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Nutritional Content, Antimicrobial Study and Isolation of Quercetin from *Ziziphus spina-Christi* Leaf

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ABSTRACT
Plant has been the major source of medicinal globally. This study aimed at determining the nutritional composition, antimicrobial activity and isolation of phytochemical from the leaf of *Ziziphus spina-Christi.* Proximate composition, elemental (Ca, Na, Mg, K, P, Cu, Cd, Fe, Ni, Zn) concentrations, phytochemical screening and antimicrobial activity were carried out using standard protocols. Column chromatographic elution process was used to fractionate the methanol extract and Preparative TLC was used to isolate a compound. The result of the proximate composition and elemental concentration levels showed that the leaf is nutritious. Phytochemical screening showed the presence of alkaloids, cardiac glycosides, flavonoids, saponins and tannins. The methanol extract inhibited the growth of *Escherichia coli*, *Salmonella typhi*, *Staphyloccocus aureus* and *Bacillus subtilis* significantly at extract concentrations of 100, 200, 300, 400 and 500mg/mL was used, thus having antimicrobial activity. The compound isolated has 114-116^{o}C melting point (uncorrected) with a single banding pattern confirmed by TLC study. Physical and chemical tests of isolated compound were conducted. By using IR,^{1}H-NMR and ^{13}C-NMR spectra of the isolated compound was confirmed as Quercetin – a flavonoid.

Keywords: *Ziziphus spina-Christi*; Phytochemical; Quercetin; Antimicrobial; Elements

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1. INTRODUCTION

Plant products have been part of phytomedicines since time immemorial. The use of plants in the management and treatment of diseases started with life (Sofowora, 2008) [1]. In Africa, knowledge of traditional medicine as part of holistic system was passed through generations by oral communication and indigenous practices (Mosihuzzaman, 2012) [2]. In more recent years, with considerable research, it has been discovered that many plants possess medicinal values (Sofowora, 2008) [1].

According to the World Health Organization (WHO), a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active (Liu, 2004) [3].

These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto-'from Greek - meaning 'plant') or phytoconstituents some of which are responsible for protecting the plant against microbial infections or infestations by pests (Doughari, Human, Bennade, & Ndakidemi, 2009) [4].

Compounds belonging to the terpenoids, alkaloids and flavonoids are currently used as drugs or to prevent various diseases (Raskin, Ribnicky, Komarnytsky, Ilic, Poulev, Borisjuk, Brinker, Moreno, Ripoll, Yakoby, O'Neal, Cornwell, Pastor,& Fridlender, 2002) [5] and in particular some of these compounds seem to be efficient in preventing and inhibiting various types of cancer(Reddy, Odhav, & Bhoola, 2003) [6].

Ziziphus spina-christi (Figure 1) commonly known as Christ's Thorn Jujube, is a deciduous tree and native to the warm-temperate and subtropical regions, including North Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and Middle East (Yossef, Khedr, & Mahran, 2011) [7]. It belongs to the Rhamnaceae family in the order of Rosales that contains about 60 genera and more than 850 species.

Figure 1: Leaf of *Ziziphus spina-Christi*

The leaves of *Ziziphus spina-Christi* plants contain betulinic and ceanothic acids, various flavonoids, saponins, sterols, tannins and triterpenes (Ali & Hamed, 2006) [8]. The extract of *Z. christi* was shown to contain butic acid and ceanothic acid (a ring-A homologue of betulinic acid), cyclopeptides, as well as saponins and flavonoids, lipids, protein, free sugar and mucilage (Adzu, Amos, Amizan, & Gamaniel, 2003) [9]. Cardiac glycosides and polyphenols (such as tannins) are also reported from the leaves (Abalaka, Daniyan, & Mann, 2010) [10].

From current pharmaceutical studies, additional pharmaceutical applications of *Z. spina-Christi* have revealed antifungal, antibacterial, antinociceptive, antioxidant, antidiabetic, antiplasmodial, anti-schistosomiasis, analgesic and anticonvulsant activities among others (Abdel-Zaher, Salim, Assaf, & Abdel-Hady, 2005 [11]; El-Rigal, Aly, Rizk, & Said, 2006 [12]; Adamu, Abayeh, Ibok,& Kafu, 2006 [13]; Adzu & Haruna, 2007 [14]; El-Kamali, & Mahjoub, 2009 [15]; Adzu, Amos, Wambebe, & Gamaniel, 2001 [16]; Adzu, Haruna, Ilyas, Pateh, Tarfa, Chindo, & Gamaniel, 2011 [17]; Abalaka, Mann & Adeyemo, 2011 [18]; Waggas & Al-Hasani, 2010 [19]).

Ziziphus spina-Christi is widely distributed in Northeastern Nigeria, especially in Borno State. Despite the popularity of the plant among rural dwellers in the state, which is majorly inhabited by local people who depend on traditional herbal system of medicine for the treatment and management of diseases, to the best of our knowledge, there is no scientific study on the nutrition, antimicrobial as well isolation and characterization of flavonoidal compound from any part of the plant using chromatographic and spectroscopic technique in the region. Thus, this study will add to the ethnobotanical records of the plant as one can be used to treat microbial related diseases as well as contains significant phytochemical of universal interest.

2. MATERIALS AND METHODS

Sample Collection and Identification

Fresh leaves of *Ziziphus spina-Christi* were collected in the month of December, 2021 in University of Maiduguri campus, Maiduguri Borno State, Nigeria. It was authenticated by a plant Taxonomist of the Department of Biological Sciences, University of Maiduguri, Nigeria. The sample was air dried under shade, rendered free of foreign materials through manual picking and ground with a wooden mortar and pestle to powder.

Sample Preparation and Proximate Analysis

Two grammes (2 g) each of the crushed samples were processed for analysis of various parameters according to the Association of Official Analytical Chemists (AOAC, 1990; AOCS, 2000 [20,21]) methods. The proximate analysis such as moisture, ash, crude lipid content, crude fibre, nitrogen free extracts, crude protein and carbohydrates (by difference of the ethanol extract) of the dried samples were determined using AOAC methods (AOCS, 2000) [21]. The moisture and ash contents were determined using weight difference method. Crude fibre content was estimated from the loss of weight of the crucible and its content on ignition. Carbohydrate was determined by differential method when the sum of the percentages of moisture, ash, crude lipid/fat, crude protein and crude fibre content was subtracted from 100 (Muller & Tobin, 1980) [22].

The nitrogen value, which is the precursor for protein of a substance, was determined by micro-Kjehldahl method, involving digestions, distillation and titration of the sample (Pearson, 1976) [23]. The nitrogen value was converted to protein by multiplying nitrogen value with a factor of 6.25. The determination of crude lipid content of the samples was done using soxhlet extractor type of the direct solvent extraction method. The solvent used was n-hexane (boiling range 40-60^{o}C). The total carbohydrate (CHO) content was determined by difference, as the sum of the % Moisture, % Ash, % Crude Lipid/fat, % Crude Protein and % Crude Fibre which was subtracted from 100 (Muller &Tobin, 1980) [22]. All the proximate values were presented as percentages (AOCS, 2000) [21].

Sample Digestion and Elemental Content Analysis of *Ziziphus spina-Christi* Leaf

The air-dried plant sample was pulverized manually in a wooden mortar and pestle into a coarse powder. 0.5 g of the sample was independently packed into an acid-washed porcelain crucible and then placed in a muffled furnace for 4 hr at 55^{o}C. The crucible was removed from the furnace and cooled. Ten (10) ml of 6 M HCl was added and then covered, and the content was heated on a steam bath for 15 minutes. One ml of HNO was later added, and 3 evaporated to dryness by continuous heating for one hour so as to dehydrate silica and completely digest organic substances. Lastly, 5 ml of 6 M HCl and 10 ml of water was added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman No. 1 filter paper into 100 ml volumetric flask and then made up to the mark with distilled water (Radojevic & Bashkin, 1999) [24]. The macro and microelements were determined using Perkin-Elmer Analyst 300 single beam Atomic Absorption Spectrophotometer (AAS) and the data were obtained in parts per million (ppm) which were then converted to mg/g. Calibration curve was established using working standards for each element. Laboratory procedures for the preparation and determination of macro and microelements were used as outlined by Radojevic and Bashkin (1999)]24] for plant samples.

Plant Extraction and Partitioning

Three hundred grammes (500 g) of the pulverized dried leaf was extracted by cold maceration method with 2.5 L of 85 %methanol in a 5L conical flask at room temperature for seventy-two hours (72 hr) in a flat bottom flask with occasional shaking in a sonicator for 6hrs daily. The soaked sample were passed through a muslin cloth to remove the vegetative debris and the liquid was filtered through Whatman No. 1 filter paper. The crude extracts were concentrated to dryness. The extract was weighed and the percentage yield was calculated, labelled as ZME and subjected to the following analysis.

The extract obtained from the leaves was exhaustively defatted in a separating funnel using absolute n-hexane (1L). The fractions obtained (n-hexane and residual portions) were concentrated to dryness under reduced pressure, encoded (ZPH and ZPR) and stored.

Preliminary Phytochemical Screening

The crude methanol, n-hexane and residual fractions of the leaf of *Ziziphus spina-Christi* were screened qualitatively for the presence of phytochemicals which include alkaloids, anthraquinones, flavonoids, saponins, tannins, terpenoids, cardiac glycosides and steroids, using methods as described by Evans (2009) [25] as adopted by Yakubu, Usman, Balami, Jonathan, Semiu, and Teri (2016) [26].

Isolation and Purification

The methanol extract was fractionated by column chromatography and thin layer chromatography for the isolation of the phytochemical. A glass tube with a diameter of 2.8 cm and a height of 90 cm with a tap at the bottom was used for the column chromatographic technique. A plug of cotton wool was well placed at the bottom of the column very close to the tap so as to prevent the stationary phase from blocking the column. About 250 g of silica gel 60-120 mesh (Quikelem, India) was used to prepare slurry by wet method. The silica gel was mixed with n-hexane and stirred with a clean glass rod until a uniform mixture was obtained. It was then packed cautiously and manually to about two third the size of the column tube using a glass funnel. The gel was then allowed to settle and pack for 24 hrs. The air bubbles were avoided and care was taken not to dry the column by maintaining the level of the n-hexane to that of the silica gel. The methanol of 10 g was mixed with 5 g of silica gel followed by the addition of 100 ml of methanol. The mixture was stirred well for proper mixing, allowed to stand overnight and was mounted through a glass funnel using a glass rod to the already equilibrated silica-fixed column on top of the stationary phase. This was topped with a small layer of cotton, then sand to protect the shape of the organic layer from the velocity of newly added eluent (stationary phase). The eluting solvent initially was 100 % ethyl acetate and the polarity was gradually increased at 90:10, ethyl acetate: methanol ratio until 0:100 ethyl acetate:

methanol ratio was used. Thirty (30) sub-fractions were collected. The column fractions were monitored for similarities of the fractions based on retardation factor (R_f) values using thin layer chromatographic (TLC) technique and afforded six (6) pooled fractions encoded Z1, Z2, Z3, Z4 and Z5.

Sephadex LH20 (10 g) [Amersham Bioscience Sweden] was mixed with methanol and allowed to swell in methanol overnight. The swollen Sephadex was packed manually into a column of 45.0 cm x 10 cm i.d. using a glass funnel. The gel was allowed to settle for 24hrs. Precaution was taken to avoid air bubbles as well as not to allow the column run dry. Fraction Z4 (0.2g) was mounted on the already equilibrated Sephadex fixed column. A total of three sub-fractions were obtained and encoded A, B and C. A preparative TLC was used to isolate a single compound from the sub-fraction. The crystalline solid obtained was soluble in methanol, ethanol, n-butanol and water but insoluble in chloroform, petroleum ether and n-hexane.

Structural Analysis

Fourier Transform Infrared (FTIR) Spectroscopic Analysis

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about how the structure of a molecule could frequently be obtained from its absorption spectrum. The fractions were mixed in dry potassium bromide (KBr). The mixture was thoroughly pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Agilent technologies Infrared Spectrometer. The sample was scanned from 4000 to 400 cm^{-1}. The peak values of the FTIR were recorded (Williams & Fleming, 2016) [27].

Nuclear Magnetic Resonance Spectroscopic Analysis

Nuclear Magnetic Resonance Spectroscopic (NMR-1HNMR and ^{13}C-NMR at 500 MHz) spectra were recorded using Bruker AVANCE (Bruker Instruments Inc. Karlsrube, Germany).

***In vitro* Antimicrobial Studies**

Test for organisms

The antimicrobial effects of the fractions were carried out on clinically isolated microorganism obtained from the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, and University of Maiduguri. This include: *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella, pneumoniae, Salmonella typhi, Bacillus subtilis,Candida albicans* and *Aspergillus niger*.

Screening for Antimicrobial activity

The crude and residual fraction of the leaf were subjected to preliminary *in vitro* antimicrobial test using the agar well diffusion technique as described by Usman, Abdulrahman, & Usman, 2009) [28]. Wells were bored on the media using 6 mm cork-borer and were filled with 0.2 mL aliquots of various concentrations of the extract (500 mg/mL, 400 mg/mL, 300, 200 mg/mL and 100mg/mL). The plates containing the agar were kept in an incubator at 37^{o}C for 24 hrs. The diameters of the zones of inhibition zone after incubation for the methanol extract and fractions were measured in millimetres using a transparent ruler calibrated in meter, and the tests were carried out in triplicates.

Statistical Analysis

The data obtained from the antimicrobial study was analysed using Graphpad Prism version 8.4.3 for windows, followed by Turkey-Kramer's multiple comparison test. Values of $p<0.05$ were considered and were expressed in mean and standard error of mean (SEM).

3. RESULTS AND DISCUSSION

Proximate Composition of *Ziziphus spina-Christi* Leaf

Figure 1 shows the proximate composition of ***Ziziphus spina-Christi*** leaf. The result of the study revealed that the leaf is composed of 5.30 %, 2.0 %, 1.0 %, 22.0 %, 3.02 % and 66.98 % of the moisture, ash content, crude lipid, crude fibre, crude protein and carbohydrate respectively.

Elemental Content Analysis of *Ziziphus spina-Christi* Leaf

The concentration levels of both macro and trace elemental analysed are shown in Table 1. The results revealed that calcium had the highest concentration (2.06E-02mg/g), while phosphorus had the least (4.50 E-06mg/g).

Extraction Profile of Leaf and N-hexane and Residual Partitioned Fractions of *Ziziphus spina-Christi*

The extraction of the leaf of *Ziziphus spina-Christi* using methanol yielded extract of 10.46 % with greenish brown colour and gummy mass in texture. The n-hexane partitioned fraction of the 31.40g of methanol crude extract yielded fractions 8.62g and the residual fraction was 22.76g. The result of the extraction profile is shown in Table 1:

Phytochemical Constituents of *Ziziphus spina-Christi* Leaf Methanol, n-Hexane and Residual Fractions

The phytochemical screening carried on the methanol leaf extract and the n-hexane and residual fractions of the methanol leaf extract revealed the presence of phytochemicals which include, flavonoid, cardiac glycosides, saponins, alkaloids and tannins. The results of this study is shown in Table 1.

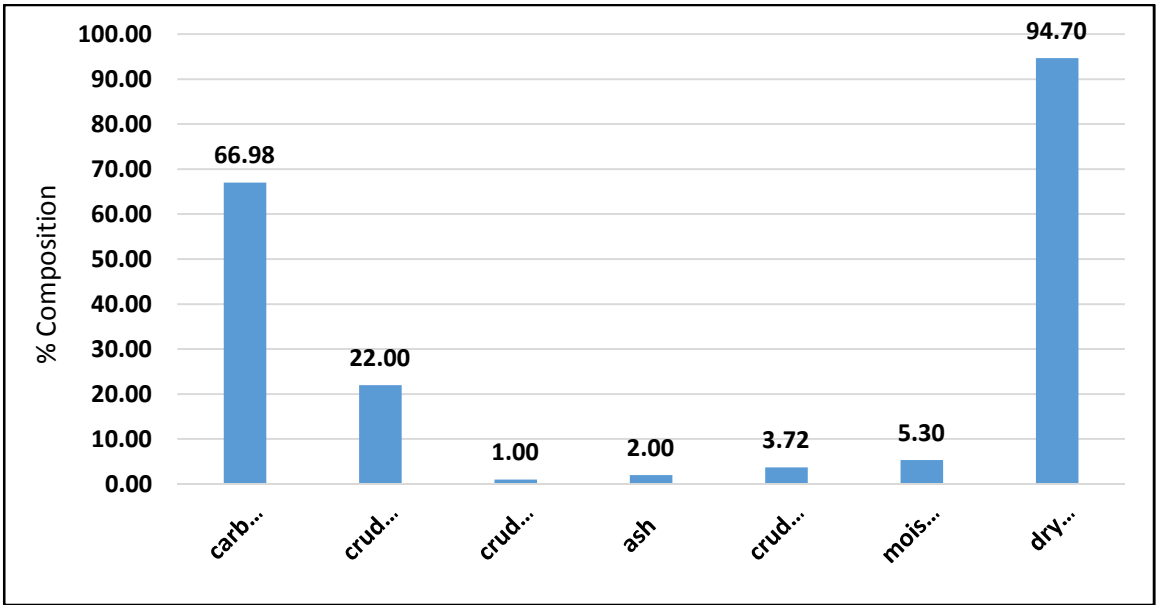

Figure 2: Proximate Composition of *Ziziphus spina-Christi* Leaf

Table 1: Elemental Concentration Levels of *Ziziphus spina-Christi* Leaf

S.N.	Elements	Concentration (mg/g)	WHO/FAO Maximum Permissible Limits (mg/g)
1	Ca*	2.06E-02	8.00E-01
2	Cd	1.00E-05	3.00E-03
3	Cr	3.00E-05	1.30E-03
4	Cu	9.80E-05	1.00E-02
5	Fe	6.50E-04	2.61E-01
6	K*	1.50E-03	1.00E-01
7	Mg*	1.16E-02	4.00
8	Na*	3.00E-02	5.00E-01
9	Ni	1.65E-05	1.00E-02
10	P*	4.50E-06	-

Key - * denotes macro-elements

Table 2: Extraction and Partitioned Profile of *Ziziphus spina-Christi* Leaf

S.N.	Sample	Mass (g)	%Yield ($^{w}/_{w}$)	Colour	Texture
1	ZME	31.40	10.46	greenish brown	gummy
2	ZPH	8.64	-	greenish	gummy
3	ZRE	22.76	-	Brown	powdery

Key: ZME: *Ziziphus spina –Christi* methanol extract; ZPH: *Ziziphus spina –Christi* N-Hexane Partitioned Fraction; ZPR: *Ziziphus spina –Christi* Residual Partitioned Fraction

Table 3: Phytochemical Constituents of *Ziziphus spina-Christi* Leaf Crude Methanol, n-Hexane and Residual Fractions

S.N.	Phytochemical Test	ZME	ZPH	ZPR
1	Test for carbohydrates	+	+	+
2	Test for Tannins	+	-	+
3	Test for cardiac Glycosides	+	-	-
4	Test for Flavonoids	+	-	+
5	Test for Saponins	+	+	+
6	Glycosides	-	-	-
7	Test for Alkaloids	+	+	-

Table 4: Summary of Isolated compound

Isolate	Mass	Colour	Texture	Melting point	Rf Value
ZI	20.24 mg	yellowish	Amorphous	314-316^{o}C	0.68

IR Spectral Analysis

The IR absorption spectrum of the isolated compound of methanol leaf extract of *Ziziphus spina-Christi* showed absorption peaks at 3387 cm^{-1} (OH_{str}), 2930 cm^{-1} (CH_{2str}), 2864.8 cm^{-1} (CH_{str}), 1734 cm^{-1} (C=O), 1454.3 cm^{-1} (C-O vibration), 1022 cm^{-1} (C-H_{def}).

Nuclear Magnetic Resonance (NMR) Spectral Analysis

1H-NMR spectra

The isolated compound encoded ZI showed the signals of 1H-NMR when DMSO D6, was used as the solvent at radio frequency of 500MHz. Signals were observed at δ 6.24 (1H, d, H-6), 6.46

(1H, d, H-8), 6.83-6.90 (1H, d, H-5′), 7.53-7.55 (1H, dd, H-6′), 7.68 (1H, d, H-2′), 9.35 (1H, broad, C14 and C15-OH), 9.59 (1H, s, C11-OH), 10.79 (1H, s, C12-OH) and 12.49 (1H, s, C13-OH). Two characteristic peaks were observed by 1H-NMR spectrum at δ 6.18-6.19 and 6.40- 6.41 which are corresponds to meta protons H-6 and H-8 on A ring and an ABX system at 6.88-6.90, 7.53-7.55 and 7.68 corresponding to the catechol protons on B ring. A hydroxyl group at 5th and 7thposition of the A ring showed a broad singlet at δ10.79 and 12.49 respectively. A hydroxyl group at 3rd and 4th position of the B ring showed a broad merged singlet peak at δ9.31. A hydroxyl group at 3rdposition of the C ring showed a singlet peak at δ12.42.

^{13}C-NMR Spectra

The isolate encoded ZI showed ^{13}C-NMR signals (DMSO D6, 120MHz spectrum, at ppm 147.30 (C-2), 135.99 (C-3), 176.15 (C-4), 161.20 (C-5), 99.05 (C-6), 164.00 (C-7), 94.10 (C-8), 156.61(C-9), 102.01 (C-10), 122.50 (C-1′), 115.62 (C-2′), 145.72 (C-3′), 148.20 (C-4′), 115.99 (C-5′) and 120.38 (C-6′). The chemical shifts were observed for carbon nucleus at 136.20 (C-3), 161.20 (C-5), 164.21 (C-7), 145.54 (C-3′) and 148.22 (C-4′) which suggested possible carbons at positions 3, 5, 7, 3′ and 4′ of oxygenated flavones nucleus. The peaks at δ147.24 (C-2), 176.33 (C-4), 99.00 (C-6), 93.77 (C-8), 156.01 (C-9), 103.52 (C-10), 122.409 (C-1′), 115.520 (C-2′), 116.055 (C-5′) and 120.43 (C-6′) were observed for other carbons.

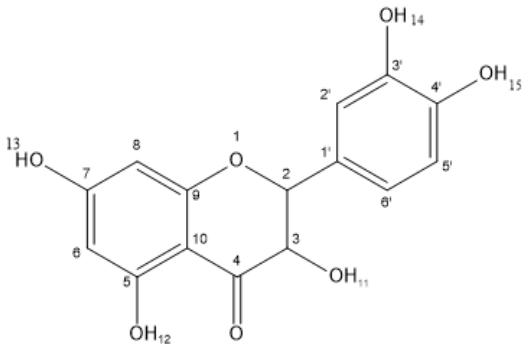

Figure 3: Compound ZI isolated from the Leaf of *Ziziphus spina -Christi*

Antimicrobial Susceptibility Study of *Ziziphus spina-Christi*

Table 5 presents the antimicrobial activity of *Ziziphus spina-Christi* methanol leaf extract. The antimicrobial susceptibility test presented as inhibition zones showed highest activity against *Salmonella typhi* (18.67±0.33mm) at 500mg/ml concentration of the extract, while it has the lowest activity against *Staphylococcuc aureus* (7.67±0.58mm) at 100mg/ml. Notably, *Ziziphus spina-Christi* had activity against *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli,* at extract concentration of 500, 400, 300, 200 and 100 mg/ml of the leaf extract was used. Other microbes which include *Streptoccocus pyogenes, Aspergillus niger, Candida albicans* and *Klebsiella pneumonia* were all resistant to the extract at all concentrations tested

Table 6 presents the antimicrobial activity of *Ziziphus spina-Christi n-* hexane partitioned methanol leaf extract. The antimicrobial susceptibility test presented as inhibition zones showed highest activity against *Escherichia coli* (14.00±0.00mm) at 500mg/ml concentration of the extract, and lowest activity with inhibition zone of 7.00±0.00mm at 200mg/ml as the only organism the n-hexane partitioned fraction inhibited its growth; while there was no observation effect of the fraction against all the other pathogen used for this study.

Table 7 presents the antimicrobial activity of *Ziziphus spina-Christi* residual partitioned methanol leaf extract. The antimicrobial susceptibility test presented as inhibition zones showed highest activity against *Staphyloccocus aureus* (15.00±0.00mm) at 500mg/ml concentration of the extract, and lowest activity with inhibition zone of 8.00±0.00mm at

200mg/ml as the only organism the n-hexane partitioned fraction inhibited its growth; while there was no observation effect of the fraction against all the other pathogen used for this study.

Table 5: Antimicrobial Activity of Methanol Leaf Extract of *Ziziphus spina-Christi*

S.N.	Concentration (mg/ml)/ Mean Zone of inhibition in diameter (mm)						
	Organism	**500**	**400**	**300**	**200**	**100**	**Control(CIP)**
1	*Staphylococcus aureus*	17.67±0.58^{a}	14.67±0.58^{b}	12.00±0.00^{c}	9.67±0.58^{d}	7.67±0.58^{e}	32.00±0.00^{f}
2	*Streptococcus pyogenes*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	30.00±0.00
3	*Bacillus subtilis*	15.00±0.00^{a}	11.68±0.58^{b}	9.00±0.00^{c}	7.00±0.00^{d}	0.00±0.00^{e}	33.00±0.00^{f}
4	*Escherichia coli*	15±0.00^{a}	12.00±0.00^{b}	10.00±0.00^{c}	8.00±0.00^{d}	0.00±0.00^{e}	29.00±0.00^{f}
5	*Klebsiella pneumonia*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	47.00±0.00
6	*Salmonella typhi*	18.67±0.33^{a}	15.67±0.33^{b}	13.00±0.00^{c}	11.00±0.00^{d}	8.00±0.00^{e}	36.00±0.00^{f}
7	*Aspergillus niger*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8	*Candida albicans*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	11.00±0.00

Key: Values across rows with different superscripts are statistically ($p<0.05$) different; n=3; disc diameter=6mm, Mean ± SEM

Table 6: **Antimicrobial Activity of n-Hexane Fraction of Methanol Leaf Extract of *Ziziphus spina-Christi***

S.N.	Concentration (mg/ml)/ Mean Zone of inhibition in diameter (mm)						
	Organism	**500**	**400**	**300**	**200**	**100**	**Control(CIP)**
1	*Staphylococcus aureus*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	32.00±0.00
2	*Streptococcus pyogenes*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	30.00±0.00
3	*Bacillus subtilis*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	33.00±0.00
4	*Escherichia coli*	14±0.00^{a}	12.00±0.00^{b}	9.00±0.00^{c}	7.00±0.00^{d}	0.00±0.00^{e}	29.00±0.00^{f}
5	*Klebsiella pneumonia*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	47.00±0.00
6	*Pseudomonas aeruginosa*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	36.00±0.00
7	*Aspergillus niger*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8	*Candida albicans*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	11.00±0.00

Key: Values across rows with different superscripts are statistically ($p<0.05$) different; n=3; disc diameter=6mm, Mean ± SEM

Table 7: Antimicrobial Activity of Residual Fraction of Methanol Leaf Extract of *Ziziphus spina-Christi*

S.N.	Concentration (mg/ml)/ Mean Zone of inhibition in diameter (mm)						
	Organism	**500**	**400**	**300**	**200**	**100**	**Control(CIP)**
1	*Staphylococcus aureus*	15±0.00^{a}	12.00±0.00^{b}	10.00±0.00^{c}	8.00±0.00^{d}	0.00±0.00^{e}	32.00±0.00^{f}
2	*Streptococcus pyogenes*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	30.00±0.00
3	*Bacillus subtilis*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	33.00±0.00
4	*Escherichia coli*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	29.00±0.00
5	*Klebsiella pneumonia*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	47.00±0.00
6	*Samonella typhi*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	36.00±0.00
7	*Aspergillus niger*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8	*Candida albicans*	0.00±0.00	0.00±0.000	0.00±0.00	0.00±0.00	0.00±0.00	11.00±0.00

Key: Values across rows with different superscripts are statistically ($p<0.05$) different; n=3; disc diameter=6mm,Mean ± S

4. DISCUSSION

The Proximate composition study revealed that the leaf of *Ziziphus spina-Christi* to be moderately nutritious. The result showed that *Ziziphus spina-Christi* is made up of 5.30% moisture. This result indicates that the plant leaf could have high shell life.

The crude protein of 3.72% of which the recommended dietary allowance (RDA) by WHO for protein is 56% for individual weighing 70kg and 46g for adult weighing 50kg, children may consume 2kg/day (Jones, Johnson, Netlerville, Wood,& Joesten, 1985) [29] is within the permissible limit of consumption. Low ash content of 2.00 % is the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid etc) have been incinerated at a temperature of about 500^{o} C. Ash content is generally taken to be a measure of the mineral content of the original food (Onwuka, 2005) [30]. Crude fat of 0.1% indicates that the leaf has very low content of fats, thus cannot contribute significantly as a good source of energy. Crude fibre in food or plant is an indication of the level of non-digestible carbohydrate and lignin. The crude fibre obtained is 22.00%. This significant is considered appropriate, because it aids absorption of glucose and fat.

The results of the elemental and anion analyses of the leaf of *Ziziphus spina-Christi* shows that calcium, cadmium, chromium, copper, iron, potassium, magnesium, sodium, nickel and zinc had concentration levels within the world Health Organization (WHO) permissible limits. Some significant numbers of trace elements are known to influence various functions due to their direct or indirect action in physiological or toxic concentration (WHO, 1999) [31]. Iron occurs as a natural constituent in plants and animals. The presence of some of these elements in the leaf of *Ziziphus spina-Christi* could be an indication of the types of mineral elements present in the soil. The toxic element cadmium (Cd) was found in very low concentration in plants (IPCS, 1994) [32] and this may be due to its low deposit in the soil. Zinc is a constituent of many enzymes and is essential for the proper function of these various enzymes. Zinc is essential for the metabolism and structural stability of nucleic acids. Zinc has been associated with a variety of bodily functions such as the wounds healing, reproduction, growth and maintenance of glucose tolerance in the body (RDA, 1989) [33]. Ca and Fe are necessary to maintain an optimal bone development, more of both minerals being required during childhood and growing stages to prevent rickets and osteomalacia (Valverd e, Periago, Santaella, & Ros, 2000) [34]. Ca also has an essential role in blood clotting, muscle contraction and nerve transmission. Iron (Fe) is an important mineral; it is required to help our red blood cells deliver oxygen to the rest of the body. Iron is essential for many proteins and enzymes that maintain good health, transporting oxygen in the blood to all parts of the body as well as proper functioning of the liver (Nuray & Ozkan, 2007) [35]. Mn is essential for normal functioning of central nervous system and is good anti-oxidant (Bibi, Dastagir, Hussain, Sanuallah & Park, 2006) [36]. Copper is a common environmental metal and is essential in cellular metabolism but at high concentrations, it can be highly toxic to fish (Grosell, Hogstrand& Wood, 1997) [37].

Flavonoids exhibit several biological effects. They are potent antioxidants and have free radical scavenging abilities (Kumar, Mishra & Pandey, 2013) [38]. Many have antiallergic, antiviral actions and some of them provide protection against cardiovascular mortality (Hertog, Feskens, Hollman, Katan & Kromhout, 1993) [39]. They have shown to inhibit the growth of various cancer cell lines *in vitro* and reduce tumor development in experimental animals (Mori, Nishinoc & Tavata, 1988) [40]. Several flavonoids such as catechin, apigenin, quercetin, naringenin, rutin, and venoruton are reported for their hapatoprotective activities (Tapas, Sakarkar & Kakde, 2008) [41]. Saponins have been reported to possess Anti-bacterial activity (Ibrahim, Khan, Tiwari, Habeeb, Khaja, & Habibullah, 2006) [42].Tannins are polyphenols that are obtained from various parts of different plants (Gajendiran & Mahadevan, 1990) [43]. In addition to its use in leather processing industries, tannins have

shown potential antiviral, antibacterial and antiparasitic effects (Yakubu *et al*., 2016) [26].

Terpenes have been reported to possess important biological activities, such as analgesic anticonvulsant, cardiovascular, antimalarial and antibacterial (Evans, 2009; Yakubu *et al*., 2016) [25, 26]. They are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties (Wagner & Elmadfa, 2003) [44].

The plant crude methanol extract showed some degree of antimicrobial activity against some tested pathogenic microbes. There was inhibition of the growth of *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus*. The result is in conformity with the report of Ali, Almagboulb, & Mohammed (2015) [45]. This justifies the use of the plant for the treatment of microbial related diseases. However, the resistance of the *A. niger*, *Candida albicans, Aspergillus niger, Klebsiella pneumonia* and *Streptococcus pyogenes* against the tested concentrations those not indicate a total inactivity of the extract, but rather a resistance of working concentrations. It was noticed that the antimicrobial activity of the residual partitioned fraction was significantly decreased when compared with the concentration of the crude methanol extract. This shows that the defatting could have reduced the potency of possible synergistic effect of the phytochemicals present in the extract. Hence, the low activity of the partitioned fractions.

The methanol extract of the leaf was partitioned with *n*-hexane. The residual fraction was purified and one compound was isolated using chromatographic technique. By means of spectroscopic analysis, it was characterized as Quercetin. Although the compound has been reported by El-Shahir, El-Wakil, Abdel Latef,& Youssef (2022) [46] in Egypt from the same plant, this is the report of its isolation from *Ziziphus spina-Christ i*in Borno state for the first time, to the best of our knowledge.

5. CONCLUSION

In the present study, the leaf of *Z. spina-christi* L. is quite nutritious, exhibited antimicrobial activity and contains class of phytochemicals of significant importance. Phytochemical investigations led to the isolation quercetin for the first time in Northeastern Nigeria. The structure of this compound was determined by IR, ^{1}H and ^{13}C NMR spectroscopy and confirmed by comparing with the previously reported values.

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