Antimicrobial Activity of Ethanolic Leaf Extract of *Lawsonia inermis* (Henna) Against Some Selected Bacteria

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**ABSTRACT**

The emerging resistance of pathogenic microorganisms against the currently available antimicrobial agent in the market demands the exploration of new antimicrobial agent. *Lawsonia inermis* (Henna) a very useful medicinal plant that has been adopted by many cultures in Africa, Asia and Latin America as source for certain illness, the leaves are very popular natural dye to color hand, finger, nails and hair. However, the antimicrobial activity of leaf extract of *Lawsonia inermis* was investigated against some selected bacteria including: *Staphylococcus aureus*, *Bacillus subtilis*, *Corynebacterium specie*, *Escherichia coli*, *klebsiella pneumoniae*, and *Pseudomonas aeruginosa* using standard method of sample collection and analysis. However, *Salmonella* and *Streptococcus pyogenes* develop resistance against all concentrations of the extract. The extract showed highest activity with *Bacillus subtilis* with zone of inhibition of 17mm; this is followed by *Pseudomonas aeruginosa* with zone of inhibition of 14mm. lowest activity was observed in *Corynebacterium* with zone of inhibition of 7mm at 300mg/ml respectively. The plant extract revealed maximum effect on *Bacillus subtilis* with inhibitory concentration of 150mg/ml and minimum effect in *staphylococcus aureus* and *Corynebacterium specie* with 300mg/ml and zone of inhibition of 7mm respectively. Several works including that of Sharma has showed similar results. The outcome of this analysis showed that *L. inermis* could be design for treatment of some infectious agents.
INTRODUCTION

The emerging resistance of pathogenic microorganisms against the currently available antimicrobial agent in the market demands the exploration of the new antimicrobial agent. The use of the natural medicinal plant as a substitute is the paramount area of research to overcome the drug resistance of infectious agent. A scientist has not made enough on the evaluation of the safety of medicinal plant yet; however, most regarded as safe and is already used as food or food additive without perceived side effect (Newman, 2000).

*Lawsonia inermis* is a scientific name of a tall shrub plant commonly known as Henna, or mehndi tree is a flowering plant, having a height of 5 meters, natural to subtropical and tropical regions of the world including South Asia, Africa, and Oases of Sahara Desert and even in Northern regions of Australia. Leaves of henna plant are Oval-shaped and smooth. Leaves have a length of 2-3cm with 1-2 cm width. Henna shrubs are highly branched and possess greyish brown barks. Main chemical constituents of henna are Lawson (2-hydroxynaphthoquinone) mucilage, Mannite, gallic acid, and tannic acid. Henna is known to be utilized as a cosmetic agent for dyeing hair, nails as well as skin (Pathirana et al, 1992; Cown, 2009). Studies has shown that L. inermis have bioactive free radical (Philip et al, 2011)

Medicinal plants are prospective renewable natural resources and are generally considered to play a favorable role in the improvement of human health care. Medicinal plants are known as nature’s best gift to cure some of diseases of men and animals. There are about 121 clinically useful prescription drugs that are worldwide derived from higher plants out of which about 70 percent of them came to the attention of pharmaceutical houses because of their use in traditional medicine (Abelson, 1990).

In the earlier period, medicinal plants were used intensively in ethnic medicine for the management of various disorders. Today, it is estimated that about 80% of people in developing countries rely on traditional medicines for their primary health care. Traditional medicines are
becoming popular, due to high toxicity and undesirable effect of conventional medicament. This has led to a sudden increase in the number of green industries in the drug market. Several plant species are used by various indigenous systems such as Siddha, Ayurveda, Unani and Allopathic for the treatment of different ailments. (Farnworkert et al., 1985).

Henna grows better in tropical savannah and tropical arid zones, in latitude between 15° and 25°N and S, produces highest dye content in between temperature 35 to 45°c.the optimal soil temperature ranges for germination are 25 to 30°c.

The seeds of henna have been reported to possess deodorant action and are used in most cases of gynecological disorder such as Menorrhagia, vaginal discharge and leucorrhoea (Newman et al., 2000). The leaves of Lawsonia inermis with these of Hibiscus rosa-sinensis, Eclipta prostrata and seeds of Abrus precatorius when they are taken in equal quantities and ground into a paste which is soaked in sesame oil for five days is used as hair oil by the tribes of Andra Pradesh. The powdered roasted seed is mixed with gingelly oil to make a paste which is used for the treatment of ringworm. A concoction of the leaves is used for aseptic cleaning of wounds and healing (Kumari et al., 2011). Flowers are used in cephalalgia, burning sensation, sardiopathy, anemia, insomnia and fever. A seed, flowers, leaves and, roots are used in ayurvedic medicinal systems in the management of a range of disease. Leaves on the other hand are valuable for the treatment of dysentery, diarrhea, leprosy, as well as boils (Prajapati et al, 2007).
METHODOLOGY

SAMPLE COLLECTION
The sample (Lawsonia inermis) were purchased from Monday Market, Nigeria and was confirmed by the Herbarium unit of the University of Maiduguri. This was ground into the powder using pestle and mortar immediately the sample was taken to the laboratory for extraction. The organisms were collected from a clinical case at the University of Maiduguri Teaching Hospital, Nigeria.

EXTRACTION
Soxhlet extraction apparatus was employed, 500g of powdered seed was used in a Soxhlet apparatus and boiled in respective solvents of ethanol for 30 minutes and extracted for 3h. After extraction, the solvents were removed by rotary vacuum evaporator at 40ºC and dried in a vacuum oven for 2h. (Udaya and Sankar, 1989)

DETERMINATION OF AN ANTI-BACTERIA ACTIVITY
The anti-Bacterial activity was determined using the disc diffusion method. A disc of blotting paper of 6mm was impregnated with Lawsonia inermi’s leave extract and place on plate of sensitivity testing agar uniformly inoculated with the test organism and place in an incubator for 24 hours. The extracted diffuse from the disc into the medium. Organism sensitive to extract are inhibited at a distance from the disc whereas resistant strains show no zone of inhibition or grow up to the edge of the disc (Monica, 2006).

MINIMUM INHIBITORY CONCENTRATION (M.I.C)
The leaf extract was made to obtain 50mg/ml 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml, 300mg/ml, 350mg/ml 400mg/ml, 450mg/ml and 500mg/ml. The various concentrations of extract was further impregnated into the 6mm disc and place on growth testing medium, it was incubated at 37ºC for 24 hours, and zone of inhibition was read in millimeter (mm). The minimum concentration that shows activity was taken as the MIC (Monica, 2006).
RESULT PRESENTATION

Antimicrobial activity of ethanolic extract of *lawsonia inermis* against some bacterial isolates

Table 1: Antimicrobial Activity.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ethanolic extract activity</th>
<th>zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>9mm</td>
</tr>
<tr>
<td><em>Streptococcus pyogens</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>+</td>
<td>17mm</td>
</tr>
<tr>
<td><em>Corynebacterium specie</em></td>
<td>+</td>
<td>9mm</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
<td>12mm</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>+</td>
<td>12mm</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>+</td>
<td>14</td>
</tr>
</tbody>
</table>

- Keys: + Effective, - Resistance
TABLE 2: MINIMUM INHIBITORY CONCENTRATION (M.I.C)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MIC (mg/ml)</th>
<th>zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>300</td>
<td>7mm</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>150</td>
<td>7mm</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>300</td>
<td>7mm</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>200</td>
<td>7mm</td>
</tr>
<tr>
<td>Klebsiella pneumonae</td>
<td>200</td>
<td>7mm</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>200</td>
<td>7mm</td>
</tr>
</tbody>
</table>

DISCUSSION

The results obtained from the antimicrobial activity of *Lawsonia inermis* by ethanol extract shows activity against six (6) out of eight (8) organisms that were analyzed, i.e., *Staphylococcus aureus, Bacillus subtilis, Corynebacterium specie, Escherichia coli Klebsiella pneumonae, and Pseudomonas aeruginosa*. However, *Salmonella* and *Streptococcus pyogenes* develop resistance against all concentrations of the extract.

The extract showed the highest activity with *Bacillus subtilis* with a zone of inhibition of 17mm, this is followed by *Pseudomonas aeruginosa* with a zone of inhibition of 14mm, lowest activities was observed in Corynebacterium and *Staphylococcus aureus* with a zone of inhibition of 9mm at the same concentrations respectively. The plant extract revealed the maximum effect on *Bacillus subtilis* with the activity of 150mg/ml and minimum effect in *Staphylococcus aureus* and *Corynebacterium species* with a concentration of 300mg/ml and zone of inhibition of 7mm respectively.

The results of this research work conform the work of (Sharma et al., 1995), states that *Lawsonia inermis* have anti-bacteria activity against both gram-negative and gram-positive bacteria. Similarly, Bhuvaneswari (2002), discovered that *Lawsonia inermis* has activity against *Staphylococcus aureus* and *Escherichia coli Klebsiella pneumonae*.
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