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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIOXIDANT PROPERTIES OF *NAUCLEA LATIFOLIA* ROOT SMITH. (RUBIACEAE)

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ABSTRACT

Nauclea latifolia is a valuable medicinal plant used in therapeutic management of different ailments. Various studies have been done to identify antioxidants from plant sources and efforts have been taken to integrate it in conventional therapy. This study describes the phytochemical analysis and antioxidant properties of the root of *N. latifolia*. Collected root samples were washed and air-dried to constant weight; pulverized and extracted via cold maceration with methanol. The methanol crude extract was fractionated into n-hexane, ethyl acetate, butanol and aqueous fractions. Phytochemical analyses and antioxidant activity of the methanol extract / fractions were assessed using DPPH free scavenging method. Four different fractions were obtained from the methanol (crude) extract. The phytochemical screening conducted showed the presence or absence of certain phytochemicals. The methanol (crude) extract and the different fractions had good antioxidant properties. *N. latifolia* root possesses phytochemicals and antioxidant effect as seen in this study.

KEYWORDS: *Nauclea latifolia*, 2, 2-diphenyl-1-picrylhyrazyl (DPPH), Phytochemicals, Antioxidant, Free radicals.

INTRODUCTION

Traditional medicine has remained the most affordable and easily accessible source for the management of diseases. It allows one utilize natural means as a way of improving health and finding relief from numerous diseases.[1] Approximately 80% of the world`s population relies mainly on traditional medicine, predominantly originated from plants, for their primary healthcare, this was estimated by the World Health Organization (WHO).[2] In recent years, considerable researchers have discovered that the medicinal values of many plants[3], composed of bioactive compounds with proven efficacy as the basic raw material of drugs.[4,5]

A plant is considered a medicinal plant only when its biological activity has been ethno-botanically reported or scientifically established.[6] Several reports have been put over the years by different researchers on the medicinal uses of various plants. One of which is the *Nuclea latifolia* plant. *N. latifolia* Smith. (Rubiaceae) is a straggling shrub with sweet scented flowers and well distributed in many parts of Nigeria. It is also known as African peach; it is a medicinal plant that may be used for traditional medicinal practices of the East and West African sub-regions of continental Africa, where various extracts of the plant are used for the therapeutic management of malaria, hypertension, prolonged menstrual flow, cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailment.[6]

An antioxidant can be defined as any substance that delays or inhibits oxidative damage to a target molecule.[7] The characteristic feature of an antioxidant is the ability to scavenge the free radicals due to their redox hydrogen donor and singlet oxygen quencher.[8,9] Free radicals can be scavenged by the natural (plants) and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol, and tetra butyl hydroquinone) antioxidants.[10,11] However, these synthetic antioxidants are now replaced because the natural antioxidants could be considered safer without any side effects.[12] Drug formulations that are antioxidant based are used in the prevention and treatment of complex diseases which include atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer.[13]

MATERIALS AND METHODS

Equipment

Grinding machine, Hot air oven (Relitech), Rotary evaporator, Refrigerator, Electrical weighing balance (OHAUS Model 2610), Laboratory oven (Relitech), Digital water bath (Sanfa-Model No DK420).

Apparatus/Glass wares

Beakers, Conical flasks, Round bottom flasks, Filter papers, Aluminum foils, Separating funnel, Test tubes, Cotton wool, Hand gloves, Facemasks, 10 ml and 0.5 ml syringes, Marker, Masking tapes, Pasteur pipettes, Measuring cylinders, Stirrer, Micro pipettes.

Chemicals/Reagents

All chemicals and reagents used were of analytical grade.

Plant Material

Samples of fresh healthy *Nuclea latifolia* roots were obtained from Nsukka, Enugu State, Nigeria; authenticated and deposited with the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam. The roots were air dried, pulverized into a fine powder, weighed and stored in an air-tight container till use.

Extraction and Fractionation

The plant materials (500 g) were air-dried, pulverized and cold macerated in methanol for 72 hrs with intermittent shaking, decanting and reconstitution with 500 ml methanol every 24 hrs. The mixture was filtered with Whatman (No.1) filter paper and evaporated using rotary evaporator at 40℃ and completely dried in a hot air oven at the same temperature. The methanol extract was subjected to liquid-liquid fractionation to obtain n-hexane, ethyl acetate, butanol and aqueous fractions respectively.

Preliminary phytochemical screening

The test was carried out on the methanol extract and fractions using standard procedures as described by.[14]

Antioxidant activity

The experiment was conducted using the DPPH (2,2-diphenyl-1-picrylhyrazyl) free radical scavenging assay as described by (Sharma *et al*., 2017). A stock solution of 5 mg/ml of each

sample extract was prepared by dissolving 100 mg of each extract in 20 ml of methanol; a serial dilution was done and the procedure was repeated for n-hexane, ethyl acetate, butanol and aqueous fractions. A 0.2 mmol DPPH solution was prepared by dissolving 8 mg of DPPH in 100 ml of methanol. It turned violet in color. The beaker was properly wrapped with aluminum foil in order to maintain a dark environment.

Methanol was used to zero the UV, absorbance of the blank/ positive control (1 ml of DPPH + 2 ml of methanol) was measured. Extracted solution of 1 ml of the different concentration of each fraction was mixed with 1 ml of DPPH solution and 1 ml of methanol. They were each incubated at room temperature and the absorbance were noted at 520 nm and percentage inhibitions were calculated using the equation:

$$\% \text{ inhibition} = ((Ac - As)/Ac)\ x\ 100$$

Where, Ac is the absorbance of the positive control and As is the absorbance of DPPH solution in the presence of the test sample. A graph of % Scavenging activity of the crude methanol extract and fractions were plotted against the concentrations. The entire procedure was repeated for n-hexane, ethyl acetate, butanol and aqueous fraction.

RESULTS

The yield from the extraction and fractionation carried out on the root of *N. latifolia* using various solvents as presented in table 1 below.

Table 1: Yield from the extraction and fractionation of *N. latifolia* root using various solvents.

Extract/ fractions	Weight of powder (g)	Yield (g)	% yield
Methanol extract	500.00	25.70	5.14
N- Hexane fraction	20.70	1.43	6.91
Ethyl acetate fraction	20.70	6.98	33.72
Butanol fraction	20.70	4.65	22.46

Table 2: Results of Phytochemical analysis of *N. latifolia* root.

Test	Methanol	N-Hexane	Ethyl acetate	Butanol
Anthraquinone	-	+	-	+
Alkaloid	+	-	+	+
Flavonoid	+	+	+	+
Phenol	+	+	+	+
Tannins	+	-	-	+
Steroids	-	+	+	-
Terpenoids	-	+	+	-
Glycoside	+	-	-	+
Saponin	+	-	-	-

Keys: - = Absent, + = Present

Antioxidant activity

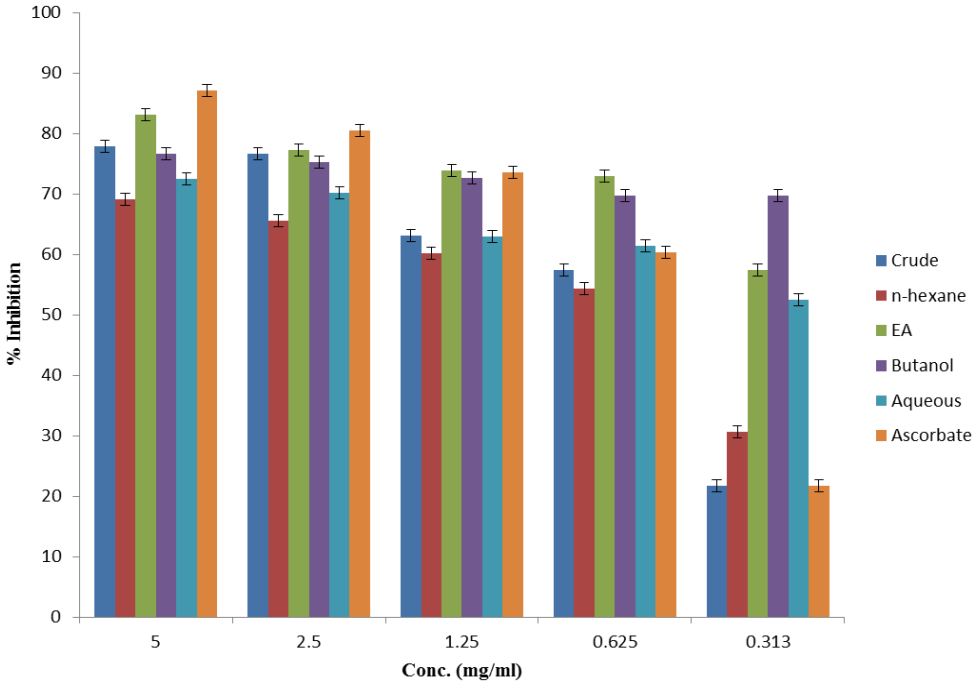

Fig. 1: % inhibition of crude methanol extract and fractions (n-hexane, ethyl acetate, butanol and aqueous) of *N. latifolia* and ascorbic acid against concentration.

DISCUSSION

From the results, 'Table 1' showed the yield and percentage yield of the methanol extract and its derived fractions. The yield ranged from 1.43 - 25.7 g while the percentage yield ranged from 5.14 - 33.72%.

'Table 2' gives the phytochemical constituents in methanol extract, n–hexane, ethyl acetate, butanol and aqueous fractions of the root of *N. latifolia*. Anthraquinone, steroids and terpenoids were absent while alkaloids, flavonoid, phenol, tannins, glycosides and saponins were present in the crude extract; anthraquinone, flavonoid, phenol, steroids and terpenoids were present whereas alkaloid, tannin, glycoside and saponin were absent in n-hexane fraction; anthraquinone, tannins, glycoside and saponin were absent while alkaloid, flavonoid, phenol, steroids and terpenoids were absent in ethyl acetate fraction; anthraquinone, alkaloid, flavonoid, phenol, tannins and glycoside were present whereas steroids, terpenoids and saponin were absent in butanol fraction; anthraquinone, alkaloids,

flavonoids, phenols, tannins, glycosides, anthraquinones, saponins were present while steroids and terpenoids were absent in aqueous fraction.

'Fig. 1' shows the percentage inhibition of different concentrations / doses of crude methanol extract, n-hexane, ethyl acetate, butanol and aqueous fractions of *N. latifolia* root and ascorbic acid (Reference). The response was dose dependent and at the highest concentration (5 mg/ml) tested, the ethyl acetate fraction showed the highest % inhibition at 83.17% comparable to ascorbic acid (87.13 %) which is the reference standard against methanol crude extract (77.89 %), n-hexane (69.15 %), butanol (76.57 %) and aqueous (72.41 %) fractions.

CONCLUSION

The methanol extract, n- hexane, ethyl acetate, butanol and aqueous fractions of *N. latifolia* root significantly inhibited DPPH free radicals at different doses with ethyl acetate fraction showing the highest percentage inhibition, thus, it is concluded that the ethyl acetate fraction of *N. latifolia* root contain most phytochemicals which are sources of natural antioxidants.

Authors' Contributions

This research idea was conceived by Ezeagha Chigozie Celestina, and the experiments were performed under the close supervision of Ezeagha Chigozie Celestina, who also performed the interpretation and analysis of data. Chinenye Henrietta Nedum wrote the first draft of the manuscript. All authors reviewed and approved the final manuscript for publication.

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None.

Conflict of Interest

The authors declare that there is no conflict of interests.

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