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Short Communication

An Evaluation of the Antibacterial Properties of *Oliveria decumbents* against Bacteria Isolated from Patients with Respiratory Infections

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Abstract

Background and Aim: Medicinal plants have long been used as an alternative or complement to the treatment and prevention of respiratory tract infections**.** The present study attempts to investigate the antibacterial effects of *Oliveria decumbents* on bacterial infections isolated from a hospitalized patient with respiratory disorders.
Materials and Methods: Twenty-five respiratory infection samples were collected from fifty-one patients that were hospitalized in the intensive care unit of the medical centers of Jahrom, Iran. Plant materials were obtained from the natural environment areas around Jahrom, and hydro-alcoholic extraction was prepared through the percolation method. The antimicrobial impacts of the plant extract were evaluated by the disc diffusion method against clinical and standard strains, and the results were compared with common antibiotics. Moreover, the minimum inhibitory concentration (MIC) of the herbal extracts was assessed by the broth macro dilution method.
Results: From the 51 clinical samples, *S. aureus* (11 cases, 21.5%), *S. pneumoniae* (8 cases, 15.6%), and *P. aeruginosa* (6 cases, 11.7%) were isolated. Furthermore, this herbal extract showed antibacterial activity by inhibiting the growth of three strains of *S. aureus* and one strain of *S. pneumoniae* in comparison to Amoxicillin and Amoxicillin-Clavulanate. Nevertheless, this extract did not affect *P. aeruginosa* clinical isolates. The MIC activity of the herbal extract for *S. aureus* with a concentration of 25 µg/ml, for *S. pneumoniae* with 50 µg/ml, and for *P. aeruginosa* with 200 µg/ml was observed.
Conclusion: Hydro-alcoholic extract of *O. decumbens* showed antibacterial effects on different bacterial strains that were isolated from respiratory infections.
Keywords: *Oliveria decumbents*, Anti-bacterial, Bacterial infections

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Introduction

Respiratory tract infections (RTIs) refer to infections of certain organs in the body, including the sinuses, throat, airways, or lungs, that have significant roles in breathing. Most RTIs pass within 1 to 2 weeks without treatment. RTIs often pass from one person to another in coughs and sneezes of someone with a microbial infection by various symptoms such as cough, sneezing, high temperature, stuffy or runny nose, headaches, and feeling generally unwell. They are categorized into distinct types. More exactly, they are usually grouped into upper (common cold, sinusitis, tonsillitis, and Laryngitis) and lower RTIs (bronchitis, bronchiolitis, chest infection, and pneumonia). Most respiratory infections are of viral and bacterial etiologies. Among bacterial infections, for example, epiglottitis and laryngotracheitis caused by *Haemophilus influenzae* type b or pharyngitis are often caused by *Streptococcus pyogenes.* Furthermore, the most common bacterial agents responsible for acute sinusitis are *Streptococcus pneumonia* and *Moraxella catarrhalis or* atypical pneumonia is caused by such agents as, *Coxiella burnetti, Chlamydia spp, Legionella,* and *Mycoplasma pneumonia* (1-3). Moreover, according to reports, acute bacterial respiratory diseases have a prevalence of approximately 3.9 million annually in infants and children worldwide. The most common antibiotic used in respiratory infections for 20 consecutive years is azithromycin, which binds to subunits of bacterial ribosomes and prevents essential translations and protein production, and ultimately has an inhibitory effect on bacterial growth (3, 4). Although many of these infections can be effectively treated with systemic or inhaled antibiotics, different lung diseases such as COPD, cystic fibrosis, or bronchiectasis cause changes in the mucous membranes of the airways and airways, which make patients susceptible and limit the effectiveness of antibiotic treatment. Hence, there is still a need for more effective treatments. Today, all over the world, one of the most effective methods of controlling and treating respiratory infections is the use of traditional and medicinal plants. Medicinal plants comprise several constituent metabolites with antimicrobial effects, which represent an efficient method of fighting pathogens (4). Due to the wide range and diversity in the genera and species of medicinal plants with antimicrobial properties, different articles are published every year in different countries. Iran is a vast country in West Asia that has a wide variety of medicinal plants. Many of these plants are used for therapeutic purposes, especially for microbial infections. One of the most famous local medicinal plants in Iran is *Oliveria decumbents*, which grows in limited areas of the southern and the western areas. This medicinal herb belongs to the Apiaceae family with voucher specimen code (No. 6637-THE) and the local name of Moshkorak" or "Denak (5). In addition to Iran, this plant grows in Iraq, Syria, and southeast Anatolia (6, 7). This medicinal plant is widely used in the treatment of fever and infections, digestive complications, abdominal infections, and diarrhea. Various studies have reported the composition of the essential oil of this plant by the GC-MS method. These compounds include P-Cymene, myristicin, Carvacrol, thymol, and limonene (5). Thymol and carvacrol are the most significant essential oil constituents of *O. decumbens.* According to studies, these compounds have anti-inflammatory antioxidant, antiprotozoal, antiviral, antibacterial, and antifungal effects (5-7). It has been shown that essential oil has inhibitory effects against different bacterial species, and antifungal activity against *A. niger* and *C. albicans* (8). Furthermore, it has been indicated that alcoholic extracts of the plant could have destructive effects on Gram-positive and Gram-negative bacteria. This antimicrobial effect is due to the accumulation of carvacrol hydrophobic compounds in the membrane of bacteria (9). Although numerous studies have shown the beneficial effects of the compounds of this traditional plant on standard bacterial, and fungal as well as parasitic strains in *in-vitro* conditions. Nevertheless, there is limited information about the impacts of the hydro-alcoholic extracts of this plant on respiratory infections in traditional medicine. Hence, this study attempted to investigate the effects of this plant on some bacterial species isolated from respiratory infections of hospitalized patients in comparison with standard strains.

Materials and Methods

Plant Materials and Extraction

Plant materials were obtained from the natural environment and mountainous areas around the city of Jahrom in Fars province, Iran. The plant samples were washed with distilled water and dried at 25°C for 72 hours. The samples were powdered and prepared for hydro-alcoholic extraction through percolation. The 50 gr of dried plant samples were submerged in 70% hydro-alcoholic solution and plant materials were placed into the percolator for 72 hours. Subsequently, the hydro-alcoholic solvent was poured in from above and passed through the plant. Once the percolation was ended, the liquid that flowed from the percolator was pale and, therefore, devoid of the plant's active ingredients. Moreover, the extract was concentrated by a rotary evaporator at a temperature of 40-500C and dried through a desiccator for 24 hours. The dried extract was solved in appropriate concentrations (µg/ml) of distilled water until further analysis (10, 11). Information about the geographical and biological distributions of *O. decumbens* (Herbarium No. 6637-THE) in Fars province, Iran has been shown in Figure 1.

Standard Strains, Antibiotic Discs, and Media

In this study, standard strains including *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, and *Streptococcus pneumonia* ATCC 33400 were prepared from the collection center of the Iranian Scientific and Industrial Research Organization as standard controls. Moreover, antibiotic discs, culture media, and chemical reagents were obtained from MAST UK and Sigma Aldrich (Germany) respectively (12).

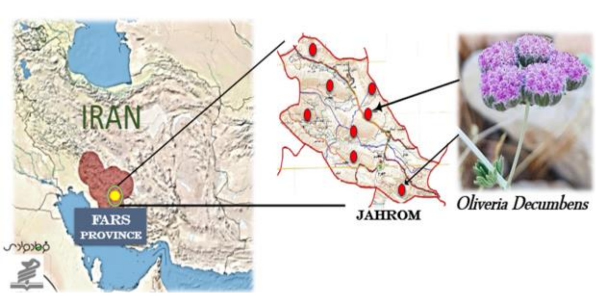

Figure 1. Information about the geographical and biological distribution of *O. decumbens* in Fars province, Iran.

Clinical Samples and Identification

Fifty-one sputum and bronco alveolar lavage samples were isolated from patients with respiratory tract infections hospitalized in medical centers in Jahrom from January to December 2020. The patients they were transferred to the diagnostic laboratory for further diagnosis according to standard protocols (13). The infected samples were analyzed through microbiological methods, including preparation of the smears and Gram staining, microscopic examination, and culturing in specific and differential media such as nutrient, sheep blood, EMB, and Mc Conkey agars. After incubation at 37°C for 48 hours, bacterial specimens were identified and verified using diagnostic tests such as catalase, coagulase, and fermentation of mannitol, DNase, and Optochin discs for Gram-positive bacteria. Furthermore, standard biochemical and differential tests such as triple sugar iron agar (TSI), sulfide indole motility (SIM), simmons citrate agar, growth at 42 C, and oxidation-fermentation (OF) test for Gram-negative bacteria. (13, 14) were performed.

Antibacterial Activity of the Herbal Extracts

The antimicrobial impacts of the plant extract were examined using the disc diffusion method. The herbal extracts were prepared (200–12.5 µg/ml), and Müller-Hinton agar (Merck, Germany) was uniformly inoculated with 1 mL of each bacterial suspension (10^8 CFU/mL) according to CLSI 2020 guidelines (15). Thus, herbal extract dilutions (10 µL) were placed onto sterile paper discs (10 mm), set on the bacterial culture plate, and incubated at 37°C for 48 hours. Hence, the antibacterial activity of the extract on clinical and standard strains was investigated in comparison with selected antibiotics from CLSI 2020 guidelines.

Minimum Inhibitory/Bactericidal Concentrations (MIC & MBC)

The MIC and MBC of the herbal extracts were assessed vis serial macro dilution assays in control and test tubes according to CLSI 2020 guidelines (15). Initially, the bacterial strains were cultivated in Müller-Hinton broth overnight at 37°C. All the extracts were primarily tested at 200 µg/ml and serially diluted twofold (control and test tube) to 0.048 µl. Each test tube contained 0.5 mL of each concentration and was inoculated with equal volumes of the microbial suspension (10^5 CFU/mL). The control tubes contained herbal extract concentration and broth medium without bacterial inoculum. In this experiment, we used microbial suspension without herbal extracts as a positive control and incubation was performed at 37°C for 24 hours. The lowest concentration of plant extracts that inhibited the bacterial growth was considered the MIC and the lowest concentration that killed microorganisms was considered as a MBC (11).

Statistical analysis

All the patients meeting the inclusion and exclusion criteria encountered during a study period of one year were recruited. Cohen's sampling was used for statistical analysis and the information was analyzed using the statistical SPSS version 21. The bacterial strains that caused respiratory infections were included in our study, and other microorganisms such as fungi were excluded.

Results and Discussion

From 51 clinical samples collected and analyzed from the patients with respiratory tract infections, *S. aureus* (11 cases, 21.5%), *S. pneumoniae* (8 cases, 15.6%), and *P. aeruginosa* (6 cases, 11.7%) were identified as bacterial infections. Other collected samples were found to be free of microbial contamination. Investigation of the diameter of the inhibition zone showed that hydro-alcoholic *O. decumbens* extracts could inhibit the growth of all three standard isolates through MIC activity on *S. aureus* with a concentration of 25 µg/ml, *S. pneumoniae* with 50 µg/ml, and for *P. aeruginosa* with 200 µg/ml. The MBCs were determined for these strains 100 µg/ml, 200 µg/ml, and 400 µg/ml respectively. Despite in-hospital resistant isolates, it was effective in inhibiting only three strains of *S. aureus* (one strain with a concentration of 50 and two strains with a MIC concentration of 25 µg/ml), and one strain of *S. pneumoniae* (with a concentration of µg/ml). However, it did not affect *P. aeruginosa* isolates (Figure 2). Moreover, the results showed that the greatest effects of the hydro-alcoholic *O. decumbens* extract were on the standard strains of *S. aureus*, *S. pneumoniae*, and *P. aeruginosa* respectively. However, the inhibitory effect of antibiotics compared with the plant extract on the growth of standard strains was determined by the disk diffusion method (Table 1, 2). Furthermore, the susceptibility patterns of the strains of *S. aureus*, *S. pneumoniae*, and *P. aeruginosa* to common antibiotics and *O. decumbens* extract have been

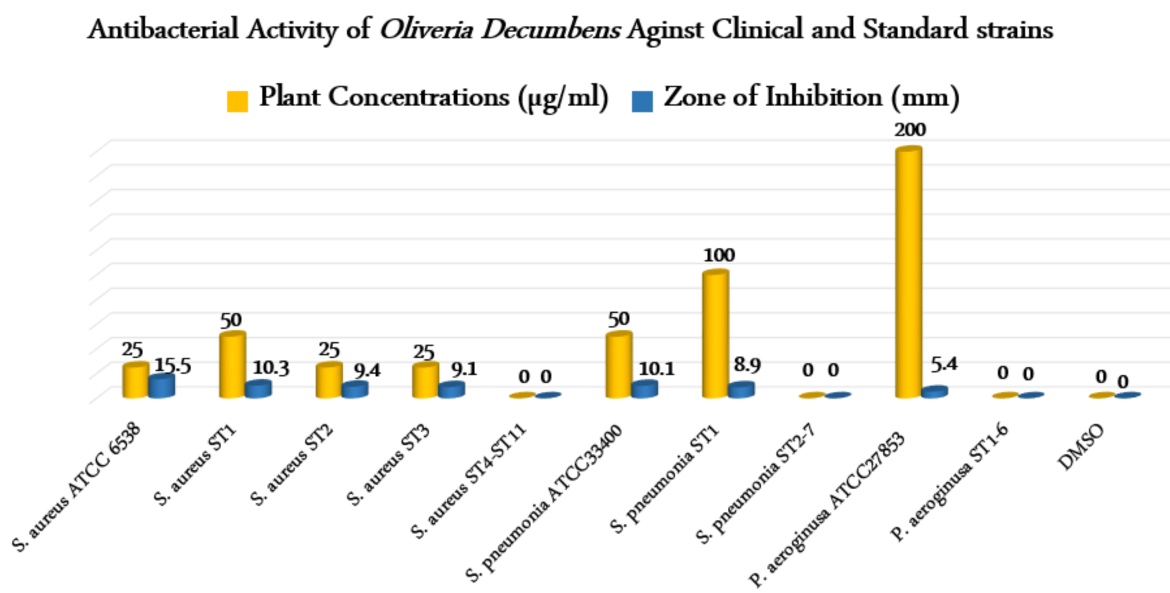

Figure 2. Antibacterial activity of *O. decumbens* extracts against standard and clinical bacterial strains that isolated from sputum sample of the patients with respiratory tract infections. (ST= Strain of bacteria isolated from patients).

Table 1: Antibacterial susceptibility test Results for clinical and standard strains of *S. aureus and S. pneumonia* in compared *O. decumbens extracts*.

Antimicrobial agents (μg)	Number of Resistance cases		Number of Intermediated Resistance cases		Number of Sensitive cases	
	S. aureus N=11 (%)	*S. pneumonia N=8 (%)*	*S. aureus N=11(%)*	*S. pneumonia N=8 (%)*	*S. aureus N=11 (%)*	*S. pneumonia N=8 (%)*
Amoxicillin (AMX 25μg)	9 (81.8%)	7 (87.5%)	2 (18.2%)	1 (12.5%)	0 (0%)	0 (0%)
Cephalexin (CN 30 μg)	8 (72.7%)	6 (75%)	0 (0%)	0 (0%)	3 (27.3%)	2 (25%)
Cefixime (CFM 5 μg)	5 (45.4%)	4 (50%)	2 (18.2%)	1 (12.5%)	4 (36.٤%)	3 (37.5%)
Azithromycin (AZM 15 μg)	8 (72.7%)	5 (62.5%)	0 (0%)	0 (0%)	3 (27.3%)	3 (37.5%)
Amoxicillin-Clavulanate (AC 25 μg)	8 (72.7%)	5 (62.5%)	3 (27.3%)	3 (37.5%)	0 (0%)	0 (0%)
Vancomycin (VM 30μg)	5 (45.4%)	4 (50%0)	2 (18.2%)	1 (12.5%)	4 (36.٤%)	3 (37.5%)
Meropenem (MRP 10 μg)	4 (36.٤%)	3 (37.5%)	2 (18.2%)	1 (12.5%)	5 (45.4%)	4 (50%0)
O. decumbens (25 μg) (against clinical *S. aureus* isolates)	8 (72.7%)	-	1 (9.1%)	-	2 (18.2%)	-
O. decumbens (100 μg) (against clinical *S. pneumonia* isolates)	-	7 (87.5%)	-	0 (0%)	-	1 (12.5%)

Table 2: Antibacterial susceptibility test Results for clinical and standard strains of *P. aeroginusa* in compared with *O. decumbens extracts*.

Antimicrobial agents (μg)	Number of Resistance cases (*P. aeroginusa*)	Number of Intermediated Resistance cases (*P. aeroginusa*)	Number of Sensitive cases (*P. aeroginusa*)
Gentamicin (GM 10μg)	3 (50%)	1 (16.6%)	2 (33.4%)
Ceftazidime (CAZ 30μg)	1 (16.6%)	3 (50%)	2 (33.4%)
Cefepime (FEP 30μg)	0 (0%)	1 (16.6%)	5 (83.4%)
Meropenem (MRP 10μg)	0 (0%)	1 (16.6%)	5 (83.4%)
Co-trimoxazole (STX 25 μg)	4 (66.6%)	0 (0%)	2 (33.4%)
O. decumbens (200 μg)	6 (100%)	0 (0%)	0 (0%)

characterized in Table 3.

Respiratory tract infection is a common medical condition that disturbs the respiratory system and can affect the sinuses, throat, lungs, or airways in children and adults who may carry bacteria and viruses. Furthermore, individuals with conditions such as weak immune systems, heart disorders or lung disease are at a higher risk of acquiring this type of infection (16). Those who suffer from a second disease may get more severe infections. Bacteria can cause upper respiratory infections, and doctors use different antibiotics to control these infections. For instance, amoxicillin and penicillin are often prescribed for strep throat (16, 17). It has been indicated that antibiotic prescription is high, and the excessive use of antibiotics may result in increased antibiotic-resistant bacterial infections (18,19). Hence, traditional and medicinal plants have long been used as alternatives or complements for the treatment and prevention of respiratory tract infections. For instance, different studies have shown that the use of Mahaenggamseok-tang, a herbal medicine in Asian countries, for lower respiratory tract infections in pediatric patients is a more effective and safer therapeutic method (17). Moreover, a herbal blend containing *Echinacea, Propolis*, and citamin C showed special activity in preventing respiratory tract infections in children, and various traditional herbs such as *Camellia sinensis*, *Panax ginseng*, and *Allium sativum* can prevent respiratory infection. Advantages of most of these herbal medicines include their inflammation-modulating effects, stimulation of the immune system to control respiratory infection, and finally prevention of cytokine storm (18-21). Iran is a vast country with rich resources of traditional and medicinal plants (22-25). In this country, people of Fars province use the aerial parts of *O. decumbens* to treat infectious diseases. Studies conducted in Iran on this traditional plant have shown the presence of valuable compounds in this plant and its extensive antimicrobial effects in vitro. In a study conducted by Mahboubi *et al*., the main components of its essential oil were shown to be thymol, carvacrol, p-cymene, and γ-terpinene (24). Furthermore, in other studies gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis revealed that carvacrol, thymol, γ-terpinene, *p*-cymene, and myristicin were the major volatile compounds of this medicine plant (26). It was shown that the essential oil of *O. decumbens* had remarkable antifungal activity against filamentous fungi and yeast. Hence, resistance degrees of microorganisms to *O. decumbens* oil are not the same. More exactly, bacteria are more resistant than fungi and Gram-negative bacteria are more resistant than Gram-positive bacteria (9). These results are completely consistent with the results and effects of the *O. decumbens* hydro-alcoholic extract achieved in this study. These results can be related to the synergistic effect between thymol and carvacrol, their effects on the cell membrane, and the change in the permeability of the H+/K+channels of bacteria and fungi. Although in our study *O. decumbens* extracts inhibited the growth of *P. aeruginosa* through 200 µg/ml MIC activity, other reports documented the low effects of this plant on Gram-negative bacteria such as *P. aeruginosa*. This result can be due to the presence of an external outer membrane in the cell wall and protection of the cell membrane. Other studies in Iran have reported that *O. decumbens* essential oil with high proportions of thymol and carvacrol could be a useful source of antimicrobial, anti-*Helicobacter* pylori (6). However, other reports have revealed that *O. decumbens* essential oil have a great degree of inhibitory impacts against all bacteria in comparable to Gentamycin and the antimicrobial activity of the oil reduced by dilution, and effect on MICs results the resulting MICs (5). In our study, hydro-alcoholic extracts of *O. decumbens* exhibited strong antibacterial properties against standard strain *S. aureus* and *S. pneumonia* in comparison with Amoxicillin and Amoxicillin-Clavulanate. Moreover, according to our results, *O. decumbens* was effective in inhibiting only three strains of *S. aureus*, and one strain

Table 3: Susceptibility patterns of the strains of *S. aureus* to common antibiotics and *O. decumbens* extract. AMX; Amoxicillin, CN; Cephalexin, CFM; Cefixime, AZM; Azithromycin, AC; Amoxicillin-Clavulanate, VM; Vancomycin, MRP; Meropenem, GM; Gentamicin, CAZ; Ceftazidime, FEP; Cefepime, STX; Co-trimoxazole.

Bacterial strains	Maximum to Minimum Susceptibility patterns
S. aureus ATCC 6538	MRP > VM & CFM > CN & AZM > *O. decumbens* > AMX & AC
S. pneumonia ATCC 33400	MRP > VM & CFM & AZM > CN > *O. decumbens* >AMX & AC
P. aeroginusa ATCC 27853	MRP & FEP > GM & CAZ & STX > *O. decumbens*

of *S. pneumonia*. Furthermore, it did not affect *P. aeruginosa* isolates. Since the hydro-alcoholic extract of *O. decumbens* showed an inhibitory effect against all of the standard strains and had antibacterial activity against clinical strains, further studies are required to investigate these effects against different clinical samples. This traditional plant can be efficient in the treatment of certain types of respiratory infections.

Conclusion

In this study, the hydro-alcoholic extract of *O. decumbens* showed beneficial antibacterial effects on different Gram-positive and Gram-negative bacteria that were isolated from respiratory infections. Although other studies have revealed the antimicrobial effects of the essential oil of *O. decumbens* on a wide range of microorganisms, it is necessary to conduct more studies on the effectiveness of this plant against other Gram bacterial strains associated with human infections. Furthermore, the ability of this extract as an antimicrobial composition should be investigated in *in-vivo* conditions, particularly in respiratory infections of animal models.

Acknowledgment

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Conflict of Interest

The authors declare that they have no conflict of interest.

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