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The inhibitory effect of aqueous and alcoholic extract of *Lavandula Officinalis* L. on Some type of Pathogenic

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Abstract

The purpose of this study is to know the inhibitory ability of alcoholic and aqueous extracts of lavender leaves *Lavandula Officinalis* L. to the growth of some human pathogenic organisms represented by two types of bacteria, Candida Albicans and Escherichia coli (E. coli) and Klebsiella pneumonia (Kleb). Ethanol and distilled water were used as solvents for extraction at concentrations of 0, 25, 50, and 100 mg ml^{-1} for each. The alcoholic and aqueous extracts showed an inhibitory effect on the growth of the three species at a concentration of 100 mg ml-1, and the aqueous extract showed an inhibitory effect on Candida and Kleb at concentrations of 25 or 50 mg mL^{-1}. The concentrations showed variation in their inhibitory effect on pathogenic organisms, as the alcoholic extract at a concentration of 100 mg ml^{-1} inhibited the growth of Candida and E. coli with a larger inhibitory diameter than the aqueous extract, and the aqueous extract at a concentration of 100 mg ml^{-1} inhibited the growth of Kleb with a larger diameter than the alcoholic extract. GC-MAS analysis of the volatile oil sample of lavender leaves showed the appearance of some compounds with an important effect on the inhibitory activity, such as 1,8-cineole, alpha-pinene, beta-pinene, Camphor, and Terpineol.

Keywords: Extract type, Lavender plant, Inhibitory activity, Candida Albicans, Escherichia coli (E. coli), Klebsiella pneumoniae, GC-MAS

I. Introduction:

The escalating global crisis of antibiotic resistance demands the urgent development of novel antimicrobial strategies (Truong and Mudgil, 2023). Although a focused area of synthetic chemistry and biotechnology is slowly dictating the practice of medicine today, the natural products, especially the medicinal plants continue to provide significant bioactive source for controlling infectious diseases. In fact, it is estimated that about 40% of the drugs that are in use today are derived from natural products where plant derived drugs still remain relevant (Srivastava and Singh, 2020). Therapeutic benefit of exercising pharmacognosy on plants As it is established that the plant kingdom is home to an estimated 500,00 species, exploring its possibility in the provision of therapeutic agents is still limited (Singh, 2015). This inherent potential is particularly apt if the target is antimicrobial drugs since the world has begun to face challenges arising from multi-drug-resistant pathogens.

Among the promising candidates for antimicrobial drug development is *Lavandula officinalis*, a Lamiaceae species with a long history of traditional medicinal and cosmetic applications (Basch *et al*., 2004; Meftahizada *et al.*, 2011; Jigău *et al.*, 2022). The essential oils of *L. officinalis* are rich in a diverse array of bioactive compounds, including phenolic compounds, monoterpenes (such as linalool and linalyl acetate), sesquiterpenes, flavonoids (including luteolin), triterpenoids (such as ursolic acid), and coumarins (including umbelliferone) (Hajhashemi *et al.*, 2003; Slimani *et al*., 2022; Rao *et al.*, 2023). These compounds have been implicated in the plant's reported antimicrobial properties, making it an attractive candidate for investigation as a source of novel antimicrobial agents.

This study evaluates the antimicrobial efficacy of both aqueous and alcoholic extracts of *L. officinalis* against a panel of clinically relevant, antibiotic-resistant pathogens. Specifically, the activity of these extracts against *Klebsiella pneumoniae*, a Gram-negative bacterium associated with various infections

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and known for its increasing antibiotic resistance (Ashurst and Dawson, 2023); *Escherichia coli*, a ubiquitous Gram-negative bacterium responsible for a wide range of infections, including urinary tract infections, and also exhibiting growing antibiotic resistance (Mueller and Tainter, 2023; Lopes and Lionakis, 2022); and *Candida albicans*, an opportunistic fungal pathogen that is becoming increasingly resistant to antifungal treatments (Lopes and Lionakis, 2022) was investigated. The results of this study will contribute to a better understanding of the antimicrobial potential of *L. officinalis* and its potential as a source of novel therapeutics to combat antibiotic resistance.

II. Materials and methods

2.1 Plant samples

Lavender plants were brought from a nursery in Baghdad province, and the herbarium of the College of Science, University of Basra, diagnosed the plants. The vegetative group was dried at room temperature, and after complete drying, the samples were ground using a Panasonic electric grinder. Figures 1 and 2 show the drying plant and dried plant samples, respectively.

Figure 1: Drying the Plant

Figure 2: Dried Plant Samples

1- Extraction of volatile oil

To know the components of dry leaves from the active substances, the volatile oil of the ground dry leaves was extracted in the laboratory of the Medicinal and Aromatic Plants Unit affiliated with the Deanship of the College of Agriculture, University of Basra, according to (Clevenger, 1928) by water distillation using the Clevenger device (Figure**,** 3) connected to a 1-liter flask. 50 g of the sample was taken from the dry and ground leaves and placed in the flask of the device, and 500 ml of distilled water was added to it. The distillation process was carried out by heating the flask continuously for three hours until the volatile oil was extracted from the sample, as two layers of oil were formed on top and water on the bottom. The water was disposed of by opening the tap of the oil receiver, collecting the oil in opaque, tightly sealed bottles and kept in the refrigerator at a temperature of 4 °C until the analysis of the quantitative and qualitative components of the oil was carried out using a gas chromatography device connected to a spectrometer Mass GC-MS.

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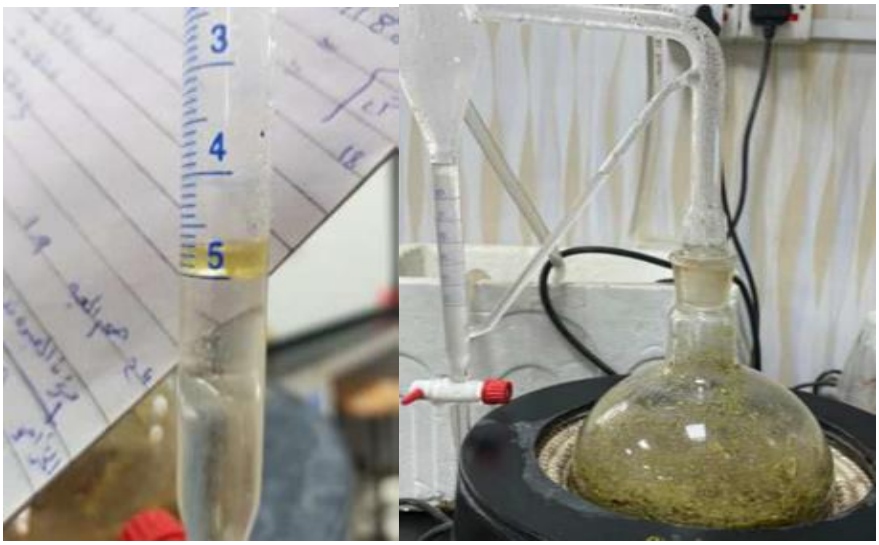
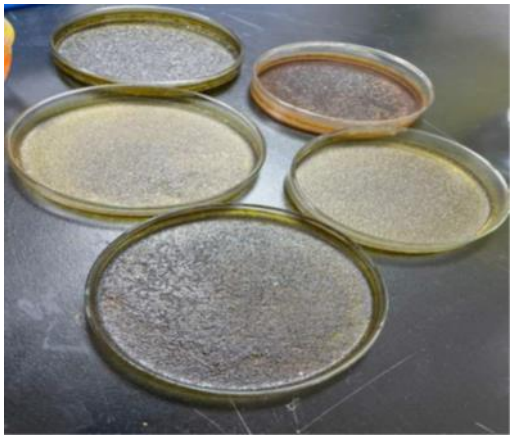


Figure 3: Clavenger Device for Extracting Essential Oil

2- Aqueous extraction

A modified Parekh and Chanda (2007) method was used for aqueous extraction. Fifty grams of air-dried leaf powder were mixed with 500 ml of distilled water in an electric mixer for 15 minutes. The mixture was then transferred to a 1-liter beaker and stirred using a magnetic hotplate stirrer at 45°C for two hours. The mixture was filtered through eight layers of gauze and centrifuged at 5000 rpm for 10



minutes. This centrifugation step was repeated twice to remove residual sediment. The resulting filtrate was placed in Petri dishes to air dry (Figure 4), after which the dried material was collected, stored in sterile bottles at 4 °C, and weighed 4.4250 g. Figure 5

3- Eethanol extraction

For ethanol extraction, 50 g of air-dried leaf powder was combined with 500 ml of 90% ethanol in a 1-liter conical flask. The flask was sealed with cotton wool and placed on a rotary shaker at 190-220 rpm for 24 hours. The solution was then filtered, and the filtrate was placed in Petri dishes to air dry. The dried material was collected and stored in sterile bottles at 4 °C, weighing 5.7274 g (Figure 6).

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Figure 4: Drying of the aqueous and alcoholic extracts

Figure 5: Dry weight of the aqueous extract

Figure 6: Dry weight of the alcoholic extract

2.2 Bacterial isolates

The bacterial isolates were obtained from the laboratories of the College of Science / Department of Life Sciences, University of Basra. Escherichia coli bacteria infect the digestive system and Klebsilla pneumonia bacteria infect the respiratory system. And Candida albican fungus, which is one of the fungi that infect the skin.

2.3 Testing the effectiveness of plant extracts against two types of bacteria

The effectiveness of four concentrations of the aqueous or alcoholic extract 0, 25, 50, and 100 mg ml^{-1} was tested on the two types of selected bacteria, as stated in Smânia *et al.,* (1999). and Berghe and Vlietinck (1991) used Muller Hinton agar to grow bacteria after placing 20 ml of it in each Petri dish. Then, the dishes were planted using the spreading method, where 50 microliters of bacterial suspension prepared from bacterial isolates were placed, spread by sterile cotton swabs, and incubated for 24 hours at 37 °C. Five wells with a diameter of 7 mm were made in the agar medium inoculated with bacteria, 20 mm apart, using a sterile cork piercer. Using a fine pipette, 50 microliters of each concentration of the plant extracts, aqueous or alcoholic, were transferred to four wells under study and 50 microliters of distilled water or sterile alcohol were placed inside the fifth well at the same time instead of the extract as a control treatment. After that, the dishes were incubated for 24 hours at 36 ± 1 °C. and the result was recorded by measuring the diameter of the inhibition zones in millimeters using a ruler.

2.4 Testing the effectiveness of extracts against fungi

Four concentrations of each of the aqueous or alcoholic extracts were used, namely 0, 25, 50 and 100 mg ml^{-1}, to determine their inhibitory effect on the fungus, as stated in Thangavelu. *et al.*, (2013) and Talibi *et al.,* (2012) using the agar diffusion technique, where the PDA medium was used, 100 microliters of the prepared fungal suspension were spread on the surface of the agar for each petri dish, and holes with a diameter of 7 mm were made inside which the samples to be tested for their inhibitory effectiveness (aqueous or alcoholic extract) were placed. The dishes were incubated for 72 hours at 28 ± 2 Cº and the results were recorded by measuring the diameter of the inhibition zone in millimeters using a ruler.

2.5 Statistical analysis

The results were analyzed using Graph Prism version 6.01, and the means were compared at the 0.05 probability level using two-way ANOVA.

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III. Results and Discussion

3.1 Essential Oil Components Analysis of Lavender Leaves Using GC-MAS Technique

Table (1) shows the results of the GC-MAS analysis of the essential oil of *Lavandula Officinalis* L. leaves, where eight main bioactive compounds were identified in the essential oil of lavender leaves according to the peak area and retention time. The analysis in Table (1) revealed the presence of 1,8-cineole at a rate of 23.04%, followed by Camphor at a rate of 10.90%, alpha-cadinol at a rate of 0.99%, Caryophyllene oxide at a rate of 0.51%, Linalool at a rate of 0.42%, Cymene at a rate of 0.27%, beta-pinene and alpha-pinene at a rate of 0.21% each, and Terpineol at a rate of 0.19%.

Table 1: Active compounds in lavender leaf oil

No.	Compound Name	R. Time	Area percentage%
1	1,8-cineole	**7.659**	23.04
2	Camphor	10.388	10.90
3	alpha-cadinol	21.098	0.99
4	Caryophyllene oxide	19.422	0.51
5	Linalool	9.242	0.42
6	Cymene	7.467	0.27
7	alpha-pinene	6.383	0.21
8	Beta-pinene	6.383	0.21
9	Terpineol	8.484	0.19

3.2 Inhibitory effect of alcoholic and aqueous extracts of lavender leaves

The antimicrobial activity of alcoholic and aqueous *Lavandula officinalis* L.leaf extracts against human pathogenic microorganisms was assessed at various concentrations. It is noted that the inhibitory effect of these extracts differed depending on the type of extract and its concentration, as well as the type of pathogenic microorganisms. Figure 7 shows the inhibitory effect of the alcoholic extract on the growth of human pathogenic microorganisms. The alcoholic extract at a concentration of 100 mg ml^{-1} significantly inhibited the growth of the three microorganisms. The most affected organisms by the alcoholic extract were Candida fungi, with an inhibitory diameter of 18 mm, followed by E.Coli bacteria, with an inhibitory diameter of 14 mm. The least affected organism by the alcoholic extract was Klab, with an inhibitory diameter of 8 mm. The comparison treatment did not affect the effectiveness of the three organisms. While we find that the alcoholic extract at concentrations of 50 and 25 mg ml^{-1} was effective in inhibiting Candida only with an inhibition diameter of 14 and 13 mm, respectively. The extract at both concentrations, in addition to the comparison treatment, did not inhibit the growth of other species.

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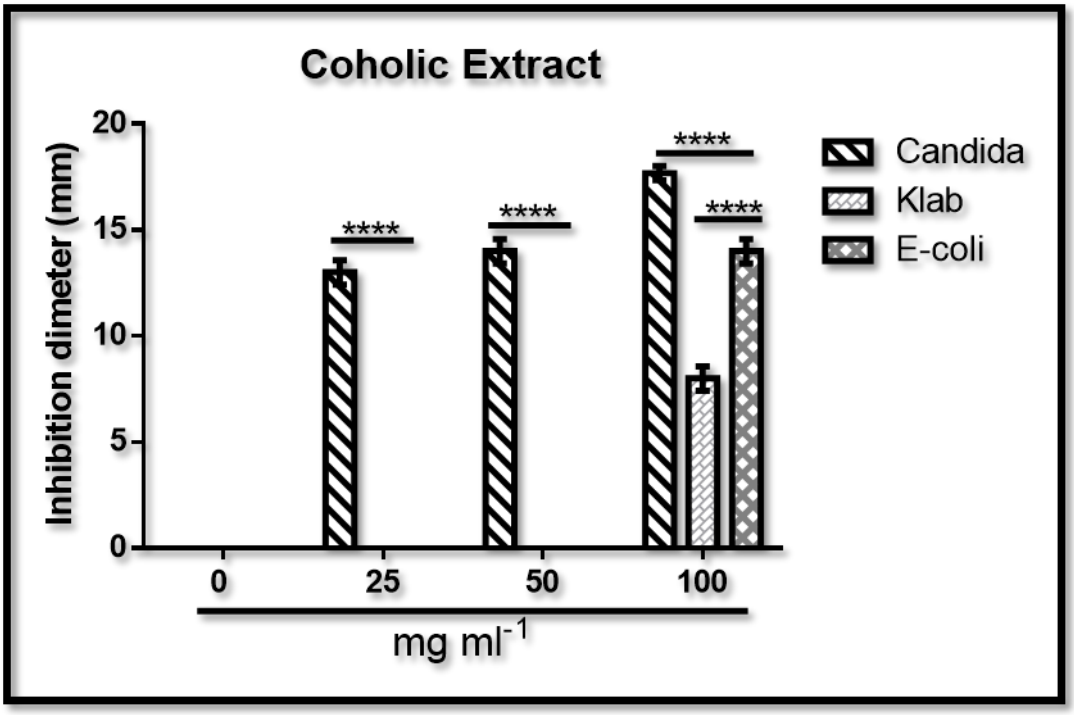

Figure 7: Effect of the alcoholic extract of lavender leaves at concentrations of 0, 25, 50, 100 mg ml^{-1} in inhibiting human pathogenic organisms *Candida albecan*, *Klebsiella pneumoniae* (Kleb), *Escherichia coli* (E. coli). Multiple analysis of variance was performed using the usual two-way analysis of variance comparisons to compare the means of the coefficients. Significance was determined as follows: **** P value greater than 0.0001.

As for the effect of the aqueous extract of lavender leaves in inhibiting the growth of the three microorganisms, it was significant, as Figure 8 shows that the aqueous extract at a concentration of 100 mg ml^{-1} had a significant effect in inhibiting the growth of the three types of pathogenic organisms with varying diameters, as its effect was effective and clear in inhibiting the growth of Candida fungi with an inhibitory diameter of 15 mm, followed by Kleb bacteria with an inhibitory diameter of 13 mm, outperforming Ecoli bacteria, which showed less effect from the aqueous extract with an inhibitory diameter of 9 mm, and the comparison treatment did not affect the inhibition of the three types. While we note that the effect of the aqueous extract at concentrations of 50 and 25 mg ml^{-1} was effective in inhibiting the growth of Candida and Kleb only with inhibitory diameters of 13 and 10 mm and 12 and 8 mm for the two concentrations, respectively, and the comparison treatment had no effect. Inhibitory to the growth of three pathogenic organisms.

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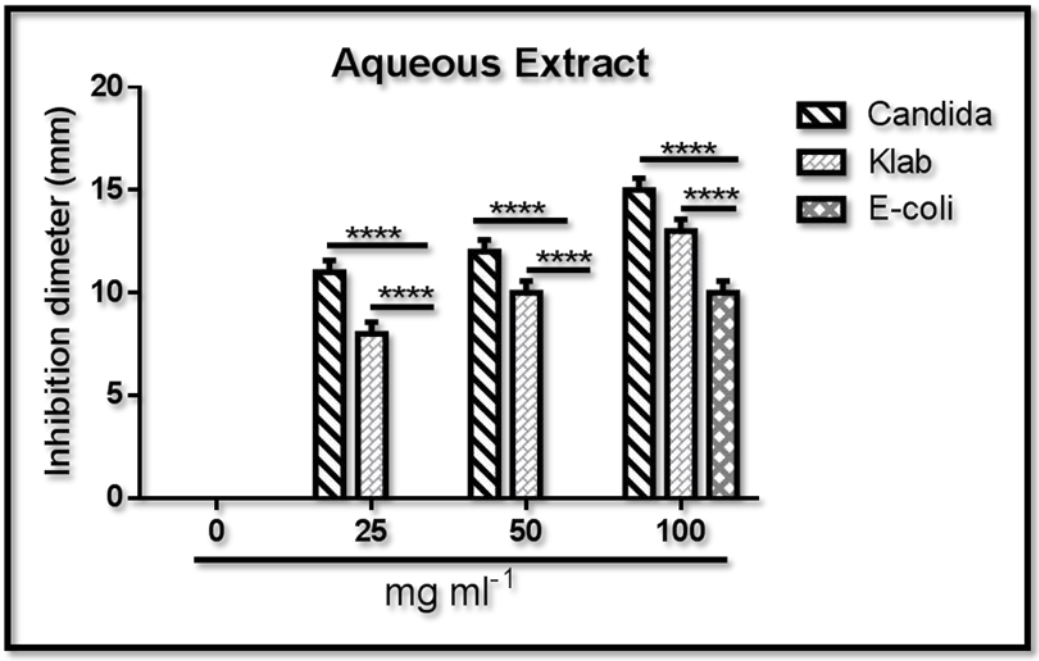

Figure 8: The effect of the aqueous extract of lavender leaves at concentrations of 0, 25, 50, and 100 mg ml-1 in inhibiting human pathogens *Candida albecan*, *Klebsiella pneumoniae* (Kleb) , *Escherichia coli* (E. coli). Multiple analysis of variance was performed using the usual two-way ANOVA comparisons to compare the means of the coefficients. The significance was determined as follows: **** P value greater than 0.0001.

As for the effect of the concentration of the alcoholic and aqueous extracts in inhibiting the growth of pathogenic organisms, Figure 9 shows that the effect of the concentration of 25 mg ml-1 of the alcoholic and aqueous extracts varied in inhibiting the growth of pathogenic species, as the alcoholic extract at a concentration of 25 mg ml^{-1} showed a significant superiority in inhibiting the growth of Candida fungi with an inhibitory diameter of 13 mm compared to the aqueous extract, which gave the lowest inhibitory diameter of 11 mm, while we note that at the same concentration, we find that the aqueous extract was significantly superior in inhibiting the growth of Kleb bacteria with an inhibitory diameter of 8 mm compared to the alcoholic extract, which did not show any inhibition in the growth of these bacteria. As for the effect of the alcoholic and aqueous extracts at a concentration of 25 mg ml^{-1}, they had no effect in inhibiting the growth of E.coli bacteria for both extracts.

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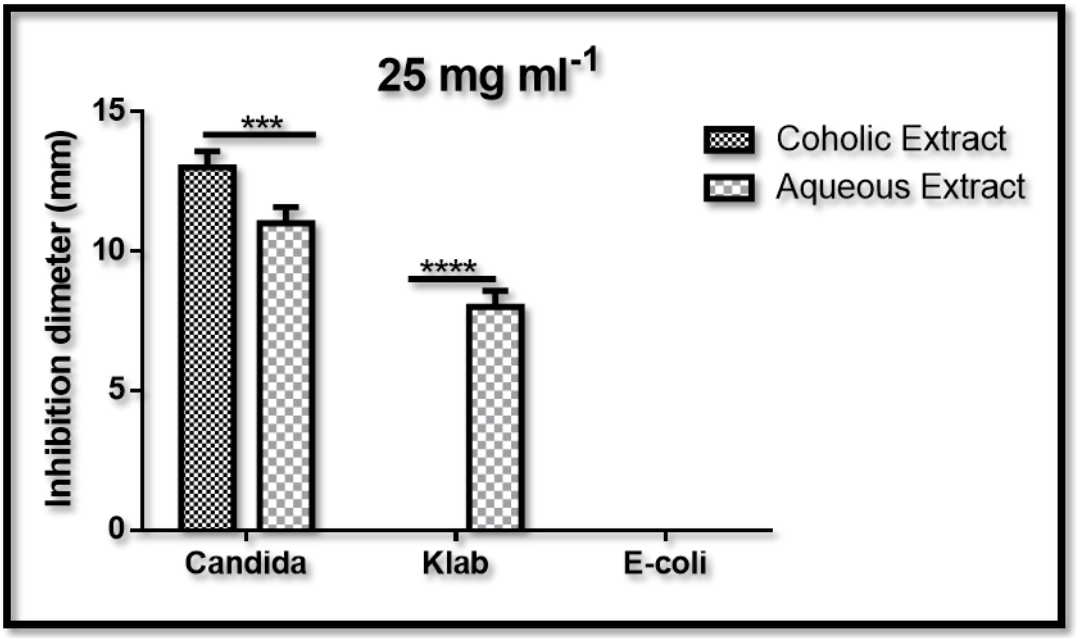

Figure 9: Effect of concentration 25 mg ml-1 of alcoholic and aqueous extracts in inhibiting the growth of human pathogenic organisms *Candida albecan*, *Klebsiella pneumonia* (Kleb), *Escherichia coli* (E.coli). Multiple analysis of variance was performed using ordinary two-way ANOVA comparisons to compare the means of the coefficients. Significance was determined as follows: *** P value greater than 0.001, **** P value greater than 0.0001.

As for the effect of concentration 50 mg ml^{-1} of alcoholic and aqueous extracts in inhibiting the growth of the three types of pathogenic organisms, the effect was similar to the effect of concentration 25 mg ml^{-1} Figure 10.

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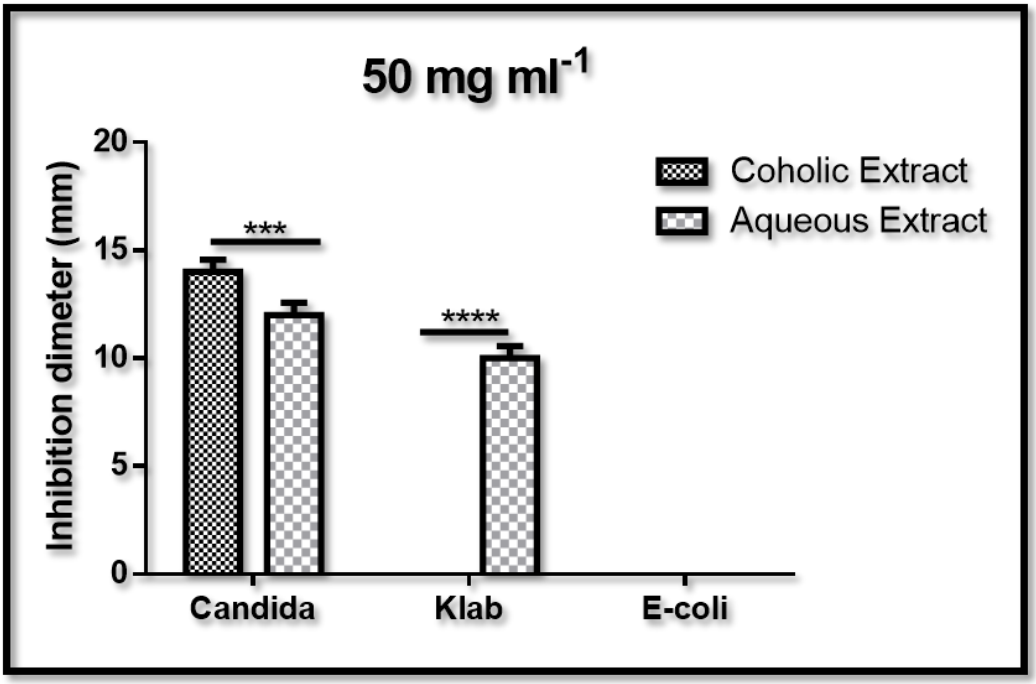

Figure 10: Effect of concentration 50 mg ml-1 of alcoholic and aqueous extracts of lavender leaves in inhibiting the growth of human pathogenic organisms *Candida albecan*,) *Klebsiella pneumonia* (Kleb), *Escherichia coli* (E. coli). Multiple analysis of variance was performed using ordinary two-way ANOVA comparisons to compare the means of the coefficients. Significance was determined as follows: *** P value greater than 0.001, **** P value greater than 0.0001.

As for the effect of the concentration of 100 mg ml-1 of the alcoholic or aqueous extracts, it significantly inhibited the growth of all species, as shown in Figure 11. The effect of the alcoholic and aqueous extracts at this concentration varied in the inhibitory ability of these organisms. It is noted from the figure that the lavender leaves alcoholic extract excelled on the aqueous extract at a concentration of 100 mg ml^{-1} significantly in inhibiting the growth of Candida and E.coli, with an inhibitory diameter of 18 and 14, respectively. As for Kleb bacteria and the extent of their influence by the alcoholic and aqueous extracts at a concentration of 100 mg ml, we find that they were affected by the aqueous extract more than the alcoholic extract, with an inhibitory diameter of 14 mm.

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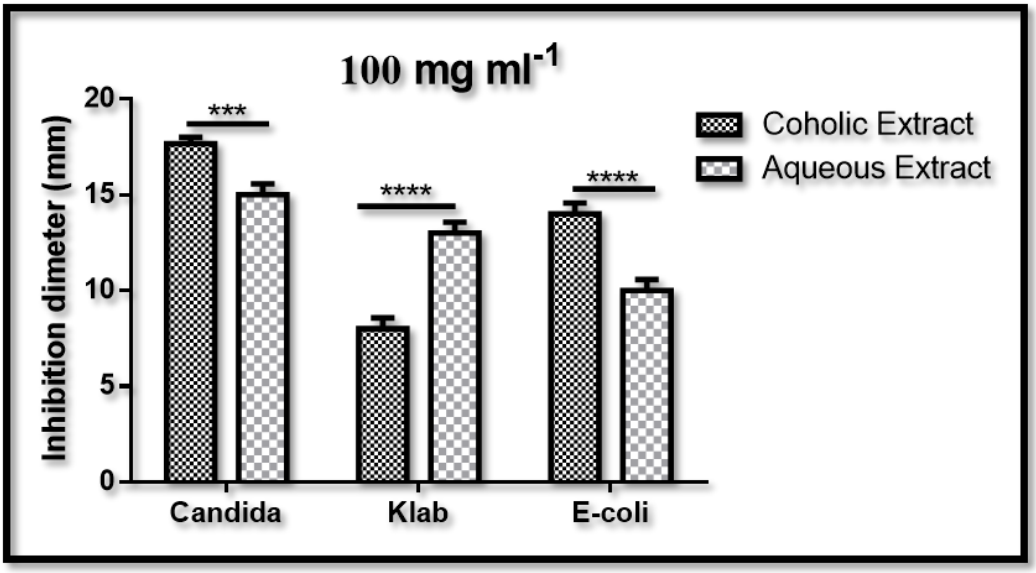

Figure 11: Effect of concentration 100 Effect of concentration 50 mg ml-1 of alcoholic and aqueous extracts of lavender leaves in inhibiting the growth of human pathogenic organisms *Candida albecan, Klebsiella pneumoniae* (Kleb), *Escherichia coli* (E. coli). Multiple analysis of variance was performed using ordinary two-way ANOVA comparisons to compare the means of the coefficients. Significance was determined as follows: *** P value greater than 0.001, **** P value greater than 0.0001.

The results obtained showed that the plant extracts of lavender leaves showed significant effects in inhibiting pathogenic organisms in some treatments. The inhibitory effects shown by the alcoholic and aqueous extracts of lavender leaves against the fungi and bacteria under study may be attributed to the presence of active compounds in these extracts, such as alkaloids, glycosides, saponins, flavonoids, tannins and phenols (Slimani et al., 2022; Roa et al., 2023). Alternatively, the reason may be attributed to the content of the lavender leaves of volatile oil and its components of active compounds (1,8-cineole, Camphor, alpha-cadinol, Caryophyllene oxide) and according to the analysis conducted by the researchers using the GC-MAS technique and the results of which are shown in Table (1), some of these compounds are monoterpenes such as 1,8-cineole, alpha-pinene, beta-pinene, Camphor, Terpineol. These terpenes have antibacterial properties due to their ability to interfere with cell membranes, as they are lipophilic and thus, their activity leads to a malfunction in the mechanism of active transport and the proton motive force and then the flow of electrons, which leads to the occurrence of coagulation of the intracellular components (Ali and Mahde, 2012). The synergistic effects of the mixture of major and minor constituents in the essential oil, which work together to disrupt the bacterial cell membrane through lipophilic sites, can be due to the mixture of major and minor constituents of the essential oil. The results showed that the alcoholic extract of lavender leaves was better than the aqueous extract in most treatments. This may be attributed to the fact that the biologically active components in the plant leaves were more soluble in organic solvents, and the high effectiveness of the ethanolic extract in inhibiting the growth of bacteria and fungi may be attributed to the dissolving power of alcohol for the active components in the plant compared to water (Olusola et al., 2020).

IV. Conclusions

By analyzing the research findings, the alcoholic and the aqueous extracts of Lavandula officinalis leaves, provided considerable inhibition against the growth of Candida albicans, Escherichia coli, and Klebsiella pneumoniae. The study also found that the effectiveness depended on the type of extract (alcoholic repeatedly proved more effective in most cases of the study), the concentration that was most

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successful at 100 mg ml^{-1} and the microorganism. The better performance of the alcoholic extract may be attributed to the high solubility of these compounds in organic solvents. Among the bioactive compounds, the alkaloids, glycosides, saponin, flavonoids, tannins, phenols and monoterpenes, including 1,8-cineole, alpha-pinene, beta-pinene, camphor and terpineol detected through GC-MAS are responsible for the inhibitory activity. These compounds are, therefore, believed to act by disrupting cell membranes. Future research should seek to purify the isolated compounds and identify their antimicrobial properties more comprehensively, determine the best extraction processes that yield the highest volume of extracts with high potency and investigate the effectiveness and safety of these extracts in treating human infections.

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