

LEPTADENIA HASTATA (PERS) DECNE: PHYTOCHEMICAL, PHARMACOLOGICAL, BIOTECHNOLOGICAL, BOTANICAL, TRADITIONAL USE AND AGRONOMICAL ASPECTS

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Article Received on 30/03/2018

Article Revised on 20/04/2018

Article Accepted on 10/05/2018

ABSTRACT

Leptadenia hastata (Pers.) Decne (Asclepiadaceae), it is a traditional tropical plant species with curative potentials. Many of this medicinal plants species are used as spices, foods and medicinal purposes. It is widely used in traditional medicine to treat various ailments such as cough, dyspnea, and fever, burning sensation, night blindness, cancer, hypertension diabetics, gonorrhoea, catarrh, sex potency, wound healing, inflammation and dysentery. The therapeutic potential of this herb is as a result of the presence of diverse bioactive compounds such as α -amyrin, β -amyrin, ferulic acid, luteolin, diosmetin, rutin, β -sitosterol, stigmasterol, hentriacontanol, a triterpene alcohol simiarenol, apigenin, reticulon, denticulatin, and leptaculatin. However, most of the studies on *Leptadenia hastata* are restricted to crude extracts, and many biologically active compounds are yet to be identified in order to claim the traditional uses of this plant as acclaimed by the herbal and traditional practitioners. The plant is threatened and endangered because of overexploitation, unscientific harvesting, and habitat loss. The increased demand from pharmaceutical, nutraceutical, academia and veterinary industries has prompted its large-scale propagation. However, its commercial cultivation is hampered because of the non-availability of genuine planting material and the lack of knowledge on its agronomical practices. However there is a need for micro propagation technique to obtain the right type of *Leptadenia hastata* through the knowledge of biotechnological approaches can guaranty conservation as well as increased metabolite production from *Leptadenia hastata*. This review summarizes scientific information on the botanical, agronomical, phytochemical, pharmacological, and biotechnological aspects of *Leptadenia hastata*. This knowledge of information will certainly provide better utilization of this industrially important herb towards the discovery of lead drug for diseases and ailments. However, most studies of this plant species are restricted to crude extracts, which gives room for yet unidentified compounds to appreciate the claim to evidence the base use of the plant by the traditional medical practitioners.

KEYWORDS: *Leptadenia hastata*, biotechnology, phytochemical, agronomical, morphology.

1. INTRODUCTION

Leptadenia hastata (Pers) Decne occurs in Africa, It is essentially an African genus both temperate and tropical species, however this study is reviewing the phytochemical, Pharmacological, Biotechnological, Botanical and, Agronomical Aspects of *Leptadenia hastata* (Pers) Decne as a tropical plant herbs with great potential. It is a traditional medicinal that has been utilized for their therapeutic value and has been known to mankind from times immemorial and has played an essential role in the various traditional systems of medicine. *Leptadenia hastata* is an edible plant with creeping latex stems, glabrescent leaves glomerulus and racemes flowers as well as follicles fruits.^[44] It is

typically grown in tropical dry lands in sandy soil. This plant is mostly used in Nigeria among Higgi's in the north east and the Hausa's in the north central as spices and sauces as well as medicine by local healers. The plant consist of about 500 genera and 600 species distributed all over the world.^[1] At present, drug discovery research is mainly focused on natural plant resources and their compounds. Most of the currently available therapeutically active drugs are discovered based on the knowledge available from various traditional practices for disease treatments.^[2, 3, 4, 5] Awareness, health consciousness, thoughts on prevention is better than cure, and natural ways of healthy living has further increased the use of herbal plants and well as

their products. The exponential growth of nutritional supplements and cosmeceutical consumers has increased the demand for plant raw materials.^[5, 7] Historically, medicinal plants have provided a good source of inspiration for novel therapeutic drugs as plant-derived medicines, this have made large contributions to the health and well-being of humans. Some traditional medicine involves using crude plant extract which may contain an extensive diversity of molecules often with indefinite biological effects.^[6] However, famine and higher market value and global demands for *Leptadenia hastata* have forced farmers and traditional herbal practitioner to consider cultivating them in recent times. Today, various medicinal plants are commercially cultivated to meet the ever increasing global demand for plant metabolites used by pharmaceutical industries,^[5] sad to note that *Leptadenia hastata* is not considered for commercial cultivation. Nevertheless, various crop improvement strategies are yet to be adopted to develop superior varieties of medicinal plants. However the use of micropropagation approach will certainly help in the large-scale production of elite genetically and chemically uniform planting material for *Leptadenia*.^[5] Thus the nutritional, academia and pharmaceutical value explored will give room for global demand which will force farmers to consider cultivating the plant in commercial purposes to meet up with the in-coming demand of plant metabolites used by pharmaceutical industries. The present review was undertaken to compile the most available information on the phytochemical, agronomical, morphology, biotechnology and biological activities of *Leptadenia hastata* for drug discovery research by scientist.

2. Botanical Aspects of *Leptadenia hastata*

2.1 Taxonomy

Leptadenia hastata (Pers) Decne is a perennial plant of the family of Asclepiaceae, the plant is edible non-

domesticated vegetable and it is collected in wild throughout Africa. Its taxonomic position is detailed as follows.^[41]

Kingdom-Plantae
Subkingdom -Viridaeplantae
Phylum - Magnoliophyta
Subphylum- Spermatophytina
Class - Magnoliopsida
Subclass- Lamiidae
Order – Gentianles
Family - Asclepiadaceae
Genus - Leptadenai
Specific - Hastata

Leptadenia hastata is referred to by many names, it is known as yahdiya in hausa Nigeria, hagalhadjai in Arabic, in Niger it is also called by vernacular names as listed in Table1 as reported by Steven *et al.*,^[41] The genus *Leptadenia* is comprised of four species namely, *L. pyrotechnica* (Forssk.), *L. arborea* (Forssk.), *L. reticulata* (Weight and Arn.) and *L. hastata* (Pers.),^[26, 27] Among them, *L. pyrotechnica* is a desert herb with straight stems and mostly leafless, while others are twining shrubs and bear leaves. Because of its taxonomic complexity, these three species are further stated to be comprised as a single species.^[27, 28] Most of these *Leptadenia* species are economically valued for their therapeutic properties. Among them, *Leptadenia hastata* is one important medicinal herbs used in Africa by the traditional healers for treatment of disease and ailment and for food by the local people in terms of hunger.^[44]

Table 1:

| Language | Vernacular name | Country |
|---------------|------------------|--------------|
| Arabic | hagalhadjar | Chad |
| Hausa | yadiya | Nigeria |
| Kusume | hayla | Ethiopia |
| Turkana | ekamongo | Kenya |
| Moore | lolongo | Burkina faso |
| Busumba-amata | tarhat or darhat | Senegal |
| Barbara | Nzongne | Mali |

2.2. Origin and Geographical Location

Leptadenia hastata is widely distributed in tropical Africa: from Mauritania and Senegal eastwards to Cameroon, Ethiopia and northern Kenya to Uganda extended to Nigeria. In some locations, e.g. locally in Ethiopia, it is also cultivated in some parts of Africa.^[26] *Leptadenia* is a genus of plant of a family Apocynaceae, it is first described as a genus in 1810. It is native to Africa including Madagascar, as well as southwest Asia and the Indian subcontinent.^[30]

2.3. Morphology

The plant is climbing, it is a latex-containing herb, becoming woody at base, with strongly branched, finely pubescent stems becoming corky with age. Leaves opposite, simple; petiole 0.5–1.5 cm long; blade variable, usually ovate, 2.5–12.5 cm × 0.5–4 cm, base rounded to cordate, apex acuminate, margin entire, both sides puberulous, often glabrescent. Inflorescence umbellate, minutely tomentose in all parts, it has many-flowered; peduncle up to 1.5 cm long. The flowers are bisexual,

regular, five-merous, yellowish, scented; pedicel up to 0.5 cm long; calyx lobed nearly to the base, lobes 2–4 mm long; corolla rotate-campanulate; tube 2 mm long, lobes linear-lanceolate, twisted, 4–5 mm long, densely bearded inside; corona of five fleshy lobes 1–3 mm long, inserted at the sinuses of the corolla, apex hairy.^[29] The fruit is a pair of follicles, each one conical, up to 10 cm long, greenish, glabrous, and many-seeded. Seeds is with a tuft of hairs at apex.^[28] It is a perennial much branched, twining, and laticiferous climber. Mature stem of the plant *Leptadenia hastata* is pale yellowish with deeply cracked barks, while the immature one is greenish glabrous. The leaves are quite big (8–10 cm long and 2–5 cm wide), simple, opposite, ovate and light green, the plant flowers profusely during the flowering time which occurs between July and October and fruiting between September and December. The flower is yellowish with lateral or sub auxiliary umbellate cymes. The Calyx is five-lobed; lobes are ovate, sub-acute, and silky with small hairs on the surface. Corolla is rotate and fleshy pubescent with short tube. Staminal column is short. Corona is five-lobed, gamopetalous, spreading with spur from the interior of each lobe. Stamens are five and adnate to the base of the corolla tube; filaments fuse with the stigmatic head to form a five-angled disc called gynostegium. Anthers are without membranous appendages. Pollen grains are arranged on the lateral side of stigma. Ovary is bicarpellary with marginal placentation. The plant roots are rough and white in color with longitudinal ridges and furrows. They are cylindrical and twisted irregularly with longitudinal ridges. Its length can reach up to one meter or more. The stem is yellowish white in color with longitudinal lenticels.^[44]

3. *Leptadenia hastata* Agronomy

3.1. Climate and soil

The plant is typically grown in tropical dry lands in sandy soil and requires moderate rainfall and relative humidity. This plant also grows in arid regions, which are characterized by sandy soil, low organic matter, and rainfall deficit. Black soil is found to be good for cultivation; however, red laterite soil is also suitable for its satisfactory growth, constant sunlight and support is necessary for healthy and vigorous growth. *Leptadenia hastata* also grows in sand dunes, they are more or less the only growth in clay flat during the dry season.^[35]

3.2. Propagation of *Leptadenia hastata*

The propagation of the plant *Leptadenia hastata* is by seed.^[22] However a study conducted by National Medicinal Plants Board^[37] reported that *Leptadenia plants* reproduce vegetatively from stem cuttings, roots, and vines. Evaluation of various planting materials such as stem cuttings, root cuttings, and vine cuttings revealed that propagation using healthy and strong stem cuttings is most successful among all parts. Maintenance of high humidity around the cutting was found to be a critical factor to reduce the evaporative loss of water from cuttings. High humidity was maintained by covering the

planting material with clear plastic bags. After rooting, the plastic bag can be removed. Treating the cuttings with root-promoting compounds is found to be a valuable tool in stimulating root formation. Newly rooted cuttings should not be planted directly. The plants can instead be transplanted into a container or a bed before transferring them to a permanent location to increase the chances of survival. Although the fruits contain fairly large quantity of seeds, the number of seedlings was less because of low germination rate and limited availability. Fruits turn ripe during November to December. Seeds are collected before the fruits dehisce, and they are dried and stored. After soaking in water for 4–5 h, the seeds are sown on nursery bed with thick layer of sand. About 1–1.5-month-old seedlings were transferred to the main field, the period of February-March is suitable for planting the cuttings.^[37]

4.0. Phytochemical

A phytochemical screening conducted by Bello *et al.*^[38] on *Leptadenia hastata* leaves indicate the presence of phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins.^[38] The total phenolic, total flavonoid and proanthocyanidin contents were in the ranges of 17-38, 10-16 and 4-10 mg/g respectively depending on the extraction solvent. In their study, they report that the acetone extract had highest content of total phenol (35.77 mg/g) than the methanol extract and aqueous extract. The flavonoids content of methanol fraction (15.85 mg/g) is higher than that of acetone and water extracts. The methanol extract (9.69 mg/g) had highest content of proanthocyanidins compared to water and acetone.

Aquino *et al.* have revealed that the chloroform extract of *L. hastata* bark contains mixtures of polyoxypregnane ester derivatives, including six novel esters, as well as the known esters 12-O-acetylsarcostin. (penupogenin), gagaminin, kidjolanin, metaplexigenin and cyanforidin.^[27] Gagaminin derivatives have been reported to have antibacterial activity.^[39]

Work done by Nikiéma *et al.* found that *L. hastata* contains triterpenes like lupeol, lupeol acetate and lupeol palmitate.^[40] In studies by Sena *et al.*^[43] and Freiberger *et al.*,^[45] reveals fatty acids (23.2 mg/g dry weight) with large amounts of α -linolenic acid, lutein (53.8 μ g/g dry weight), β -carotene (50.8 μ g/g dry weight), protein, and eight essential amino acids.^[41,43] *Leptadenia hastata* is also found to be a rich source of copper, calcium, and phosphorus.^[43]

5.0. Nutritional properties of *Leptadenia hastata*

The leaves of *Leptadenia hastata* can be used to form part of regular human diet since the leaves contain significant amount of nutrients and minerals required for human nutrition. Reported by Hassan, *et al.*, Their results obtained indicated that all the parameters (carbohydrate, crude lipid, crude protein, ash content, crude fiber, moisture content and minerals) determined were present

at varying concentrations. Their results indicated high moisture content of 78.85 ± 0.32 %, ash content of 8.73 ± 0.14 %, crude protein content of $4.65 + 0.09$ %, available carbohydrate of $6.50 + 0.43$ %, energy content of $49.79 + 1.89$ kcal/100 g, but low in crude fiber and crude lipid contents of $0.18 + 0.02$ % and $1.10 + 0.03$ % respectively. The results of the mineral analyses showed higher levels of calcium ($43,086.67 + 270.00$ mg/kg), magnesium ($94,325.00 + 330.00$ mg/kg), potassium ($1,160.00 + 165.13$ mg/kg) and iron ($1,322.50 + 28.40$ mg/kg). Low levels of zinc ($53.00 + 5.35$ mg/kg), manganese ($29.50 + 1.91$ mg/kg), copper ($12.80 + 1.89$ mg/kg) and phosphorus (3.59 ± 0.56 mg/kg) This indicates that the leaves of *Leptadenia hastata* is a potential source of iron for anaemics as well as a source of calcium for the development and maintenance of strong bones and teeth in both children and adults.^[64]

Yirankinyuki, *et al.*, studies on chemical composition and nutritive value of *Leptadenia hastata* reported that *L.hastata* proximate analysis shows moisture, ash, lipid, crude protein, crude fiber and carbohydrate contents to be (7.67, 17.67, 5.0, 14.88, 9.33, and 45.45) % respectively. The high percentage of carbohydrates indicates that *Leptadenia hastata* leaves could be served as a good source of energy to the body. The elemental analysis was carried out using Atomic Absorption Spectrophotometer (AAS). Calcium recorded the highest concentration of 1845.66 mg/kg and Zinc with the lowest concentration of 15.27mg/kg. Other elements includes; Na, Fe, K and Mg with concentrations of (72.54, 148.94, 1245.34 and 275.87) mg/kg respectively. These results also agrees with the study of Hassan, *et al*, suggest that *Leptadenia hastata* leaves could be served as good source of minerals such as Ca, Fe, and K that are essential for human and livestock.^[65]

Umaru, *et al.*, in their recent study reported also the nutritional potential of *Leptadenia hastata* on Metabolic Profile of Pregnant Albino Rats, this study was undertaken as a result of the widely use of *Leptadenia hastata* by pregnant women in northern Nigeria with so much nutritive and therapeutic claims attributed to it. The effect of different doses of the plant on metabolic profile of pregnant rats was determined. Results obtained showed significant decrease ($p \leq 0.05$) in Fasting Blood Sugar (FBS), Total Cholesterol (TC), Triglycerides (TG) and Low Density Lipoprotein (LDL) values. Urea, creatinine and alanine aminotransferase (ALT) levels increased significantly ($p \leq 0.05$) in a dose depended manner while aspartate aminotranferase (AST) activity showed little or no change in activity. The study proved the hypolipidemic effect of *L. hastata* and provided a scientific support for the ethno medical uses of the plant in the management of pre eclampsia in the study area. Pregnant women in Adamawa state depend on vegetables to satisfy a substantial part of their nutritional requirement. This plant *L. hastata* is particularly used during pregnancy for both nutritional and therapeutic purposes.^[58]

6.0. Pharmacological properties of *Leptadenia hastata*

6.1. Cytotoxicity and safety

Cytotoxicity of *Leptadenia hastata* using brine shrimp was reported by Ikwueme, *et al.*, The results of the brine shrimp lethality assay for chloroform, ethanol and aqueous extracts of *Leptadenia hastata* showed 148ppm, 70ppm, and 294ppm respectively. The leaf extracts of *L. hastata* exhibited good brine shrimp larvicidal activity. In The observed lethality of the plant extracts to brine shrimps indicated the presence of potent cytotoxic, bioactive and probably antitumor components of the plants. In view with the report of Godman, *et al.*, that "cytotoxic action of a drug is believed to be provided by disturbing the fundamental mechanisms associated with cell growth, mitotic activity, differentiation and function".^[69] The observed cytotoxic activity for these extracts may be due one of these mechanisms. Hence, the ethno-pharmacological activities of these plant species might be due to the different bioactive compounds present in these plants.

According to Owolarafe *et al.*,^[54] and Meyer *et al.*^[55], crude plant extract is active if it has an LC₅₀ value of less than 1000 ppm or $\mu\text{g/mL}$ while it is considered inactive if it's LC₅₀ value is greater than 1000 ppm or $\mu\text{g/mL}$. From the result, all the plant extracts possessed cytotoxic activity against the brine shrimp and considered as containing active or potent components. This is because their LC₅₀ values are less than 1000 ppm. From their studies they conclude that *Leptadenia hastata* may have curative properties against several human pathogens and suggest its importance in traditional medicine.^[1]

Aquino *et al.*^[27] tested isolated compounds from the bark chloroform-methanol fractions for their cytotoxic activity on Raji cells (a human lymphoblastoid cell line from Burkitt's lymphoma). However, their results shown no activity at concentrations evaluated (0.5 and 1.0 $\mu\text{g/ml}$) after six hours of incubation.^[27]

Tamboura *et al.*,^[25] conducted their experiments by the means of male albino mice using concentrations 1000-2000 mg/kg body weight of *L. hastata* aqueous extract (leaves and stems). The mice were injected with the extract intraperitoneally and were observed during 48 to 72 hours. According to Tamboura *et al.*, *Leptadenia hastata* is considered safe to use due to its high LD quotient value of 0.78.^[46]

Umaru, *et al.*,^[71] also conducted a study on brine shrimp, the result showed that the leaf extracts of *Leptadenia hastata* were virtually non-toxic on the shrimps. They exhibited very low toxicity with LC₅₀=346 $\mu\text{g/ml}$. given the LC₅₀ value greater than 100 $\mu\text{g/ml}$. this only provides a proof of cytotoxicity effects of the plant extracts which might suggest a base line information in understanding the potential medicinal value of *Leptadenia hastata*.

6.2. Galactagogue activity

Leptadenia hastata extract provides a good remedy for new mothers suffering from breast milk deficient or absence. The plant is said to have galactagogue effect and also useful in the treatment of habitual abortions.^[41] It has been concluded that *Leptadenia hastata* extract therapy when done alone proved beneficial for the management of threatened abortion.^[43]

6.3. Anti-inflammatory activity

Nikiéma *et al.* 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.^[42] All the triterpenes tested at a dose of 0.42 µmol/ear induced a significant reduction of oedema. Lupeol exhibited 80% inhibition of oedema and was found to be more active than indomethacin (73%). Nikiéma also found that lupeol hemisuccinate, a synthetic derivative of lupeol exhibited a higher activity than lupeol, in the oedema test. They also study and verify the topical use of *L. hastata* latex in wound-healing. Their experiments examined an in vitro model of human skin keratinocytes (epidermal explants) cultured at an air-liquid interface on a de-epimerized human dermis (DED) to investigate the effects of lupeol esters on skin repair and when compared with the control, lupeol acetate and lupeol palmitate improved keratinocyte proliferation at a concentration of 5µM in the culture medium. However, lupeol hemisuccinate obtained from the *L. hastata* extract also induced a sufficient differentiation of keratinocytes with a well-formed stratum corneum without parakeratosis.^[42]

6.4. Anti-androgenic activity

Bayala, *et al.* demonstrated a competitive effect of the aqueous extracts of *L. hastata* leaf steams and the testosterone propionate (TP) on castrated immature Wistar rats.^[8] They found that the anti-androgenic effect of the extract of *L. hastata* is expressed when the testosterone propionate (TP) amounts are weak. Concentrations of TP ranged from 0.04-1,000µg/ kg of TP. At low doses of TP, *L. hastata* (at 200 mg/kg) inhibited TP effects, whereas at high doses of TP, *L. hastata* extracts potentiated TP effects. To further evaluate the competition between TP and *L. hastata* extracts, Bayala *et al.* conducted a follow-up study.^[8] In their 2012 studies they reported that *L. hastata* aqueous extracts reduced significantly the weight of androgen-dependent sex glands, the level of phosphatase acid prostatic (PAP) and fructose in seminal vesicles and prostate, and the serum testosterone level^[9], within the ranged of 100-400 mg/kg. This study showed that the low doses of *L. hastata* increased TP activity and the high doses inhibited its action. These results confirmed the anti-androgenic effects of *L. hastata* extracts and have implications on prostate cancer treatment and reproductive health.^[10, 11]

6.6. Antibacterial activity of *Leptadenia hastata*

Leptadenia hastata is reported to possess a wide range of pharmacological activities and is used in many preparations for both human and veterinary usage. Aliero and Wara investigated the effect of *Leptadenia hastata* leaf extracts on *Bacillus metagarium*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi* and *Pseudomonas aeruginosa*.^[18] Aqueous extract markedly inhibited the growth of *S. paratyphi* and *E. coli* at 30mg/ml and *P. aeruginosa* at 60 mg/ml. The activity exhibited by the methanol extract was generally low and acetone extract did not show any activity against the tested organisms. Aliero and Wara^[18] also examined in the same study the antifungal activity of *L. hastata* extracts with *Aspergillus niger* and *Fusarium oxysporum* as a potential anti-fungal medicinal plant because of the clear zone of inhibition by the extracts.

Aliero and wara studied the antibacterial activity *Leptadenia hastata* leaves extract against *Bacillus metagarium*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi* and *Pseudomonas aeruginosa* strains. They observed clear zones of inhibition after the incubation period.^[15] Were the diameters of the zones of growth inhibitions were measured for each concentration of the extract using a metre rule and diameter < 8.0 mm indicates low sensitivity, while > 8 mm indicates high sensitivity.^[9] While acetone extract showed no activity against all the tested Gram-positive Patrick, *et al.*,^[23] studied antibacterial activities of three latex plants of Asclepiadaceae family used in traditional medicine in South Togo against Gram-positive (*Staphylococcus aureus* ATCC 29213 and clinical strain of *Staphylococcus aureus*), and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and clinical strains *Salmonella typhi*, *Klebsiella pneumoniae*) bacteria. Were he reported the efficacy of *Leptadenia hastata*. In the study which was undertaken to evaluate the in vitro antibacterial potentials of the extracts the results indicated that all *Leptadenia hastata* extracts exhibited antibacterial activities.^[23] The activity of this plant *Leptadenia hastata* was may be as a result of the presence of; alkaloids, saponins, phenolic glycosides, tannins, flavonoids, proanthocyanidins and triterpenes.^[41] They made to conclude that the antibacterial activities observed can be due to the presence of these components obtained from the plant extracts. In agreement with their results, Raghavamma *et al.*, also reported the antibacterial activities of *Leptadenia hastata*.^[24]

Umaru, *et al.*, studied the antibacterial activities of chloroform crude extract of *Leptadenia hastata* against *Escherichia coli* (ATCC©25922), *Salmonella typhi*, (ATCC©14028), *Staphylococcus aureus* (ATCC©25923) and *Kliebselia pneumonia*, (ATCC©19155) were he reported the extract of chloroform of *Leptadenai hastata* were active against these bacteria with increase in concentration.^[71]

6.7. Antifungal activities

Aliero and wara reported the effect of this plant *Leptadenia hastata* against the cultures of *Aspergillus niger* and *Fusarium oxysporum*. The diameter of the fungal growth was measured and expressed as percentage growth inhibition. The results of antifungal assays of *L. hastata* extracts did not show any activity on the growth of fungal species studied at 5 mg/ml. However, methanol extract suppressed the growth of *F. oxysporum* and *Aspergillus niger* at 80 mg/ml with inhibition percentages ranging from 58.89 to 73.30%.^[18] The activity of acetone extract on the growth of *A. niger* and *F. oxysporum* was low with 40 and 50% inhibition respectively. The activity exhibited by the methanol extract of this species is instructive, as the fungus is known to have high resistance to most fungicides.^[15] This support the claimed ethno medical uses of aqueous extracts of *Leptadenia hastata* potential as antifungal agent.

Umaru, *et al.*, studies on antifungal activity of *Leptadenia hastata* (Pers) Decne leaf extract, results obtained revealed that all the fungal species were affected by the administration of *Leptadenia hastata* in a dose dependent manner. The concentration of extract at day four and five, 25ppm, 50ppm and 100ppm showed the highest activities against *Aspergillus flavus* and *Aspergillus niger*. While the inhibition of *Candida tropicalis*, and *Fusarium oxysporum* was very slow when compared with the other two at the same concentration same days. The activity of the extracts was determined against different pathogenic fungi including. *Aspergillus flavus*, *Aspergillus niger*, *Candida tropicalis*, and *Fusarium oxysporum*. Extracts at 50ppm and 100ppm were the most effective followed by 500ppm which showed moderate activities. The lowest activity was recorded for 1000ppm. The Four fungi differed with regard to their susceptibility to the plant concentration per days. *Aspergillus niger*, was the most susceptible, followed by *Aspergillus Flavus*, *Candida tropicalis* and *Fusarium oxysporum*.^[72] the result suggests that hexane extracts at a given concentration possess an antifungal potential which is effective at 25ppm, 50ppm and 100ppm, hence should be used to treat infections with pathogenic fungi.

6.8. Antidiabetic activity

Effect of *Leptadenia hastata* on glucose: The rate of glucose transport across cell membrane in yeast cells system at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively was reported by Ukwuani and Igbokwu,^[19] in their studies that glucose uptake rate increased with increasing concentration of the plant fraction which was comparable to the standard. Glucose transport across yeast cell membrane occurs via facilitated diffusion down the concentration gradient. The glucose transport occurs only if the intracellular glucose is efficiently reduced or utilized which was agreed by the work of Abirami, *et al.*,^[20] The results obtain suggests that fractions of *Leptadenia hastata*

leaves were capable of enhancing glucose uptake there by controlling the blood glucose level.

Ukwuani and Igbokwu studies on Alpha amylase inhibitory effect of *L. hastata* leaves fraction agrees with the work of Abirami, *et al.*, which state that Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha linked polysaccharide such as starch to yield high levels of glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide.^[20] Ukwuani and Igbokwu went further in their study to acclaim that there was a dose-dependent increase in percentage inhibitory activity against α -amylase enzyme which shows at a concentration 100 μ g/ml. *L. hastata* indicated high percentage inhibition of 87%. Recent advances in understanding the activity of intestinal enzymes (α -amylase and α -glucosidase both are important in carbohydrate digestion and glucose absorption) have led to the development of newer pharmacological agents this agrees with the report of Narkhede, *et al.*, that α - amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates.^[21] They revealed that *Leptadenia hastata* leaves has potential effect on α -amylase enzyme *in vitro*, this agrees with the work of.^[22]

In the study of Bello, *et al.*, on Hypoglycaemic and Hypolipidaemic Effects of *Leptadenia hastata* (Pers.) Decne in Alloxan Induced Diabetic Rats, they reported that diabetic rats treated with methanol and water extracts showed a significant ($p < 0.05$) increase in body weights compared to the diabetic untreated groups which may be due to protein sparing effect.^[38] They went further that the Changes in serum glucose and tissue glycogen content of alloxan-induced diabetic rats treated with methanol and water extract of the leaf of *L. hastata* indicated an elevation in blood glucose level when compared to normal control rats. Thus shows a significant decreased in blood glucose level was observed in diabetic rats treated with methanol and water extracts. The Liver and muscle tissue glycogen level decreased significantly ($p < 0.05$) in diabetic rats when compared to normal control rats. Administration of methanol and water extracts significantly ($p < 0.05$) increased the liver glycogen level, but not muscle tissue glycogen level. However caused a significant decrease ($p < 0.05$) in triglycerides and VLDL-C levels and a significant increase ($p < 0.05$) in HDL-C level in diabetic treated rats. This study indicated that *Leptadenia hastata* reduce the blood glucose level in alloxan induced diabetic rats. Thus becomes a led compounds in the management of diabetes mellitus Sanda, *et al.*, in their study, was designed to evaluate the antidiabetic profile and the hypoglycaemic activity of aqueous root extracts of *Leptadenia hastata* in normal and alloxan-induced diabetic rats model. Eighty five albino rats were used for this study out of this thirty five were used subjected to experimental diabetes by the use of alloxan at a dose of 160 mg kg(-1) body weight. Seven experimental groups

of five rats per group (A-G) were used for this study. A standard antidiabetic drug (insulin) group (B) and normal saline group (G) serves as positive control. The blood glucose lowering activity of the extract, insulin and normal saline groups were monitored at 0, 1, 3, 6, 12 and 18 h post extract administration. On the other hand the remaining fifty albino rats were used to determine the acute toxicity and the hypoglycemic activity of the extract. The blood glucose levels of the rats were monitored at 0, 7, 14, 21 and 28 days post extract administration. Oral administration of aqueous root extract at 600 and 800 mg/kg/bwt have significantly ($p < 0.05$) decreased the blood glucose in diabetic albino rats. On the other hand the hypoglycemic activity of the aqueous root extract on normal rats at dose of 1000 mg/kg/bwt have significantly ($p < 0.05$) decreases blood glucose level in normal albino rats. The results of the current study have demonstrated the antidiabetic and hypoglycaemic effects of *Leptadenia hastata* aqueous root extracts and underscore its potentials in the management of diabetes mellitus especially following prolonged use in days.^[53]

6.9. Antioxidant activity of *Leptadenia hastata*

Abubakar, *et al.*, used the stable radical 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and butylated hydroxyl anisole (BHA) and Ascorbic acid, which are known antioxidants model to evaluate the antioxidant potential of *Leptadenia hastata* solvent extract. The result of the antioxidant activity of *L. hastata* leaves ethanolic extract exhibited a strong antioxidant activity at 0.5, 0.125, and 0.25 concentrations respectively as such 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]

Antioxidative properties of methanol extract and its dichloromethane and ethyl acetate fractions were assessed by using the 1, 1-diphenyl-2-picrylhydrazyl method. The methanol extract showed the stronger radical scavenging activity than dichloromethane and ethyl acetate fractions, with an antiradical power of 5, 3.5 and 2 respectively. The main components isolated from these extracts as friedelin, lupeol and epicathechin were responsible of these activities.

Umar, *et al.*, in their studies on In vitro anti trypanosomal activity, antioxidant property and phytochemical constituents of aqueous extracts of nine Nigerian medicinal plants they reported that *L. hastata* have relatively high percentage of Thiobarbituric acid scavenging activity.^[67] The results of the study suggest the protective mechanism of this plant against various oxidative stresses.

6.10. Antiulcer activity of *Leptadenia hastata*

The aqueous leaf extract (100 mg/kg/bwt-500mg/kg/bwt) of *L. hastata* was evaluated for antiulcer activity in rats. The results revealed a significant reduction in total

acidity, acid volume, and ulcer indices when compared to control animals, suggesting the potential of *Leptadenia hastata* leaves in the treatment of ulcer.^[73]

7.0. Phytochemistry of *Leptadenia hastata*

Leptadenia hastata and other medicinal plants gain recognition as they are the source of a wide range of bioactive chemical entities. The enormous chemical diversity within each plant draws the attention of pharma sector and leads to the development of many unique drugs. The literature survey revealed diverse chemical profile of *Leptadenia hastata*, which included a total of 48 chemical compounds. Following the characterization and isolation of *Leptadenia hastata* extracts, Aquino, *et al.*, in their study on new polyoxyypregnane ester derivatives from *Leptadenia hastata* they reported that plants of the family Asclepiadaceae are known to contain cytotoxic and tumoricidal C/D-cis-polyoxyypregnane esters and glycosides.1-4 during the course of their investigation of new potentially bioactive principles from *Leptadenia hastata* Decne. (Asclepiadaceae), they reported the isolation and structural elucidation of six new C/D-cis-polyoxyypregnane ester derivatives1-6 together with five known esters 10-14 from the less polar fractions of the chloroform extract of *Leptadenia hastata*. Three new glycosides 7-9 which linked one or two sugar units such as D-cymarose and D-oleandrose at C-3 of the aglycon were also present in the more polar fraction of the same extract. 34 out of 48 were isolated from the more polar extracts. They possess sarcostin or deacetylmetaplexigenin as the aglycons and acetyl, benzoyl, cinnamoyl, nicotinoyl, and m-hydroxybenzoyl residues as the ester moieties linked at C-12 and C-20 of the aglycons. The oligosaccharide moiety linked to C-3 of the aglycons was made up of three to five 2, 6-dideoxy-3-O-methylpyranoses, 6-deoxy-3-O-methylpyranoses, or glucose. Compound 3, was identified as 12-O-benzoyl-20-O-cinnamoylsarcostin.^[27]

Leptadenia hastata provided three new C/D-cis-polyoxyypregnane esters 15, 16, 17 and were indicated as unidentified derivatives of the C/D-cis-polyoxyypregnane sarcostin (3â, 8â, 12â, 14â, 17â, and 20-hexahydroxyypregn-5-ene). They reported, compound 17 structure as 20-di-O-acetylsarcostin, 15, as (C₃₇H₄₄O₈) 12-O-cinnamoyl-20-O-benzoylsarcostin and 16 as 12-O-nicotinoyl-20-O-cinnamoylsarcostin (isogagaminin (C₃₆H₄₃O₈N).

Compound 18 and 19 were determined to be penupogenin 3-O-â-D-oleandropyranosyl-(1f4)-â-D-cymaropyranosyl-(1f4)-â-D-cymaropyranoside and compound and 12-O-benzoyl-20-O-cinnamoylsarcostin 3-O-â-oleandropyranosyl-(1f4)-â-D-cymaropyranosyl-(1f4)-â-D-cymaropyranoside.^[27]

Compounds 20, 21, 22, and 23 with molecular formula's as follows C₅₈H₈₈O₂₀, C₆₅H₉₂O₂₁, C₆₃H₉₀O₂₁, and C₆₀H₉₀O₂₁, respectively are tetrasaccharides made up of three 3-O-methyl-2,6-dideoxyhexopyranosyl and a 3-O-

methyl-6-deoxyhexopyranosyl units based on their studies they came with the structure of these compound (20-23) as sugar chain compound determined to be 6-deoxy-3-*O*-methyl- α -D-allopyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranosyl-(1f4)- α -D-cymaropyranoside.

Compound 34-30 were identified from NMR data as five sarcostin derivatives, penupogenin (10) as 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin (3), 12-*O*-acetylsarcostin (1), 12-*O*-nicotinoyl-20-*O*-acetylsarcostin (5), and the new aglycon 12-*O*-benzoylsarcostin and the two deacetylmetaplexigenin derivatives, kidjolanin (12) and metaplexigenin (13). ¹H and ¹³C NMR data suggested that 24-30 are pentasaccharide derivatives by the presence of five anomeric carbon and proton signals, more than one was observed in compounds, as such came up with the structure of the sugar moiety of compounds 24-30 as α -D-glucopyranosyl-(1f4)-6-deoxy-3-*O*-methyl- α -D-allopyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranosyl-(1f4)- α -D-cymaropyranoside.

Compound 31 (Chain L). was assigned the structure 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin 3-*O*- α -D-glucopyranosyl-(1f4)- α -D-thevetopyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranosyl-(1f4)- α -D-cymaropyranoside.

Compounds 32 and 33 were identified as metaplexigenin (13) and cyanforidin (14) by NMR data (1), confirmed 32 was metaplexigenin 3-*O*- α -D-oleandropyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranoside and 33 was cyanforidin 3-*O*- α -D-oleandropyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranoside.^[27]

Compounds 34-39 (Chain G). The aglycon moiety of compounds 34-38 was determined to be that of the known compounds 12-*O*-benzoyl-20-*O*-cinnamoylsarcostin (3), 12,20-*O*-dibenzoylsarcostin (2), 12-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin (11), cyanforidin (14), and 12-*O*-nicotinoyl deacetylmetaplexigenin (6), respectively. The aglycon moiety of compound 39 was a new sarcostin derivative. They have the same tetrasaccharide sugar.

Compounds 40-47 (Chain W). The NMR spectra revealed that the aglycon moieties of 40-46 were 1 (12-*O*-acetylsarcostin), 10 (penupogenin), 5 (12-*O*-nicotinoyl-20-*O*-acetylsarcostin), 15 (12-*O*-cinnamoyl-20-*O*-benzoylsarcostin), 14 (cyanforidin), 13 (metaplexigenin), and 12 (kidjolanin), respectively. The genin of compound 47 presents an unusual acetylation at C-17 and was identified as 12-*O*-nicotinoyl-17-*O*-acetyl-20-*O*-cinnamoylsarcostin.

Compound 40-47 based on ¹H and ¹³C NMR and FABMS spectra revealed that they are pentasaccharides having an identical sugar chain made up of D-cymarose, D-oleandrose, 3-*O*-methyl-6-deoxy-D-allose, and D-

glucose. Thus compound 40-47 was established as α -D-glucopyranosyl (1f4)-6-deoxy-3-*O*-methyl- α -D-allopyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranoside. Were as 48 was penupogenin 3-*O*- α -D-glucopyranosyl-(1f4)-6-deoxy-3-*O*-methyl- α -D-allopyranosyl-(1f4)- α -D-oleandropyranosyl-(1f4)- α -D-cymaropyranoside.^[27]

8.0. Traditional uses

The plant is known to have effective antifungal and antibacterial properties.^[56, 57] The juice of the plant is applied to ring worm and other fungal diseases. The crushed leaves are used as dressing for fresh cuts, wounds and ulcers. Decoction of the leaves of *L. hastata* with the bark of *Erythrina senegalensis* is either taken orally or used as a medicinal bath to treat onchocercosis in Mali.^[46] In Chad, the roots are used to treat scabies.^[47] This plant is commonly used in Hausa-speaking communities in Nigeria as a spice and used in sauces.^[48] Also in Nigeria, local healers use the plant for hypertension, catarrh and skin diseases.^[49] In Burkina Faso, locally it is used for sexual potency (chewing leaves), trypanosomosis (decoction of leaves), skin diseases and wound-healing (application of latex).^[25] In Senegal, the leaves have been reportedly used for lactation and as a purgative by Kerharo and Adam, Arbonnier.^[50, 51] Senegalese healers also use the *L. hastata* for prostate and rheumatism complaints.^[52]

In Chad, the roots are used to treat scabies.^[47] This plant is commonly used in Hausa-speaking communities in Nigeria as a spice and used in sauces.^[48] Also in Nigeria, local healers use the plant for hypertension, catarrh and skin diseases.^[49] In Burkina Faso, it used locally for sexual potency by chewing the leaves. Decoction of the leaves is used in the treatment of trypanosomosis. It is also useful in the treatment of skin diseases and in wound-healing.

9.0. Biotechnology aspects of *Leptadenia hastata*.

A specific strategy approach toward the disappearance of this medicinal plant as a result of misuse or improper way of utilization and because of well-defined pharmacopeia, growing urbanization, indiscriminate collection, and overexploitation of natural resources, many of the important medicinal plants are facing extinction. In order to arrest this alarming situation biotechnology have to come in to salvage the situation such as plant tissue culture as biotechnological approach, this is widely employed as an alternative source to obtain sufficient genuine planting materials for commercial cultivation. In addition, many endangered medicinal plant species can be conserved^[59, 4, 5, 60, 61] and there is a possibility of an increased production of plant secondary metabolites useful in pharmaceuticals, cosmeceutical, and food industries.^[5] A specific strategy is required to produce active principles from *in vitro* cultured cells, as reported by Swammy, *et al.*^[5, 64] The *in vitro* culture system will be very useful for the genetic manipulation studies or large-scale secondary metabolites production.

The expanding interest in the therapeutic potential of *L.hastata* around the globe will result in several biotechnological research activities.

The demand for medicinal plants is growing at the rate of 15 to 25% annually. Currently, the global market for herbal medicinal products is approximately USD 62 billion. It is speculated that this demand will grow up to USD 5 trillion by the year 2050.^[62] Demand for some important medicinal plants has increased to an extent that it has resulted in the rapid loss of biodiversity as well as the extinction of such important species. In recent years, the demand for medicinal and aromatic plants has grown rapidly because of accelerated local, national, and international interest notably from the pharmaceutical, nutraceutical and aroma industries. As such the natural and current practices of propagation are unable to meet the huge demand for this plant, and the species is currently threatened in nature, however a need to the biotechnological studies to increase the production of this plant is paramount.^[63]

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