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HPLC-UV ANALYSIS AND THE ANTIOXIDANT ACTIVITY OF THE CRUDE METHANOL EXTRACT AND THE SOLVENT FRACTIONS OF THE PERICARP OF *LANDOLPHIA OWARIENSIS* FRUIT USING DPPH.

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ABSTRACT

The pericarp of *Landolphia owariensis* was collected from the ripe fruit, air dried, pulverized and extracted exhaustively with soxhlet extractor using methanol as solvent. The methanol extract was fractionated using four solvents of different polarity (hexane, ethylacetate, butanol and water). Phytochemical analysis was conducted on both the extract and the fractions using standard procedures. The crude methanol extract and solvent fractions were subjected to HPLC-UV (High Performance Liquid Chromatography coupled with Ultra-Violet Spectrophotometer) analysis. Antioxidant analysis was carried out on both the crude methanol extract and the solvent fractions using 2,2-diphenyl-1-picrlhydrazyl(DPPH).

The phytochemical analysis of the concentrated crude methanol extract showed the presence of flavonoids, alkaloids, saponins, protein and glycosides. The HPLC-UV analysis of the crude methanol extract and the solvent fractions showed that they contained many compounds with the ethylacetate fraction containing more phenolic compounds. The ethylacetate fraction showed the highest antioxidant activity at the concentrations of 250.00 µg/ml, 125.00 µg/ml, 62.50 µg/ml and 31.25 µg/ml respectively.

Key words:*Landolphia owariensis*, Phytochemical analysis, Antioxidants Phenolics, DPPH

INTRODUCTION

Phyto-compounds are important and integral part of pharmaceutical industries because of their structural diversity and pharmacological activities [1]. Plants which are the source of these compounds have played important roles globally in treating and preventing human diseases. Generally, medicinal plants are believed to be safer and less damaging to the human body than synthetic drugs [2]. Plants that contain phenolic compounds such as flavonoids are known to exhibit high level antioxidants activity because of their ability to scavenge free radicals and reactive oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals [3].

Landolphia owariensis belongs to a genus of flowering plants found mainly in Africa and was identified and described first in Nigeria as a genus in 1804 as *Landolphia* P. Beauv. The plant was later given the botanical name, *Landolphiaowariensis* P. Beauv [4].It is found either as a woody plant with tendrils or a sprawling bush, associated with tropical forest, coastal lowland and sub-tropical bush [5]. This plant is popularly known as "White rubber vine, Vine rubber" (English)"Utu/Eso"(Igbo)"Mba"(Yoruba)"Ciwo" (Hausa) [6]. Different parts of *Landolphia owariensis* such as the leaves and seeds have been reported to possess antioxidant, antimicrobial and hepato-protective [7, 5, 9, 10]. The pericarp of the

fruits has not been of particular interest because it is not usually consumed and so regarded as not edible. The need to know the constituent as well as the medicinal uses were the major interest propelling the conduct of this research.

MATERIALS AND METHODS

Sample collection and preparation

The ripe fruits of *Landolphiaowariensis* were collected from Aria Market, Ngwo in Enugu North Local Government Area, in the months of April through July. The plant was identified by Prof. J. C. Okafor a renowned taxonomist.The fruits were broken in order to separate the pericarp from the seeds and pulp. The pericarp of the fruit was separated from the seeds and pulp. The separated pericarp was dried at room temperature for two weeks. The dried pericarp was pulverized using mechanically driven pulverizing machine and stored in an air tight container before use.

Extraction

The pulverized plant material (520.4 g) was extracted using the soxhlet extractor with analytical grade methanol at a temperature of 35˚C. The reddish-brown extract was allowed to cool after which, it was concentrated using a rotary evaporator at a temperature of 40 ˚C followed by boiling in a water–bath at a temperature of45˚C. The concentrated semi-dried crude methanol extract was weighed using an electronic chemical balance.

The percentage extractive was calculated using the equation:

$$\%\ extractive = \frac{Weight\ of\ Crude\ extract}{Sample\ weight} \times 100 \quad \ldots \text{ Equation 1}$$

Phytochemical analysis of the crude methanolic extract

Qualitative phytochemical analysis of the concentrated crude methanol extract was determine using standard procedure as described by Harbone 1973 [10].

Fractionation of the crude methanol extract

The solvent-solvent partitioning was carried out following standard protocol[13,14]. All the solvent fractions were dried and kept for other analyses.

High performance liquid chromatography (HPLC-UV) of the crude methanol extract and the solvent fractions

The crude methanol extract and the solvent fractions were subjected to HPLC-UV analysis

The crude methanol extract (2 mg) and the dried solvent fractions (hexane, ethyl acetate, butanol and aqueous fractions 2 mg each) were reconstituted with 2 mL of HPLC grade methanol soncated for 10 min, centrifuged and filtered. The filtrate (100 µL) containing dissolved samples were each transferred into HPLC vials containing 500 µL of HPLC grade methanol. HPLC-UV analysis was carried on the samples with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany). Detection was at 235, 250, 280 and 340 nm. The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nano-pure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent.

Antioxidant testof the crude methanol extract and the solvent fractions

The crude methanol extract and the solvent fractions were dissolved with ethanol to form different concentrations ranging from 31.25 µg/mL-500 µg/mL.

The stock solution of the radical was prepared by dissolving 23.3 mg of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in 100 mL of methanol (MeOH). Different concentrations of the crude methanol extract and the solvent fractions (0.5 mL each) were dispensed into test tubes in duplicates. 0.5ml of the prepared DPPH was also dispensed into each test tube containing the extract and the solvent fractions. Then 4 mL of ethanol was added and the absorbance was read immediately at a wavelength of 500nm using visible spectrophotometer. The blank absorbance was measured and its reading was 0.704. The work was done in low illuminated place. The absorbance values of the crude methanol extract and the solvent fractions were taken trice and the average sample absorbance for their different concentrations was calculated [14].

The percentage scavenging activity was calculated using the formula:

$$100 - \left(\frac{100}{Blank\ absorbance} \times Sample\ absorbance\right) \ldots \text{Equation 2}$$

RESULTS AND DISCUSSION

Phytochemical analysis of the crude methanol extract

The concentrated crude methanol extract was reddish-brown in colour and gave a semi-solid of mass 162.8 g with percentage yield of 31.28 %. This extract (20 g) was analyzed for some phytochemicals present as shown in table 1.

Table: 1 Phytochemical analysis of crude methanol extract of the pericarp of*LandolphiaOwariensis*fruit

Phytochemical Constituents	Relative Abundance
Alkaloids	++
Saponins	+++
Tannins	-
Flavonoids	++
Steroids	-
Terpenoids	-
Cardiac glycoside	-
Protein	++
Glycosides	+++

Key

- Absent ++ Moderately present

+ Mildly Present +++Abundantly Present

From table 1, it was observed that the crude methanol extract gave positive results for alkaloids, saponins, flavonoids, protein and glycosides. The presence of flavonoids shows that it possesses anti-oxidant properties. Plants containing flavonoids have been shown to have anti-spasmodic, anti-hypertensive, anti-inflammatory and anti-oxidant activities [7].

Fractionation of the crude methanol extract

The fractionation of the crude methanol gave four solvent fractions(hexane,ethylacetate,butanol and water) which were subjected to HPLC-UV analysis

High performance liquid chromatography (HPLC-UV) of the crude methanol extract and the solvent fractions of the pericarp of *landolphia owariensis* fruit.

The crude methanol extract and the solvent fractions were subjected to HPLC-UV analysis and the result produced the following chromatograms with different peaks, retention time and the UV detector showed different compounds present in the crude methanol extract and the solvent fractions according to their wavelengths match with that in the library of the HPLC-UV spectrophotometer.

Table 2: Retention time (minutes) and library compounds found in HPLC-UV of crude methanol extract

Peak No.	Retention Time in minutes	Compound in the UV Library
1	3.25	Isofistularin
2	4.42	Septicine
3	11.83	Protocatechus
4	15.44	Caulerpin
5	16.15	Aloesin
6	17.89	Corynesidone
7	18.28	Hydroxyanthranilic acid
8	19.67	Acteosid
9	20.84	9 alpha-OH-Pinoresinol
10	22.23	Quercetin-3-o-gall
11	23.62	Querce-3-o-arab-furano
12	23.95	Citreodrimene
13	25.76	Isorhamnetin-diglycoside
14	38.57	No spectra library hits found
15	48.57	Alterporriol
16	56.38	Scopularide

Table 3: Retention time (minutes) and library compounds found in HPLC-UV of hexane fraction

Peak No.	Retention Time in minutes	Compound in the UV Library
1	3.06	Septicine
2	10.96	Septicine
3	23.57	Citreodrimene
4	28.89	(z)-2-decylpent-2-endioic acid
5	33.76	Cyclopenol
6	37.14	Lutein
7	37.78	Sarasiniside
8	38.20	Naamine
9	38.32	No spectra library hits found
10	38.85	No spectra library hits found
11	39.76	No spectra library hits found
12	40.22	No spectra library hits found
13	40.59	Cerebroside
14	40.71	HBI-305 (steroid)
15	42.03	No spectra library hits found
16	42.48	No spectra library hits found
17	44.15	Cytreo-α-pyrone
18	46.13	No spectra library hits found
19	48.50	No spectra library hits found
20	48.77	No spectra library hits found
21	57.02	No spectra library hits found

Table 4: Retention time (minutes) and library compounds found in HPLC-UV of ethylacetate fraction

Peak No.	Retention Time in minutes	Compound in the UV Library
1	3.04	Isofistularin
2	4.87	Epicatechin-o-3,4-dimethylgallate
3	5.69	Catechin-o-3,4-dimethylgallate
4	6.38	Methoxygallate
5	11.21	3,4-o-dimethylgallic acid
6	13.03	(-)-epicatechin-3-(3,4,5-trimethoxybenzoate)
7	14.82	12-α,β-hydroxyrot-2′-enonic acid
8	15.59	3,4-dicaffeoylchina
9	16.55	3,4-dicaffeoylchina
10	17.29	3,4-o-dimethylgallic acid
11	17.69	4-(4-hydroxyl-phenyl0-but-3-en-2-one
12	19.27	Pyranopyrrol
13	20.34	Vermistatin
14	22.00	Kampferol-/quercetin-3,4′-o-triglycoside
15	22.68	Aloeresin
16	23.45	Quercitrin
17	24.12	Que-3-o-(2″-o-mal)-gallact
18	25.70	Quercitrin
19	26.60	Kampf-3-o-rhamnoglucoside
20	26.89	Fasiculatin
21	27.85	Chrysin-6-C-(2″-o-α-L-rhamnosyl)-β-D-glucopyranoside
22	48.53	Piperchabamide

Table 4 above showed that polyphenolic compounds such as epicatechin-o-3,4-dimethylgallate, methoxygallate, 3,4-o-dimethylgallic acid, 3,4-dicaffeoylchina and kampf-3-o-rhamnoglucoside were present in ethylacetate fraction.

Table 5: Retention time (minutes) and library compounds found in HPLC-UV of butanol fraction

Peak No.	Retention Time in minutes	Compound in the UV Library
1	2.96	Isofistularin
2	4.05	Septicine
3	15.97	Bastadin
4	17.37	Cyclopenol
5	19.93	Waol
6	40.14	Di-iso-octylphtalate
7	48.48	No spectra library hits found
8	48.78	No spectra library hits found
9	56.07	Septicine

From the table above, peaks could be seen at various retention times but probably the quantities of the components were small. This could be that most components were non-polar in nature.

Table 6: Retention time (minutes) and library compounds found in HPLC-UV of aqueous fraction

Peak No.	Retention Time in minutes	Compound in the UV Library
1	3.00	Cyclopenol
2	4.10	Septicine
3	17.44	3,4-dihydroxybenzaldehyde
4	20.51	Septicine
5	37.52	No spectra library hits found
6	44.14	No spectra library hits found
7	50.02	No spectra library hits found
8	50.26	No spectra library hits found
9	56.49	Enniatin

The table showed that the aqueous extract contained small quantities of compounds at the various retention times indicated in the spectrum above as the peaks were not legible at various retention times. This could be that water was not a suitable solvent or that most

of the compounds were extracted by the non-polar ethylacetate.

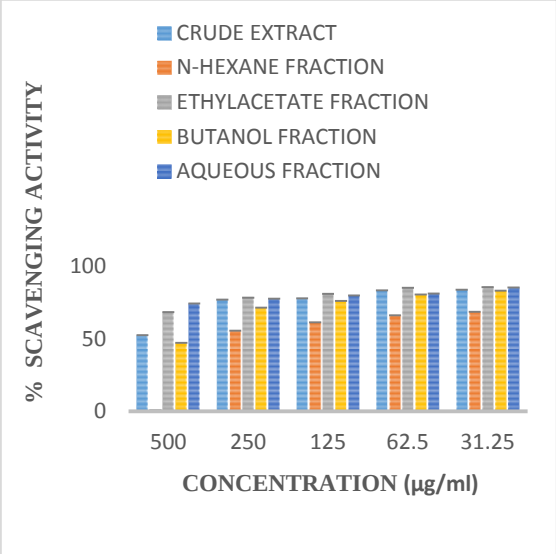

Fig.1: Multiple bar chart representing percentage free radical scavenging activity against concentration

From figure 1, the result indicated high percentage of free radical scavenging activity of the crude methanol extract and the solvent fractions. The high percentage free radical scavenging activity obtained may be attributed to the high reducing power of the crude methanol extract and the solvent fractions [14]. It was evident that the crude methanol extract and the solvent fractions had good free radical scavenging activity but the ethylacetate fraction had the highest with respect to its percentage free radical scavenging values at concentrations 250.00 µg/mL, 125.00 µg/mL, 62.50 µg/mL and 31.25µg/mLrespectively. Other fractions, the aqueous and butanol also possess anti-oxidant property but to a less extent. The percentage free radical scavenging activity of the ethyl acetate fraction was as a result of the compounds such as epicatechin-o-3,4-dimethylgallate,catechin-o-3,4-dimethylgallate, methoxygallate, quercetrin , and 3,4-o-dimethylgallic acid found in abundance in this fraction. These polyphenolic compounds have been ascertained to be good anti-oxidants as they donate electrons or hydrogen atoms to quench the rampaging free radicals.

CONCLUSIONS

The phytochemical analysis result showed the presence of alkaloids, saponins, flavonoids, protein and glycosides while steroids, tannins and cardiac glycosides were absent. The HPLC-UV analysis of the crude and the fractions indicated that methanol and ethyl acetate were better solvents as they extracted more compounds than the butanol and aqueous fraction. It also indicated that the pericarp of *Landolphia owariensis* contained mostly polyphenolic compounds such as quercitrin, quercetin-3,4′-o-triglycoside, 3,4-o-dimethylgallic acid, methoxygallate, catechin-o-3,4-dimethylgallate and kampf-3-o-rhamnoglucoside.The result of the anti-oxidant test using DPPH showed that the crude methanol extract and the solvent fractions had good free radical scavenging activity while the ethyl acetate fraction had the highest activity at concentrations of 250.00 µg/mL, 125.00 µg/mL, 62.50 µg/mL and 31.25 µg/mL when compared with other solvent fractions from the crude methanol extract. This implies that the pericap of the fruit *Landolphia owariensis* could be a useful source of antioxidants.

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