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Inhibitory Effect of *Tamarix aucheriana* Aqueous and Alcoholic Extracts against Klebsiella Pneumonia Which Isolated from Patients in Nasiriya City, Southern Iraq

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ABSTRACT

The present study was included to determine the inhibitory activity of Tamarix aucheriana extracts on the drug-resistant Klebsiella pneumoniae. Plant samples were collected from Nasiriyah region, southern Iraq. It was dried and ground, then extracted from the sample (the aerial parts of the stem and leaves) aqueous and alcoholic extracts, and qualitative chemical tests were conducted for the extracts to know the active components in them. The efficacy of these extracts was tested at concentrations (100%-75%-50%-25%-15%-5%) on Klebsiella pneumoniae isolated from respiratory patients at Al-Hussein Teaching Hospital in Dhi Qar. The results showed a significant effectiveness of the alcoholic extract and less than that of the aqueous extract The extracts with concentrations (100% and 75%) recorded a higher inhibition rate than (50% and 25%) and the minimum inhibitory concentration (MIC) was 5%.

Keywords
Tamarix aucheriana, Klebsiella Pneumonia, aqueous and alcoholic extracts

Introduction

The *Tamarix aucheriana*. plant is a member of the *Travaux* family, and grows into a medium-sized evergreen with small leaves that look like art forms. The tamarisk tree grows in warm and humid climates, such as Western Asia, Yemen and the countries of the Mediterranean basin (Sherry et al., 2016). Bedouins eat the sugary liquid that flows from the stem of the tree as food, especially during the summer, and this liquid or sugary juice contains glucose, fructose and dextrin, and provides the body with heat energy, and the Bedouins use it to be called red steel (Drus, et al., 2014). The leaves contain tamarexin and several minerals. Sodium, calcium and potassium are beneficial to human health. Treating fever and heat stroke using sugary juice(Bean, 2013). Its roots go back to the countries of West Asia, the countries of the Mediterranean basin, and Yemen, and the plant is distinguished by its white flowers, medium height, evergreen, a source of shade, and it participates in the treatment of some diseases. . The plant of the thyme contains: sodium, magnesium, calcium, potassium, minerals, tamarxin, afsip substances, and sugars such as glucose, dextrin, fructose, sugary juice, and phenol (Van Rieber et al., 2008). Near the waters, rivers and valleys.The stems are made of hard wood, the leaves are very delicate and the flowers are cluster pink.The ships are made of stems especially because they are located near seas and valleys,(Harms, Hebert, 2006) *Klebsiella* is a genus of Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. *Klebsiella* species are found everywhere in nature. This is thought to be due to distinct subspecies that develop specific specialized adaptations, with associated biochemical adaptations

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that make them more suitable for a particular environment (Chen, et al. 2014). The cells are rods in shape and are generally 0.3 to 1.5 µm in width and 0.5 to 5.0 µm in width. They can exist singly, in pairs, in chains or connected end-to-end. Klebsiella can grow on normal laboratory medium and has no special growth requirements, like other members of Enterobacteriaceae. (Yigit, 2001). Aerobic species but optionally anaerobic. Its ideal growth temperature is 35° to 37°C, while its ideal pH level is around 7.2. A type of bacteria normally found in nature. In humans, bacteria are often found in parts of the digestive system where they generally do not cause problems. In the United States, *Klebsiella pneumoniae* and *Klebsiella oxytoca* are the two strains responsible for most human diseases. (Humphreys et al., 2010). Some *Klebsiella* bacteria have become very resistant to antibiotics. When bacteria like *Klebsiella pneumoniae* produce an enzyme known as carbapenemase (referred to as KPC-producing organisms), a class of antibiotics called carbapenems will not work to kill the bacteria and treat the infection. (Satellin et al. 2017).

Aim of the Study Evaluation of the effect of medicinal plant extracts and *Tamarix aucheriana*. on drug-resistant bacteria isolated from respiratory tract infections

Materials and Methods

2.1 Collection of Plant Samples

Plant samples for this study were collected in Nasiriyah, Dhi Qar Governorate, in southern Iraq, in October 2020. Dr. Haider Radi, a professor at Dhi Qar University's Faculty of Science, found and diagnosed the plants. They were thoroughly cleaned of contaminants and processed into a fine powder using an electric mill before being stored in sterile glass bottles until use (Ghazanfar and Edmondson, 2001).

2.2. Preparation Aqueous Extract

Water extract from the plant was prepared by weighting 50g of plant powder in a flask, and 0250ml of distilled water was added then mixed with a magnetic stirrer for two hours. The mixture was filtered through filter paper (Wattman No. 1). The supernatant was evaporated to dryness in a rotary evaporator at 40oC under reduced pressure, and the aqueous extract was left at room temperature to remove any excess water (Harborn, 1982)

2.3. Preparation Alcoholic Extract

The alcoholic extract of plant powders used in the study was prepared by A quantity of 50g of plant powder was mixed with 250ml of absuolute ethanol by Soxhlet apparatus for 8 hours at (40-60) ºC. The solution was evaporated to dryness in a rotary evaporator (Harborn, 1984).

2.4. Stock Solution

Prepare the concentrated solution by dissolving (1) g of extracted in (10) mL of Dimethyl sulfoxide to obtain a solution with a concentration of 100 mg/mL, sterilizing the concentrated solution for each extract using microfiltration filters Millipore filter with a diameter of (0,22) mm, and then repeating the concentration of 3 mg/ml (Foroughi et al., 2016).

2.5.Sample Culture

Sputa and different samples collected from the respiratory tract are polluted with typical verdure. Additionally, sputum is a gooey non-homogenous sample. Both of these realities make it difficult to culture sputum without first preparing the sample. Sputum samples are treated with a mucolytic specialist to separate the bodily fluid and a disinfecting operator to stifle the development of the typical verdure present in the sample. On the off chance that this was not done, the ordinary vegetation would congest the more gradually developing types of mycobacteria, making their disengagement from clinical examples inconceivable. Sputum

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samples are culture on the media of blood agar, McConkey, and chocolate for 24 hrs, at a temperature of 37° C.(Oliver, 2013).

2.6.Disc Diffusion Method

determinationof minimum bacteriocidal concentration(MBC) On Mueller-Hinton agar and punched with 6-mm diameter wells for the bacteria, respectively. Then 100 µl plants extracts were added to the wells, while 10% DMSO was used as the negative control. The antimicrobial activity was estimated, after incubation of the plates at 37 °C for 18 to 24 hrs, by calculating the diameter of inhibition zones (mm). Each test was done three times and the mean of the results was determined. The extraction solvents were used as negative controls were assessed (Al-Massarani and El-Dib, 2015).

3.Results and discussion

3.1.Identification of Infectious Bacteria

A total of 104 clinical samples were collected from patients with respiratory tract infection. The samples were collected from AL-Husain Teaching Hospital in Al-Nasiriyah City/Iraq. The result of the current study indicated that 41.3% had positive growth for pathogenic bacteria, while 58.7% had negative growth. The reason may be due to the different locations, time of collection and the number of samples (Hassan and Mosa, 2019).

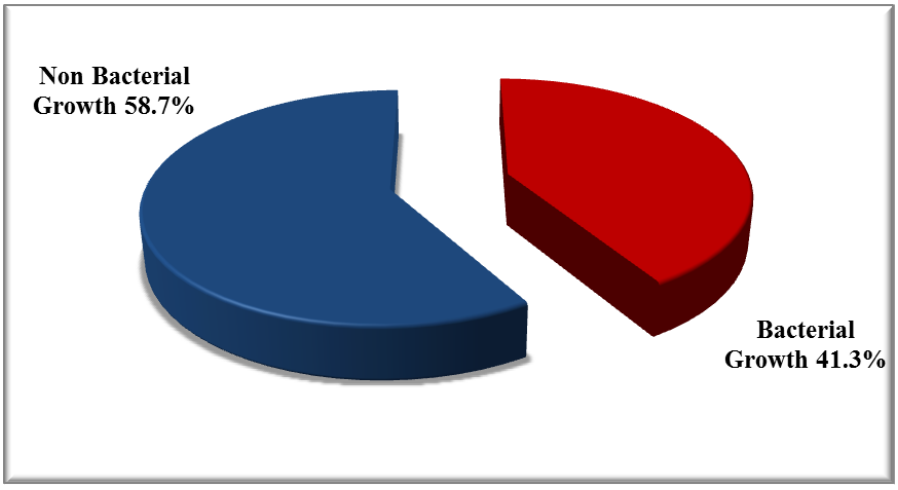

Figure 1: Identification of infectious bacteria

3.2.Bioactivity for Plants Extract:

Tamarix is a highly effective antibacterial plant incl. Two types of alcoholic and aqueous extracts were prepared and these extracts were tested on the most common pathogenic bacteria Klebsiella pneumoniae in the respiratory tract which possess many virulence factors. And resistance to many antibiotics, which has become one of the challenges facing the world, is among the species isolated in the study (Al-Harbi et al., 2017).

3.3., Aqueous And Alcoholic Extract Of Tamarix Aucheriana

Both the aqueous and alcoholic extract were highly effective against bacteria, and the alcoholic extract was more effective than the aqueous extract, as they were effective at concentrations (100,75,50,25,15,5) according to Table (1and2) and with different areas of inhibition. The statistical analysis showed a relationship between the increase in concentration and the large size of the inhibition zone, and this result is consistent with what was mentioned

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(Aneja et al., 2010) The alcoholic extract is more effective. The plant contains multiple organic components including phenols and alkaloids. It is known that these substances are soluble in polar solvents according to indication (Indu et al., 2006; Aneja et al., 2010), and it has been observed that the antimicrobial effect of plant extracts varies from plant to plant. The plant undergoes different research in different regions of the world, due to many factors such as the influence of climate, soil composition, age and stage of the vegetation cycle on the quality, quantity and composition of the extracted product, different bacterial strains Moreover, different studies have found that the type of solvent has an important role in the extraction process. (Akrayi, 2014).. This result was consistent with what was mentioned (Al-Tememy,2013) where it showed that ethanol extract is the most effective among other alcoholic extracts. On K. pneumoniae extracts of Tamarix possess antibacterial activity against the strain tested in this study, this may be due to the presence of alkaloids with phenols and tannins. These types of compounds have antibacterial activity (Almazini, 2007). The alkaloid contained in Tamarix is responsible for its anti-inflammatory, anti-asthmatic, anticonvulsant and antibacterial activity (Mishra, 2010). The alkaloid also has the ability to bind to bacterial DNA causing its death, while the phenolic compound has a precipitating activity on the microbial enzyme and leads to its inhibition and loss of function (Al-Tememy,, 2013).

Table (1): Activity of Tmarix alcoholic extract against isolated bacteria

Pathogens	***stem extract for K. pneumoniae***	***leaf extract for K. pneumoniae***
CON	**Inhibition Zone/ mm^2 Mean + SD**	**Inhibition Zone/ mm^2 Mean + SD**
5%	3.33 ± 0.5^{a}	6.00 ± 1.0^{a}
15%	5.33 ± 0.5^{b}	8.00 ± 1.0^{a}
25%	8.33 ± 0.5^{c}	14.0 ± 1.0^{b}
50%	10.3 ± 0.5^{d}	17.6 ± 1.5^{c}
75%	12.6 ± 0.5^{e}	22.6 ± 1.5^{d}
100%	15.0 ± 1.0^{f}	24.6 ± 1.5^{d}
P. Value	**< 0.01**	**< 0.01**
LSD	**1.18**	**2.29**

Table(2): Activity of *Tmarix* aquatic extract against isolated bacteria

Pathogens	**leaf extract for K. pneumoniae**	**stem extract for K. pneumoniae**
CON	**Inhibition Zone/ mm^2 Mean + SD**	**Inhibition Zone/ mm^2 Mean + SD**
5%	2.66 ± 0.5^{a}	1.66 ± 0.5^{a}

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15%	6.66 ± 0.5^{b}	3.66 ± 0.5^{b}
25%	11.6 ± 1.5^{c}	6.00 ± 1.0^{c}
50%	14.0 ± 1.0^{d}	8.33 ± 1.0^{d}
75%	17.6 ± 0.5^{e}	10.3 ± 0.5^{e}
100%	18.3 ± 1.5^{e}	13.3 ± 1.5^{f}
P. Value	**< 0.01**	**< 0.01**
LSD	**1.87**	**1.18**

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