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Determination of Ziziphus Spina-christi leaves extracts Antibacterial activity against some pathogenic bacteria

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Abstract Medicinal plants are traditionally used for the treatment of human infections. The present study was undertaken to investigate Ziziphus spina-christi leaves for their potential activity against human bacterial pathogens. The Aqueous extracts in 50 µg/ml didn't appear any effect on all bacterial isolates and the maximum Zone Inhibition appeared in100 µg/ml was 9 mm against *Klebsieela .oxytoca* and *Proteus .mirabilis* ,*Pseudomonas .aeruginosa* and *Staphylococcus. aureus* appeared slow responses toward this extract with zone of inhibition 6 mm; 4 mm and 4 mm for above isolates respectively in 100 µg/ml . The zone inhibition of 50 µg/ml of Ethanol extract were follow . *K. oxytoca* (17 mm), *P.mirablis* (15 mm), *P. aeruginosa* (13mm)and *S. aureus* (16 mm), . but when used 100 µg/ml form Ethanol extract the results appeared the inhibition zone of bacterial growth were 26mm for *K.oxytoca* ,28 mm for *P. mirabilis*,23mm for *Ps. aeruginosa* and 24mm for *Staph. aureus*. In other side the results listed in same table showed the Methanol extract were more effective as follow in diameter of zone inhibition for *K.oxytoca* (19 mm),*P. mirabilis* (16 mm), *Ps.aeruginosa* (14 mm) and *Staph. aureus* (18mm) when used 50 µg/ml .but when used 100 µg/ml the diameter of zone inhibition were increased to reach 30mm for *K.oxytoca* ,28mm for *P.mirabilis* ,26mm for Ps. *aeruginosa* and 25mm for *Staph. aureus* .All above results were compared with antibacterial activity of Gentamycin (10 µg) and Amikacin (30 µg) . ***Key word*:** Ziziphus spina-christi; Antibacterial activity; Ethanol and Methanol Extract.

Introduction Global prevalence of infectious diseases caused by bacteria is a major public health problem **(Zhang *etal*.,2006; Paterson ,2008**). The bacterial agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections (**Peirano,2008**). Recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents (**Eggleston,2010**) and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy **(Alviano and Alviano,2009**). In Pakistan, a diverse flora of medicinal plants is grown naturally **(Hussain etal.,2011).**

While synthetic antibiotics has undoubtedly recorded significant successes in the management of diseases and infections through their static and cidal effects (but not without limitations like side effect and microbial resistance), nature as it were, has been a source of medicinal agents for thousands of years**.(Eggleston *etal*.,2010)**

According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. In developed countries about 80% of plants are used in traditional medicine. Therefore, such plants have been investigated for better understanding their medicinal properties. The antimicrobial properties of many plants have been investigated by a number of researcher's in worldwide Plants have

been a source of medicine in pharmacopoeia. (**Adamu *et al.*, 2005**). Herbal medicine can be used as an alternative to some commercial drugs Medicinal plants are defined as a group of plants that possess some special properties that qualify them as articles of drugs and therapeutic agents and provide inestimable projections for new drug discoveries because of the matchless availability of chemical range. (**Anyamene and Ezeadila, 2010).**

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Plants are prospective source of antimicrobial agents in different countries (**Alviano and Alviano,2009)**. About 60 to 90% of populations in the developing countries use plant-derived medicine. Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases **(Zhang *etal*.,2006**). Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial properties (**Dorman and Deans, 2000; Talib and Mahasneh ,2010**). Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses (**Ruberto *etal*.,2000; Cruz *etal*.,2007).**

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory study (**Kumar *etal*., 2006 ;Bibi *etal*.,2011).**

Aim of study

This study was aimed at investigating the antimicrobial characteristics of extracts of Ziziphus spina-christi leaves, and to screen for its phytochemical compositions responsible for its antimicrobial activity

Material and Method

1-Collection of and preparation of powder of Ziziphus spina-christi leaves.

Ziziphus Spina-christi leaves were collected from different local gardens in Al-Diwaniyah city, during March /2020. The collected leaves were dried under shade and then mashed with the help of mortar and pestle.

- Sub culturing of bacterial strains2

The antibacterial activity of Ziziphus spina-christi leaves was carried out against pathogenic bacterial strains (*Proteus mirabilis*, isolated from urinary tract infection) and *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* isolated from Burn infection).

Nutrient broth was used for sub-culturing of bacterial strains. The media was prepared according to manufacturer's instructions (Oxoid, UK). Bacterial cultures were inoculated on nutrient broth and incubated overnight at 37 °C and its turbidity compared with that of 0.5 Mac‑Farland to standardize each culture to 106cfu/ml.(**Usman *et al*. 2013).**

3 -Extraction procedure of leaves extracts

Fresh leaves of Ziziphus spina-christi leaves were thoroughly washed using tap water and rinsed with distilled water. The leaves were dried for 5 min in an oven at 28 C for one week and then pulverized to a fine powder with the aid of a Star lite blender. Three solvents were used for the preparation of the extracts, namely Distilled water, ethanol 60% conc. and methanol 60% conc. These extracts prepared as follow

• **A-The aqueous extract**

was prepared by weighing out (100 g) of the milled powdered leaves were soaked in 1 L of distilled water in a conical flask and was stirred and heated for about 4 hours.

After cooling, the extract was filtered by using Whatman No.1 filter paper. The filtrate was collected and frozen in ice cube container. The frozen ice cube was freeze-dried (i.e. lyophilisation) to obtain concentrated, aqueous extracts in powder form. **(Juvatkar *etal.*, (2012).**

• B-The ethanol and methanol extracts

Were obtained by weighing out same fraction 100 g of the pulverized powdered leaves of Ziziphus spina-christi and soaking in 1 L from each the 60% ethanol and 60% ethanol . The extracts were then filtered using Whatman no.1 filter paper. and then followed by rotor- evaporated the supernatant by using the Switzerland Rotary Evaporator to remove the ethanol and to obtain concentrated, oily extract. The crude extracts were then kept at -20 ºC in sterile universal bottles. (**Alo *etal*.,2012).**

4-Preparation of concentrations

Stock solution of Ziziphus spina-christi Leaves extracts was prepared by dissolving 100 mg of extract with 1 ml of solvents (sterile distilled water and 99.9% dimethyl sulfoxide. 50 µg/ml and 100 µg /ml of concentration have then used.

5-Determination of microbial activity of the extracts.

Antibacterial activity of these extracts against *K. oxytoca , P. mirabilis, P. aeruginosa, and S. aureus* was determined by agar well diffusion method by using Muller –Hinton Agar . (**Perez,. 1990).** Amikacin and Gentamycin were incorporated as positive control

while distilled water and dimethyl sulfoxide without Leaves extract was incorporated as negative control.

Agar well diffusion technique as described by **Cheesbrough (2006)** was used to determine the antibacterial activity of the extracts. An 18 ml of Muller Hinton agar plates that has been checked for sterility were seeded with 2 ml of an overnight broth culture of each bacterial isolate in sterile Petri -dish. The seeded plates were allowed to set after a uniform distribution of the bacterial isolate following slow rotation of the Petri dish. A standard sterile cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells filled with 2 ml of each extracts were with the aid of a sterile syringe. One of the well in each Muller Hinton agar plate is left unfilled as a control. The plates were then allowed to stand for 1 hour at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 37 oC for 24 hours and observed for zones of inhibition. A zone of clearance round each well signifies inhibition and the diameter of such zones were measured in millimeter (mm).

6-Data analysis. All the experiments were independently repeated three times, and average zone of inhibition of test extracts relative to negative were calculated as mean of 'repeats'.

Results and Discussion In the present study, Ziziphus spina-christi leaves extract dissolved in Distilled water, Ethanol and Methanol showed variable antibacterial activity against *K. oxytoca , P. mirabilis, P. aeruginosa, and S. aureus* . The zones of inhibition (mm) exhibited by leaves extracts are listed in *Table 1*. Showed the Aqueous extracts in 50 µg/ml didn't appear any effect on all bacterial isolates and the maximum Zone Inhibition appeared in100 µg/ml was 9 mm against *K.oxytoca* and *P.mirabilis* ,*Ps.aeruginosa* and *Staph. aureus* appeared slow responses toward this extract with zone of inhibition 6 mm; 4 mm and 4 mm for above isolates respectively in 100 µg/ml . The zone inhibition of 50 µg/ml of Ethanol extract were follow . *K. oxytoca* (17 mm), *P.mirablis* (15 mm), *P. aeruginosa* (13mm)and *S. aureus* (16 mm), . but when used 100 µg/ml form Ethanol extract the results appeared the inhibition

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Table (1): Comparison of Aqueous, Ethanol and Methanol Ziziphus spina-christi leaves extract Activities of different extracts against human pathogenic bacterial isolates.

Bacterial isolates	Zone of Inhibition in (mm) ±SD								
	Aqueous (µg/ml)		Ethanol (µg/ml)		Methanol (µg/ml)		CN (µg)	AM (µg)	D.W
	50	100	50	100	50	100	10	30	Free
K. oxytoca	0±0.0	9±0.2	17±0.2	26±0.2	19±0.3	30±0.3	15±0.5	18 ±0.2	0±0.0
P. mirabilis	0±0.0	6±0.3	15±0.3	25±0.1	16±0.2	28±0.4	16±0.4	17±0.7	0±0.0
Ps. aeruginosa	0±0.0	4±0.1	13±0.4	23±0.2	14±0.1	26 ±0.1	14±0.2	14 ±0.4	0±0.0
Staph. aureus	0±0.0	4±0.3	16±0.3	24±0.1	18±0.2	25±0.2	16± 0.3	19±0.3	0±0.0

SD =Standard Division ….CN=Gentamycin…...AM=Amikacin

Global burden of infectious diseases caused by bacterial agents is a serious threat to public health **(Eggleston *etal*.,2010).** Antibiotic treatment is a preferred choice to treat bacterial infections; however, emergence of antimicrobial resistance and toxicity issues subside the use of antibacterial agents (**Zhang *etal*.,2006; Malini *etal*.,2013**). Safety- and efficacy-related limitations to antibiotics augment biological research on the antimicrobial role of plants due to comparable toxicity and efficacy (**Alviano and Alviano ,2009).**

In the present study, we have investigated the antibacterial activity Ziziphus spina-christi leaves, by using three types of solvents: Water, Ethanol and Methanol. biological activity of these extracts was tested against human bacterial pathogens. *K.oxytoca,P.mirabilis;Ps. aeruginosa* and *Staph. aureus*.

Results of this study showed that Methanol extract of Ziziphus spina-christi leaves, has potential inhibitory effects on all tested bacteria and more efficient use as antibacterial agent in both 50 µg/ml and 100 µg/ml , this might indicate that the methanol and Ethanol probably extract different antimicrobial agents from leaves, followed by the Ethanol extracts with concentration 100 µg/ml and in 50 µg/ml show limited inhibitory effects against tested bacteria. Aqueous extract showed weak activity against tested bacteria in 100 µg/ml while in 50µg/ml this extract didn't appear any effect on bacterial growth .

This work indicated that ethanol and methanol are better solvent than water for the extraction of the active ingredients of these plant.

Our results are supported by the study of **Al-Bayatti, *et al.* (2011),** in which he reported that, methanol and water extracts Ziziphus spina-christi seeds and leaves can

be effective against five different species of bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Acinetobacter* spp. and *Enterococcus* spp. isolated from skin infection.

Al-Saimary ,2007; Al-Saimary ,2009 studied the effects of Zizyphus leaf aqueous extracts on Staphylococcus aureus and found that (100 mg/ml) and (250 mg/ml) have no effects on the growth of Staphylococcus aureus, while higher concentrations (500 mg/ml) and (750 mg/ml) inhibit the growth by about (9.00 mm) and (12 .00 mm) respectively.

This bacterial activity of may be belong to aromatic substances such as phenolic, e.g. phenolic acids, flavonoids, quinones, coumarins, The flavonoids from plant extracts have been found to possess antimicrobial and antioxidants properties in various studies.

The problem of antibiotic resistance in both hospital- acquired (nosocomial) and community- acquired bacterial infection have made many antibiotics virtually obsolete, and also , it is well known that no antibiotics can last effective too long. Therefore, **Al-Bayatti, *et al.* (2011)** depending on their results of him study recommend to using of all the parts of Zizyphus spina-christi plants (leaves, seeds, fruits ,barks and root bark) and at the same time searching through Iraqi flora extensively to investigate the bioactive compounds for use as therapeutic agents for treatment of inflammatory and infectious diseases and this effort would support confidently the fact that the herbal remedies play a fundamental role in traditional medicine. we agreement with this recommendation.

Conclusions

It can be concluded that Methanol Ziziphus spina-christi leaves extract showed potential antimicrobial activities against the tested bacterial. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids. Results of our findings confirmed the use Ziziphus spina-christi leaves as traditional medicine. However, Extract of leaves may be subjected to detailed phytochemical and pharmacological studies in order to find out new drugs against pathogenic bacterial strains.

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