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Evaluating the Antibacterial Activity Against *Escherichia coli* Using Aqueous Extracts of (Mint, Wormwood, Rosemary, and Clove) Plants

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Abstract

Background: *Escherichia coli* is an opportunistic bacterium that can become a serious pathogen if conditions are favourable, this study aimed to find biologically effective alternatives to inhibit the growth of this bacterium.
Materials and Methods: Using aqueous extracts of mint (*Mentha piperita*), wormwood (*Artemisia herba-alba*), rosemary (*Rosmarinus officinalis*), and clove (*Syzygium aromaticum*) at concentrations of 40% and 60%, compared to a control (distilled water only). The agar well diffusion method was employed on Nutrient Agar (NA) medium.
Results: The results demonstrated that all plant extracts exhibited inhibitory activity against the tested bacterium, though to varying degrees. Clove extract achieved the highest mean inhibition zone of 21.17 mm at 60% concentration, followed by mint extract at 40% concentration with a mean of 19.17 mm, then rosemary extract at 40% concentration with a mean of 16.50 mm, and finally wormwood extract with a mean of 15.00 mm.
Conclusions: This study concluded that aqueous extracts of mint, wormwood, rosemary, and clove plants possess varying inhibitory activity against Escherichia coli. The results showed that these extracts can form clear inhibition zones depending on the plant type and extract concentration.

Keywords: *Escherichia coli*, *E. coli*, Plant extracts, Clove extract.

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INTRODUCTION

Escherichia coli is an opportunistic pathogenic bacterium that can cause disease when favorable conditions are present. It is a normal inhabitant of the intestinal tract and can cause gastrointestinal infections, gastric ulcers, diarrhea, and may extend to urinary tract infections.[1] *E. coli* is a rod-shaped organism measuring approximately 2-3 micrometers in length and 0.5-1 micrometer in width, occurring singly or in pairs, and rarely in chains. It is Gram-negative. Due to the increasing rate of bacterial resistance to antibiotics, researchers have turned to plants as the most promising source for discovering new antimicrobial agents. Plants have historically served as a source of medicine for humans, both in old and modern times, within the field of healthcare.[2]

Plants produce different groups of bioactive compounds as secondary metabolites, including glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes.[3] These compounds can function as drugs against pathogenic microorganisms[4] by disrupting or decreasing certain metabolic pathways within bacterial cells.[5] The use of plants and medicinal herbs in healthcare does not cause side effects, provided that patients adhere to the recommended conditions of use. Researchers have recently turned to treating many diseases using plant-derived pharmaceuticals due to their inhibitory

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effectiveness against pathogens,[6] in addition to their minimal side effects, safety, and lower cost compared to chemical treatments.

A study done on wormwood plant extract showed that ethanol, hexane, and propanol extracts of wormwood at various concentrations had a strong effect on different bacterial types, with propanol extract showing the highest effect against all tested bacterial species: *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* spp., and *Klebsiella pneumoniae*.[7]

A study at the University of Misurata showed that aqueous extracts of rosemary leaves were able to stop the growth of *Staphylococcus aureus*. The level of inhibition depended on where the plant grew.[8] Likewise,[9] assessed the antibacterial properties of rosemary and discovered that the plant shown significant inhibitory effects on some pathogenic bacterial strains used in the study.

The aim of this study was to determine the inhibitory effect of aqueous extracts of wormwood, mint, rosemary, and clove on *E. coli* bacteria in an attempt to find therapeutic alternatives to decrease the excessive use of antibiotics, which has led to the spread of resistance traits.

MATERIALS AND METHODS

Plant samples of mint, clove, wormwood, and rosemary were collected from local markets for use as extracts. The samples were washed several times with water, air-dried in the shade, ground into powder, and stored in a fridge until use, as shown in (Table 1).

Table 1: Plants used as extracts and their utilized parts.

Common Name	Scientific Name	Family	Part Used
Mint	Mentha piperita	Lamiaceae	Leaves
Wormwood	Artemisia herba-alba	Asteraceae	Aerial parts
Rosemary	Rosmarinus officinalis	Lamiaceae	Aerial parts
Clove	Syzygium aromaticum	Myrtaceae	Dried flower buds

Preparation of Aqueous Extract

To obtain a 10% aqueous extract, 20 grams of powdered plant material from each of wormwood, mint, rosemary, and clove were separately mixed with 200 ml of sterilized distilled water. The mixture was agitated using a hot plate shaker. After 24 hours, the solution was filtered using filter paper to remove excess plant material and residual fibers. The extracts were then placed in tightly sealed bottles and stored in a fridge until use.[10] This concentration served as the standard stock solution, from which concentrations of 40% and 60% were prepared during the experiment.

E. coli Isolate

The bacterial isolate was obtained from the Serology Laboratory at the Forensic Evidence and Laboratory Administration, Criminal Investigation Department, Tripoli.

Assessment of Inhibitory Activity by Agar Well Diffusion Method

Following the manufacturer's recommendations, 28 grams of nutrient agar medium were added to one liter of distilled water and then sterilized in an autoclave for 20 minutes at 121°C. The medium was put into sterilized Petri plates and let to cool and harden. To make the bacteria active, they were put into

sterilized nutritional broth and kept at 37°C for 16 to 18 hours.[11] A sterile swab was used to extract the bacterial inoculum from the nutrient broth and spread it on the surface of the prepared Petri dishes. A sterile cork borer with a 5 mm diameter was then used to make wells in the agar. We used a micropipette to add 0%, 40%, and 60% concentrations of the plant extracts we had made earlier to the wells. After that, the plates were put in an incubator at 37°C, and the sizes of the inhibitory zones were determined in millimeters.[7]

Statistical Analysis

Data were analyzed using the SPSS statistical analysis program to calculate the mean values for the replicate sets used in the experiments.

RESULTS

Effect of Aqueous Extracts on E. coli Growth

This study evaluated the effectiveness of aqueous extracts of mint, wormwood, rosemary, and clove on inhibiting the growth of *Escherichia coli* (Table 2).

The control (0%) recorded zero values in all treatments. Clove extract at 60% concentration achieved the highest mean inhibition of 21.17 mm. Mint extract at 40% concentration showed a mean of 19.17 mm, which decreased to 14.33 mm at 60% concentration. Rosemary extract at 40% concentration recorded a mean of 16.50 mm, with 16.17 mm at 60% concentration. Wormwood extract recorded the lowest means across all concentrations (12.67 mm at 40% and 15.00 mm at 60%) (Figure 1).

The overall mean values varied by extract type: mint recorded the highest overall mean (11.17 mm), followed by clove (11.11 mm), rosemary (10.89 mm), and wormwood (9.22 mm).

Table 2: Mean values for all concentrations (mm).

Plant Extract Type	Mean at 0% (Control)	Mean at 40%	Mean at 60%	Overall Mean
Mint	0	19.17	14.33	11.17
Wormwood	0	12.67	15.00	9.22
Rosemary	0	16.50	16.17	10.89
Clove	0	12.17	21.17	11.11

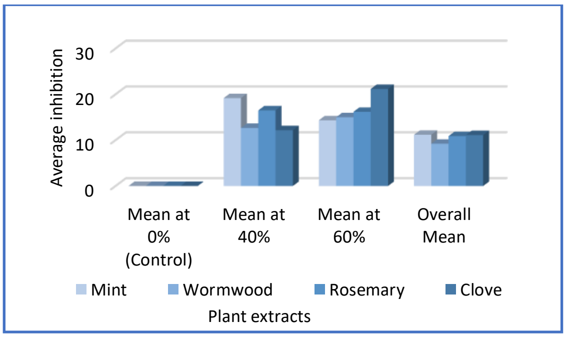

Figure 1: shows the mean inhibition of the plant extracts used at concentrations of 0%, 40%, and 60%.

The results of the Two-Way ANOVA demonstrated a statistically significant effect of both extract type and concentration on the dependent variable. The significance level for extract type was 0.007 (F = 8.42). The concentration factor showed a highly significant effect (F = 145.20, Sig = 0.000). The interaction between extract type and concentration was also significant (F = 12.15, Sig = 0.004) (Table 3).

Table 3: Mean values for all concentrations (mm).

Source of Variation	Degrees of Freedom (df)	Calculated F-value	Significance Level (Sig)
Extra Type	3	8.42	0.007
Concentration (0,40,60)	2	145.20	0.000
Interaction (Extract X Concentration)	6	12.15	0.004

DISCUSSION

The observed inhibitory effects are attributable to the addition of plant extracts rather than other factors, as confirmed by the zero values in all control treatments. This agrees with findings reported by ALAbani and Ali (2024) and Al-Ghbeini et al. (2024), [3,5] who showed that plant extracts have inhibitory effects on the growth of different pathogenic bacterial genera.

The inhibitory activity recorded by clove extract can be attributed to its high eugenol content, which reaches up to 85%.[12] Several studies have confirmed the ability of clove oil to inhibit *E. coli*. [13] The inhibitory effect of clove is attributed to the presence of phenolic compounds known for their effectiveness against bacteria. [14]

Following clove, mint extract at 40% concentration showed strong inhibition; however, this decreased at 60% concentration, suggesting the possible existence of an optimal inhibitory concentration near 40%. This agrees with the study by Al-Araibi et al,[2] which showed that mint extract possesses antibacterial properties against *E. coli* at concentrations of 20% and 50%.

Rosemary extract at 40% concentration recorded inhibition with a mean of 16.50 mm. This can be attributed to the presence of rosmarinic acid in rosemary, which exhibits activity within a specific concentration range. These findings are consistent with Al-Ghbeini et al,[5] who demonstrated that rosemary extract has effectiveness against *Staphylococcus aureus* at 80% concentration. Additionally, Salem [15] indicated the potential use of rosemary oil against bacteria isolated from chronic wounds in diabetic patients, including *E. coli*. [16] Ahmed et al, [1] also showed that the effectiveness of alcoholic rosemary extract in inhibiting microorganisms is attributed to its content of tannins, flavonoids, and saponins, which have inhibitory activity against a wide range of microorganisms, including bacteria and fungi.

In contrast, wormwood extract recorded a relatively lower mean across all concentrations compared to the other extracts, indicating that the rate of concentration increase was insufficient to enhance the antimicrobial activity, despite its content of artemisinin compounds known for their antimicrobial properties. This suggests that the concentrations used may not have been sufficient to demonstrate its full effectiveness. This is consistent with ALAbani and Ali, [3] who indicated the potential use of wormwood extract against *E. coli* as an alternative to antibiotics.

The variation in overall means among the studied extracts reflects differences in chemical composition and active compounds, which explains the disparity in bioactivity levels.

The interaction results between extract type and concentration showed significant differences (F = 12.15, Sig = 0.004), indicating that the response depends not only on each factor independently but is also affected by the combined interaction between extract type and concentration level. This means that extracts exhibit higher activity at certain concentrations compared to others, which supports the descriptive results presented in the means table (Table 2).

CONCLUSION

This study concluded that aqueous extracts of mint, wormwood, rosemary, and clove plants possess varying inhibitory activity against *Escherichia coli*. The results showed that these extracts can form clear inhibition zones depending on the plant type and extract concentration. Clove extract at 60% concentration achieved the highest inhibitory activity at 21.17 mm, followed by mint extract at 40% concentration with a mean inhibition zone diameter of 19.17 mm; however, its effectiveness decreased when the concentration was increased to 60%, suggesting the existence of an optimal inhibitory concentration that warrants further investigation in future studies.

Rosemary extract showed moderate activity of 16.50 mm at 40% concentration, while wormwood extract recorded relatively lower effectiveness.

The statistical analysis results using Two-Way ANOVA highlight the importance of both extract type and concentration individually, as well as their interaction, confirming that inhibitory effectiveness is not the result of a single factor, but rather the outcome of multiple integrated variables. These findings hold significant importance in light of the global challenge of increasing bacterial resistance to antibiotics, opening promising avenues for developing natural pharmaceuticals of plant origin with high efficiency, low cost, and fewer side effects compared to chemical antimicrobials.

RECOMMENDATIONS

• It is recommended to conduct further research on these plants to determine the optimal concentration for clove and mint extracts through graduated concentration studies, along with quantitative chromatographic analysis (HPLC/GC-MS) of active compounds such as eugenol, menthol, and rosmarinic acid to directly correlate them with biological activity and standardize dosages.

• Study their effects on the normal microbiome to avoid potential gastrointestinal disturbances and determine the daily safe dose (Daily Safe Dose).

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