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ORIGINAL ARTICLE

Estimation of some Essential oils and study Antibacterial activity of *Rosmarinus officinalis* Extract

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Abstract

The study was done to look into the antibacterial activity of equeous and methalonic extracts of the *Rosmarinus officinalis* leaves on the growth repression of Staphylococcus aureus and Escherichia coli in vitro. The disc diffusion method was employed to determine the susceptibility of the bacteria tested to the two rosemary extracts. In order to compare the activity of two extracts, gentamicin (300 mg/ml) was used as a positive control. The results demonstrated that the two extracts exhibit antibacterial efficacy in inhibiting the development of tested microorganisms. In examined bacteria, the methalonic extract inhibited growth more than the equeous extract, and S. aureus was more susceptible to the action of the two extracts than E. coli. Some of the medically important oils in rosemary have been estimated (Cineole, Camphor, Linalool)%. The results showed that the alcoholic extract gave higher results than the aqueous extract

Keywords: *Rosmarinus officinalis*, pathogenic bacteria, disc diffusion method.

1 Introduction

Bacteria that cause disease have long been regarded as a significant source of illness and deaths in humans. The resistant bacteria paved the way for an increase in the prevalence of illnesses that can only be treated with a limited number of antimicrobial medications. The advent of resistance in gram-negative bacteria poses a significant challenge to antimicrobial curative of infectious illnesses and raises the frequency of complications and death. Antimicrobial resistance among bacteria is a medical issue having public health, societal, and even political implications [1]. Plants have been utilized for millennia as cures and therapies for ailments. With over 2,600 plant species, the Middle Eastern Mediterranean area is rich in plant species, many of which are claimed to have medicinal characteristics. Yet, there has been few research on therapeutic herbs in this region [2]. Plant-derived antimicrobials offer a large untapped supply of medications, and greater research into plant antimicrobials is required as a result. Plants are increasingly being seen as the foundation of contemporary medicine and antibiotic manufacture [3]. Rosemary (Rosmarinus officinalis) is native to southern Europe. Because of its attractive flavor, strong antioxidant activity, and recent usage as an antibacterial agent, its oil and herbs are widely used in culinary preparation as spices and flavoring elements. [4] Rosemary plants have been proven to be high in phenolics with antibacterial activity both against gram-positive as well as gram-negative bacteria. They ascribed a high portion of the antibacterial action to carnosic acid and carnosol. Although rosemary extracts clearly have bioactive characteristics, their antibacterial actions have not been It's widely investigated. The essential oils of plants have been recognized to have antimicrobial properties for millennia,

but their strong flavor has limited their usage in food [5]. Although the antibacterial capabilities of essential oils and their constituents have previously been discussed, the action mechanism has not been thoroughly researched. Given the huge number of distinct chemical groups included in these oils, it's most likely that their anti-bacterial effect is due to several targets in the cell rather than a single mechanism [6]. Rosemary plants' antibacterial efficacy against both gram-positive as well as gram-negative bacteria was also verified. The study aimed to evaluate the antibacterial activity of rosemary leaf extract against a variety of pathogenic isolates.

2 Materials and Method

1. Clinical simples: Thirty urine and blood samples were taken from Yarmook hospital's outpatient clinic.

2. Bacterial isolates: All bacterial organisms were isolated and identified to the species level using various available procedures such as Gram stain and other phenotypic methods in accordance with standardized methods and identified as E. coli and S. aureus, which were tested microorganisms in this study.

3. Plant preparation: Rosmary leaves were acquired from a local market and processed to a fine powder in a combination. The particles were kept in the freezer at -20 ∘C until they were used.

4. Plant extract preparation: 10 g of dry rosemary powder were steeped in 250 ml of 95% methanol and placed in a conical flask for two weeks. Filter sheets were used to filter the entire mixture. The supernatant was taken and stored at 4 ∘C until utilized. The equeous extract made in the same way, but with distilled water rather than methanol.

5. Antibacterial properties of Rosmary leaf extract: The antibacterial activity of Rosemary leaves crude extract was investigated using the standard disc diffusion susceptibility test solid media [7]. A pure bacterial cell culture of each clinical isolate E. coli and S. aureus was produced and was streaked throughout nutritive solid medium in 100 microliters. A 6-mm diameter Whatmann No.5 sterile filter paper was saturated with methalonic and equeous extracts before being placed over the culture media and incubated at 37 degrees Celsius for 24 hours The zone's diameter of growth inhibition surrounding the filter disc was measured and reported in millimeters. Gentamicin (300 mg/ml) solution was soaked in sterile filter sheets. A negative result was defined as any inhibition zone of 7 mm around the filter paper.

6. Determination of certain vitamins in Rosmary aqueous and alcoholic extracts: Using commercial materials and 25ug/ml, an HPLC (High Performance Liquid Chromatography) was utilized to determine the vitamin A and vitamin B content of Rosmary as shown in Table 1 .

Table 1: The time of retention and active compound area.

Subject	Minutes of retention	Area	Concentration
Cineole	3.57	58435	25ug/ml
Camphor	6.33	144393	25ug/ml
Linalool	8.56	188573	25ug/ml

3 Results

Rosmary extracts were investigated for antibacterial efficacy against (E. coli) a G-ve as well as (*S. aureus*) a G+ve bacteria. According to Tables 2 and 3, the two extracts exhibit a broad spectrum of inhibitory action against both bacteria. In G+ve bacteria, the two extracts are more active than in G-ve bacteria. The suppression of growth by two extracts was compared to gentamicin, a standard antibiotic. (1), (2), (3), and (4) are diagrams.

Table 2: Antimicrobial activities of methalonic extract of Rosmary leaves in examined bacteria.

Rosmary extracts	Tested bacteria	Inhibition zone (mm)
	S. aureus	19
	E.coli	15
	S. aureus	24
	E.coli	22

Table 3: The antibacterial activity of a rosemary leaf equeous extract against the microorganisms examined.

Rosmary extracts	Tested bacteria	Inhibition zone (mm)
Equeos extract	S. aureus	17
	E.coli	14
Gentamicin (300mg/ml)	S. aureus	24
	E.coli	22

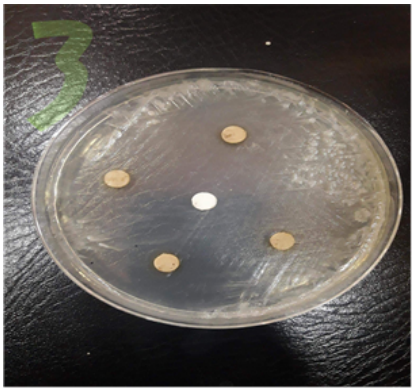

Figure 1: Zone of *S. aureus* growth suppression by Rosmary Methalonic extract

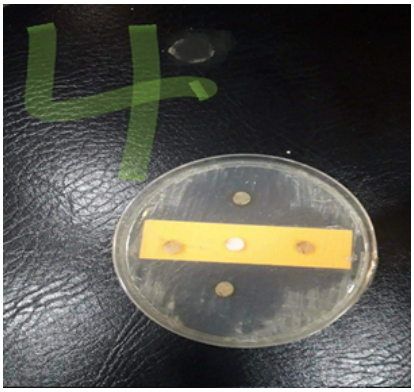

Figure 4: Rosmary equeous extract inhibits *E. coli* growth zone.

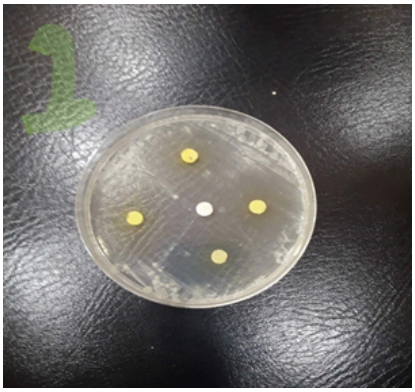

Figure 2: Growth inhibition Zone of *S. aureus* by Rosemary aqueous extract.

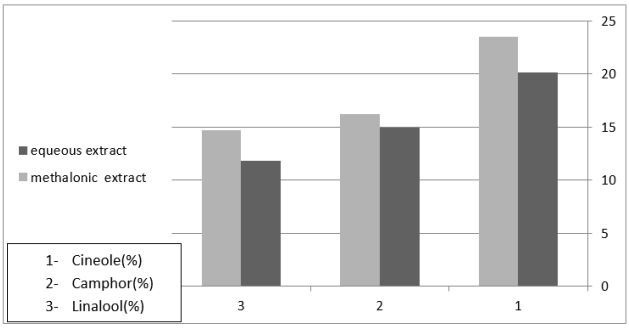

Figure 5: Concentration of certain essential oils in Rosmary aqueous and alcoholic extracts

The results shown in Figure 5 indicate that the alcoholic extract of rosmary gave the highest concentrations of Essential oils Cineole, Camphor and Linalool reached (23.5 ,16.2,14.7) % sequentially The aqueous extract gave the lowest concentrations of (20.12,14.89,11,82) % sequentially

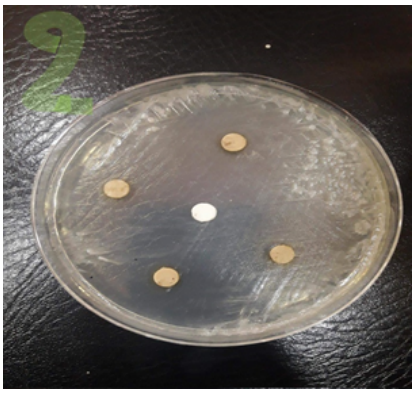

Figure 3: Rosmary Methalonic Extract inhibits the growth zone of *E. coli.*

4 Discussion

The diffusion results confirm and expand prior discoveries that rosemary contains multiple biologically active chemicals, some of which are commonly utilized in folk medicine for their antibacterial qualities. The presence of diterpenes (carnosol) might explain rosemary's biological action against the tested microorganisms [8, 9]. authors [10–12] have found that rosemary has excellent to moderate antibacterial activity because the extract of rosemary leaves includes α-pinene and compher, which are responsible for antimicrobial action [13]. Rosmarinic leaf extracts had greater antibacterial activity. As previously stated, carnosic acid is the primary bioactive antibacterial component of rosemary [4]. This might explain this for gram-negative bacteria. Carnosic acid

is more effective than rosmarinic acid against gram-positive bacteria [14] Based on this data, it is obvious that rosmarinic leaf extract has many modes of action and greater biological activity towards gram-positive bacteria. In gram-negative bacteria, the outer membrane enclosing the cell wall and the per plasmatic area holding enzymes capable of breaking down foreign compounds given from outside may be the basic rationale for differences in bacterial sensitivity [15]. Natural medicines such as It has been demonstrated that rosemary oil is considerably safer than traditional antibiotics. Another benefit of essential oils is their extensive antimicrobial action [16]. The acquired results may differ from prior publications due to variances in extract composition, which might be attributable to seasonal fluctuation, the extraction techniques, as well as the environmental conditions, or to the plant's nutritional status. Moreover, E. coli, which is resistant to several antibiotics, is susceptible to both rosemary extracts; hence, the extracts containing the essential oils of this plant can be employed by the pharmaceutical industry to develop innovative synthetic medications for the treatment of infectious diseases. These are consistent with the findings[17–19].

5 Conclusion

Based on the study's findings, it can be inferred that the alcoholic extract was more successful in suppressing and increasing the amount of rosemary in essential oils.

Conflict of Interest: No conflicts of interest exist between the authors and the publication of this work.
Ethical consideration: The study received approval from the university's ethical council at Medical Technical Institute-Mansour, Middle Technical University, Baghdad, Iraq.

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