INNOVARE JOURNAL OF LIFE SCIENCE

Vol 10, Issue 6, 2022

ISSN - 2321-550X

Research Article

IN VITRO ANTIBACTERIAL ACTIVITY ROSEMARY OIL AGAINST *ESCHERICHIA COLI* ISOLATED FROM CLINICAL SAMPLES IN SYRIA

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Received: 01 October 2022, Revised and Accepted: 17 October 2022

ABSTRACT

Objective: The aim of this research is to determine the antibacterial activity of rosemary oil against *Escherichia coli* that causes the urinary tract infection) UTI).

Methods: An *in vitro* study was carried out using the following bacterial strains involved in UTI diseases using well diffusion (WD) testing: *E. coli* (ATCC 25922) and 25 strains were compiled from Aleppo Hospital. It was from people and has UTI. The antibacterial activity of rosemary oil was determined in the form of inhibition zone using agar WD testing.

Results: The obtained results indicated that rosemary oil has an inhibitory effect on *E. coli* (ATCC 25922) and 10 strains. This study showed that rosemary oil has antibacterial activity.

Conclusion: Rosemary oil has shown an antibacterial effect that causes UTIs, so we suggest using this oil in the treatment of UTIs.

Keywords: *Escherichia coli*, Rosemary oil, Well diffusion testing.

 DOI: http://dx.doi.org/10.22159/ijls.2022v10i6.46501. Journal homepage: https://innovareacademics.in/journals/index.php/ijls

INTRODUCTION

Escherichia coli is an important cause of urinary tract infections (UTIs). It is one of the main causes of nosocomialacquired UTIs (30–50%). *E. coli* is a normal intestinal and reproductive flora, but it can ascend the urethra and enter the urinary tract. These strains harbor a variety of virulence factors that allow them to establish an infection, including adhesions and toxins [1].

UTIs are one of the most common bacterial infections worldwide. It is estimated that the number of cases is increasing annually all over the world. Although this infection is treatable, organismal multidrug resistance leads to complications, treatment failure, and increased mortality and morbidity [1].

The choice of medication to treat a UTI and the duration of treatment depend on the patient's history and the type of bacteria causing the infection. Traditional medicines for treating UTIs are associated with serious side effects on human health. It is therefore necessary to explore an alternative antibacterial drug that can be used to treat UTIs with less drug resistance, more efficacy, and fewer side effects [2].

In recent studies, there is an interest in the introduction of medicinal plants and their extracts as an alternative treatment to traditional medicines, especially antibiotic-resistant treatments.

Therefore, in this research, the effect of rosemary oil as an antibacterial for *E. coli* isolated from samples of people with UTIs was studied.

The rosemary oil is one of the most popular and widely used essential oils, mostly because of its main components, camphor. The previous studies have shown antibacterial, antifungal, antibiofilm formation, and antioxidant activities [3,4]. The rosemary oil is reported to have strong antibacterial activity against many species. The main goal of this study is to examine the antibacterial activity of rosemary oil against *E. coli* isolated from urine samples of Syrian people with UTIs.

METHODS

Materials

The rosemary oil was purchased from AL-Asi laboratory. Ceftriaxone (CET) was a kind gift from Alpha Pharmaceutical Company, Syria. Dimethyl sulfoxide (DMSO) was purchased from Sigma.

Samples

A total of 25 urine sample were collected using sterile urine cap from people with renal failure who visited Aleppo University Hospital in Aleppo city, and belonging to different age groups ranging from 25 to 65 years. All specimen was transported immediately to the laboratory and culturing within the 2 h after the sample collection. *E. coli* (*ATCC 25922*) was kindly donated by Aleppo University Hospital.

E. coli isolation

Urine samples were cultured on eosin methylene blue agar (EMB) plates and incubated at 37°C and examined for its growth at 24 h. The culture plates were examined for the appearance, size, color, and morphology of the colonies. Fig. 1 is shown the colony's *E. coli* in Agar EMB agar.

Identification of *E. coli* isolates

E. coli was identified by the shape and color of the colonies, in addition to using biochemical tests, and the following Table 1 shows the biochemical test results.

Preparation of rosemary oil solution

In order to assay the antibacterial activity of rosemary oil on *Escherichia coli*, 100 µl of rosemary oil is prepared at a concentration of 30 µg/well. Ceftriaxone (CET) (100 µL at a concentration of 3 mg/10 mL, equivalent to 30 µg/well) was used as positive control and DMSO as a negative control.

Antibacterial test using the agar diffusion method (well)

The well diffusion test was carried out with Muller-Hinton agar (MHA). The inoculum was prepared using 24-h plate cultures of *E. coli*.

The colonies were suspended in 0.85% saline, and the turbidity was compared with the 0.5 McFarland standard (equal to 1.5×10^8 colony-forming units/ml). The suspension was loaded on a sterile cotton swab that was rotated several times and press firmly against the inside wall of the tube to remove excess inoculum from the swab. The dried surface of an MHA agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated 2 more times, rotating the plate approximately 60° each time to ensure a uniform distribution of inoculum. Next, where 7 mm wells were cut and filled with 100 µL of rosemary oil at a concentration 30 µg/well. Ceftriaxone (CET) (100 µL at a concentration of 3 mg/10 mL, equivalent to 30 µg/well) was used as positive control and DMSO as a negative control. The Petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples [5]. Then, the plates were incubated at 37°C±1°C for 24 h. The antibacterial activity was determined by measuring of inhibition zone diameters (mm) and was evaluated according the parameters suggested by [6]: Inhibition zones <9 mm, inactive; 9–12 mm, less active; 13–18 mm, active; >18 mm, very active [7]. All assays were performed in triplicate and repeated at least 3 times.

Fig. 1: *Escherichia coli* colonies on EMB agar

Table 1: Biochemical identifications of the isolated *E. coli*

Biochemical test	*E. coli*
Indole	Positive
Methyl red test	Positive
Urease	Negative
Catalase	Positive
Oxidase	Negative
Nitrate	Positive

Statistical analysis

The statistical study of the collected data was carried out using the Excel program and the alpha value was calculated for these data.

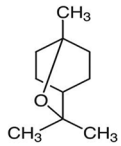
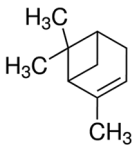
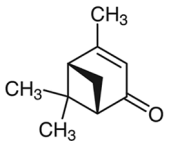
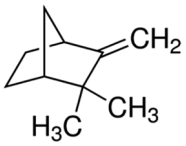
Chemical composition of rosemary oil

A total of 30 constituents, representing 96.2–98.2% of the total oil composition, were identified. Major constituents of the oil were camphor (23.9–35.8%), 1,8-cineole (18.0–23.9%), α-pinene (4.5–14.4%), verbenone (6.5–12.4%), camphene (2.5–6.9%), limonene (2.1–2.8%), bornyl acetate (1.1–4.1%), α-terpineol (1.9–3.6%), and β-pinene (2.1–3.3%) [8]. The following Table 2 provides information about the three most important components of rosemary oil [9-11].

RESULTS AND DISCUSSION

Essential oils are classified as "Generally Recognized as Safe" by the food and drug administration, and therefore are not harmful and, due

Table 2: The chemical structure of some compounds in rosemary oil

Chemical name	IUPAC name	Information	Compound structure
Camphor	1,7,7trimethylbicyclo [2.2.1]heptan-2-one	MW: 152.23 g/mol MF: C10H16O H-bond donor: 0 H-bond acceptor: 1 XLog p: 2.2	
1,8-cineole	4,6,6-trideuterio-1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane	MW: 157.27 g/mol MF: C10H18O H-bond donor: 0 H-bond acceptor: 1 XLogP: 2.5	
α-pinene	2,6,6-trimethylbicyclo [3.1.1]hept-2-ene	MW: 136.23 g/mol MF: C10H16 H-bond donor: 0 H-bond acceptor: 0 XLogP: 2.8	
verbenone	4,6,6Trimethylbicyclo [3.1.1]hept-3-en-2-one	MW: 150.22 g/mol MF: C10H14O H-bond donor: 0 H-bond acceptor: 1 XLogP: 1.6	
camphene	2,2-dimethyl-3-methylidenebicyclo [2.2.1]heptane	MW: 136.23 g/mol MF: C10H14O H-bond donor: 0 H-bond acceptor: 1 XLogP: 1.6	

to their natural origin, are more widely accepted by consumers than "synthetic" agents.

In this study, we conclude from the statistical study that there is no significant difference between the effect of rosemary oil and ceftriaxone as an inhibitor of *E. coli*, as the alpha value appeared to be 0.26, and this means that the difference is not significant, as shown in Diagram 1.

Table 3: Antibacterial activity of rosemary oil against *E. coli*

***E. coli* isolates**	**Diameters of inhibition zone (mm)**	
	Rosemary oil	**Ceftriaxone**
ATCC 25922	40	35
1	32	30
2	35	30
3	38	32
4	35	32
5	25	20
6	35	35
7	30	30
8	38	35
9	32	30
10	30	30
11	33	32
12	30	28
13	12	0
14	28	25
15	3	25
20	10	0
21	22	0
22	35	32
23	15	5
24	20	10
25	10	0

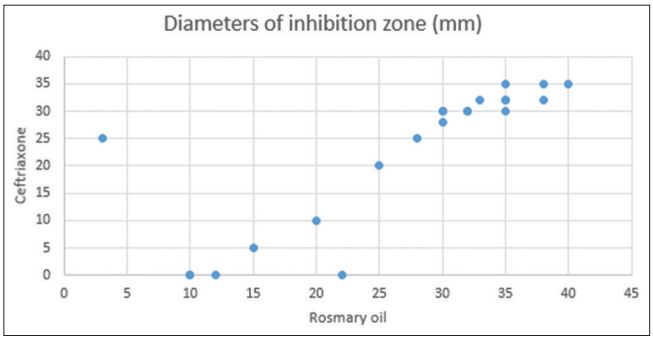


Diagram 1: Means the difference is not significant

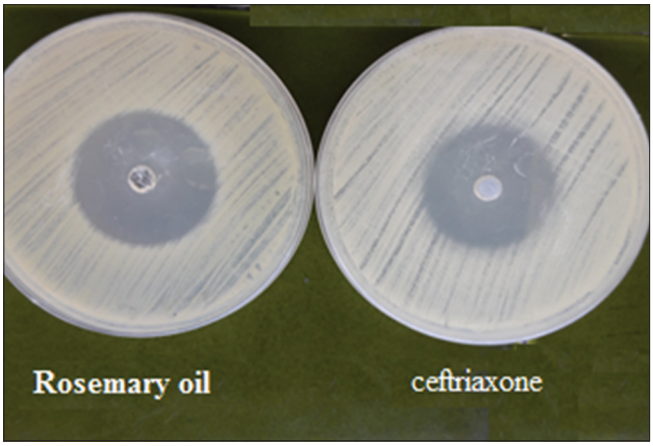


Fig. 2: Agar well diffusion assay showing inhibition zones, antibacterial activity of rosemary oil, and ceftriaxone against *Escherichia coli (ATCC 25922)*

It was also found that rosemary oil had an effect on bacteria that were resistant to ceftriaxone, as shown in Table 3.

Therefore, we conclude from this study that rosemary oil has a good effect as an inhibitor of *E. coli*, which is close to the effect of ceftriaxone and we suggest that antibacterial activity of rosemary oil is largely also due to presence of camphor, which is the major component of rosemary essential oil. Many reports suggest that the Antibacterial mechanism of camphor is due to membrane damage. In addition, camphor, known to be a lipophilic compound, can enter between the fatty acid chains that make up the membrane lipid bilayers, thus altering the fluidity and permeability of cell membranes. Our results assess a direct and a clear sight at the role of rosemary oil as shown in Fig. 2.

This supposes that cell lethality was a consequence of cellular lysis. The results of our study strongly support the idea that the Antibacterial activity of rosemary oil is due to the disruption of the membrane, leading to cell death [12,13].

CONCLUSION

Rosemary oil shows antibacterial activity against *E. coli*.

We assume that rosemary oil, due to the properties of its lipophilic components, penetrates the cell wall and membrane and interacts with it, causing the destruction of the cell wall and membranes, which then leads to the loss of vital intracellular substances, which leads to the death of the germ cell.

Therefore, rosemary oil can be considered as a new and promising alternative treatment against bacterial agents, which deserves further studies using quantitative methodology and clinical laboratory correlations to better define sensitivity limits and appropriate treatment protocols.

ACKNOWLEDGMENT

We would like to thank Dr. Samer Haj Kaddor for generous help on research. Microbiology Laboratory (Aleppo University Hospital, Syria) and Ebla Private University supported this work.

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