



An experimental assessment of several medicinal plant extracts' antibacterial activity against harmful microorganisms

Shaymaa Hamood Karakosh

Office of the President of Wasit University

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Abstract

The hunt for substitute antimicrobial agents made from natural items has been brought on by the emergence of antibiotic-resistant bacterial species. The present experimental laboratory study was designed to investigate the role of the extracts of ethanol, garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), oregano (*Origanum vulgare*) and neem (*Azadirachta indica*) against some harmful microorganisms such as *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Disc diffusion was used to assess plant extracts that were made by ethanol extraction. procedures, agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). Garlic extract exhibited inhibition zones ranging from 21-29 mm and oregano extract exhibited maximum antibacterial activity (25-32 mm) and Neem, turmeric exhibited moderate antibacterial activity and ginger exhibited comparatively lower activity. In general, Gram-positive bacteria were more susceptible than Gram-negative bacteria. The results showed that the plant extracts differed statistically significantly ($P < 0.05$). The findings demonstrate that the medicinal herbs have strong antibacterial properties and may be used in place of manufactured antibiotics.

Keywords: Medicinal plants, antibacterial activity, phytochemicals, bacterial growth, agar diffusion, natural antibiotics, microbiology.

Introduction

Although antimicrobial drugs are widely used, bacterial infections still have a high prevalence as a global public health problem. The problem of new treatment techniques has grown because of the rapid evolution of multi-drug resistant strains of bacteria, making many of the traditional antibiotics less effective. The medicinal plants have received a lot of scientific attention due to the presence of biologically active compounds which have a wide range of antimicrobial inhibitory activity (Silva & Fernandes Júnior, 2010); (Upadhyay et al., 2014); (Srivastava et al., 2014).

For thousands of years, traditional medicine has employed medicinal herbs to treat skin conditions, gastrointestinal issues, wounds, and respiratory infections. In the modern investigations, it has been

confirmed that many plants have an antibacterial activity due to the presence of phytochemical components like phenolic, flavonoids, alkaloids, terpenoids, tannins and saponins Nielsen et al., 2012; (Billah et al., 2013); (Górniak et al., 2019).

Garlic, ginger, turmeric, neem, oregano are certain medicinal plants have demonstrated strong antibacterial action against clinically significant microorganisms, including *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*. These phytochemicals disrupt the metabolic pathways and cellular structure of bacteria and result in the inhibition of bacterial growth and even death (Eloff, 2019); (Gupta et al., 2013); (Biswas et al., 2002); (Burt, 2004); (Ali et al., 2008); (Ankri & Mirelman, 1999).

Using common microbiological laboratory methods, the current study sought to evaluate the antibacterial efficacy of some therapeutic plant extracts versus pathogenic microorganisms.

Materials and Methods

2.1 Study Design

This was an experimental laboratory study done in the Microbiology Laboratory of the Department of Biology from January to March 2026.

2.2 Collect plant materials

Fresh bulbs of garlic, rhizomes of ginger and turmeric and leaves of neem and oregano were collected from local herbal markets. After properly cleaning the plant materials with distilled water, they were allowed to air dry for seven days at room temperature (Naeim et al., 2020); (Dar et al., 2017); (Kuethe, 2010).

2.3 Plant Extracts preparation

Sterile electric grinder was used to finely grind the dried plant materials. All powdered samples (50g) were steeped for seven days in 250 ml of 95% ethanol with shaking every few hours. The extracts were concentrated in a 40°C rotary evaporator after being filtered with Whatman No. 1 filter paper. The raw extracts were stored at 4°C in sterile containers prior to usage Khameneh et al., 2019); (Cheesman et al., 2017); (Mostafa et al., 2018).

2.4 Test Microorganisms

Pseudomonas aeruginosa, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* were the microbes employed in this investigation. The strains were isolated from clinical specimens and identified using the traditional biochemical tests (Dar et al., 2017).

2.5 Preparation of Bacterial Inoculum

Inocula of bacteria were prepared from fresh cultures grown on nutrient agar plates. A few isolated colonies of each strain of bacteria were aseptically placed on 0.85% NaCl sterile saline solution. The bacterial suspensions' turbidity was modified to the 0.5 McFarland value, which is the same as the approximate value of 1.5×10^8 CFU/mL. The standardized inocula were immediately used to perform antimicrobial susceptibility testing such as MIC, MBC, agar well diffusion, and disc diffusion assays to obtain uniform bacterial density during the experiments (Dar et al., 2017); (Ríos & Recio, 2005).

2.6 Disc Diffusion Method

The antibacterial activity of plant extracts was evaluated by the Kirby-Bauer method of disc diffusion. Mueller-Hinton agar plates were uniformly inoculated with bacterial solutions using sterile cotton brushes. The contaminated area of the agar was covered with sterile discs of filter paper soaked in 100 µL of every plant extract. The plates were incubated at 37°C for 24 hours. The diameter of inhibitory regions was measured in mm using a digital calliper (Dar et al., 2017); (Ríos & Recio, 2005); (Bassolé & Juliani, 2012); (Hemaiswarya et al., 2008); (Dorman & Deans, 2000).

2.7 Method of Agar Well Diffusion

Mueller-Hinton agar plates were bored using a sterile cork borer to produce wells that were 6 mm in diameter. Each well received 200 μ L of the plant extract. Following a 24-hour incubation period at 37°C, inhibition zones were examined (Dar et al., 2017); (Bassolé & Juliani, 2012).

2.8 The Minimum Inhibitory Concentration (MIC)

The MIC values were calculated using the broth dilution technique. Plant extracts were serially diluted from 12.5 to 200 mg/ml in nutrient broth-based tubes seeded with a suspension of bacteria. The minimal inhibitory concentration (MIC) was considered as the lowest concentration that did not show any visible bacterial growth after incubation (Eloff, 2019); (Bassolé & Juliani, 2012); (Compean & Ynalvez, 2014).

2.9 The Minimum Bactericidal Concentration (MBC)

Samples from MIC tubes with no apparent growth were streaked out on nutrient agar plates. The MBC was found to be the smallest concentration that totally inhibited the development of bacterial colonies (Eloff, 2019); (Bassolé & Juliani, 2012).

2.10 Analysis of Statistics

There were three versions of the trials. The data were shown using the mean $\pm$ standard deviation (Mean $\pm$ SD). One-way ANOVA and Tukey's post hoc test were used for statistical analysis. The p-values were considered statistically significant if they were less than 0.05 (Dar et al., 2017).

Results and Discussion

3.1 Antibacterial Activity of Medicinal Plant Extracts

When tested against the identified bacterial strains, all plant extracts showed various degrees of antibacterial activity.

Table 1. Mean inhibition zones (mm) of medicinal plant extracts against pathogenic bacteria.

Plant Extract	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
Garlic	29 $\pm$ 1.2	25 $\pm$ 1.0	21 $\pm$ 0.8	24 $\pm$ 0.9
Ginger	22 $\pm$ 0.9	19 $\pm$ 1.1	17 $\pm$ 0.7	18 $\pm$ 0.8
Turmeric	24 $\pm$ 1.0	21 $\pm$ 0.9	19 $\pm$ 0.6	20 $\pm$ 0.7
Neem	26 $\pm$ 1.1	23 $\pm$ 0.8	20 $\pm$ 0.7	22 $\pm$ 0.8
Oregano	32 $\pm$ 1.3	28 $\pm$ 1.1	25 $\pm$ 0.9	27 $\pm$ 1.0

According to the findings, ginger extract had the lowest antibacterial activity while oregano extract had the highest activity against all examined microorganisms Burt, 2004); (Ali et al., 2008); (Dar et al., 2017); (Khameneh et al., 2019).

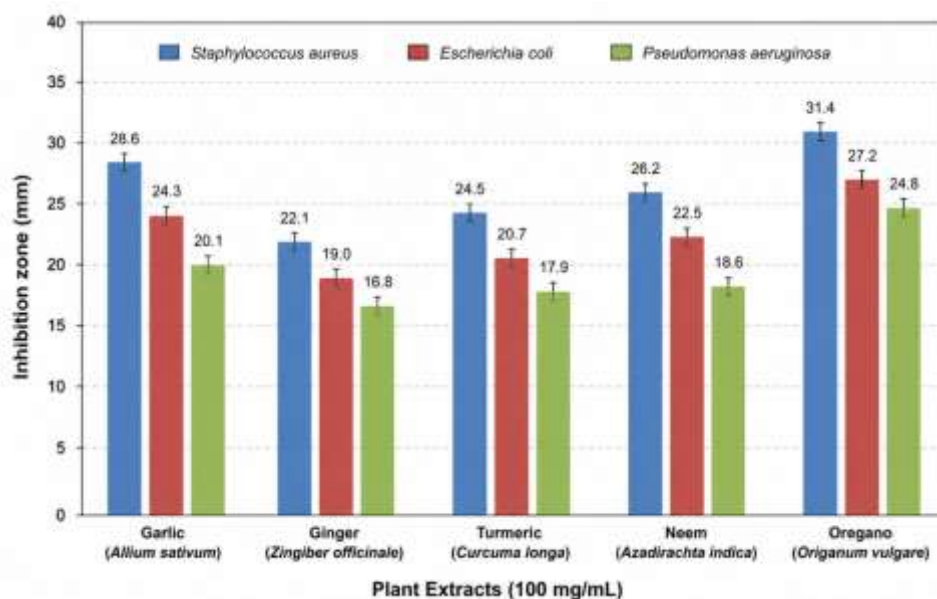


Figure 1. Comparative inhibition zones produced by medicinal plant extracts against pathogenic bacteria.

The Figure illustrates the antibacterial effectiveness of garlic, ginger, turmeric, neem, and oregano extracts against *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. typhi*. Oregano showed the largest inhibition zones, particularly against *S. aureus* (Burt, 2004); (Ali et al., 2008); (Ankri & Mirelman, 1999); (Dar et al., 2017); (Khameneh et al., 2019).

3.2 Determination minimum inhibitory concentration or MIC

Bacterial species and type of extracts influenced the MIC values of the plant extracts.

Table 2. Medicinal plant extracts' minimum inhibitory concentrations (MICs).

Plant Extract	<i>S. aureus</i> mg/ml	<i>E. coli</i> mg/ml	<i>P. aeruginosa</i> mg/ml	<i>S. typhi</i> mg/ml
Garlic	25	50	50	50
Ginger	50	100	100	100
Turmeric	50	50	100	50
Neem	25	50	50	50
Oregano	12.5	25	25	25

Oregano exhibited the lowest MIC values, indicating stronger antibacterial potency.

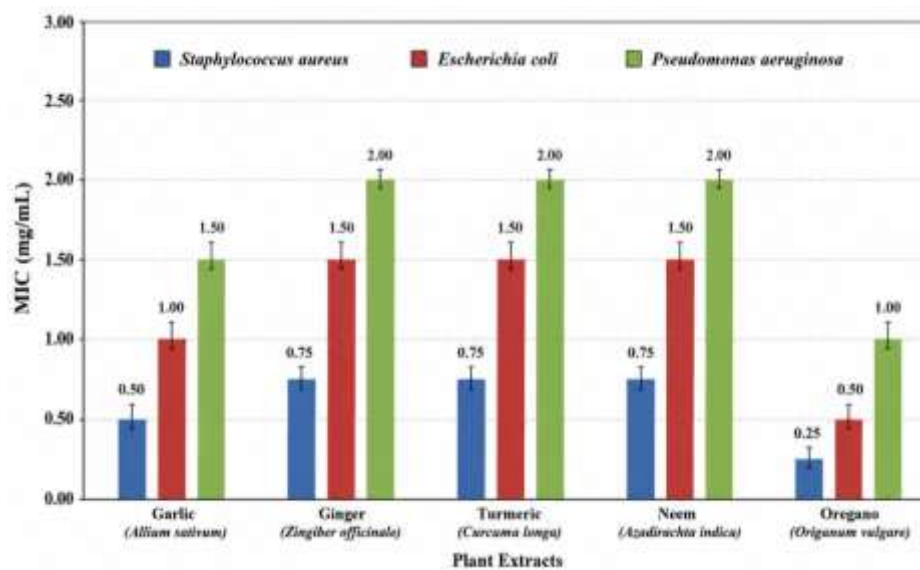


Figure 2. Medicinal plant extracts were evaluated against the tested bacterial strains using MIC.

The minimum inhibitory concentrations of each medicinal plant extract that inhibits bacterial growth is shown in the Figure 2 (Eloff, 2019); (Ankri & Mirelman, 1999); (Mostafa et al., 2018); (Bassolé & Juliani, 2012); (Compean & Ynalvez, 2014).

3.3 Minimum Bactericidal Concentration (MBC)

The bactericidal activity of oregano and garlic extracts was strong as revealed by the MBC results.

Table 3. The minimum bactericidal concentration (MBC) values of medicinal plant extracts.

Plant Extract	<i>S. aureus</i> mg/ml	<i>E. coli</i> mg/ml	<i>P. aeruginosa</i> mg/ml	<i>S. typhi</i> mg/ml
Garlic	50	100	100	100
Ginger	100	200	200	200
Turmeric	100	100	200	100
Neem	50	100	100	100
Oregano	25	50	50	50

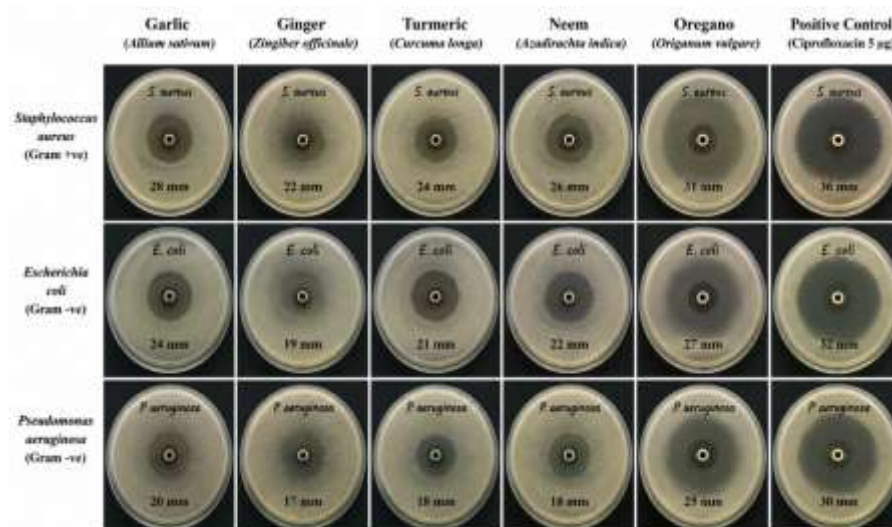


Figure 3. The antibacterial activity of extracts obtained from medicinal plants against pathogenic microorganisms under test are indicated by agar diffusion plates

Representative agar-plate images showing clear inhibition area around the wells filled with extracts of oregano, garlic, turmeric, neem and ginger (Eloff, 2019); (Burt, 2004); (Dar et al., 2017); (Kuate, 2010).

3.4 Statistical Analysis

Results of statistical analysis revealed that there were significant differences between plant extracts in antibacterial activity ($P < 0.05$). Oregano extract was found to have significantly higher antibacterial activity than the ginger extract and turmeric extract (Burt, 2004); (Khameneh et al., 2019); (Mostafa et al., 2018).

Table 4. Statistical analysis of inhibition zone diameters.

Plant Extract	Mean Inhibition Zone (mm)	Standard Deviation	P-value
Garlic	24.8	± 1.0	<0.05
Ginger	19.0	± 0.8	<0.05
Turmeric	21.0	± 0.8	<0.05
Neem	22.8	± 0.9	<0.05
Oregano	28.0	± 1.1	<0.05

In the present study, the schematic experimental flow of the work carried out in the extraction of plants, conducting anti-bacterial assays, determination of MIC/MBC, and statistical analysis is illustrated (Figure 4).

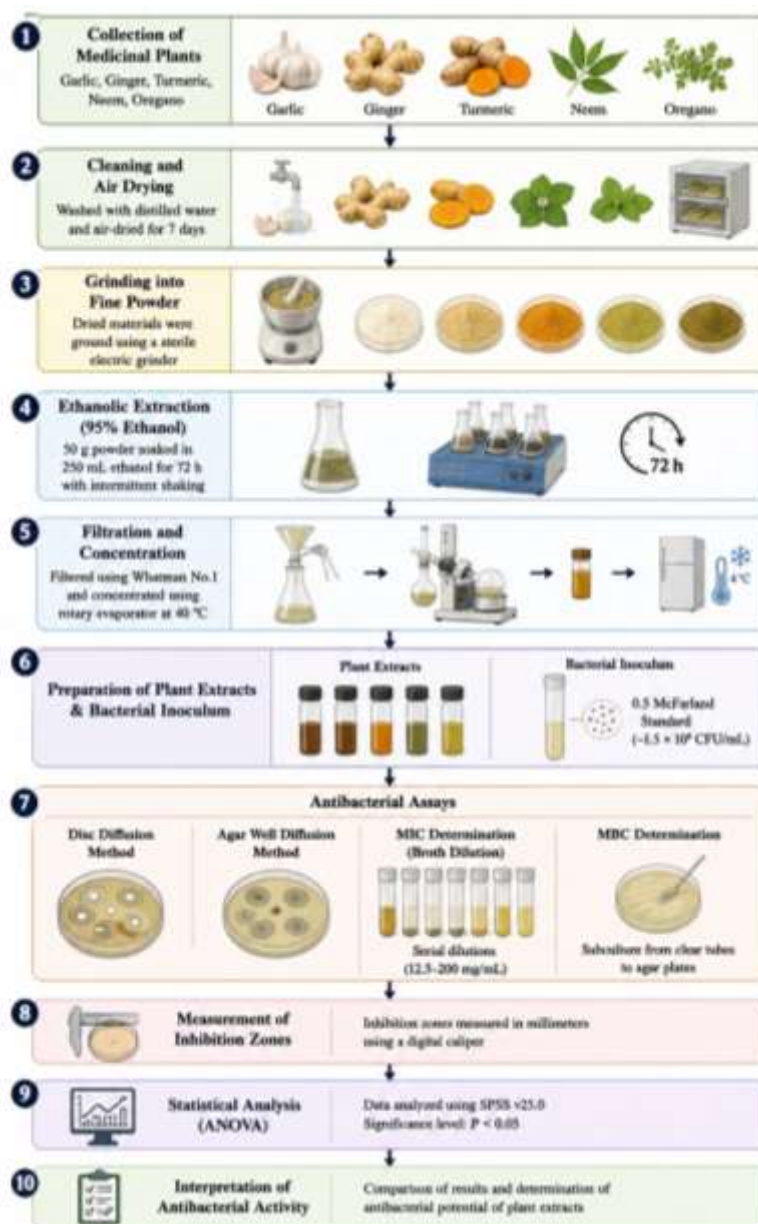


Figure 4. Overview of the Experimental Design and Antibacterial Screening Procedures of Medicinal Plant Extracts Against Clinically Important Bacterial Pathogens

In the present study, the antibacterial activity of some medicinal plant extracts against pathogenic bacteria has been investigated by an experimental method, which is schematically summarized in Figure 4. Collection and processing of plant materials, extraction of phytochemicals by using ethanol, preparation and standardization of bacterial suspension, assessment of antimicrobial activity using conventional disc diffusion and diffusion agar well methods, determination of MIC and MBC values, and statistical analysis and interpretation of the results were carried out (Eloff, 2019).

The current experimental investigation shown that medicinal herbs have strong antibacterial action against pathogenic microorganisms that are clinically meaningful. The antibacterial activity was maximum in oregano followed by garlic and neem. They argue that the high effect of oregano is likely due to the presence of carvacrol and thymol, compounds with known membrane disrupting activity and

known to increase the membrane permeability. Garlic extract exhibited strong inhibitory activity as a result of allicin which also has an inhibitory effect on enzyme systems and protein synthesis of bacteria. Overall, the plant extracts had more impact on Gram-positive than on Gram-negative bacteria. This difference could be because Gram-negative bacteria have an outer membrane that protects them from antimicrobial drugs. The current study's findings are consistent with earlier research showing that medicinal herbs have strong antibacterial action against harmful germs. The observed antimicrobial activity thus indicates that medicinal plants can be used as a potential source of novel therapeutic agents especially for multidrug resistant organisms (Burt, 2004); (Ankri & Mirelman, 1999); (Khameneh et al., 2019); (Cheesman et al., 2017); (Mostafa et al., 2018); (Ríos & Recio, 2005); (Bassolé & Juliani, 2012).

4. Study Limitations

In the present study, a number of limitations were observed in the ability of the medicinal plant extracts found to have a promising antimicrobial activity. The study was carried out in laboratory (in vitro) conditions, which may not fully represent the complex biological environment in humans. Secondly, chemical characterization and isolation of the active phytochemicals of the plant responsible for the reported antibacterial activity was not carried out. Third, no toxicity and safety evaluations were done and so the adverse effect of these plants is unknown. Lastly, there were no studies in vivo or clinical studies to validate the therapeutic effectiveness of the extracts in humans. Further investigations are recommended for extraction of bioactive compounds, analysis of the toxicity profile and the therapeutic application of antimicrobial medicines derived from medicinal plants (Eloff, 2019); (Khameneh et al., 2019); (Cheesman et al., 2017); (Compean & Ynalvez, 2014).

Conclusion

The present laboratory study proved that the plant extracts have good antibacterial activity against the pathogenic bacterial species. Oregano and garlic extracts were found to be the most antibacterial with ginger showing comparatively less antibacterial activity. Mainly bioactive phytochemicals are responsible for the antibacterial activity that interferes with the cellular functions of bacteria.

Medicinal plants could be a good alternative to synthetic antibiotics; they may be helpful to develop new antibiotics. Active compound(s) should be isolated, toxicity data should be collected and clinical trials should be conducted for future use (Silva & Fernandes Júnior, 2010); (Upadhyay et al., 2014); (Srivastava et al., 2014); (Burt, 2004); (Khameneh et al., 2019); (Cheesman et al., 2017); (Mostafa et al., 2018); (Ríos & Recio, 2005); (Bassolé & Juliani, 2012); (Compean & Ynalvez, 2014).

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