



RESEARCH ARTICLE

COMPARISON OF THE PROXIMATE COMPOSITION AND THE ANTI- MICROBIAL PROPERTIES OF ETHANOL AND AQUEOUS EXTRACTS OF THE STEM-BARK OF *LEPTADENIA HASTATA* (RUBIACEAE)

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Abstract

Stem - bark of the plant *Leptadenia hastata* (Rubiaceae) was extracted with ethanol and water and the proximate composition of both solvents compared, showed presence of anti-nutrients; phytate and oxalate content less than is nutritionally significant. It was rich in protein but low in crude fats & ash. The anti-microbial activity of the extract using agar diffusion method showed zones of inhibition (mm) against some gram positive and gram negative bacteria; *Streptococcus pyogenes* (12.00±0.01, & 13.00±0.01), *Staphylococcus aureus* (12.00±0.01 & -), *Escherichia coli*, (6.00±0.02 & 13.0±0.03) and *Shigella dysenteriae* (11.00±0.01 & 06.3±0.02), respectively. It showed water as a better extraction solvent confirming its usage in local / crude indigenous extraction /herbal preparations, hence, the use of aqueous decoctions and concoctions of *yadiya* in diseases treatment in Adamawa State.

Keywords: Nutrients, Anti-nutrients, *Leptadenia hastata* (Rubiaceae), Stem –bark, Antimicrobial

Introduction

Medicinal plants are used for therapeutic purposes or are precursors for the synthesis of useful drugs used in forms of decoction, concoction, infusion, sedative etc , (Bulus; 2009). The increasing demand for plants which have healing prowess triggered the scientific investigation of *leptadenia hastata* used by traditional healers to cure various ailments, (UNESCO; 1996). Since this plant has both nutritional and medicinal values it has been used for centuries as remedies for human diseases because of these components, (Nastro; 2001). More-so, the search for a novel, high quality, cheap and readily available source of plant protein to replace Soya-beans is now a major concern of livestock nutritionist in most of the developing world,(Adeniji, *et al.*; 2005, Obum & Ayanwale; 2006). One of such legumes with great prospects

as alternative and replacement for Soya-beans is *Leptadenia hastata* which has been reported to contain comparatively high amounts of vitamin A and C and other antioxidant micronutrients (Shulz., *et al.*; 2001; Jimoh, *et al.*; 2008), which promote good health by assisting in preventing cancer and high blood pressure, stimulating the immune system, improving drug metabolism and tissue regeneration (Krebs, *et al.*; 2001). Food contains a variety of nutrients, but there is less awareness that many foods contain small amounts of potentially harmful substances. These are toxins commonly referred to as anti-nutrients, (Ken Adachi; 2008). Since anti-nutrients are found in some levels in almost all foods for a variety of reasons, it is necessary to test for their presence in *L. hastata*, (Balogun; 1988). The awareness of the importance

of medicinal plants in human health care in developing countries has resulted in research into traditional medicine with a view to integrating it with modern Orthodox medicine and they have been of great importance to the health of individuals and communities, (Sofowora; 1993 and Edeoga, *et al.*; 2005). Like in the words of Norman J. Temple, in Strategies for Disease Prevention, "The needed transformation in thinking on transport, environment, work facilities, education, health and food policies, and perhaps in social and economic policies is unlikely when governments are wedded to individualism, but without these changes to enhance physical activity and alter food quality, societies are doomed to escalating obesity rates" (Norman; 2001).

The plant *Leptadenia hastata* belongs to the kingdom plantae, family Rubiaceae with vary ethnic names; Hausa - Yadiya, (Ghana) Grus -Benaduru, Iran-aji-igbo. It is distributed in tropical Africa from Mauritania, Senegal, Eastwards to Cameroon, Ethiopia, Northern Kenya to Uganda. *Leptadenia hastata* has characteristics of dry savanna vegetation and semi-arid zones. Propagation of *Leptadenia hastata* is by seeds and sometimes it is intentionally sown near houses so that it is available when the need arises. In some parts of Ethiopia the fresh vegetable is marketed as an ingredient for soup. In the northern parts of Cameroon and Nigeria it is used for the treatment of ear infection, blood replenishing, constipation, urethral discharge, gonorrhoea, stomachache, diarrhea, milk drying, sexual-impotence, trypanosomiasis, acute rhinopharyngitis, wounds and as fodder for ruminants. For the research scientist who penetrates the unknown of medicinal plants alone, no guides are possible because the territory they travel is uncharted.

This piece of research is aimed at filling the knowledge gap in our social communities, since the need for the integration of local knowledge for a sustainable management and conservation of natural resources receives attention much too often (Posey. 1992). With this in mind, the research authenticates the claims of the herbalists by investigating and comparing the proximate composition and the anti- microbial properties of ethanol and aqueous extracts of the stem bark of *L. hastata* thus exploring the fauna and flora of Adamawa highland in order to add to the compendium of our indigenous medicinal / nutritional plants. This will also pave the way in the selection of the extracting solvent(s) when the need

arises in the preparation of decoctions, concoctions, infusions, and sedatives for maximum and effective yield.

Materials and Methods

Collection of plant materials

The stem bark of *Leptadenia hastata* was collected from Michika on 12th December, 2010, authenticated by Mr. Ibrahim T. Yusuf of Divisional Forest Office, Mubi North Local Government Area, Adamawa State. The FHI number is 52 and a specimen of the plant was kept at the Divisional forest office, Mubi North LGA. The plant stem- bark was air dried in the laboratory (Eloff, 1998), and pounded using laboratory mortar and pestle to powder.

Extraction Procedure

Powdered *Leptadenia hastata*, stem- bark, 150g each was accurately weighed and percolated with 2.0L each of distilled ethanol and water for 72hrs. After which there was decantation, filtration, and concentration using rotary evaporator (R110) at 35°C to obtain ethanol & aqueous soluble fractions, (F_E¹), [38g] and F_W¹. [43g]. This gave 25.3% & 28.7% stem extracts respectively. The fractions were kept for further analysis.

Anti-nutrient Determination

Tannin

Tannin was determined according to the method by Trease and Evans, (1989). 0.5g of the dry sample was boiled with 20ml of water. 0.1% FeCl₃ was added to observe for brownish green or blue-black colouration.

Oxalate

Oxalate was determined according to the method by Day and Underwood, (1986). 1.0g of the sample was dissolved in 100ml of 0.75M H₂SO₄. The solution was then carefully stirred with a magnetic stirrer for 1hr and filtered. 25ml of the filtrate was pipetted and titrated hot (80—90°C) against 0.1M KMnO₄ to an end point of a faint pink colour that persisted for more than 30 seconds. Result was calculated as follows:

T x constant (0.225), where T = Titre value

Phytate

The method by Reddy and Kove, (1999), was adopted for the determination of phytate. 4.0g of sample was soaked in 100ml of 2% HCl for 5hrs and filtered. 25ml of the filtrate was pipetted into a conical flask and 5ml of 0.3% ammonium thiocyanate (NH₄SCN) solution was added. The mixture was titrated against 0.1M FeCl₃ until a brownish yellow colour end point that persisted for 5mins was obtained. The result was calculated as:

T x constant (0.1635), where T = Titre value

Determination of Nutritive Value.

Determination of Protein using micro kjedahl method

Sample (1g) was taken in 250ml Pyrex digestion tubes and 30ml of conc. H₂SO₄ carefully added. Then 10g potassium sulphate and 14g copper sulphate, were added and the mixture placed on sand bath at a low flame to boil the solution. This was heated further till the solution became colorless and clear and was allowed to cool. It was then diluted with distilled water and transferred into 800ml kjeldahl flask. The digestion flask was washed, four (4) pieces of granulated zinc and 100ml of 40% caustic soda added, and the flask was connected with the splash heads of the distillation apparatus. 25ml of 0.1N sulphuric acids was taken in the receiving flask and distilled: it was tested for completion of reaction. The flask was removed and titrated against 0.1N caustic soda solution using methyl red indicator for determination of Nitrogen, which in turn gave the protein content (Jayaraman, 2005).

Determination of crude fat

Crude fat was determined by extracting 1g each of moisture free sample with diethyl ether in a soxhlet extractor, heating the flask on sand bath for 1h till a drop taken with care so that the dripping leaves no greasy stain on the filter paper. The residual diethyl ether was filtered using Whitman No 01 filter paper and the filtrate evaporated in a pre- weighed clean beaker.

Determination of Crude Fiber

2g of moisture and fat free material was treated with 200ml of 1.25% H₂SO₄. After filtration and washing, the residue was treated with 1.25 NaOH, and then

washed with hot distilled water again. The residue was ignited and the ash weighed to give the weight of crude fiber (Watanable *et al.*, 1965).

Determination of ash content

The ash content was determined as described by (Sadasivam *et al.*, 1996). 5g of sample was weighed and taken in silica crucible and heated, first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 5 hours at 600°C. Then the sample was cooled in a desiccator and weighed again to ensure completion of ashing. It was again heated in muffle furnace for 1h, cooled and weighed. This was repeated consequently until the weight of the sample became constant (Ash became grayish white weighed of [(Ash + crucible) – (Weight of crucible)] = Ash content.

Microorganisms

Organisms used for this study were gram-positive, (*Streptococcus pyogenes* SHY3001, *Staphylococcus aureus* SHY 3004,) and gram-negative (*Escherichia coli* SHY 3007, *Shigella dysentryae* SHY 3001, SHY 3002) bacteria. These organisms were clinical isolates obtained from Yola Specialist Hospital, 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 10⁷ – 10⁸ 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 0.025 mg / mL. The antibiotic Ciprofloxacin at concentration of 10mg /mL was used as positive control and distilled water as negative control. The plates were incubated at 37°C for 24 h after which the diameter of the zones of inhibition were measured. Each treatment was conducted in triplicate. (Fereshteh *et al.*, 2005).

Results and Discussion

Tannin detection in the aqueous sample was in traces and BDL in the ethanol sample. This therefore implies no interference with digestion and absorption in monogastric animals, (Back-knudson,

Table 1. Some Anti-Nutrients in *Leptadenia hastata* (mg/100g)

Solvent	Tannin	Oxalate	Phytate
Ethanol	BDL	0.138 ± 0.02	0.150 ± 0.01
Aqueous	Traces	0.153 ± 0.03	0.155 ± 0.02

BDL = Below detectable levels

Table 2. Nutrients in *Leptadenia hastata*

Nutrients (g)	Ethanol	Aqueous
Crude fat	10.3 ± 0.01	14.4 ± 0.02
Crude fibre	2.00 ± 0.02	3.40 ± 0.01
Ash	0.99 ± 0.01	1.12 ± 0.02
Protein	27.86 ± 0.04	30.20 ± 0.03

Table 3. Antimicrobial activity of the stem bark extracts.

Bacteria	Mean diameter of zone of inhibition (mm)		
	Ethanol	Aqueous	Standard Sparfloxacin
<i>Streptococcus pyogenes</i>	12.0 ± 0.02	13.0 ± 0.01	13.0 ± 0.02
<i>Staphylococcus aureus</i>	12.0 ± 0.01	-	15.0 ± 0.00
<i>Escherichia Coli</i>	6.0 ± 0.03	13.0 ± 0.02	0 8.0 ± 0.01
<i>Shigella dysenteriae</i>	11.0 ± 0.02	06.3 ± 0.03	15.0 ± 0.02

Key: - Results: Mean of three trials ± Standard error.

Sparflo = Sparfloxacin (Bacteria Control): 10µg/ml

1988). The value of phytate and oxalate obtained in both solvents are very low 0.138mg/100g, & 0.150mg/100g, and 0.153mg /100g & 0.155mg/100g respectively. These values are low compared to values reported in other vegetables e.g. (Penuel *et al.*, 2013), testified of 0.315mg /100g oxalate & 0.229mg / 100g phytate ,in defatted *Citrullus vulgaris (guna) seed*, (Oladele *et al.*, 2009), reported 21.42mg/100g phytate and 1.12mg/100g oxalate content in tiger nuts, Pigeon pea has oxalate content of 310.50mg/100g, (Oloyo, 2002). (Eka, 1977), reported that phytate and oxalate levels in traditional foods of the Northern Nigeria were below toxic level, which is in line with the values gotten from *L. hastata*

An oxalate diet limits the ingestion of oxalate to 40 – 50mg a day. Higher oxalate content contains more than 10mg per serving, while low content has less than 2mg per serving, http://www.botanical-online.com/oxalatecontent_of_foods.htm 2011. The minimum amounts of phytic acid to cause negative

effect on iron and zinc absorptions are 10 – 50mg per meal, (Sanberg, 1991). In view of the aforementioned, the phytate and oxalate of '*L. hastata*' pose no danger in diet, as, Siddhuraja & Beekers, (2001) reported a safe or normal range of 4 – 9mg/100g for phytate and oxalate, (Table.1).

From table 2, the protein values 27.86% for ethanol and 30.20% for water, compete favourably with those of protein rich food such as "Guna seeds" ranging from 36.58 – 83.56%, (Penuel *et al.*, 2013), ebony seeds (36.10%) (Gonzalez-Quijada, 2002), pumpkin, water melon and other melon varieties which range between 25.8 – 38.1%, (Osagie, 1998), cowpea, lima bean, pigeon pea with protein values ranging between 23.1 – 33.0%, (Olaofe, 1994) and the value is above that of some Nigerian legumes such as Lima bean (20.20%). It has comparatively low fat and low crude fibre. The plant thus, has good nutritive value which supports their use as medicine, fodder and a good source of important nutrient for livestock.

Ethanol extract of the stem bark were active against the four clinical isolates, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Shigella dysenteriae*. The tested organisms used in this study are associated with various forms of human infections. From a clinical point of view, *S. pyogene* causes diseases such as sour throat, scarlet fever and kidney disease, *S. aureus* causes minor skin infection such as impetigo boils, scalded skin syndrome and abscesses, to diseases such as pneumonia and meningitis, *S. dysenteriae*, causes dysentery / diarrhea and *E. coli*, septicemia and can infect the gall bladder, meningitis, surgical wounds, skin lesions and the lungs, especially in debilitate and immune-deficient patients (Black., 1996). The demonstration of activity against all tested bacteria is an indication that, the plant can be a source of bioactive substances, Robert, F. Boyd, (1995). The plant being active on these clinical isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in our environments.

From the analysis, it was realized that in comparing the variables of the control (Sparfloxacin) with the variables water and ethanol, the results were all statistically significant ($p < 0.005$). This shows that water is the best proposed solvent of extraction - the much advocated "green chemistry paradigm" (Eric and Denis, 2006).

This is a confirmation of the usage of *L. hastata* by the indigenes in forms of concoctions and decoction using the universal solvent, water as the medium of extraction. It equally shows that water is a more polar solvent, good for the extraction of polar molecules than ethanol. This corroborates the adage "like dissolves like" in chemistry showing improved protective potential with increased concentration of the plants extracts in the aqueous fraction.

Conclusion

L. hastata contains chemical constituents nutritionally valuable for life sustenance though with few anti nutritional traces. These secondary metabolites could also be responsible for the antibacterial activities. The plant could therefore be used as a medicinal herb and nutritional vegetable. It also confirms that in its extraction, aqueous extraction has an edge over other solvent(s) extraction.

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