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Original article

Antimicrobial Effect of Aqueous Extract of *Moringa oleifera* and *Juniperus oxycedrus*

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Abstract
The present study set out with the objective of investigating the effect of aqueous extracts of *Moringa oleifera* and *Juniperus oxycedrus*. leaves on four isolates of bacteria: two Gram-negative (*Escherichia coli* and *Salmonella*) and two Gram-positive (*Streptococcus* and *Staphylococcus aureus*). The estimation of antibacterial activity was conducted by utilising the Disc Diffusion Technique. The aqueous extracts of *Moringa oleifera* and *Juniperus oxycedrus*. leaves, when desiccated, are the subject of this study. The extract of Juniperus exhibited sensitivity at rates ranging from 0.2 to 0.7 mm to bacterial isolates of the gram-positive *Streptococcus* and *Staphylococcus aureus*, respectively. Furthermore, an inhibitory zone of 0.4 mm was demonstrated for both gram-negative *E. coli* and *Salmonella*. The aqueous extract of *Moringa oleifera* leaves demonstrated antibacterial properties, exhibiting inhibitory zones of 0.6 mm, 0.7 mm, and 0.1 mm against bacterial strains *Streptococcus, Salmonella,* and *E. coli,* consecutively. However, no inhibitory effect was observed against *Staphylococcus aureus*. A comprehensive investigation into the impact of varying concentrations of *Moringa oleifera* and *Juniperus oxycedrus* extracts on bacterial isolates, employing a range of solvents, is warranted.
Keywords. *Moringa Oleifera*, *Juniperus Oxycedrus*, Inhibition Zone, Gram-Positive and Gram-Negative Isolates, Aqueous Extract.

Introduction

Medicinal and aromatic plants and herbs represent some of the most ancient botanical specimens known to humankind. The intensive and prolonged use of synthetic drugs, including antibiotics, has been demonstrated to engender a plethora of deleterious side effects on human health, including allergies and poisoning. Moreover, the misuse of these antibiotics has resulted in the emergence of numerous symptoms, ultimately leading to the development of resistance in disease-causing microbes through mutations [1]. The *Moringa oleifera* and *Juniperus* trees are utilised in their entirety, encompassing the leaves, branches, seeds, fruits, and roots. The leaves constitute the most frequently utilised component. *Moringa oleifera* has been employed since antiquity in dietary regimens, owing to its numerous biological properties, including its capacity to reduce inflammation, prevent tumours, and combat cancer, as well as its anti-diabetic and antimicrobial properties [2]. The present study set out to investigate the antimicrobial action of *M. Oleifera* on *Bacillus subtilis*, *Serratia marcescens,* and *Mycobacterium phaeum*. The resultant data demonstrated that B. subtlis was completely inhibited at a concentration of 56 µmol/L, while M. pheli was inhibited at a concentration of 40 µmol/L. In the concentration range under consideration, only partial inhibition was observed for *S. Macesscens* [3]. As demonstrated by Thilza et al. (2010), Moringa leaves exhibited a mild degree of activity in the presence of E. coli and Enterobacter. Moreover, it has been demonstrated that the extraction of chloroform and ethanol from Moringa seeds has a suppressive effect on the proliferation of *E. coli* [5]. The extraction of methanol from *Juniperus* has been demonstrated to exert an inhibitory effect on the growth of a multitude of strains, encompassing many distinct bacterial strains belonging to *Acinetobacter, Bacillus, Enterobacter, Escherichia, Pseudomonas*, and *Staphylococcus*. The present study has been conducted for the purpose of investigating the inhibitory effect of aqueous extracts of *Moringa oleifera* and *Juniperus* spp. leaves Against four isolates of bacteria: two Gram-negative (*Escherichia coli* and *Salmonella*) and two Gram-positive (*Streptococcus* and *Staphylococcus aureus*).

Methodology

Bacterial isolates

Five bacterial isolates, which were identified and tested in the microbiology laboratory at the Joint Diseases Unit, Faculty of Medicine, University of Sirte, are shown in (Table 1).

Table 1. Bacterial Genera and with indication of their isolation source

Site of isolation	Bacterial Isolates
The presence of the infection was localised to the nasal cavity of an infected person.	Staphylococcus aureus
This genus was isolated from the oral environment.	Streptococcus
The isolation of *Salmonella* was performed in the bathroom environment.	Salmonella
The isolate was derived from a specimen obtained from a bathroom environment.	E. coli

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Antibiotic
In order to establish a comparison between the effectiveness of plant extracts and some commonly used commercial antibiotics in medical centres, the antibiotic Ceftriaxone was utilized.

Preparation of aqueous plant extracts
The plants selected for the study were collected, thoroughly washed with distilled water, and then dried by spreading them out on paper and placing them in a shaded area for 10 days. Subsequently, the plants were subjected to a grinding process using an electric grinder, following the removal of the hard, woody parts. This procedure was undertaken to yield a powder form of the plants. The plant extracts were prepared by infusion [6]. Forty grams of each of the plant powders under study were placed in 250 ml of distilled water and left to infuse for 24 hours in a shaker. The precipitate was then removed by filtering the plant extracts through sterile gauze. The extracts were then subjected to a centrifugal process at a speed of 4000 rpm for a duration of ten minutes. The filtrate was collected, transferred into sterile bottles, and stored in the refrigerator until use [7].

Gram stain test
Gram staining is a technique used to differentiate between two types of bacteria: those that are positive (purple) and those that are negative (red). This discrepancy can be attributed to the variation in the structure and chemical composition of the cell wall [8].

Preparing the culture medium
A quantity of 10 g of Mueller Hinton II Agar medium is to be placed into 250 ml of distilled water. The solution should then be transferred to a heating device (Heter) until the powder has fully dissolved. Following this, the item should be placed in the autoclave for a period of 15 minutes at a temperature of (121 °C), which is equivalent to (1) atmosphere pressure, for the purpose of sterilization. Subsequently, permit the substance to cool to a slightly lower temperature before decanting it into dishes in a sterile medium and allowing it to solidify. The bacterial species targeted in the study were cultivated on the nutrient medium using the planning method and then incubated at a temperature of 37°C for 24 hours.

Performance of the sensitivity test
The Disc Diffusion Technique [9] was utilised in this study. Nutrient agar was dispensed into three separate plates for each extract. A bacterial suspension was then prepared from a 24-hour culture by collecting 4-5 pure single colonies from the surface of the medium using a sterile loop. These colonies were placed into a test tube containing 2 ml of distilled water, and the mixture was agitated using a vortex mixer. Subsequently, bacterial species were cultivated on the surface of the medium through the application of a sterile cotton swab. This process entailed the removal of excess bacterial suspension by firmly pressing the swab against the interior walls of the test tube. The cultures were then distributed equally using an agar strip technique. Filter paper discs (5 mm in diameter) were immersed in the plant extracts for a period of one hour until they had been saturated. Subsequently, three discs of each extract were meticulously placed into each plate using sterile forceps. In a similar manner, tablets were immersed in the antibiotic (Ceftriaxone) until they were saturated as an indicator on the surface of the prepared plates. Subsequently, all the plates were placed in the incubator at a temperature of 37°C for 24-72 hours, depending on the growth period of each bacterium.

Results and Discussion
The differentiation of the isolates was achieved through the implementation of a Gram stain, which was based on the observed variation in the structure of the bacterial cell wall, as determined through microscopic examination. The gram-positive isolates, namely *Staphylococcus aureus* and *Streptococcus*, exhibited a purple staining reaction and were thus designated as gram-positive. Conversely, the gram-negative isolates, comprising *Salmonella* and *E. coli*, exhibited a pink staining reaction and were designated as gram-negative.

Table 2. The mean diameters of the inhibition zones of the tested bacteria (in millimeters)

	average diameters of inhibition zones (in mm) for the tested bacteria		
Bacterial isolates	**Ceftriaxone**	**Juniperus**	**Moringa**
Staphylococcus aureus	1.9	0.2	_*
Streptococcus	1.9	0.7	0.6
Salmonella	1.9	0.4	0.7
E. coli	1.9	0.4	0.1

**(-) means no inhibition*

Malu et al. (2009) reported the findings of research which indicated that extracts of Juniperus phoenicea possess antibacterial properties and could be used for the treatment of bacterial infections. The isolates that

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were the subject of this study demonstrated sensitivity to juniper (*Juniperus*) extract at rates ranging from 0.2 mm to 0.7 mm (see Table 1 and Figure 1). These results were consistent with the aqueous and methanol extracts of Juniperus oxycedrus, which have been demonstrated to exert an inhibitory effect on the growth of *Acinetobacter, Bacillus cereus, Bacillus substilis, Escherichia coli,* and *Pseudomonas aeruginosa* [11]. The extraction of Juniperus phoenicea has been shown to significantly inhibit the growth of E. coli, with a greater effect observed against Staphylococcus aureus and Pseudomonas aeruginosa bacteria [12]. The essential oil of *J. phoenicea* contains chemical compounds, including α-pinene, terpinolene, and carene, which have the capacity to induce an antimicrobial effect against a wide range of bacterial strains [13].

The Moringa (Moringa oleifera) extraction exhibited no inhibitory activity against the growth of *Staphylococcus aureus*, yet it demonstrated efficacy against the other isolates, with inhibition diameters ranging between 0.1 mm and 0.6 mm (see Table 1 and Figure 2). As demonstrated by Abdul-Razzaq et al. (2015), the methanolic and aqueous extracts of dried Moringa leaves exhibited the highest inhibition rates against all Gram-negative strains of *E. coli* and *Pseudomonas,* as well as the Gram-positive bacteria *Staphylococcus aureus*. In addition, *Moringa oleifera* exhibited antimicrobial activity against E. coli, *Staphylococcus aureus,* and *Bacillus subtilis* through water extraction [15]. These findings are consistent with those of Singh demonstrated in (2013), aqueous, ethanolic, methanolic, and hexane extracts of Moringa leaves exhibited an inhibitory effect against several bacterial strains, namely *Staphylococcus* aureus, *Pseudomonas*, and *E. coli.* The concentration of 5 mg/ml of leaf acetone extract of *Moringa oleifera* exhibited antibacterial activity against *E. coli*, *Proteus vulgaris,* and Staphylococcus aureus [17].

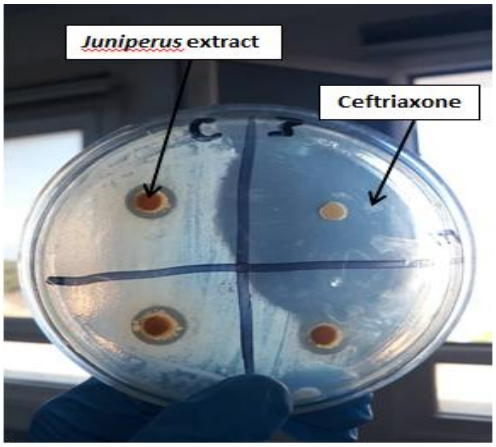

Figure 1. the impact of Juniperus extract on the proliferation of E. coli in the presence of the antibiotic Ceftriaxone

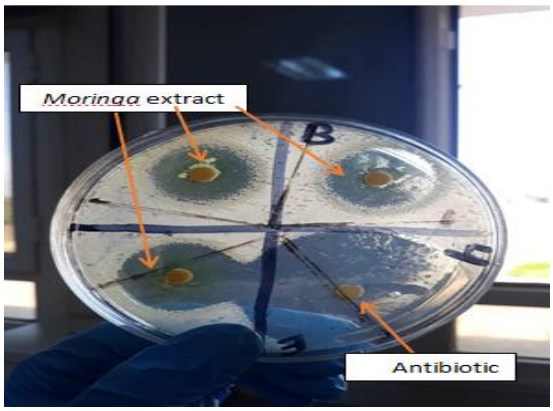

Figure 2. the impact of Moringa extract on the proliferation of E. coli in the presence of the antibiotic Ceftriaxone

Conclusion

The present study investigates the effect of cold aqueous extracts of moringa and juniper on the inhibition of the growth of *Streptococcus, Salmonella, Escherichia coli*, and *Staphylococcus aureus*. Juniper and Moringa extracts demonstrated the least potent inhibitory effect on *Staphylococcus aureus* and *E.coli*, exhibiting a zone of inhibition measuring 0.2 mm and 0.1 mm, respectively. The present study provides evidence to demonstrate the effectiveness of aqueous extracts of *Moringa oleifera* and *Juniperus oxycedrus* in combatting selected bacterial isolates. Further research is required to investigate the impact of different concentrations of *M. oleifera* and *J. oxycedrus* extracts, as well as the use of alcohol solvents such as ethanol and methanol, on the various isolates of Gram-negative and Gram-positive bacteria.

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Conflict of interest. Nil

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