

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.605.005>

Antibacterial Activity of Fruit Methanol Extract of *Ziziphus spina-christi* from Sudan

Emad Mohamed Abdallah*

Department of Laboratory Sciences, College of Sciences and Arts,
Qassim University, Al-Rass, Saudi Arabia

*Corresponding author

ABSTRACT

The fruit (Nabag or Christ's Thorn Jujube) of Sidir tree (*Ziziphus spina-christi*) is a native snack food in Sudan. The antibacterial properties of methanol fruit extract of the Sudanese *Ziziphus spina-christi* was investigated against different ATCC strains of Gram-positive and Gram-negative bacteria. The agar-well diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were used to evaluate the antibacterial activity. In general, the study revealed that the fruit extract has no antibacterial activity against the Gram-negative bacteria (*Escherichia coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603). While, moderate antibacterial activities against the Gram-positive bacteria were recorded. The most sensitive bacteria were *Staphylococcus epidermidis* ATCC 12228, followed by *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876, respectively. The least sensitive bacteria were *Enterococcus faecalis* ATCC 29212. However, the detected activities were not significant compared to the antibiotic (Chloramphenicol 5mg/ml). Further studies using different solvents for extraction alongside with phytochemical and pharmacological studies are necessary to determine the bioactive compounds of the fruits of *Ziziphus spina-christi*.

Keywords

Antibacterial activity,
Agar-well diffusion,
MIC, MBC,
Ziziphus spina-christi,
Sudan.

Article Info

Accepted:
04 April 2017
Available Online:
10 May 2017

Introduction

Plants have been used in the human activities since ancient times as a basic source for food, fuel, shelter, clothing, cosmetics, ceremonies and medicine. People in the current industrial civilization tend to use synthetic medication as well as fast food, rich in nutrients such as carbohydrates, proteins, and fats; while very poor in bioactive phytochemical compounds. Such behavior from the modern humans let them less immunity against pathogenic diseases caused by microorganisms. Nowadays, infectious diseases are the second leading cause of mortality worldwide, where more than 25% of deaths are caused by

infectious diseases (Fauci *et al.*, 2005). Accordingly, human should go back to extract pharmaceuticals from plants and not from synthetic drugs and consume food rich in bioactive compounds. The WHO estimated that up to 80% of the world population particularly in the developing countries relies mainly on traditional medicine, which is mostly derived from plants (Arvigo and Balick, 1993). Most people in the rural areas in Sudan cannot afford the expenses of modern medicine and totally depend on traditional medicinal plants (Robinson, 2006). Although there are up to 2000 medicinal

plants in use in Sudanese traditional medicine, However, scientific investigations on their bioactivity are very modest (Abdalla and Abdallah, 2016). Plants are able to produce numerous secondary molecules as part of its survival strategy (Houghton, 2001). The antimicrobial potential of some plants is attributed to the presence of some secondary molecules called phytochemicals such as alkaloids, flavonoids, terpenoids, tannins, saponins, anthraquinones and glycosides (Omojate *et al.*, 2014). The tree of *Ziziphus spina-christi* L. Desf., family: Rhamnaceae, named in Sudan and the Middle East as Sidr or Sidder (In English: Christ's Thorn Jujube). It is an evergreen spiny shrub (5-10 meters tall), drought and heat tolerant, distributed throughout tropical and subtropical countries in North Africa, Middle East and North West India, this plant is native to Sudan and widespread in central and western parts of the country (Saied *et al.*, 2008).

Ziziphus spina-christi is well known since ancient pharaonic civilization, its fruit is known in hieroglyphic language as nebes (Similar to the current local name in Sudan: Nabag), where it was mentioned in ancient papyri for wound healing and anti-swellings (Kadioglu *et al.*, 2016). In Sudan, the fruits of *Ziziphus spina-christi*, which is known locally as Nabag are eaten to treat diarrhea and malaria and as an antispasmodic (El-Kamali and El-Khalifa, 1999). Different parts of this plant have been investigated for its bioactivity such as leaves, stem bark and roots, which showed antimicrobial, antioxidant, antinociceptive and antidiabetic effects (Asgarpanah and Haghghat, 2012). However, studies on the fruit of Sudanese *Ziziphus spina-christi* are not adequate. The present study aimed at evaluating the *in vitro* antibacterial activity of fruit of *Ziziphus spina-christi*, widely consumed in Sudan as a popular snack food.

Materials and Methods

Plant material and extraction

Dry fruits of *Ziziphus spina-christi* were purchased from local markets in Khartoum, Sudan. Washed with distilled water, dried with towels and the inner seed was removed, the fruit pulp was separated and kept in a clean dark cupboard for 3 days, ground to a fine powder and macerated in 80% methanol for up to a week with frequent shaking. Then, filtered twice with Whatman filter paper, the macerate was kept in an incubator at 40 °C to evaporate the solvent for up to 2 weeks until getting dry extract. The crude dry extract was reconstituted in 10% Dimethyl sulfoxide (DMSO) to make two different concentration 500 mg/ml and 250 mg/ml.

Microorganisms

Six referenced bacterial strains representing different Gram-positive and Gram-negative bacteria were used in this study. The tested Gram-positive bacteria were *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* ATCC 10876. While the tested Gram-negative bacteria were *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 35218.

Antimicrobial activity

The antibacterial activity of the methanol extract of *Ziziphus spina-christi* fruit was evaluated by means of the agar-well diffusion assay as mentioned in (Güven *et al.*, 2006). 20 ml of molten Mueller-Hinton agar (45°C) was poured into sterile Petri dish. 100 µl of the microbial suspension containing approximately 10⁸ CFU/ml bacteria was loaded on the plate and spread using a sterile cotton swab. The plate was left to dry

aseptically at room temperature for up to 15 minutes. A 6 mm diameter wells were made on the agar plate using a sterile cork borer and subsequently 50 μ l of the methanolic extract that was reconstituted before in 10%DMSO at two different concentrations; 500 mg/ml and 250 mg/ml were placed into the wells. Chloramphenicol 0.5% w/v (5 mg/ml) was also loaded in wells and used as standard antibiotic. The pre-experimental test showed that 10%DMSO has no inhibitory effect on bacterial growth. The plates were then incubated at 37°C for 24 h. The experiment was repeated twice and the mean zone of inhibition against the test organism was calculated.

Determination of minimum inhibitory concentration (MIC)

MIC test was performed on bacteria, which showed sensitivity against the methanol extract from above 10 mm zone of inhibition using the broth dilution method as reported by El-Mahmood and Ameh (2007) with minor modifications. Firstly, test tubes containing 1 ml of Mueller-Hinton broth were prepared and autoclaved. Then, 1 ml of the plant's extract at a concentration of 200 mg/ml was poured into a test tube. Subsequently, serial two-fold dilutions were made by transferring 1 ml from the first tube after shaking well to another 1 ml Mueller-Hinton broth tube and so on, giving extract concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.123 mg/ml. Another 1 ml of fresh referenced microbial strain was poured to each concentration. In addition, to another two Mueller-Hinton broth tube 1 ml of antibiotic (Chloramphenicol 5mg/ml) was poured to serve as a positive control, and 1 ml of 10% DMSO to serve as a negative control. All test tubes were incubated at 37°C for 24 h. The tube with the lowest dilution with no visible growth was considered as the MIC.

Determination of minimum bactericidal concentration (MBC)

The MBC test was evaluated following the method reported by Doughari (2006) with slight modification. Directly after the MIC testing, 100 μ l from the MIC tubes that showed no visible growth was decanted on the surface of plates containing autoclaved Mueller-Hinton agar. To another 2 Mueller-Hinton plates, 100 μ l from the MIC tube containing chloramphenicol was decanted on the surface of Mueller-Hinton agar plate to serve as negative control and 100 μ l from the MIC tube containing 10% DMSO was decanted on the surface of another Mueller-Hinton agar plate to serve as negative control. All inoculated plates were then incubated for about 18 hours at 37 °C and investigated for bacterial growth. Plates with no visible growth were considered to be the MBC for the plant extract.

Statistical analysis

Data were expressed as Mean \pm standard error of means; the statistical software used was SPSS (v.16.0).

Results and Discussion

Results of the agar-well diffusion test showed that the methanol fruit extract of *Ziziphus spina-christi* has varied degrees of antibacterial effects on the Gram-positive bacteria and no effect on the Gram-negative bacteria (Table 1 and Figures 1 and 2). At the concentrations 500 mg/ml and 250 mg/ml, the most sensitive bacteria was *Staphylococcus epidermidis* ATCC 12228 (11.0 \pm 1.0 and 10.5 \pm 0.5 mm), followed by *Staphylococcus aureus* ATCC 25923 (11.0 \pm 0.0 and 7.7 \pm 0.7 mm), and *Bacillus cereus* ATCC 10876 (10.5 \pm 0.5 and 8.2 \pm 0.7 mm), respectively. The least sensitive bacteria was *Enterococcus faecalis* ATCC 29212 (8.0 \pm 1.0 and 6.7 \pm 0.2

mm), respectively. However, the detected activities were not significant compared to the antibiotic (Chloramphenicol 5mg/ml) which showed obvious inhibitory effects against all the tested bacteria. On the other side, Gram-negative bacteria was not susceptible toward the fruit extract but sensitive to the antibiotic, where both of *Escherichia coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603 recorded 6.0 ±0.0 mm against the fruit extract (the diameter of the hole is 6 mm). Leaves and some other parts of *Ziziphus spp.* were exhaustively studied, but studies on the fruits are scant. The findings of the current study are in agreement-in partial-with the results of (Ali *et al.*, 2015), both showed antibacterial activity against the Gram-positive bacteria, but our study did not detect antibacterial effect against the Gram-negatives. Actually, varied results of antibacterial and antifungal

activity of the fruit of *Ziziphus spina-christi* were published. Tom *et al.*, (2009) cited that the aqueous extract of the pulp (fruit) of *Ziziphus spina-christi* has antibacterial potential against *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negatives) and antifungal potency against *Candida albicans*. While, our study showed no effect against *Escherichia coli* (Ali *et al.*, 2015) mentioned that methanol extract of *Ziziphus spina-christi* has no antifungal effects against either *Aspergillus niger* or *Candida albicans*. Geographical and environmental variations may affect on the bioactive constituents of the same plant species. Therefore, on the lack of adequate information, consecutive studies on the fruit of *Ziziphus spina-christi* using different solvents and antibacterial methods would clarify the contradicted findings.

Table.1 The antibacterial activity of the methanol fruit extract of *Ziziphus spina-christi*

Tested Compound	Mean zone of growth inhibition in mm*					
	Gram-positive bacteria				Gram-negative bacteria	
	Sa	Se	Ef	Bc	Ec	kp
Methanol extract 500 mg/ml	11.0 ±0.0	11.0 ±1.0	8.0 ±1.0	10.5 ±0.5	6.0 ±0.0	6.0 ±0.0
Methanol extract 250 mg/ml	7.7 ±0.7	10.5 ±0.5	6.7 ±0.2	8.2 ±0.7	6.0 ±0.0	6.0 ±0.0
Chloramphenicol 5 mg/ml	40.5 ±0.5	29.5 ±0.5	31.0 ±1.0	31.0 ±0.0	22.5 ±0.5	23.5 ±0.5
10% DMSO	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0

*Disc diameter=6.0 mm, 6.0±0.0= No activity (the disc diameter), Zone of inhibition is the mean of two replicates ±standard error of means, Sa=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 12228, Ef=*Enterococcus faecalis* ATCC 29212, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 35218, Kp=*Klebsiella pneumoniae* ATCC 700603, DMSO= Dimethyl sulfoxide.

Table.2 The MIC and MBC tests of the methanol fruit extract of *Ziziphus spina-christi*

Microorganism	MIC (mg/ml)	MBC (mg/ml)
<i>Staphylococcus aureus</i> ATCC 25923	100	200
<i>Bacillus cereus</i> ATCC 10876	100	< 200
<i>Staphylococcus epidermidis</i> ATCC 12228	50	200

Fig.1 Representative Photo showing fruits of the Sudanese *Ziziphus spina-christi* and antibacterial effect; A: The fruits (Nabag), B: Its antibacterial activity

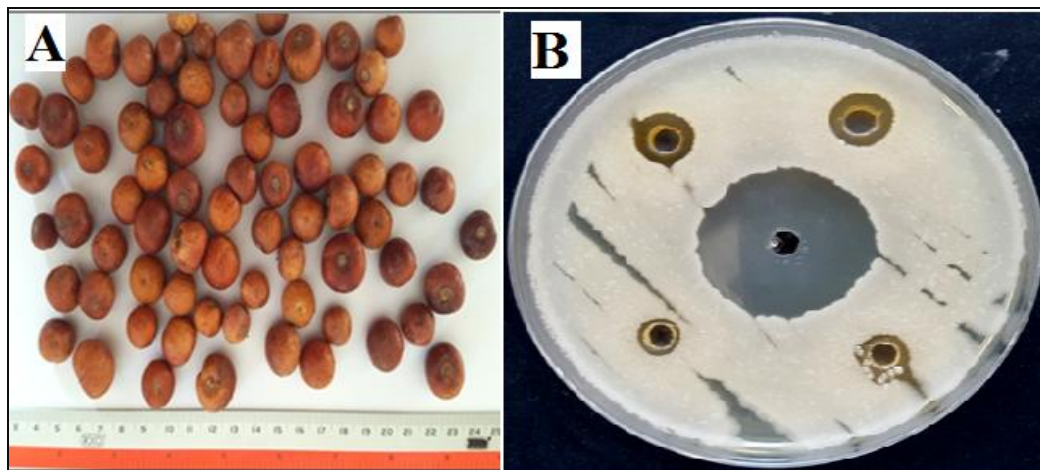
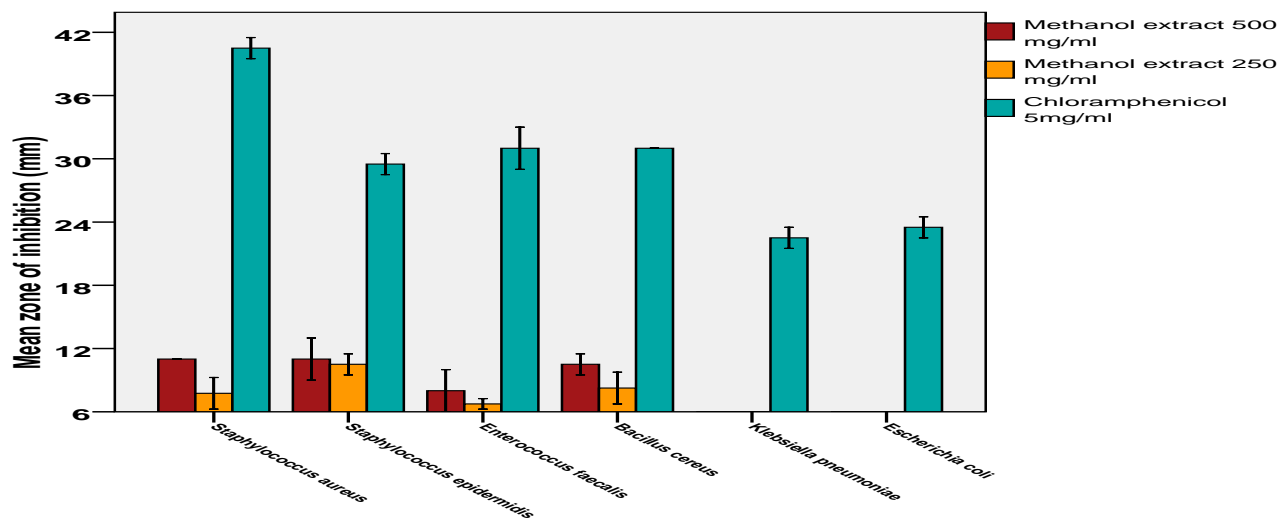


Fig.2 Sensitivity of bacterial strains toward fruits of *Ziziphus spina-christi* compared to chloramphenicol



The results of the MIC and MBC values are shown in (Table 2). Only bacteria that showed inhibition zone with the cup-plate diffusion assay were tested. Low MIC values indicate high antibacterial effect (Rios *et al.*, 1988). Accordingly, The MIC values showed that the lowest level of antibacterial agent from the fruit extract of *Ziziphus spina-christi* that inhibit the growth of bacteria was ranging between (50-100 mg/ml). The most affected bacteria was *Staphylococcus epidermidis*

ATCC 12228 (50 mg/ml), followed by both of *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876 which recorded (100 mg/ml). The MBC values demonstrate the lowest level of antimicrobial agent that results in microbial death, meaning that, even if a particular MIC shows inhibition, plating the bacteria onto agar might still result in organism proliferation because the antimicrobial did not cause death. Antibacterial agents are generally considered

as bactericidal if the MBC is no more than four times the MIC. Accordingly, the methanol fruit extract of *Ziziphus spina-christi* has a bactericidal nature. Although, the results of the extract was not comparable to the antibiotic, as the extract contains many unknown compounds. Accordingly, identification, isolation and separation of this antibacterial agent may lead to the discovery. The methanol fruit extract of *Ziziphus spina-christi* was found to have good quantities of flavonoids which have possible protective effects on human health (Pawlowska *et al.*, 2009), it was also reported that fruits of *Ziziphus spina-christi* have good antioxidant and anti-inflammatory effects and it was rich in bioactive phytochemicals such as tannins, steroids, flavonoids, alkaloids, glycosides and terpenoids (Alhakmani *et al.*, 2014).

In conclusion, the findings of this result showed that the methanolic fruit extract of the Sudanese *Ziziphus spina-christi* has some degree of antibacterial effects and support the traditional medicinal use as a topical antimicrobial in wound healing process, although it was not comparable to the antibiotic (Chloramphenicol). However, the current study gives medical value to that fruit in addition to its nutritional importance. Since investigations on the fruit are scant, more phytochemical and pharmacological studies on the fruits of are *Ziziphus spina-christi* recommended in order to explore the bioactivity of this plant product.

Conflict of interest statement: We declare that we have no conflict of interest.

References

- Abdalla, W.E. and Abdallah, E.M. 2016. Promising Sudanese medicinal plants with antibacterial activity-a review Article. *Biol. Forum*, 8(1): 299-323.
- Alhakmani, F., Khan, S.A. and Ahmad, A. 2014. Determination of total phenol, in-vitro antioxidant and anti-inflammatory activity of seeds and fruits of *Zizyphus spina-christi* grown in Oman. *Asian Pac. J. Trop. Biomed.*, 4(S 2): S656-S660.
- Ali, A.B., Almagboul, A.Z. and Mohammed, O.M. 2015. Antimicrobial activity of fruits, leaves, seeds and stems extracts of *Ziziphus spina-christi*. *Arabian J. Med. Arom. Plants*, 1(2): 94-107.
- Arvigo, R. and Balick, M. 1994. Rainforest remedies: One hundred healing herbs of Belize. Twin Lakes: Lotus Press. New Delhi, India.
- Asgarpanah, J. and Haghghat, E. 2012. Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. *Afri. J. Pharm. Pharmaco.*, 6(31): 2332-2339.
- Doughari, J.H. 2006. Antimicrobial Activity of *Tamarindus indica* Linn. *Trop. J. Pharma. Res.*, 5(2) 597-603.
- El-Kamali, H.H. and El-Khalifa, K.F. 1999. Folk medicinal plants of riverside forests of the Southern Blue Nile district, Sudan. *Fitoterapia*, 70: 493-497.
- El-Mahmood, A.M. and Ameh, J.M. 2007. *In-vitro* antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microorganisms associated with Urinary tract infections. *Afri. J. Biotech.*, 6(11): 195-200.
- Fauci, A.S., Touchette, N.A. and Folkers, G.K. 2005. Emerging Infectious Diseases: a 10-Year Perspective from the National Institute of Allergy and Infectious Diseases. *Emerg. Infect. Dis.*, 11(4): 519-525.
- Güven, K., Yücel, E. and Cetintaş, F. 2006. Antimicrobial activities of fruits of *Crataegus* and *Pyrus* species. *Pharma. Boil.*, 44 (2): 79-83.
- Houghton, P.J. 2001. Old Yet New—Pharmaceuticals from Plants. *J. Chem.*

- Edu.*, 78(2): 175-184.
- Kadioglu, O., Jacob, S., Bohnert, S., Naß, J., Saeed, M.E.M., Khalid, H., Merfort, I., Thines, E., Pommerening, T. and Efferth, T. 2016. Evaluating ancient Egyptian prescriptions today: Anti-inflammatory activity of *Ziziphus spina-christi*. *Phytomedicine*, 23:293–306.
- Omojate, G.C., Enwa, F.O., Jewo, A.O. and Eze, C.O. 2014. Mechanisms of Antimicrobial Actions of Phytochemicals against Enteric Pathogens – A Review. *J. Pharma. Chem. Biol. Sci.*, 2(2):77-85.
- Pawlowska, A.M., Camangi, F., Bader, A. and Braca, A. 2009. Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits. *Food Chem.*, 112: 858–862.
- Robinson, J. 2006. Useful wild tree resources of southern Sudan: a review. *Plant Genet. Resour.*, 4:188–197.
- Rios, J.L., Recio, M.C. and Villar, A. 1988. Screening methods for natural products with antimicrobial activity: A review of the literature. *J. Ethnopharma.*, 23(2-3): 127-149.
- Saied, A.S., Gebauer, J., Hammer, K. and Buerkert, A. 2008. *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. *Gen. Resour. Crop Evol.*, 55:929–937.
- Tom, G.M., Yesufu, H.B. and Abdulrahman, F.I. 2009. Antimicrobial screening and effect of the pulp extracts of *Zizyphus spina-christi* (Linnaeus Desf) on some biochemical parameters in rats. *J. Pharm. Biores.*, 6(2): 58-64.

How to cite this article:

Emad Mohamed Abdallah. 2017. Antibacterial Activity of Fruit Methanol Extract of *Ziziphus spina-christi* from Sudan. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 38-44.
doi: <http://dx.doi.org/10.20546/ijcmas.2017.605.005>