A comparative study of the antimicrobial properties of the ethanolic extracts of *Landolphia owariensis* leaf and root


Department of Biochemistry, School of Science, Federal University of Technology, Owerri, Nigeria.

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The leaf ethanolic extract (LEEX) and root ethanolic extract (REEX) of *Landolphia owariensis* were comparatively subjected to phytochemical and antimicrobial screening. Susceptibility of some clinical isolates (*Staphylococcus* spp., *Proteus* spp. and *Escherichia coli*) to the extracts was determined using dehydrogenase assay method. The phytochemical screening indicated the presence of alkaloids, flavonoids, saponins and tannins in both the leaf and root extracts with cyanogenic glycosides present in the root extract only. The response of the bacterial strains to the leaf and root extracts was concentration dependent. Dehydrogenase activity was progressively inhibited by the root extract at a higher concentration range (150 – 2500 µg/ml) than the leaf extract (20 – 2000 µg/ml). LEEX gave an IC$_{50}$ of 20, 200, and 550 mg/ml against *Staphylococcus* spp., *Proteus* spp. and *E. coli*, respectively, while REEX gave an IC$_{50}$ of 340, 320, and 1560 mg/ml against the organisms, respectively. The LEEX exhibited higher antibacterial activity than the REEX. Susceptibility of the organisms to the extracts showed: *Staphylococcus* spp. > *Proteus* spp. > *E. coli*. The observed antimicrobial properties could be due to the presence of alkaloid, flavonoids and tannins, which were identified in both extracts. The findings may be of clinical relevance and further substantiates the traditional use of *L. owariensis* leaves and roots as antimicrobial agents.

**Key words:** Ethanolic extract, *Landolphia owariensis*, dehydrogenase activity.

**INTRODUCTION**

There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing countries (Murray, 1998). The use of plants and their natural products in Nigeria as either extract or infusion is a widespread practice in the treatment and management of diseases (Iwu, 1982).

Medicinal plants contain accumulated natural products, biologically active materials and ingredients which have various effects. These active ingredients representing the value in use are produced by biological synthesis in the plant in very small concentrations of the dry material content of the plant (Hornok, 1992). Some of these active ingredients accumulate in certain parts of the plant. It is only those portions of these plants that contain active ingredient that are used in therapeutic purposes. The part that contains the active ingredient is taken in the form of extract, infusion and decoction (Odebiyi and Sofowara, 1979).

*Landolphia owariensis* belong to the family Apocynaceae commonly called vine rubber and known locally by various names; Eso/Utus (Ibo language), Mba (Yoruba language) and Ciwa (Hausa language). It is commonly found in the rain forest region of Nigeria and other parts of African. *L. owariensis* is one of the plants whose leaves and roots are used in herbal medical practice. Different parts of this plant are used for the treatment of many ailments. The decoction of leaves is used as purgative and to cure malaria. The root is soaked in local gin for a week and the extract given two glasses a day to cure gonorrhea (Gill, 1992). Owoyele et al. (2002) also reported that aqueous, methanol and chloroform extracts of *L. owariensis* leaves have anti-inflammatory and anal-
gesic activities. Lewis and Lewis (1977) also reported the use of stem bark as vermifuge. The latex is used as an enema for intestinal worms in parts of Ivory Coast. The latex is also used as a natural preservative (Anthony, 1995).

This work was undertaken to compare the phytochemical and antimicrobial properties of the leaf and root extracts of *L. owariensis* on three clinical bacterial isolates so as to validate or otherwise the claim of the herbalists who use them as antimicrobial agent. The study will make for more economic and optimal use of the plant in alternative medicine.

**MATERIALS AND METHODS**

**Preparation of plant materials**

The leaves and roots of *L. owariensis* were collected from their natural habitat in Otulu Ahiazu Mbaise, Imo State, Nigeria, in the month of April, 2006. The plant was identified by Dr. S. E. Okeke, a plant taxonomist of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria.

**Extract preparation**

The fresh leaves and roots of *L. owariensis* were sun dried for ten days to a constant weight. The dried leaves and roots were separately ground into powder using a mechanical grinder. One hundred grams each of the leaves and root powder were weighed and soaked in 500 ml of 95% ethanol in a conical flask. This was covered, shaken every 30 min for 6 h and then allowed to stand for another 48 h. The solution was subsequently shaken and filtered using Whatman number 1 filter paper. The filtrate was evaporated to dryness using a rotary evaporator (Model type 349/1, Corning Limited). The extracts were stored at 4°C in a refrigerator before use.

**Phytochemical studies**

Phytochemical test for the presence of alkaloids, flavonoids, saponins, tannins and cyanogenic glycosides were carried out as described by Trease and Evans (1989).

**Isolation of test organisms and cultural conditions**

Three clinical bacterial isolates of *Escherichia coli*, *Staphylococcus* spp. and *Proteus* spp. obtained from the Pathology Department of the Federal Medical Centre, Owerri, Nigeria were used for the study. They were isolated and purified on nutrient agar plates and characterized by the use of standard microbiological and biochemical methods as described by Holt et al. (1994).

The bacterial isolates were grown to mid exponential phase in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2°C). The cells were later harvested by centrifugation at 1000 g for 10 min and washed thrice in distilled water. The washed cells were re-suspended in distilled water and the turbidity adjusted to an optical density of 0.85 at 420 nm. An aliquot of 0.2 ml of the cell suspension was used as inoculum in the dehydrogenase assay. The dry weight of cells was determined by drying a 20 ml aliquot of cell suspension in a pre-weighted crucible to constant weight in an oven at 110°C.

**Evaluation of antimicrobial activity**

Dehydrogenase assay method as described by Praveen-Kumar (2003) was adopted for the study. The dehydrogenase activity (DHA) was determined using 2, 3,5-triphenyl tetrazodium chloride (TTC) as the artificial electron acceptor, which was reduced to the red coloured triphenylformazan (TPF). The assay was done in 5 ml volume of nutrient broth – glucose – TTC supplemented with varying concentrations of the ethanolic leaf and root extracts respectively in separate screw-capped test tubes. About 0.2 ml volume of the bacterial suspension was inoculated into triplicate glass tubes containing 3.7 ml of phosphate buffered (pH 7.2) nutrient broth – glucose medium supplemented with varying concentrations of the leaf and root extracts stock solutions. The volumes were made up to 4.9 ml with distilled water. They were incubated in a rotary incubator (150 rpm) at room temperature for 30 min thereafter, 0.1 ml of 1.5% (w/v) phosphate buffered distilled water was added to each tube to obtain final concentrations in both the leaf and root extracts of 0, 20, 50, 100, 200, 500, 1000, and 2000 µg/ml in the different tubes, respectively. The process was carried out on the three bacterial isolates of *E. coli*, *Staphylococcus* spp. and *Proteus* spp. The control tubes consisted of the bacterial isolates, the media and the TTC without the leaf and root extracts. The reaction mixtures were incubated under aseptic conditions at room temperature (28 ± 2°C) for 14 h. The TPF produced was extracted in 4 ml of ethyl acetate and determined spectrophotometrically at 460 nm.

The amount of formazan produced was determined from standard dose response curve, 0 – 200 µg/ml TPF (Sigma on ethyl acetate). Dehydrogenase activity was expressed as milligrams of TPF formed per milligram dry weight of each biomass per hour. Extract inhibition of dehydrogenase activity was calculated relative to activity in the control. Inhibition data (% inhibition) were plotted against the concentration of the leaf and root extracts and the total inhibition concentration extrapolated.

**Statistical analysis**

Data obtained from the study were analysed using Analysis of variance (ANOVA). Fshers least significant Difference was used to separate the means and values for P < 0.05 were regarded as significant (Sanders, 1990).

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and saponins in both the leaf and root ethanolic extracts but with cyanogenic glycosides also present in the root extract (Table 1). Secondary plant metabolites exhibit varied biochemical and pharmacological actions in animals and microorganisms when ingested (Trease and Evans, 1989). Flavonoids, alkaloids, and tannins have been associated with antimicrobial effects in various studies using plant extracts (Nweze et al., 2004; Abo et al., 1999). Many plants containing alkaloids and flavonoids have been shown to have diuretic, antispasmodic, anti-inflammatory and analgesic actions (Oyowele et al., 2002). This confirms the anecdotal use of *L. owariensis* leaves decoction as cure for malaria as reported by Gill (1992), since pain mostly accompany malaria fever.

Three clinical isolates comprising two gram negative (*E. coli,* and *Proteus* spp.) and one gram positive (*Sta-
Table 1. Phytochemical profile of the leaf and root extracts of *L. owariensis*

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Cyanogenic glycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Root</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++ = Highly present, + = present, ND = Not detected.

Table 2. Dehydrogenase activity (mg formazan/mg cells dry weight/h) in the test clinical isolates.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Dehydrogenase Activities* (mg formazan/mg cells dry weight/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em> spp.</td>
<td>0.660 ± 0.058</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>0.509 ± 0.056</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.184 ± 0.041</td>
</tr>
</tbody>
</table>

*Data represent mean ± standard deviation of triplicate determinations.

Figure 1. Effect of varying extract concentrations on dehydrogenase activity in *Staphylococcus* spp. subjected to dehydrogenase assay showed that these organisms were able to reduce TTC to the red formazan at variable rates. The dehydrogenase activity in the clinical isolates showed that *E. coli* with 0.184 ± 0.041 mg formazan/mg cell dry weight/h had the least dehydrogenase activity, followed by *Proteus* spp. at 0.509 ± 0.056 and *Staphylococcus* spp. at 0.660 ± 0.058 mg formazan/mg cell dry weight/h, respectively (Table 2). This differed with previous studies in which gram-negative bacteria have been shown to have higher rate of dehydrogenase activity than the gram positive ones (Nweke et al., 2006). This variation may be due to difference in cell wall components (Matthew and Obdard, 2001).

The response of the bacterial dehydrogenase activities to *L. owariensis* leaf and root extracts was concentration dependent and varied among the organisms (Figures 1 - 3). There were initial increases in the dehydrogenase activities of all the bacterial isolates when treated with 20
the root is the main route of absorption of these elements. These increases in enzyme activity have recently been attributed to presence of the phytochemical constituents especially tannins which were detected more in the leaf extract. A similar initial increase in dehydrogenase activity was observed in the leaf extract, given the fact that the root is the main route of absorption of these elements in plants. These initial increases in dehydrogenase activities of the organisms notwithstanding, higher concentrations of both extracts inhibited the organisms’ enzyme activities.

A further study of the inhibitory concentrations of the leaf and root ethanolic extracts of the plant against these clinical isolates (Table 3) indicated that the Staphylococcus spp. was inhibited by the least concentration of both the leaf and root extracts (Table 2). This may explain the reported traditional use of both L. owariensis leaf and root decoction for the treatment of venereal diseases (Gill, 1992), since Staphylococcus spp. is among the major causative agents of venereal diseases. On the other hand, E. coli was least affected by the varied concentrations of both plant extracts. In general however, both extracts showed a concentration dependent inhibitory effect on all the bacteria species. This finding corroborates the reports of Esimone et al. (1998) and Osadebe and Ukwueze (2004) who independently found that various plant extracts inhibit the growth of some hospital bacteria isolates.

In conclusion, the ethanolic leaf and root extracts of L. owariensis have antimicrobial activity against Staphylococcus spp., Proteus spp. and E. coli, with lower concentrations of the leaf extract inhibiting the dehydrogenase activities of the organisms more than corresponding concentrations of the root extract. This higher inhibitory action of the leaf extract can be attributed to presence of the phytochemical constituents especially tannins which were detected more in the leaf than in the root extract.

REFERENCES


