ELEMENTAL ANALYSIS AND COMPARISON OF THE WATER/ ETHANOL ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM THE FRUITS OF *Ziziphus mucronata*

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Abstract

The elemental, phytochemical analysis and antibacterial comparison of Water and Ethanol extracts of the fruits of Ziziphus mucronata used locally for treatment of certain diseases in humans/animals was investigated. The elemental analysis showed the presence of some mineral elements: Cu, Ca, K, Mg, Na and Mn. The phytochemical tests showed the presence of bioactive constituents: phenols, flavonoids, alkaloids, tannins, saponins, resins and terpenoids. Steroids and cardiac glycosides were absent. The presence of these bioactive constituents made the ethanol and water extracts active against: Staphylococcus aureus, Streptococcus aerugenosa, Pseudomonas spp and Salmonella typhi. The comparison of their activity indicated that there is a statistical difference, which is statistically significant. It thus agrees with the potential therapeutic significance of the plant as a natural source of drug development and equally that the solvent for extraction be carefully selected.

Keywords: phytochemical, antibacterial, *zizipus mucronata*, saponin, *staphylococcus aureus*.

Introduction

Ethnic diversity in the temperate zone reflects an untapped wealth of indigenous uses of medicinal plants for the treatment of various health problems (Counteix, 1961). Their secondary metabolites (Active ingredients) is a source of our sustenance (Ghani, 1990; Dobelis, 1993; Fatope, 2001; and Kubmarawa *et al*., 2007) and are due to the presence of some valuable phytochemicals that combat most of the human maladies (Chidambara, *et al*; 2003).

Local indigenes of the Adamawa highlands, Nigeria, choose faith healing first, traditional herbal medicine next and modern medicine only when the first two have failed (Shariff, 2001, Sudhakar, 2007). The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996)

Increasing resistant isolates during antibacterial therapy is on the rise throughout the world (Gerding *et al*., 1991, Gold *et al*., 1996, Archibald *et al*., 1997, O`Brien *et al* 1999, Cookson 2000, WHO, 2001, Cohen, 2002, Fridkin *et al*., 2002). One of the measures to minimize the increasing rate of resistance in the long-run is to have continuous in-depth investigation for new safe and effective antimicrobials as alternative agents to substitute the non effective ones. Natural resources, especially plants and microorganisms are potent candids for such purposes.

The plant *Zizipus mucronata* is a tree with alternate compound leaves and yellow flowers. The decoction of the glutinous roots is commonly administered as a painkiller for all sorts of pains as well as dysentery. A concoction of the bark and the leaves is used for respiratory ailments and other septic swellings of the skin. Pastes of the roots and leaves can be applied to treat boils, swollen

glands, wounds and sores. Steam baths from the bark are used to purify and improve the complexion. In East Africa, roots are used for treating snake bites, (Bekele-Tesemma1993). It is a medicinal plant used for stomach ailments, ulcers and chest problems.

The fruits can be eaten and are also used for making porridge and flour (Wynn 2006). The seeds are roasted as a coffee substitute. The fruits are also eaten by many animals including impala, warthog, baboon, monkey nyala and black rhino (Baquar, (2001). The medicinal uses are attributed to the peptide alkaloids and antifungal properties isolated from the bark and leaves (Hutchinson et al, 1973). The berries are edible and are used by residents in the former Transvaal in making beer when properly fermented (Bekele-Tesemma1993). Africans have many beliefs and superstitions attached to the tree. Zulus and Swazis use the buffalo thorn (*Ziziphus mucronata*) in connection with burial rites. It was once customary that when a Zulus chief dies, the tree were planted on his grave as a reminder or symbol of where the chief lies. Hence, the name Umlahlankosi (that which buries the chief). A twig from the tree was and is used to attract and carry the spirit of the deceased from the place of death to the new resting place. When a stock owner dies and is buried according to custom, within the cattle or goat kraal, some branches will be placed on the grave so that the animals nibbled on leaves and twigs so as to understand that their master had died (Bekele-Tesemma, 1993).

In other parts, Afrikaans drag a branch round the village to protect it from evil spirits as it is believed to keep evil spirits away. In Botswana as well as most parts of West Africa, the residents believe the buffalo thorn to be immune against lightening; anyone standing under one in a storm would be safe. It is also believed that, if it is felled in summer, a drought, hail or lightening will certainly follow (Beentje, 1994 and Wynn, 2006). The English name of buffalo thorn is so called because it is said that when a buffalo is defending itself from Uris, it can reverse into a buffalo thorn. Protecting that flank and then only has defend the front (Hutchison *et al*, 1973, Simpson, *et al*,1997) The young twigs zigzag indicates that life is not always straight forward. The two thorns that are found at the nodes are also important because, the one facing backward represents where we come from and one facing forward represents where we are going. The presence of the tree is said to indicate the presence of underground water (Beentje, 1994).

The genus of *Z. mucronata* has historical and a Biblical importance. Christ's crown of thorn when He was crucified is said to have been made from *Ziziphus* spines. Although the fruits of *Z. mucronata* cannot be counted as very tasty, the tree itself plays an important role ecologically.

According to the information obtained from the inhabitants of Mubi Area of Adamawa State, the fruits are used (decoctions and concoctions) for treatment of bilharzias and are administered to those who urinate on bed to instantly stop the urination.

Common names: Buffalo thorns (Eng.), blinnkblaar-wag-n-bietjie(Afr.),umphafa, umlahlankkosi, isilhla(Isizulu), umhafa (Isixhosa), umlahlabbantu (Swazi), Mokgalo (Tswana), Mutshhete (Venda), Mphasamhala (Tssonga), mokgalo, moonnaona (Nsotho), Huyamapulao (Kilba) (Hutchinson *et al*, 1973, Baquar, (2001).

This work is aimed at determining the elemental and phytochemical constituents of fruits extracts of *Z. mucronata*, analyzing and comparing the antibacterial activity of the water and ethanol extracts, with the view of checking their statistical difference. This will form the basis for the prediction and subsequent advice on what solvent(s) to use to get maximum yield of the plant metabolites. This is also prompted by the strong push in the chemical industries to move away from the use of large amounts of organic solvents and when possible, to perform chemical reactions in water so that

there is lees organic waste, a paradigm of *green chemistry* (Anastas1998 and 2000).

MATERIALS AND METHODS

Collection of plant materials:

Fresh samples of the fruits of the indigenous plant Buffalo thorns were collected in Mubi North LGA, Adamawa State and were identified in the Biological Sciences Department Adamawa State University Mubi. The FHI number is 0202 and a specimen of the plant was deposited in the herbarium. The samples (1.00kg) each were air dried in the laboratory before pounding to a fine powder using pestle and mortar to about 70 mesh sizes and then stored in dry containers.

Extraction

150g each of the powdered sample was accurately weighed and percolated with 2.0L each of water and distilled ethanol for 72hrs. After which there was decantation, filtration, and concentration using rotary evaporator (NYC R-205D) at 35^0C to obtain water and ethanol soluble fractions, (F_W^1), and (F_E^1) labeled, F_W^F [11g], and F_E^F [14.7g], for water and ethanol fractions respectively. These fractions were divided into two portions each for phytochemical screening and the biological evaluation.

Elemental analysis

Analysis for K, Na, and Ca

A small quantity of the powdered sample was placed in a crucible and heated in muffle furnace at 400ºC till there was no evolution of smoke. The crucible was cooled in a desiccator at room temperature; the ash totally free from carbon moistened with conc. Sulfuric acid was heated on hotplate till fumes of sulfuric acid evolved. The crucible with sulphated ash was heated at 600 ^{O}C in muffle furnace till weight of sample became constant (3hrs 30mins). 1g sulphated ash was taken into a beaker and dissolved in 100ml 5% conc. HCl and the standard solutions of K, Na and Ca were determined through Flame emission photometer.

The respective concentrations of the elements were obtained from standard calibration curves (Vogel 2000).

Determination of Cu, Pb and Mn

About 1.25g powdered sample was weighed into destruction tube (kjeldal flask), 25ml nitric acid was added, boiling chips were added and a funnel was placed on top of the destruction tube. The tube was heated to 100 ^{O}C, 125 ^{O}C and 150^0C and was maintained at each interval for 60min. 75min and 90 min respectively. The tube was heated to 200 ^{O}C and 5ml of nitric acid was added. The mixture was concentrated to about 5ml. After cooling, 1ml, 3% hydrogen peroxide was added and was destructed for 10min. 25ml distilled water was added and heated till boiling. The whole sample was cooled and transferred to a 250ml volumetric flask, filled up to mark and left to settle for about 6hrs.

The absorbance of the clear supernatant was measured and the various concentrations were determined from the standard calibration curves (Vogel, 2000).

Qualitative Chemical test

Standard methods described by (Sofowora 1993, Evans 2000, Abulude *et al*., 2001 and Abulude 2007) were used to test for the presence of phytochemical compound(s) [Tannins, Saponins, Steroids, Alkaloids, Resins, phlobatannins, Terpenoids, Flavonoids, Phenols and Cardiac glycosides] in the fractions.

Microorganisms

Organisms used for this study were, gram- negative (*Pseudomonas auroginosa* GHM3005, *Salmonella typhi* GHM3002) and gram-positive (*Staphylococcus aureus* GHM 3004, *Streptococcus pyogene* GHM3001) bacteria. These organisms were clinical isolates obtained from General Hospital Mubi, Adamawa State, Nigeria.

Determination of Antibacterial Activity

The antibacterial activity of the extracts was determined using the agar well diffusion technique (Adeniyi *et al.*, 2008).Sensitivity

test agar plates were seeded with 0.1 mL of an overnight culture of each bacterial isolate (equivalent to 10^7–10^8 cfu mL^{-1}). The seeded plates were allowed to set and a standard cork borer of 8mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.1 mL of each extract at a concentration of 0.02 mg/mL. The antibiotic Ampiclox at concentration of 0.01g/mL was used as positive control and distilled water as negative control. The plates were incubated at 37^{O}C for 24 h after which bacteria and their comparison and statistical analysis in *figure (2)* the diameter of the zones of inhibition were measured. (Fereshteh *et al*., 2005)

RESULTS AND DISCUSSION

The elemental and phytochemical analysis of the powdered sample from the fruits of the indigenous plant *Ziziphus mucronata* are shown in *Figure (1)* and *Table (1)*. The antimicrobial activities of the water and ethanol fractions against some gram-positive and gram-negative.

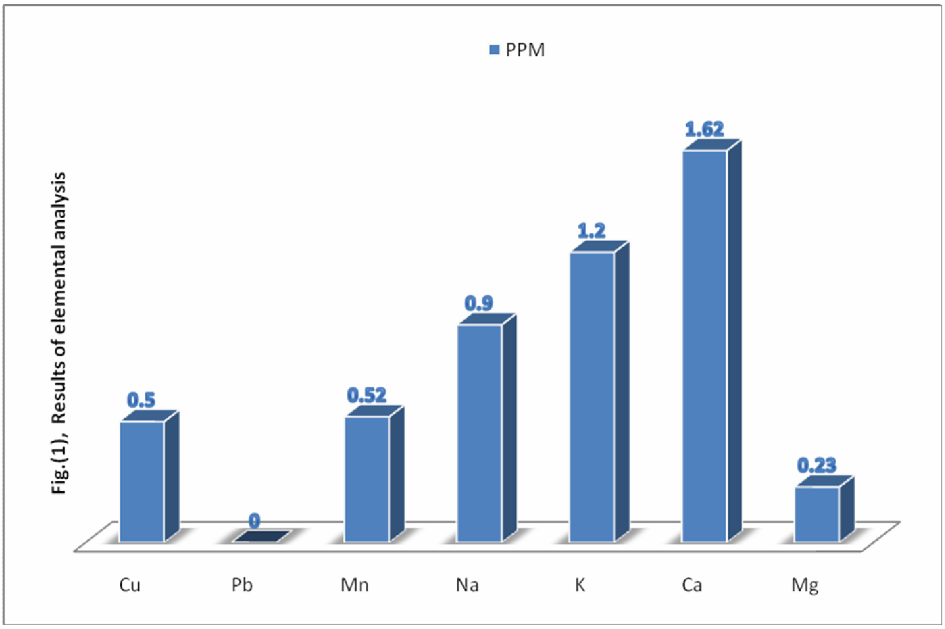

Figure (1)

Table 1: Result of phytochemical analysis

Tannins	Saponnins	Steroids	Alkaloids	Resins	Phlobatannins	Terpenoids	Flavonoids	Phenols	Cardiac glycosides
+	+	-	+	+	-	+	+	+	-

Key - = not present + = Present

n	15	(ETHANOL & WATER EXTRACT)

Ethanol and water extracts	n	Mean	SE	Pooled SE	SD
Control (2mg Ampiclox)	5	15.00	0.707	0.992	1.58
Water Extract	5	10.44	1.181	0.992	2.64
Ethanol Extract	5	13.40	1.030	0.992	2.30

Source of variation	Sum squares	DF	Mean square	F statistic	p
Ethanol and water extracts	53.53	2	26.76	5.44	0.0209
Residual	59.07	12	4.92		
Total	112.60	14			

Dunnett Contrast	Difference	95% CI		
Water Extract v Control (2mg Ampiclox)	-4.56	-8.07	to -1.05	(significant)
Ethanol Extract v Control (2mg Ampiclox)	-1.60	-5.11	to 1.91	

Figure 2

Discussion

Data obtained were subjected to statistical analysis using coupled (MS-Excel-Analyse-it® 2010). Independent Student's *t*-test at $p < 0.05$ was considered significant in results of both mineral contents and comparison of antibacterial assay solvents.

Mineral constituents

Fig. 1 shows the results of mineral constituents in the fruits of *Z. mucronata.* Lead was not detected in the fruits. It is known to cause respiratory problems and restricts the flow of blood into the body systems. Magnesium Copper and Manganese were present in very small concentrations. Calcium has the highest concentration (1.62 µ g /g).The presence of Cu is an important component of many enzyme systems such as cytochrome oxiadse, lysyl oxidase and ceruloplasmin, an iron oxidizing enzyme in blood. Cu deficiency has been associated with cardiac abnormalities in humans and animals such as anemia and neutropenia. Mg. Plays a vital role in the formation and function of bones muscles and prevents high disorders, high blood pressure and depression in enzyme activities. Its deficiency interferes with transmission of nerve and muscle, impulses, causing irritability and nervousness, thus, preventing heart diseases. Mn is the key component enzyme systems including oxygen. Handling enzymes support brain function and reproduction required for blood regulation part of bone structure. Na and K take part in ionic balance of human body and maintain tissue excitability, carry normal muscle contraction, help in formation of gastric juice in stomach. K helps in the release of chemicals which act as nerve

impulses, and regulate heart rhythms. Their deficiency causes nervous irritability, mental disorientation, low blood sugar, insomnia and coma. Ca plays an important role in building and maintaining strong bones and teeth, large part of human blood and extra cellular fluids. It is also necessary for normal functioning of cardiac muscles, blood coagulation, milk clothing and regulation of cell permeability. Ca deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus (Shariff Z. U. 2001).

From *Table 1*, the phytochemical screening of chemical constituents of *Z. mucronata* showed that the fruits are rich in alkaloids, tannins, resins, terpenoids, phenols, flavonoids, while steroids, phlobatannins and cardiac glycosides were absent. The presence of terpenoids is widely used in herbal medicine (Hayashi *et al*, 1993). The presence of tannins and alkaloids makes the fruit to be used in the treatment of ulcer, bilharzias, cough and hay fever (Rahila *et al*, 1994 and Gill 1992). Flavonoids present in the fruit serve as anti-viral, anti-inflammatory, antioxidants and anti-allergic in the human body Epidemiological studies have shown that intake of flavonoids is inversely related to mortality from coronary heart diseases and to incidence of heart attack.

Figure 2: Antibacterial activity of the fruits extracts against bacteria could be attributed to the presence of phytochemical constituents mentioned. These phytoconstituentes are associated with the antibacterial activities of the extracts based on the fact that certain types of phytochemicals have been previously reported to be responsible for antibacterial activity (Fillipos, 2007). According to (Lin *et al*, 2006) hydrolysable tannins have significant antibacterial and antifungal activities. Saponins have been reported to have antibacterial activity against *Streptococcus aeruginosa, Escherichia coli,* Salmonella *typhi,* and *staphylococcus aureus* [gram-positive bacteria]. From their comparative classical statistical analysis of variance and comparing values of the Kruskal Walis (post hoc) test, it was realized that comparison of the variables with the control (Ampiclox) was statistically significant. This shows that water is the best proposed solvent of extraction. It also confirms the usage of water by Mubi indigenes in concoctions and decoctions with water as the extracting solvent. The much advocated for, *green chemistry* paradigm (Anastas *et al*., 1998 and 2000)

Conclusion

Elemental analysis of the fruits of *Z. mucronata* confirms the presence of most of the tested elements, only lead was absent. It contains chemical constituents responsible for antibacterial activities. The water extract is more active than the ethanol extracts. This confirms the scientific basis for the use of *Z. mucronata* in traditional system of medicine for treatment of diseases such as bilharzias, diarrhea and fevers (Wynn 2006). Hence their ethno-medical claims in the management of some human diseases are justified. It is suggested that more research be conducted that will further elucidate the effective components and possible mode of actions involved in the use of these plants in ethno-medical practices.

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