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Full Length Research Paper

Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf

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Ethanolic extract of the leaves of *Landolphia owariensis* were subjected to phytochemical screening and antimicrobial activities on *Escherichia coli, Staphylococcus* sp. and *Proteus* sp. using dehydrogenase assay method. The phytochemical screening indicated the presence of alkaloids, flavonoids, tannins and saponins. The bacterial isolates were exposed to different extract concentrations (20 – 2000 μg/ml) of the extract in nutrient broth, and their response was concentration dependent. In all three bacteria, dehydrogenase activity was progressively inhibited at concentrations of about 20 μg/ml, with total inhibition observed with 700 μg/ml for *Staphylococcus* sp., 1000 μg/ml for *Proteus* sp. and 1800 μg/ml for *E. coli*.

Key words: Phytochemical activity, ethanolic extract, *Landolphia owariensis*, dehydrogenase assay.

INTRODUCTION

The medicinal use of plants leaves and roots in the management and treatment of diseases have been an age long practice (Sofowara, 1982). The continued investigation into the secondary plant metabolites has led to important breakthroughs in pharmacology. This has also helped, in no small measure, in the development of modern pharmacotherapeutics in Africa and other parts of the World (Doerge et al., 1971). The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agents currently in use. Therefore, the search for new drugs from novel sources, such as plant, is necessary (Fransworth and Morris, 1976).

Many plant species have been found to have one or more medicinal properties. Majority of medicinal plants are flowering plants (angiosperms) and are readily available in rural areas (Fransworth and Morris, 1976). In southeastern Nigeria, many fruits, spices, herbs and leafy vegetables used as food and for medicinal purposes are obtained from wild tropical forest where they may be as many as a thousand species (Ibe and Nwufor, 2005). To date, plants continue to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products (Sofowara, 1982).

Landolphia owariensis P. beaur (Family: *Apocynaceae*) commonly called vine rubber and known locally by various names (Eso/Utu in Ibo, Mba in Yoruba and Ciwa in Hausa) is widely used for the treatment of many ailments (Owoyele et al., 2002). The decoction of its leaves is used as a purgative, and to cure malaria (Gill, 1992). The extract of the root is also used to treat gonorrhoea infection (Gill, 1992). Further reports have tried to validate the folkloric use of the plants extract as an antimicrobial agent (Ebi and Ofoefule, 1997). Lewis and Lewis (1977) also reported the use of the stem bark as vermifuge. The latex is drunk or used in French Equatorial Africa as enema for intestinal worms (Irvine, 1961). The latex is also used as a natural preservative (Anthony, 1995).

This study is aimed at investigating the antimicrobial properties of the ethanolic extract on three bacterial isolates so as to validate or otherwise the claim of the herbalists who use it as an antimicrobial remedy. The study will also expose new frontiers or improve on the current applications of the plant extract.

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Table 1. Phytochemical profile of the leaf extract of *Landolphia owariensis*.

Plant	Saponins	Tannins	Alkaloids	Flavonoids	Cardiac glycoside	Cyanogenic glycoside
Landolphia owariensis leaf extract	+	++	+	+	-	-

++ = highly present; + = present; - = not present.

MATERIALS AND METHODS

Preparation of plant materials

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

Extract preparation

The fresh leaves of *L. owariensis* were sun dried for ten days to a constant weight. The dried leaves were ground into powder using a mechanical grinder. 100 g of the leave powder was 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 about 48 h. The solution was subsequently shaken and filtered using Whatmann number 1 filter paper. The filtrate was evaporated to dryness using a rotary evaporator (Model type 349/2, Corning Ltd). A yield of 11% of aqueous extract was obtained. The extract was stored at 4°C.

Phytochemical studies

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

Isolation of test organisms and culture conditions

Three clinical bacteria strains made up of *Escherichia coli, Staphylococcus* sp. and *Proteus* sp. obtained from the Pathology Department of Federal Medical Centre Owerri, Nigeria were used for the study. They were isolated and purified on nutrient agar plates and characterised 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 resuspended 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 activity assay. The dry weight of cells was determined by drying a 0.2 ml aliquot of cell suspension in a preweighed 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-triphenyltetrazolium 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 extract in separate screw-capped test tubes. About 0.2 ml volume of the bacterial suspensions was inoculated into triplicate glass tubes containing 3.7 ml of phosphate buffered (pH 7.2) nutrient broth-glucose medium supplemented with varying volumes of the extract stock solution. 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) TTC in phosphate buffered distilled water was added to each tube to obtain final extract concentration of 0, 20, 50, 100, 200, 500, 1000, and 2000 µg/ml in the different tubes. The process was carried out on the three bacterial isolates of *E. coli, Staphylococcus* sp. and *Proteus* sp. The control tubes consisted of the bacterial isolates, the media and the TTC without the leaf extract. 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 mg/l TPF (Sigma) in 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 controls. Inhibition data (% inhibition) were plotted against the concentrations of the extract and the total inhibition concentrations extrapolated.

Statistical analysis

Data obtained from the study were analysed by the use of one-way analysis of variance (ANOVA) and values for $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins (Table 1). Flavonoids, alkaloids and tannins have been associated with antimicrobial effects in various studies using plant extracts (Nweze et al., 2004; Abo et al., 1999; Corthout et al., 1991). However, more research is required to determine the role of flavonoids, alkaloids and tannins in the antimicrobial activity of *L. owariensis*.

The dehydrogenase assay of the control showed that *E. coli* at 0.184 ± 0.041 had the least dehydrogenase activity, followed by *Proteus* sp. at 0.509 ± 0.056, and *Staphylococcus* sp. 0.660 ± 0.058 mg formazan per mg cell dry weight per hour respectively (Table 2; $P < 0.05$). *E. coli* and *Proteus* sp. are gram negative organisms while *Staphylococcus* sp. is gram positive. Gram-negative bacteria have been shown to have higher rates of dehydrogenase activity than the gram positive ones

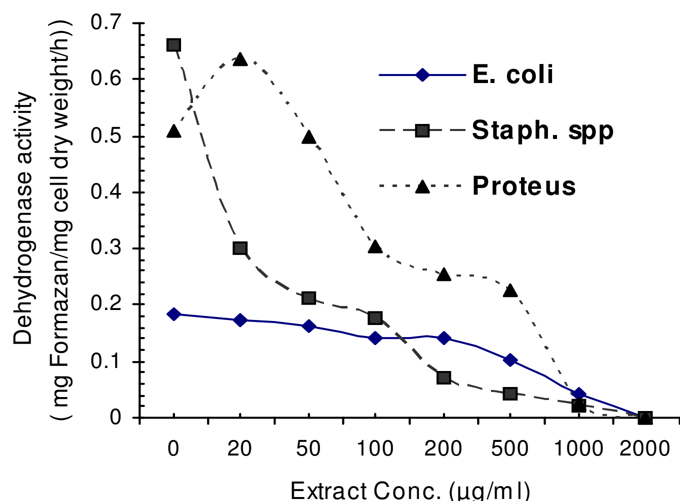

Figure 1. 2,3,5-triphenyltetrazolium chloride (TTC) reduction activity in response to various concentrations of *Landolphia owariensis* ethanolic leaf extract by the bactera.

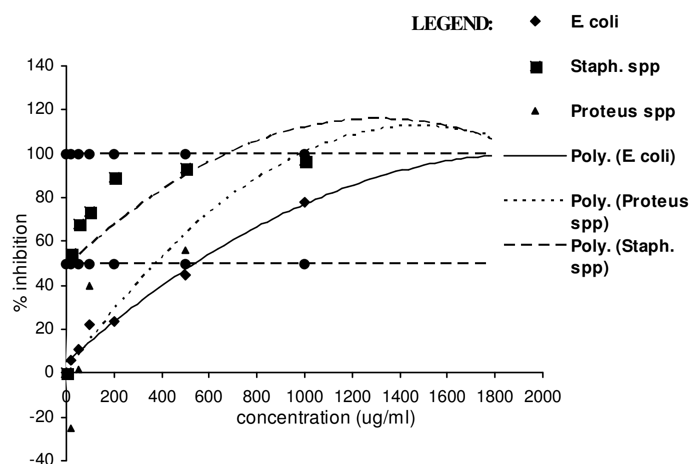

Figure 2. Polynomial regression of % inhibition of dehydrogenase activity in the organisms against graded concentrations of ethanolic extract of *Landolphia owariensis* leaf.

Table 2. Dehydrogenase activities in the control bacterial tests.

Test organism	Dehydrogenase activity* (mg formazan/mg cell dry weight/h)
Staphylococcus sp.	0.660 ± 0.058
Proteus sp.	0.509 ± 0.056
E. coli	0.184 ± 0.041

*Values represent mean ± standard deviation of triplicate tests.

(Nweke et al., 2006). On the other hand, the results of the present study show that *Staphylococcus* sp., a gram positive bacterium, had the highest dehydrogenase activity. These variations may be due to difference in cell wall components or dehydrogenase systems, since different microorganisms have been reported to have different dehydrogenase systems (Proveen-Kumar, 2003). The responses of the bacterial dehydrogenase activities to *L. owariensis* leaf extract were concentration dependent and vary among the organisms (Figure 1). The rise in dehydrogenase activity of *Proteus* sp. using the extract at 20 µg/ml is a form of stimulation, and this may be attributed to the use of the leaf components such as trace elements by the bacterium. It has been observed that dehydrogenase activities in *Proteus* sp. and *Micrococcus* sp. are stimulated by low concentrations of trace elements such as Zn^{2+} (0.2 mM). However, higher concentrations inhibit the organisms' dehydrogenase activities. Although, *E. coli* showed the least level of dehydrogenase activity, it was also least affected by the varied concentrations of the extract. *Staphylococcus* sp. had the highest percentage inhibition than other organisms (Figure 2). Thus, 50% inhibition of dehydrogenase activity was achieved on *Staphylococcus* sp. with an extract concentration less than 20 µg/ml, while same was achieved on *E. coli* at about 475 µg/ml. Similarly, 100% inhibition was achieved with about 700, 1000 and 1800 µg/ml of extract on *Staphylococcus* sp., *Proteus* sp. and *E. coli*, respectively. This result further supports the folkloric use of *L. owariensis* leaf decoction for the treatment of venereal disease (Gill, 1992), since *Staphylococcus* sp. are among the major causative agents of venereal diseases.

In conclusion, ethanolic extract of *L. owariensis* leaf has antimicrobial activity against *Staphylococcus* sp., *Proteus* sp., and *E. coli*. Although the exact active components of the extract that showed this effect were not identified, but antimicrobial active plant principles such as flavonoids, alkaloids and tannins were observed in the extract.

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