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Comparative Phytochemical Analysis of *Ziziphus mauritiana* Lam, *Ziziphus mucronata* Willd. and *Ziziphus spina-christi* (L.) Desf.

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Abstract
The three Ziziphus species namely *Ziziphus mauritiana, Ziziphus mucronata and Ziziphus spina-christi* (all belong to the Rhamnaceae family) are used by traditional medicine men in Yobe state to treat various medical conditions. The present study was undertaken in order to determine the presence of some major phytochemicals in the roots and leaves of the three Ziziphus species and to compare the distributions profile according to polarity.
The root and leaf samples were dried under shade in the laboratory at ambient temperature, pulverised and extracted with n-hexane, ethyl acetate and methanol using Soxhlet method. The extracts were concentrated in vacuo and percent yield determined.. Results showed that there is no significant difference in the extraction yields (0.71 to 1.32%) and the main phytochemicals found in all plant species were alkaloids, cardiac glycosides, flavonoids, reducing sugars, saponins, steroids, tannins and terpenoids. Comparative studies on the distribution of these phytochemicals (except for reducing sugars which were detected in all samples analysed) has revealed species variations in phytochemicals especially in the n-hexane and ethyl acetate crude extracts of the roots and leaves of the three plants. The types of secondary metabolites detected in both *Z. mucronata* and *Z. Mauritiana* put together almost matched those found in *Z. spina-christi*. This species' difference can be exploited in the treatment of various diseases and ailments.

Keywords: *Ziziphus mauritiana, Ziziphus mucronata and Ziziphus spina-christi,* Rhamnaceae, phytochemicals, secondary metabolites and screening

Introduction
Plants are continuously used as important sources of food (to support growth, development, reproduction, etc.) and medicine for the prevention of illness and maintenance of human and veterinary health. The plants used in traditional medicine practice come in many forms including herbs, shrubs, climbers, grasses and trees (Joy *et al*, 2001) [14]. Different parts of these plants, like the leaves, roots, stem barks, flowers, seeds, etc. are being used for the treatment of ailments and diseases affecting the respiratory system, gastrointestinal system, urinary system and many other metabolic and non-metabolic disorders (Umar and Babalola, 2014) [22]. These researchers have also reported that some traditional medicine men in Yobe State use different parts of *Ziziphus mucronata*, *Ziziphus mauritiana* and *Ziziphus spina-christi* in treating the same disease or entirely different diseases. For instance the seeds and fruits of *Z. mucronata* and the leaves of *Z. spina-christi* are used in the treatment of ring worms while the leaves of *Z. mauritiana* is employed for treating toothaches, mouth sores and wound healing; and also used as blood tonic.
Other workers have earlier reported the use of the three *Ziziphus* species for the treatment of some diseases, such as digestive disorders, weakness, liver complaints, obesity, urinary problems, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anaemia, diarrhoea, and insomnia (Han and Park, 1986; Kirtikar and Basu, 1984) [9, 16]. Their leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu *et al*., 2001) [4]. The seeds are sedative and are taken sometimes with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy (Kaaria, 1998) [15]. They are also used to treat respiratory ailments and fevers and to promote the healing of fresh wounds, for the treatment of dysentery (Abalaka *et al*., 2010) [1].
These uses and many others not mentioned here including those yet to be yet to be identified may be attributable to some bioactive metabolites with variable activities that may be present in the these species. From literature generally the *Ziziphus* species are reported to contain cyclopeptide and isoquinoline alkaloids, flavonoids, terpenoids and their glycosides (Asgarpanah and Haghighat, 2012) [5]. On basis of individual species Abalaka *et al*. (2011) [2] and Ads, Rajendrasozhan, Hassan, Sharawy and Humaidi (2017) [3] have reported the presence of five different phytochemicals, namely cardiac glycosides, polyphenols, resins, saponins tannins and terpenoids in *Z. spina-christi* L. Jain *et al*, (2012) [12] have also reported the presence of alkaloids, flavonoids, glycosides, phenol, lignins, saponins, sterols and tannins in *Z. Mauritiana*. Information on the phytochemical composition of *Z. Mucronata* as reported by Ngaradoum *et al*., 2017, includes the presence of flavonoids, glycosides, saponins, steroids and tannins. It was based on the fact that these *Ziziphus* species are used to treat many diseases (in traditional medicine) that the present study was undertaken to analyse the phytochemical composition of the three Ziziphus species and to compare their distributions among the species.

Materials and Methods
Sample collection and preparation
Fresh samples of leaves and roots of *Ziziphus mucronata*, *Ziziphus mauritiana* and *Ziziphus spina-christi* were collected within and around the Yobe State University

Damaturu campus in the month of October, at the end of the rainy season when samples are in abundance. The plants were identified by experts in the Ministry of Environment, Biology Department of the University and confirmed by the traditional healers. Both roots and leaves were washed with water and allowed to dry to constant weight under shade at ambient laboratory temperature. The dried materials were then ground to coarse and then to fine powder using local wooden mortar and pestle.

Successive solvent extractions were carried out on 450g (in three equal batches) of the powdered samples using the Soxhlet method beginning with n-hexane, ethyl acetate and finally methanol (all are products of Loba Chemie PVT Ltd, Mumbai India). The resulting extracts were filtered with Whatman No. 1 filter papers (Whatman International Ltd, Maidstone, England) and the volume was reduced to about 50ml using rotary evaporator (Medica Instruments Mfg Co, Berlin Germany). The concentrates were then transferred into clean, dry and weighed evaporation basin and finally dried to constant weight in a thermostatic oven (Model DNG 9202, OHAUS, USA) maintained at 35ºC.

Phytochemical Screening Tests

The Phytochemical Screening of the three *Ziziphus species'* extracts were performed using an adaptation of the standard procedures outlined by Harborne (1973) [10], Sofowora (1993) [20] and Trease and Evans (2005).

Test for Alkaloids

Dragendorff's Test: A small amount of the dried crude extract (0.2g) was dissolved in 1 ml of methanol in a test tube and then 2 - 3 drops of Dragendorff's reagent was added. The appearance of orange-red precipitate was taken as positive test for the presence of alkaloids.

Mayer's Test: another 0.2g of the crude extract was taken in 1 ml of methanol and 1 ml of Mayer's reagent was added. The development of dull white precipitate indicated the presence of alkaloids.

Test for Flavonoids

Alkali Test: About 0.2g of the dried crude extract in 1 ml of methanol was treated with 1 ml of 10% NaOH. The appearance of yellow colouration which disappeared upon addition of dilute acid indicated the presence the presence of flavonoids.

Test for Saponins

Frothing Test: About 1g of the crude extract was boiled in 5 ml of distilled water and filtered into a boiling tube. Additional 3 ml of water was added to the filtrate and shaken vigorously for 5 minutes. The appearance of froth which persisted on warming was taken to indicate the presence of saponins.

Test for Steroids

*Chloroform Test***:** 2 ml of chloroform is added to 2 ml extract followed by same quantity (2 ml) of concentrated H_2SO_4, Formation of red colour in chloroform layer shows the presence of steroids

Test for Tannins

Ferric chloride test: A solution of 0.2g of the crude extract in 1 ml of methanol was treated with 2 ml of distilled water followed by 2 - 3 drops of fresh 2% ferric chloride solution. The appearance of blue-green colour was indicative of the presence of tannins.

Test for Terpenoids

Chloroform test: To 0.2g of the extract dissolved in 1ml of methanol, 2ml of chloroform was added followed by few drops of conc. H_2SO_4 were added carefully along the side of the tube. The appearance of a reddish-brown colouration at the interface between chloroform and conc. sulphuric acid layers indicated the presence of terpenoids.

Test for Glycosides

Ferric chloride test: To 0.2g of the extract dissolved in 1ml of methanol was added1ml of ferric chloride solution (prepared by mixing 1 volume of 5% ferric chloride, w/v and 99 volumes of glacial acetic acid). Then few drops of conc. sulphuric were added carefully. The appearance of greenish blue colour was taken to indicate the presence of glycosides.

Test for Reducing Sugars

Fehling's Test: Fehling's solutions A and B were prepared fresh according to the methods outlined by Harborne (1973) [10] Sofowora (1993) [20] and Jain (2011). Equal volumes of Fehling's solutions A and B were mixed when required for use. Two millilitres (2ml) of this mixture was added to 0.2g of the extract and boiled gently. The appearance of a brick red precipitate was taken to indicate the presence of reducing sugars.

Results

Three solvents of different polarities namely, n-hexane, ethyl acetate and methanol were used to successively extract phytochemicals from the root and leaf samples of three Ziziphus species according to increasing polarity, (nonpolar, semi-polar and polar respectively). The results from the extraction yields (Table 1) indicated that low amounts of crude non-polar, semi-polar and polar phytochemicals were generally found in *Z. mucronata, Z. mauritiana and Z. spina-christi* roots and leaves. The calculated percentage yields for the three species ranged from 0.71% to 1.32% of the total 450g of samples extracted. The methanolic extracts of both roots and leaves generally showed relatively higher percentage yields (1.2 - 1.3% and 0.97 - 1.32% respectively) as compared to the n-hexane and ethyl acetate extracts.

The extracts came in various colours and appeared to show some kind of pattern. For instance, n-hexane root extracts of the three *Ziziphus* species were all pale yellow in colour whereas the corresponding leaf extracts were dark green except for *Z. Spina-christi* that appeared yellow. These findings were taken to indicate the presence of different types of non-polar, semi-polar and polar phytochemicals in the roots and leaves of the three *Ziziphus* species studied. The importance of this finding is not clear at this moment but comparative phytochemical analyses of the extracts had revealed the presence of some different secondary metabolites (Table 2). These include alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids and reducing sugars. The distributions of these compounds in roots and leaves are described below.

The Roots

N-Hexane crude root extracts

The results for the comparative study are shown in Table 3. It revealed that crude non-polar alkaloids, saponins and terpenoids were not detected in all the n-hexane root extracts of the three *Ziziphus* species screened. On the other hand non-polar glycosides and tannins were detected only in the crude root extracts of *Ziziphus mucronata;* and non-polar flavonoids and steroids were present only in roots of *Ziziphus spina-christi*.

Ethyl acetate crude root extracts

The ethyl acetate crude extracts (containing semi-polar components) of the roots (Table 3) showed a different pattern to those of the non-polar components in that alkaloids and terpenoids were detected in the three species investigated. Tannins and cardiac glycosides were detected in the semi-polar root extracts of *Z. mucronata* and *Z. spina-christi* but not in *Z. mauritiana*; flavonoids and steroids were detected in *Z. mauritiana and Z. spina-christi* but not in *Z. Mucronata;* and interestingly saponins were found only in *Z. Mucronata.* It is also interesting to note that within limits of phytochemicals studied the types of compounds found in both *Z. mucronata* and *Z. Mauritiana* put together matched those found in *Z. spina-christi*, as shown below:

a. *Z. mucranata*: alkaloids, glycosides, reducing sugars, saponins, tannins and terpenoids.
b. *Z. mauritiana*: alkaloids, flavonoids, reducing sugars, steroids and terpenoids.
c. *Z. spina-christi*: alkaloids, flavonoids, glycosides, reducing sugars, steroids, tannins and terpenoids.

The only exception to this generalization is the presence of saponins in *Z. mucronata.* These results showed differences in the distribution of phytochemicals in the root extracts of the three Ziziphus species.

Methanol Crude Root Extracts

The crude methanolic root extracts of the three Ziziphus species, on the other hand, showed the presence of all the phytochemicals analysed (Table 3). Going by the intensity of the colour reaction, one can assume that they are available in varying concentrations. Based on this assumption for example, *Z. mauritiana* and *Z. spina-christi* are richer in flavonoids and steroids; more glycosides and saponins in *Z mucronata* and more alkaloids in *Z. mauritiana*. The results of this study suggested that species similarities and differences existed in the presence and distribution of the phytochemicals studied in the roots of the three *Ziziphus* species.

The leaves

The leaves of the three Ziziphus species were also extracted in identical manner to those of the roots in order to reveal the presence and distribution of non-polar, semi-polar and polar phytochemicals. The results of the screening tests were presented in Table 2. The distributions of phytochemicals detected are also given in Table 3 for comparative purposes. It indicated that glycosides, saponins, tannins and terpenoids were not detected in all the n-hexane extracts of the three Ziziphus plants studied. Non-polar alkaloids and flavonoids were detected only in *Z. spina-christi* leaves and steroids were found only in *Z. mauritiana* thus indicating a different distribution pattern to the corresponding root extracts. More striking differences were detected in the ethyl acetate extracts of the three plants studied. Thus semi-polar alkaloids, flavonoids and reducing sugars were detected in the three species and saponins were not detected in any of them. Furthermore, tannins were detected in *Z. mucronata* and *Z. spina-christi* and not in *Z. mauritiana*. Terpenoids were present in *Z mucronata* only. These findings represent differences in the distribution of the non-polar and semi-polar phytochemicals in the three Ziziphus trees investigated.

The methanolic extracts of the leaves showed the presence of all the phytochemicals tested except for *Ziziphus spina-christi* in which polar saponins were not detected indicating another point of species difference. In addition saponins were not detected in the crude leaf extracts of the three solvents used in the extraction. It should be also recalled that saponins were found in the methanolic root extracts of *Z. spina-christi* (Table 3). Based on the intensity of the colours produced during the screening exercises, it can speculate that the amounts of these phytochemicals, (especially alkaloids, flavonoids and reducing sugars) may differ among the species. Similar analogy may be extended to glycosides in *Z. mucronata*, steroids in *Z. mauritiana* and tannins in *Z. mucronata* and *Z. spina-christi.*

Total number of phytochemicals extracted

The numbers of different types of phytochemicals extracted by the three solvents were also compared as presented in Table 4. It showed that the number of phytochemicals extracted by the three solvents was methanol > ethyl acetate > n-hexane. In both the roots and leaves methanol extracted about half of the total number of secondary metabolites thus indicating that the three Ziziphus plants studied contained more polar compounds than the other two polarities.

Table 1: The Extraction Yields of phytochemicals Extracted with n-Hexane, Ethyl Acetate and Methanol from the roots and leaves of *Z. Mucronata, Z. mauritiana* and *Z. Spina-christi*

Plant Spp	Plant Part	Solvent	Wt of Sample (g)	Wt of Extract (g)	Percent Yield (%)	Colour of Extract
Z. mucron.	Root	n-Hexane	450	3.34	0.74	Yellowish
Z. maurit.	Root	n-Hexane	450	3.32	0.72	Yellowish
Z. spina-c.	Root	n-Hexane	450	3.1	0.71	Yellowish
Z. mucron.	Root	Ethyl Acetate	450	3.54	0.79	Yellow
Z. maurit.	Root	Ethyl Acetate	450	4.1	0.91	Yellow
Z. spina-c.	Root	Ethyl Acetate	450	4.15	0.95	Yellow
Z. mucron.	Root	Methanol	450	5.41	1.2	Red
Z. maurit.	Root	Methanol	450	5.79	1.29	Red
Z. spina-c.	Root	Methanol	450	5.8	1.3	Yellow

Z. mucron.	Leaf	n-Hexane	450	3.23	0.72	Dark green
Z. maurit.	Leaf	n-Hexane	450	3.24	0.82	Dark green
Z. spina-c.	Leaf	n-Hexane	450	3.33	0.74	Yellowish
Z. mucron.	Leaf	Ethyl Acetate	450	4.1	0.91	Black
Z. maurit.	Leaf	Ethyl Acetate	450	3.9	0.89	Black
Z. spina-c.	Leaf	Ethyl Acetate	450	4.17	0.97	Yellow
Z. mucron.	Leaf	Methanol	450	5.79	1.29	Black
Z. maurit.	Leaf	Methanol	450	4.23	0.97	Black
Z. spina-c.	Leaf	Methanol	450	5.9	1.32	Yellow

Table 2: Phytochemical Screening of n-Hexane, Ethyl Acetate and Methanol Extracts of *Z. Mucronata*, *Z. mauritiana* and *Z. Spina-christi*.

S/No	Phytochemical	Root Extracts			Leaf Extracts		
		n-Hexane	Ethyl Acetate	Methanol	n-Hexane	Ethyl Acetate	Methanol
Ziziphus mucronata							
1	Alkaloids	–	+	+	–	+	++
2	Flavonoids	–	–	+	–	+	++
3	Glycosides	+	++	++	–	+	++
4	Red. Sugars	++		++	++	++	++
5	Saponins	–	++	++	–	–	+
6	Steroids	–	–	+	–	–	+
7	Tannins	+	+	+	–	+	++
8	Terpenoids	–	+	+	–	+	+
Ziziphus mauritiana							
1	Alkaloids	–	+	++	–	+	++
2	Flavonoids	–	+	++	–	+	++
3	Glycosides	–	–	+	–	+	+
4	Red. Sugars	++	++	++	++	++	++
5	Saponins	–	–	+	–	–	+
6	Steroids	–	+	++	+	+	++
7	Tannins	–	–	+	–	–	+
8	Terpenoids	–	+	+	–	–	+
Ziziphus spina-christi							
1	Alkaloids	–	+	+	+	+	++
2	Flavonoids	+	+	++	+	+	++
3	Glycosides	–	+	+	–	–	+
4	Red. Sugars	++	++	++	++	++	++
5	Saponins	–	–	+	–	–	–
6	Steroids	+	+	++	–	–	+
7	Tannins	–	+	+	–	+	++
8	Terpenoids	–	+	+	–	–	+

Table 3: Comparison of Phytochemicals Extracted by n-Hexane, Ethyl Acetate and Methanol from *Z. Mucronata*, *Z. mauritiana* and *Z. spina-christi*.

Ziziphus Species	Phytochem. Tested	Root Extracts Phytochemicals			Leaf Extracts Phytochemicals		
		n-Hexane	Ethyl Acetate	Methanol	n-Hexane	Ethyl Acetate	Methanol
mucronata	Alkaloids	–	+	+	–	+	++
mauritiana	,,	–	+	++	–	+	++
spina-christi	,,	–	+	+	+	+	++
mucronata	Flavonoids	–	–	+	–	+	++
mauritiana	,,	–	+	++	–	+	++
spina-christi	,,	+	+	++	+	+	++
mucronata	Glycosides	+	++	++	–	+	++
mauritiana	,,	–	–	+	–	+	+
spina-christi	,,	–	+	+	–	–	+
mucronata	Red. sugar	++	++	++	++	++	++
mauritiana	,,	++	++	++	++	++	++
spina-christi	,,	++	++	++	++	++	++
mucronata	Saponins	–	+	++	–	–	+
mauritiana	,,	–	–	+	–	–	+
spina-christi	,,	–	–	+	–	–	–
mucronata	Steroids	–	–	+	–	–	+
mauritiana	,,	–	+	++	+	+	++
spina-christi	,,	+	+	++	–	–	+
mucronata	Tannins	+	+	+	–	+	++
mauritiana	,,	–	–	+	–	–	+
spina-christi	,,	–	+	+	–	+	++

mucronata	Terpenoids	–	+	+	–	+	+
mauritiana	„	–	+	+	–	–	+
spina-christi	„	–	+	+	–	–	+

Table 4: Comparison of the number of different types of phytochemicals extracted by n-Hexane, ethyl acetate and methanol extracts of *Z. Mucronata*, *Z. mauritiana* and *Z. Spina-christi*.

Ziziphus Species	Root Extracts			Total	Leaf Extracts			Total
	n-Hexane	E. Acetate	Methanol		n-Hexane	E. Acetate	Methanol	
mucron.	3	6	8	17	1	6	8	15
Maurit.	1	5	8	14	2	5	8	15
Spina-c.	3	7	8	18	3	4	7	14

Discussion

There is a wide usage and dependence on medicinal plants for primary health care worldwide, which motivated the World Health Organization (WHO) to encourage the use of medicinal plants in primary health care (Shoeb, 2006). The presence of various phytochemicals in the three Ziziphus species may be a major factor for their use in traditional medicine. Most of these constituents are potent bioactive compounds found in medicinal plant parts which may serve as precursors for the synthesis of useful drugs (Sofowora, 1993) [20]. These secondary metabolites are reported to have varied uses such as antimicrobial and other physiological activities (Sofowora, 1980) [20]. In specific terms the three Ziziphus species of *Ziziphus mucronata*, *Ziziphus mauritiana* and *Ziziphus spina-christi* are used in traditional medicine for the treatment of many diseases such as digestive disorders, body weakness, liver complaints, obesity, urinary problems, diabetes, skin infections, wound healing, loss of appetite, fever, pharyngitis, bronchitis, anaemia, diarrhoea, and insomnia among others (Han and Park, 1986; Kirtikar, Basu, 1984, Orwa *et al*, 2009; (Abalaka *et al*., 2010). and Umar & Babalola, 2014) [22].

As a consequence of the numerous shortcomings of the semi-synthetic and synthetic drugs coupled with their high costs, many people in rural communities and urban areas of Nigeria (including Yobe State) have heeded to the call of the WHO (Shoeb, 2006) in patronizing the services of traditional medicine men. At least in Yobe State, this traditional system of medication is very popular but it is not well organised or backed by scientific knowledge and standardized procedures. The present study was carried out to throw more light on the presence or absence of some important phytochemicals in the three Ziziphus species and to determine the nature of their distribution. It has presented evidence for the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids and reducing sugars in the three Ziziphus species. Although there is limited data on the phytochemical compositions of the three Ziziphus plants, the results of this study compares with previous works on *Z. spina-christi* leaves by Dangoggo *et al*, (2012) [7] and Jain *et al* (2012) [12] on *Z. mauritiana*.

However, in this study's comparative analysis on the presence and distribution of these phytochemicals (except for reducing sugars which were detected in all samples analysed) has revealed marked species variations especially in the n-hexane and ethyl acetate crude extracts of the roots and leaves of the three plants. It is also interesting to note that within limits of compounds studied, the types of secondary metabolites detected in both *Z. mucronata* and *Z. Mauritiana* put together almost matched those found in *Z. spina-christi*. This species can be exploited in the treatment of various diseases and ailments. For example where saponins are not required as active ingredients, *Z. spina-christi* can be used in place of either *Z. mucronata* or *Z. mauritiana.* The issue of identifying the components with bioactive activities will be left for future investigations on antimicrobial, anti-inflammatory, anticancer, etc. activities of the various crude and fractionated components. However, a review carried out by Asgarpanah and Haghighat, (2012) [5] on *Z. spina-christi* has shown the plant to exhibit antibacterial, antifungal, antioxidant, anti-hyperglycaemic, and antinociceptive activities. They reported the presence of only flavonoids, alkaloids and saponins from this plant previously. There is the need for further fractionation of extracts and to carry out studies especially on the antioxidant and antimicrobial activities as well as determining other factors responsible for the therapeutic benefits of these *Ziziphus* plants.

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