Preliminary phytochemical screening and antibacterial activity of ethanolic and aqueous extracts of Sudanese medicinal plant *Ziziphus spina-christi* L leaves

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In the current work ethanolic and aqueous extracts of *Ziziphus spina-christi* leaves were investigated for their phytochemical and antibacterial activity. Phytochemical screening was conducted using standard qualitative methods and the antibacterial activity was investigated using disc diffusion method. The microorganisms employed were six pathogenic bacteria; three *Gram*-positive: *Staphylococcus aureus* (ATCC1026), *Bacillus subtilis* (ATCC19659) and *Enterococcus faecalis* (ATCC29212); and three *Gram*-negative: *Salmonella typhi* (ATCC14038), *Pseudomonas aeruginosa* (ATCC15442) and *Escherichia coli* (ATCC10536). Phytochemical analyses revealed the presence of alkaloids, flavonoids, saponins, phenolics, terpenoids and tanins in ethanol extratct. Aqueous extract showed the presence of alkaloids, flavonoids, saponins and tanins. Both extracts demonstrated varying levels of activity against *Gram*-positive bacteria, whereas all *Gram*-negative bacteria completely resistance to the extracts. Ethanol extract dominated aqueous extract in inhibiting the growth of the pathogenic bacteria under study. Highest antibacterial activity was observed with ethanol extract against *S. aureus* (18.1 mm), while minimum activity was observed with aqueous extract against *B. subtilis* (13.3 mm). The findings of this study indicated that the leaves of *Z. spina-christi* possess various secondary metabolites having the potential for the developing pharmaceutical drugs, especially antibacterial ones.

Keywords: *Ziziphus spina-christi*, Phytochemical screening, Antibacterial activity.

1. Introduction

In recent years, a large number of antibacterial drugs were produced in the world with an aim of eradicating the bacteria strains which were responsible for many infections (Al-Juraifani, 2011). However, these drugs induced mutations in the genetic composition of these microorganisms rendering them resistant to several antibacterial drugs (Cohen, 1992). Furthermore, the side effects associated with the extensive use of the chemical drugs may lead to serious damages to many of human organs (Divya et al., 2016).

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Therefore, to solve this limitation of chemical drugs, scientists have shifted their focus towards medicinal plants which are recognized as rich sources of antibacterial drugs and are widely used by various communities for medicinal purposes (Iris et al., 2005 and Demetrio et al., 2015).

Ziziyphusspina-christi (Family: Rhamnacese) is a plant that grows wild in Asia and tropical Africa. The plant is originally of the Middle-east south of the Euphrates and spread to Saharan Oases across Africa into the Sahel (kafamiya et al., 2013). The Genus *Zizyphus* has wide ranging pharmacological applications. Various *Zizyphus* species are frequently used 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, rheumatics disease from the intestine (Adzuet al., 2003). Besides, people from various regions believe that the species is used as a source of food (Al- Ghamdi, 2001; El Dakhakhny et al., 2000). *Z. spinac-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 (Moodi et al., 2016; Mohammed et al., 2012). *Z. spina-christi* was shown to contain betulic and ceanothic acid, three cyclopeptide alkaloids as well as four saponin glycosides (Mahranet al., 1996) and several flavonoids have been isolated from the leaves of *Z. spina-christi* (Amos et al., 2001). This study aimed to investigate the phytochemical and antibacterial activity of ethanolic and water extracts of *Z. spina-christi* leaves grown in Sudan on selected clinically pathogenic bacteria isolates.

2. Materials and methods

2.1. Plant material and preparation of extracts

The fresh leaves of *Ziziphus spina-christ* were collected on May, 2016 from Doka, Al-Gedarif state, Sudan. A voucher specimen (K978) was deposited at the Department of Chemistry of the Medicinal and Aromatic Plants Research Institute. Khartoum, Sudan. The sample was dried for 15 days in a dark and ventilates room at 25-30 °C, then grounded and the powder (50 g) stored at -20 °C. Equal amounts (20 g) of powdered sample were extracted using ethanol and distilled water for three days by the plant tissue homogenization method as previously described. The extracts were then

concentrated using rotary evaporator and further dried individually on Petri dishes. These leaves crude extracts (ethanol and water) were subjected to phytochemical and antibacterial activity experiments.

2.2 Phytochemical screening of the extracts

Phytochemical screening was done using standard procedures as previously described (Harborne, 1973). *Z. spina-christi* leaves extracts (ethanol and water) were screened for the following phytoconstituents: Alkaloids, flavonoids, saponins, phenolics, terpenoids and tanins.

2.3 Antibacterial assay

The ethanol and water crude extracts of leaves have been tested against six bacteria strains. Three *Gram*-positive bacteria (*Staphylococcus aureus* (ATCC1026), *Bacillus subtilis* (ATCC19659) and *Enterococcus faecalis* (ATCC29212); and three *Gram*-negative bacteria (*Salmonella typhi* (ATCC14038), *Pseudomonas aeruginosa* (ATCC15442) and *Escherichia coli* (ATCC10563)). The strains of bacteria were obtained from Microbiology Department, Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The agar disc diffusion method was [need] used to investigate the antibacterial potential of the extracts. Briefly, sterilized discs (filter paper Whatman. No 1, 6 mm in diameter) were impregnated with 100 mg/mL of each sample, and dried. Standard discs of gentamycin (100 µl) were used as positive controls. The discs of the samples and the standard antibiotic were carefully placed onto the previously marked zones on the agar plates pre-inoculated with test organisms. The plates were incubated at 37 °C for 24 hours in upright position. All the experiments were conducted in triplicate and the data were presented as the mean values with their standard deviation.

2.4 Analytical methods

The results obtained in the current work were analysed using SPSS software (SPSS Statistical Version 22). All values presented are mean values ± standard deviation of triplicates (n=3), obtained from three separate experiments. The one-way ANOVA was performed to examine the differences among the groups. A P value of < 0.05 was considered to be statistically significant.

3. Rresult and discussion

3.1 Phytochemical profile

The result of the phytochemical analysis of both solvent extracts (ethanol and water) showed the presence of various secondary metabolites with varied degree such as alkaloids, flavonoids, saponins, phenolics, terpenoids and tannins. Even though, ethanol extract showed a considerably broader phytochemical than water extract (Table 1). The potency of the plant extracts depends on the solvent used. This may be due to the degree of solubility of the bioactive constituents. It has been documented that different solvents have diverse solubility capacities for different phytochemical (Rawani et al. 2011).

Table 1. Qualitative phytochemical parameters of *Z. spina-christi*

S. No	Compound	ethanol extract	Water extract
1	Alkaloids	+++	++
2	Flavonoids	++	+
3	Saponins	++	+++
4	Phenolics	+++	–
5	Terpenoids	+++	–
6	Tanins	+++	++

+: presence; –: absence

3.2 Antibacterial study

The pharmacological action of the plant cannot be ascertained by the result of phytochemical profile only. Thus the antibacterial activity on six pathogenic bacteria was also investigated. The results indicated that its antibacterial activity of the extracts was only against *Gram*-positive bacteria. The results are in agreement with previous studies of *Gram*-negative bacteria were more resistant to most plant extracts than *Gram*-positive bacteria (Saraste et al., 2000). The ethanolic extract showed highest antibacterial activity against *S. aureus* (18.1 mm) followed by *B. subtilis* (16.1 mm) and *E. faecalis* (15.4 mm).

The aqueous extracts showed highest antibacterial activity against *S. aureus* (14.2 mm) followed by *E. faecalis* (14.1 mm) and *B. subtilis* (13.3 mm). Whereas, all *Gram*-negative bacteria (*S. typhi*, *P. aeruginosa* and *E. coli*) completely resistance to the extracts and not observed any inhibition zones (Table 2).

Table 2: Antibacterial activity of *Z. spina-christi* leaves extract at 100 mg/mL

	Zone of inhibition (mm)[a]		
Microorganism	Ethanol extract	Aqueous extract	Gentamycin (100 µl)
S. aurous (G^+)	18.1 ± 0.1	14.2 ± 0.4	11.6
B. subtilis (G^+)	16.1 ± 0.3	13.3 ± 0.2	14.1
E. faecalis (G^+)	15.4 ± 0.4	14.1 ± 0.1	13.2
S. typhi (G^-)	0.0	0.0	12.9
P. aeuroginosa (G^-)	0.0	0.0	–
E. coli (G^-)	0.0	0.0	12.8

[a]Inhibition zone diameter; –: no antibacterial activity; G^+: *Gram*-positive; G^-: *Gram*-negative; values were means of three replicates.

Although further work is needed to precisely locate the active principles in both extracts, the ethanol extract showed highest antibacterial values than aqueous extract, suggesting that this extract would contain the most active antibacterial agents. These results were in accordance to Rawani et al. 2011 who had reported that organic extract has better antibacterial activity as compared to aqueous extract against *B. subtilis*, and *S. aureus*. Similarly, another report has also shown that the organic solvents had better results as compared to water (Edayadulla et al., 2012).

The antibacterial activity in the leaves extracts may be due to the presence of various secondary metabolites such as alkloids, flavonoids, saponins, phenolics, terpenoids and tannins. These medicinally bioactive compounds exert antibacterial action through different mechanism. Alkaloids, including quaternary alkaloids, are known to affect biological functions at very low concentrations. Many alkaloids are known to be

antimicrobial and an aesthetic (Mukeshwar, et al., 2011). Flavonoids which have been found to be effective antibacterial substances against a wide array of microorganisms *in-vitro* are known to be synthesized in response to microbial infection by plants. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999). The saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz et al., 1996). Phenolics compounds are known for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes (Epand et al., 2007). Terpenoids are responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue (Hemandez et al., 2000). Isolated terpenoids from plant showed antimicrobial properties (Shahidur Rahman et al., 2009). Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with prolene rich protein (Mamtha et al., 2004). Sodipo et al. (1991) reported that tannins prevent the development of microorganisms by making useful proteins unavailable to the organism and facilitate the precipitation of microbial protein.

4. Conclusion

In the current investigation, the overall findings from the preliminary phytochemical screening of the leaves extracts (ethanol and water) of *Z. spina-christi* indicated the potentials for developing antibacterial agents from them against certain type of bacteria. The phytochemical detected in these extracts are alkloids, flavonoids, saponins, phenolics, terpenoids and tannins. Both extracts of *Z. spina-christi* leaves were evaluated on six species of bacteria (*S. aureus*, *B. subtilis*, *E. faecalis*, *S. typhi*, *P. aeruginosa* and *E. coli*). Result indicated that the antibacterial effect of both extracts demonstrated varying levels of activity against *Gram*-positive bacteria, whereas all *Gram*-negative bacteria completely resistance to the extracts and not observed any inhibition zones.

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