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Assessment of Antimicrobial and Antioxidant Efficacy of Methanol Extract of Ziziphus Spina-Christi against Some Pathogenic Micro-Organisms

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Abstract

The antimicrobial activity of methanolic extracts of Ziziphus spina-christi leaves were examined by using agar well diffusion method against five bacteria *Staphylococcus aureus* (ATCC653-8), *Pseudomonas aeruginosa* MTCC2453, *Bacillus cereus* (ATCC6633), *Escherichia coli* MTCC739, *Staphylococcus epidermides*(local isolate) in addition to *Candida albicans* (ATCC2019). The results indicated that the Ziziphus spina-christi leaves methanol extract is effective against tested gram positive bacteria in addition to Candida albicans at all concentration used(50 mg/ml to 300 mg/ml) while don't have any effect against tested gram negative bacteria. In general, all concentration used given an effect on the gram positive bacteria only. The highest inhibition zone given by the high concentration of Ziziphus spina-cristi acetone extract 300 mg/ml on *Bacillus cereus*. While the lower inhibition zone given by third concentration 100 mg/ml against B.cereus. The results of the DPPH scavenging activity of methanol extract possess high antioxidant activity at different concentrations with IC50 value equal to 166.4698 ug / ml compared to the IC50 value of control sample (L-Ascorbic acid) that equal to 145.8838 ug / ml .

Keywords: Antimicrobial activities Antioxidant activity; Ziziphus spina-christi; Pathogenic microorganism

Introduction

Genus Ziziphus belongs to the family Rhamnaceae. This genus comprises of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world Genus Zizyphus has medicinal importance as all parts of the plant are used by the local Arab people to help maintain a healthy life style [1]. Ziziphus spina-hristi has been used in folk medicine as a depurative, demulcent, anodyne, stomach-ache, for toothaches, emollient, astringents, antibacterial, antifungal and as a mouth wash [2]. The leaves of these plants contain betulinic and ceanothic acids, various flavonoids, saponins, erols, tannins and triterpenes. Plant products are rich sources of a variety of biologically active compounds, mainly phenolics, and these phytochemicals have been found to possess various biological properties like antioxidant and antimicrobial potentials [3].The leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases. Zizyphus Spina-Christi L has been reported to have activity against bacterial and fungal pathogens that are normally quite resistant to modern medications [4]. The methanol extract of sider could be used not only as a safe potential natural functional food ingredient or as therapeutic drug in the treatment of diabetes, but also it is effective in reducing both hyperlipidemia and oxidative stress accompanying diabetes.

The main objective of this research was evaluation of antimicrobial activity of methanolic extracts of leaves and seeds of Zizyphus spina-christi against bacterial strains (including *Staphylococcus aureus* (ATCC653-8), *Pseudomonas aeruginosa* MTCC2453, *Bacillus cereus* (ATCC6633), *Escherichia coli* MTCC739, *Candida albicans* (ATCC2019) and *Staphylococcus epidermides* (local isolate).

Materials and Methods

Sample collection

The useful plant leaves were identified according to instructions of the Ministry of tourism and environment [5].The collected plant material was dried in an open air protected from direct exposure to sun light. All dried plant samples were finely ground by using an electrical grinder to a fine powder and made ready for extraction.

Preparation of extract

Powdered of Z. spina-christi leaves plant was macerated in ratio 1:10 in 80% methanol at room temperature for 48 hours with occasional shaking [6]. Then the resulting extract was filtered by using filter paper. The solvent extract was evaporated to dryness in oven at 45Cº 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 include)

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

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The bacterial strains were maintained on Trypton Soya Agar Medium (TSDA), and the fungal strains were maintained on Sabouraud Dextrose Agar (SDA) medium [7].

Antimicrobial and Antibiotic assays

The Antimicrobial and Antibiotic assay was performed by using Agar Well Diffusion Method.

Antimicrobial assay

Four different concentrations of each extract of selected plants (300, 200, 100 and 50 mg/ml) were dissolved in 10% DiMethylSulfOxide (DMSO) in Phosphate buffered saline to be used in antimicrobial activity 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 on Tryptone Soya Agar (TSA) [8].

In each of these plates four wells were cut out by using a standard corn 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 HI Media & Oxoid Company [9].

Results and Discussion

Antimicrobial Activity of methanol extract

The test of antimicrobial activity of Ziziphus spina-christi methanol extract was conducted on five types of positive and negative bacterial strains of Gram stain, which were tested. *Staphylococcus aureus* (ATCC653-8) , *Bacillus cereus* ATCC6633, *Staphylococcus epidermides* (local isolate), *Escherichia coli* MTCC739 and *Pseudomonas aeruginosa* MTCC2453 in addition to *Candida albicans* (ATCC2019); Comparing with the effect of some standard antibiotics used (in pharmacy) which are Ceftazidin (CAZ30), Aztreonam (AT30), Norfloxacin (NOR5), Gentamycin (Gen10), Co-Trimoxazole (COT25), Vancomycin (VA30), Nystatin (100IU)) Against those tested microorganisms.

The results showed appeared that the methanol extract of Zizyphus spina-christi have antimicrobial activity against tested gram positive bacteria as well as C.albicans with the inhibition zone diameter wobble between 11mm and 16.5mm at different concentrations (50mg/ml to 300mg/ml) were S.aureus was more sensitive than other tested gram positive bacteria and C.albicans with the inhibition zone diameter equal to 17mm at high concentration 300 mg/ml while B.cereus was less sensitivity than others at the same concentration with the inhibition zone equal 14 mm whereas that the methanol extract of ziziphus – spina Christi don't have any antimicrobial effect against gram negative bacteria(*E. coli & P. aeruginosa*) at all concentration used This variation in sensitivity response between gram positive and negative bacteria perhaps to difference bacterial cell wall composition where The gram-negative bacteria requires a pathway through the lipopolysaccharide outer membrane to entrance of antibiotics as well as other complex molecules such as plant extracts **Table 1**. This pathway is provided by protein channels called porins. The ability of molecules to entrance through these channels is affected by their size, shape, and electrical charge **Figure 1**. It has been established that porins serve as major entry gates for antibacterial compounds in these organisms. These membrane proteins were originally believed to be absolutely responsible for the inherently higher resistance of gram-negative bacteria to antibiotics. Decreased entry of antibiotic into the bacterial cell is not important in gram-positive bacteria because they lack a lipopolysaccharide outer membrane. Although the peptidoglycan layer of gram-positive bacteria is thicker than that of gram negative bacteria, it does not pose a significant barrier to antibiotic entry [10]. This antimicrobial vigorous of methanol extract of Ziziphus spina Christi leaves probably return to possess **Figure 2** phytochemicals compounds such as tannins, flavonoids, alkaloids and many secondary metabolites of plants that perform as defensive mechanics Against many microorganisms, insects and herbivores [11-13]. In the regarding the effective effect of the methanol extract of Ziziphus spina Christi leaves plant on S.aureus bacteria this results agree with the results studies by and disagree with the results study regarding to *E.coli & P.aeruginosa*[14].

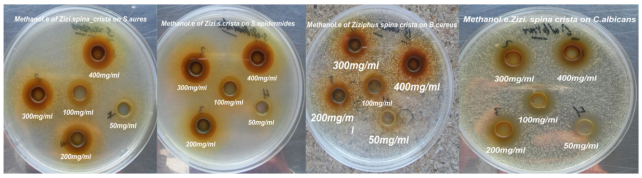

Figure 1: Appear Inhibition zone diameters of Ziziphus spina-christi methanol extract against tested microorganisms.

DPPH radical scavenging activity

DPPH scavenging activity of Zizyphus spina-cristi methanol extract					
Type of extract	Radical scavenging effect %				
	concentration				
	250µg/ml	500µg/ml	1000µg/ml	means	IC50 µg/ml
acetone	93.67743	94.07963	94.8599	94.205	166.4698
L-ascorbic acid	96.76391	96.83067	96.88698	96.827	145.8838
L.S.D at(P<0.05)for extracts = 0.0057					

Table 1: Illustrate the direct relationship between the concentration and antioxidant activity the Zizyphus spina-cristi methanol extract and comparison with the positive control (L-Ascorbic acid).

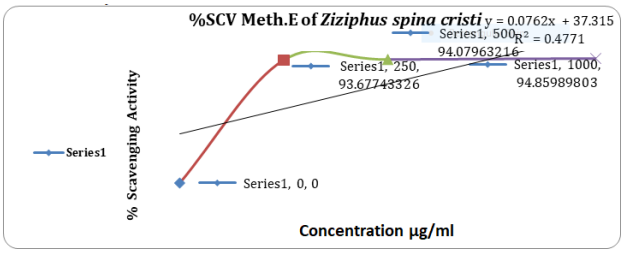

Figure 2: Illustrate the direct correlation between increased concentration of Zizyphus spina-cristi methanol extract and antioxidant activity

Conclusion

The results of the DPPH scavenging activity of methanol extract referred to that the Ziziphus spina-cristi methanol extract contains highly active antioxidants equal to 94.8599% at higher concentration (1 mg/ml) in comparison with those for sample control (L-ascorbic acid) equal 96.827% and with inhibitory concentration 50% (IC50) value equal to 166.4698 ug/ml compared to the IC50 value of control sample (L-Ascorbic acid) that equal to 145.8838 this results of is agreement. The results of the DPPH scavenging activity of methanol extract possess high antioxidant activity at different concentrations with IC50 value equal to 166.4698 ug/ml compared to the IC50 value of control sample (L-Ascorbic acid) that equal to 145.8838 ug/ml .

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