

Antioxidant and Biological Activities of *Leptadenia Hastataethyl* Acetate Roots Extracts

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Abstract

Background: *Leptadenia hastata* (Pers) Decne is one of the most important medicinal plants in West African traditional medicine for the treatment of inflammation, Ant gonorrhea, stomach ache, hypertension, catarrh, skin diseases, trypanosomiasis and Wound healing. Much attention has been paid to this medicinal plant because of its traditional uses. The aim of the study was to evaluate antioxidant and biological activity of the extract obtained from the ethyl acetate extract of the roots of *Leptadenia Hastata* (Pers.) Decne.

Material and Method: Antioxidant activity of extracts was evaluated by DPPH and the bacterial effect of the plant *Leptadenia hastata* was evaluated for antimicrobial activity in vitro by agar desk diffusion method with four standard bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Kleibilia pneumonia*).

Result: The ethyl acetate possessed strong radical scavenging activity (IC₅₀1.889). The extracts were also effective against all Gram-positive and Gram negative bacteria tested, especially for *Staphylococcus aureus* and *Escherichia coli* with an inhibition potential of 1.03 ± 0.12 and 1.30 ± 0.10 respectively at 500ppm.

Conclusion: These findings give a scientific basis to the traditional usage of *Leptadenia hastata* (Pers) Decne, also showing its potential as rich sources of natural antioxidant compounds. Thus is a potential candidate to be used as an antioxidant agent, for treatment of other ailment as well as infectious diseases.

Keywords: Antioxidant; Roots; DPPH; *Leptadenia hastata*; Bacterial

Introduction

The multiple roles of wild traditional vegetables as both food and medicinal sources have been widely used for health care today, the search for natural compounds rich in antioxidant and antimicrobial properties is escalating due to their medicinal importance in

controlling many related chronic disorders such as cancer and cardiovascular diseases.

Antioxidants aid in the prevention by scavenging the excess free radicals in the body which has increased the interest in the use of antioxidative nutritional substances [1,2] body against some diseases caused by oxidative

imbalan. Numerous studies showed that using of some vitamins, minerals, and other food constituents with antioxidant activity can protect our ce and free radicals such as cardiovascular disease and the aging process [3-6]. Antioxidants can decrease. Natural products are very rich sources of natural antioxidant agents

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. It is a voluble herb with creeping latex stems, glabrescent leaves, glomerulus and racemes flowers as well as follicle fruits. The leaves are up to 10cm long, mostly ovate and light green. The flowers are cream or yellowish green [7]. *Leptadenia hastata* is typically grown in tropical dry lands in sandy soil. Wild foods like this plant provide food security during seasonal changes and are used medicinally in many areas. The plant belongs too many of the root-type famine-food plants; they are drought tolerant and can stay in the soil intact for a long time.

The vernacular names for *Leptadenia hastata* include; hagalhadjar in Arabic and in Chad, yadiya in Hausa in Nigeria and Niger, while in kusume hayla in Ethiopia, ekamongo from Turkana in Kenya, Moore people call it lolongo in Burkina Faso, tarhat or darhat from Wolof in busumba, amata from Jola in Senegal, and Bambara's call it nzongne in Mali [8]. It is a very drought-resistant plant, and can grow with 100 to 450 mm/year. This plant can tolerate high pH and high exchangeable sodium and potassium within the environment [9]. The leaves are edible, young leaves are collected, washed and cooked before consumption. In Alduba village, Hamer-Benaworeda and South-Omo Farmers deliberately grow the species in their homes as food readily available [10].

Local healers use the plant for Ant gonorrhoea, stomach ache, hypertension, catarrh, skin diseases, trypanosomiasis, Wound healing and as vegetable for nutrient [11]. The breeders commonly used the leaf and stems for the treatment of parasites and against placental retention among their animals [12,13]. Most important effects of these plants are because of compounds, such as alkaloids, tannins, flavonoids, terpenoids, saponins, and phenolic compounds. The plants are now in great demand in the developing world for primary health care not because they are inexpensive but because of their therapeutic, performance, better compatibility and acceptability with human body with low toxicity and minimal side effects [14]. The aim of the study was to evaluate antioxidant and biological activity of the extract

obtained from the ethyl acetate extract of the roots of *Leptadenia Hastata* (Pers.) Decne.

Material and Methods

Chemicals

All chemicals used in this research were obtained from SIGMA. Standard antibacterial agent (30µg) tetracycline, antimicrobial susceptibility test discs and Nutrient agar (CM0003) were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG2 8PW, UK.

Preparation of Test Samples

The crude extracts (Ethyl acetate) of *Leptadenia hastata* was used in antibacterial and antioxidant activity, the ethyl acetate crude extracts were tested by disc diffusion method on nutrient agar medium as described by Pundir & Jain [15]. Exactly 3 mg of the crude sample was dissolved homogeneity in 3mL of methanol giving a stock solution of 1000µg/ mL. Different volumes from the stock solution were taken, amounted to 25, 50, 100, 250, 500, 1000ppm each, and dissolved in 5mL of methanol to make final concentration respectively.

Preparation of Agar Plates

Preparation of agar plates was performed based on method described by Pundir & Jain [15] as reported by Umaru IJ, et al. [14]. Nutrient agar was prepared according to manufacturer's instruction with 14 g of dried agar dissolved in 500mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at 121°C. The agar solution was then poured into a sterile petri plate and allowed to cool down and forming a gel. The plate was divided into eight sections by making a line marking on the outside surface of the plate. The eight sections were for each test samples namely the 25, 50, 100, 250, 500, 1000ppm samples, tetracycline 30µg (positive control) and methanol (negative control). The plate was sealed using parafilm and keep chilled at 4°C upon bacteria inoculation.

Preparation of Bacteria Broth

Several selected bacteria were used to evaluate the antibacterial activities of the crude extracts of *Leptadenia hastata*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Kliebselia pneumonia* were obtained from the stock culture provided by Virology Laboratory, University Malaysia Sarawak, the nutrient broth was prepared according to manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200mL distilled water followed

by sterilization in autoclave at 121°C. The bacterial was sub cultured in a 10mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with shaker at 37°C [16]. After 16 hours' incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use [17]. Measurement was performed at wavelength 575 nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

Plate Inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on method described by Umaru IJ, et al. [14]. Approximately 1mL of the ready bacterial broth was transferred into mini centrifuge tubes. A sterile cotton swap was dipped into the mini centrifuge tube containing bacteria broth and streaked over entire of the agar plate surface, performed in 4 different directions. The agar plate was then left for 5-10 minutes before applying the test samples. The disc used was 6 mm diameter. A volume of 10µL of the test samples of concentration 25, 50, 100, 250, 500, 1000ppm were each pupated onto the discs and placed onto the agar plate by using sterile forceps and gently pressed to ensure contact. Next to be placed on the agar plate was the disc pupated with methanol as negative control, followed by 30µg of tetracycline as standard antibacterial agent (positive control). The plates were left at room temperature for 10 minutes to allow the

diffusion of the test samples and the standards into the agar. Each crude extract was tested in triplicate for each bacterium used. The plate samples were then incubated at 37°C for 24 hours before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter to indicate the presence of antibacterial activity for each sample, as compared to the positive control.

Statistical Analysis

Experimental results are expressed as means \pm S.D. All measurements were in triplicate. The analysis was done using the software-SPSS one-way ANOVA. The IC₅₀ values were calculated using Prism 6 software.

Result and Discussion

Results

The issue of antibacterial resistance emanating has led to the search for new antimicrobial agents. Due to the problem of microbial resistance to antibiotics, attention is given toward biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antimicrobial activities [14]. Our search for antioxidant and antibacterial activity from this plant *Leptadenia hastata* ethyl acetate extract revealed an antioxidant and antibacterial activity. The results of antioxidant and antibacterial tests of the plant extracts are as listed in Tables 1 & 2 below.

Results

Plant parts	Crude Extracts	Calibration equation	R ²	IC ₅₀ (µg/mL)	Log IC ₅₀
Roots	Control	2.638x - 0.03159	0.9937	57.89	1.763
	Ethyl acetate	7.435x - 0.02965	0.9886	79.34	1.889

Table 1: IC₅₀ value of Roots.

Extract	Control	25ppm	50ppm	100ppm	250ppm	500ppm	1000ppm
<i>Staphylococcus aureus</i>	2.05 \pm 0.02	0.47 \pm 0.06	0.60 \pm 0.10	0.77 \pm 0.06	0.90 \pm 0.00	1.03 \pm 0.12	1.20 \pm 0.10 ^b
<i>E. coli</i>	2.06 \pm 0.01	0.77 \pm 0.06 ^a	0.90 ^a \pm 0.00	0.83 ^a \pm 0.06	1.07 \pm 0.15	1.30 \pm 0.10 ^a	1.23 \pm 0.12 ^a
<i>Salmonella typhi</i>	2.06 \pm 0.10	0.43 \pm 0.06	0.50 \pm 0.10	0.43 \pm 0.06	0.57 \pm 0.06	0.67 \pm 0.06	0.73 \pm 0.06
<i>Kleibsilia pneumonia</i>	2.07 \pm 0.03	0.45 \pm 0.07	0.53 \pm 0.06	0.57 \pm 0.06	0.77 \pm 0.06	0.77 \pm 0.06	0.80 \pm 0.10 ^b

Table 2: Effect of Ethyl-acetate Root Extract of *Leptadenia hastata*.

Values are Mean \pm SD for three determinations

a. Significantly (p < 0.05) higher compared to different extract at the same concentration

b. Significantly (p < 0.05) lower compared to the control

Discussion

The study revealed in DPPH radical scavenging activity model, ethyl acetate extracts showed high activity than standard compound. IC50 values of the samples was 1.889µg/mL while the IC50 values for ascorbic acid, was 1.763µg/mL. The polyphenolic content in the extracts may correlate to their good DPPH radical scavenging activity. It has been assumed that phenol and flavonoid can quench DPPH radical via hydrogen- or electron-donation mechanism and changes its colour from violet to yellow. Antioxidant is defined as any substances which are able to perform this reaction.

The antibacterial activity of the extract against the tested species of microorganisms (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsella pneumonia*) between concentration ranges for 25 to 1000ppm. The studies revealed that the extracts possess antibacterial activity in a concentration dependent manner against the test organisms and were comparable with the standard drug.

According to the results of disc diffusion assay, this root extract of the plant *Leptadenia hastata* has active compounds that are effective for the prevention of infections caused by these bacteria *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Klebsella pneumonia*. The inhibition yielded at concentration 25-250ppm was found to be active against all tested strains under study. However, the extract showed higher significant antibacterial activity against *Escherichia coli*, and *Staphylococcus aureus* over the other pathogens with an inhibition value of $1.20 \pm 0.10\text{mm}$ and $1.23 \pm 0.12\text{mm}$ respectively. Though, the extract showed moderate activity at all concentration on the bacterial species, the potency exhibited less significant activity against *Escherichia coli* and *Klebsella pneumonia*. However, the antioxidant potential of this which showed to be high than the control may account for the potent antibacterial activity of the extract. This implies that it may also possess potent antibacterial activity.

Conclusion

The result of this study showed that *Leptadenia hastata* (Pers) Decne extract contains bioactive components. Potentially, these compounds have the most important applications against human pathogens. The results of tests from various concentration and the presence of antioxidant component of the leaf extract indicate that the leaves have some measurable inhibitory action against gram-positive and gram negative bacteria.

Conflict of Interests

All authors have none to declare.

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