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Evaluation of phytochemical and potential antibacterial activity of *Ziziphus spina-christi* L. against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri, Borno State – Nigeria

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Abstract
Plants form an integral part of life in many indigenous African communities. Due to either limited availability or affordability of pharmaceutical medicines about 80% of the rural population in Sub-Saharan Africa (SSA) depends on traditional herbal remedies for primary health care (PHC) and veterinary use. The aim of the study is to determine the phytochemical and antibacterial activity of the seed oil extracts of *Z. spina-christi Ziziphus spina-christi* L. found to have potential antibacterial activity against four medically important bacteria (Staphylococcus aureus, Escherichia coli, Shigella spp and *Pseudomonas aeruginosa*). The antibacterial activity of seed oil extracts of *Ziziphus spina-christi* was examined using agar well diffusion method. The result obtained showed that the essential oil was active against Gram-positive more than Gram-negative bacteria, the essential oil had strong antibacterial activity against *Staphylococcus aureus* (zone of inhibition of growth is 11 mm, *E. coli* (zone of inhibition of growth is 10 mm), *Shigella* spp (zone of inhibition of growth is 8mm) and *P. aeruginosa* (zone of inhibition of growth is 8 mm). Physiochemical screening of the oil extract of *Ziziphus spina-christi* revealed presence of different types of secondary metabolites such as glycosides, tannins and alkaloids. The seed oil extract of *Ziziphus spina-christi* showed significant activity against *Staphylococcus aureus* and *Escherichia coli* that suggest that the chemical components that exist in the extract such as glycosides, alkaloids and tannins have the powerful antibacterial effects on the bacterial cell wall and DNA. The results of this study have provided scientific validity for the use of this seed oil in the treatment of bacteria-related infections in herbal medicine.

Keywords: Phytochemical screening; *Ziziphus spina-christi*; Antibacterial; Medicinal plants.

1. Introduction
Plants have a great importance in our lives because they fulfill our basic needs for food, shelter, clothing, fuel, ornamentals, flavoring and medicine. 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 [1]. *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 [1]. Some species, like *Ziziphus mauritiana* Lam. and *Ziziphus spina-christi* (L.) wild occur on nearly every continent. *Ziziphus mauritiana* and *Ziziphus spina-christi* have very nutritious fruits and are usually eaten fresh. *Zizyphus spina-christi* L. known as "Kurna" in Kanuri or Hausa, or Jerusalem-thorn or Christ-thorn is a member of the Rhamnaceae family. The majority of the rural population in Northern Nigeria use *Zizyphus spina-christi* extensively for its medicinal and economic importance [2]. Medicinal plants are plants that have recognized medicinal uses [3, 4]. They range from plant which is used in the production of mainstream pharmaceutical product to plant used in herbal medicine preparation [4]. Medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs [4].
The plant *Zizyphus spina-christi* is readily distributed in the Sahara and Sahel, from Senegal to the Sudan and Arabia where the annual rainfall is about 50 – 300 mm or on periodically inundated sites [4, 5]. It grows in dry conditions and are conspicuously thorny, often the thorns in pairs one being straight and other curved. It has numerous small, sometimes minute teeth

like leaves [5]. The 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. *Zizyphus spina-christi* has been reported to have activity against bacterial and fungal pathogens that are normally quite resistant to modern medications [5, 6]. It is used extensively for the treatment of ulcers, wounds, eye diseases, bronchitis febrifuge, diuretic and as anti-inflammatory agent for healing skin diseases such as atopic dermatitis [6]. Similarly, different parts of the plant are used for various medicinal purposes among the local populace of Northern Nigeria. It is used for the treatment of wounds, burns, stomach discomfort and urinary infections [7]. Previous studies suggest that *Zizyphus spina-christi* can be very useful in the control of hepatic and nephrotic abnormalities. The present study was undertaken to provide scientific validity for the folkloric use of *Zizyphus spina-christi* as an antimicrobial agent [7, 8].

The seeds are sedative and are taken sometime with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy [8]. The extract of *Z. spina-christi* found was shown to contain beutic acid and ceanothic acid, cyclopeptides, as well as saponin glycoside and flavonoids, lipids, protein, free sugar and mucilage [9]. Plant materials are cheap and significantly contribute to the improvement of human health in terms of cure and prevention of diseases. Plants have been useful as food and medicine and a few have been studied especially African medicinal plants. They contain vitamins needed by human body for healthy living [9].

The *Z. spina-christi* seed were potentially a good source of antimicrobial compound. The use of these compounds for application in crop production was not as many as in medicinal field [9, 10]. *Z. spina-christi* seed (extract) was found to contain beutic acid, ceanothic acid, cyclopeptide, as well as saponin glycoside, flavonoids, lipid, protein, free sugar and mucilage [10].

2. Materials and Methods

2.1 Sample Collection

Sample of fruits of *Zizyphus spina-christi* Linn was purchased from the Monday Market Maiduguri, Maiduguri Metropolitan Council Area of Borno State, Nigeria. The fruits material was identified and authenticated by a plant Botanist at the Department of Sciences Laboratory Technology, Ramat Polytechnic, Maiduguri Borno state. The fruits were thoroughly washed with tap water followed by sterile distilled water and then allowed to air dried at room temperature.

2.2 Procedure for Phytochemical extraction of seed Oil from *Zizyphus spina-christi*

Zizyphus spina-christi seed oil was extracted with organic solution Hexane by Soxhlet units. The fruits of *Z. spina-christi* was cleaned, air-dried and pulverized with a motor to obtain a meal. The oil extract that obtained was evaluated for antibacterial activities [10, 11].

2.3 Phytochemical screening of the seed oil extract

Phytochemical screening for some constituents of seed oil extracts were under taken using standard method as described by various authors [14, 15, 16]. The seed oil extract was screened for the presence of biologically active chemical constituents like glycoside, alkaloids, tannins and Flavonoids.

Glycosides - 10 ml of 50% H_2SO_4 was added to 1 ml of the extract and the mixture heated in boiling water for about 15 min. 10 ml of Fehling's solution was then added and the mixture boiled. A brick - red precipitate was confirmatory for the presence of glycosides.

Alkaloids - 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered. Then 2 drop of Mayer's reagent to 1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

Tannins - 1 ml of freshly prepared 10% KOH was added to 1ml of the extract. A dirty white precipitate showed the presence of tannins.

Flavonoids - 1 ml of 10% NaOH was added 3 ml of the extract. There was no yellow colouration which is indicative of the absence of flavonoids.

2.4 Determination of Antibacterial activity

The antibacterial activity of the *Zizyphus spina-christi* seed oil extracts was determined using agar well diffusion method. The agar plates were swabbed with the test organisms. Five wells (5 mm diameter) were punched in the agar with a sterile cork borer. With a micropipette, 40 µL of the oil was measured and dripped directly into the first well until a concave rim is achieved; 30 µL and 20 µL were also dripped directly into the 2nd and 3rd well respectively. Sterilized distilled water was used as a negative control which was introduced into the 4th well instead of plant extract. Antibiotic Chloramphenicol 30 mg was used as the positive control which was introduced into the 5th well instead of plant extract. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hours at 37 °C, the plates were observed. The antibacterial activity was present on the plates; it was indicated by an inhibition zone surrounding the well containing the oil seed extract. The experiment was performed under strict aseptic conditions and the relative antibacterial potency of the oil preparation was measured and recorded in millimeters using a ruler [12, 13].

Table 1: Phytochemical constituents of hexane extract obtained from seed oil of *Ziziphus spina-christi* Linn

S. No.	Phytochemical parameters	Reagent Used	Hexane extract
1	Alkaloids	HCl + Mayer's	+
2	Flavonoids	NaOH	_
3	Glycosides	H2SO4 + Fehling's solution	+
4	Tannins	KOH	+

(+) = Present, (-) = Absent

Table 2: Diameter zone of inhibition (mm) produced by hexane extracts of the seed oil of *Ziziphus spina-christi Linn* various concentrations

S. No.	Test organisms	1st well 40 µL (mm)	1st well 30 µL (mm)	1st well 20 µL (mm)	1st well Negative control	1st well Positive control
1	*Escherichia coli*	10.00	8.00	6.00	0.00	10.00
2	*Pseudomonas aeruginosa*	8.00	7.00	5.00	0.00	11.00
3	*Staphylococcus aureus*	11.00	10.00	8.00	0.00	12.00
4	*Shigella spp*	8.00	7.00	6.00	0.00	8.00

Key: positive control = Chloramphenicol 30 mg

3. Results

The phytochemical analysis carried out of water extractives for tannins and saponins and on alcoholic extract for flavonoids, glycosides, alkaloids and phenol. The detailed of phytochemical screening is given in the table 1. revealed the presence of some secondary metabolites like tannins, glycosides, alkaloids and flavonoids in the study oil. The result of bacterial activity of the seed oil extracts of *Zizyphus spina-christi* are indicated in table 2. The results have showed that the essential oil has very good activity against all bacteria: Gram-positive more than Gram-negative bacteria. The essential oil of *Zizyphus spina-christi* has maximum zone of inhibition against *Staphylococcus aureus* (11 mm) and *Escherichia coli* (10 mm) while the minimum zone of inhibition was against *Shigella spp* and *Pseudomonas aeruginosa* (8 mm), the most significant results were recorded for the concentration of 40 µL. The results of the antimicrobial assay of the methanolic extract of *Ziziphus spina-christi* indicated that the plant exhibited antimicrobial activity against the tested microorganisms at three different concentrations of 40, 30 and 20 µL and the zone of inhibition was recorded and presented above in the tabulation drawn (Table 2).

4. Discussion

The current study was initiated because of the increasing antibacterial resistance to most of the standard antibiotics. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents. As a result, the antimicrobial activity of seed oil extract of *Ziziphus spina-christi* was screened against the most common clinically important bacterial pathogens. However, the curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides and tannins [13].

In the present research work, the active phyto components of *Ziziphus spina-christi* was studied and further the antimicrobial activity of the life extract of *Ziziphus spina-christi* was also tested against *S. aureus, E. coli Shigella* spp and *P. aeruginosa* at different concentrations of the extract. The maximum zone of inhibition was obtained at a concentration of 40 µL. *P. aeruginosa* was least susceptible to the essential oils. The weak antibacterial activity against Gram-negative bacteria was ascribed to the presence of their cell wall, lippolysaccharide [14].

Presence of tannins suggests the ability of this seed oil to play a major role as antidiarrhoec and antihemorrhagic agent [14, 15, 16]. Tannins are present in many plants and have various physiological effects like antimicrobial and antiparasitic. Tannins have general antimicrobial and have been reported to prevent the development of bacteria by precipitating microbial protein and making nutritional proteins unavailable for them [16, 17]. The growth of many bacteria and was inhibited by tannins [17]. According to this study, tannins can be toxic to bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity [17]. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA of both gram positive and negative bacteria. Alkaloids also interfere with cell division [18]. Saponins are a special class of glycosides which have soapy characteristics [18]. The secondary metabolites identified in the *Ziziphus spina-christi* could be responsible for antimicrobial activity exhibited by this plant.

5. Conclusion

In developing world countries, including Nigeria, where contagious diseases are common, it is important to search out and promote medicines that are plant-based. This work will help to identify active ingredients for the treatment of bacterial diseases. Additional studies are needed to assess the effects of the selected plants on other pathogenic organisms.

6. Acknowledgements

References made to published literature have been duly acknowledged.

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