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Nutritional and Pharmacological Potentials of *Leptadenia Hastata* (pers.) Decne. Ethanolic Leaves Extract

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Abstract The work focused on the nutritional and pharmacological potential of *Leptadenia hastata* (pers.) Decne. ethanolic leaves extract. The proximate compositions of the leaves shows the values of moisture content (7.63 ± 0.07), Ash content (17.19 ± 0.05), Carbohydrate (47.13 ± 0.17), Crude protein (20.85 ± 0.07), Crude fibre (7.50 ± 0.05), and Crude lipid (2.70 ± 0.06). The Phytochemistry of the ethanolic leaves extract divulged the presence of Tannins, Glycosides, Alkaloids, Carbohydrates and Flavonoids. The leaves extract tested possess some decree of antimicrobial activities on *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis* at 200 mg/ml and 100 mg/ml respectively. The mineral composition analysed (using Shimadzu AA 6800 Atomic Absorption Spectrophotometer) of the leaves quantified the nutritive elements such as Calcium (23.90 ± 0.01), Magnesium (147.45 ± 0.01), Sodium (1.26 ± 0.01), Iron (0.82 ± 0.00), Zinc (2.30 ± 0.05), Copper (2.86 ± 0.01), Manganese (1.80 ± 0.00), Chromium (0.25 ± 0.00) and potassium (0.18 ± 0.00). While Lead and Cadmium are absent. Nevertheless, the invitro antioxidant potentiality was evaluated using 2, 2-Diphenyl-1-Picrylhydrazyl radical (DPPH Sigma-Aldrich), which shows promising antioxidant activity of the plant leaves extract.

Keywords: *antibacterial, antioxidant, Elemental analysis, Leptadenia hastata, Phytochemistry, proximate composition*

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1. Introduction

It is highly valuable to preserve knowledge of leafy vegetable plants, both cultivated, semi cultivated and wild due to their nutri phamacological potentials as well as their central role in household food security in rural areas of developing countries. Leafy vegetables are important valuable sources of nutrients especially in rural areas where they contributes substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets [1]. It is worthwhile to note that consumption of numerous types of edible vegetable plants as sources of food could be beneficial to nutritionally marginal population especially in developing countries where poverty and climate is causing havoc to the rural populace [2].

Leptadenia hastata (Pers.) Decne is a wild plant used as vegetable by many African populations and medicine due to its nutritive and therapeutic properties. Despite the fact that in some part of Nigeria the plants are classified as underutilized vegetables while in the other parts the high rate of consumption is well pronounced [3]. *L. hastata* is belongs to Class: Magnoliopsida, Order: Gentiananae, Family: Apocynaceae and Genus: Leptadenia. Is a climber plant, with pale white, soft, groved stem. Few drops of yellow liquid exude from cut stem. Simple leaves with pale undersurface. Latex white, paired dehiscent fruits. According to the useful Plants of West Tropical Africa by [4], in Nigeria, the leaves of *L. hastat*a are boiled and the liquor is drunk for treating gonorrhea. "Hausa tribe in Northern Nigeria gives this liquor to cure stomach-ache in children [5]. Also in Nigeria, local healers use the plant for hypertension, catarrh and skin diseases [6]. The sap, or the root in decoction is used for ophthalmia in Senegal. In association with other plants, it is used in Senegal for suckling babies with green diarrhoea, Senegalese healers also use the *L. hastata* for prostate and rheumatism complaints [7]. In Burkina Faso, locally it is used for sexual potency (chewing leaves), trypanosomosis (decoction of leaves), skin diseases and wound-healing (application of latex) [8,9] examined triterpenes isolated from *L. hastata* latex for their anti-inflammatory activity. Lupeol, lupeol acetate and lupeol palmitate were found to be the main anti-inflammatory constituents in the croton oil-induced ear oedema test. The aim of this research work is to evaluate the proximate constituents, and pharmacological potentials of *L. hastata* ethanolic leaves extract.

Figure 1. *L. hastata* plant

Figure 2. *L. hastata* leaves powder

2. Materials and Methods

2.1. Sample Collection and Processing

The *L. hastata* leaves were collected in uncultivated land at Sheda Science and Technology Complex, located at Kwali area council Abuja, Federal Capital Territory of Nigeria, which lies between latitude 8.9 degrees south and longitude 78 degrees east. The leaves are collected in polyethene bag, washed in order to remove the dust and debris, followed by shade drying. Using Mortar and Pestle the powder were obtained and stored for various analysis. The *L. hastata* was identified in the Department of Biological Science (botanical garden) University of Abuja, Federal Capital Territory of Nigeria. By Mr. O. Segun and Authenticated by Professor O. Olorode Botanist/Taxonomist, where voucher specimen was deposited.

2.2. Proximate Analysis

Standard methods of the Association of Official Analytical Chemists [10] were used to determine the moisture, crude protein, crude fat, total ash and crude fibre contents of the powder. Moisture content was determined by heating 2 g of powdered plant material to a constant weight in a crucible placed in an oven maintained at 105 EC. The dry matter was used in the determination of other parameters. Crude protein (% total nitrogen x 6.25) was determined by the Kjeldahl method, using 2 g of powdered plant material; crude fat was obtained by exhaustively extracting 5 g of powdered plant material in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. Ash was determined by incineration of 10g of powdered plant material placed in a muffle furnace maintained at 550°C for 5 h. Crude fibre was obtained by digesting 2 g of powdered plant material with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5 h. Each analysis was carried out in triplicate.

2.3. Elemental Analysis

About 2 g of powdered plant material was digested with (Aqua regia) 3 ml of HCL to 1 ml OF HNO_3 until the clear solution was observed; the suspension was then filtered into a 100 m volumetric flask and made it up to the mark (100 ml) using deionized water. Atomic absorption spectrophotometer (Shimadzu AA 6800 Atomic Absorption Spectrophotometer) was used for the determination.

2.4. Phytochemistry of the Leaves Powder

A small portion of the dry extract was subjected to the phytochemical test using [10,11] methods, to identify the active constituents such as alkaloids, flavonoids, steroids, phenols, glycosides, resins, Saponins balsams, volatile oil and tannins.

2.5. Antioxidant

The free radical scavenging activity was measured in terms of hydrogen donation or radical scavenging ability using the stable radical 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) [12]. About 0.1mM of DPPH in methanol was prepared and 1ml of this solution was added to 3.0ml of extract solution in ethanol at different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The same experiment was carried out on buthylated hydroxyl anisole (BHA) and Ascorbic acid, which are known antioxidants. All tests and analyses were run in triplicate and the results obtained were averaged. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \ inhibition = \frac{(Ab - Aa)}{Ab} x100$$

Where Ab was the absorbance of the control (blank, without extract) and Aa was the absorbance in the presence of the extract. All these tests were performed in triplicate and the barchart was plotted with the mean values.

2.6. Antibacterial Activity

Agar well diffusion technique as described by [13], was used to determine the antibacterial activity of the extracts. The test organisms were collected from Bayero University

Kano, department of microbiology. The four clinical organisms are *Staph. Aureous, E. coli, kleb. pneumonia* and *Proteus mirabilis*, both are characterized base on the standard procedure adopted from [13].

Prior to the screening 0.5% McFarland equivalent standard was prepared [14]. Both the 0.5% McFarland and the suspension of test organisms were Spectophoto metrically analysed using (Cecil, C-7500, Cambridge England). 20 ml of Mueller hinton agar plates that has been checked for sterility were seeded with 2 ml of an overnight broth culture of each bacterial isolate in sterile Petri-dish .The seeded plates were allowed to set after a uniform distribution of the bacterial isolate following slow rotation of the Petri dish. A standard sterile cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar. The wells filled with 0.5 ml of each prepared extract concentrations of (300, 200 and 100 mg/ml) with the aid of a sterile syringe. One of the well in each Mueller hinton agar plate was filled with 0.5 ml of Chloramphenicol to served as a positive control. The plates were then allowed to stand for 1 hour at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 37°C for 24 hours and observed for zones of inhibition. A zone of clearance round each well signifies inhibition and the diameter of such zones were measured in millimeter (mm).

3. Result and Discussion

Table 1. Proximate analysis of *L. hastata leaves* powder

Constituents (%)	Leaves powdered.
Moisture content	7.63 ± 0.07
Ash Content	14.19 ± 0.05
Crude lipids	2.70 ± 0.06
Crude fibre	7.50 ± 0.05
Carbohydrate	47.13 ± 0.70
Crude protein	20.85 ± 0.07

The values ± are means standard deviation of triplicate

The proximate analysis results of *L. hastata* leaves powder Table 1, shows low moisture content (7.63 ± 0.07) compared to other cultivated vegetable plants reported by [2], such as *Telfaira occidental* (12.05), *Amaranthus hybridus* (10.00) and *Vernonia amygdalina* (10.02), while (7.00 ± 1.0) moisture content was reported by [15], for *Moringa oliefera*. The low moisture content will afford a long shelf life of *L. hastata* leaves powder. Ash content, which is an index of mineral contents in biota is high (14.19 ± 0.05) compared to other cultivated leafy vegetables repoted by [16], such as *Talinum triangulare* (1.6), *Telferiria occidentalis* (2.2), and *Vernonia amygdalina* 2.5. [17] also reported the ash content of (13.80) for *Amaranthus hybridus*.The leaf of *L. hastata* possessed law lipid content (2.70 ± 0.06) which qualified the leaf as good vegetable for obesity patient. The crude fibre of (7.50 ± 0.05) was also low when compared with other leafy vegetables reported by [2] such as *Vernonia amygdalina* (12.08), *Telfaira occidental* (11.05) *Hibiscus sabdariffa* (12.04), and *Moringa oleifera* (20.0 ± 0.03) which was reported by [15]. The carbohydrate content of (47.13 ± 0.70) is higher than 23.6 ± 0.20 reported for *Moringa oleifera* (15), *Telfaira occidental* (4.4), *Vernonia amygdalina* (5.00) and *Talinum triangulare*, (4.3), reported by [16]. The crude protein content (20.85 ± 0.07) was favourably higher than Nigerian cultivated leafy vegetable *Amaranthus hybridus* (17.92) [17].

The qualitative preliminary phytochemical screening in Table 2, shows the present of active pharmacological components such as tannins flavonoids, phenols, alkaloids, carbohydrate and glycosides which are known to be biologically active, they protect the plant against infections and predations by animals and also serve as therapy in various human ailment. Tannins are plant metabolite well known for antimicrobial properties [18]. Alkaloids generally exert pharmacological activity particularly in mammals such as humans and many of our most commonly used drugs are alkaloids from natural sources [19]. Flavonoids have both antifungal and antibacterial activity. They possessed anti inflammatory Property [20]. Phenols possess antioxidant potential that could enhance the body defense against pathology induced free radical generation [21]. Ethnomedicinally, *L. hastata* plays important roles for the treatment of children green diarrhea, stomach-ache in children, and anti inflammation, this can serve as the scientific justification of the use of *L. hastata* in traditional medicine.

Table 2. Phytochemistry of Ethanolic extract of *L. hastata* leaves

Test	Result
Steroids	-
Tannins	+
Saponins	-
Flavonoids	+
Phenols	+
Balsams	-
Alkaloids	+
Carbohydrate	+
Glycosides	+
Volatile oil	-

+, Indicates present. -, Not detected.

The antibacterial susceptibility test in Table 3 shows that the activity of the extract are more pronounced on *E. coli* (16 mm) at 200 mg/ml, *Kleb. Pneumonia* and *proteuos mirabilis* shows inhibition zone of (13 mm) each at 200 mg/ml while *Staph aureus* shows the least activity (11 mm) at 200 mg/ml respectively. The activity of the extract on *E. coli* and other gram negative microorganisms is in line with the work carried out by [22], despite the variation in the concentration and solvent of extraction. This is a clear indication that *L. hastata* ethanolic extract possessed more activity on gram negative organism then gram positive. In comparison with the positive control Chloramphenicol the activity of the extract is low which may be related to the fact that the standard antibiotic is in its refined form while the *L. hastata* leaves ethanolic extract contain both active and inactive substances.

Table 3. Antimicrobial screening of *L. hastata* ethanolic leaves extract

Test organisms	Concentration in Mg/ml 300 200 100 Zone of inhibition in (mm)			+control CHL(1 mg/ml)	-control DMSO + water (1: 1)
Staph. aureus	11	9	NA	22	NA
E. coli	16	12	NA	21	NA
Kleb. Pneumonia	13	11	NA	21	NA
Proteous mirabilis	13	11	9	23	NA

Key: CHL – Chloramphenicol, DMSO - Dimethylsulphoxide + Positive, - Negative

The minerals determined in *L. hastata* ethanolic leaves extract are iron, magnesium, calcium copper, zinc, cadmium, lead, manganese, potassium, and sodium. Minerals are natural compounds formed through geological processes. In human, minerals are nutrients needed by the body in small amounts to help it function properly and stay strong. Humans need small amounts of about 25 minerals to maintain normal body function and good health, 16 of which are essential nutrients and must be supplied by the diet [23]. All the minerals analysed from the Leaves of *L. hastata* are within the safety limit and also compares favourably with the value reported in some cultivated and semi-cultivated edible leafy vegetable in Nigeria and other part of Africa. The Iron content of the extract 0.82 ± 0.00 is low compared to the total iron intake range 14.4-20.2 mg/day [24]. Iron is an important trace element in the human body, which plays crucial roles in haemopoiesic, control of infection and cell mediated immunity [25]. The Magnesium content of *L. hastata* leaves ethanolic extract (147.45 ± 0.01) was lower compared to (231.22) recorded for the *Amaranthus hybridus* [17], 288.65 for *Telfaria occidentalis* [2]. But is higher than (120.04) reported for *Hibiscus sabdariffa*, 61.08 for *Vernonia amygdalina*, and 27.51 for *Basella alba* (17). Magnesium plays a role in the contraction and relaxation of muscles and in the production of protein. Most dietary magnesium comes from vegetables, such as dark green leafy vegetables, fruits, nuts, peas and beans, whole grains etc. Deficiency of magnesium can occur in people who abuse alcohol or in those who absorb less magnesium. The deficiency symptoms include anorexia, apathy, confusion, fatigue, insomnia, irritability, rapid heartbeat, continued muscle contraction, delirium, hallucination, tingling etc. The recommended dietary intake of magnesium for adults is 400 mg per day [26]. Calcium is a mineral needed for strong bones and teeth, good posture, walking and they are believe to be beneficial until old age. Foods rich in calcium are fish, meat, green vegetables etc [27]. The value of calcium 23.90 ± 0.01 reported is lower compared to other cultivated vegetables consumed in Nigeria. Copper helps to keep blood vessels, nerves, immune system and bones health. It is important for infant growth, brain development and for strong bones, the Cu content of 2.86 ± 0.01 is higher than that of *Amaranthus hybridus* 0.08, *Telfaria occidentalis* 0.33 and *Vernonia amygdalina* 1.06) reported by [2]. The Zinc level (2.30 ± 0.50) is lower compared to other cultivated leafy vegetable consumed in Nigeria such as *Hibiscus sabdariffa* (15.43), *Telfaria occidentalis* (24.46) [2]. The dietary chromium as needed by the human body, are refer to the trivalent form (Cr^{3+} or Cr (III)), the (0.25 ± 0.00) chromium leveled in *L. hastata* leaves is within the safety limit. Cadmium and Lead are not detected in the leaves extract. The manganese level of (1.80 ± 0.00) is within the safety limit. Manganese helps the body form connective tissues, bones, blood clotting factors, and sex hormones. Manganese is also necessary for normal brain and nerve function. The recommended dietary intake of manganese for adult is 2 mg per day. Good sources of manganese include nuts, seeds, whole grains, leafy green vegetables and tea [28]. The value of potassium (0.18 ± 0.00) is low compared to others values of cultivated green leafy vegetables consumed in Republic of Niger, *Moringa oleifera* (9120 µg/g), *Adansonia digitata* 5830 µg/g and *Hibiscus sabdariffa* 19,700 µg/g, [29]. The extract shows promising result on sodium (1.26 ± 0.01) Compared with daily recommended per day for adult and children 1.5 g each [23]. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction [30].

Table 4. Elemental analysis of *L. hastata leaves* ethanolic extract

Test Elements	Concentrations in Mg/100
Fe	0.82 ± 0.00
Mg	147.45 ± 0.01
Ca	23.90 ± 0.01
Cd	Nil
Cu	2.86 ± 0.01
Zn	2.30 ± 0.50
Cr	0.25 ± 0.00
Pb	Nil
Mn	2.80 ± 0.00
K	0.18 ± 0.00
Na	1.26 ± 0.01

All values are mean standard deviation ± of triplicate

The result of the antioxidant activity is shown in Figure 3. The *L. hastata* leaves ethanolic extract exhibited a strong antioxidant activity at 0.5, 0.125, and 0.25 concentrations respectively, however the extract showed a weak activity in comparisons with the standard antioxidant Vitamin C and Buthylated Hydroxyl Anisole (BHA). Epidemiological studies have shown that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species [31]. Many plants including fruits and vegetables are recognized as sources of natural antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species [32,33,34]. These positive effects are believed to be attributable to the antioxidants; particularly the carotenoids, flavonoids, lycopene, phenolics and β-carotene [35]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are suspected to be tumourigenic [36], Therefore, there is a need to search for potential antioxidant compounds especially from herbs that can replace their synthetic counterparts. The highly antioxidant activity of the *L. hastata* leaves ethanolic extract at low concentration shows that the plant could be very useful for the treatment of ailments resulting from oxidative stress such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections [37].

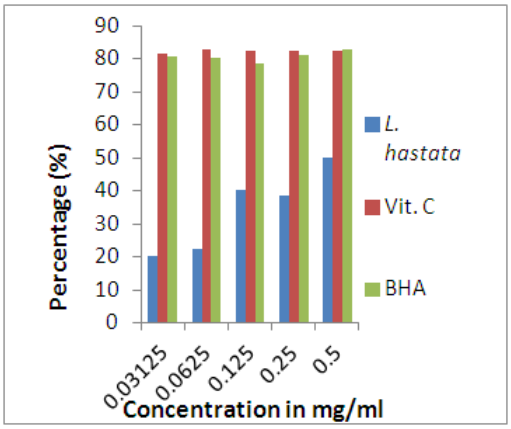

Figure 3. Antioxidantactivity of *L. hastata* leaves compared with other standard antioxidant

4. Conclusion

The present study has shown that the leaves of *L. hastata* examined have high content of crude protein with low lipids content and moderate crude fibre. The value of Ash contents were also pronounced, the leaves extract also contained good minerals within safety limit such us Magnesium, Manganese, Calcium, Chromium and Copper while Lead and Cadmium are absent. The results suggest that if the leaves consume wisely would contribute greatly towards meeting human nutritional requirement for normal growth and prevent any diseases arising from malnutrition. The leaves could also be a good therapeutic preparation in treatment of various infections.

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