IJBPAS, April, 2014, 3(4): 490-500

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COMPARING THE ANTIFUNGAL EFFECTS OF FIVE ESSENTIAL OILS PLANTS EUCALYPTUS, CINNAMON, WORMWOOD, SAGEBRUSH, AND IRANIAN ROSA DAMASCENA ON THREE STANDARD STRAINS OF *CANDIDA ALBICANS* IN VITRO

DOUDI M[1*] SETORKI M[2] AND HOVEYDA L[1]

1: Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

2: Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

***Corresponding Author: E Mail: Monirdoudi@yahoo.com; Doudi@iaufala.ac.ir**

ABSTRACT

Dangers of using drugs and increasing acquired resistance of Candida species to antifungal drugs has led producing medicinal plants, especially essential oils, to become a good platform for research. The purpose of this study was to investigate the effects of applying essential oils of Eucalyptus, Cinnamon, Wormwood, Sagebrush and the Iranian Rosa damascena on three standard strains of *Candida albicans*.

The plants used in this experimental study were collected during the year 2011 from different regions of Iran. Then, the extracts were prepared by water distillation by Clevenger apparatus. Serial dilutions of each extract were prepared in 96 micro-titer plate. In each well of the plate, 200 ml sabouraud dextrose broth (SDB) medium, with 10 µl of cell suspension with a density of 1×10^6 cell per ml and various amounts 1, 5, 10, 15, 20, and 50 µl of produced essences were added. This test was performed in three repetitions. The diameter of inhibition zone for minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were measured and use from three species of *Candida albicans* (PTCC: 1167, 5027, 62061).

The inhibition zone diameter for fluconazole, for all three *Candida albicans* species relatively 35 mm; and for the essential oils of Cinnamon, Eucalyptus, Rose, Wormwood, and Sagebrush, the diameters were 30, 25, 21, 19, and 16 mm, respectively. The mean MIC of essential oils against three species of *Candida albicans* was between 15 and 420 µg/ml and the mean MFC was between 30 and 620 µg/ml. The strongest antifungal effect was of cinnamon and the weakest was of Wormwood.

In this study, the essential oils of Cinnamon, Eucalyptus, Iranian Rosa damascena, Sagebrush and Wormwood made appropriate antifungal activity compared with chemical drug Fluconazole, against standard strains of *Candida albicans*. So, these essential oils after completing studies, can be an excellent alternative for drugs to treat candidiasis and could be used in medical, pharmaceutical, veterinary, food, aroma therapy, cosmetic applications.

Keywords: Herbal Essences, Anti-fungal, *Candida albicans*, Fluconazole

INTRODUCTION

The history of herbal medicine in the East, specialists reproaching herbal medicine, surveys carried out on Iranian essential oils, and their effective biological activities **[1]** motivated us to conduct this study. Increase in fungal infections caused by pathogenic and opportunistic fungi, especially in patients with weakened immune systems has been one of the major causes of death from the early nineties, especially in hospitalized patients. Important opportunistic pathogens such as Candida albicans and other Candida species play an important role in this regard **[2, 3]**. However, it is worth noting that Candida species were the fourth factor of bloodstream infections in hospitalized patients, responsible for approximately 40% of mortality rate **[4]**.

Systemic infections caused by Candida in patients with AIDS have increased. These types of infections are treated with antifungal azoles, especially Fluconazole. Prolonged and repeated treatment with these drugs caused the emergence of Fluconazole-resistant isolates among Candida species. On the other hand, conventional antifungal therapies using conventional antifungal drugs are not completely effective; and this is due to the numerous side effects and drug resistance pathogenic agents after long-term use of the drugs. On the other hand, due to the appearance of the undesirable side effects of synthetic compounds, lack of compatibility with human nature, the high cost of drugs, and the inability of many Third-World countries to buy such drugs, particular attention has been made to the provision of safe and inexpensive drugs from plants with proven therapeutic effect **[5]**.

Using herbal medication together or with modern medicine to reduce the side effects of the drug has been well received as combination for treatment [6]. The use of antifungal drugs in combination with herbal extracts can prevent or delay the development of resistance to antifungal agents against antifungal drugs. In addition, using combined amounts of antifungal drugs reduces the toxicity of high doses and single dose of each compound. Reports on the use of plants such as Garlic, Onions, Eucalyptus, Bitter olive, Balsam, Cedar, Thyme, etc. in the treatment of fungal diseases exist. Therefore, the use of these compounds with fewer side effects and lower costs than antifungal drugs are considered **[7-10]**.

Considering the above information and several compounds found in essential oils such as Eucalyptus, Cinnamon, Wormwood, Sagebrush, and Iranian Rosa damascena, the present study was conducted to examine the treatment effects of anti-fungal properties of these essential oils.

METHODS

Preparation of Plant Materials and Essential Oils

Medicinal plants used in this study were collected from different regions of Iran, and dried correctly. Eucalyptus, with its Latin name Red Gum, is a plant from Myrtaceae family, a fast growing large tree of Oceania, and has spread to different parts of the world. Vast forests have been created of it in India and Morocco. Eucalyptus camaldulensis species, with the synonym Eucalyptus rostrata, is a tree that blooms in northern Iran in early August. It has lemon color flowers and its seeds grow early in winter. In this study, essential oil has been extracted from its leaves **[11]**.

The Cinnamomum zeylanicum is only the part from which cinnamon essence is produced from it. The English name is Cinnamon which is derived from the Arabic word Kinnamon. Its essence if is fresh has a yellowish bright color. Nevertheless, over time due to oxidation it turns to golden yellow and eventually to reddish brown. This species is available in northern of Iran **[11]**.

Iranian Artemisia plant has the scientific name of Artemisia Persica. It is from the Anthemideae tribe and Compositae (Asteraceae) family. The plant reaches a height of 60 cm and its leaves have multiple divisions, yellow flowers, and inflorescences of the Capitol **[11]**. The plant was obtained during blooming in early July 2011 in Yazd province in central Iran.

Sagebrush with the scientific name Aniethum graveolens is from Umbellifera family, and lasts for one to two years. It sometimes reaches a height of one meter. It has a straight cone-shaped root and is white. This plant in most places of the world, including parts of southern Europe, America and China have been isolated. In this study, the essential oil of aerial branches was used that contain 28%-

45% ketone compounds as Karoon. But depending on the harvesting season it is different. Sampling from the main branches of this plant was performed in early July 2011 in Isfahan in central Iran **[11]**.

Iranian rose, with the scientific name Rosa damasena, has a furry bush (Decideous) and rarely is evergreen. Rosa has 250 species, which its strongest is rose. From the early sixteenth century, rose was distributed from Asia, more likely from Damascus to Europe. These shrubs produce highly fragrant flowers that have medicinal properties. The flowers bloom in early May to early July. Sampling from this flower was performed in early July 2011 in Kashan located in Isfahan province in central Iran.[11] Then to detect the correct genus and species of these plants, they were approved in Herbarium laboratory of Islamic Azad University, Falavarjan branch, and by a postgraduate of plants systematics and ecology via scientific approach, the genus and species were detected and identified. These plants were selected based on information obtained from traditional medicine, Prophetic Medicine and considering their growing areas **[11,12]**.

After grinding the plants, their essential oils were prepared using Clevenger machine and water distillation (Hydro distillation); then, dehydrated with anhydrous sodium sulfate and collected in brown vial with 10 ml volume. After sealing vial caps, they were kept in refrigerator with noting the specifications of the essence until being used.

Preparation of Standard Solution (Stock) from Essential Oils

In this study, 25 g of leaves, flowers, or bark of the plants were naturally dried and grinded. The powder obtained was poured in a one-liter flask. Then, 300 ml of sterile distilled water was added. Using a Clevenger machine, essence was extracted. Produced essences were mixed with one to one ratio in Tyrode buffer containing salts KCl and NaCl, HEPES, NaH_2PO_4, H_2O, and glucose and were sterilized by 0.4 micrometer filtering. Until use, they were kept in refrigerator with a temperature of 4°C.

To determine the concentration of each extract tested in this study, they were separately poured into pre-weighed containers with amounts of 1 ml, and after 24 hours of incubation and drying with temperature of 50°C, their weight were again measured. The mean of three times of repeating the experiments was considered as the dry weight of the essences and their concentrations were calculated per ml **[11]**.

Yeast Suspension for Inoculation

First, 3 standard fungus *Candida albicans* strains, *Candida albicans PTCC 1167,* *Candida albicans PTCC 5027, and Candida albicans PTCC 62061*, were used from the Iranian Research Organization for Science and Technology (center for collection of fungi and bacteria) **[13]**.

Then, they were cultured on Sabourau Dextrose Agar (SDA,Merc) containing cholramphenicol antibiotics, and it was kept for 24- 48 hours in incubator with 30-35°C.

After the incubation period, and the growth of Candida yeast, the cell suspension was prepared and poured in a microtube of 1 ml sterile Buffered Saline Phosphate (PBS: pH = 7.2). Then, with a sterile loop, a small amount of grown colonies on SDA in PBS was suspended. After mixing the yeast in the buffer, using Neubauer lam, the number of yeast cells was counted under a light microscope, and a cell suspension with a density of 1×10^6 cell per ml was prepared **[14]**.

Determining the Susceptibilities of *Candida albicans* Compared to Herbal Essences and Fluconazole (Disk Diffusion Method)

To evaluate the susceptibility of yeasts to Fluconazole and essential oils, first the standard strains of *Candida albicans* in the culture of SDA was cultured via pour plate. In this method, after the preparation of plates containing culture medium, with temperature of 45°C, 500 microliters of the prepared suspension with density of 1×10^6 cell per ml was inoculated into the plates. After 15-20 minutes, amount of 1.2 of the minimum inhibitory concentrations (MIC) of the tested essential oils was added to the blank discs and Fluconazole disk was placed on a plate containing SDA. After 24- 48 hours of incubation at 30-35°C, zone of growth inhibition compared to control (sick without oil) was measured.

MIC and Minimum Fungicidal Concentration (MFC) Determination Via Dilution Method in Liquid Medium

In this experiment, a 96 micro-titer plate was used. In each well of the plate, 200 ml sabouraud dextrose broth (SDB) medium, with 10 µl of cell suspension with a density of 1×10^6 cell per ml and various amounts 1, 5, 10, 15, 20, and 50 µl of produced essences were added. This test was performed in three repetitions. Plates with 96 titers were placed for 24- 48 hours in 30-35°C shaking incubator with speed of 150 rpm **[15, 16]**. After the incubation period, wells opacity using colony counts and yeast growth or no growth was assessed. Well with minimum inhibitory concentration of yeast was regarded as MIC of that essence.

To evaluate and determine the minimum fungicid concentration (MFC), all the contents of the wells was cultured on the SDA medium. Thus, 10 ml of the solution in the wells was removed by sampler, transferred to solid SDA plates containing chloramphenicol, and incubated for 24- 48 hours at 30-35°C. The highest dilution of the extract, which lacked growth of *Candida albicans* on SDA medium, was regarded as MFC of that essence. All experiments were repeated three times.

Data Analysis

The statistical calculations were performed using t-test.

RESULTS

Concentration and dry weight of the essential oil of Cinnamon, Eucalyptus, Rose, Wormwood, and Sagebrush were 30, 27, 25, 23, 20 mg/m, respectively.

The measurements of inhibition zone diameter showed that for Fluconazole, the diameter for all three *Candida albicans* species of the study was relatively 35 mm; and for the essential oils of Cinnamon, Eucalyptus, Rose, Wormwood, and Sagebrush, the diameters were 30, 25, 21, 19, and 16 mm, respectively **(Figure 1)**.

The MIC and MFC values of essential oils and Fluconazole are seen in **Table 1**.

The t-test showed that results of mean MIC and MFC of essential oils of cinnamon, eucalyptus, rose, sagebrush and some what wormwood showed significant differences ($P < 0.05$) compared with fluconazole on three standard strains of *Candida albicans*(PTCC: 1167, 5027, 62061)

DISCUSSION

Due to the use of herbal treatment for many diseases, the medicine approach towards this area, the harmful effects of drugs on human body, and effects becoming clearer every day, the results of this study and other similar studies would implicate a variety of effects, including germicidal properties of these plants.

This study showed that Cinnamon essential oil at concentration of 15 µg/ml, had the most inhibitory effect on the growth of different strains of standard *Candida albicans* fungi, and at concentration of 30 µg/ml, it had cytotoxic effect (MFC) on three strains of *Candida albicans*. The results were consistent with Lopez et al. Study in Spain that was performed via disk method on Candida fungus **[17]** and Rukayadi et al. Study in Korea using Cinnamon extract on six species of Candida with micro dilution method **[18]**.

Cinnamon plant contains chemicals such as amidon, aldehyde; mucilage, calcium oxalate, cinnamic aldehyde, cinnamomin, manit, and 4% of phenols specially eugenol with safrole, phellandrene. The phenol available in the essence has antibacterial, antiviral, anti-parasitic and anti-fungal effects **[11]**.

Therefore, it can be inferred that the powerful properties of essential oil of this plant against many other fungi, especially the three strains of *Candida albicans* in this study, could be related to the above-mentioned compounds.

In this study, it was found that eucalyptus essential oil in a dilution of 70 µg/ml had inhibitory effect on fungi (MIC) and in the dilution of 142 µg/ml had fungicide effect. This result was consistent with the results of Wilkinson et al. Study in Australia with agar disk diffusion method **[19]**.

The chemical composition of Eucalyptus essential oil are valeric aldehyde, butyric aldehyde, straight pin, camphene, terpinic alcohol named pinocarveol, small amount of ethyl alcohol, amyl acetate, a crystallizable substance called edesmol, that is found in most species of eucalyptus, and 60-80 percent of the essence is related to cineol with formula $C_{10}H_{18}O$, with the names eucalyptol and cajeputol which are the main ingredient in eucalyptus essential oil **[11]**. It seems that many of the chemical compositions of the essential oil of this plant has phenolic and phenolic-like compounds with antifungal properties especially against the three standard *Candida albicans* used in this study.

Our results showed that Iranian Rosa damascena in a dilution of 130 µg/ml had inhibitory effect (MIC) on the three fungus of the study and at dilution of 260 µg/ml, it had fungicide effect (MFC). This result was consistent with Aynehchi and Salehi-Sormaghi study on Iranian plants and antimicrobial activity (anti-fungal, anti-parasitic, anti-bacterial and anti-viral) **[3]**.

Iranian Rosa damascena essential oil contains citronellol (34-55%), geraniol (30-40%), strapunin (22-26%), and phenyl ethyl alcohol (3-5%) **[11]**. It can be concluded that if phenol is used in the formulation of chemical compounds, it can be a very important factor for the antifungal activity, especially against the three standard *Candida albicans* in this study.

In this study, it was found that the essential oil of Artemisia at a dilution of 312 µg/ml had inhibitory effect (MIC) on the three fungus of the study and at dilution of 520 µg/ml, it had fungicide effect (MFC). So far, there have been numerous studies on the antimicrobial properties of this essential oil **[7-10]**. The results of the antimicrobial effects of Artemisia hauss kenechtii showed that the essence of this plant prevents the proliferation of pathogenic microorganisms **[7]**. Even in some cases, its antimicrobial activity is more that some of the antibiotics such as nalidixic acid, gentamicin, cephalothin, amoxicillin, and chloramphenicol. The main components of this essential oil are cineol, camphor, Artemisia ketone and borneol. Many of these compounds have antimicrobial properties **[11]**.

The compounds of the Iranian Artemisia persica essential oil showed are scopodrinal, scopofarnol, cymene, sabinene, cineol, linalool, eugenol, borneol, farnesol, ester, alcohol, and etc. that have antibacterial, anti-fungal, anti-viral, anti-parasitic and anti-cancer effects **[7]**. Also studying the components of Artemisia sieberi have showed that this essential oil has main compounds such as camphor, limonene, cineol, camphene and pinene, many of them have antimicrobial properties **[11]**.

Investigating the chemical constituents of tarragon essential oil (*Artemisia dracumculus*) showed that antibacterial (*Klebsiella pneumoniea*), antifungal and antiseptic effects are according to compounds such as borneol, cymene, cineol, and camphor **[20, 21]**.The effects of Iranian Artemisia compounds in this study can be attributed to a part of combinations of this essence.

The results of this study showed that Wormwood essence at a dilution of 420 µg/ml had inhibitory effect (MIC) on three standard strains of *Candida albicans* and at dilution of 620 µg/ml, it had fungicide effect (MFC) on three standard strains of *Candida albicans*. According to the statistical calculation t-test method it showed that there was no significant differences compared with fluconazole ($P > 0.05$), and it cannot be scientifically interpreted.

CONCLUSION

According to antifungal effects of the five essential oils in the present study, and admissibility of using these plants in our society, not only they are useful for the production of new herbal antibiotics with minimal side effects, they can also help to promote health whether in healthy subjects or in patients with immunodeficiency.

ACKNOWLEDGMENT

Appreciation goes to Mrs. Maryam Valivand, a postgraduate of plants systematics and ecology and the authority of Herbarium Laboratory of Islamic Azad University, Falavarjan branch, and Mrs. Shadi Shahsar, the specialist in university research laboratory, who assisted in the performance of this research.

REFERENCES

[1] Alitonou GA, Avlessi F, Sohounhloue DK, Agnaniet H, Bessiere JM, Menut C, Investigations on the essential oil of *Cymbopogon giganteus* from Benin for its potential use as an anti-infalmmatory agent, Int. J. Aromather., 16, 2006, 37-41.

[2] Anaissie EJ, Ginnis MR, Pfaller MA, Clinical Mycology, United Kingdom, 2003, 195-196.

[3] Aynehchi Y, Salehi Sormaghi MH, Screening of Iranian Plants for Antimicrobial Activity, Acta Pharm. Suec., 17, 1980, 341-346.

[4] Bannerman RH, Burton J, Wen C, Traditional Medicine and health Care Coverage, England MAC Millan/Spoottis Wood., 1983, 9-17.

[5] Bently R and Triman H, Medicinea Plants. Indian (Eehli). Reptint I Davendra Gahlot, 3, 1981, 172.

[6] Beth DS, Robert G. Basic and Clinical Biostatistics. Translated by Sarafraz AA, Ghaffar-zadegan K. Ferdowsi University press, Mashhad, Iran., 1997.

[7] Brodin K, Alahyar H, Hedner T, Sterner O, Faergemann J, Invitro activity of *Artemisia abrotanum* extract against *Malassezia Spp*. *Candida albicans* and *Staphylococcus aureus*, Acta dermato Venereologica Sweden., 87 (6), 2007, 540-542.

[8] Carta C, Moretti MDL. And peana AT, Activity of the oil *Salvina officinali L*. against *Botrytis Cinerea*. J Essencial oil Res., 8, 1996, 399-440.

[9] Kantarcioglu AS, Yucel A. The presence of fluconazole-resistant *candida dubliniensis* strain among *candida albicans* isolated from immunocompromised or otherwise debilitated HIV negative Turkish patients. *Rev Iberoam Mycol* 2002; 19: 44-48.

[10] Kim JH. Anti-Bacterial action of onion (Alliumcepa) extracts against oral pathogenic bacteria. *J Nihon Univ Sch Dent* 1997; 39: 136-41.

[11] Shams-Ghahfarokhi M, Shokoohamiri MR, Amirrajab N, Moghadasi B, Ghajarh A, Zeini F, Sadeghi G and Razzaghi-Abyaneh M, In vitro antifungal activities of *Allium cepa*, *Allim sativum* and ketoconazole against some pathogenic yeasts and dermatophytes, Fitoterapia., 77, 2006, 321-323.

[12] NCCLS document M27-A, Reference method for broth dilution antifungal susceptibility testing of yeasts, Approved, 17, 1977, 1-29.

[13] http://ptcc.irost.org

[14] NCCLS document M38-P, Reference method for broth dilution antifungal susceptibility testing of condition-forming filamentous fungi, Approved standard, 22, 1998, 1-29.

[15] Royo P, Martin-Casabona N, Martinez E, Andonegui M, In vitro susceptibility of *Mycobacterium kansaii* to the difluorinated quinolone of spoufloxacin Using a broth microdilution and macrodilution MIC system, Int J Tuberc Lung Dis., 3(4), 1999, 349-353.

[16] Takahama U, Hirota S, Deglucosilation of quercetin glucosides to the aglycone and formation of antifungal agents by peroxidase-dependent oxidation of quercetin on browning of onion scales, Plant Cell Physiol., 41, 2000, 1021–1029.

[17] Lopez P, Sanchez C, Batlle R, Nerin C, Solid and Vaporphase antimicrobial activites of Six essential oils, susceptibility of selected food borne bacterial and fungal strains, J Agric food chem., 53(17), 2005, 46-69.

[18] Rukayadi Y, Yong D, Hwangy J, In vitro anticandidal activity of xanthorrhizol isolated from curuma xanthorriza roxb, Antimicrob chemother, 57(6),2006, 237-234.

[19] Wilkinson JM, Cavanagh MM. Antibacterial activity of essential oils from Australian native plants, Phytother., 19 (7), 2005, 43-46.

[20] Yin M, Cheng W, Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and species, J Food Protect., 61, 1998, 123-5.

[21] Zaini F, Mahbod ASA, Emami M, Comprehensive Medical Mycology, First edition, Tehran., 1377, 330-348.

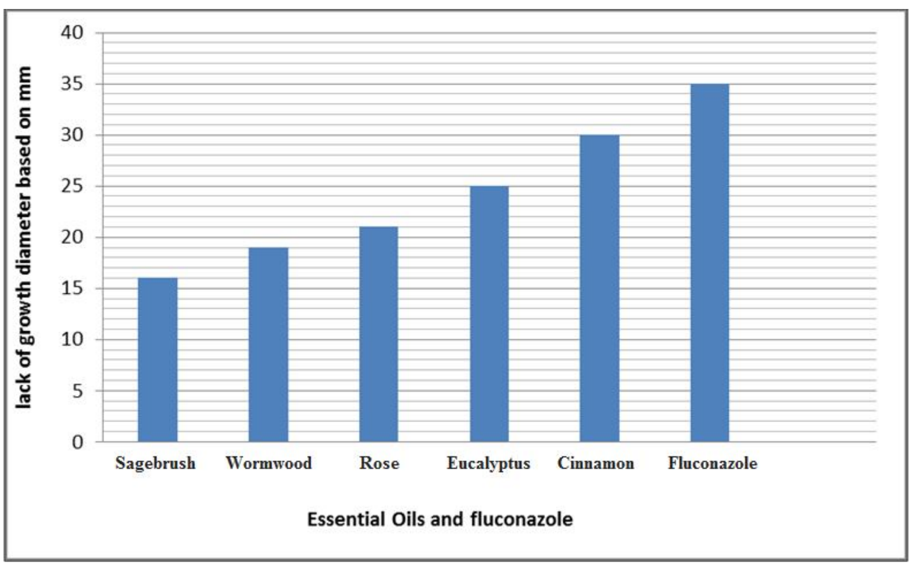

Figure 1: The Mean Diameter of Inhibition Zone of Essential Oils on Three Standard *Candida albicans* (PTCC: 1167, 5027, 62061) Base on Millimeters

Table 1: Results of the Mean Values of MIC and MFC Essential Oils and Fluconazole on Three Standard *Candida albicans* (PTCC: 1167, 5027, 62061)

Essence name	MIC (μg/ml)	MFC (μg/ml)
Cinnamon	15	30
Eucalyptus	70	142
Damask rose	130	260
Artemisia	312	520
Wormwood	420	620
Fluconazole	1	2

MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration