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# ADMIRABLE *IN VITRO* ANTIBACTERIAL AND ANTI-*CANDIDA* ACTIVITY OF PREFERRED NATIVE ESSENTIAL OILS OF SAUDI ARABIA

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## ABSTRACT

Four essential oils (from Lavender, Basil, Rosemary and Turmeric) native to Saudi Arabia have been selected to test the antimicrobial activity. Antimicrobial activity was revealed using standard pathogenic (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) bacteria and fungus (*Candida albicans)*. Lavender, Basil and Rosemary (native to the Albaha province) showed very strong antibacterial activity against all tested strains at both full strength and reduced concentrations. These essential oils showed different bactericidal and fungicidal activities when tested by direct application. These results suggest that all the tested essential oils might be used as broad-spectrum anti-microbial agents for decontaminating an indoor environment. Furthermore; in the present study a large variability in the potential antimicrobial

activity has also been observed. It has been demonstrated that the Lavender, Basil and Rosemary exhibited exceptional antibacterial and anti-candida properties. However; least sensitivity of strains was found towards the turmeric oil. Moreover; the higher susceptibility of Gram-positive bacteria when compared with Gram-negative strains was found.

**Keywords: Antibacterial; Anti-*Candida*; Essential oils; Clinical strains; Saudi Arabia**

## INTRODUCTION

Ever growing awareness of consumers about the negative effects of synthetic preservatives has resulted interest in essential oils and their multifaceted applications such as in food preservation and as antimicrobial agents against resistant bacteria, fungi and viruses etc. Essential oils attained from plants are aromatic in nature due to multiple compounds originating from diverse chemical families, such as aldehydes, ketones, terpenes, alcohols, esters, phenolic as well as ethers etc (Galvão et al. 2012; Swamy and Sinniah 2016). The essential oils have remarkable potential due to their distinctive flavor, aroma as well as marvelous biological activities. Nevertheless the essential oils have been applied in aromatherapy and for the cure of a number of serious ailments such as diabetes, cardiovascular problems, Alzheimer's disease, and even for cancer (Said et al. 2016) treatment. Furthermore; long-ago the antimicrobial properties of essential oils as well as their constituents/or compounds have been documented by numerous scientists (Duschatzky et al. 2005; Galvão et al. 2012). In addition, various studies have even clearly demonstrated the synergistic impact of two or more components of essential oils against a variety of environmental microbes as well as human pathogens (Nazzaro et al. 2013). Medicinal herbs as well as their essential oils (EOs), have been utilized since the commencement of human history for flavored foods, beverages; and to manage fitness related issues, therefore signifying the cultural and fiscal significance (Bauer et al. 2008). The EOs can be synthesized by different

parts of the plant; for instance; flowers; buds, flowers, leaves, stems, branches, seeds, roots, epidermal cells or trichomes etc (Bakkali et al. 2008). Temporal and spatial difference in total content of secondary metabolites of plants take place at special points ensuing due to physicochemical, ecological as well as evolutionary communications (Gobbo-Neto and Lopes 2007). For the industrial purpose, the EOs are usually extracted by steam distillation (Mazutti et al. 2006). These EOs have quite a lot outstanding biological characteristics; for instance; these oils possess larvicidal activities (Govindarajan 2010), antioxidant properties (Wannes et al. 2010), analgesic and anti-inflammatory actions (Mendes et al. 2010), fungicide (Carmo et al. 2008) and antitumor activities (Bou et al. 2013) etc.

Taking into account the versatile properties of EOs; this study was aimed to screen the preferred essential oils which are native to Saudi Arabia especially the Albaha province. Herein we selected four essential oils such as Lavender, Basil, Rosemary and Turmeric to check the *in vitro* antibacterial and antifungal activity using pathogenic and environmental bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa).*We have selected *Candida albicans* as model fungus because. *C. albicans* is an opportunistic pathogen, frequently accountable for most of the yeast diseases in human beings. The antimicrobial activity revealed that Lavender, Basil and Rosemary possess very strong antibacterial activity against all tested strains at both full strength and reduced concentrations. Currently, increased multidrug resistance has led to the severity of diseases owing to pathogens (Raut and Karuppayil 2014). This has provoked the biological scientists to look at the substitutes which are effective against resistant bacterial and fungal strains(Galvão et al. 2012). In this regard, plant essential oils and their major chemical constituents are potential candidates.

## MATERIALS AND METHODS

### Essential oils

Pristine plant essential oils were procured from Diar Almadina Company

in Jeddah, Saudi Arabia and were evaluated against the common human and environmental pathogens. Four concentrations (25, 50, 75 and 100 µl/tube) of four oils in order to check the MIC of different oils against different strains has been used as described earlier elsewhere(Amna et al. 2014).

**Strains**

The bacterial and fungal pathogenic strains were the kind gift from King Fahad Hospital-Al-Baha, Saudi Arabia.

**Culture medium**

The Mueller Hinton agar (MHA) medium used for antibacterial assay was purchased from Micro-media, Medical Disp. Manufacturing, Kingdom of Saudi Arabia. The potato Dextrose agar (PDA) medium used for antifungal assay was purchased from Pharmacopeia, Europe.

**Screening of preferred native Saudi essential oils for antibacterial activity**

The bacterial strains from the American Type Culture Collection (ATCC) standard strains (*E. coli ATCC 25922*, *S. aureus ATCC 25923* and *P. aeruginosa ATCC 27853*), as well as fungus (*C. albicans ATCC* 10231), were selected from strains stored at −80°C in the Department of Microbiology. Prior to make use of, the lyophilized strains were revived on the agar medium to check viability and purity of the selected strains. Furthermore the revived axenic cultures were maintained on nutrient agar and potato dextrose agar for use in bacterial as well as fungal susceptibility tests respectively. The antibacterial activity of EOs has been assayed in Mueller Hinton broth as described elsewhere (Amna et al. 2014). The aforementioned pathogenic strains were grown ($10^6$ CFU) with specific quantity (twofold dilution) of selected EOs in order to verify the minimum inhibitory concentration (MIC) of the native Saudi EOs. The experimental concentrations applied herein were as such 25, 50, 75, and 100 µl/tube respectively. The kinetics was studied at 37 °C with rpm of 150, maintaining a regular time interval (4 h) by examination of OD with spectrophotometer. The alteration in the absorbance was calculated at 600 nm by Ultra violet (UV) spectrophotometer.

The *Candida albicans ATCC 10231* was cultured and maintained in

the potato dextrose agar. Briefly, after spreading the plates with representative fungus, the inoculated plates were kept in incubator for 30 minutes for incubation. For the fungal culture the growth was monitored after 72 h incubation period. The experiments were executed in set of three.

**RESULTS AND DISCUSSION**

The *in vitro* antibacterial as well as anti-*Candida* activities of chosen native oils (Lavender, Basil, Rosemary and Turmeric oils) have been assessed against three bacteria among which one is Gram-positive and two Gram-negative bacteria utilizing MIC method as demonstrated in Figures 1-3 of this manuscript. The anti-*Candida* and antibacterial action was confirmed taking four diverse strengths of oils as aforementioned in this (50, 100, 150 and 200µl/tube). The results of this study suggested that Lavender, Basil and Rosemary showed very strong antibacterial activity against all tested strains at both full strength and reduced concentrations. The observed MIC of Lavender, Basil and Rosemary oils was 25 µl for Gram positive strains (*S. aureus*) whereas for Gram negative strains (*E. coli* and *P. aeruginosa*) it was found to be 25 and 50µl. The susceptibility of Gram positive strain *Staphylococcus aureus* towards the native oils was in the following order: Lavender > Rosemary > Basil > turmeric oil. The paramount of bacteriostatic efficiency was revealed by Lavender oil and least effect was seen in case of turmeric oil. In case of Gram negative strain, *Escherichia coli the* susceptibility towards selected oils was in the following order: Lavender > Rosemary > Basil > turmeric oil. However; the susceptibility order in case of *Pseudomonas aeruginosa* was in such a manner that Lavender ≥ Rosemary > Turmeric oil > Basil. The *Pseudomonas aeruginosa* depicted least susceptibility towards Basil oil. The difference in the sensitivity of strains towards the selected oils is attributed to the difference in the structure of cell membrane(Dorman and Deans 2000; Stefanakis et al. 2013). The antibacterial properties are due to the chemical and volatile constituents harbored in the EOs. Earlier reports

have also reported the antimicrobial action of EOs against diverse bacteria (*E. coli, L. monocytogenes, S. enterica, C. jejuni, S. enteritidis, and S. aureus* etc) (Ouhayoun 2003). Thus; our study is consistent with the earlier findings. Furthermore; in this study we evaluated the native oils for the anti-*candida* activity. *C. albicans* is significant organism causes majority of infections. The EOs derived from aromatic plants, are well-known for their broad spectrum activities, counting antifungal characteristics as well. Interestingly in this study all the native oils depicted excellent anti-*candida* action. The anti-*candida* activity was observed in the following order Lavender ≥ Rosemary > Basil ≥ turmeric oil. The results of this study are similar to the reported reports. Conclusively, it has been observed that the Lavender oil possesses excellent antibacterial as well as anti-*candida* activity. However, the least antimicrobial action was depicted by turmeric oil. In case of fungi the Basil and turmeric oil depicted similar anti-*candida* activity. Commonly, the biological activities of the EOs are indomitable to their key compounds (Bowles 2003; Pichersky et al. 2006). Generally the terpenes and terpenoids were found the major compounds while aromatic and aliphatic compounds have also found (Bassolé and Juliani 2012). In the present study it was also noted that the *S. aureus* is more sensitive to the tested oils as compared to the other tested strains. The more sensitivity of *S. aureus* has been credited to the difference in the cell organization of the strains. Typically the permeability, composition and ionization pattern on the outer configuration of the microorganisms are essentially responsible for differences in their sensitivity (Paduch et al. 2007).

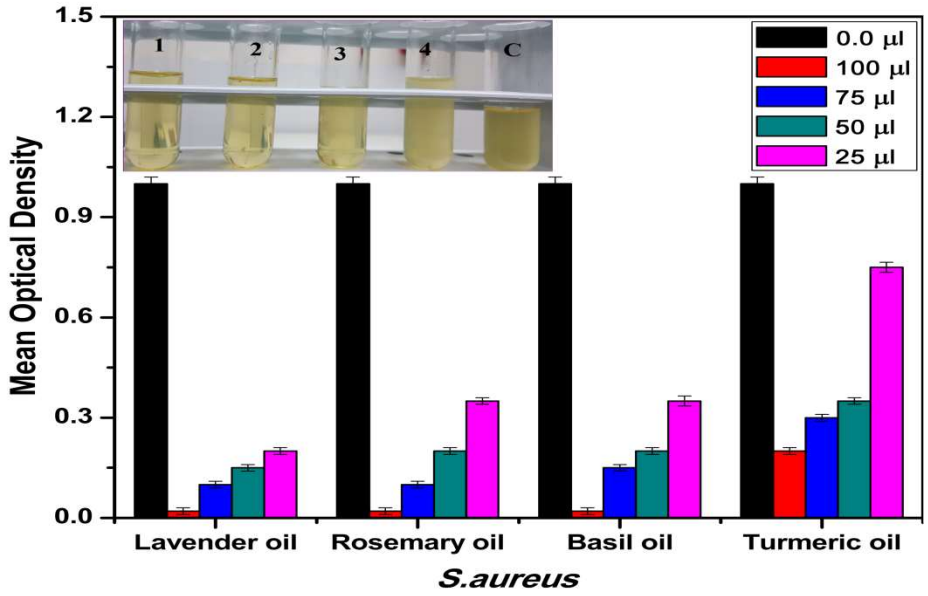


**Figure 1: Bar graph illustration of culture broth of *S. aureus* exponentially inoculated and treated with different concentrations (0, 25, 50, 75, 100µl) of Lavender, Basil, Rosemary and Turmeric EOs.**

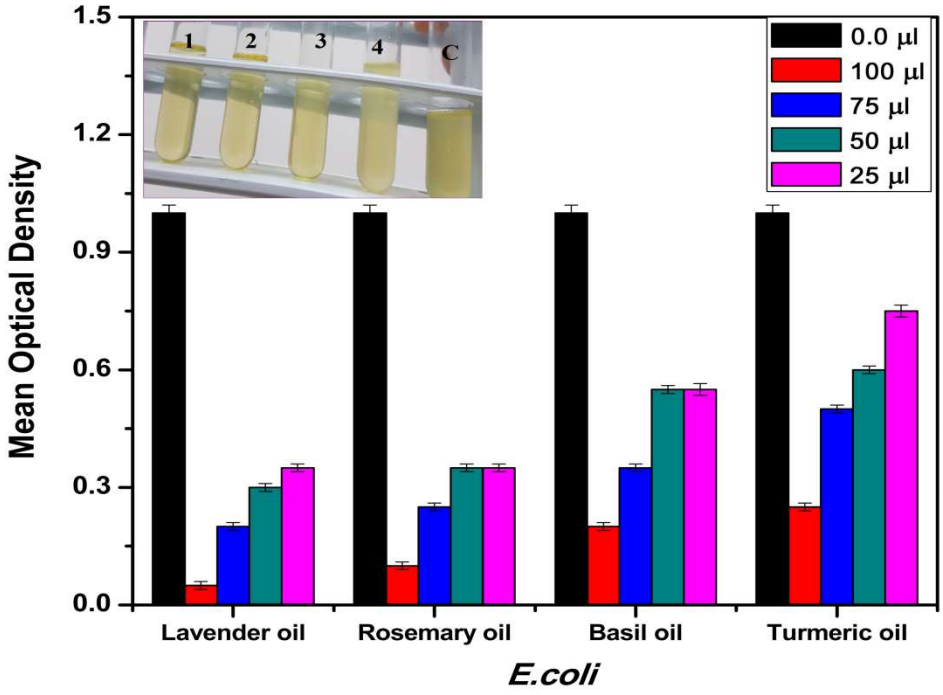


**Figure 2: Bar graph illustration of culture broth of *E. coli* exponentially inoculated and treated with different concentrations (0, 25, 50, 75, 100µl) of Lavender, Basil, Rosemary and Turmeric EOs**

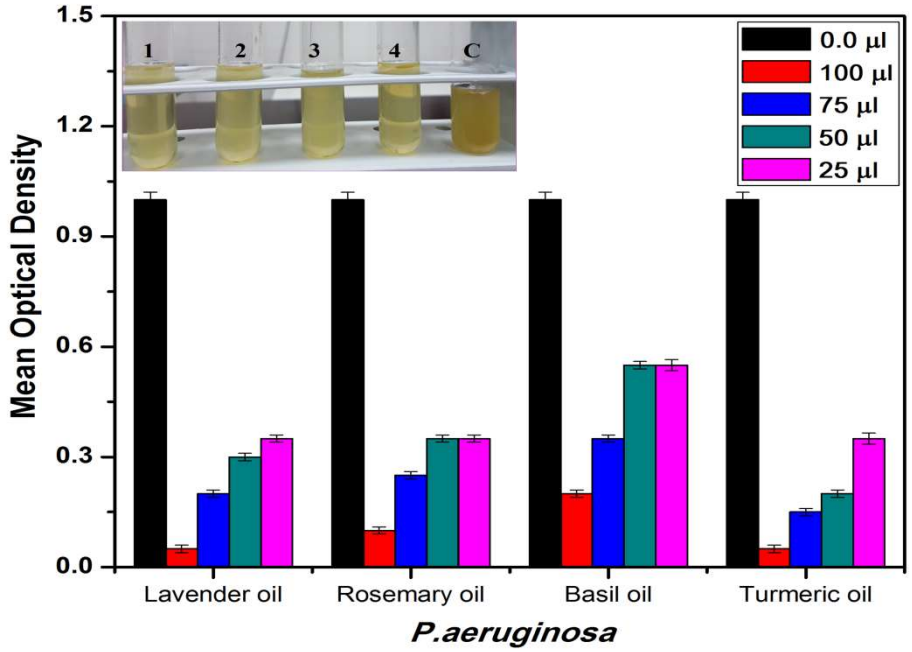


**Figure 3: Bar graph illustration of culture broth of *P. aeruginosa* exponentially inoculated and treated with different concentrations (0, 25, 50, 75, 100µl) of Lavender, Basil, Rosemary and Turmeric EOs**

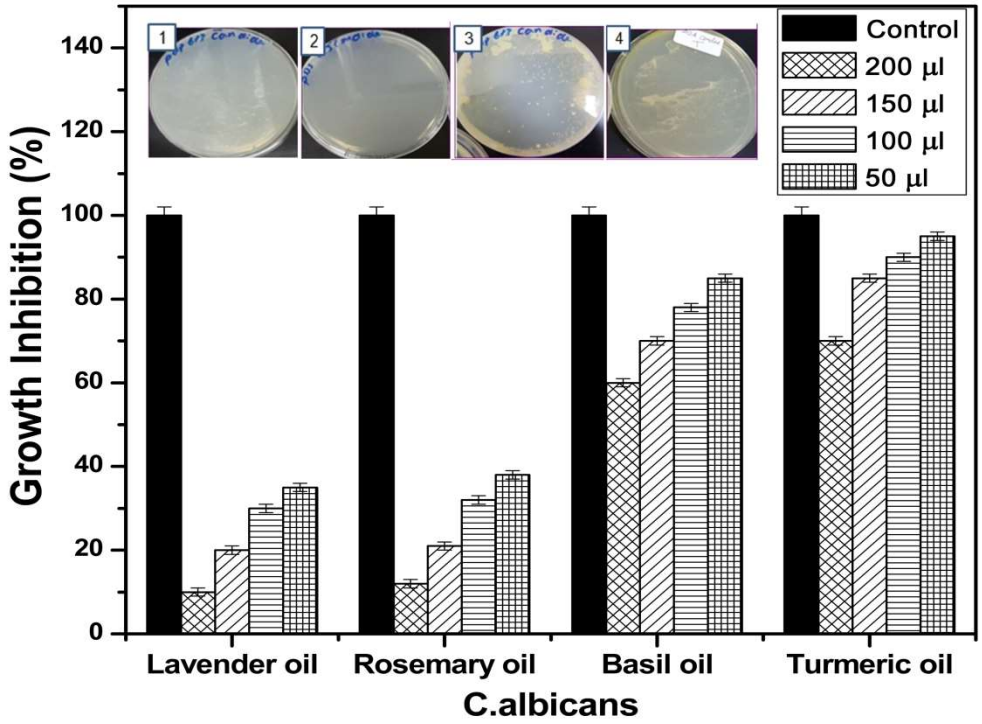


**Figure 4: *In vitro* antifungal activity of Lavender, Basil, Rosemary and Turmeric EOs against *C. albicans* treated with different concentrations (0, 50, 100, 150, 200µl) of Lavender, Basil, Rosemary and Turmeric EOs. The fungal enlargement was expressed as percent of control (experimentally 100% growth, is fungal enlargement in potato dextrose medium without the supplementation of aforementioned EOs)**

## CONCLUSION

In current times the antimicrobial activity, mechanism and prospective exploitation of EOs has received huge eminence. In EOs, the natural phytochemicals are gifted with a variety of useful applications. These EOs have been utilized from ancient times as traditional medicine for the treatment of many diseases worldwide. It has been ruled out with evidence that there are negligible/or no side-effects of EOs when compared to the analogous synthetic drugs. With the alarming increase in human infectious diseases; there is enormous demand of natural medicines (which is effective and safe), to treat threatening fungal and bacterial pathogens. This study provides useful information about the antimicrobial activities of native EOs of Saudi Arabia. Therefore; the results of this investigation recommend the use of native EOs to control various human and environmental infections; but only after accurate *in vitro* trials.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Ethical Statement

There is no use of Animal models for the experiments. All authors approve the submission.

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