

Phytochemical Screening, Antimicrobial Properties and Proximate Analysis of *Landolphia owariensis* P. Beauv Seeds

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Abstract

Phytochemical screening, antimicrobial properties and proximate analysis of the seeds of *Landolphia owariensis* P. Beauv were investigated. Dried seeds of *Landolphia owariensis* P. Beauv were pulverized and extracted with 96.5% ethanol using soxhlet extraction method. The phytochemical screening of the extract revealed the presence of glycosides, saponins and phenols while tannins, flavonoids, alkaloids and steroids were not detected. The crude ethanol extract showed antimicrobial potency against *Candida albicans*, *Streptococcus faecali*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Coliform bacilli* at the concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL and 16.5 mg/mL with inhibition zone diameter (IZD) values ranging between 16 mm-30 mm. The crude sample was partitioned into acidic, basic and neutral metabolites which showed varying degrees of antimicrobial potency at the concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL and 16.5 mg/mL against the same organisms. The acidic metabolite showed pronounced antimicrobial activity against these organisms compared to the basic and neutral metabolites. Amoxycillin (amoxyl) and orazone were used as standard antibiotics and levofloxacin as a standard fungicide. The proximate analysis of the untreated seed sample gave 2.27% ash content, 15.32% moisture content, 15.70% crude fiber, 28.06% crude protein and 48.45% carbohydrate.

Keywords: ethanol extract, metabolite, antimicrobial, proximate analysis, *Landolphia owariensis*

1. Introduction

Naturally occurring useful substances could be sourced from plants, animals or from minerals (Sofowora, 1993). These could be obtained from both primary and metabolic processes and have provided and still provide sources of medicine and pharmaceuticals for ages (Gills, 1992). Medicinal plants and the use of herbs are now sources of many important drugs and food supplements of the modern world and have been documented by some researchers (Harborne, 1973; W. H. Lewis & M. P. E. Lewis, 1977; Holetz, Pessini, Sanches, Cortez, Nakamura, & Filho, 2002). They are the bioactive constituents in form the of steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, glycosides, saponins, phenols and so on (Addae-Mensah, 1992).

Antimicrobial substances like saponins, glycosides, flavonoids, alkaloids are found well distributed in plants and they are the active principles used as antioxidants, antifungals, antibiotics, anti-inflammatory, hemolytic, antipyretic, analgesics, diuretics, molluscicidal (Ojinnaka & Nwokonkwo, 2012; Nwokonkwo, 2009; Akinyele, Adu, & Ayeni, 1996). In herbal and traditional medicine, the part of the plant that contains the active ingredient; leaves, stem, root, seeds and so on is taken in form of an extract, infusion or decoction (Dhir, Craig, & Berman, 2002; Burkhill, 1985; Dazi, 1993; Ake-Assi & Guinko, 1991).

Landolphia owariensis P. Beauv belongs to the family *apocynaceae* commonly called vine rubber and known in the Ibo language of South Eastern Nigeria as *Utu*. Different parts of this plant are used for the treatment of several ailments. The decoction of the leaves is used as a cure for malaria. The root is soaked in local gin and the extract given in the treatment of gonorrhoea (Obute, 2005). The aqueous, methanol and chloroform leaf extracts have shown anti-inflammatory, analgesic and antisecretory properties (Owoyele, Olaleye, Oke, & Elegbe, 2001). The stem bark had been reported as a vermifuge (Odugbemi & Akinsulere, 2006) while the latex was used as an enema for intestinal worms and as a natural preservative (Obitte, Chukwu, & Onyishi, 2010). The ethanol extract of the leaf and root showed antimicrobial activities (Nwaogu, Alisi, Igwe, & Ujowundu, 2008). Okonkwo (2013) reported the isolation, characterization of bioactive compounds of *Landolphia owariensis* P. Beauv.

There is a great need for new antimicrobials as a result of high incidence of multidrug resistant microorganisms. For an example, *Staphylococcus aureus* was discovered to be resistant to ciprofloxacin, erythromycin and clindamycin while *Escherichia coli*, *Pseudomonas aeruginosa* and *Kllessiella* species were implicated in drug resistant bacteria septicaemia in Nigeria (Adegoke, Iberi, Akinpelu, Aiyegoro, & Mbotu, 2010)

In this article, the proximate analysis of the seed of *Landolphia owariensis* P. Beauv, the phytochemical screening and the antimicrobial and antifungal activities of the ethanol crude extract of the seed as well as the antimicrobial and antifungal activities of the acidic, basic and neutral metabolites of the ethanol crude extract of the seed were considered.

2. Materials and Methods

2.1 Plant Material and Preparation

The fruits of *Landolphia owariensis* P. Beauv were collected in February of 2012 from Enugu, Enugu State in Nigeria and authenticated at the Department of Plant Sciences and Technology, Federal University of Technology Owerri Imo State, Nigeria. The seeds were removed from the pulp, washed with distilled water, sun dried for seven days and pulverized. The ground sample was stored in a sterile cellophane bag until needed.

2.2 Extraction

Approximately 650 g of the ground sample was weighed on analytical weighing balance Kern Germany Model 770 and extracted by soxhlet in 1000 mL ethanol (BDH chemical Ltd. England) for 24 h. The solvent was distilled off to yield 150 g of brown gummy like substance.

3. Phytochemical Screening

Preliminary phytochemical tests were carried out on the crude ethanol extract (Obi & Onuoha, 2000; Ejele & Alinor, 2010; Ejele & Nwokonkwo, 2013).

3.1 Test for Tannins

About 2 mL of the filtrate which was prepared by dissolving 2 g of the crude sample in 20 mL of ethanol was added to 5 mL distilled water and 2 drops of FeCl_3 (dissolved in dilute HCl). There was no formation of coloured precipitate.

3.2 Test for Flavonoids

Approximately 1 mL of the stock solution was dissolved in 5 mL 0.1 M solution of NaOH. To this was added 0.5M solution of H_2SO_4 until the solution became acidic (tested with litmus paper), there was no colour change.

3.3 Test for Alkaloids

Wagner's reagent was prepared and 2 mL of it was added to 2 mL of the plant sample and shaken vigorously, there was no presence of coloured precipitate.

3.4 Test for Saponins

To 2 mL of the plant sample was added 2 mL of distilled water and shaken, the agitation produced frothing of the solution. Also to a fresh 2 mL sample was added 2 mL of olive oil and shaken vigorously this also produced frothing.

3.5 Test for Steroids

Chloroform 2 mL was added to 2 mL of the plant sample solution. To this mixture was added carefully 2 mL conc. H_2SO_4 . This reaction produced two layers; the upper organic layer and the aqueous lower layer. The organic layer was removed, evaporated to dryness and 5 mL conc. H_2SO_4 added to it. The whole reaction mixture was heated for 10 min in a water bath, allowed to cool; there was no visible colour change.

3.6 Test for Glycosides

To 2 mL solution of the sample was added 2 mL chloroform and 2 mL ice-cold acetic anhydride in 2 mL conc. H_2SO_4 . A pink colour which developed slowly into purple colour was observed.

3.7 Test for Phenols

Approximately 0.5 mL of the sample solution was mixed with 4 mL of distilled water and heated, allowed to cool and filtered. 0.5 mL FeCl_3 was added to the filtrate and a dark-brown precipitate was observed.

4. Preparation of Metabolites

4.1 Basic Metabolite

3.0 g of the sample was treated with 20 mL of 0.01 M HCl and extracted with 30 mL of chloroform in a separatory funnel and allowed to stay for 3 h (Ejele & Akujiobi, 2011). The lower chloroform layer was removed and reserved for the preparation of neutral metabolite. The acid layer was treated with 10 mL of 0.01 M solution of NaOH until the mixture became basic. The resulting solution with or without precipitate was allowed to evaporate completely to reveal a gel like substance which was dissolved in 95% ethanol and filtered. This filtrate was used, without further purification for antibacterial analysis.

4.2 Neutral Metabolite

The chloroform layer obtained above was treated with 30 mL of 0.01 M solution of NaOH. The mixture was allowed to equilibrate for 3 h. The chloroform layer was removed and allowed to dry to give a gel like compound which was dissolved in 95% ethanol and filtered. The filtrate was used for antimicrobial susceptibility test.

4.3 Acidic Metabolite

The aqueous alkaline layer obtained from above was treated with 50% conc. HCl, the substance obtained was used for antibacterial screening.

5. Antibacterial Properties of the Extract and the Metabolites

This analysis was done in Microbiology Department, Federal Medical Center Owerri, Imo State, Nigeria. Test organisms used were *Candida albicans*, *Streptococcus faecali*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Coliform bacilli*. Microorganisms culture were maintained in Nutrient medium (Merck) for bacteria, and Sabouraud dextrose (Merck) for fungus. The agar-diffusion method was used (George & Roger, 2002), the microorganisms were maintained on agar slants, and sub-cultures were freshly prepared before use. The bacteria for inoculation were made in 5 mL of nutrient agar and grown for 24 h at 37 °C while the fungus was inoculated 5 mL Sabouraud broth and grown for 48 h at 25 °C. The final inoculating agents were made with Nutrient agar medium or Sabouraud agar seeded with the test microorganisms. Plates were prepared by pouring 20 mL of freshly prepared Nutrient agar or 20 mL of Sabouraud agar into 20 mm × 100 mm petri dishes and adjusted to 45 °C. The inoculums (5 mL) were poured directly over the surface of the prepared plates, allowed to solidify for 5 min; a cork borer of 1 cm was used to penetrate the medium. 1 mL of the ethanol plant extract was inoculated into each borer, and the plates incubated at 35 °C for both the bacteria and fungus. After 24 h incubation, inhibition zones were recorded as the diameter of the growth free zones. Amoxicillin and Orazone at 30 mg/mL respectively were used as positive controls for bacteria and 30 mg/mL Levofloxacin for the fungus.

6. Proximate Analysis

The proximate analysis was carried out using the methods of Association of Official Analytical Chemist, AOAC (2005). The parameters considered for proximate analysis included sample ash content, moisture content, crude fiber, crude protein and carbohydrate.

7. Results

The result of the phytochemical screening of the crude ethanol extract is shown on Table 1. Antibacterial properties of the crude extract, the basic, neutral and acidic metabolites against the microorganisms at different concentrations are shown on Tables 2- 6 while the proximate analysis result is shown on Table 7.

Table 1. Phytochemical screening of the crude extract

Phytochemicals	Result
Tannins	-
Flavonoids	-
Alkaloid	-
Steroids	-
Saponins	+
Glycosides	+
Phenols	+

- (Absent), + (Present).

Table 2. Antibacterial properties of the crude extract

Test Organisms	Diameter of Zone of Inhibition(mm)				
	Concentration (mg/mL)				
	100	50	25	12.5	6.25
<i>Candida albicans</i>	25	19	15	9	-
<i>Escherichia coli</i>	30	23	16	10	-
<i>Pseudomonas aeruginosa</i>	20	14	10	-	-
<i>Streptococcus faecalis</i>	15	10	-	-	-
<i>Staphylococcus aureus</i>	20	14	-	-	-
<i>Coliform bacilli</i>	-	-	-	-	-

- (no activity), 9 -19 (ineffective), 20- 23 (effective), 25- 30 (strongly effective).

Table 3. Antibacterial properties of the basic metabolite

Test Organisms	Diameter of Zone of Inhibition(mm)				
	Concentration (mg/mL)				
	100	50	25	12.5	6.25
<i>Candida albicans</i>	30	20	15	10	-
<i>Escherichia coli</i>	30	25	20	10	-
<i>Pseudomonas aeruginosa</i>	30	24	16	12	-
<i>Streptococcus faecalis</i>	20	14	10	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-
<i>Coliform bacilli</i>	-	-	-	-	-

- (no activity), 10 -16 (ineffective), 20- 24 (effective), 25- 30 (strongly effective).

Table 4. Antibacterial properties of the neutral metabolite

Test Organisms	Diameter of Zone of Inhibition(mm)				
	Concentration (mg/mL)				
	100	50	25	12.5	6.25
<i>Candida albicans</i>	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	30	23	17	10	-
<i>Streptococcus faecalis</i>	22	15	10	-	-
<i>Staphylococcus aureus</i>	25	19	12	6	-
<i>Coliform bacilli</i>	30	24	16	10	-

- (no activity), 6- 19 (ineffective), 22- 24 (effective), 25- 30 (strongly effective).

Table 5. Antibacterial properties of the acidic metabolite

Test Organisms	Diameter of Zone of Inhibition(mm)				
	Concentration (mg/mL)				
	100	50	25	12.5	6.25
<i>Candida albicans</i>	30	24	16	10	-
<i>Escherichia coli</i>	25	18	13	6	-
<i>Pseudomonas aeruginosa</i>	34	27	20	13	-
<i>Streptococcus faecalis</i>	30	25	19	12	-
<i>Staphylococcus aureus</i>	35	28	20	14	-
<i>Coliform bacilli</i>	30	23	15	-	-

- (no activity), 6-18 (ineffective), 20- 23 (effective), 25- 35 (strongly effective).

Table 6. Antibacterial properties of the control drugs

Test Organisms	Diameter of Zone of Inhibition(mm)		
	Concentration (30 mg/mL)		
	Amoxyl	Orazone	Levofloxacin
<i>Candida albicans</i>	-	-	-
<i>Escherichia coli</i>	30	5	30
<i>Pseudomonas aeruginosa</i>	22	-	17
<i>Streptococcus faecalis</i>	-	-	-
<i>Staphylococcus aureus</i>	10	15	20
<i>Coliform bacilli</i>	-	10	7

- (No activity), 5-17 (ineffective), 20- 22 (effective), 30 (strongly effective).

Table 7. Proximate analysis of the seeds of *Landolphia owariensis*

Parameters	Result (%)
Ash	2.27 ± 2
Moisture	15.32 ± 4
Crude Protein	28.06 ± 3
Fiber	15.70 ± 2
Carbohydrate	48.45 ± 2

8. Discussion

Preliminary phytochemical screening result revealed the presence of saponins, glycosides and phenols in the ethanol seed extract of *Landolphia owariensis* P. Beauv, tannin, alkaloids and steroids were absent, Table 1.

The crude ethanol extract showed antimicrobial potency against *Candida albicans* and *Escherichia coli*, moderate activity against *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in the concentrations of 100 mg/mL. At 50 mg/mL, the extract was effective against *Escherichia coli*. There was inhibition against *coliform bacilli* and no inhibition at 25 mg/mL and 12.5 mg/mL concentrations and no activity at all at 6.25 mg/mL, Table 2.

In Table 3, the basic metabolite inhibited the growth of *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* at 100 mg/mL concentration with inhibition zone diameters of 30 mm respectively and bacteriastatic against *Streptococcus faecalis* at the same concentration. At 50 mg/mL the extract was bacteriastatic against the same pathogens even at 25 mg/mL where the extract showed some activity against *Escherichia coli*. The basic metabolite did not show any inhibitory activity against *Staphylococcus aureus* and *Coliform bacilli*.

The neutral metabolite showed strong antimicrobial sensitivity at 100 mg/mL for *Pseudomonas aeruginosa* and *Coliform bacilli* mildly active against *Streptococcus faecalis* and *Staphylococcus aureus*. There was considerable activity against *Pseudomonas aeruginosa* and *Coliform bacilli* at 50 mg/mL concentration, Table 4.

The acidic metabolite showed strong inhibition against five of the six pathogens at 100 mg/mL with IZD of 30 mm and above, it showed mild inhibitory activity against *Escherichia coli* with IZD value of 25 mm. Even at 50 mg/mL and at more dilute concentration of 25 mg/mL; the extract still exhibited antimicrobial sensitivity.

At more dilute concentrations of 12.5 mg/mL and 6.25 mg/mL of all the metabolites, no significant sensitivity was observed. Comparatively, the acidic metabolite was effective against all the pathogens, suggesting that this metabolite could be used as a source of effective and potent antimicrobial/antifungal drugs. Comparison of the inhibitory activity of the extract with the control antibiotic drugs (amoxyl, orazone and levofloxacin) in table 6 revealed that the metabolites of the ethanolic extract of *Landolphia owariensis* P. Beauv seed showed significantly higher inhibitory activity than these drugs.

The antimicrobial potency of this extract was attributed to the presence of saponins, glycosides and phenols and supports the use of this species of plant in traditional or herbal medicine. The partitioning of the seed extract into the different metabolites had provided an easy method for carrying out a bio-assay guided isolation, purification and characterization of the acidic metabolite.

The results obtained from the proximate analysis of the crude plant extract in Table 7; gave ash content 2.27±2%, moisture content 15.32±4%, crude fiber 15.70±3%, crude protein 28.06±3% and carbohydrate 48.45±2% respectively. The crude fiber, crude protein and carbohydrate composition of the seed extract are important because of their nutritive values; this showed that the plant seeds could be a good source of nutrition for body building.

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