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RESEARCH ARTICLE

Estimation of Invitro Antibacterial Activity of Ethanolic Extract of *Olea Europaea* (Olive) and *Rosmarinus Officinale* (Rosemary) Plant Leaves

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ABSTRACT

Medicinal use of plants for the treatment of various diseases has existed since prehistoric era. From ancient times these plants were used in different traditional systems such as traditional chinese medicine (TCM) Ayurveda and homeopathy. Until now a generous portion of the world's population still uses them to treat their aliments. In this study, the antibacterial activity of ethanolic extract of olive and rosemary leaves was investigated against *(E. coli, S. aureus, B. subtilis* and, *Pseudomonas aeruginosa)* by disk diffusion method and correlated the results with the inhibitory zone of gentamicin as standard. The results show that the ethanolic extract of olive and rosemary leaves has antibacterial activity against all four of these bacteria. For olive leaves *E.coli* shows the zone of inihibiton of 11 mm, *S. aureus* shows 11.5 mm, *B. subtilis* shows 7 mm and *P. aurgenosa* shows the zone of 10 mm. For Rosemary the zones of inhibiton for *E.coli* and *S. aureus* was 8.5 mm, *B.subtilis* shows 11mm and *P. aurgenosa* shows zone of 15 mm. These results show that both olive and rosemary leaf extracts have the potential to (A.H) develop new antibacterial agents.

1. Introduction

The use of plants for the treatment of various ailments dates back to the prehistoric age. Therapeutic knowledge of these medicinal plants was spread in different parts of the world through ancestral and experienced healers who transferred their knowledge to their students and so on, with time by the advancement of medicinal science, these plants became the center of attention for various traditional systems of medicine such as Chinese traditional medicine, ayurvedic medicines and allopathic system of medicine [1]. The medicinal value of plants is due to the presence of certain metabolites which possess some therapeutic effects. Due to these active ingredients, about 80% of the world's population still use these plants extracted for the treatment of their diseases [2].Medicinal plants act as a natural source of many active ingredients that can be used as a source of active ingredients for drug development. Not only do these plants serve as a great source of nutrition which further aids in human health. Some of these plants are ginger, olives, rosemary, and many more. These plants show antiviral, antibacterial, cardioprotective, antihypertensive, and antiatherogenic properties [3]. Like all of these plants olive and rosemary are those plants that show some great therapeutic potential. *Olea europaea* L., which is typically known as an olive tree is a woody green tree having fusiform coriaceous leaves which are grayish green in color and about 1 to 1.5 cm wide and 5 to 6 cm long. The main reason for these beneficial effects of olive leaf extract is the presence of its phenolic compounds known as bio phenols.

Among them, the most important bio phenol is oleuropein, which belongs to a group of coumarin-like substances known as secoiridoids. Oleuropein is responsible for antioxidants, antimicrobial, antiviral, antihypertensive, cardioprotective, and antiatherogenic properties of olive lef extract [4]. It also has hypocholesterolemic and hypoglycemic properties which further aid the beneficial effects on human health [5]. Other than all these health benefits, olive leaf extract shows potent antibacterial activity against *Staphylococcus aureus*, *pseudomonas aeruginosa*, *B. subtilis*, and *E. coli* [6]. *Rosmarinus officinalis* is commonly known as rosemary, a bushy evergreen shrub that belongs to the sub-Himalayans and Mediterranean region used in traditional medicines for the treatment of pain, spasms, headaches, neuralgia, insomnia, migraine, and depression. Other than this rosemary shows significant antioxidant, anti-bacterial, anti-inflammatory, anti-apoptotic, antinociceptive, and antitumorigenic properties. It also shows beneficial effects on mood, sleep, memory, anxiety, and pain [7] Rosemary leaf extract is enriched with many active ingredients like phenolic acids such as 4-hydroxybenzoic acid, rosmarinic acid, caffeic acid, and coumaric acid, flavonoids like diosmetin, luteolin, diosmetin, apigenin and hesperidin [8]. The major active ingredients of rosemary responsible for antibacterial activity are rosmarinic acid, epirosmanol, rosmaridiphenol, and isorosmanol, camphor, α-pinene, carvacrol and bornyl acetate and the ingredients responsible for the antioxidant activity are carnosol [9]. The antibacterial effect of rosemary occurs when these substances interact with the cell membranes and cause changes in their genetic material and nutrition. They further change the electron transport chain and alter the proteins in the membrane which affects the structure and function of bacteria [10] Olive leaf extract (OLE) serves as an antimicrobial plant and antioxidant, because of its catastrophic effects on *Staphylococcus aureus Pseudomonas aeruginosa*, and *E.coli.* It is proven that the oleuropein shows complete antibacterial activity against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*, at the concentration of 0.4%[11]. OLE (olive leaf extract) is also prove to be most effective against *Staphylococcus aureus* including *methicillin-resistant S. aureus* (MRSA) with a MIC value of 0.31% to 0.78% [12]. Ethanoic extract of rosemary leaf shows great antibacterial activity against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa*, and *bacillus cereus* [13]. Besides all, still there is a need to study these plant extract at various conditions, concentrations, and on different microorganisms to fully understand the efficacy of these plant extract and to qualify them to be used in future pharmaceutical industries. The aim of this study is to evaluate the antibacterial efficacy of olive and rosemary leaf extract against *Staphylococcus aureus, Escherichia coli, B.subtilis,* and *Pseudomonas aeruginosa.*

2. Materials and Methods

2.1. Place of study

This study was conducted in the pathobiology lab of Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan, Quetta.

2.2 Collection of ethanolic extracts of plants

The olive and rosemary plants will be collected from the Balochistan Agriculture Research and Development Center (BARDC), Quetta, and will be processed for ethanolic extraction in the nutrition and Toxicology lab (CASVAB) University of Balochistan Quetta. After collection, the leaves of plants were placed separately on a paper at 35℃ for about 72 hours. After drying the powder was made by crushing the plant leaves in a mortar. Then the powder was mixed with 80% ethanol by using a modified massage method at 1:10 m/v for 18 minutes at 40℃. Then the extract was condensed through a rotary apparatus at 50℃ at the speed of 80rmp for about 10 minutes to get the desired condensed form of extract.

2.3 Preparation of bacterial culture

Bacterial strains of *staphylococcus aureus, E. coli, Pseudomonas aeruginosa,* and *B. subtitles* were obtained from the seed bank of CASVAB. The culture strains were pre characterized. After collection, the bacterial strains were inoculated into double strength brain-heart infusion and incubated them for 24 hours.

2.4 Comparison with McFarland standard

0.5 McFarland standard was made by mixing 0.5 ml 1.17% of barium chloride with 99.5 ml of 1% sulfuric acid. Then the turbidity of bacterial culture was set by comparing bacterial culture with McFarland.

2.5 Preparation of plates for antibacterial

The plates were autoclaved for about 15 minutes at a set temperature of 121℃ to prepare the plates for antibacterial susceptibility testing. After cooling we labeled the plates with the name of bacteria, positive control, negative control, and the plant extract. To prepare the media Mueller Hinton agar (MHA) was made by mixing 38 g of Muller Hinton agar powder with 1 liter water and autoclaving it for 15 minutes at 121℃. After cooling down the media the plates were filled at 4mm of depth. After that, the plates were rested on laminar flow to solidify and then we put the plates in an incubator to remove any excess moisture. A total 8 plates were made 2 for each bacterium as per previously established protocol.

2.6 Transfer of bacterial pathogen to MHA plates

To transfer bacterial pathogens on sterile plates. The loopful culture was taken with a cotton swab and streaked onto the agar plate. Then the plates were allowed to dry for a few minutes under laminar flow.

2.7 Antibacterial susceptibility testing.

Paper disks were made through Whatman filter paper which was about 2mm in size. Then the disks were dipped in the extract and then allowed to dry under laminar flow. The sterile forceps were used to transfer the disks on the plates to their designated areas. The olive and rosemary extracts were placed on their portions and control disks of antibiotic (gentamicin) and a disk that was not dipped in any extract were placed on their portion. After that, we slightly press them to ensure complete contact. The plates were then incubated for 24 hours at 37℃. After 24 hours we checked the results. To measure the zone of inhibition we took the diameter of a clear zone of inhibition. The size was measured using a ruler. The zones were measured in mm. Then the zones were compared with the zone of inhibition of gentamicin.

3. Results

In this study, we use olive and rosemary leaf extracts for the comparative analysis of their antibacterial activity with gentamicin. All the bacterial pathogens (*Staphylococcus aureus, E. coli, P. aeruginosa,* and *B. subtilis*) were exposed to plant extracts of olive and rosemary leaves. They showed varied antibacterial activity. The highest value of antibacterial activity was shown by rosemary extract on *P. aeruginosa* which is 15mm±0.21. The olive extract shows the highest antibacterial activity against *S. aureus* which is 11.5mm±0.21(Table.1; Fig.1). The p value of the data was calculated using MS Excel. The p-value of the data is ($p>0.05$). For statical analysis, the numeric data were presented in the form of standard deviation and mean. One-way ANOVA test was performed to find out the differences among the groups. Bar graphs were also made using MS Excel. The comparative data of olive leaf extract with gentamicin is given below.

The mean value of olive leaf extract (OLE**)** is 11±1.8mm which when compared to gentamicin is less than 15mm which is the minimum inhibitory concentration of gentamicin. This result shows that olives show antibacterial activity against *E. coli* but are not as effective as gentamicin. For *Staphylococcus aureus* the olive leaf extract shows a mean value of 11.5±2.0mm which shows a stronger effect on bacterial pathogen but still the MIC of gentamicin is more than the value of OLE. The zone of inhibition of olive leaf extract against *B. subtilis* was 7 ±2.8mm which shows less antibacterial activity of OLE against *B. subtilis.* The olive leaf extract shows the inhibitory zone of 10±2.1mm against *p. aeruginosa* which shows moderated antibacterial activity. Gentamicin with the zone of inhibition of 15 mm indicates superior antibacterial activity against *p, aeruginosa.*

Table 1: Comparison of antibacterial activity of olive and rosemary leaf extract.

Bacterial pathogen	Inhibitory zone of Olive (mm)	Inhibitory zone of Rosemary (mm)	Gentamicin (mm)
Escherichia coli	11±1.8	8.5±1.8	15
Staphylococcus aureus	11.5±2.0	8.5±2.1	15
Bacillus subtilis	7 ± 2.8	11±2.8	15
Pseudomonas aeruginosa	10±2.1	15±2.1	15

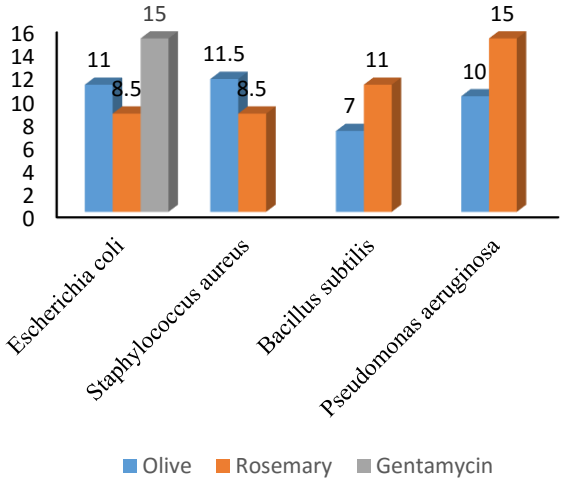

Fig.1. Antibacterial activity of *Olive and Rose Mariam* leaf extract against selected pathogens measured in milli meters

Rosemary leaf extract shows moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* which is 8.5±1.8mm which is far less than the activity of gentamicin. This result shows that the antibacterial activity of rosemary leaf extract is less than gentamicin. The antibacterial activity of rosemary leaf extract against *Bacillus subtilis* is 11±2.8mm which shows strong activity but less than gentamicin. Rosemary leaf extract shows strong antibacterial activity against *Pseudomonas aeruginosa* which is 15±2.1mm. The activity of rosemary leaf extract is comparable with gentamicin. The results show moderate to high antibacterial activity of olive and rosemary leaf extract. When compared to gentamicin no

extract shows the same zone of inhibition as gentamicin (Table.1).

4. Discussion

Plant-based antimicrobial agents play a vital role in the treatment and prevention of many diseases. Due to their antibacterial and antioxidant activities, scientists now turn their attention to these plant extracts which were initially used in folk medicines. Biological screening of these plant extracts makes it more vulnerable to find out their active ingredients and use them to make new antimicrobial agents. The main purpose of the study is to evaluate the antibacterial activity of olive and rosemary leaves against gram-positive and gram-negative bacteria to find out their potential as a natural antibacterial agent. Our results show that the ethanolic olive and rosemary leaves in their concentrated form prevent the growth of both gram-positive and gram-negative bacteria. Thus, this research shows the antibacterial effect of these medicinal plants on *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa.* The zone of inhibition of rosemary on *E. coli* and *S. aureus* is 8.5±1.8mm whereas for *B. subtilis* and *P. aeruginosa,* it is 11±2.8mm and 15±2.1mm, respectively. For olives the zone of inhibition for *E. coli* is 11±1.8mm, for *S. aureus* it is 11.5±2.0mm for *B. subtilis* the value is 7 ± 2.8mm and for *P. aeruginosa* it is 10±2.1mm. when compared to the inhibitory zone of gentamicin all these zones are less than the zone of gentamycin. There are a few differences between these results and other similar studies. The results of our study correspond with the study of Rimawi et al. which shows that the olive leaf extract shows complete antibacterial activity against *E. coli, p. aeruginosa,* and *S. aureus* [14]. Another study by Elnahas et al. shows that the olive leaf extract show antibacterial activity against *S. aureus, B. subtilis* and *E. coli.* Their antibacterial activity is mainly due to their phenolic compounds [15]. A study conducted by Golshani et al (2013) show that the rosemary leaf extracts shows strong antibacterial activity against *P. aeruginosa, S. aureus, B. subtilis,* and *E. coli* which completely corresponds to our current study [13] Another study that corresponds with our results was conducted by Walid et al.(2022) according to their results ethanolic extract of rosemary leaves show antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Campylobacter jejuni,* and *Salmonella enterica* [16]. Both the medicinal plants have important and potential value in the cure of different aliments.

5. Conclusions

Olive and rosemary leaf extract have great antibacterial potential *against E. coli, S. aureus, B. subtilis,* and *P. aeruginosa.* Thus, they can be used in the treatment of various diseases of these bacterial pathogens. More research is needed for their potential use in pharmaceutical industries for the development of new antibiotics.

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Authors Contribution:

F.U.R and Y.T: Study design and experimentation. H.W: Supervised the laboratory work and facilitation. A.H.K and F.S: Critical review and statistical analysis. S.A.K and Y.T: manuscript drafting. All authors have read and approved the final manuscript.

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