

RESEARCH ARTICLE

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Antibacterial, Antioxidant and Cytotoxicity Activities of *Ziziphus spina-christi* n-hexane and Methanol Extracts

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Abstract

This study was carried out in Gezira State, during March, 2023. The plant of *Ziziphus spina-christi*, locally known Sider or Nabag, it was chosen for this study because of it is being used traditionally in treatment of many diseases. The objective of the present study was to evaluate the *in-vitro* antibacterial and antioxidant activity of n-hexane and methanolic extracts of leaves *Ziziphus spina-christi*. In addition, cytotoxicity of these extracts were determined. Extracts from each plant were prepared by sequential maceration of dried leaf powder in solvents of increasing polarity (n-hexane and methanol). The antibacterial activity was evaluated against Gram-positive and Gram-negative bacteria. Antioxidant activity were assessed based on the scavenging activity of the stable 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH). Cytotoxicity via brimp shrimp. Generally, the results of antibacterial activity showed the highest in methanolic extract against *Pseudomonas aeruginosa* (20±2.01mm) flowed methanol extract against *Bacillus subtilis* (19±3.09mm). The highest scavenging radical activity was obtained from the methanol extracts of leaf *Ziziphus spina-christi* (50 ±0.01%) while the n-hexane extract gave lowest than the methanol extract (32 ±0.02%). While the results of cytotoxicity of all extracts (n-hexane and methanol) shown not toxic against *Artemia salina*, lethality. In conclusion, the studied of plant was rich in bioactive agents with antioxidant and antibacterial activities potential and could have interesting pharmaceutical and cosmetic applications.

KEY WORDS: *ziziphus spina-christi*, antibacterial activity, antioxidant activity, cytotoxicity, crude extract.

1. INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the system of useful drug[1]. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day-to-day practice. Among the plants known for medicinal value[2].

The flora of Sudan consists of 3137 documented species of flowering plants belonging to 170 families and 1280 genera[3]. It is estimated that 15% of these plants are endemic to Sudan. The intersection of cultures and the unique geographical position of Sudan hold great potential for research in many fields, the most important of which is medicinal and aromatic plants the diversity of climates in Sudan results in a rich variety of flora species corresponding to the wide range of ecological habitats and vegetation zones. Though not yet investigated systematically or in depth, there are clues in literature about the bio-activity of the Sudanese medicinal plants and their chemical constituents[4].

The 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. *Ziziphus* species can grow either as shrublets, shrubs or trees with thorny branches and are used as a hedge to form defensive fences for animals[5]. Since ages extracts of *Ziziphus spina-christi* have been used as inflammatory treat toothache, analgesic, pectoral, astringent (LF), anti-rheumatic, purgative (FR), for stomach pain, anti-helminthic[6,7]. Some species, like *Ziziphus mauritiana* Lam. and *Ziziphus spina-christi* (L.) wild occur on nearly every continent.

2. MATERIAL AND METHODS

Plant material

The Plant *Ziziphus spina-christi*, leaves were collected in March, 2023 from Abu-haraz region, in Gezira State. The plant material was taxonomically identified by Dr. Ahmed Ali Mustafa, University of Gezira, Faculty of Science, Department of Botany and Microbiology. Plants was dried for 5 days in the herbarium and after drying plant sample was the homogenized in a pistil and mortar.

Preparation of extracts

Separately, (50g) of dried powdered leaf of each species were extracted consecutively by maceration in n-hexane and methanol (400 mL each) using a shaker apparatus, for about 24 h at room temperature, filtered and then solvents were evaporated under vacuum using a rotary evaporator. The resultant dry extracts from each sample were weighted and stored at 4°C until used.

Antimicrobial activity

The bacterial cultures used were *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 10145. Each extract (10mg/disc) was tested using the disc diffusion method as described by Mbavenge and coworkers[8]. 20 µg from each extract was then used to impregnate a blank sterilized disc and were left to dry. A bacteria culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Muller Hinton agar plates evenly using a sterile swab. The standards; gentamicin at a concentration of 10 mcg/disc served as the positive control for evaluation of the antibacterial activity and DMSO (100%) as the negative control. Plates were then incubated at 37° C for 24 h for bacteria. Results were documented by measuring the zone of inhibition in mm.

Antioxidant activity

The antioxidant activity of the extracts was evaluated using the *in vitro* 2,2-Diphenyl- 1-picrylhydrazyl (DPPH) radical scavenging method[9]. The reaction mixture consisted of 1.0 ml of DPPH in methanol (0.3 mM) and 1.0 ml of the extract (1.56 - 50 µg/ml). Thereafter, it was incubated in the dark for 10 min, after which the absorbance was measured at 517nm. Propyl gallate (1.56-50 µg/mL) was used as a positive control.

Cytotoxicity (brine shrimp) assay

The plant extracts (20 mg) were dissolved in 2 ml of respective solvent (methanol/ hexane). From this stock solution 5, 50 and 500µl was poured separately in, per marked at 5 ml; 20 ml vials (3vials/concentration) to attain final concentration 10, 100, 1000 ppm respectively. The vials were kept open over night with continuous air flow to evaporate the solvent. Artificial sea water (3 ml) was poured

in each vial and 10 matured brine shrimp larvae were added with the help of pasture pipette. Final volume in each vial was increased at marked level by adding sea water[10]. The vials were kept under illumination and after 24 h, survived nauplii were counted macroscopically.

Statistical analysis

All the procedures for extraction antibacterial antioxidant and cytotoxicity studies were repeated in triplicate and LD₅₀ value was calculated by probit analysis in a finny computer program. The descriptive analysis (mean and standard deviation) was used to discuss the results, assuming the normal distribution of the studied variables.

3. RESULT AND DISCUSSION

Antimicrobial activity

Sudan is the largest country in Africa with rich plants due to the climate diversity which are used in traditional medicine to treat several diseases[11,12]. The antibacteria effects n-hexane and methanolic extracts of leaves *Ziziphus spina-christi* were determined against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* microorganism bacteria. Results are depicted in Table 1 and Figure 1.

Extracts from the was studied plant displayed variable antibacterial activity. The highest antibacterial activity was recorded against *S. aureus* exerted by methanolic extract of *Ziziphus spina-christi* (20mm) and methanolic extract against *Bacillus subtilis* with inhibition zone (IZ) of (19mm) flowed n- hexane extract against *Bacillus subtilis* gave intermediate (IZ 15mm). This compared with assays reported by El-kamali and Mahjoub, 2020[13].

While n-hexane and methanol extracts against *P. aeruginosa* and *S. aureus* showed the resistant bacteria (12 and 10mm respectively). Also the n- hexane extract against *E.coli* and *S. aureus* not active. Generally, extracts of the *Ziziphus spina-christi* exhibited better methanolic extract than n-hexane extract. Our findings can be used to enhance the potential of currently used antibacterial agents[14]. Further studies needed to ensure the safety of *Ziziphus spina-christi* leaves of safer total phenolic content from this natural source.

Table 1. Antibacterial activity of *Ziziphus spina-christi*.

Name of plant	Extract	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Z. Spina-christi	N- hexane	NA	15 ±4.03	12 ±3.66	NA
	Methanol	9 ±5.74	19 ±3.09	20 ±2.01	10 ±1.8
Gentamicin	10 mcg/disc	19±1.01	23±4.02	22±0.34	20±0.00

IZD (mm): > 18mm: Sensitive; 14-18 mm: intermediate : < 14mm: Resistant.

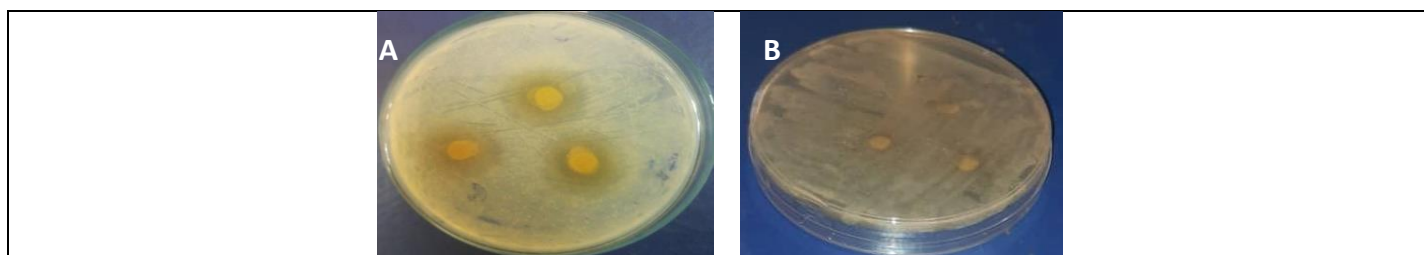


Figure 1. Antibacterial activity of methanol extract of leaves *Ziziphus spina-christi* against (a). *P. aeruginosa* and (b). *B. subtilis*

Antioxidant activity

Antioxidant activity of extracts from the *Ziziphus spina-christi* was determined by evaluating their capacity to scavenge the DPPH free radicals and results are presented in Table 2. The highest scavenging radical activity was exerted by the methanolic extract of leaves *Ziziphus spina-christi*

gave highest activity (50%), while the n-hexane extract lowest than the methanol extract (32%), this study provided by Al-zaemey et al., 2021[10]. The antioxidant activity of *Ziziphus spina-christi* attribute to its high quantity of phytochemical compounds (phenols, flavonoids, cardiac glycosides, polyphenols, saponins and tannins)[16].

Table 2. Antioxidant activity of *Ziziphus spina-christi*

Plant name	Organ	Extract	% RSA±SD (DPPH)
<i>Z. spina-christi</i>	Leaves	N- hexane	32 ±0.02
		Methanol	50 ±0.01
Standard	SD	Propyl gallate	89 ±0.01

Cytotoxicity assay

Brine shrimp assay (*Artemia salina*) is suggested to be a convenient probe for the pharmacological activities in plant extract[16]. Historically, different scientist worked out to access the cytotoxic activity of different plant extracts by using different testing. Results pertaining to cytotoxicity are shown in Table 3. None of the plant extracts were found highly effective ($P > 0.05$) however, at higher concentration plant extracts was effective at probability level 0.05 LD₅₀

values describe the n-hexane extract of leaves *Ziziphus spina-christi* was most effective with LD₅₀ (3162µg/ml). The methanol extract of leaves *Ziziphus spina-christi* was effective with mortality value maximum LD₅₀(10000µg/ml). while control gave the value highly toxic (LD₅₀= 7.463µg/ml). A previous report shows that hexane and methanol extracts of leaves *Ziziphus spina-christi* has moderate nontoxic activity against Brine shrimp assay (*Artemia salina*)[10].

Table 3. Cytotoxicity of *Ziziphus spina* on brine shrimp

Name of Plant	Solvents	LD ₅₀ (µg/ml)	The degree toxicity
<i>Z. spiana</i>	n-hexane	3162	Non toxic
	Methanol	10000	Non toxic
Etoposide	Control	7.463	Highly toxic

Interpretation: Key: n: number of replicates, ND: not determined, LD₅₀ < 249 µg/ml: were considered as highly toxic; 250-499 µg/ml: as moderately toxic; 500-1000 µg/ml: as lightly toxic; > 1000 µg/ml: Non-Toxic [17].

4. CONCLUSIONS

The high antimicrobial and antioxidant clear efficiency to methanolic and n- hexane extracts 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 methanolic extracts in primary health care.

REFERENCES

- [1]. Mohamed AY, Mustafa AA. Gas chromatography-mass spectrometry (GC-MS) analysis of essential oil *Salvia officinalis* in Sudan. J. Multidis. Res. Rev. 2019;1:43-5.
- [2]. Ramesh B, Satakopan VN. In vitro Antioxidant Activities of Ocimum Species: *Ocimum basilicum* and *Ocimum sanctum*. Journal of Cell & Tissue Research. 2010 Apr 1;10(1).
- [3]. Khalid H, Abdalla WE, Abdelgadir H, Opatz T, Efferth T. Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. Natural products and bioprospecting. 2012 Jun;2:92-103.
- [4]. Ahmed EH, Nour BY, Mohammed YG, Khalid HS. Antiplasmodial activity of some medicinal plants used in Sudanese folk-medicine. Environmental Health Insights. 2010 Jan;4:EH1-S4108.
- [5]. Cherry M. The needs of the people. In; Wickens, GE, Goodin, JR, Field DV (Eds). Plants for arid lands.
- [6]. Tounekti T, Mahdhi M, Khemira H. Ethnobotanical study of indigenous medicinal plants of Jazan region, Saudi Arabia. Evidence-based complementary and alternative medicine. 2019 Jun 2;2019.
- [7]. Alzahrani AM, Alzahrani AA, Alsharm AA. The use of *Ziziphus spina-christi* extract in treating erlotinib (Tarceva®) associated rash: a case report. Case Reports in Oncology. 2020 Dec 6;12(3):909-12.
- [8]. Amokaha RA, Ubwa ST, Otokpa M, Shenga G. Phytochemical Screening of *Danta Stramo niumlinn* (Silanaceae) Seeds. Journal of Chemical Society of Nigeria. 2002;27(1):105-7.
- [9]. Aliyu AB, Musa AM, Oshanimi JA, Ibrahim HA, Oyewale AO. Phytochemical analyses and mineral elements composition of some medicinal plants of Northern Nigeria. Nigerian Journal of Pharmaceutical Sciences. 2008 Mar;7(1):119-25.
- [10]. Al-zaemey A, Abdullah AB, Al-Husami R.(2021). A study of antimicrobial and antioxidant activity to ethanolic extract of sider (*Ziziphus spina-christi*) leaves against some Pathogenic microorganisms. J Microbiol Exp. 2021;9(5):172–176.

- [11]. Bhadoriya SS, Ganeshpurkar A, Bhadoriya RP, Sahu SK, Patel JR. Antidiabetic potential of polyphenolic-rich fraction of Tamarindus indica seed coat in alloxan-induced diabetic rats. Journal of basic and clinical physiology and pharmacology. 2018 Jan 26;29(1):37-45.
- [12]. Koko WS, Mesaik MA, Ranjitt R, Galal M, Choudhary MI. Immunosuppressive phenolic compounds from Hydnora abyssinica A. Braun. BMC complementary and alternative medicine. 2015 Dec;15(1):1-9.
- [13]. El-Kamali HH, Mahjoub SA. Antibacterial activity of Francoeuria crispa, Pulicaria undulata, Ziziphus spinachristi and Cucurbita pepo against seven standard pathogenic bacteria. Ethnobotanical Leaflets. 2009;2009(6):6.
- [14]. Harakeh S, Khan I, B Almasaudi S, I Azhar E, Al-Jaouni S, Niedzweicki A. Role of Nutrients and Phyto-compounds in the Modulation of Antimicrobial Resistance. Current drug metabolism. 2017 Sep 1;18(9):858-67.
- [15]. Elaloui M, Ennajah A, Ghazghazi H, Youssef IB, Othman NB, Hajlaoui MR, Khouja A, Laamouri A. Quantification of total phenols, flavonoides and tannins from Ziziphus jujuba (mill.) and Ziziphus lotus (l.)(Desf). Leaf extracts and their effects on antioxidant and antibacterial activities. International Journal of Secondary Metabolite. 2017 Jan 7;4(1):18-26.
- [16]. Marwah RG, Fatope MO, Al Mahrooqi R, Varma GB, Al Abadi H, Al-Burtamani SK. Antioxidant capacity of some edible and wound healing plants in Oman. Food chemistry. 2007 Jan 1;101(2):465-70.
- [17]. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. Drug information journal. 1998 Apr;32(2):513-24.