Journal of Biological Sciences and Bioconservation
Volume 11, Number 2, 2019
ISSN: 2277-0143

PHYTOCHEMICAL AND ANTI BACTERIAL ACTIVITY OF *ZIZIPHUS MUCRANATA LEAVES EXTRACT*

Garba U., Gayari, M.S., Boyi, M.Y and S/Dogarai, B.B
Department of Chemistry
Shehu Shagari College of Education Sokoto
Email: umar.g4000@gmail.com

ABSTRACT

This research work investigate on the major active chemical constituents present in the leave of the ziziphus mucranata (Ramnaceae) and anti-bacterial activity of different extracts of the plant leaves. Ziziphus mucronata (Rhamnaceae), commonly known as buffalo thorn, is native to Northern Nigeria. The percentage yield (%) of the extracts were quantified, whereby Aq, MeOH, Ethyl acetate and DCM extracts possessed 0.92%, 2.20%, 1.98% and 1.65% respectively. Conclusively, Leaves of Ziziphus mucronata reveal some degree of anti-bacterial activities against staphylococcus and E.coli, these activities may be traced to either alkaloids or steroids. These indicate that most of medicinal plants used in ethnomedicine are potentially useful pharmacological and nutraceutical in the treatment of some pathogenic microorganisms. This research may contribute to the clear understanding of antibacterial activities of the plant. Health benefits and biodiesel/biofuel of the plant should be tested.

Keywords: *Phytochemical, Antibacterial, Extraction, Ramnacea, ethnomedicine, biofuel.*

INTRODUCTION

Plants have been used for health and medical purposes for several thousands of years. The number of higher plant species on earth is about 250 000. It is estimated that 35 000 to 70 000 species have, at one time or another, been used in some cultures for medicinal purposes. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years (WHO, 1998).

Medicinal plants are important sources for pharmaceutical manufacturing. Medicinal plants and herbal medicines account for a significant percentage of the pharmaceutical market (WHO, 1998). Plants have provided man the essential of life since time immemorial. The healing potentiality of the plant progressively plays a vital role in the primary health care of about 80% of the world's population, even today in western medicine, and despite progress in synthetic chemistry, some 25% of prescriptions medicines are still derive either directly or indirectly from plants (Farnsworth & Soejarto, 2009)

Ziziphus mucronata (*Rhamnaceae*), commonly known as buffalo thorn is native to Northern Nigeria. It is also distributed throughout the summer rainfall areas of sub-Saharan Africa, extending from South Africa northwards to Ethiopia. The genus *Ziziphus* found in desert areas (Jawanda and Bal, 1978)

belongs to the *Rhamnaceae family*. Ziziphus species are important versatile fruit trees in many arid countries and are planted as hedges to protect live stocks from predators (Cherry, 1985).

The plant decoction is used traditionally in the treatment of diabetes mellitus (Etuk *et al*., 2010) among the rural populace of Northern Nigeria. Its bark and roots are used medicinally for the treatment of various ailments, including rheumatism, gastrointestinal complaints, and snake bites (Burkhill, 1985). Warm bark infusions are used to relieve body pains. It is also used as expectorants for cough, medicine for respiratory infections and for chest problems. The root infusions are used for treating gonorrhea, diarrhea and dysentery. Decoctions of roots and leaves are applied externally to treat sores and glandular swellings (Burkhill, 1985 and Amusan *et al*., 2005). While the fruits are sometimes sucked by small children, some rural dwellers believe that the tree serves as a protection against lightning and others cultivate this species to mark burial sites.

Ziziphus mucronata (*Rhamnaceae*), commonly known as buffalo thorn, is native to Northern Nigeria. It is also distributed throughout the summer rainfall areas of sub-Saharan Africa, extending from South Africa northwards to Ethiopia. The genus *Ziziphus* found in desert areas (Jawanda and Bal, 1978) belongs to the *Rhamnaceae* family. The members of the taxon are known to be drought tolerant and very resistant to heat. *Ziziphus* species are important versatile fruit trees in many arid countries and are planted as hedges to protect live stocks from predators (Cherry, 1985).

The plant decoction is used traditionally in the treatment of diabetes mellitus (Etuk *et al.*, 2010) among the rural populace of Northern Nigeria. Its bark and roots are used medicinally for the treatment of various ailments, including rheumatism, gastrointestinal complaints, and snake bites (Burkhill., 1985). Warm bark infusions are used to relieve body pains. It is also used as expectorants for cough, medicine for respiratory infections and for chest problems. The root infusions are used for treating gonorrhea, diarrhoea and dysentery. Decoctions of roots and leaves are applied externally to treat sores and glandular swellings (Burkhill., 1985; Amusan *et al.*, 2005). While the fruits are sometimes sucked by small children, some rural dwellers believe that the tree serves as a protection against lightning and others cultivate this species to mark burial sites.

The antibacterial activity of crude methanolic extract of *Ziziphus mucronata*, Willd. Subsp. Mucronata Willd against both gram-positive and gram-negative bacteria was investigated using agar and macro broth dilution methods. The extract had effective activities against both Gram-positive and Gram-negative bacteria. The research revealed the broad spectrum potential of the plant as well as established its ethno botanical relevance in the traditional treatment of diarrhea and dysentery (Olajuyigbe, and Afolayan., 2012).
The ethanolic stems bark extract of *Z. mucronata* against medically important pathogens was investigated for the antimicrobial activity by the agar dilution method while the degree of its antimicrobial activity was determined by the macro broth dilution method. The extract showed good antibacterial and antifungal activities. Both Gram-negative and Gram-positive bacteria were highly inhibited by the

extract at very low concentrations. The antimicrobial activity of the extract indicated a broad spectrum, great therapeutic potential and justified the use of this plant by the rural communities in the treatment of microbial infections (Olajuyigbe and Afolayan., 2012).

MATERIALS AND METHOD

The Instruments Used for this analysis includes; 25ml Measuring cylinder, Triple beam balance, Round bottom flask, Beaker, Funnel, Filter paper no1, Retort stand, Dropper, Stirrer, Test tube , Capillary tube, U.V spectrophotometer, Autoclaving machine, conical flash, Petri dish, Cork borer and test organisms (E.Coli and *Staphylococcus*). While the chemicals used includes; Sulphuric acid, Benzene, Ferric chloride, Lead acetate, Ammonium solution, Sodium hydroxide, Hydrochloric acid, Acetic acid, Sodium carbonate, Chloroform, Wagners reagents, Mayer's reagents, Dragendoff's reagent and Olive oil.

The leaves sample of *ziziphus mucranata* (Buffalo thorn) was freshly collected in the month of March, 2017 from Yabo Local Government, Sokoto State, Nigeria. The plant leaves were collected and identified at the Botany Unit, Usman Danfodiyo University, Sokoto, Nigeria. The leaves were immediately washed with distilled water to remove debris, dried under the shade for about two weeks and then grinded into fine powder using a kitchen blender and stored in air tight bottle inside a desiccators till use.

Extraction of Plant Materials

Fifty (50) gram powdered material was extracted with 200cm^3 of different solvents, 200 cm^3 of Dichloromethane

(DCM), 200 cm^3 of Ethyl acetate, and 200 cm^3 of methanol except Aqueous extract which was extracted with 400 $cm^3$of distilled water, for 48hours in a laboratory oven. The mixture was filtered and different filtrates were concentrated and kept for preliminary photochemical test and antifungal analysis. The percentage yield and other physical properties were recorded. The evaporated residue was used for Antibacterial activities.

Plate 1.1: Preparation of extracts.

Phytochemical Screening of *Ziziphus mucronata*

Tests for Alkaloid

- **Mayer's Test:** Five drops of the Mayer's reagent was added into 2cm^3 of each extract in a test tube, appearance of yellowish precipitate was taken as indication for presence of alkaloid. (El-Olemay *et al,* 1994).
- **Dragendroff's Test:** Five drops of the dragendroff's reagent were added to 2cm^3 of each extract in a test tube formation of brown precipitate was taken as indication of the presence of alkaloid (El-Olemay *et al,* 1994).
- **Wagner's Test:** Five drops of the Wagner's Regent were added into 2cm^3 of each extract in test tube formation of dark brown precipitate indicated the presence of alkaloids. (El-Olemay *et al*, 1994).

Test for Saponins

Two ($2cm^3$) of each filtrate was diluted with $5cm^3$ of distilled water and vigorously shaken then allowed to stand for 30 minutes; persistent frothing indicated the presence of saponins. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for formation of an emulsion (Sofowora, 1984)

Test for Phlobatannins

Two ($2cm^3$) of the filtrate was added to 5cm of 1%HCl and heated, deposition of red precipitate was an indication of presence of phlobatannins (Sofawara, 1984).

Test for Steroids

- **Liebenmann's Test**: five (5) cm^3 of anhydrous acetic acid and $5cm^3$ of chloroform were combined together with the extract and cooled in ice, then few drops of concentrated sulphuric acid was added down the side of the test tube. A violet or pink colour which gradually changes to blue and blue-green was taken as positive test (Abubakar, 2009).
- **Slakowski Test**: A little quantity of each of the extracts was dissolved in $2cm^3$ of chloroform and few drops of concentrated H_2SO_4acid were added into the test tube to form two layers. A red or yellow colour was taken as indication of the present of sterol (Abubakar, 2009).

Test for Cardiac Glycosides

Five ($5cm^3$) of the extract was dissolved in $2cm^3$of glacial acetic acid containing few drops of ferric chloride solution. A

reddish brown ring obtained at the interface, indicates the presence of a Cardiac glycoside (Trease and Evans, 1989).

Test for Flavonoids

- **Sodium Hydroxide Test**: Two ($2cm^3$) of the each filtrate was acidified with 1% HCl followed by drops of 20% NaOH. Canary yellow colour indicates presence of flavonoids (Ganapathi *et al*, 2011)
- **Lead Acetate Test**: Two ($2cm^3$) of each of the filtrates was treated with 3 drops of 10% lead acetate solution. A light yellow milky coloured precipitate indicates the presence of flavonoid (Harbone, 1984)

Test for Anthraquinones

Two ($2cm^3$) of each filtrate was treated with 5cm of benzene. This formed two layers. The colourless upper layer was pipette and treated with $3cm^3$ of 10% Ammonia. Formation of pinkish colour indicates presence of Anthraquinones (Trease and Evans, 1989).

Test for Tannins

Two ($2cm^3$) of each filtrate was treated with 3 drops of 5% ferric chloride solution. A dark-black coloured precipitate which gives green-black to blue-black colouration on dilution indicates the presence of tannins (both hydrolysable and condensed) (Sofowora, 1984).

Antibacterial Activity

Sample Standardization of Innoculum

The overnight suspensions of the test organisms (*Staphylococcus* and *E.coli*) were prepared by transferring several identical colonies of each organism into 2mls of

nutrients drops transferred into a sterilized test-tube containing 2ml of nutrient drops. The tubes were capped with aluminum foil and incubated overnight at 37°C. The overnight bacteria suspensions were transferred into a glass cubic and placed inside a U.V spectrophotometer. The absorbance was measured and adjusted to 0.5 (McFarland standard).

Preparation of Several Concentrations of Plants Extracts

A concentration of 100mg/ml of each extract was prepared by weighing 0.1g of the extract and dissolved in 1ml of 10% dimethyl sulphate oxide (DMSO). It was then preserved at 4°C until use.

Media Preparation

Nutrient agar and molar Hinton agar were prepared according to the manufacturer instructions and Autoclaved at 121°C at 15 pound per square inch. In Molar Hinton agar was allowed to cool to 5°C and dispersed on sterilized Petri-dishes, subsequently allowed to solidify. In agar well diffusion method (ditch method) on the other hand, a sterilized 6mm cock borer was used to make 5 wells of equal distance apart. 200 micro litres of each concentration was transferred into allocated wells using a 500 micro litres micro pipette. A concentration of 20% dettol (v/v) was used as positive control on the centre of each plate allocated to each extract. The test-organisms were inoculated in the media using spread method. The plates were covered and to stand for 30mins before being transferred to the incubator and incubated at 37°C for 24hours. After 24hours of incubation, Zones of clearance (milli metres) were observed. The diameter of zone of inhibitions was measured using a ruler. The results were

recorded and any zone of inhibitions greater than 6mm was considered susceptible.

RESULTS

Table 1.1: Preliminary Phytochemical Screening of *Ziziphus mucranata*.

Phytochemical	Dichloromethane (DCM)	Ethyl acetate	Methanol	Aqueous
Alkaloids				
Dragendroff's reagent	++	++	-	++
Mayer's Reagent	-	-	-	-
Wagner's Reagent	++	++	-	++
Steroid				
Liebermann's test	++	++	++	-
Salkowski Test	-	-	-	++
Saponins test	-	+	++	-
Cardiac glycoside	++	-	-	-
Flavonoid				
NaOH Test	-	-	-	-
Lead acetate test	-	-	-	+
Tannins	+	+	++	++
Anthraquinone	-	-	+	-
Phlobatannins	-	-	+	-

Key: + = present ++ = appreciable present - = absent

Table 4.2: TLC Results for aqueous, dichloromethane, ethyl acetate and methanol extracts of the leaves of *Ziziphus mucranata*

Extract	Solvent system	Number of components	Distance of spot (cm)	Solvent front (cm)	RF value
Aqueous	MeOH:H_2O(1:1) =($5cm^3$:$5cm^3$)	2	6.30cm	7.10cm	0.89
			5.70cm	7.10cm	0.80
Methanol	MeOH:Ethylacetate:DCM(1:2:3) =($2cm^3$:$4cm^3$:$6cm^3$)	10	7.70cm	8.00cm	0.96
			7.30cm	8.00cm	0.91
			6.00cm	8.00cm	0.75
			5.60cm	8.00cm	0.70
			5.30cm	8.00cm	0.66
			4.50cm	8.00cm	0.56
			3.10cm	8.00cm	0.39
			2.10cm	8.00cm	0.26
			1.1cm	8.00cm	0.14
			0.8cm	8.00cm	0.10
Ethyl acetate	MeOH:Ethylacetate :DCM(1:2:3) =($2cm^3$:$4cm^3$:$6cm^3$)	8	7.30cm	8.00cm	0.91
			6.30cm	8.00cm	0.79
			5.90cm	8.00cm	0.74
			5.50cm	8.00cm	0.69
			5.20cm	8.00cm	0.65
			4.70cm	8.00cm	0.59
			1.00cm	8.00cm	0.13
			0.50cm	8.00cm	0.06
DCM extract	MeOH:Ethyl acetate: DCM(1:2:3) =($2cm^3$:$4cm^3$:$6cm^3$)	5	7.50cm	8.00cm	0.94
			7.00cm	8.00cm	0.88
			5.70cm	8.00cm	0.71
			5.30cm	8.00cm	0.66
			4.80cm	8.00cm	0.60

Table 4.3: Zones of inhibition of *Staphylococcus and E. Coli* millimeter)

SN	Sample (Extract)	Test Organism	100mg	50mg	Control
1	Aqueous	*Staph*	25 + 25mm	00 00	30 + 28mm
2	Aqueous	*E.Coli*	20 + 20mm	00 00	15 + 15 mm
3	Methanol extract	*Staph*	11 + 11mm	00 00mm	30 + 30mm
4	Methanol extract	*E.Coli*	10 + 10mm	00 00mm	15 + 15mm
5	DCM extract	*Staph*	00 + 00mm	00 +00mm	26 + 30mm
6	DCM extract	*E.Coli*	00 + 00mm	00 + 00mm	15 + 17mm
7	Ethyl acetate	*Staph*	09 + 09mm	00 + 00mm	27 + 26mm
8	Ethyl acetate	*E.Coli*	10 + 10mm	00 + 00mm	14 + 14mm

Diameter (6mm) of the well was subtracted from the zone of inhibition

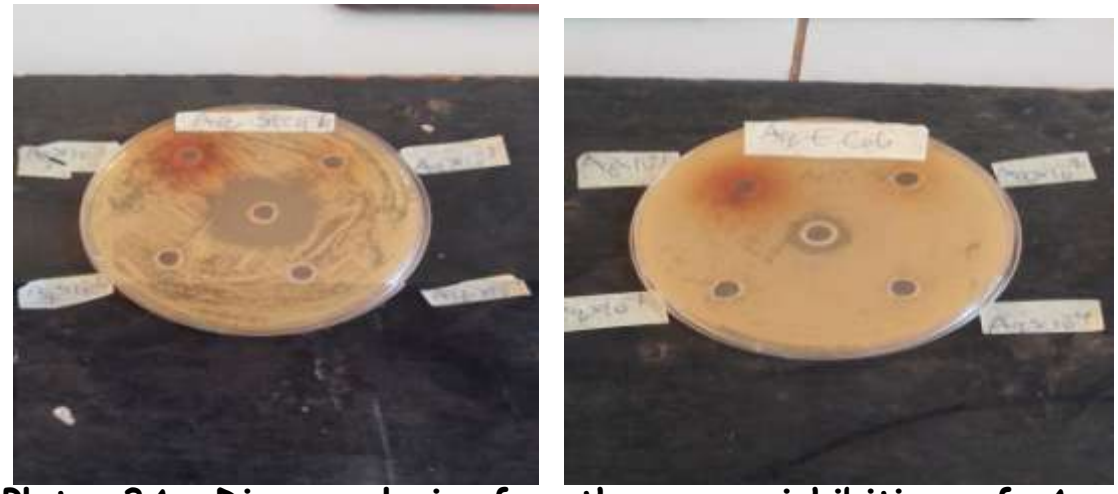

Plate 3A: Disc analysis for the zone inhibition of Aq extract against *Staphylococcus* and *E.Coli*.

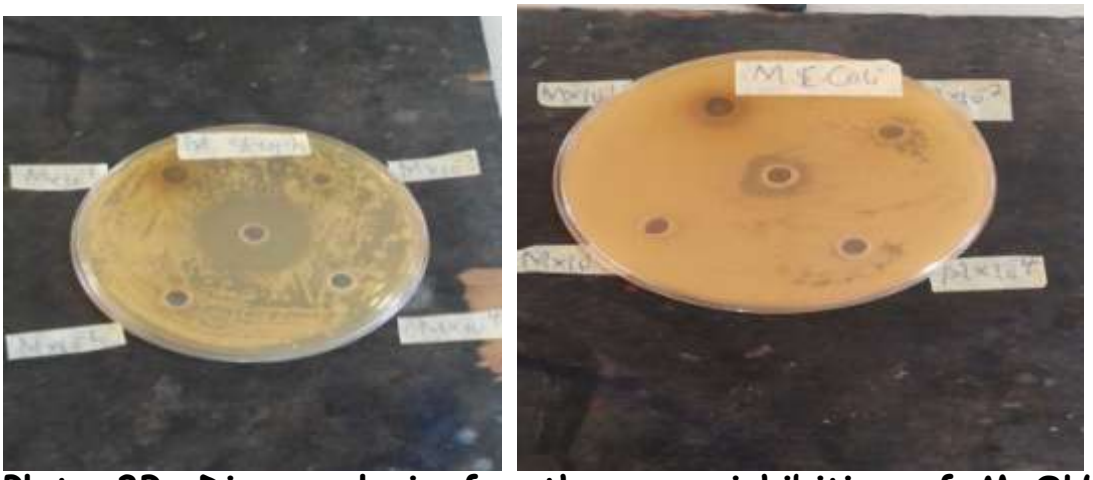

Plate 3B: Disc analysis for the zone inhibition of MeOH extract against *Staphylococcus* and *E.Coli*

Plate 3C: Disc analysis for the zone inhibition of Ethyl acetate extract against *Staphylococcus* and *E.Coli.*

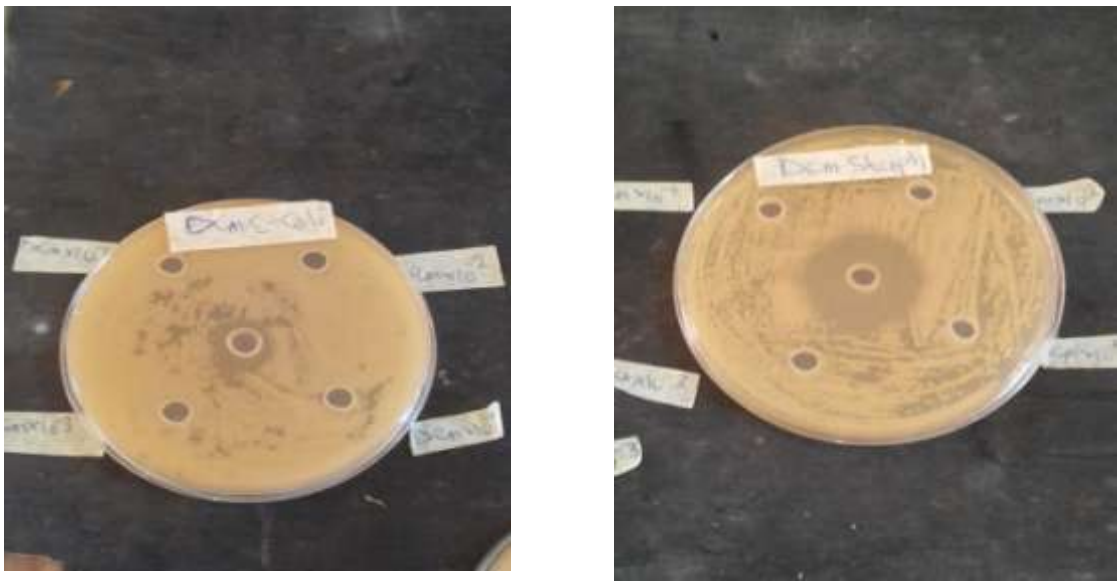

Plate 3D: Disc analysis for the zone inhibition of DCM extract against *Staphylococcus* and *E. Coli*

DISCUSSION

The preliminary Phytochemical screening of the leaves of *Ziziphus mucronata* indicates the presence of steroids and tannins in all the extracts (Aqueous (Aq), methanol (MeOH), Ethyl acetate and dichloromethane (DCM). Alkaloids were only detected in water (Aq), Ethyl acetate and DCM extracts. Flavonoids were detected in aqueous extract. Furthermore, saponins were present in MeOH and Ethyl acetate extracts. Anthraquinone and Phlabotannins were detected in MeOH extract, while cardiac glycosides found present only in DCM extract. The presence of these secondary metabolites which possess various pharmacological importance draw the attention for further study on the leaves so as to evaluate its medicinal usage. It is pertinent that Alkaloids and Steroids are presence in appreciable quantity as indicated in table 1.The phytochemical analyses illustrate natural plant products

with potentially useful pharmacological and nutraceutical activities as compounds such as sex hormones, antioxidants, antimicrobial, anti-inflammatory agents, antihistamines and health promoting agents. Thin Layer Chromatography was used to identify numbers of components presence in the sample extracts, in which two (2) spots were detected in the chromatogram clearly for aqueous extract using methanol (MeOH): water (H_2O) in the ratio of $5cm^3$:$5cm^3$as solvent system and the R_f values were found to be 0.89 and 0.80 respectively. Furthermore, ten (10) spots were detected in methanol extract using methanol (MeOH): Ethyl acetate: Dichloromethane (DCM) in the ratio of $2cm^3$:$4cm^3$:$2cm^3$as solvent system and the R_f values were found to be 0.96, 0.91, 0.75, 0.70, 0.66, 0.56, 0.39, 0.26, 0.14 and 0.10 respectively.
Ethyl acetate extract was detected to have eight (8) spots using methanol (MeOH): Ethyl acetate: Dichloromethane (DCM) in the ratio of $2cm^3$:$4cm^3$:$2cm^3$as solvent system and the R_f values were found to be 0.91, 0.79, 0.74, 0.69, 0.65, 0.59, 0.13, and 0.06 respectively. Dichloromethane extract on the other hand was detected to possesses five (5) spots using methanol (MeOH):Ethyl acetate: Dichloromethane (DCM) in the ratio of $2cm^3$:$4cm^3$:$2cm^3$as solvent system and the R_f values were found to be 0.94, 0.88, 0.71, 0.66 and 0.60 respectively as shown in table 2 and figure 2.
The percentage yield (%) of the extracts were quantified, whereby Aq, MeOH, Ethyl acetate and DCM extracts possessed 0.92%, 2.20%, 1.98% and 1.65% respectively. They were then subjected to anti-bacterial activity to ascertain their efficiency.

Table 4 shows zone of inhibition values of the active extracts on all the two tested microorganisms. The zone of inhibition

values obtained in this study from the plant extracts tested ranged from 100 to 50 mg/ml. The highest zone of inhibition value of 100 was observed for aqueous, methanol, ethyl acetate and DCM extracts from *ziziphus mucronata*, against staphylococcus and *E.Coli*. Aqueous crude extracts had the highest zone of inhibition values of 25mm and 20mm against *staphylococcus* and *E.Coli* respectively. It was then followed by methanol extract with values of 11mm and 10mm against *staphylococcus* and *E.Coli* respectively. Ethyl acetate had zone of inhibition values of 09mm and 10mm against *staphylococcus* and *E.Coli* respectively with no activity on DCM extract on both bacterial strains.

CONCLUSION

Leaves of *Ziziphus mucronata* reveal some degree of anti-bacterial activities against *staphylococcus* and E. coli and these activities may be traced to either alkaloids or steroids. These indicate that most of medicinal plants used in ethno medicine are potentially useful pharmacological and nutra ceutical in the treatment of some pathogenic microorganisms. This research may contribute to the clear understanding of antifungal activities of the plant. On account of this vital information therefore, there is need to call for thorough investigation to isolate and characterize the active compounds responsible for these and other unexplored activities.

REFERENCE

Abubakar, M.S., (2009): Practical Manual of Pharmacognosy and Ethnomedicine. Sokoto: Usmanu Danfodiyo University press pp.15 – 44.

Amusan, O.O., Sukati N.A., Hlophe, F.G. (2005). Herbal Remedies from Shiselweni Region of Swaziland. Phytotherapeutics, Rec. Prog. Med. Plants, **10**: 451-471.

Cherry M (1985). The Needs of the People in Plants for Arid Lands. Eds. G.E. Wickens, J.R. Goodin and D.V. Field. Unwin Hyman Ltd.,London, pp. 1-8.

El-Olemy, M.M., Almuhtadi, F.J. and Afifi, A.A (1994): Experimental Phytochemistry, a Laboratory Manual, King Saud University press Pp 8 – 9.

Etuk, E.u, Bello, S.O, Isezuo, S.A, Mohammad B.J. (2010).Ethnobotanical Survey of Medicinal Plants Used for the Treatment of Diabetes Mellitus in the North Western Region of Nigeria. *Asian J. Exp. Bio. Sci*. **1**:55-59

Farnsworth, N.R. & Soejereto, D.D.,(2009).USODE plants Medicinal Plants, Et O. Akerele V. Heywood & H. Synge (eds). 7425 Conservation of Medicinal Plants, Pp. 25-51.

Ganapathi, S.R., Kumar, D.S., Harami, A., Pathiban, M.P., Venkateshwarlu, G. (2011),Comparison Studies of Phytochemical Screening and Antibacterial Activities of Allium cepa Bulb and Allium sativum Bulb Extracts, Asian *Journal of Pharmaceutical and Health Sciences*. **1**(3): 133 – 134.

Harbone, J. B. (1984). Phytochemical Methods.A Guide ToMordern Techniques of Plant Analysis. 3rd Edition. Chapman and Hall London. Pp. 42-60.

Jawanda, J.S, and Bal J.S (1978). The berry, highly paying and rich in food value. Indian Hortic, Oct–Dec, pp. 19-21.

Olajuyigbe, O.O. and Afolayan, A.J., (2012). Antimicrobial potency of the ethanolic crude bark extract of Ziziphus mucronata Willd. subsp. mucronata Willd. *African Journal of Pharmacy and Pharmacology* **6**(10):724-730.

Sofowora, E.A. (1984), Medicinal plant and Traditional medicine in Africa, Wiley and Sons Chi Chester. pp 178.

Trease, C. and Evan, C.W. (1989): A Text book of Pharmacology 13th edition Brilliers Tindal Limited London pp 40-58.

WHO (1998) World Health Organization Guidelines for the Appropriate use of Herbal Medicines Pp 1- 88 Available online at http://apps.who.int/medicinedocs/en/d/Jh2945e/2.1.html

Reference to this paper should be made as follows: Garba,U et al.,(2019), Phytochemical and Anti Bacterial Activity of *Ziziphus Mucranata Leaves Extract. J. of Biological Science and Bioconservation,* Vol. 11, No. 2, Pp. 1 - 18