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Comparative study of the effect of Eucalyptus extract on *Candida albicans* and human pathogenic bacteria

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ABSTRACT

Background & Aim: During recent decades, infections disease resulting from opportunistic fungi such as *Candida albicans* and resistance increasing of human pathogenic bacteria to current antibiotics has led to many problems for treating of these diseases. The aims of the present study was to investigate anti-fungi and anti-bacterial effects of Eucalyptus extract on *Candida albicans* species isolated from clinical samples and some standard human pathogenic bacteria.

Experimental: Eucalyptus extract was provided using rotary apparatus and maceration method. Thirty isolates of *Candida albicans* were isolated from referred patients to gynecologist and then were purified and identified by valid keys. Finally, 30 isolated were used to growth inhibitory activity assay. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of eucalyptus extract against 5 standard bacteria inculding: *Staphylococcus aureu, Shigella dysenteriae, Listeria monocytogenes, Vibrio cholera* and *Bacillus cereus* were evaluated using micro broth dilution method.

Results: Our results indicated that in compare to bacterial strains, fungus isolates showed more sensitivity to eucalyptus extract. The highest and lowest MIC of extract was recorded at 12.5 and 3.1 ppm for *S. aureus* and *B. cereus*, respectively. As *MIC*, the maximum MBC (20ppm) and minimum MBC (5ppm) of extract was recorded fro *S. aureus* and *B. cereus* respectively. Minimum inhibitory concentration of extract to fungal growth inhibitory was 50 ppm, whereas the maximum inhibiting concentration was 150 ppm.

Recommended applications/industries: The positive and interesting results suggest the essential oil of *E. globulus* could be exploited antibiotic for the treatment of candidiasous disease caused by C*andida albicans* fungi, and some human pathogenic bacteria studied in this work.

1. Introduction

Although, fungal infections compared to bacterial and virus infections, was not much prevalent in the past, but they have been responsible for considerable increase of diseases during recent decades (Baker *et al.*, 2012). Risk factors such as long and wide use of antibiotics, croton drugs, immuno suppressants medicament and basic disorders such as diabetes, AIDS and malignancies caused by fungal diseases, especially increased more than in the past (Goswami *et al.*, 2000; Merenstein *et al.*, 2013). Vaginal yeast infections known as candidiasis, is a disease caused by growth of more yeast around genital aerea of women (Consolaro *et al.*, 2004; Giraldo *et al.*, 2000). This is an important disease during the years in women who are sexually acitve, and it is increasingly spreading (Hay and Ashbee, 2010). *Candida albicans* species is the main causal agent that generates candida virginities disease with growth in two biological forms: yeast and mycelia forms in medium culture (De Vos *et al.*, 2005). This dimorphic fungus growth mainly in the human body as digestive and genital systems, and culture of, and also is present on vaginal secretion of 10-25% of women without symptoms disease (Cates *et al.*, 1990; Mathema *et al.*, 2001). The sexual contact is not usually the main cause of fungus spread in candidiasis or yeast infection. Antibiotics, birth control pills, steroid consuming and also tight clothing, severe obesity and warm weather, cause increase numbers of yeast.

One of the most widespread methods of candidiasis controlling is the use of fungicides such as fluconazole, ketoconazole, itraconazole (Amber *et al.*, 2010; Schuster *et al.*, 2008). Resistant of *C. albicans'* isolates to normal treatment by topical medications such as miconazole and fluconazol antifungal cream has been reported (Hoehamer *et al.*, 2010; Moosa *et al.*, 2004). Bactreia are another pathogenic factor in human. Currently, human pathgenic bacteria are controlled using chemical drugs including different antibiotics. Regarding to broad-spectrum and fast-acting of antibiotics, they are the first choice of patients (Biyela *et al.*, 2004; Levy, 1998; Neo, 2011). Continous and immethodical consumption of chemical drugs cause resistance to these combinations which result in weakened or neutralized effect of drugs and finally leads to raising the drug doze and tendency to apply new and more powerful formulae. Another disadvantage of using these drugs is their side effects which lead to emergence of diseases more dangerous than the primary ones (Pinto *et al.*, 2009). During recent years, medical plants are being used as an appropriate alternative for chemical drugs without their negative side effects. Eucalyptus plant (*Eucalyptus globule*s, *Myrtaceae*) is a flowering tree and shrub which have been adapted to hot and humid climate of Iran and is frequently used as medical plants to cure for the common cold. Antifungal and antibacterial effects of eucalyptus extract in human (Cermelli *et al.*, 2008; Hammer *et al.*, 1999; Saeeidi *et al.*, 2014) and plant diseases (Ghorbani *et al.*, 2014; Wilson *et al.*, 1997) have been assessed, and it has been shown that in most cases in exprimental condition, eucalyptus has had good inhibiting effects on the growth of tested fungi and bacterial. Extract of Eucalyptuse has anti-inflammatory, antiseptic, antibacterial and expectorating properties and has resulted to inhibit inflammation of nasal mucosa, pharyngeal mucosa, otitis, sinuses inflammation and vaginitis (Noumi *et al.*, 2011). Analysis of chemical compound of eucalyptus extract content has shown that these compounds contain cineol, cytrol (Maciel *et al.*, 2010), piperitone, α-Phellandrene and trepene (Gilles *et al.*, 2010) . Because of biological and pharmacological properties of essential oil of Eukalyptus species and its applications as anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, in the international pharmacopeia and marke we were firstly interested, to investigate antifungal effects of eucalyptus extract on *C. albicans* isolates obtained from clinical samples and secondly to compare this with anticacterial effects of this extract against some human pathogenic standard bacteria.

2. Materials and Methods

2.1. Sample preparation

The plant used in this study was purchased from commercial source and based on morphological and botanical descriptions were identified as Eucalyptus *globules* by an expert botanist. The leaves of plant were dried in the shadow under the appropriate conditions, were grinded in a grinder and homogenized to a fine powder and then were maintain at room temperature for further studies.

2.2. Plant extraction

Plant extraction, was carried out using maceration method. For this propose, 10 gr dried powder of the eucalyptus leaf was put in 0.5 lit Erlenmeyer flask containing 100 ml ethanol (96%) and water. The flask content was mixed using a shaker in the room temperature with the speed of 130 rpm for 24 h, and then was filtered from Whatman No. 2. The solvent was separated and concentrated from the extract using a rotary evaporator (Heidolph, Germany), and was dried after being passed from a microbial filter with 0.45 micro meter pores in 60 °C temperature for two days (Saeedi et al., 2012). The obtained extract was wieghted and solved in DSMO solvent and was kept in refrigrator in 4°C until being used in anti-microbial tests. To determine the Maximum Inhibiting Concentration (MIC) of the extracts, was done using dilution method

2.3. Fungal isolates

Fungi isolates were isolated from patients' vaginas referred to the medical center using a sterile swab, by a gynecologist and then were quickly transferred to a sterile falcon tube containing sterile water for further culture and purification. Two solid culture medium including: Sabouraud Dextrose Agar and Sabouraud Dextrose Broth (Merck, Germany) were used for fungal culture and purification. One drop of fungi suspension was spread in a line across over the solid SDA medium in plates. All plates were transferred to an incubator at 37°C and then were examined after 24 hrs incubation. Growth individual colonies were cultured again on the solid medium and fresh colonies were used for purification and identification. Each purified colony was identified according to the standard keys based on morphological critari. Isolates were kept for further studies in -20°C (Otang et al., 2012). To prepare a suspension of fungal spores, a piece of *C. albicans* cultured on Sabouraud Dextrose Agar medium were transferred to liquid physiologic serum and kept in 37°C. After fungi growth in liquid medium, the concentration of fungal suspension was measured using a spectrophotometer (Unico, USA) in 530 µm wavelength. Based on the obtained light absorption, a suspension with a concentration of 10^6 cells/mL was adjusted.

2.4. Bacterial strains

Standard bacteria strains including: *Staphylococcus aureus* ATCC1189, *Shigella dysenteriae*, ATCC1188, *Listeria monocytogenes* ATCC1298, *Vibrio cholera* ATCC1611, *Bacillus cereus* ATCC1015 were used to determine inhibiting effects of eucalyptus extract. After preliminary preparations, a colony of bacteria was cultured in 10 ml Tryptic Soy Broth (TSB, Merck, Germany) medium and was incubated in 37 °C for 18 hrs; then, 100 ml of the fresh grown suspension of each bacterium was transferred to the solid TSB medium and was incubated in 37 °C for 18 hours.

2.5. Preparing McFarland microbial suspension

To prepare a microbial suspension, one day before experiment, a piece of stock culture (solid medium) was transferred to nutrient agar medium (Merck, Germany). After growth of bacterial cells on the surface of culture medium, the surface was washed using a normal salt solution and concentrated microbial suspension was obtained. A drop of bacterial suspension was poured in leaded sterile tubes containing normal saline and its turbidity was measured using a spectrophotometer in 530 µm wavelength and was diluted with normal saline and then the turbidity of a bacterial suspension was equal to the turbidity of 0.5 McFarland suspension. The final concentration of the bacterial suspension was adjusted to 1.5×10^8 CFU.

2.6. The Minimum Inhibitory Concentration (MIC) Method

The minimum inhibitory concentration (MIC), is the lowest concentration of an antibiotic or plant extract that can inhibits the growth of a particular

organism as bacteria or fungi. The MIC of eucalyptus extract was determined using broth micro-dilution method. For this proposes, one drop of each bacterial suspension (1.5×10^8 CFU) was added to well of micro-titer plate containing Muller liquid Hinton nutrient medium (MHB). A serial dilution (ppm) of plant extract was done for each bacterial strain. Micro-titer plates were incubated at 37°C and the MIC was recorded after 24 h.

2.7. The Minimum Bacterial Concentration (MBC)

The lowest concentration of plant extract which is able to reduce the viability of the initial bacterial inoculums by ≥99.9% is called MBC. The MBC is complementary to the MIC. MBC was determined by sub-culturing the test dilutions on to a fresh solid medium and incubated further for 18- 24 h. The highest dilution that yielded no bacterial/fungal growth on solid medium was taken as MBC.

2.8. Statistical analysis

The mean values of studied parameters were taken from the measurements of three replicates and the "Standard Error" of the means was calculated. One-way ANOVA was applied to determine the significance of the results between different treatments and then Duncan multiple range tests ($p < 0.05$) were performed. All the statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (version 18.0).

3. Results and discussion

The result of this study showed that various concentrations of eucalyptus extract can inhibit the growth rate of *Candida albicans* isolates in excremental condition. Increase of plant extract concentration resulted to high growth inhibitory effect on solid medium. Minimum inhibitory concentration was 50 ppm, whereas the maximum inhibiting concentration was 150 ppm (Fig. 1).

The results of investigating the effect of eucalyptus extract on bacteria indicated that eucalyptus extract was able to inhibit growth of all tested bacteria. The maximum inhibiting concentration (MIC) and maximum bacterial concentration (MBC) (MBC) was recorded for *staphylococcus aureus* strain and the minimum inhibitory concentration (MIC) minimum bacterial concentration (MBC) was recorded for *Bacillus cereus* strain, and (Fig. 1).

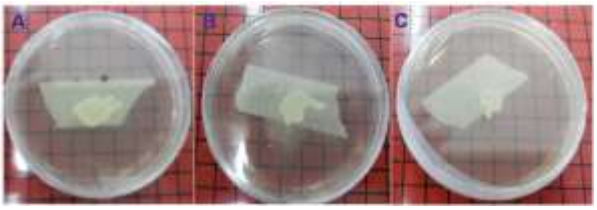

Fig. 1. the control sample of candida albicans in the medium without extract (A): isolate of candida albicans in 100 ppm concentration of eucalyptus essence, (B): isolate of candida albicans in 150 ppm of eucalyptus extract (C).

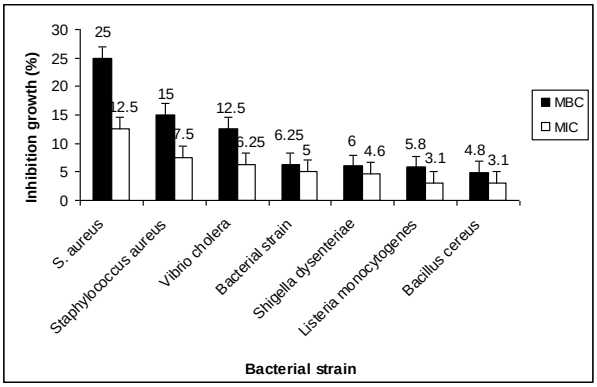

Table 1. The results of minimum inhibiting concentration and minimum bacterial concentration of eucalyptus extract against human pathogenic bacteria.

The growth inhibition effect assay showed that the sensitivity of 30 tested isolates varied from 50-150 ppm of eucalyptus extract. The mean of inhibitory effect of eucalyptus extract was calculated to be 120 ppm.

Although aggressive and systemic fungal diseases are hardly prevalent (2% of the patients), the mortality rate resulting from these infections have been reported to be 26-56 percent. Regarding to, resistance of fungi including different species of Candida to current fungicides, many scientists have been interested to develop new treatment with the least side effects on humans to disease control (Patterson *et al.*, 1996). In recent years, many human pathogenic fungi have been

resisted to antibiotics. So, many laboratories have attempted to find alternative drugs. Currently, medicinal plants are one the most appropriate alternative controlling factors. The provenance of medicinal plants using is traditional medicine that has resulted from experimental activities. So, based on these experiments, use of medicinal plants has open up new perspective in order to apply them in pharmaceutical industries for treatment of infectious disease

The present study, investigated the inhibitory effect of eucalyptus extract on *Candida albicans* isolates, as well as its comparative effect on inhibitory of some human pathogenic bacteria. The study of inhibitory effect of two essential oil of eucalyptus and lavender plant on the growth of clinical strains of *Candida albicans* isolated from animals infected by mucosal and cutaneous infections and comparing them with *C. albicans* resistant and sensitive to Itraconazole fungicide showed that the highest effect of Eucalyptus essential oil was at concentrations of 32 and 64 pm while, the highest effect for lavender essential oil was at concentrations of 8-16 ppm. Based on the obtained results of the present study and compared to effective concentrations, of plant extract, these values are lower than the ones for our data. This fact could be resulted from higher permeable nature of essential oil than extract.

Application of eucalyptus extract for male rats having diabetes led to reducing diabetes symptoms and indictors such as hyperglycemia and polyphagia as well as considerably reducing *Candida albicans* in the liquid around kidney and liver (Bokaeian et al., 2010). Uses of medical plants as synthetic medicines and determining their inhibitory effect has been investigated in various plants. The effect of plant extract of *Myrtus communis* as topical ointment application to inhibit the growth of *C. albicans* and comparing its effect with clotrimazole ointment shows that like clotrimazole, *M. communis* extract reduced itching in vaginal area of women having candidacies and this effect is similar to the effect of antibiotics (Genani *et al.*, 2011). Antibacterial effect of essential oil related to 26 species of eucalyptus species on six strains of gram-positive bacteria showed that all species were able to inhibit the growth of bacteria in concentrations of 15-250 ppm under experimental condition, but none of them had any effects on gram-negative bacteria (Takahashi, 1966). In the present study, all gram-positive bacteria showed sensitivity to eucalyptus extract. Seed oil of eucalyptus has fungicide effect on *Fusarium oxysporum* species wich produce canceous toxin in human (Kottearachchi *et al.*, 2012). These results confirm effectiveness of eucalyptus extract in inhibiting the growth of fungi. The results of our research showed that with increase of the extract, the inhibitory effect against fungi and bacteria was increased. The reason for this increase might be the change in effective material of plants due to alterations in plant culture condition and climate and this may consequently affect microorganisms differently. The inhibitory effect of essential oil of Eucalyptus plant on gram-positive bacteria S*taphylococcus aureus* and gram-negative bacteria E*scherichia coli* has been proved (Bachir and Benali, 2012). The result of Saeidi et al's study showed that Eucalyptus extract has considerable MIC in 5 mg/ml against S*taphylococcus aureus* , and the highest amount of MBC is observed in 10 mg/ml concentration (Saeeidi *et al.*, 2014). The minimum inhibitory concentrations of eucalyptus extract against:S. *aureus, B. cereus, E. faecalis, Alicyclobacillus acidoterrestri* and *Propionibacterium acne* were recorded as 3.9, 3.9, 7.8, 31, and 7.8 mg/ml, respectively (Takahashi *et al.*, 2004) which is in accordance with the results of the present study. Findings of another study showed that inhibition zone diameters of ethanolic extract of eucalyptus at concentrations of 5, 7.5, 10 and 20 µl/ml was observed 1.4, 1.9, and 3.2 mm against *staphylococcus aureus* and 1.3, 1.5, 2, and 2.4 mm inhibitory zone against E*scherichia coli* (Ghalem and Mohamed, 2008).

4. Conclusion

It should be noted that during recent years, several reports about resistance of a series of *C. albicans* species-to chemical fungicide have been released. Based on the obtained data in this study, the results indicate that the extract of eucalyptus plays an important role in inhibiting the growth of *C. albicans*

isolates and tested bacteria, and thus it can be used as an appropriate alternative for chemical anti-fungal medicine. Performing complementary tests with standard fungal isolates and various species of eucalyptus plants can be effective in introducing proper medicine in pharmaceutical industry.

5. References

Amber, K., Aijaz, A., Immaculata, X., Luqman, K., and Nikhat, M., 2010. Anticandidal effect of Ocimum sanctum essential oil and its synergy with fluconazole and ketoconazole. *Phytomedicine*. 17 (12): 921-925.

Bachir, R.G., and Benali, M., 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against Escherichia coli and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*. 2 (9): 739-742.

Baker, R.D., Angulo, O., Barroso-Tobila, C., Carbonell, L.M., Cespedes, R., Chick, E.W., Clark, B.M., Duque, O., Edington, G.M., and Fetter, B.F., 2012, The pathologic anatomy of mycoses: human infection with fungi, actinomycetes and algae, Springer Science & Business Media.

Biyela, P., Lin, J., and Bezuidenhout, C., 2004. The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. *Water Science & Technology*. 50 (1): 45-50.

Bokaeian, M., Nakhaee, A., Moodi, B., and Khazaei, H.A., 2010. Eucalyptus globulus (eucalyptus) treatment of candidiasis in normal and diabetic rats. *Iranian Biomedical Journal*. 14 (3): 121-129

Cates, W., Rolfs, R.T., and ARAL, S.O., 1990. Sexually transmitted diseases, pelvic inflammatory disease, and infertility: an epidemiologic update. *Epidemiologic Reviews*. 12 (1): 199-220.

Cermelli, C., Fabio, A., Fabio, G., and Quaglio, P., 2008. Effect of eucalyptus essential oil on respiratory bacteria and viruses. *Current Microbiology*. 56 (1): 89-92.

Consolaro, M.L., Albertoni, T.A., Yoshida, C.S., Mazucheli, J., Peralta, R.M., and Svidzinski, T.E., 2004. Correlation of Candida species and symptoms among patients with vulvovaginal candidiasis in Maringá, Paraná, Brazil. *Review Iberoam Micol*ogy 21(4): 202-205.

De Vos, M., Cuenca-Estrella, M., Boekhout, T., Theelen, B., Matthijs, N., Bauters, T., Nailis, H., Dhont, M., Rodriguez-Tudela, J., and Nelis, H., 2005. Vulvovaginal candidiasis in a Flemish patient population. *Clinical Microbiology and Infection*. 11(12): 1005-1011.

Genani, F., Akbari, S., Delfan, B., Tolabi, T., Ebrahim zadeh, and Motamedi, M., 2011. Coamparsion study of effect of Myrtus communis vaginal -cream and clotrmazol on treatment of vaginal candisiosis *Yafteh*. 13(1): 35-43.

Ghalem, B.R., and Mohamed, B., 2008. Antibacterial activity of leaf essential oils of Eucalyptusglobulusand Eucalyptus camaldulensis. *African Journal of Pharmacy and Pharmacology*. 2(10): 211-215.

Ghorbani, F., Panjehkeh, N., Nasrollanejad, S., Salari, M., and Sabagh, S., 2014. Inhibitory effect of essential oils of Eucalyptus sp. and Zataria multiflora on fungal growth of Macrophomina phaseolina. *Archives of Phytopathology and Plant Protection*. 47(19): 2410-2413.

Gilles, M., Zhao, J., An, M., and Agboola, S., 2010. Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. *Food Chemistry*. 119(2): 731-737.

Giraldo, P., von Nowaskonski, A., Gomes, F.A., Linhares, I., Neves, N.A., and Witkin, S.S., 2000. Vaginal colonization by Candida in asymptomatic women with and without a history of recurrent vulvovaginal candidiasis. *Obstetrics & Gynecology*. 95(3): 413-416.

Goswami, R., Dadhwal, V., Tejaswi, S., Datta, K., Paul, A., Haricharan, R., Banerjee, U., and Kochupillai, N., 2000. Species-specific prevalence of vaginal candidiasis among patients with diabetes mellitus and its relation to their glycaemic status. *Journal of Infection*. 41(2): 162-166.

Hammer, K.A., Carson, C., and Riley, T., 1999. Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*. 86(5): 985-990.

Hay, R., and Ashbee, H., 2010. Mycology. *Rook's Textbook of Dermatology, Eighth Edition*.1-93.

Hoehamer, C.F., Cummings, E.D., Hilliard, G.M., and Rogers, P.D., 2010. Changes in the proteome of Candida albicans in response to azole, polyene, and echinocandin antifungal agents. *Antimicrobial Agents and Chemotherapy*. 54: 1655-1664.

Kottearachchi, N.S., Sammani, A., Kelaniyangoda, D.B., and Samarasekara, R., 2012. Anti-fungal activity of essentialoils of Ceylon Eucalyptus species for the control of Fusarium solaniandSclerotiumrolfsii. *Phytopathology and Plant Protection*. 45(3): 2026-2035.

Levy, S.B., 1998. The challenge of antibiotic resistance. *Scientific American*. 278: 32-39.

Maciel, M., Morais, S., Bevilaqua, C., Silva, R., Barros, R., Sousa, R., Sousa, L., Brito, E., and Souza-Neto, M., 2010. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. *Veterinary Parasitology*. 167(1): 1-7.

Mathema, B., Cross, E., Dun, E., Park, S., Bedell, J., Slade, B., Williams, M., Riley, L., Chaturvedi, V., and Perlin, D.S., 2001. Prevalence of vaginal colonization by drug-resistant Candida species in college-age women with previous exposure to over-the-counter azole antifungals. *Clinical Infectious Diseases*. 33(5): e23-e27.

Merenstein, D., Hu, H., Wang, C., Hamilton, P., Blackmon, M., Chen, H., Calderone, R., and Li, D., 2013. Colonization by Candida species of the oral and vaginal mucosa in HIV-infected and noninfected women. *AIDS Research and Human Retroviruses*. 29(1): 30-34.

Moosa, M.-Y.S., Sobel, J.D., Elhalis, H., Du, W., and Akins, R.A., 2004. Fungicidal Activity of Fluconazole against Candida albicans in a Synthetic Vagina-Simulative Medium. *Antimicrobial Agents and Chemotherapy*. 48(1): 161-167.

Neo, H., 2011. Worlds of food: Place, power, and provenance in the food chain. *Asian Journal of Social Science*. 39(3): 409-410.

Noumi, E., Snoussi, M., Hajlaoui, H., Trabelsi, N., Ksouri, R., Valentin, E., and Bakhrouf, A., 2011. Chemical composition, antioxidant and antifungal potential of Melaleuca alternifolia (tea tree) and Eucalyptus globulus essential oils against oral Candida species. *Journal of Medicinal Plants Research*. 5(17): 4147-4156.

Otang, W.M., Grierson, D.S., and Ndip, R., 2012. Ethnobotanical survey of medicinal plants used in the management of opportunistic fungal infections in HIV/AIDS patients in the Amathole District of the Eastern Cape Province, South Africa. *Journal of Medicinal Plnat Research. 6 (11): 2071*. 2080.

Patterson, T.F., Revankar, S.G., Kirkpatrick, W.R., Dib, O., Fothergill, A.W., Redding, S.W., Sutton, D.A., and Rinaldi, M.G., 1996. Simple method for detecting fluconazole-resistant yeasts with chromogenic agar. *Journal of Clinical Microbiology*. 34(7): 1794-1797.

Pinto, R.J., Marques, P.A., Neto, C.P., Trindade, T., Daina, S., and Sadocco, P., 2009. Antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers. *Acta Biomaterialia*. 5(6): 2279-2289.

Saeedi, S., SABBAGH, S., and SABORI, R.E., 2012. A Study of antibacterial activity of plant extract and essential oil of Myrtus communis against resistant strains of *Staphyloccocus aureus* bacteria to selective antibiotics. Rostamineh. 4(1): 21-32.

Saeeidi, S., Sabbagh, S.K., and Bazi, S., 2014. Antibacterila Effect of Eucalyptus globules Againts Resistance Staphyloccocus aureus. 5(4): 50-58.

Schuster, M.G., Edwards, J.E., Sobel, J.D., Darouiche, R.O., Karchmer, A.W., Hadley, S., Slotman, G., Panzer, H., Biswas, P., and Rex, J.H., 2008. Empirical fluconazole versus placebo for intensive care unit patients: a randomized trial. *Annals of Internal Medicine*. 149(2): 83-90.

Takahashi, E., 1966. Effect of silicon on resistance of rice to radiation. *Jpn J Soil Sci Plant Nutr*. 37: 183-188.

Takahashi, T., Kokubo, R., and Sakaino, M., 2004. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from Eucalyptus maculata. *Letters in Applied Microbiology*. 39(1): 60-64.

Wilson, C., Solar, J., El Ghaouth, A., and Wisniewski, M., 1997. Rapid evaluation of plant extracts and

essential oils for antifungal activity against Botrytis cinerea. *Plant Disease*. 81(2): 204-210.