

A study of antimicrobial and antioxidant activity to ethanolic extract of sider (*Ziziphus spina-christi*) leaf against some Pathogenic microorganisms

Abstract

The antimicrobial activity of ethanolic extracts of *Ziziphus spina-christi* leaves were examined by using agar well diffusion method against five bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* and *Staphylococcus epidermidis*) in addition to *Candida albicans*. Results of this present study indicated that the ethanol extract of *Ziziphus spina-christi* leaves was effective against tested gram positive and negative bacteria whereas the *C. albicans* was showed resistance to this extract. Results showed that the gram positive bacteria more sensitivity to ethanolic extract of *Ziziphus spina-christi* leaves at all concentration used (from 5% to 30%) than gram negative bacteria. The highest inhibition zone was by a high concentration of *Ziziphus spina-christi* ethanolic extract 30% against *S. aureus* with inhibition zone equal (19mm) while, the lower inhibition zone given by second concentration 20% against *P. aeruginosa* with inhibition zone equal to (9mm). The results of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of ethanolic extract possess high antioxidant activity at different concentrations with IC_{50} value equal to 176 μ g/ml compared to the IC_{50} value of control sample (L-Ascorbic acid) equal to 145.88 μ g/ml.

Keywords: antimicrobial activity, antioxidant activity, *Ziziphus spina-christi*, ethanolic extract, pathogenic microorganisms

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Introduction

Medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs.¹ The medicinal plants have been used for traditional health remedies and considered the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects.² In recent years, there is most interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly commercial synthetic drugs that have adverse effects.^{3,4}

The family of Rhamnaceae included many genres that have medicinal importance. One of these genres is *Ziziphus* that comprises about 100 species of deciduous or evergreen trees and shrubs. distributed in the tropical and subtropical regions of the world. All parts of the plant *Ziziphus* has medicinal importance and used by the local Arab people to help maintain a healthy life style.⁵ Various species of *Ziziphus* are used frequently in traditional medicine in the middle-east, Africa and some Asian countries for acquiring good health and treating of many ailments; including headache, fever common cold, asthma, pulmonary ailment, malaria, wounds, burns, stomach discomfort and urinary infections.⁶

Ziziphus spina-christi has been used in folk medicine as a depurative, demulcent, anodyne, stomach-ache, toothaches, emollient, astringents, antibacterial, antifungal and as a mouth wash.^{7,8} Plant products are rich sources with different biological compounds, mainly phenolics, various flavonoids, saponins, tannins, betulinic, ceanothic acids, erols, and triterpenes. These phytochemicals have been found to possess different biological properties like antioxidant and antimicrobial effect.^{9,10} The leaves are used to sores remedy, and the roots are used to cure and prevent skin diseases.⁵ The seeds are sedative and are taken sometimes with buttermilk to halt nausea, vomiting and abdominal

pains associated with pregnancy.¹¹ *Ziziphus spina-christi* (L.) has been reported to have activity against bacterial and fungal pathogens that are normally quite resistant to modern medications.¹²

The main objective of this study to investigate potential uses of *Ziziphus spina-christi* as antimicrobial and antioxidant against some foodborne pathogens and using ethanolic extract of *Ziziphus spina-christi* as alternative food preservative agent.

Materials and methods

Preparation of sample

Sample collection

The useful plant leafs were collected from Yemen - Taize government - Bani Alhusam and identified according to guideline of the.^{13,14} The collected plant material was dried in an open air protected from direct exposure to sun light. All dried plant sample was finely ground by using an electrical grinder to a fine powder and made ready for extraction.

Preparation of extract

The sample of *Ziziphus spina-christi* leafs was extracted by using 1:10 in ethanol 80% at room temperature for 48 hours with occasional shaking. Then filtered by using filter paper (Whatman No 1.5, whatman Ltd., England). The extract was evaporated to dryness in oven at 45°C to yield dry crude extract. The obtained crude extract was filled to amber tight closed bottle glass in refrigerator until uses.

Antimicrobial assay

Microorganisms used

The microbial strains were get from faculty of science, Sana'a university, republic of Yemen in April-2018. These microorganisms include:

Pseudomonas aeruginosa MTCC2453 Mar.2001, *Escherichia coli* MTCC739 Mar.2001, *Staphylococcus aureus* (ATCC653-8), *Staphylococcus epidermidis* (local isolate), *Bacillus cereus* (ATCC6633) and *Candida albicans* (ATCC2019).

The bacterial strains were maintained on tryptone soya agar (TSA) and the fungal strains were maintained on Sabouraud dextrose agar (SDA).

Antimicrobial and Antibiotic assays

The Antimicrobial and Antibiotic assay was performed by using Agar Well Diffusion Method according to.^{15,16}

Antimicrobial assay

Four different concentrations of each extract of selected plants {300mg/ml (30%), 200mg/ml (20%), 100mg/ml (10%) and 50mg/ml (5%)} were dissolved in 10% {dimethylsulfoxide (DMSO) in Phosphate buffered saline} to be used in antimicrobial assay test. Extract solutions were prepared just before carrying out the test. Antimicrobial activity of the extracts was determined by agar well diffusion method as described by^{15,16} on Tryptone soya Agar (TSA).

In each of these Plates four wells were cut out by using a standard cork borer (8 mm). About 60µl of each extract was added into different wells (triplicate each concentration). DMSO was used as a negative control. A positive control antibiotic disc was placed in the other plate. All the plates were incubated for 24h at 37°C. After incubation bioactivity was evaluated by measuring the zone of inhibition. All the experiment was performed in triplicates.

Antibiotic assays

The Antibiotic assay was performed by using antibiotic standard disc diffusion. The antibiotic standard disc was get from HiMedia and oxoid company which are Aztreonam (AT₃₀), Cefotaxime (CAZ₃₀), Co-Trimoxazole (COT₂₅), Gentamycin (Gen₁₀), Nystatin (100IU), Norfloxacin (NOR₅) and Vancomycin (VA₃₀)

Determination of DPPH radical scavenging activity:

The antioxidant activity of the ethanolic extract, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, with some modification according to.¹⁷ The antioxidant activity of the plant extract was estimated for a typical reaction, 1 ml of 0.2mM DPPH solution in methanol was mixed with 1 ml of (1, 0.5, 0.25 mg/ml) of plant extract. The reaction mixture was incubated in the dark for 15 min. L-ascorbic acid was used as standard antioxidants and methanol was used as the control. An aliquot of 2 ml of a methanolic solution of DPPH (0.2mM DPPH) was added into the different concentration (1, 0.5, 0.25 mg/ml) of each extract and ascorbic acid as long as control sample (both extract and ascorbic acid were dissolved in methanol). All samples were incubated in the dark at room temperature for 15 min before absorbance values were read at 517 nm (PerkinElmer UV. visible spectrophotometer). The decrease in absorbance was calculated as an IC₅₀ and expressed as µg/ml, which is the concentration of sample required for 50% scavenging of DPPH radicals in the specified time period. The radical scavenging effect was calculated as follows:¹⁸

$$\text{Radical scavenging effect (\%)} = \frac{Ac - As}{Ac} \times 100$$

Ac = absorbance of control

As = absorbance of test sample.

Where control is the absorbance of the DPPH radical + methanol.

Results

Antimicrobial activity of ethanol extract

The results showed in Table 1 explain the efficacy of the difference in the concentration as well as the type of tested microorganisms on the antimicrobial efficacy of the ethanol extract of the *Ziziphus spina-christi* which is directly proportional to the increase in concentration, that ranged between 5% and 30% where it was found that the ethanolic extract have antimicrobial activity against all tested microorganisms except *C. albicans*, which was resistance to this extract. Results also referred that the gram positive bacteria more sensitive than gram negative bacteria where the higher diameter of inhibition zone was 19 mm against *S. aureus* at the highest concentration 30%. While the lowest value of inhibition zone diameter was 9 millimeters against *P. aeruginosa* at the second concentration 20%. The results of sensitive and resistant of antibiotics (standards) was high with COT₂₅ (35mm) with gram-negative bacteria *E. coli* whereas the results showed resistance of *P. aeruginosa* to antibiotic (COT₂₅ and VA₃₀) respectively (Figure 1).

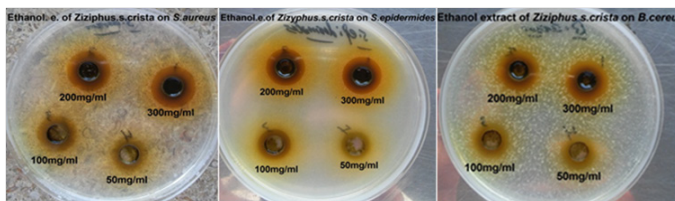


Figure 1 Inhibition zone diameters of *Ziziphus spina-christi* ethanolic extract against tested microorganisms.

DPPH radical scavenging activity

Results in table 2 revealed that the high antioxidants activity of *Ziziphus spina-christi* ethanol extract equal 92.32% at higher concentration (1 mg/ml) in comparison with those for sample control (L-ascorbic acid) equal 94.09% and with inhibitory concentration 50% (IC₅₀) value equal 176µg/ml compared to the IC₅₀ value of control sample (L-Ascorbic acid) that equal 145.88. The free radical scavenging efficacy was increasingly with the increased in concentration (Figure 2).

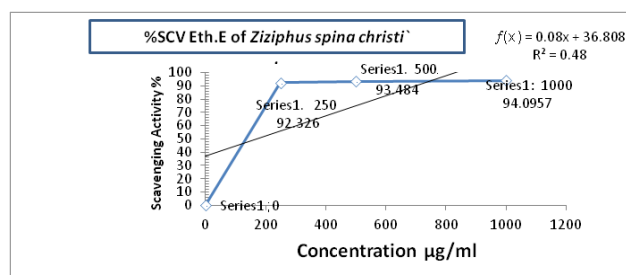


Figure 2 Correlation between concentration of *Ziziphus spina-christi* ethanolic extract and antioxidant activity.

Discussion

Antimicrobial Activity of ethanol extract

In ethanolic extract the highest antimicrobial activity was at 30% and 20% against all tested microorganisms except *C. albicans* that displayed resistance to all tested concentrations. The lower concentrations (10 and 5%) revealed antimicrobial effect only against

gram positive bacteria (*S. aureus*, *S. epidermidis* and *B. cereus*) While, gram-negative bacteria (*P. aeruginosa* and *E. coli*) resistant to the lower concentrations.

This Findings were in agreement with previous studies conducted by^{8,19-24} who reported that there was antimicrobial activity against *S.aureus*. Results conducted by² that was found the alcohol extract of *Ziziphus spina- christi* leaves is high antimicrobial effect against bacterium *B. cereus*. As for the activity ethanol extract of the *Ziziphus spina- christi* plant against *E. coli* bacterium the results were confirmed with the previous studies^{2,8,21,25,26} who mentioned that the ethanol extract of the *Ziziphus spina- christi* leaves have inhibitory efficacy against *E. coli* bacterium. Results conducted by^{24,27} display the antimicrobial activity against *P. aeruginosa* this results agreed with the present study. On the regarding to effect of ethanol extract against *E. coli* the results disagree with study of.²⁴ On the other hand

the results disagree with results of^{20,2} which showed a significant effect of the ethanol extract against the *C. albicans*. Study by² concluded that the antimicrobial activity of *Ziziphus spina-christi* leaves extract on microorganisms increase with the increased in concentration. The activity of some extracts may be returned to presence of some effected compounds such as phytochemicals (a type of phenolic compound that has the ability to break down molecular biofilms). This efficacy was related to the high level of not only by the monoterpene hydrocarbons²⁸ but also by the tannins which bind cell walls of ruminal bacteria.²² The high activity of the alcoholic extract is due to the low value of acidity (pH) compared to some other extracts.²⁹ The same studies indicated to that *Ziziphus spina-christi* leaves contain several organic acids such as malic, citric, tannic, acetic and other acids that lead to a decrease in the acid function (pH) which increases the antimicrobial efficacy of the extract.

Table 1 Ethanolic extract activity of *Ziziphus spina – christi* against tested microorganism at different concentrations

Type microorganism	Concentration mg/ml(%)				Name of antibiotic standard							
	5%	10%	20%	30%	AT ₃₀	CAZ ₃₀	NOR ₅	Gen ₁₀	COT ₂₅	VA ₃₀	Nys ₁₀₀ IU	
	Zone of inhibition in mm											
<i>Staphylococcus aureus</i>	15	15.5	18.5	19	0	23	20	22	28	21	NT	
<i>Staphylococcus epidermidis</i>	12.5	14	16	17	0	19	20	23	28	22	NT	
<i>Bacillus cereus</i>	13	14.5	16.5	17	0	24	30	27	34	30	NT	
<i>Pseudomonas aeruginosa</i>	R	R	9	12	24	21	12.5	21	0	0	NT	
<i>Escherichia coli</i>	R	R	9.5	12	29	27	26.5	19.5	35	12	NT	
<i>Candida albicans</i>	R	R	R	R	0	0	0	0	0	0	13.8	

NT=not test R=resistance

Table 2 DPPH scavenging activity to ethanolic extract of *Ziziphus spina-christi* leaves

Type of extract	Radical scavenging effect %				
	Concentration				
	250µg/ml	500µg/ml	1000µg/ml	means	IC ₅₀ µg/ml
ethanol	92.326	93.484	94.0957	93.302	176
L-ascorbic acid	96.76391	96.83067	96.88698	96.827	145.8838
L.S.D at (P<0.05)for extracts = 0.0057					

Antioxidant activity

The results of this study was appeared high antioxidant activity to ethanolic extract of *Ziziphus spina- christi* leaves comparing to results of standard (Ascorbic acid). The results was in agreement with the results of previous study by²³ about quantification of total phenolic, flavonoids and tannins in ethanol extract of other genus of *Ziziphus* (*Ziziphus lotus* and *Ziziphus jujube*) from many regions in Tunisia and their antimicrobial and antioxidants activity. The antioxidant activity of *Ziziphus spina-christi* attribute to its high quantity of phytochemical compounds (phenols, flavonoids, cardiac glycosides, polyphenols, saponins and tannins).^{23,30} The phytochemicals activity was correlated with their chemical structures and degrees of polymerization.³¹ Sterols like β-sitosterol, terpenoid, phytosterols, triterpenoids, alkaloids, saponins, flavonoids, glycosides and tannins have been reported to have antioxidant activity.^{32,33} This antioxidant activity attribute to structure of phytochemicals content where flavonoids contain conjugated ring structures and hydroxyl groups that have the probable to function as antioxidants in vitro or cell free systems by scavenging lipid peroxyradicals, superoxide anion, singlet oxygen and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species.^{34,35} Flavonoids can be act by

various ways to prevent injury caused by free radicals. Through in vitro experiments, it has been found that flavonoids possess anti-inflammatory, antiviral, antiallergic and anticarcinogenic properties.³⁶ The flavonoids prevent synthesis of prostaglandins that suppress T-cells by forming prominent inhibitors of cyclooxygenase and lipoxygenase. The immune cells communicate with chemical signals called cytokines, which are controlled by flavonoids.³⁷ Flavonoids directly act as antioxidant by reducing free radicals to produce a more stable and less reactive radical (oxidize the flavonoids).³⁸⁻⁴¹

Conclusion

The high antioxidant and antimicrobial clear efficiency to ethanolic extract of *Ziziphus spina Christi* leaves against many of microbial strains that causes different important diseases to human and animal confirming the traditional herbal medicines usages and provide a scientific basis for traditional medicine uses of *Ziziphus spina Christi* leaves ethanolic extract in primary health care.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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