Antioxidant Properties and Antibacterial Activities of *Leptadenia Hastata* Leaves Extracts on *Staphylococcus Aureus*

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**Abstract**

*Leptadenia hastata* extracts were evaluated for antibacterial activity and antioxidant properties. The leaf of the plant was extracted with n-hexane, dichloromethane, ethyl acetate, chloroform and methanol to give respective extracts. The Antibacterial activity against *staphylococcus aureus*, was determined by disc diffusion method. Antioxidant activity was assayed by the DPPH radical scavenging activity mechanism. The results showed that of n-hexane, ethyl acetate, dichloromethane, chloroform and methanol extracts of *Leptadenia hastata*, methanol extract and chloroform extracts displayed more activity with 1.10 ± 0.10ab and 0.97 ± 0.06ab than others at 25-1000ppm/desk of the extracts tested. The most antioxidant activities against DPPH were displayed by the hexane and methanol extracts of *Leptadenia hastata* leaf, exhibiting IC50 (63.44µg/mL) and IC50 (46.80µg/mL) respectively followed by chloroform, dichloromethane and lastly ethyl acetate crude extract. The present results showed potential of the medicinal plant used by traditional herbal medical practitioners as natural anti-microbial, antioxidant and potentially anti-inflammatory agents. The results confirm that *Leptadenia hastata* leaves extracts can be used as a source of drugs to fight infections caused by susceptible bacteria.

**Keywords:** *Staphylococcus aureus; Leptadenia hastata; 2, 2-diphenyl-1-pycryl-hydrazyl*

**Introduction**

In recent years, there has been increasing awareness about the importance of medicinal plants. Drugs from these plants are easily available, inexpensive, safe, efficient, and rarely accompanied by side effects. Plants which have been selected for medical use over thousands of years constitute the most obvious starting point for new therapeutically effective drugs such as anticancer drugs [1] and antimicrobial drugs [2]. Recently, medicinal plants usage has increased in spite of the advances made in the field of chemotherapy. The reasons proposed [3] are the use of medicinal plants as materials for the extraction of active pharmacological agents. There is also the increased use of medicinal plants in industrialized countries for medicinal purposes. *Leptadenia hastata* despite the extensive uses, there have been only limited attempts to explore the biological activities of the plants in relation to their medicinal uses. Here, we present data on antibacterial activities and antioxidative potentials of the different extracts obtained from n-hexane dichloromethane, ethyl acetate, chloroform and methanol. *Leptadenia hastata* (Pers.) Decne, which belongs to the family Asclepiadaceae, is a wild plant used as vegetable by many African populations and medicine due to its nutritive and therapeutic properties for the treatment of wounds and stomach upset conditions in children [4,5]. The plant *Leptadenia hastata* is an edible non-domesticated valuable herb with creeping latex stems, glabescent leaves, glomerulus and recemes flowers as well as follicle fruits. It is typically grown in tropical dry land in sand soil [6].
The plant is commonly used in the north Nigeria as spices and used as sauces [7], as a vegetable in Niger republic [8-11]. Local healers also use the plant for hypertension, catarrh and skin diseases [12]. In certain areas of West Africa, breeders claimed the antifertility effect of their animals after consumption of Leptadenia hastata leaf [13] this paper presents an investigation of Leptadenia hastata (Pers) Decne, antibacterial and Antioxidant. Which is one of the contributing factors responsible for the antimicrobial activity of the extracts of the leaves on Staphylococcus aureus species? The findings in this study may contribute to the present literature in understanding the bioactive value of the crawling plant Leptadenia hastata.

Material and Method

Sample Collection

Leptadenia hastata leaves: freshly leaves of Leptadenia hastata were collected from the uncultivated farmland of the Federal University Wukari Taraba State, Nigeria and was authenticated at Ahmadu Bello University Zaria and Voucher No PU: 2 ABU Herbarium No 900220. The plant Leptadenia hastata (yadiya) was dried under room temperature.

Preparation of Samples

Fresh leaves of the plant Leptadenia hastata was washed with distilled water to remove the soil and dust particles, they are thoroughly air dried and powdered using laboratory grinder machine (FGR-350, Quest Scientific). Extraction using hexane by placing 150g of the powdered samples into an Erlenmeyer flask and hexane three times the weight of the extracts was added, the solution was covered and shaken at an interval of an hour and then allowed at room temperature to stand for 7 days. The mixture was then filtered using Whatman filter paper No.4 the residue was re-extracted with fresh hexane for another 72 h and filtered. Both extracts were combined and concentrated with a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure to obtain the hexane crude extract. The residues were re-extracted using a similar procedure with dichloromethane (CH2CLl2), followed by ethyl acetate (C2H5COOH), chloroform (CHCL3), and methanol (MeOH) to obtain dichloromethane, ethyl acetate, chloroform and crude extracts, respectively. The dry weight and yield of each crude extracts were determined. It was then stored under a frozen condition until required.

DPPH (2, 2-diphenyl-1-pyrryl-hydrazyl) Free Radical Scavenging Assay

The free radical scavenging assay of compound 2, 2-diphenyl-1-pyrryl-hydrazyl (DPPH) was used to evaluate the antioxidant properties of the crude extract and the essential oil. The measurement was based on the method described by [14]. The sample was prepared by diluting 6 mg of crude extract into 6 mL of methanol, producing a concentration of 1000 μg/mL. The stock solution was sonicated to ensure the homogeneity of the sample. Five other concentrations were prepared at 10, 50, 100, 500 and 1000 μg/mL, diluted from the 1000 μg/mL stock solution. Sample of 5000 μg/mL was prepared separately by diluting 25 mg of crude extract into 5mL of methanol. Approximately 3 mL of 0.1 mM solution of 2, 2-diphenyl-1-pyrryl-hydrazyl (DPPH) in methanol was each added into six series of prepared concentrations (10, 50, 100, 500, 1000 and 5000 μg/mL) of sample solutions (1mL). Analysis was done in triplicate. The solution was mixed vigorously and left to stand at room temperature for 30 minutes in the dark after which its absorbance was measured spectrophotometrically at 517nm using Jasco ultra violet spectrophotometer model V-630. Methanol was used as blank (only methanol) and negative control (1mL methanol mixed with 3mL DPPH), while ascorbic acid (vitamin C) as the standard. The concentration of the sample required to inhibit 50% of the DPPH free radical was calculated as IC50 and the value was determined using Log dose inhibition curve which performed by using PRISM version 3.02 software, based on the calculated values of the DPPH scavenging activity (%) of the sample [15]. DPPH scavenging activity (%) was calculated with formula = A0-A1 / A0 x 100, where A0 was the absorbance of the control, while A1 was the absorbance in presence of the sample.

Preparation of Test Samples

The crude extracts of Leptadenia hastata was used in antibacterial assay namely the hexane, dichloromethane, ethyl acetate, chloroform and methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by Ram Kumar & Pranay [16]. The plant exactly 5mg of each crude sample was dissolved homogeneity in 5mL of methanol giving a stock solution of 1000 μg/mL. Lower concentration of 25, 50, 100, 250, 500, and 1000ppm, i.e. six different volumes from the stock solution were taken for the studies.

Preparation of Agar Plates

Preparation of agar plates was performed based on method described by Ram Kumar & Pranay [16]. Nutrient agar was prepared according to manufacturer’s instruction with 14g of dried agar dissolved in 500 mL 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 six sections were for each test samples namely the 25ppm, 50ppm,100ppm, 250ppm, 500pp and 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

The selected bacteria were used to evaluate the antibacterial activity of the crude extracts of Leptadenia hastata, Staphylococcus aureus (ATCC©25923) were obtained from the stock culture
provided by Virology Laboratory, Universiti Malaysia Sarawak. The nutrient broth was prepared according to manufacturer’s instruction, with 2.6g of the dried broth dissolved in 200 mL distilled water followed by sterilization in autoclave at 121°C. The bacterial was sub-cultured in a 10 mL of broth, each in universal glass vail bottle for 16 hours inside an incubator equipped with shaker at 37°C [17]. 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 [18]. The measurement of the optical density was performed at wavelength 575nm 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 Ram Kumar & Pranay [16]. Approximately 1mL of the ready bacterial broth were 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 10 μL of the test samples of concentration 10, 25, 50, 100, 250, 500 and 1000μg/mL 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 the 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 (mm) to indicate the presence of antibacterial activity for each sample, as compared to the positive control.

**Statistical Analysis**

Statistical analysis for antibacterial activities was performed using SPSS programme. The IC50 for DPPH free radical scavenging assay was statistically determined using Log dose inhibition curve in PRISM programme.

**Calibration equation = hillslope value X - bottom value**

\[
\% \text{ (Radical scavenging activities)} = \left[ \frac{1 - (\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} \right] \times 100
\]

**Results and Discussion**

The development of antibacterial resistance to presently available antibiotics has led to the search for new antimicrobial agents [19]. 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 [20]. Our search for antioxidant potential and antibacterial bioactivity from tropical medicinal plant revealed the antioxidant and antibacterial activity of five different solvent leaf extracts of *Leptadenia hastata*. Results of antioxidant and antibacterial tests of the plant extracts are as listed in Tables 1 & 2 above.

**Table 1:** IC50 value of Leaf of *Leptadenia hastata*.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Crude Extracts</th>
<th>Calibration equation</th>
<th>R2</th>
<th>IC50 (µg/mL)</th>
<th>Log IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Control (Ascorbic acid)</td>
<td>3.629x + 0.04310</td>
<td>0.9657</td>
<td>61.96</td>
<td>1.792</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>1.668x + 0.02519</td>
<td>0.9128</td>
<td>63.44</td>
<td>1.802</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>2.037x + 0.02548</td>
<td>0.9070</td>
<td>43.63</td>
<td>1.640</td>
</tr>
<tr>
<td></td>
<td>Ethylacetate</td>
<td>2.952x - 0.02048</td>
<td>0.9703</td>
<td>41.97</td>
<td>1.623</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>2.474x - 0.00846</td>
<td>0.9929</td>
<td>44.75</td>
<td>1.651</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>2.517x + 0.01401</td>
<td>0.9923</td>
<td>46.80</td>
<td>1.670</td>
</tr>
</tbody>
</table>

**Table 2:** Effect of Leaf Extract of *Leptadenia hastata* on *Staphylococcus aureus*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Control</th>
<th>25ppm</th>
<th>50ppm</th>
<th>100ppm</th>
<th>250ppm</th>
<th>500ppm</th>
<th>1000ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>2.04±e02</td>
<td>0.73±.06</td>
<td>0.93±.15</td>
<td>0.97±.06</td>
<td>1.03±.06</td>
<td>1.10±.10</td>
<td>1.27±.06b</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>2.05±e01</td>
<td>0.60±.00</td>
<td>0.90±.10</td>
<td>1.00±.10</td>
<td>1.10±.10</td>
<td>1.23±.06</td>
<td>1.33±.06b</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>2.03±.02</td>
<td>0.67±.06</td>
<td>0.77±.06</td>
<td>0.87±.6</td>
<td>0.97±.06</td>
<td>1.03±.06</td>
<td>1.10±.10</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2.04±e01</td>
<td>0.67±.06</td>
<td>0.80±.10</td>
<td>0.90±.20</td>
<td>1.03±.12</td>
<td>1.03±.06</td>
<td>1.13±.15</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.06±e01</td>
<td>0.73±.23</td>
<td>0.60±.00</td>
<td>0.83±.06</td>
<td>1.03±.06</td>
<td>1.06±.06</td>
<td>1.16±.06b</td>
</tr>
</tbody>
</table>

Values are Mean ± SD for three determinations

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

bSignificantly (p< 0.05) lower compared to the control.

Discussion

According to the results of disc diffusion assay, this leaf of the plant *Leptadenia hastata* has active compounds that are effective for the prevention of infections caused by *Staphylococcus aureus*. There are a number of factors that could influence the results of the disc diffusion assay. Firstly, the diameter of the zones is affected by the rate of diffusion of the antimicrobial compound [21,22] and thus may not exactly represent the potency of the extract's antimicrobial activity. Where studies of plant extracts are concerned, the disc preparation technique could present with another problem wherein the extract was not properly and evenly impregnated into the paper discs. Another important factor is the standardization of the inoculum size to 0.5 McFarland turbidity. This inoculum size is important to ensure confluent or almost confluent lawn growth as a smaller inoculum size (such that single colonies are seen) may produce falsely large inhibition zones while a bigger inoculum size (thick bacterial lawn) may produce falsely smaller zones instead [23]. However, in the assay against *Leptadenia hastata* leaf extracts, i.e. n-hexane dichloromethane, ethyl acetate chloroform and methanol it displayed progressive activity at 25-1000 ppm. The methanol extract of *Leptadenia hastata* showed the high effect with activity indices of 0.73 ±0.23 at 25ppm and 1.16 ± 0.06b 1000ppm but was highest with hexane extract 1.27±0.06b at 1000ppm against *staphylococcus aureus*, relative to the control tetracycline, a standard drug (Table 2). This plant *Leptadenia hastata* has also been reported to play an important role in other human diseases, the plant is a good source of various secondary metabolites, which shows growth inhibition effect against bacteria, fungi, viruses, and tumours [24]. It is also used as laxative, antipyretic and expectorant, and in the treatment of infantile diarrhea and malarial intermittent fevers [25]. Stomach problems, diarrhea, gonorrhea, malaria, cough, catarhral conditions, diabetes, and as galactogogue [26]. In the management of onchodercosis, scabies, hypertension, catarh, skin diseases, sexual potency, and wound-healing [27].

These studies is in agreement with the report of Alero and wara [28] that Methanol and water extracts from the leaves of *Leptadenia hastata* showed antibacterial activities. However, the activity observed from the hexane, dichloromethane, ethyl acetate and chloroform were all active in ascending order when compared with the drug control tetracycline. The action of *Leptadenia hastata* on *staphylococcus aureus* is instructive.

The growth of *staphylococcus aureus* was inhibited by these extracts at the tested concentration as shown in Table 2. The activity of this plant extracts against the gram positive bacteria is quite responsive the methanol and hexane extract were more active than the chloroform, dichloromethane and ethyl acetate extracts with increase in concentration. This result is however suggesting the possibility for the treatment of diseases caused by this microorganism *staphylococcus aureus* with increase in the concentrations. Antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of samples [29,30]. The antioxidant activities of leaf extracts of medicinal plants are given in Table 1. The results are shown as the relative activities against the standard ascorbic acid. The results showed that for n-hexane extracts tested it has the highest antioxidant activity of the leaf extract with DPPH inhibition of IC50 (63.44µg/mL).

The second most antioxidant activities against DPPH were displayed by the methanol extracts showing IC50 (46.80µg/mL) which followed by chloroform, dichloromethane and lastly ethyl acetate crude extract. In comparison, the standard ascorbic acid showed IC50 (61.96µg/mL) DPPH inhibition in the assay. The screening and characterization of antioxidants derived from natural sources has gained much attention and efforts have been put into identifying compounds as suitable antioxidants to replace synthetic ones [31]. The presence of *Leptadenia hastata* leaf extract with potent antioxidant as shown in Table 1 continues to be of great importance in the search for remedies against free radical-mediated diseases, prevention of oxidative reactions in foods, protection against DNA damage, anti-bacterial, and anti-fungal properties

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 bacteria such as *Staphylococcus aureus*.

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References


