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Original

Phytochemical Screening, Antimicrobial and Antioxidant Activities of *Ziziphus spina-christi* (L.)Leaves extracts

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Abstract

This study aimed to explore the phytochemical profile, antimicrobial and antioxidant activities of n-hexane, ethyl acetate and methanol extracts. Plate agar diffusion method was used to assess the antimicrobial activity of these extracts using four bacterial species*;* two Gram positive; *Bacillus subtilis*, *Staphylococcus aureus,* two Gram negative; *Escherichia coli*, *Pseudomonas aeruginosa* and two fungal species; Aspergillus *niger* and *Candida albicans*. The methanol extract showed a significantly higher antibacterial activity in comparison to the other extracts. No antifungal activity was detected in this research with all extracts of *Ziziphus spina-christi* leaves.

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Keywords: Antimicrobial, Antioxidant, Phytochemical, Sider, Rhamnaceae, *Ziziphus spina-christi*.

Introduction:

Ziziphus spina- Christi of the family Rhamnaceae, known as (Sedra, Sidr, Sider, Nbag, Nebeq, Jabat, Zejzaj, Zefzoof, Ardeg , Christ`s thorn and Jujube plant. [1,2].*Ziziphus spina-christi* is wide spread in tropical and subtropical region. The genus *Ziziphus*is widely distributed in the Middle East. Since ages extracts of *Ziziphus spina – christi* have been used in folklore and traditional medicine to treat toothache, analgesic, pectoral, astringent, antirheumatic, purgative, for stomach pain, anti-helminthic [3,4,5]. *Z. spina-christi* has very nutritious fruits that eaten. *Ziziphus* used for treatment of pneumonia, dysentery, scorpion stings, cough, constipation, intestinal worms, and fever are some of indications for application of this plant [6,7]. Its leaves extracts has antimicrobial, anti-nociceptive, anti-diabetic, and anti-hyperglycemic effects [8]. *Ziziphus spina-christi* contains antioxidants [9]. In addition, *Ziziphus spina-christi* L. is prescribed as an anti-microbial agent for relieving digestion disorders, obesity and urinary troubles, and for skin care [10]. Its leaves were also used for treatment of liver, asthma, fever, antidiabetic, hypoglycemic, antimicrobial, anti-inflammatory and as an immune system stimulant [11].

Materials and Methods:

Collection of Plant sample

The leaves of *Ziziphus spina-christi*were collected from North Kordofan State-Sudan in 2019. The plant material was authenticated by Dr. Yahiya Suleiman taxonomist of the Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan. The powder of dry leaves was extracted by Soxhlet extraction method with n-hexane, ethyl acetate and methanol.

Preparation of crude extracts

Ziziphus leaves powder (100 gram) was extracted with n-hexane in Soxhlet apparatus. The n-hexane extract was filtered and evaporated under reduced pressure using Rota- Vap. The extract was dried on air, and repacked in the Soxhlet and exhaustively extracted with ethyl acetate for 20 hours. This extract was filtered and evaporated under reduced pressure using Rota-vap. The same procedure was applied for methanol extract. Each residue was weighed and the yield percentage was determined. [12]

Phytochemical screening

Phytochemical screening of *Ziziphus spina-christi* extracts were assessed by the standard method as describe by [13].

Test for Steroids and Triterpenoids:

Liebermann Burchard test:

Crude extract of *Ziziphus spina-christi* Leaves was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green color of the upper layer and the formation of deep red color in the lower layer indicated a positive test for steroids and triterpenoids respectively.

Test for Glycosides:

Keller Killiani Test

0.5g of the extract was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Saponins:

Foam Test

0.5g of the extract was mixed with water and shaken and observed for the formation of froth, which was stable for 15 minutes for a positive result.

Test for Alkaloids:

Mayer,s reagent

0.5g of the extract was heated with 5 ml of 2N Hcl in water bath and stirred for about 10minutes, cooled filtered and divided into two test tube, to each test tube few drops of Mayer,s reagent (Potassium Mercuric Iodide) was added, A slight turbidity or heavy precipitate was taken as presumptive evidence for the presence of alkaloids.

Hager's Test:

0.5g of the extract was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate showed a positive result for the presence of alkaloids.

Test for Flavonoids:

Alkaline reagent Test:

0.5g of the extract when treated with sodium hydroxide solution, showed increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test:

0.5g of the extract when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

Test for Tannins: Gelatin Test:

The extract when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Ferric chloride test:

The extract (0.5g) was treated with few drops of neutral ferric chloride, solution (5%). A bluish black color indicates the presence of tannins.

Lead acetate test: The extract was treating with few drops of neutral Lead acetate solution (10%). The formation of yellow precipitate indicates the presence of tannins.

Antimicrobial activity

Gram positive bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC25923), gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and two fungal strains *Apergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596) were used. Bacterial and fungal strains used in the study were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute. Thebacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

In vitro testing of extracts for antimicrobial activity

Testing for antibacterial Activity

The cup-plate agar diffusion method [14] was adopted with some minor modifications to assess the antibacterial of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8–10^9 C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the extracts using automatic micro-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for the extracts against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured.

Testing for antifungal activity

The same method as for bacteria were adopted, instead of Nutirent agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25ºC for three days for the *Aspergillus niger* and two days for *Candida albicans*. After incubation, the

diameters of the resultant growth inhibition zones were measured.

Antioxidant Activity:

DPPH (radical scavenging assay):

DPPH radical scavenging was determined according to the methods of [15]. In 96-wells plate the test samples were allowed to react with 2.2Di (4-tert- octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 ºC. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol after incubation; decrease in absorbance was measured at 517nm using multiplate reader Spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Results and Discussion:

Phytochemical Screening of Plant Extracts

Phytochemical screening of *Ziziphus spina-christi* (Leaves) consisted of the following metabolites in Table (1).

Phytochemical screening for major constituents was performed according to standard qualitative methods of [13]. The plant extracts were screened for the presence of Tannins, Alkaloids, Saponins, Flavonoids, Steroids and Triterpenes, Glycosides and Coumarins.

Table (1): The Chemical Constituents of *Ziziphus spina-christi* (Leaves)

Constituents	**Reagent used**	**Results**
Tannins	Gelatin Test Ferric chloride test	+++
Alkaloids	Mayer reagent Hager reagent	+++
Saponins	Foam test	+++
Flavonoids	Alkaline reagent Test	+++

Steroids and triterpenes	Liebermann Burchard test	+++
Glycosides	Keller Killiani Test	++
Coumarins	Florescence test	++

Key: ++ Moderate, +++ High

Antimicrobial activities

The methanol extract obtained a higher inhibition zone against all bacterial strains tested ranging between (21-14m) in comparison to other extracts.

Table (2): Antimicrobial activity of *Ziziphus spina-christi* (Leaves)

Solvent used	**Standard bacterial strains**						
	Concentration in mg/ml	*B.s*	*S.a*	*E.c*	*Ps.a*	*As.n*	*C.a*
n-Hexane	100	12	16	17	13	-	-
	50	10	14	16	12	-	-
	25	-	12	14	9	-	-
	12.5	-	-	13	-	-	-
Ethyl acetate	100	14	15	18	17	-	-
	50	-	14	15	15	-	-
	25	-	13	11	-	-	-
	12.5	-	12	10	-	-	-
Methanol	100	20	20	21	18	-	-
	50	17	18	20	19	-	-
	25	15	17	17	14	-	-
	12.5	14	16	15	15	-	-

Key:

**B.s, Bacillus subtilis; S.a, ;Staphyllococcus aureus; E.c, Escherichia coli; Ps.a, Pseudomonas aeruginosa, As.n, Aspergillus niger* and *C.a, Candida albicans*.

*Concentration of extracts (100, 50, 25, 12.5mg/ml) .

*Zone of inhibition in (mm), - No inhibition, <9mm inactive, 9-12mm partially active, 13-18mm active, >18mm very active.

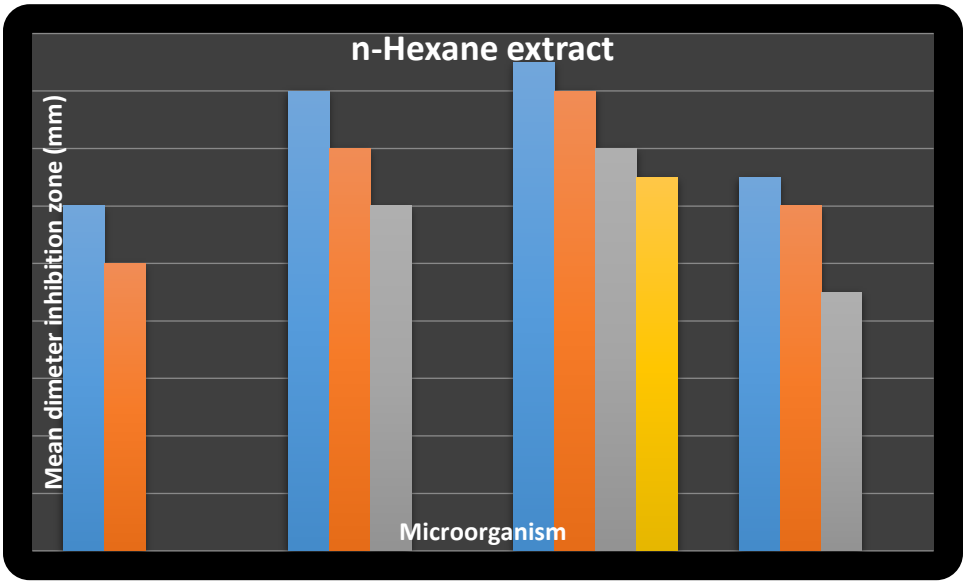

Figure 1: Antibacterial activity of n-hexane extract against (*B.s,S.a, E.c* and *Ps.a*) at Concentrations (100, 50, 25, and 12.5).

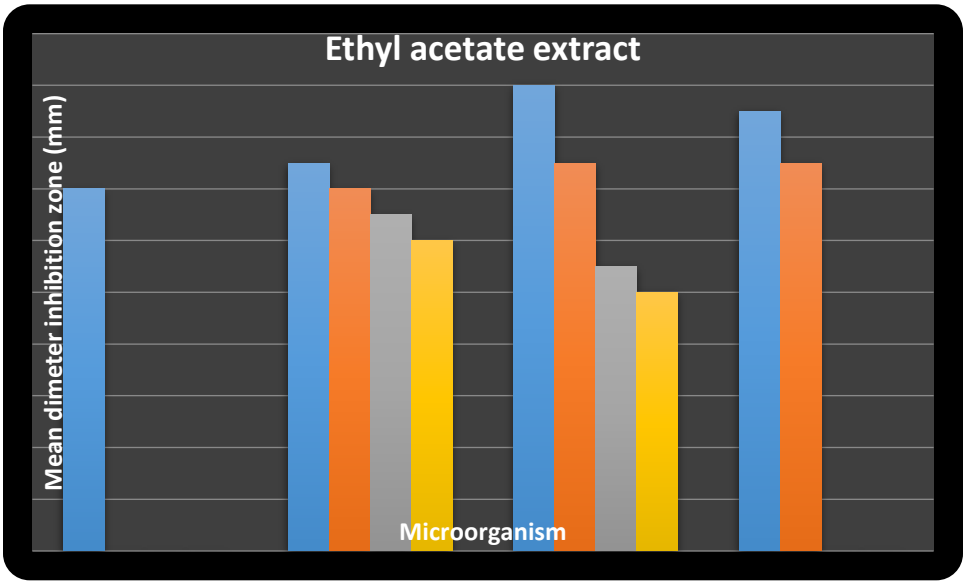

Figure 2: Antibacterial activity of ethyl acetate against (*B.s*,*S.a*, *E.c* and *Ps.a*) at Concentrations (100, 50, 25, and 12.5).

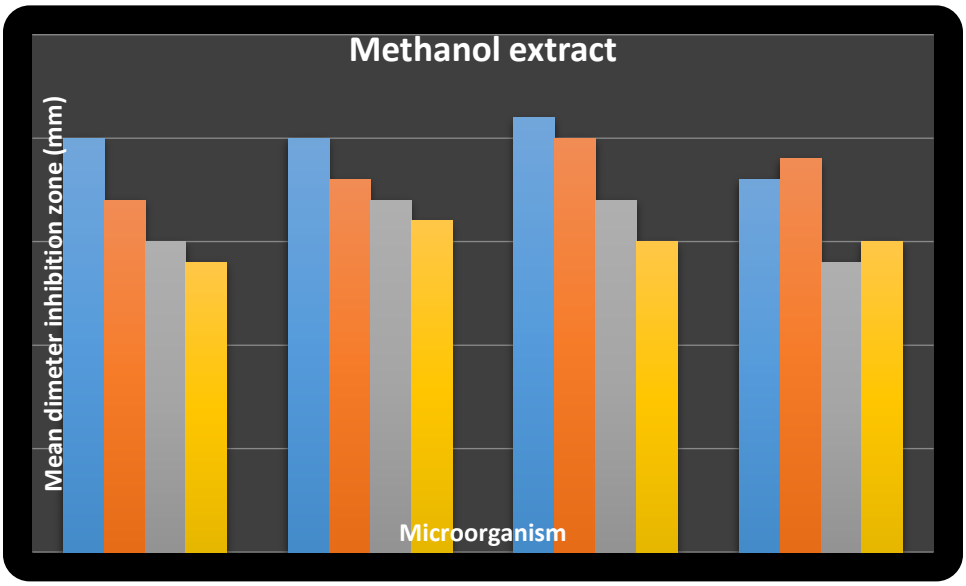

Figure 3: Antibacterial activity of methanol extract against (*B.s*,*S.a*, *E.c* and *Ps.a*) at concentrations (100, 50, 25, and 12.5).

Antioxidant activity

As indicated in Table (3), the most potent activity on the DPPH scavenging activity was obtained by the methanol extract (46.±0.09) in comparison to other extracts.

Table (3): The results of antioxidant activity:

NO	Extracts	% RSA ± SD (DPPH)
1	n-hexane	07 ± 0.02
2	Ethyl acetate	18 ± 0.04
3	Methanol	46 ± 0.09
Standard	Propyl Gallate	91 ± 0.01

Discussion:

Phytochemicals organic compounds are classified as primary or secondary constituents, depending on their role in plant metabolism [16]. Plant produces these chemicals to protect itself but recent research emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [17]. *Ziziphus spina-Christi* leaves are rich in secondary metabolites such as flavonoids, alkaloids,

tannins, glycosides, steroids and triterpenoids, coumarins and saponins. Our results were similar to [18]. The present study showed that the leaves extracts were more effective against *Pseudomonas* species (pneumonia). This aimed to assess the antibacterial and fungal activities of methanolic and ethanolic extracts of *Z. spina-christi* leaves for treatment for respiratory disorders as COVID-19. Corona viruses are positive-stranded, large RNA, enveloped viruses infecting numerous avian and mammalian species, meanwhile capable of causing gastrointestinal and respiratory diseases [19, 20]. The n-hexane, ethyl acetate and methanol extracts from *Ziziphus spina-Christi* leaves, were reported to inhibit the growth of most Gram-positive and negative bacteria. In this study, methanol extract at a conc. of 100mg/ml, showed antibacterial activity at all concentrations against Gram positive and Gram negative bacteria, than other extracts. This study revealed high antibacterial activity of methanol extract (21 mm) against *Escherichia coli, S. aureus, B. subtilis* (20mm) and *Pseudomonas aeruginosa* (18mm) The n-hexane extract of leaves exhibited high activity (17 mm) against *Escherichia coli* (16mm), against *S. aureus* , moderate activity (13mm) against *Pseudomonas aeruginosa* and (12mm) against *B. subtilis* . The ethyl acetate extract showed high activity (18mm) against *E.coli* (17mm) against *Pseudomonas aeruginosa* moderate activity (15mm) against *S. aureus* and (14mm) against *B. subtilis.* The methanol extract showed high activity against *Bacillussubtilis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, this result was similar to [21]. Methanolic extract of *Z. spina-christi* leaves was reported to have significant antibacterial activity against *E. coli* and *S. aureus* [22]. The high activity of the methanol extracts of leaves could be due to the presence of secondary metabolites. *Z. spina-christi* leaves also serve as a good source for antioxidant.Plant materials significantly lead to improving human health by the prevention of diseases.

Conclusion:

In this study the phytochemical screening of the leaves parts of *Ziziphus spina –Christi* confirmed the presence of tannins, alkaloids, saponins, flavonoids, steroids and triterpenes, glycosides and coumarins. Methanolic extracts of *Ziziphus spina – christi* leaves exhibited higher antibacterial activity against Gram positive and Gram

negative organisms than the other extracts. Accordingly, this study recommended using *Ziziphus* leaves methanolic extract for treatment of bacterial diseases caused by; *Bacillus subtilis*, *Staphyllococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. In contrast, this study provides that all extracts of *Ziziphus* leaves did not have any antifungal activities against *Aspergillus niger* and *Candida albicans.*

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Disclosure of conflict of interest

There is not any conflict of interest in this study.

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