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Phytochemical, Antimicrobial, Elemental and Anionic Analysis of Methanol Leaf Extract of *Ziziphus mauritiana* LAM

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ABSTRACT

Ziziphus mauritiana is used as a medicinal plants especially its fruit and leaf. In this work the leaf of Z. *Mauritiana* was screened, investigated qualitatively for its Phytochemical constituent and antimicrobial study carried out using disc diffusion method on some selected microorganisms. Elemental and anionic contents were carried out to determine the concentration levels of Zn, Pb, K, SO_4^{2-}, Cl^- and PO_4^{3-}. The result of the phytochemical screening of the methanol leaf extractof *Z. mauritiana* revealed the presence of carbohydrates, tannins, saponins, cardiac glycoside and flavonoids. While alkaloids, terpenoids and phlobatannins were absent. The antimicrobial study revealed significant activity concentration-dependent at $P<0.05$ against *Bacillus subtilis, Corynebacteria* spp, *Staphylococcus aureus*, and *Candida albicans,* with highest zones of inhibitions of 14.50±0.71, 16.00±0.00, 18.00±0.00, and 14.50±0.71 respectively, although less effective than a standard drug (ciprofloxacine 500mg), While *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichiacoli*, *Klebsiella pneumonia* and *Streptococcus pyrogenes* are resistant to the extract. Elemental and anionic contents indicate concentration levels of Zn, Pb, K, SO_4^{2-}, Cl^- and PO_4^{3-} which were all within/below WHO/FAO permissible limits. Thus, the study has justified the traditional use of the plant for the management of microbial related diseases and could be safe and nutritious for consumption.

Keywords: *Ziziphus mauritiana*; Phytochemicals; Antimicrobial; Elements; Anions

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INTRODUCTION

The relationship between Man and plants has always been very intimate throughout the development of human culture. No doubt the herbalist is probably one of the first professionals in the evolution of human culture [1]. The natural remedies have recently gained enormous popularity in developed as well as developing countries because they are less toxic, less expensive and often more accessible than manufactured or chemical drugs. Interestingly, the World Health Organization has recommended the integration of traditional medicines that proved to be useful into the national health programs [2].

Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavour and cosmetic industries, thus helping the country earn foreign exchange [3]. Some of plants have antimicrobial and antifungal activities and are used for the treatment of different diseases in traditional[4].The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all countries have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes [5]. Plants are potent healers because they promote the repair mechanisms in the natural way. Plant based therapy not only accelerates healing process, but also maintains the aesthetics. More than 70% of wound healing pharma products are plant based, 20% are mineral based and remaining contains animal products as their base material. The plant base materials are used as first aid – antiseptic coagulants and wound wash. In recent times, focus on plant research has increased all over the world and large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5-year period6.Plants have been used for medicinal purposes for as long as history has been recorded.

The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [7], secondary metabolites grouped as alkaloids, glycosides, flavonoids, saponins, tannins and essential oils. Primary metabolites are compounds such as sugars, proteins and chlorophyll; Primary metabolites are directly involved in metabolic process while secondary metabolites are not involved directly and work as catalyst, saponins and tannins compounds possess abroad spectrum of pharmacological activities and are of a great economic value. The bioactive properties of some plants are attributed to the secondary or phytochemical compounds [8].

Ziziphus mauritiana is a fruit tree belonging to the family Rhamnaceae. English common names include; Chinese apple, Chinese date, Indian cherry, jujube [9]. It is called "Magarya"in Hausa language or "Huhue" in Bura among the northern people of Nigeria. It is widely grown in mild-temperate region and is adapted to warm climates. *Ziziphus mauritiana* can grow either as shrub let, shrubs or trees with thorny branches. *Ziziphus mauritiana* was first named as *Z. jujube*. Two varieties are recognized in india var. hysudrica. Which are wild or cultivated with large fruit, and var. fruitcosa, which is a small shrub in the sub-Himalayan tract, with small fruit. Numerous identifiable cultivars have also been developed for fruit production.

Ziziphus mauritiana is a plant claimed to have a lot of economic value such as medicinal and nutritional values. These claims have not been fully scientifically justified and information regarding its medicinal efficacies is scanty. This research and experiment is therefore centred on investigating, analysing and justifying the claims made on the plant (leaf). And also to know the chemical composition responsible for the medicinal value of these plant (leaf)

Figure 1: **Leaf of *Ziziphus mauritiana***

MATERIALS AND METHODS

Sample Collection and Identification

The leaves of *Z. mauritiana* were collected from the environ of Mashidimami Company in Maiduguri, Borno State. It was identified by a plant Botanist of the Department of Biological Science, Faculty of Veterinary Medicine University of Maiduguri, and then brought to the department of Chemistry for analysis.

Sample Preparation

The leaf was dried under shade for some days and was grinded using mortar and pestle.

Extraction of *Z. mauritiana* leaf

About 300g of the air dried powdered plant material was extracted exhaustively with 85%methanol in distil water using soxhlet extraction. The combined methanolic extract was concentrated to dryness on a tray. After dryness the extract was then subjected to qualitative phytochemical screening and *in-vitro* antimicrobial susceptibility.

Elemental Content Analysis

Sample Digestion and Preparation for Analysis

The air-dried plant sample was pulverized manually in a wooden mortar and pestle into a coarse powder. 0.5 g of each sample was independently packed into an acid-washed porcelain crucible and then placed in a muffled furnace for 4 hr at 550^{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_3 was later added, and 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 [10].

Elemental Analysis

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 [10] for plant samples.

Determination of Sulphate (SO_4^{2-})

The spectrophotometer was calibrated using distilled water. To 10ml of sample water, 0.1g of sulfate reagent was added and scanned using smart spectrophotometer. The result was recorded.

Determination of Phosphate (PO_4^{3-})

The spectrophotometer was calibrated using 10ml of sample water. It was removed and 1.0ml pipette was used to add 1ml of phosphate acid reagent, it was then capped and mixed. Using 0.1g spoon 1ml of phosphate reducing agent was added, capped and shaken to mix until powder dissolves. After for 5 minutes full colour develops, solution turns blue.

Determination of Chlorine (Cl^-)

To a clean test tube 10ml sample water was added, using 0.1g spoon 0.1g of DPD powder was added, it was capped and gently mixed until powder dissolved completely and solution turns red. To a direct reading titrator filled with ferrous ammonium sulfate, it was titrated using previously prepared solution by inserting the tip in the solution and withdrawing the plunger. While gently swirling tube, sample is titrated with ferrous ammonium sulfate color changes from red to colorless. Result was read directly from titrator scale as ppm free chlorine. The cap and titrator was carefully removed and one chlorine DPD tablet was added. It was capped and mixed until tablet dissolves. Then titration continues until color changes from red to colorless. Result was read directly from titrator scale and recorded as ppm total residual chlorine. Combined chlorine was determined by subtracting free available chlorine from total residual chlorine, and record as ppm combine chlorine.

Combined Chlorine (ppm) = Total Residual Chlorine – Free Available Chlorine.

Preliminary Phytochemical Screening

The extract of the *Ziziphus mauritiana* screened qualitatively for phytochemical constituents using standard procedures [11].

Antimicrobial Studies

Test Organisms

Nine organisms were considered for this study, bacteria and fungi. The Gram positive bacterial were *Bacillus subtilis, Staphylococcus aureus, and Streptococcus pyogenes*. While the Gram negative bacteria were *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas*

aeruginosa. The fungi species was *Candida albicans.* The bacteria were clinical isolates obtained from the Department of Medical Microbiology and Department of Veterinary medicine, University of Maiduguri, Maiduguri-Nigeria.

Preparation of Nutrient Agar

The nutrient agar used was general purpose media for the growth of microorganism. 28g of the nutrient agar powder was suspended in 1000ml of distilled water. Heated to boiling to dissolve the medium completely, sterilized with autoclave at (120ºC) for 15 minutes, cooled to 45-50^{0}C and was poured on the petri dish waiting inoculation.

Disc Diffusion Technique

The method adopted was that of Volleková *et al*. [12] which was later modified by Usman *et al.* [13]. The test was carried out using a stock concentration of 400mg/ml, 200mg/ml, and 100mg/ml by dissolving 1g, 2g, and 4g of the crude extract in 5ml of methanol respectively. Three holes were bored in each plate (6mm) using sterile cork-borer. The isolated organisms were introduced into the petri dish differently as labeled using poured plate method. About 0.2ml of the extract solution was inoculate across the well and incubated overnight at 37^{o}C for 24 hrs. After incubation, the average diameter of three readings of the clear zone surrounding the hole was taken as the measure of the inhibitory level of the plant extract against the bacteria on test and recorded as mean ± SD.

RESULTS

Extraction profile of *Ziziphus mauritiana* Leaf

Extraction profile of *Z. Mauritiana*s hown in Table 1 presents the % yield of the methanol leaf extract with a dark green colouration and gummy texture.

Phytochemical Screening of the Leaf Extract of *Ziziphus mauritiana*

The phytochemical screening of *Z. mauritiana* revealed the presence of carbohydrates, tannins, saponins, Cardenolides, cardiac glycosides and flavonoids. While alkaloids, terpenoids and phlobatannins are absent. Table 2 shows the result for the phytochemical analysis.

Antimicrobial Study of Methanol Leaf Extract of *Ziziphus mauritiana*

The activity of the extract was tested against eight different bacteria namely; *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes*, *Bacillus subtilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa, Salmonella typhi, Corynebacteria* spp. The fungi species was *Candida albicans.* Table 3 shows the results for the antimicrobial tests.

Elemental and Anionic Analysis Result

Elemental and anionic contents indicate the presence of zinc, lead, potassium, sulphate, chloride and phosphate in different concentration with chloride having the highest concentration of 93.0 mg/l and the lowest is lead with a concentration of -0.03.

Table 1: Extraction profile of *Ziziphus mauritiana* leaf

Plant part	Weight of extract (g)	% yield	Colour	Texture
Leaf	16.0	**5.**33	Dark Green	Gummy

Table 2: Phytochemical analysis of methanol leaf extract of *Ziziphus mauritiana*

S.N.	Tests	Code
1	**Carbohydrates**	
a.	Molish's Test	_
b.	Barfoed's Test	**+**
c.	Fehling's Test	**+**
d.	Test for Combined Sugar	**+**
2	**Tannins**	
a.	Ferric chloride	**+**
b.	Lead ethanoate	**+**
3	**Phlobatannins**	

4	**Anthraquinones**	
a.	Free Anthraquinones (Borntrager's Test)	–
b.	Combined Anthraquinones (Borntrager's Test)	–
5	**Cardiac Glycosides**	
a.	Salkowski's Test	**+**
b.	Liberman	–
6	**Saponins**	+
7	**Flavonoids**	
a.	Shinoda's Test	–
b.	Ferric Chloride Test	–
c.	Sodium hydroxide test	**+**
d.	Lead ethanoate test	**+**
8	**Alkaloids**	
a.	Dragendoff's Test	–
b	mayer's Test	–
9	**Terpenoids**	–
10	**Cardenolides**	
a.	Keller killiani's test	+
11	**Soluble Starch**	–

KEY: The positive sign (+) represents presence while the negative sign (-) represents not detected.

Table 3: Antimicrobial Activity of methanol leaf extract *Z. mauritiana*

Micro-organisms	Concentration (mg/ml)			
	400	**200**	**100**	**Cip (5mg)**
	Zone of inhibition (mean±SEM)			
Corynebacteria. Spp	16.00±0.00	13.00±0.00	10.50±0.71	–
S. aureus	18.00±0.00	15.00±0.0	12.50±0.71	22.67±0.58
S.pyogenes	0.00±0.00	00.00±0.00	00.00±0.00	24.67±0.58
B.subtilis	14.50±0.71	12.00±0.00	10.00±0.00	30.00±0.00
E. coli	0.00±0.00	0.00±0.00	0.00±0.00	20.00±0.00
P.aeruginosa	0.00±0.00	0.00±0.00	0.00±0.00	49.0±1.0
S. typhi	0.00±0.00	0.00±0.00	0.000±0.00	44.33±1.5
K. pneumonia	0.00±0.00	0.00±0.00	0.00±0.00	40.00±0.00
C.albicans	14.50±0.71	10.00±0.00	7.50±0.71	24.00±0.00

KEY: Data are presented as Mean ± SEM; *C. spp= Corynebacteria specie; E.coli = Escherichia coli; S.typhi= Salmonella typhi; S.aureus= Staphylococcus aureus; S.pyogenes= Streptococcus pyogenes; K. pneumonia= Klebsiella pneumonia*

Table 4: Elemental and anionic analysis Result of *Z. Mauritiana* leaf

Contents	Concentration in mg/L	W.H.O Standard (mg/kg)
Zinc(Zn)	0.18	0.60
Lead (Pb)	0.00	2.00
Potassium (K)	0.26	1.0
Phosphate (PO_4^{3-})	0.03	0.40
Chloride (Cl^-)	93.0	250.00
Sulphate (SO_4^{2-})	26.0	30.00

DISCUSSION

The result of the phytochemical screening of the methanol leaf extract of *Z. mauritiana* revealed the presence of carbohydrates, tannins, saponins, cardiac glycoside and flavonoids. While alkaloids, terpenoids and phlobatannins are absent.

The presence of these chemical components indicates that this plant has some medical properties according to literatures [14]. The findings of this study agree with Najafi [15] who reported that leaf of *Ziziphus mauritiana* has saponins, phenolic compounds, tannins and glycosides. Parmar *et al.* [16] stated that leaves of *Ziziphus mauritiana* contain glycosides, saponins, phenols, lignins and tannins. Plants rich in saponins have anti-inflammatory activity and strengthen the immune system, Tannins are antibacterial compounds which damage the bacterial cell wall Mainasara *et al.* [17].

Flavonoids detected have been known to provide protection against oxidative stress induced diseases and are major responsible for anti-oxidative activity of the leaf. Saponins have antifungal properties. Flavonoids, tannins, saponins and glycosides are good antioxidant compounds and controls the oxidative stress related disorders [18]. These contents show different type of activity against different pathogens. Therefore, it can be used in the treatment of diseases. The methanol leaf extracts of *Ziziphus mauritiana* exhibited significant activity against *Bacillus subtilis, Corynebacteria* spp and *Staphylococcus aureus*, also the fungi specie *Candida albicans.* There is no activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichiacoli*, *Klebsiella pneumonia* and *Streptococcus pyrogenes.*

Elemental and anionic contents indicate the presence of zinc, lead, potassium, sulphate, chloride and phosphate; 0.18, 0.00, 0.26, 0.03, 93.0, 26.0 mg/kg respectively. Zinc is important for growth and for the development and health of body tissue. Chloride maintains fluid balance in the body.

CONCLUSION

The methanol leaf extract of *Ziziphus mauritiana* contains pytochemicals such as carbohydrates, tannins, saponin, cardenolides, cardiac glycoside and flavonoids; it also has activity against some microorganisms such as *Bacillus subtilis, Corynebacteria* spp, *Staphylococcus aureus*, and *Candida albicans.* The activity could be due to the presence of the phytochemicals. Elemental and anionic contents indicate concentration levels of Zn, Pb, K, SO_4^{2-}, Cl^- and PO_4^{3-} which were all within WHO/FAO permissible limits. Thus, the study has justified the traditional use of the plant for the management of microbial related diseases.

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