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Investigation of Antifungal and Antibacterial Effect of *Lavandula angustifolia* Against *Candida albicans* and *Escherichia coli*

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Abstract

Essential oils are well-known antiseptics and antibacterial agents used in traditional medicine, possessing extensive activities, including tasks hostile to both bacteria and fungus. Infections caused by both *Candida albicans* and *Escherichia coli* are of high significant issue in medical management, since some strains have proven to be resistance to antibiotics. Our aim is to investigate the antimicrobial activity of the essential oil *Lavandula angustifolia* and its effect on both *C. albicans* ATCC 10231 and *E. coli* ATCC 25922 by examining its MIC, MFC, and MBC to investigate if it can be used as a supportive or protective natural complementary herbal medication. In this experiment, our *C. albicans* isolates were then taken subcultures and incubated at 37°C for 24 hours, then different dilutions of *Lavandula angustifolia* were prepared as; 100%, 50%, 25%, 12.5%, and 6.5% diluted concentrations. Kirby- Bauer disc diffusion method was used to determine the resistance of *Lavandula angustifolia* against both *C. albican*s and *E. coli*, as well as broth microdilution method was used to determine the Minimal Inhibitory Concentration (MIC), Minimal Fungicidal Concentration (MFC) and Minimal Bactericidal Concentration (MBC). Comparing the resulted obtained showed that lavender had a significant antibiosis effect to both *C. albicans* and *E. coli* under different concentrations. Although, our results lead to establish that *Lavandula angustifolia* possess both antifungal and antibacterial activity, it is yet to be proven as for its use as both antifungal and antibacterial agent in medical care with more clinical studies necessary to validate it.

Keywords: Antimicrobial, *Lavandula angustifolia*, *Candida albicans*, *Escherichia coli*, MIC, MFC, MBC.

1. Introduction

Essential oils are well-known antiseptics and antibacterial agents used in traditional medicine, they have extensive activities including, tasks hostile bacteria, fungi and viruses. These organic chemicals can be a viable alternative to synthetic medications when administered topically. However, they are usually not acceptable for systemically use due to both their chemically and physically features, as well as their potentially irritating effects. *L. angustifolia* Mill also identified as lavender oil, belonging to the family of *Lamiacea*, is an essential oil that is usually primarily employed in aromatherapy as relaxant, carminative, as well as soothing agents. It is also conventionally employed as sanitizer for injuries, incineration, arthropod bites, lice killing, and other parasites present in animals in veterinary practice which has shown to be quite efficient. However, extensive studies on antibacterial action caused by lavender oil have only arise in the last few years (D'Auria, 2005).

The comprehensive numeral species of eukaryotes in the world has latterly been approximated to reach eight point seven million, including fungus accounting for roughly 7% of that number (611,000 species). Only about 600 species of fungus are human pathogens, some of which includes: Fungi's that lead to mild skin inflammations (*Dermatophytes* as well as *Malassezia* species, as an example), fungi that lead to critical cutaneous infections

(*Sporotrix schenkii,* as an example), and fungi that could lead to diseases that are systemically which may cause lifelong threats (*Sporotrix schenkii*as an example) make up this relatively small group (*Aspergillus fumigatus*, *Histoplasma capsulatum, Cryptococcus neoformans,* as well as *Candida albicans*). *Candida's* the 4th widely associated indicator of systemic infection usually obtained from hospitals in USA, accompanied with unrefined death measures as high as 50 percent (Mayer, 2013). *Candida albicans* can lead to 2 types of infections in humans: the first type is the superficial infections like the vagina or oral *Candidiasis* and the second type is the systemic infections which are life threating (Wilson, 2012).

After some time after birth, *E. coli* occupies alimentary tract as well as digestively tract of neonate human. *E. coli* along with their humane host that normally survive for tens of years in healthy individuals are said to present common benefit, excluding immunosuppressed hosts or when the usual fences of the gastrointestinal are prevailed over, such example occur in peritonitis, which are strains of commensal *E. coli* that hardly ever lead to diseases. The mucosal coating of the colons of mammalians becomes a place of residence for commensal *E. coli*. This bacteria, is one of the most unrestricted bacterium that is anaerobically facultative in such a sense that it has the ability to multiply in or in the deprivation of oxygen in the microflora of the human gut, which is usually identified as competitors that are fierce in this packed environment. *E. coli* which is a bacterium that is gram-negative has a fine wall of peptidoglycan as well as an external membrane containing lipopolysaccharide, whereby appears pink after staining under the microscope, which is usually due to its cell wall structure which prevents it from retaining crystal violet upon staining but instead gets stained by safranin counterstain giving it its pink colour. It usually develops as fermenting colonies with some beta haemolysis in the early isolates. It may also grow when oxygen is present or absent, indicating it to be facultative anaerobic. It usually consists of a membrane containing cytoplasm, which consists of enzymes, ATP, sugar, and other molecules floating freely inside the liquid section. *Escherichia coli* can be passed from person to person in the following ways: Ground beef, unpasteurized milk, and fresh produce are the most commonly contaminated foods (Kaper, 2004). Other means of transmission include contaminated water, animals, and humans (i.e., personal contact). *E. coli* is known to be a bacterium that dwells interiorly the intestines of both humans as well as animals causing no harm. However, if a person consumes *E. coli*-contaminated food, the bacteria can enter the body's tissues and cause injury. Food or water infected with *E. coli* can make a person sick if it comes into touch with excrement from a human or animal and is consumed. It is critical to thoroughly wash vegetables, fruits, and cooked meats, as well as your hands after using the restroom (Kaper, 2004).

The susceptibility testing of antimicrobials in clinical labs of microbiology is crucial in the authentication of vulnerability to empiric antimicrobial medications or identifying hostile particular isolates of bacteria. Because resistance mechanisms have yet to be uncovered, empirical therapy remains successful for some bacterial infections, such as *Streptococcus pyogenes* continued penicillin susceptibility. The agar disc diffusion method and the broth/dilution method are two of the most widely used methods for assessing the antibacterial activities of essential oil extracts. The disc agar diffusion procedure is very useful, however, it is limited to qualitative data due to the weak soluble components of essential oils, which makes it unlikely to spread on agar Medias and may evaporate throughout the incubation period, unlike the agar disc diffusion method, the broth dilution approach provides a quantitative investigation of antimicrobial sensitivity by finding the minimum inhibitory concentration of the reagent employed to limit microbe growth (abbreviated as MIC) (Reller, 2009).

The objective of this study is assessing the antifungal as well as antibacterial capabilities of *Lavandula angustifolia* against *C. albicans* and *E. coli* by examining its MIC, MFC, and MBC to establish if it can be used as a complementary or alternative topical medication of the natural herbal chemical based on microbiological susceptibility tests.

2. Methods or experimental section

Materials

Solutions used in this experiment were Lavender Oil (concentrations used were 100%, 50%, 25% and 12.5%) obtained only from producers specializing in the production of this plant. Saline, Ethanol (99%), Distilled Water, and Disinfectants. Medias used were Mueller Hinton Agar, DiaTek®Türkiye, Mueller Hinton Broth, DiaTek®Türkiye, and Blood Agar, DiaTek®Türkiye. Both microorganisms used in this experiment were *C. albicans* ATCC10231 and *E. coli* ATCC25922.

Methods

Multiplying

To use the fresh bacteria of standard strains in experiments, *C. albicans* and *E. coli* were cultivated and incubated in 35ºC overnight.

***Lavandula angustifolia* Dilutions**

In our study, 100%, 50%, 25%, 12.5%, and 6.25% diluted concentrations of lavender were prepared with ethanol 99%. 200 µl of lavender oil was used in order to perform the dilutions of lavender concentrations which was placed in 100% concentration.

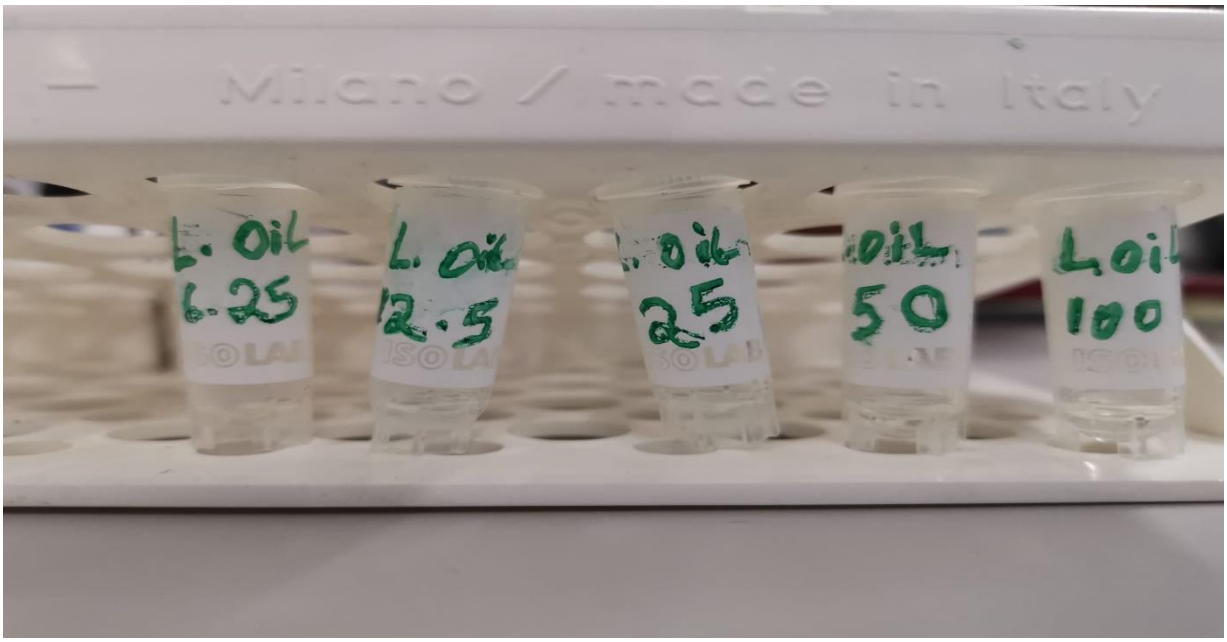

Figure 1: Diluted concentrations of *Lavandula angustifolia*. at 100%, 50%, 25%, 12.5% and 6.25% (Source: Medical Microbiology Laboratory, Altınbas University**).**

As shown above, we prepared dilutions of lavender concentrations of 100%, 50%, 25%, 12.5% and 6.25% concentrations. We added 200µl of lavender in 100%, then took 100 µl from 100% and added 100 µl ethanol giving us the 50% diluted concentration, we vortexed it then took 100 µl from the 50% and added it to100 µl ethanol giving us the 25% diluted concentration, we then vortexed it and took 100 µl from 25%, added 100µl ethanol to it giving us the 12.5% diluted concentration and also vortex it, we then finally took 100µl from 12.5%, added to it 100µl ethanol, vortexed it, and we removing 100µl disposing it giving us the 6.25% diluted concentration.

McFarland Standard

On a test tube 2 ml of saline which is isotonic was added, then *C. albicans* and *E. coli* colonies were taken to the tubes to arrange the turbidity to 0.5 McFarland standard by using McFarland densitometer.

Disc Diffusion Method

An isolate taken from the test tube we prepared from the McFarland 0.5 turbidity, which taken using a cotton swap was spread onto Mueller Hinton Agar plates. Afterwards we added 4 blank discs to each plate. The 1st plate contained 2 discs containing 15µl of 100% concentration of lavender oil, 1 disc containing15µl of 50% diluted concentration of lavender oil and the last disc containing 15µl of the control which was Ethanol 99%. The 2nd plate contained 1 disc containing 15µl of 50% diluted concentration, 2 discs containing 15µl of 25% diluted concentration of lavender oil and the last disc containing 15µl of the control. The 3rd plate contained 2 discs containing 15µl of 12.5% and 2 discs containing 15 µl of 6.25% diluted concentrations of lavender oil. These plates were then incubated for 24 hrs. This was done for both *C. albicans* and *E. coli.*

Macro Dilution

In this study, there were both a negative and positive control tubes. The negative control consisted of 500 µl of standard medium and 500 µl lavender oil, while the positive control consisted of 500 µl medium and 500 µl of *C. albicans* or 500 µl of *E. coli* (taking from McFarland standard). We then prepared 4 tubes: At first we added 1000 µl of medium to all tubes which was used as the diluting agent in this method. Tube 1 contained 1000 µl lavender oil, and 1000 µl of medium given use our 50% diluted concentration. Then we vortexed it and took 1000 µl from the 50% tube and added it to tube 2 which contained1000 µl medium, given us our 25% diluted concentration. The we vortexed it and took 1000 µl to tube 3 which contained 1000 µl medium given us our 12.5% diluted concentration. We then vortexed it and also took 1000 µl to tube 4 which contained1000 µl medium given us our 6.25% diluted concentration, vortexed it and took 1000 µl from tube 4 content and disposed it. Then finally we added 10µl of *C. albicans* concentration to all 4 tubes and incubated them for 24hrs. Same process was performed for *E. coli.*

MFC (Minimal Fungicidal Concentration) and MBC (Minimal Bactericidal Concentration)

In our study the MFC and MBC is further confirmed when grown on Blood Agar and incubated at 37°C for 24hrs.

3. Results

MFC and MBC Results

In order to confirm our MBC and MFC concentrations we sub cultured from the tubes we suspected and grew them on blood agar and incubated for 24hrs. (Figure 2).

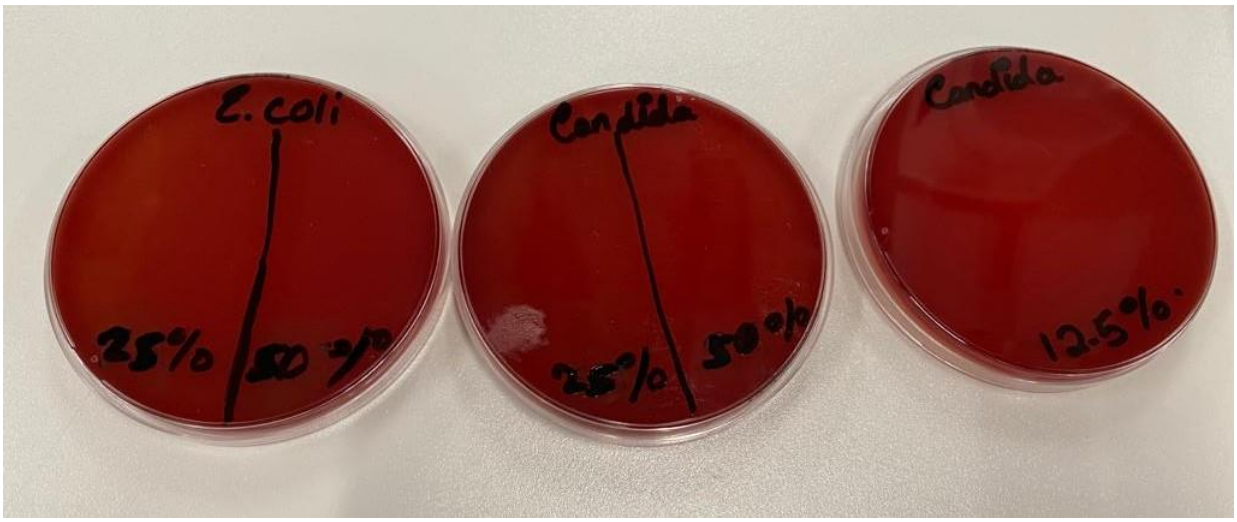

Figure 2: Suspected MFC Diluted Concentrations of 50%, and 25% of *C. albicans* and Suspected MBC Diluted Concentrations of 50% and 25% of *E. coli.* (Source: Medical Microbiology Laboratory, Altınbas University).

Figure 3: MFC of *Lavendula angustifolia* Against *Candida albicans*. (Source: Medical Microbiology Laboratory, Altinbas University).

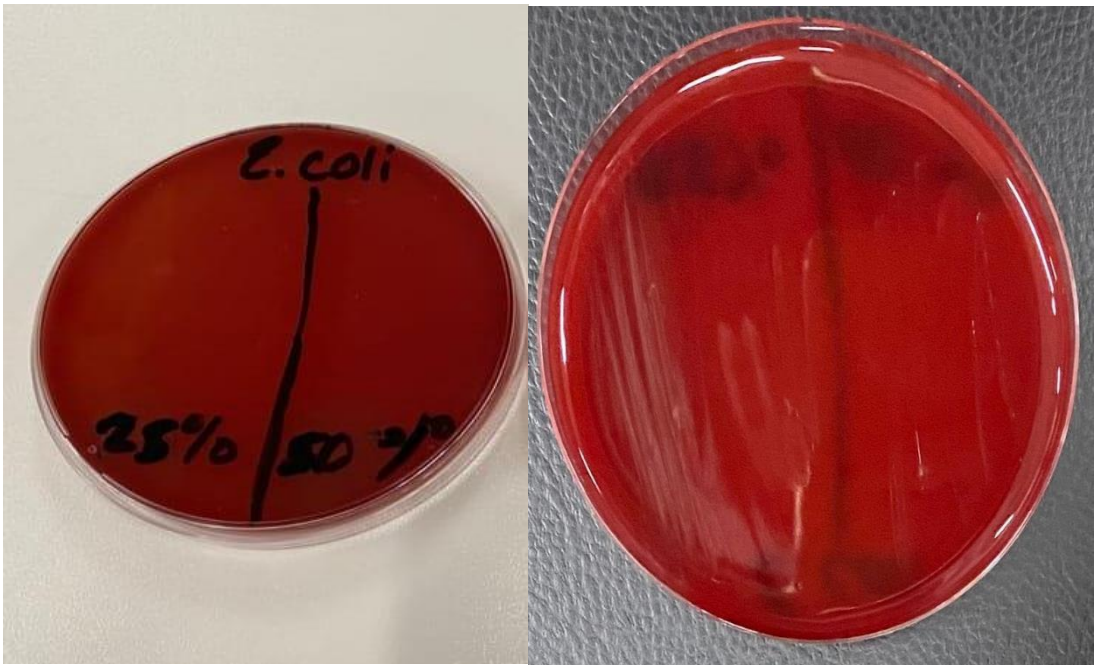

Figure 4: MFC of *Lavandula angustifolia* Against *Escherichia coli*. (Source: Medical Microbiology Laboratory, Altinbas University).

As we can see from Figure 3 and 4 there was growth on 25% diluted concentration of *Lavandula angustifolia* on both *Candida albicans* and *Escherichia coli.* While our 50% diluted concentration showed no growth, leading us to establish our MBC and MFC to be at 50 % diluted concentration of *Lavandula angustifolia*.

4. Discussion

According to our study, lavender oil possessed an antifungal and antibacterial effect on both *C. albicans* and *E. coli*. Our MIC was concluded to be 25% and both our MFC and MBC was 50%. In a study Lavender oil was discovered to exhibit fungicidal and fungistatic properties against *C. albicans* species in the investigation by D'Auria et al., on the antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. According to their study the MIC for *C. albicans* both from oral and vaginal sites was 0.64 percent and 1.04 percent, respectively (D'Auria, 2005).

According to Rapper et al. (De Rapper, 2013), study for *C. albicans, S. aureus, and P. aeruginosa*, respectively, the mean MIC values found for *L. angustifolia* essential oil against the investigated pathogens were 3.00 mg/mL, 2.00 mg/mL, and 2.00 mg/mL. This was in contract to our study for *C. albicans*.

In the study by Man et al. (Man, 2019), they established that essential oils aqueous extract had a stronger bacteriostatic impact against *Pseudomonas aeruginosa* (MIC = 6.3 percent) than the micellar suspension (MIC > 50 percent) in a group of studies. In two other situations, the aqueous extract had a better antifungal and antibacterial impact (MIC/MBC = 6.3 percent) on *Klebsiella pneumoniae* than the micellar suspension (MIC/MBC = 12.5 percent). Furthermore, Man et al., established that the good antibacterial activity of lavender in micellar form is explained by the high concentration of linalyl-butyrate that we discovered (26.5%), and the good inhibitory action

of lavender in aqueous extract may be explained by the high concentration of linalool (25%) and its solubility in water. Their study was also in contrast with our study (De Rapper, 2013).

The MBC and MFC for this study were both 50% for the fungi and bacteria employed in the experiment. This is in contrast to D'Auria et al., were the MFC for oral and vaginal *C. albicans* was 1.1 percent and 1.8 percent, respectively. The MBC for *E. Coli*, according to Man et al., is 6.3 percent which was also in contrast with our study.

5. Conclusion

C. albicans and *E. coli* infections are one of the most common infections with variety of effects, trying to find an alternating drug using natural herbs can be challenging and yet to be proven effective fully. Our results lead to establish that *L. angustifolia* possess both antifungal and antibacterial activity. Further research into the mechanisms by which this oil works could have significant clinical implications. When developing this oil for medicinal use in the treatment of infectious diseases, this research will be a fantastic place to begin. As we can conclude from our study, it is encouraging to note that lavender oil is both fungicidal and bactericidal to both *C. albicans* and *E. coli*. Not much information is available on the mode of action of the natural products that inhibit *C. albicans* and *E. coli* growth. However, if they are to be considered in topical preparation a careful exploration of their probable irritating and other undesirable effects in humans need to be undertaken. More studies should be done to confirm our results.

CONFLICT OF INTEREST

The authors declare that they have any conflict of interest.

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