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The Activity of Aquatic Extract of Ziziphus Spina-christi against Bacteria, an in Vitro Study

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Abstract--- Background: *Medicinal plants such as Ziziphus spina-christi, was described as one plant in which one of its parts contain compounds that used for chemotherapeutic purposes or medical crude for the synthesis of useful drug. So this useing of plants in treating ailments has been in practice since long time ago.*

Aims: *The present paper aimed to evaluate the antibacterial activity of (leaves, stem bark and the combination of leaves + stem bark) aqueous extracts of Z. spina–Christi .in vitro*

Methods: *Antibacterial effects of(aquatic leaves, aquatic stem park and combination of leaves + stem bark) extracts against five Gram-positive and eight Gram-negative bacteria- including Staphylococcus aurus, Staphylcoccus epidrmidis, Streptococus pyognes, Staphylcocus saprphyticus, Streptococus pnemniae, Pseudomnas aeruginsa, Enterbacter sp, Klebiella pneuoniae, Eschricha coli, Salmonlla typehi, Protus mirabilis, Seratia marcecans, Acinetbacter.*

Results: *Antibacterial actity of these extrats by well-diffusion method were measured by inhibition zones of five Gram-positive, eight Gram-negative pathognic microbes. All organisms of this study were highly sensitive to comination of leaves + stem bark extract, then stem bark aqueous extract, finally all tested organisms were slight sensitivity to leaves aqueous extract.*

Conclusion: *Sidir aquatic extracts (leaves, stem bark and combination of leaves + stem bark) had an inhibitory effect against all tested bacterial species. This plant also need further work to discover new broad spectrum bioactive compounds.*

Keywords--- *Plant Extract, Stem Bark Extract, Leaves Extract and Antimicrobial Activity*.

I. INTRODUCTION

The use of drugs to control it has led to high prevalence of side effects, and emergency of bacterial strains resistant. Herbal remedies used in the folk traditional medicine provided interesting and still largely unexplored source for the synthesis and development of potent new drugs for chemotherapy which may help to overcome the problem of resistance and also the toxicity of the currently antibiotics [1,2].

The use of medical plants, as traditional health remedies have been most popular for 80% of world population in Latin America, Asia and Africa and is reported to have minimal side effects [3]. The *Ziziphus* species pain, dandruff, wounds and ulcers, inflammatory conditions, asthma and to cure eye diseases. *Z.spina-christi* has recently appeared

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to have antibacterial, antifungal and antioxidant properties [6, 23]. Flavonoids, alkalds, triterpenoids, saponins, lipids, proteins, free sugar and mucilage are the main important compounds characterized in this plant [7,8]. *Z. spina-christi*was alkaloids also four saponin glycosides [9], and several flavonoids have been isolated from the leaves of *Z. spina-christi* [10].

II. Materials & Methods

Plant Collection

To make an aqueous extracts from stem bark and leaves of *Z. spina-christi*. Plant parts were collected from house garden, in Al-Hilla City at July 2016, then the stem bark and leaves; next ground it by blender, placed in clean container. Aqueous extract was soaked 50 g of stem bark and leaves powder by 100 ml of boiling distillated water, and allowed to stand for 72 hr, sterilized by filtration (using Millipore 0.45µm filter paper).This extract considering as the 50% present [11].

Bacterial Isolates

Different thirteen clinical bacterial isolates (listed at table1), were isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France)[12]and cultivated in pure culture, at Microbiology Laboratory\ College of Nursing\ University of Babylon.

In vitro Antibacterial Activity Testing use Well-Agar Diffusion Assay NCCLS [13]

Table 1: Bacterial Isolates Used in tis Study

Gram positive bacteria	Gram negative bacteria
Staphylococcus aureus	*Pseudomonas aeroginosa*
Staphylococcus epidermidis	*Proteus mirabilis*
Streptococcus pneumoniae	*Klebsiella pneumoniae*
Streptococcus pyogenes	*Enterobacter spp.*
Staphylococcus saprophyticus	*E. coli*
	Serratia spp.
	Salmonella typhi
	Acinetobacter spp.

Loop full growths from bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hrs. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield auniform suspension containing 1.5×108 CFU/ ml. Cotton swab was dipped and streak into adjustment suspension the entire Mueller-Hinton agar (for all tested bacteria) surface of plates and the plates were left for one 5-15 minutes on temperature of room temperature to dry.

Media were cut into four wells (5mm diameter) by cork borer and add 20µof leaves extracts solutions or stem bark solution or combination of leaves + stem bark extract (The plates were performed intriplicates). All plate of the

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tested organisms was then allowed to incubate at 37°C for overnight. After 24 hr. of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm) (24,25,26).

Statistical Analysis

Bonferroni test recommended by Danial [14], was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences between results of aqueous leaves, aqueous stem bark and combination of leaves + stem bark.

III. RESULTS

The screening of the activity against microbes of aqueous leaves, aqueous stem bark and combination (leaves + stem bark) of *Ziziphus spina-christi* extracts, were carried out using the well-agar diffusion test and results shown in figures (1-4).

These results shows that the combination (leaves + stem bark) extract produced the highest inhibition activity against all bacterial isolates, within inhibition zone range (25-35) mm, followed by stem bark extract, also inhibit growth of all isolates, with inhibition zone (20-30) mm, while the lowest effect obtained from leaves extract, which have the lowest inhibition on bacteria, with inhibition zone range from (10-15) mm.

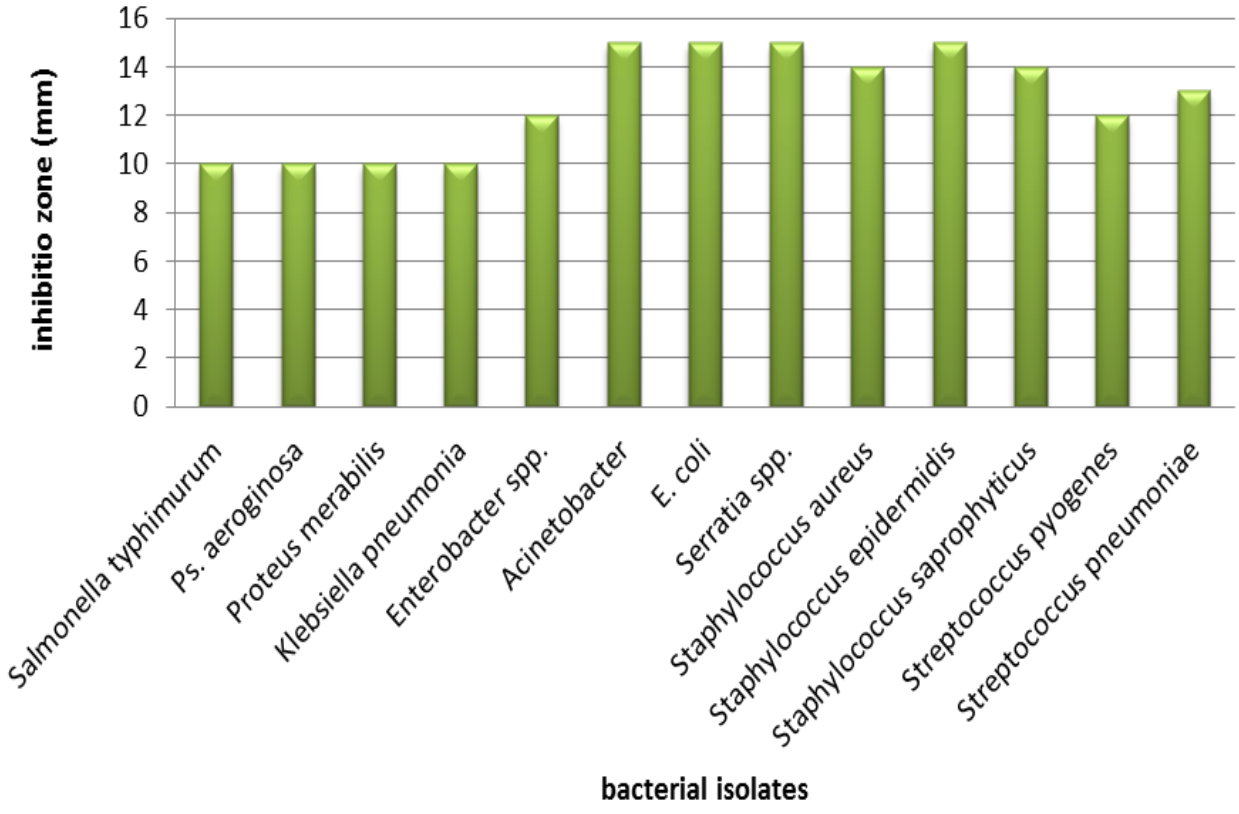

Figure 1: Antibacterial Activity of Aquatic Leave Extract against Bacterial Isolates

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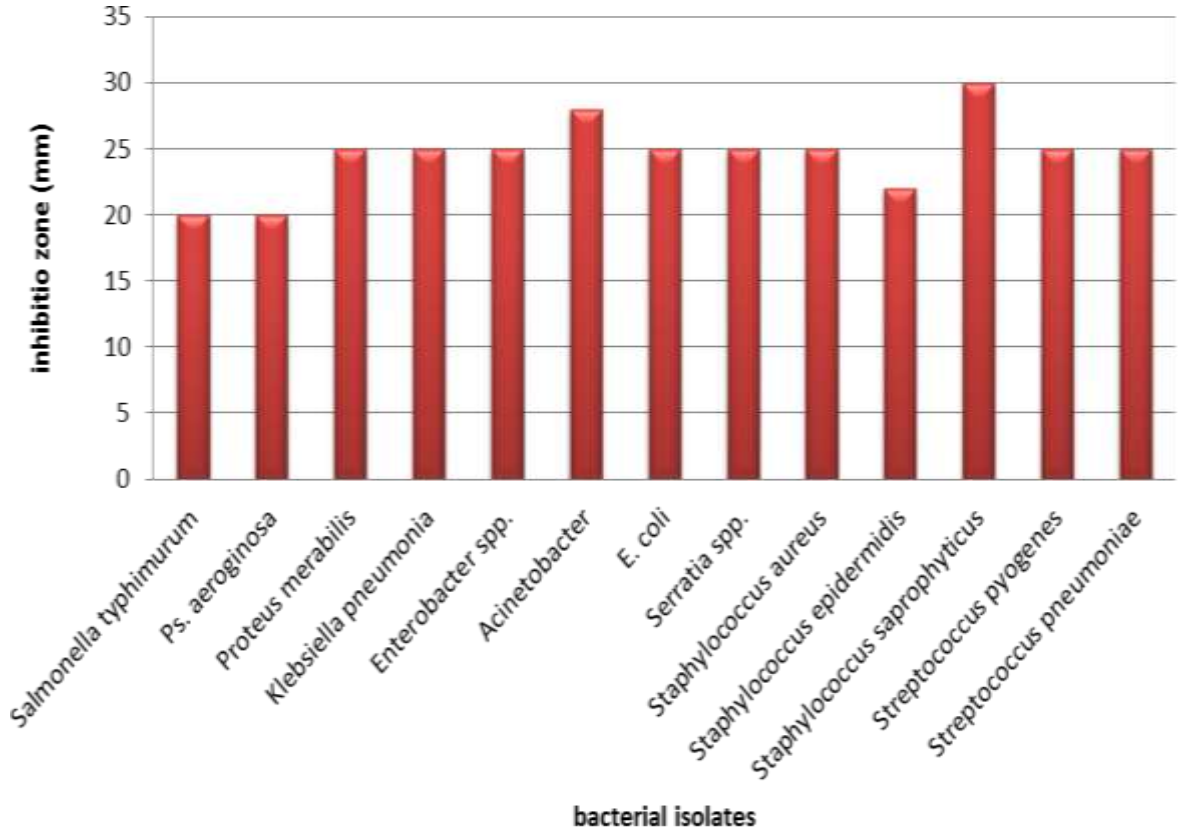

Figure 2: Antibacterial Activity of Aquatic Stem Bark Extract against Bacterial Isolates

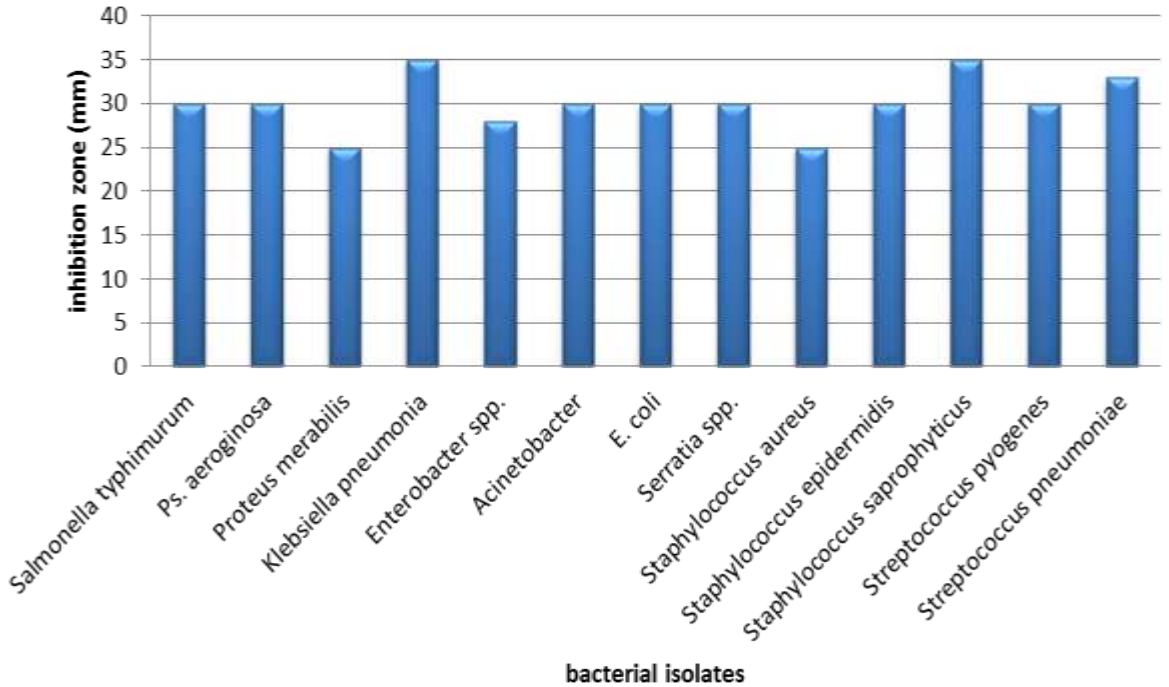

Figure 3: Effect of Combination (Leaves + Stem Bark) Aqueous Extract against Bacterial Isolates

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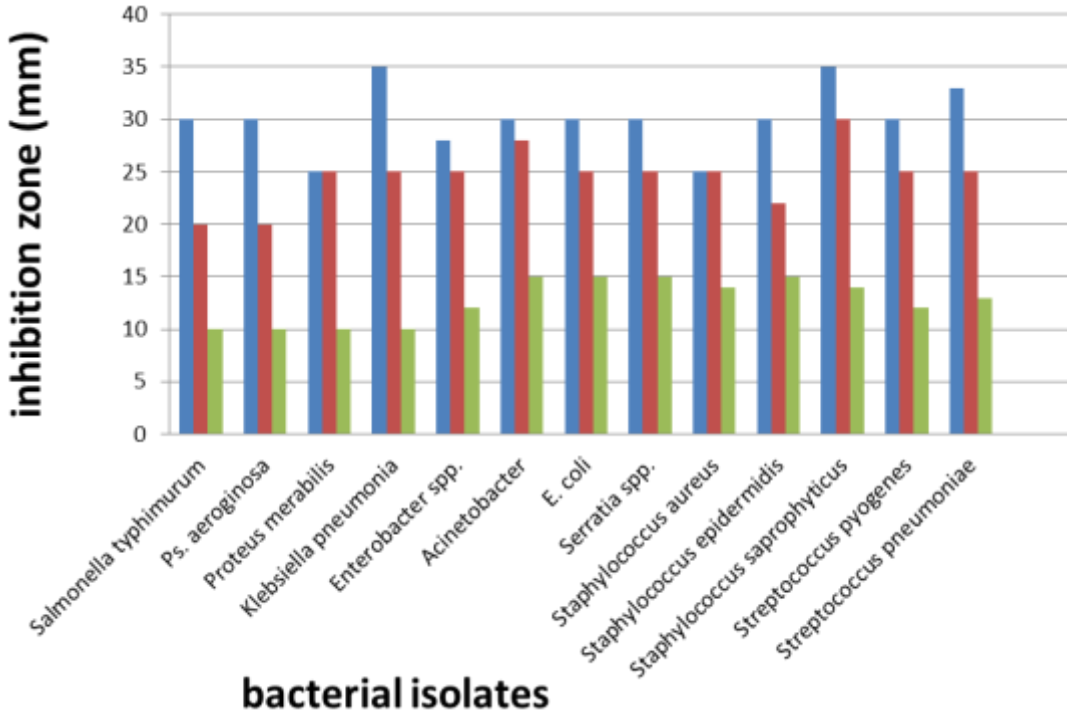

Figure 4: Comparision among Effects of Leave, Stem Bark and (Leave + Stem Bark) Aqueous Extracts against Bacterial Isolates

IV. DISCUSSION

This study aims to give preliminary information about the in vitro antimicrobial activity of *Ziziphus spina-christi* on thirteen pathogenic isolated bacteria, the agar- well diffusion test was preferred to be used in this study.

The result reveals that the aqueous extracts of both (leaves + stem bark) have an activity against both (Gram negative and positive) bacterial isolates above.

The results of the physiochemical analysis of both extract of *Z. spina-christi* showed that it contained carbohydrates, flavonoids, saponins and tannins. These compounds are known to have curative properties against several pathogens [15]. So, it's possible use in treatment a number of ailments traditionally.

The antibacterial screening records express inhibition zones on most of the tested organisms. However, it has been suggested that plant extracts exhibiting zone of inhibition diameters (>10) mm were considered active [16].

The results of this study reveals that the various aqueous extracts (leave, stem bark, leave+ stem bark) of *Z.spina-christi* have antibacterial properties especially when used in combination (leaves + stem bark) on pathogenic bacterial isolates.

The obtained inhibition zone diameters was sorted desendingly as follows; Klebsiella pneumoniae, Staphylococcus saprophyticus, Streptococcus pneumonia ,Acinetobacter spp., Escherichia coli, Serratia spp, Salmonella typhi, Pseudomonas aeroginosa, Staphylococcus epiderdimis, Streptococcus pyogenes, Staphylococcus aureus, Proteus mirabilis and Enterobacter spp.

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So the greatest result obtained from use the combination (leaves + stem bark) extract followed by stem bark and finally leaves extract. This result agreed with [17, 18, 19] results. Result of this paper also resembles hose, which show the effects of leave aqueous extract the aqueous extracts of *Z. spina-christi*shows antimicrobial activity against gram+/-vebacteria, especially *Klebsiella*, then use ager-well diffusion method to measure inhibition zone diameters, which may due to the ability of these extracts to inhibit nitrate reductase production by these microorganisms [20].

Moghdam et al. (2010) [21] showed *Z.spina-christi* extract have antimicrobial activity against MRSA, which cause serious diseases as a result to present cyclo peptide alkaloids, several flavoid and tannin. So, this work agreed with El-kamali &Al-tayabmahgob (2009) [22] that revealed the sensitivity of gram negative bacteria, especially those multidrugs resistance (MDR) pathogens like *P. aurogenosa* to stem bark extract.

Recently with an increasing multi-drugs resistance pathogen problem and threats, Silver nanoparticles (AgNPs) have become an attractive alternative to antibiotics, and creation silver nanoparticles from plant species are toxic to multidrug resistant microorganisms. So Mousod *et al*., (2016) [17] demonstrates the ability to synthesize silver nanoparticles using *Z.spina-christi*aqueous leaves extract.

V. CONCLUSION

This study appeared that combination (leaves + stem bark) extract exhibited strong antibacterial activities followed by the activity of aqueous stem bark extract of Z. spina-christi and aqueous leaves.

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