

*Full Length Research Paper*

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

L. A. Nwaogu\*, C. S., Alisi, C. U. Igwe and C. O. Ujowundu

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

Accepted 14 January, 2008

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<sub>50</sub> of 20, 200, and 550 mg/ml against *Staphylococcus* spp., *Proteus* spp. and *E. coli*, respectively, while REEX gave an IC<sub>50</sub> 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 gonorrhoea (Gill, 1992). Owoyele et al. (2002) also reported that aqueous, methanol and chloroform extracts of *L. owariensis* leaves have anti-inflammatory and anal-

\*Corresponding author. E-mail: [nwogulinus@yahoo.com](mailto:nwogulinus@yahoo.com).

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 ( $28 \pm 2^\circ\text{C}$ ). 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-weighed crucible to constant weight in an oven at  $110^\circ\text{C}$ .

## 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 tetrazolium 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  $\mu\text{g/ml}$  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 ( $28 \pm 2^\circ\text{C}$ ) 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  $\mu\text{g/ml}$  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). Fishers 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*

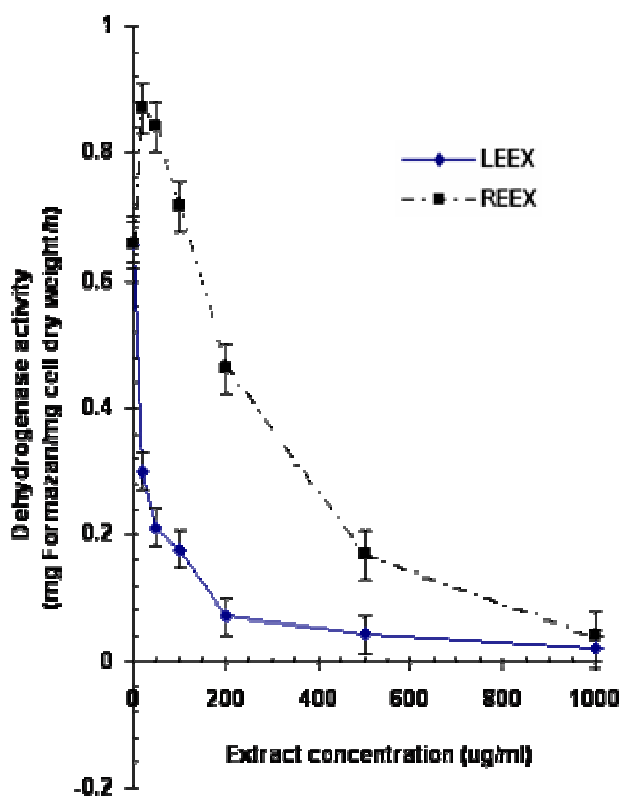
Plant part	Saponins	Tannins	Alkaloids	Flavonoids	Cyanogenic glycosides
Leaf	+	++	+	+	ND
Root	+	+	+	+	+

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

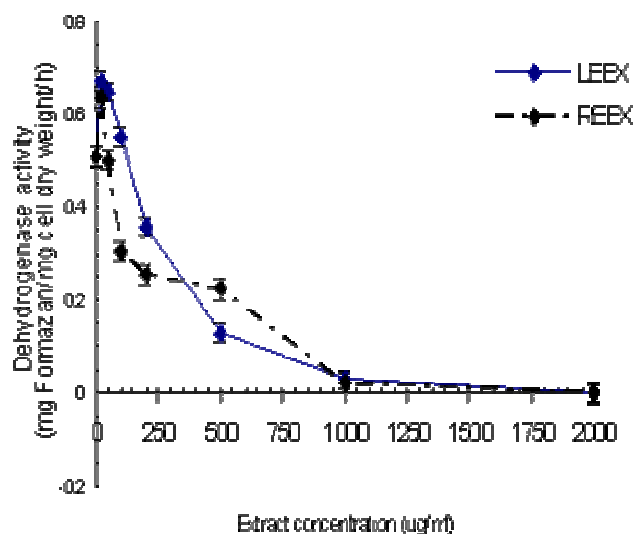
**Table 2.** Dehydrogenase activity (mg formazan/mg cells dry weight/h) in the test clinical isolates.

Test organisms	Dehydrogenase Activities* (mg formazan/mg cells dry weight/h)
<i>Staphylococcus</i> spp.	0.660 ± 0.058
<i>Proteus</i> spp.	0.509 ± 0.056
<i>E. coli</i>	0.184 ± 0.041

\*Data represent mean ± standard deviation of triplicate determinations.

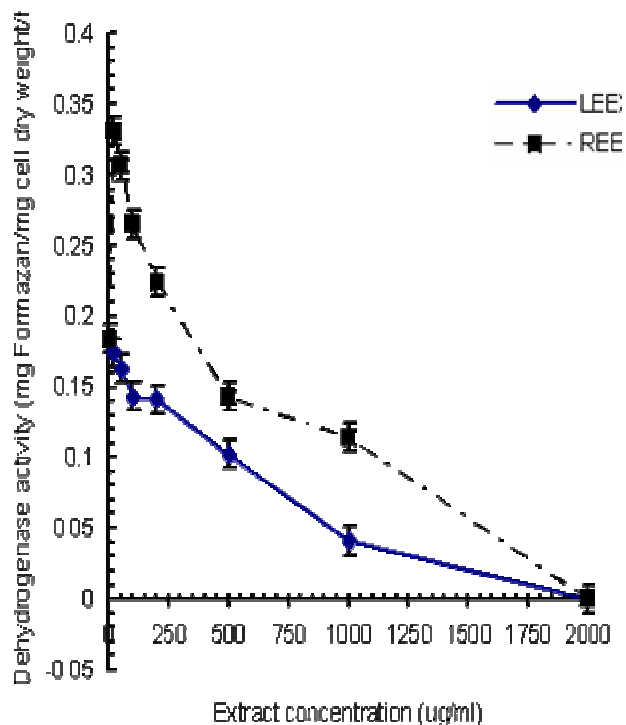
**Figure 1.** Effect of varying extract concentrations on dehydrogenase activity in *Staphylococcus* spp.

*phylococcus* 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

**Figure 2.** Effect of varying extract concentrations on dehydrogenase activity of *Proteus* spp.

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



**Figure 3.** Effect of varying concentrations of extract of dehydrogenase activity of *E. coli*.

µg/ml of the root extract. A similar initial increase in dehydrogenase activity was observed in the *Proteus* spp. upon treatment with 20 µg/ml of the leaf extract. These initial increases in the enzyme activity have recently been attributed to a form of stimulation due to the use of the plant's components such as trace elements by the bacterium (Nwaogu et al., 2007). This may further explain why this observation occurred in all the bacterial isolates upon treatment with the root 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 ex-

**Table 3.** Inhibitory concentrations of leaf and root ethanolic extracts of *Landolphia owariensis* against some clinical isolates.

Organism	LEEX (mg/ml)		REEX (mg/ml)	
	IC <sub>50</sub>	IC <sub>100</sub>	IC <sub>50</sub>	IC <sub>100</sub>
<i>Staphylococcus</i> spp.	20	1200	340	1580
<i>Proteus</i> spp.	200	1830	320	1600
<i>E. coli</i>	550	1350	1560	2000

tracts 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

- Abo KA, Ogunleye VO, Ashidi JS (1999). Antimicrobial Potential of *Spondias mombin*, *Croton zambesicus* and *Zygotritoma crocea*. *Phytothe. Res.* 13: 494-497.
- Anthony CD (1995). Natural Preservatives from *Landolphia owariensis*. *Afr. Dev. J.* 2(1): 21-22.
- Esimone CO, Adikwu MU, Okonta JM (1998). Preliminary Antimicrobial Screening of ethanolic extract from lichen usnea subflondans. *J. Pharm. Res. Dev.* 3(2): 99-101.
- Gill LS (1992). Ethnomedical use of plants in Nigeria. Uniben Press. pp. 145-146.
- Holt JG, Krieg NR, Sneath PHA, Williams ST (1994). *Bergey's Manual of Determinative Bacteriology* (9<sup>th</sup> edition). Williams Wilkins, Baltimore. pp. 38-39.
- Hornok L (1992). Cultivation and processing of medicinal plants. John Wiley and sons Ltd. Baffinslane United Kingdom. (3-7):19-22.
- Iwu MM (1992). Perspective of Igbo tribal ethanomedicine. *Ethnomed.* 2 (1-4):45.
- Lewis WH, Lewis MPE (1977). *Medical Botany*. John Wiley and sons Publishers New York pp. 21-22.
- Matthew M, Obdard JP (2001). Optimization of the Dehydrogenase Assay for measurement of Indigenous Microbial activity in beach sediments contaminated with Petroleum, *Biotechnol. Lett.* 23: 227-230.
- Nweke CO, Okolo TC, Nwanyanwu CE, Alisi CS (2006). Response of Planktonic Bacteria of New Calabar River to zinc stress. *Afr. J. Biotechnol.* 5(8): 653-658.
- Nwaogu LA, Alisi CS, Ibegbulem CO, Igwe CU (2007). Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf. *Afr. J. Biotechnol.* 6(7): 890-893.
- Murray ND (1998). *African Indigenous Medicinal, African Medical and research function*, Nairobi Kenya. pp. 37-38.
- Odebiyi OO, Sofowara EA (1979). *Phytochemical Screening of Nigerian Medical Plants Pub. 1 2<sup>nd</sup> ONA/STRC Inter African Symposium and traditional Pharmacopecia Pub. Lagos, Nigeria.* p. 115,
- Owoyele BY, Olayele SB, Elegba RA (2002). Anti-Inflammatory and Analgesic Activities of Leaf Extract of *Landolphia owariensis*. *Afr. J. Biomed. Res.* 4(3): 131-133.
- Osadebe PO, Ukwueze SE (2004). A Comparative Study of the Phytochemical and Antimicrobial properties of the Eastern Nigerian

- Species of African mistletoe (*Loranthus micronthus*) sourced from different host trees. J. Biol. Biotechnol. 2(1): 18-23.
- Praveen-Kumar JC (2003). 2, 3, 5 – Triphenyltetrazolium Chloride (TTC) and Electron Acceptor of Culturable cell Bacteria, fungi and Actinomycetes. Biol. Fert. Soil. 38: 186-189.
- Sanders DH (1990) Statistics. A Fresh Approach. (4<sup>th</sup> edition) McGraw Hill Inc. Singapore, pp. 380-385.
- Trease GE, Evans WC (1989). Pharmacognosy. 13<sup>th</sup> edition, Bacilliere Tinnall Ltd., London, pp. 5-9.