. The MICs of eugenol ranged from 6.25 to 100 mM while for linalool, MICs ranged from 12.5 to 100 mM. For all Candida strains and most bacterial strains, MBCs and MFCs were equal to or two-fold greater than the MICs. However, the MBCs of eugenol against *S. aureus* and *K. pneumonia*e and MBCs of linalool against *K. pneumonia*e were four times greater than that of the MICs (Table 1). The ratios of MBC to MIC and MFC to MIC were ranging from 1 to 4. In most cases, MBC and MFC were close to the MIC, indicating a good bactericidal or fungical activity against the tested strains. Other authors revealed the antibacterial activity of eugenol against various pathogens such as *E. coli*, *Bacillus cereus*, *Helicobacter pylori*, *S. aureus*, *S. epidermidis*, *Streptococcus pneumoniae* and *S. pyogenes* (van Zyl et al., 2006; Leite et al., 2007). Previous studies confirmed the antifungal activity for clove oil and eugenol against yeasts and filamentous fungi, such as food-borne fungal species (López et al., 2005) and human pathogenic fungi (Chaieb et al., 2007). Antifungal and antibiofilm activity of linalool against *C. tropicalis* was previously reported (Souza et al., 2016). Researchers found that linalool compound and basil oil which contains high amounts of the monoterpene linalool had antimicrobial activity against *S. aureus, B. subtilis, E. coli* and *Aspergillus niger* (Hussain et al., 2008). Consistent with our results, Hsu et al. (2013) demonstrated the fungicidal effects of linalool against *C. albicans* clinical isolates and non- *C. albicans* Candida spp.

The antimicrobial activity of EOs depends on the composition and percentage content of active constituents in EOs, which have been found to have an important role in slowing down or stopping the bacterial growth or killing the bacteria (Bozin et al., 2006). Eugenol belongs to a class of phenylpropenes. Free hydroxyl groups confer the antimicrobial activity of this class (Laekeman et al., 1990). The antimicrobial activity of eugenol can be attributed to the presence of a double bond in α, β positions of the side chain and to a methyl group located in the γ position (Jung and Fahey, 1983). Linalool is one of the most common terpenoids, which are terpenes with added oxygen molecules or that have had their methyl groups moved or removed by specific enzymes (Caballero et al., 2003).

Effect of eugenol and linalool on germ tube formation by *C. albicans*

Germ tube formation is one of the important mechanisms

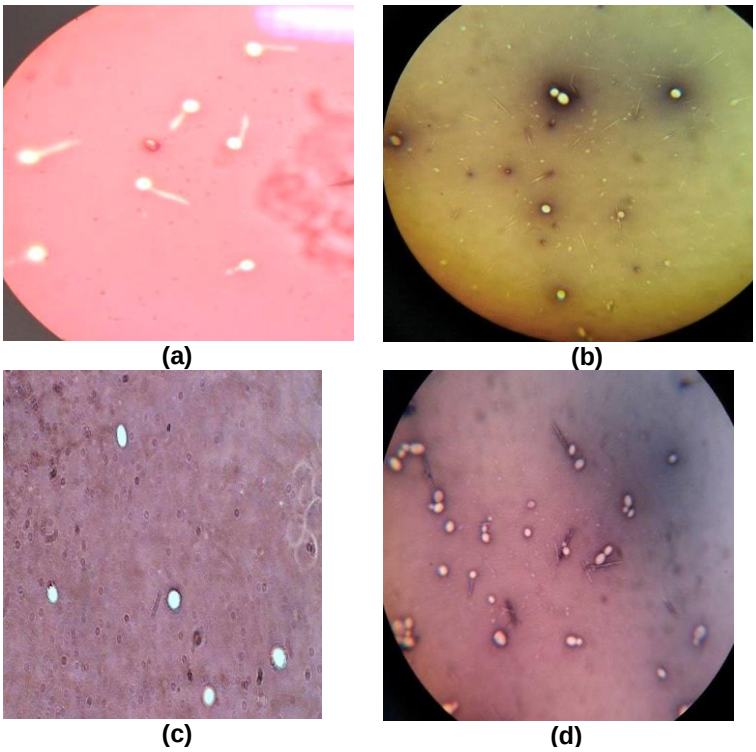
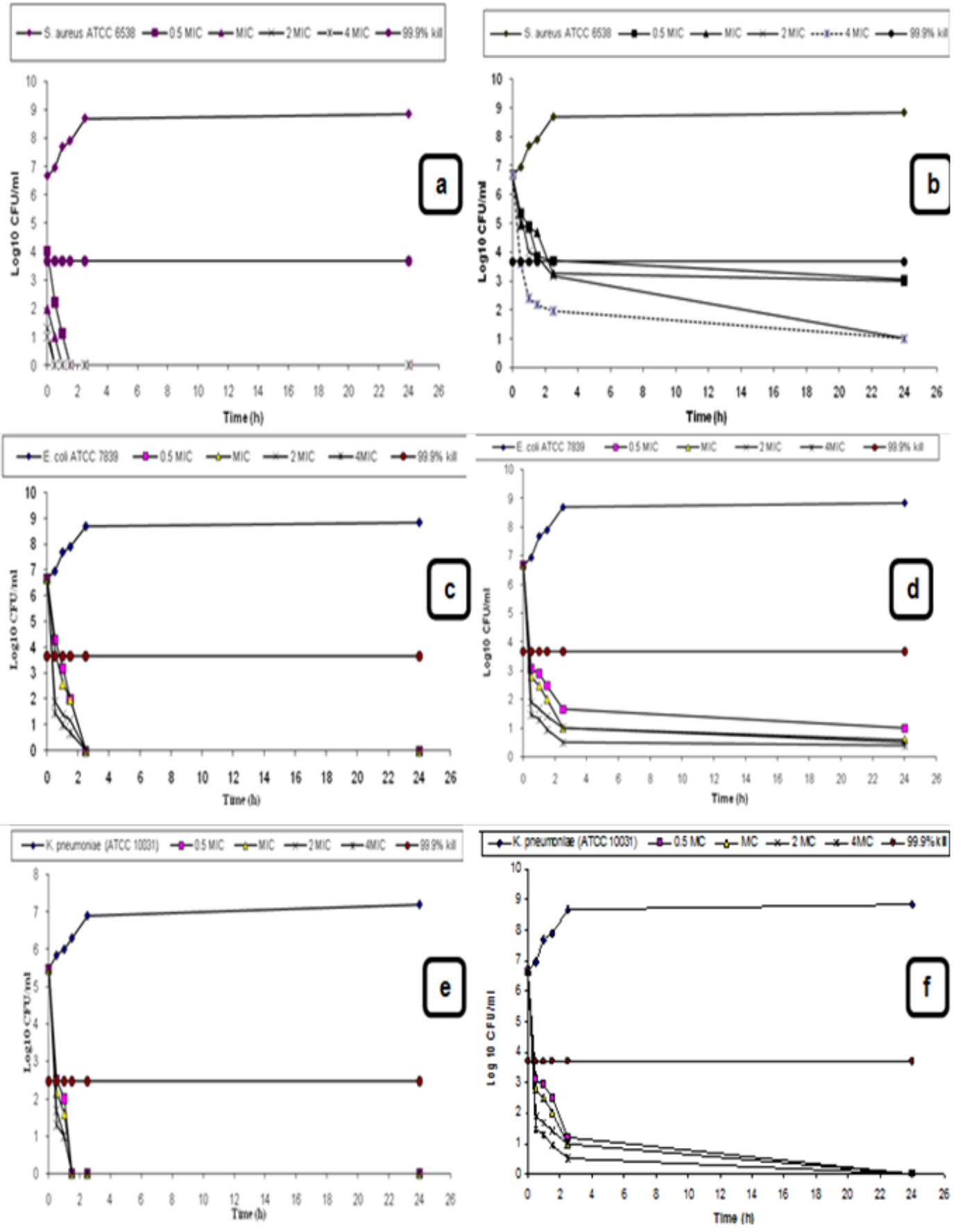


Figure 1. Effect of eugenol and linalool on germ tube formation by *Candida albicans* strains. a. Untreated *C. albicans* (ATCC 10231) (Control); b. *C. albicans* (ATCC 10231) treated with eugenol; c. *C. albicans* pathogenic strain treated with eugenol; d. *C. albicans* (ATCC 10231) treated with linalool.

of pathogenicity of *C. albicans* (Pinto et al., 2008). Most antifungal drugs are germ tube formation inhibitors (Ellepola and Samaranayake, 1998). By testing the effect of eugenol and linalool at sub-inhibitory concentrations on the germ tube formation of *Candida albicans* strains, it was found that eugenol completely inhibited serum induced hyphae in *C. albicans* (ATCC 10231) and *C. albicans* clinical strain at Sub-MICs. The inhibitory effect was dose-dependent. Linalool reduced the germ tube production significantly, rendering the cells less pathogenic as compared to control but to lesser extent compared to eugenol (Figure 1). Consistant with this, Pinto et al. (2009) found that eugenol's sub-inhibitory concentrations completely inhibited germ tube formation by *C. albicans*. Chami et al. (2004 and b), revealed the antifungal activity of eugenol in the treatment and prophylaxis of animal models of oral and vaginal candidiasis. Also, Hsu et al. (2013) demonstrated that linalool exhibited dose-dependent inhibitory activity against germ tube formation.

Time-kill assay

The results of time kill kinetic studies are illustrated in Figures 2 and 3. Eugenol was effective against all bacterial and fungal strains. It inhibited most isolates at half MICs either within 1.5 h or 2.5 h exposure, while standard *C. albicans* and *C. krusei* required MICs for inhibition. Linalool showed less activity. It had slower kill rate at 24 h of incubation either at 0.5 MIC in case of *K. pneumonia*e or at MIC as shown by *P. aeruginosa* and *C. albicans*. *C. glabrata* and *P. mirabilis* required two fold higher concentration and four fold MICs respectively. Also, linalool showed no activity against *S.aureus* and *E.coli.* Only MIC was fungicidal to *C.krusei* at 2.5 h of incubation. Kill curve assays of *C. albicans* standard strain was performed by Hsu et al. (2013) using the broth macrodilution method. Linalool showed its fungicidal effect at MIC, and the fungicidal endpoint was achieved within 1 h. Zore et al. (2011) demonstrated that MFC of linalool and eugenol killed 99.9% of *C. albicans* inoculum within 7.5 min of exposure. Recent investigations about the anti-candidal action of terpenoids showed that they modulate mevalonate pathway (MP), alter cellular levels of intermediate molecules and associated functions in eukaryotic cells (Mo and Elson, 2004). Other researchers reported that terpenoids destabilize membrane and modulate its associated functions like permeability, cell signaling, etc., leading to cell death and ascribed the


S. aureus ATCC 6538
0.5 MIC
MIC
2 MIC
4 MIC
99.9% kill
Log10 CFU/ml
Time (h)
a
b
E. coli ATCC 7839
c
d
K. pneumoniae (ATCC 10031)
e
f

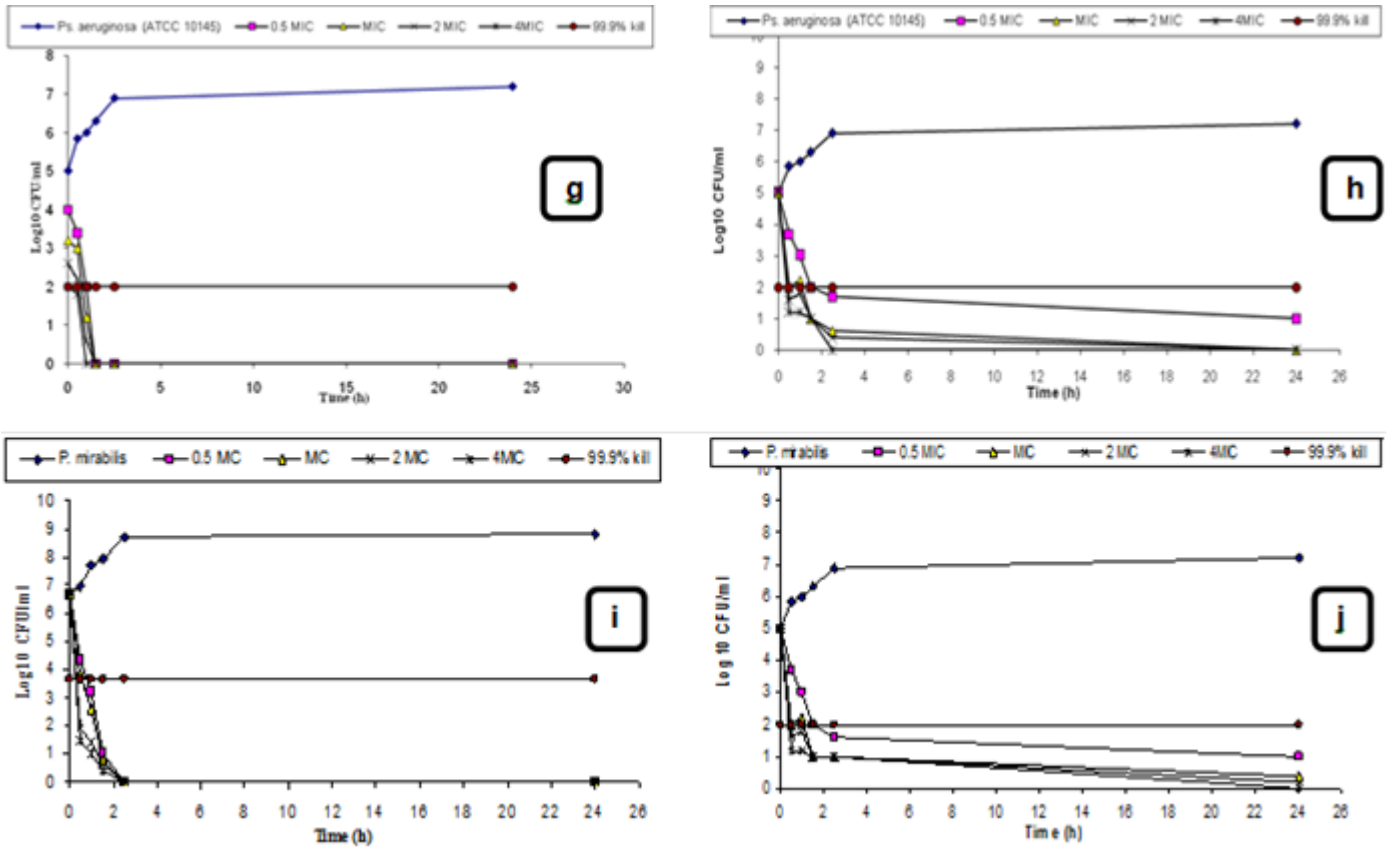


Figure 2. Time-kill curves with different concentrations of eugenol (Left panels) and linalool (Right panels) for bacterial species: (a and b) *S. aureus* (c and d) *E. coli* (e and f) *K. pneumonia*e (g and h) *P. aeruginosa* and (i and j) *P. mirabilis.*

antibacterial activity of linalool to its membrane destabilizing activity, sensitization of bacteria and enhancement of drug sensitivity (Trombetta et al., 2005). Regarding eugenol, hydroxyl group contributes to its inhibitory effect at sub-lethal concentrations as it binds to and affect the properties of proteins, thus inhibits the activity of some enzymes such as ATPase which may be important for cell killing at high eugenol concentrations because energy generation needed for cell recovery is impaired (Gill and Holley, 2006b).

Effect of eugenol and linalool on release of cytosolic material

To confirm the results of kill kinetics and for further understanding the mechanism of antimicrobial action of eugenol and linalool we evaluated the lysis of microbial cells, by treatment with different concentrations of the two compounds compared with levofloxacin and ketoconazole and measure the release of substances absorbing at 260 nm. The evaluation of the loss of cell contents contributes to demonstrate the severity of the cell membrane damage.

Our study shows that no significant absorbance was obtained on treatment with linalool. Concerning *C. glabrata,* slight increase obtained but still much lesser compared to eugenol and ketoconazole. Figures 4 and 5 show that the absorbane and accordingly the intracellular constituents release was increased in linear pattern with the increase of eugenol concentration. With all bacterial strains and at all tested concentrations, eugenol showed higher absorbance than levofloxacin. On the other hand, release of cellular constituents obtained with ketoconazole was higher than eugenol after 6 mM with standard *C.albicans* strain and at 12 mM with *C. glabrata* strain. Also, sudden increase was obtained with *S. aureus* after 1.5 mM. These results can be attributed to the eugenol's ability to disrupt the membrane and allowing the leakage of cellular content.

A study performed by Oyedemi et al. (2009) on some Gram-negative and Gram-positive bacteria revealed that eugenol induced cell lysis by damaging the cell wall and membrane caused leakage of protein and lipid contents after 120 min of exposure.

Also, it was reported that eugenol's action on membranes occurs mainly by a non-specific permeabilization which increase the transport of potassium and ATP out of the cells (Gill and Holley, 2006a).

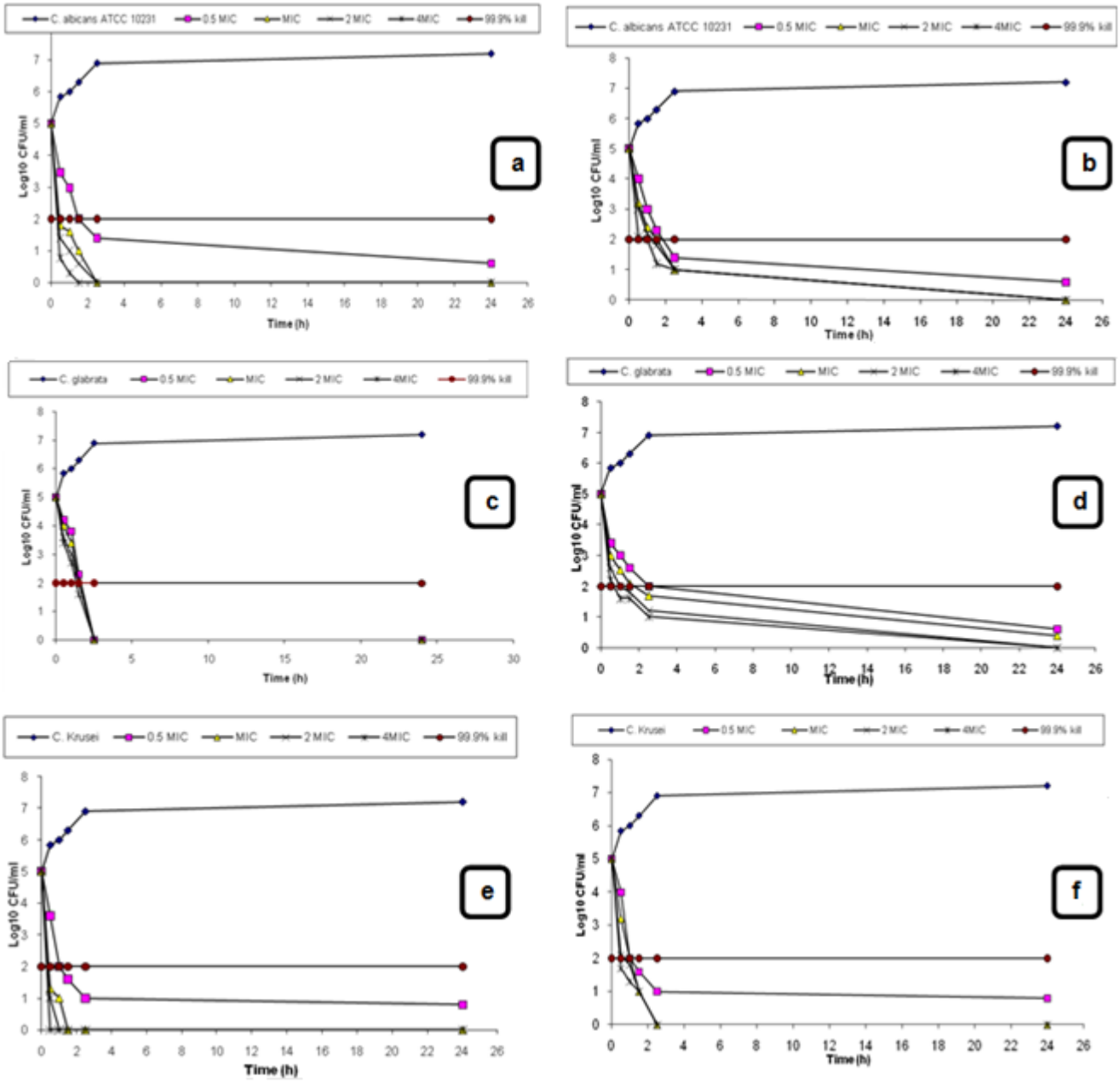


Figure 3. Time-kill curves with different concentrations of eugenol (Left panels) and linalool (Right panels) for *Candida* species: (a and b) standard *C. albicans* strain (c and d) *C. glabrata* and (e and f) *C. Krusei*.

Effect of the tested compounds on the morphology of microorganisms using scanning electron microscopy (SEM)

We studied the action of eugenol and linalool on the cell morphology using SEM. Linalool treated *S. aureus* cells showed disrupted membranes and appeared as a mass of cells (Figure 6b). While in case of *P. aeruginosa* treated cells no significant changes were obtained compared to the non treated control (Figure 6f). Concerning *E. coli* cells treated with eugenol visualized by SEM, significant deformities, irrigularities and holes in the envelope were observed (Figure 6d).

Regarding *C. albicans* (ATCC 10231), Figure 7a demonstrated that the action of linalool on cells appeared as cell wall deformity and holes. While in case of eugenol treated cells, the cell wall is different compared to that of the control cells as treated ones shows many pores on the membranes (Figure 7b). SEM observations revealed that both compounds affect not only the membrane but all the envelope of bacterial and fungal cells. Bennis et al. (2004a) studied the effect of eugenol on *Saccharomyces*

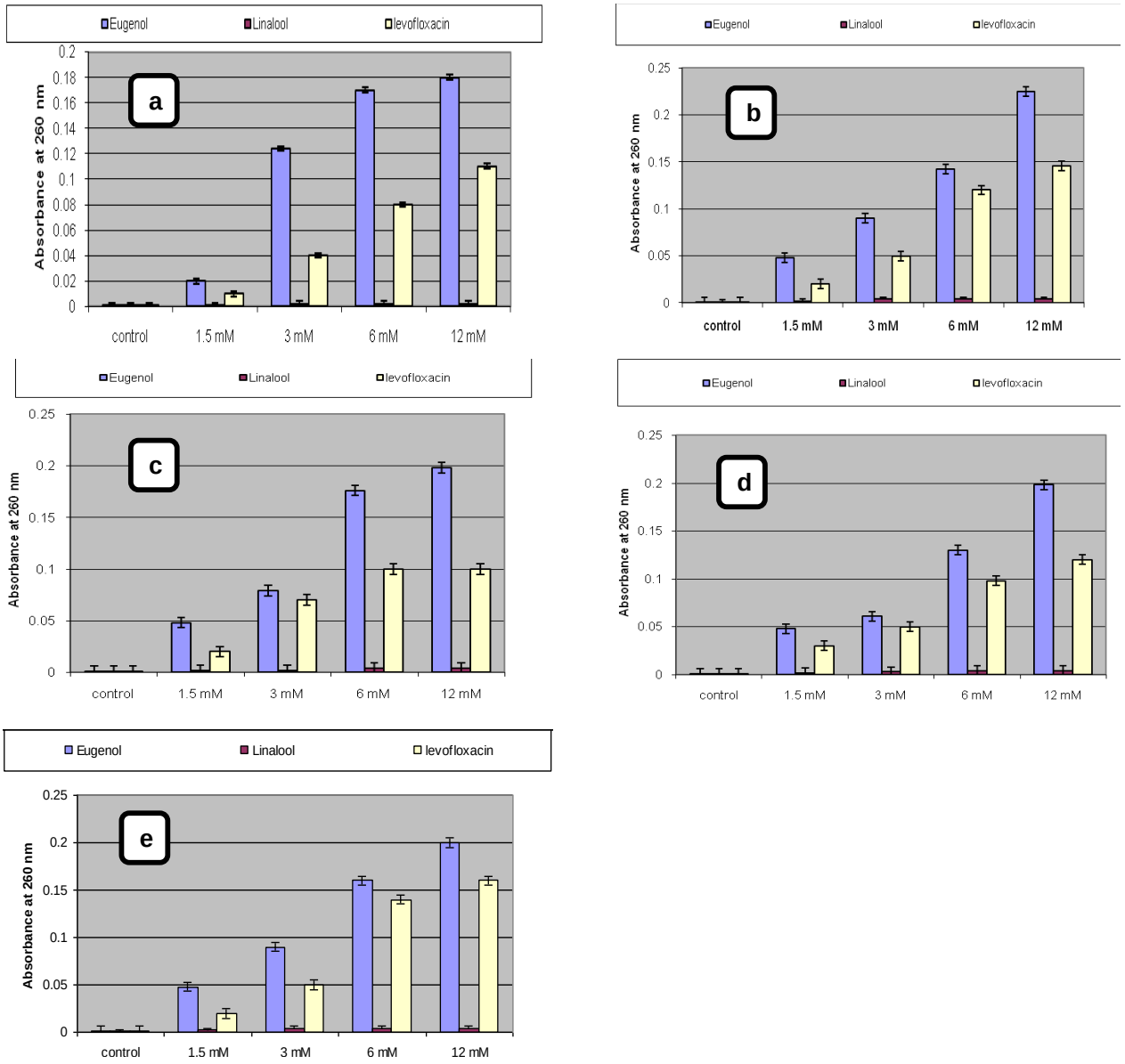


Figure 4. The effect of eugenol, linalool and levofloxacin on the release of 260 nm absorbing material from bacterial strains: (a) *S. aureus* (b) *E.coli* (c) *K. pneumoniae* (d) *P. aeruginosa* (e) *P. mirabilis.*

cerevisiae, B. subtilis and *E. coli* cells. Eugenol induced important morphological damages in *S. cerevisiae*. Also, the difference in the eugenol's action on the *B. subtilis* and *E. coli* was suggested to be the result of the difference in structure between Gram-positive and Gram-negative bacteria. Nazzaro et al. (2013) reported that rod shaped bacterial cells are more sensitive to EOs than coccoid cells. Results obtained by Di Pasqua et al. (2007) revealed alterations in the composition of the fatty acids and the morphology of the cells when treated with eugenol. They suggest that eugenol may disrupt the *E. coli* membrane and allow the leakage of intracellular contents.

Screening of β-lactamase production, urease production and motility

Other possible antibacterial mechanisms of eugenol and linalool were investigated. Two methods were used for detection of β-lactamase production in all strains; iodometric and acidometric methods. Both methods showed that linalool had no significant activity on β-lactamase producing strains, while β-lastamase production was inhibited on treatment with eugenol (Table 2). Previous report of Dhara and Tripathi (2013) demonstrated that eugenol and cinnamaldehyde possess strong antibacterial activity against extended-spectrum β-

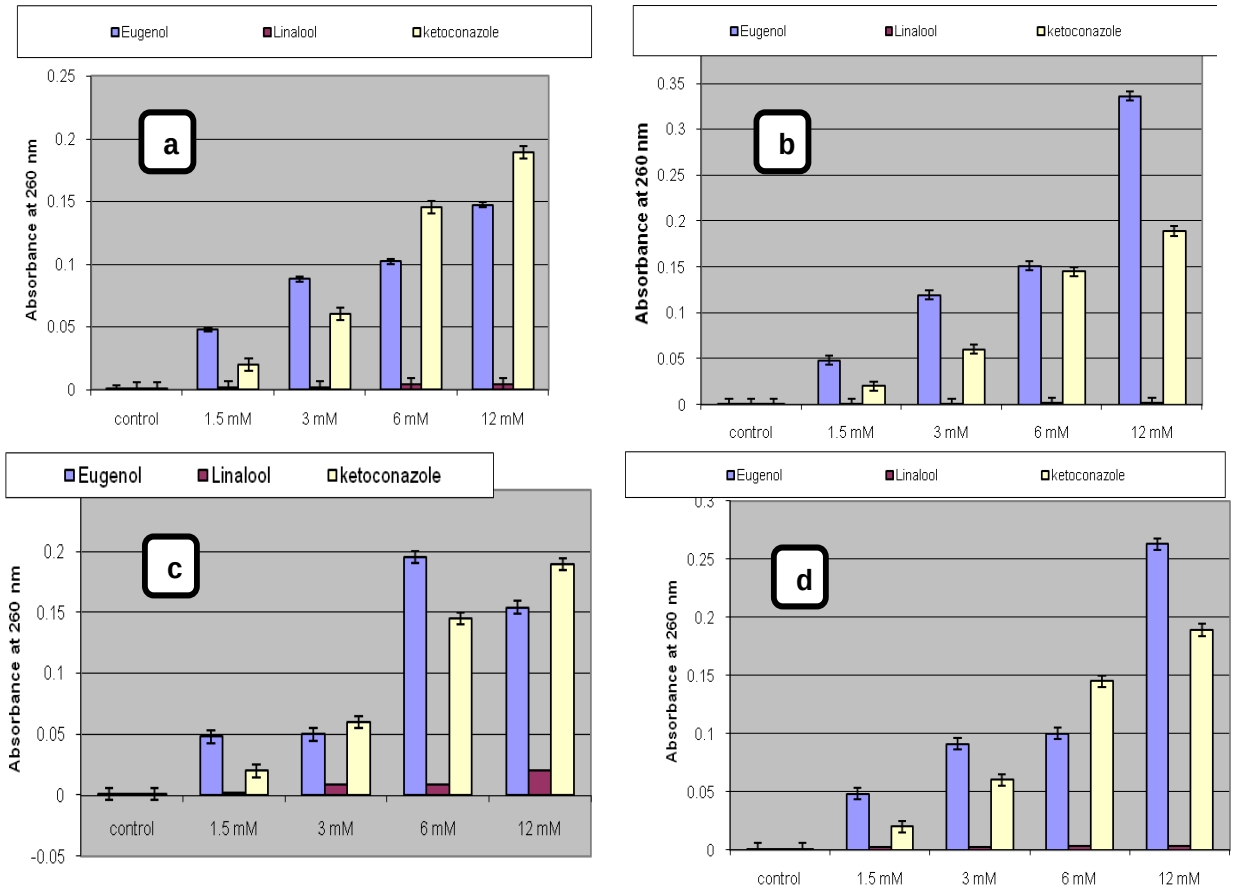


Figure 5. The effect of eugenol, linalool and ketoconazole on the release of 260 nm absorbing material from *Candida* strains: (a) *C. albicans* (ATCC 10231) (b) *C. albicans* clinical strain (c) *C. glabrata* (d) *C. krusi.*

lactamase (ESBL) positive strains. These compounds are hydrogen bonded with catalytic and crucial amino acid residues of ESBL proteins.

Bacteria need urease in order to obtain nitrogen for their growth. Urease inhibitors are potential alternatives for the prevention of *K. pneumoniae* from colonizing the gastrointestinal tract (Maroncle et al., 2006). Our data showed that eugenol inhibited urease production and arrest motility of all treated cells. On the other hand, linalool failed to inhibit bacterial urease enzyme and motility as summarized in Table 2.

The effect of terpenes on *Proteus* spp. swarming behavior was indirectly reported by Mansouri et al. (2005). Echeverrigaray et al. (2008) evaluated the effect of 17 monoterpenes on *P. mirabilis* swarming and they found that 8 compounds inhibited swarming significantly. They revealed that linalool had no activity on the *P. mirabilis* swarming. The monoterpenes antimicrobial activity increases with the presence of an oxygen containing functional group (Naigre et al., 1996). Effect of other EOs on bacterial motility was studied. Gabel and Berg (2003) reported that carvacrol inhibited flagellin.

Synthesis and decreased the proton motive force required for flagellar movement of flagellated cells.

Conclusion

In conclusion, our results indicate that eugenol and linalool exhibit promising antimicrobial activities against Gram-positive, Gram-negative and Candida pathogenic strains and could be used as natural alternatives for application in medical field. It was concluded that the antibacterial activity of linalool was much weaker than the antifungal activity. Also, the antimicrobial activity of eugenol is more powerful than linalool. These results suggest that a number of the components of the EOs such as eugenol and linalool may have potential clinical applications in treating microbial infections alone or in combination with other EO constituents or with conventional antibiotics.

Conflict of Interests

The authors have not declared any conflict of interests.

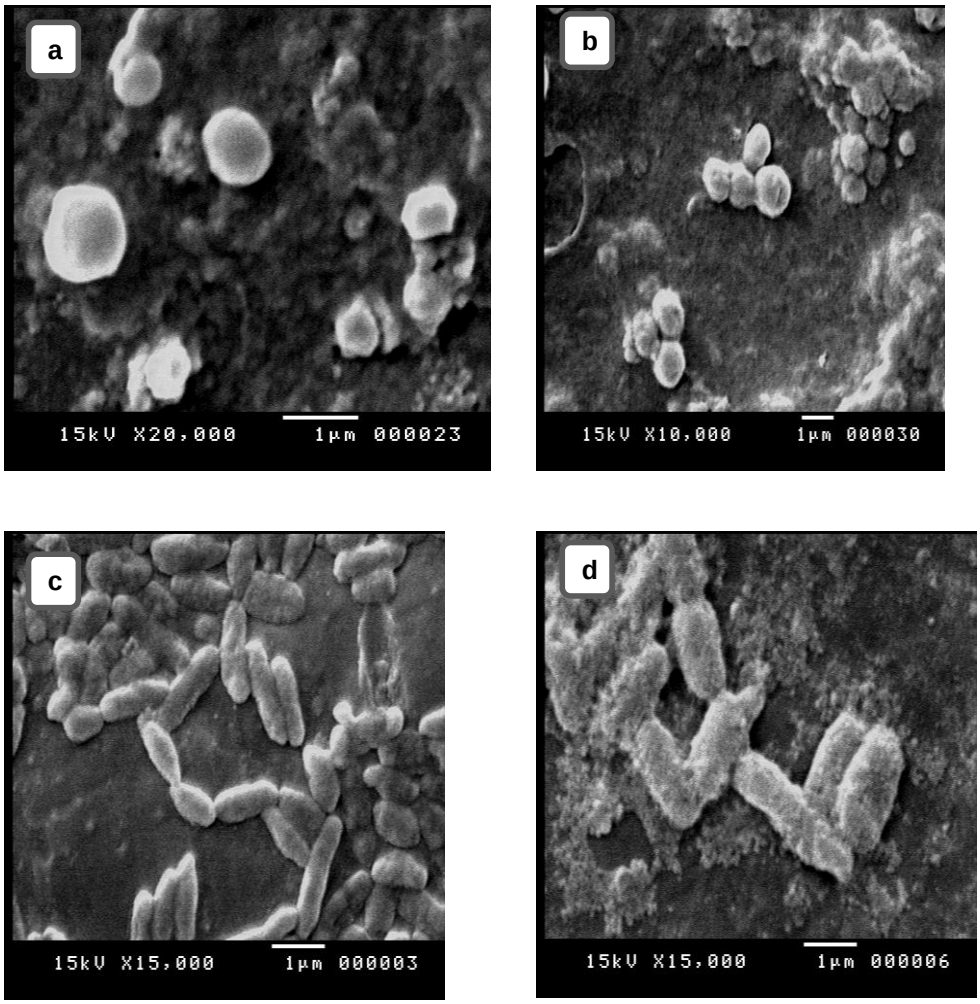


Figure 6. Scanning electron microscopy images showing: (a) *S. aureus* untreated cells (Control), (b) *S. aureus* cells treated by linalool, (c) *E. coli* untreated cells (Control), (d) *E. coli* cells treated by eugenol, (e) *P. aeruginosa* untreated cells (Control) and (f) *P. aeruginosa* treated by linalool.

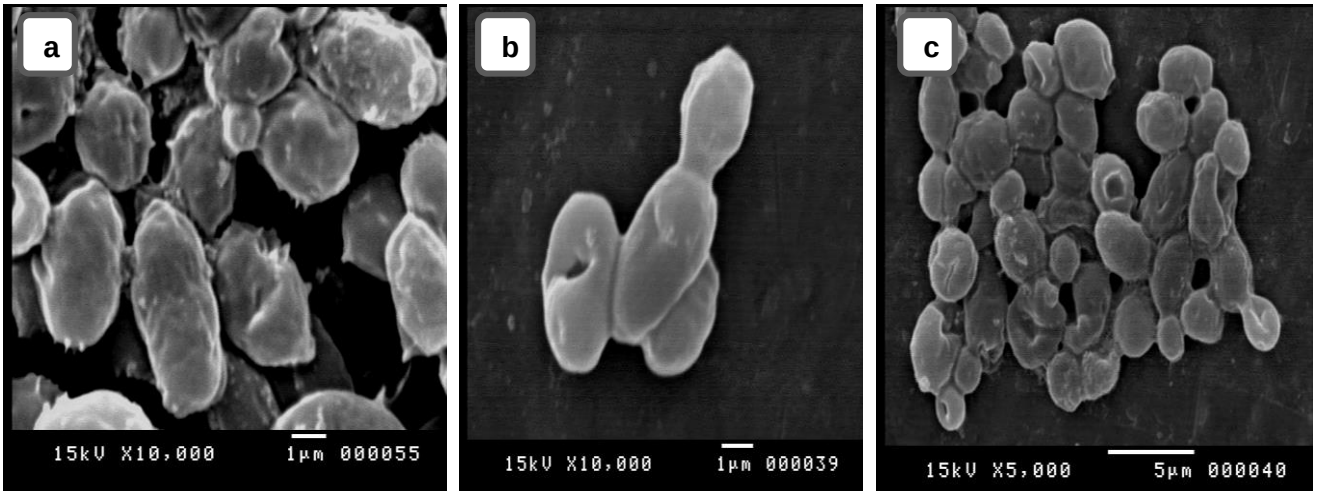


Figure 7. Scanning electron microscopy images of *C. albicans* (ATCC 10231) cells treated by (a) linalool and (b and c) eugenol**.**

Table 2. Effect of eugenol and linalool on β- lactamase production, urease production and motility of the tested strains.

Strains	β- lactamase production		Urease production		Motility	
	Eugenol	Linalool	Eugenol	Linalool	Eugenol	Linalool
S. aureus	-	+	-	+	NT	NT
E. coli	-	+	NT	NT	-	+
K. pneumoniae	-	+	-	+	NT	NT
P. aeruginosa	-	+	-	+	-	+
P. mirabilis	-	+	-	+	-	+

-, Negative; +, positive; NT, not tested.

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