

Anti-Nutritional/Nutritional Analysis and Anti-Microbial Investigation of the Ethanol Extract of the Stem Bark of *Leptadenia Hastata* (Asclepiadaceae)

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Abstract

The work seeks to determine the nutritional / anti-nutritional profile and ascertain the folkloric potential and the societal usage and value / properties of *Leptadenia hastata*. *Leptadenia hastata* (Asclepiadaceae) stem - bark extracted with 95% ethanol, showed presence of anti-nutrients; phytate and oxalate content less than is nutritionally significant, with traces of tannins while rich in protein and fats but low in 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±0.1), *Staphylococcus aureus* (12.00±0.1), *Escherichia coli*, (6.00±0.02) and *Shigella dysenteriae* (11.00±0.01), respectively. The mineral concentration indicated [in mg / kg], Cu, 0.088, Mn, 0.173, Fe, .0275, Mg, 1.96 and Pb, 0.002; therefore justifies the use of the plant in folklore medicine in North Eastern Nigeria for treatment of such diseases, as ear infection, blood replenishing, constipation, urethral discharge, gonorrhoea, stomachache, diarrhea, against milk drying, sexual-impotence, trypanosomiasis, acute rhinopharyngitis, wound and as fodder for ruminants. It thus attests to the efficacy of the plant on the management of local ailments and its inclusion in the preparation of local drugs for the above diseases.

Key words: *Leptadenia hastata* (Asclepiadaceae), anti-nutrients, anti-microbial, mineral concentration.

1 Introduction

In Nigeria and the West African regions, herbal medicine practitioners have made several claims regarding the diverse pharmacological properties of several plant species, which is often the rationale behind their usage in the management of disease conditions and the lure to access them in pharmaceutical researches for the development of new drugs, Juliani, (2009). Consequently, scientists from divergent fields are investigating plants with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as species of plants are continually being exhausted. It is therefore necessary to identify the phytochemical components of plants because it is likely that these photochemical will find their way into the arsenal of antimicrobial drugs prescribed by physicians and later to the human system, (Owolabi; (2007).

“The greatest service which can be rendered to any country is to add a useful plant to its culture”. Plants have forever been catalysts for our healing. In order to halt the trend of increasing emerging and resistant infectious diseases, it will require a multi-pronged approach that

includes the development of new drugs, or fine turning old ones using plants as the inspiration. Evaluating plants from the traditional African system of medicine, provides us with clues as to how these plants can be used in the treatment of diseases Deeni, (1999)

Medicinal plants are used for therapeutic purposes or are precursors for the synthesis of useful drugs used in forms of decoction, concoctions, infusion, sedative etc, Bulus., (2009). The increasing demand for plants which have healing powers triggered the scientific investigation of *leptadenia hastata* used by traditional healers to cure various ailments, UNESCO, (1996).

Soya beans are the main conventional plant protein source for livestock diet in Nigeria. Soya beans also serves as human food. The competition between human and livestock for the consumption of Soya beans and the increasing role of Soya-bean in the world as a biodiesel, feed stock Cotula., *et al.*, (2008) has increased its cost and demand and heightened the competition between human and beast for soya-beans. Consequently 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 even more than before Adeniji, *et al.*, (2005) , Obum & Ayanwale, (2006). One of such legumes with great prospect 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),

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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). Due to its taste and nutritional value it is considered appropriate and further research seems worthwhile to determine superior genotypes and possibilities for commercial cultivation. This is confirmed by an investigation of other species of *Leptadenia*, such as *Leptadenia pyrotechnica* said to contain various flavonoids, which are anti-oxidants Amal, (2009).

This research work is aimed at filling the knowledge gap in this important sub-area of cultural biodiversity, directly relevant to the livelihood of the tribal communities, since the need for the integration of local knowledge for a sustainable management and conservation of natural resources receives attention on a daily bases Posey, (1992). Based on this, the research authenticates the claims of the traditional healers by investigating the anti-nutrients, antimicrobial activity and elemental compositions of the ethanol extracts of the stem bark of *L. hastata* with the mind of exploring the fauna and flora of Adamawa highland so as to add to the compendium of our indigenous medicinal / nutritional plants and advise locals adequately after toxicological analysis.

2 Materials and Method

Collection and extraction of plant materials: The stem bark of plant was collected from Michika on 12th December, 2010, authenticated by Mr. Ibrahim Yusuf T. of Divisional forest office, Mubi North Local Government Area, Adamawa State and the FHI number is 52. They were air dried in the laboratory (Eloff., 1998), and pounded using laboratory mortar and pestle to powder and 150g was accurately weighed and percolated with 2.0L of distilled ethanol for 72hrs. After which there was filtration, and concentration using rotary evaporator (R110) at 35^oC to obtain ethanol soluble fraction, (F_E^1), [38g]. This gave 25.3% stem extract. The fraction was divided into two portions for phytochemical screening and the biological evaluation.

Experimental: The anti-nutrient, nutrient determination and the mineral elements compositions were evaluated thus:-

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% $FeCl_3$ 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 H_2SO_4 . 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^oC) against 0.1M $KMnO_4$ to an end point of a faint pink colour that persisted for more than 30 seconds. Result was calculated as follows:

$T \times \text{constant} (0.225)$, where T = Titre value

Phytate: The method by Reddy and Kove, (1999), was adopted for the determination of phytate. 4.0g of sample as 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 (NH_4SCN) solution was added. The mixture was titrated against 0.1M $FeCl_3$ until a

brownish yellow colour end point that persisted for 5mins was obtained. The result was calculated as:

$T \times \text{constant} (0.1635)$, where T = Titre value

Determination of Protein using micro kjedahl method: 1g sample was taken in 250ml Pyrex digestion tubes and 30ml of conc. H_2SO_4 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 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 40 filter paper and the filtrate evaporated in a pre- weighed clean beaker.

Determination of Crude Fiber: 2 g of moisture and fat free material was treated with 200ml of 1.25% H_2SO_4 . 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^oC. 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.

Elemental Analysis: 5g of pounded stem bark was taken in pre-cleaned and constantly weighed silica crucible and heated in muffle furnace at 400^oC until there was no evolution of smoke. The crucible was cooled in a desiccator to room temperature. The ash totally free from carbon was moistened with concentrated H_2SO_4 and heated on a hot plate till fumes of sulphated evolved. The silica crucible with sulphated ash was again heated at 600^oC in muffle furnace till weight of sample was constant (4hours). 1 g sulphate ash was taken in a beaker and dissolved in 100ml of 5% concentrated HCl to obtain the solution for determinations of Fe, Mg, Cu, Mn, and Pb. The Atomic Absorption Spectrometer (A.A.S.) and titration method respectively were used Indrayan *et al.*, (2000), Shivraj & Khob Sragade, (2009).

Determination of Antibacterial Activity: 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, Adeniyi *et al.*, (2008). 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)

3 Results and Discussion

Tannin was not detected in any of the samples. This therefore implies no interference with digestion and absorption in monogastric animals, Back-knudson, (1988). The value of phytate and oxalate obtained in the samples are very low 0.138mg/100g, and 0.150mg/100g, respectively. This is in consonance with the work done by Aliero *et al.*, (2009) & Bello *et al.*, (2011). These values are low compared to values reported in other vegetables e.g. Pigeon pea has oxalate content of 310.50mg/100g, Oloyo, (2002). Oladele *et al.*, (2009), reported 21.42mg/100g phytate and 1.12mg/100g oxalate content in tiger nuts. 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 2 mg per serving, (<http://www.botanical-online.com/oxalatecontentoffoods.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).

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

Sample	Tannin	Oxalate	Phytate
Powdered <i>L.hastata</i>	ND	0.138	0.150

ND = Not detected

From table 2, the protein value (27.86 mg) of the vegetable competes favourably with those of protein rich food such as guna seeds 36.58mg – 83.56 mg /100 g), Penuel *et al.*, (2013), ebony seed (36.10mg/100g) Gonzalez-Quijada, (2002), pumpkin, water melon and other melon varieties which range between 25.8 – 38.1mg/100g, Osagie, (1998), cowpea, lima bean, pigeon pea with protein values ranging between 23.1 – 33.0 mg/100g, Olaofe, (1994) and the value is above that of some Nigerian legumes such as Lima bean (20.20 mg/100 g). It has comparatively low fat and low crude fibre. Crude fibre is largely indigestible plant matter considered to play a role in the prevention of many diseases of the digestive tract. High dietary /crude fibre content therefore acts to dilute the

energy content of a food. Non-starch polysaccharides are generally not digested by the human amylases because the enzymes cannot break the beta linkages between the molecules, and are part of what has more commonly been known as dietary / 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, Vaughan & Judd, (2005).

Table 2: Nutrients in stem bark (*Leptadenia hastata*)

Nutrients (mg)	Concentrations (mg)
Crude fat	10.3
Crude fibre	2.00
Ash	0.99
Protein	27.86

Results of mineral elements in stem bark of *Leptadenia hastata* are given in table (3). This is in consonance with other results from *L. hastata* from other parts of the Northern Nigeria, (Julia *et al.*, 2000, Glew *et al.*, 2004 and Hassan *et al.*, 2007). Copper is one of the least elements in this plant. Its content falls within the values reported by Ilelaboye & Pikuda, (2009), for *Jatropha curcas* (0.16mg/kg) and *Trichosanthes cucumerina* (0.29mg/kg). Copper is a mineral that facilitates the absorption of iron and its value may account for the value of iron in the samples, Clifton, (1971). Copper is also required in the body for enzyme production and biological electron transfer. Thus, its value is within the recommended dietary allowance of 3mg/day for adult and 2mg for children. Magnesium has a very low concentration. It is very important in calcium metabolism in bones and also involved in prevention of circulatory diseases. It helps in regulating blood pressure and insulin release Umar *et al.*; (2005). The high concentration of Mg contributes in muscle tone and activates enzymes in the human body and also for the proper growth and maintenance of bones, for the function of nerves, muscles and many other parts of the body. Deficiency causes neutral value prolapse, migraines, attention disorder, fibromyalgia, asthma, etc. Iron is one of the elements with high content. These value 0.275mg /kg is moderate and within some under-exploited leguminous seeds in Nigeria as reported by Balogun & Fetuga, (1986), which range between 0.200 – 0.310mg/kg. The recommended dietary allowance for iron in adult and children is 10mg/day while female adult is 15mg/day. The results indicate that *L. hastata* is rich in iron and its value is far above the recommended standard. Iron is required for blood haem formation, Adeyeye & Fagbohon, (2005). Cu, Mn and Pb are heavy elements or "heavy metals." Small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amount of up to 0.5mg/kg of any of them may cause acute or chronic toxicity (poisoning). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lung, kidney, liver and other vital organs, Njoku & Ezeibe; (2007). Pb, if present leads to toxicity in the body; causing respiratory problems and restricts the flow of blood into the body system. The Environmental Protection Agency (EPA) has detected and declared that there is no safe level of lead intake. It can cause high blood

pressure and reduce haemoglobin production, necessary for O₂ transportation, thus interfering and can interfere with normal cellular Ca metabolism, Montague & Peter; (2010).

Table 3: Concentrations of various mineral elements (mg/kg)

Mineral	Cu	Mn	Mg	Fe	Pb
Concentration	0.088	0.173	1.958	0.275	0.002

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. A higher diameter zone of inhibition for the standard control could be as a result of impurities in the crude extracts of the plant. 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, and thus showing that the plants materials possess effective restoring prowess against those microbes.

Table 4: Antimicrobial activity of the stem bark extracts.

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

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

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

4 Conclusions and Recommendations

L. hastata contains chemical constituents responsible for antibacterial activities. This therefore justifies the use of the plant in folklore medicine for treatment of some of our common environmental maladies; as ear infection, blood replenishing, constipation, urethral discharge, gonorrhea, stomachache, diarrhea, milk drying, sexual-impotence, trypanosomiasis, and acute rhinpharyngitis, wound, impetigo, sore-throat and as fodder for ruminants. Mineral content evaluation showed presence of moderate quantities of Cu, Mn, Mg, Fe, & Pb. The plant could therefore be used as a medicinal herb and nutritional vegetable.

It is advised that toxicological analysis be done on this plant and if found safe, then its inclusion in the list of excellent medicinal and vegetable sources in the community could be contemplated.

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