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Bioactive Screening and *In Vitro* Antioxidant Assessment of *Nauclea latifolia* Leaf Decoction

Franklyn Nonso Iheagwam^{1, 2, a)}, Emmanuel Israel Nsedu¹, Kazeem Oyindamola Kayode¹, Opeyemi Christianah Emiloju^{1, 2}, Olubanke Olujoke Ogunlana^{1, 2} and Shalom Nwodo Chinedu^{1, 2}

¹Department of Biochemistry, College of Science and Technology, Covenant University, Canaanland, P.M.B. 1023 Ota, Ogun State, Nigeria

²Covenant University Public Health and Wellness Research Cluster (CUPHWERC), Covenant University, Canaanland, P.M.B. 1023 Ota, Ogun State, Nigeria.

^{a)}Corresponding author: franklyn.iheagwam@covenantuniversity.edu.ng.

Abstract. The phytochemical constituents and antioxidant properties of *Nauclea latifolia* leaf decoction were investigated. Dried leaves were extracted in ethanol. Qualitative and quantitative phytochemical analysis was determined spectrometrically. The antioxidant activities were examined *in vitro* using 2,2-diphenyl-1-picrylhydrazyl radical, total antioxidant capacity and ferric reducing antioxidant power assays. Phytochemical screening confirmed the presence of flavonoids, alkaloids, anthocyanins, betacyanins, phenols, saponins, terpenoids, cardiac glycosides and quinones. The total lycopene, β -carotene, phenolics, flavonoid and alkaloid content were found to be 0.038 ± 0.01 mg CAE/g, 0.120 ± 0.04 mg CAE/g, 58.08 ± 0.58 mg GAE/g, 10.75 ± 0.17 mg RE/g and $0.32 \pm 0.08\%$ respectively. *N. latifolia* ethanol leaf extract demonstrated effective antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl with an IC_{50} of 2.58 ± 0.08 mg/mL compared to 0.86 ± 0.02 mg/mL and $< 0.01 \pm 0.01$ mg/mL for butylated hydroxytoluene and ascorbic acid respectively. Total antioxidant capacity and ferric reducing antioxidant power of the extract were 73.81 ± 2.27 and 1314.45 ± 197.64 mg AAE/g respectively. Excellent positive correlations between the phenolic content and antioxidant activities of the extract were observed. The leaf of *N. latifolia* is of therapeutic value and may be exploited for its rich antioxidant components.

INTRODUCTION

There is an intensified research on medicinal plants due to various therapeutic potentials these herbal plants possess. The medicinal properties of these plants are believed to be conferred by phytochemicals present in the plant [2]. Phytochemicals are known as natural organic compounds/substances that are found in plants which gives them their characteristic colour, flavour, smell and texture in addition to their potential in treating, managing and preventing diseases such as cancer and cardiovascular diseases [1]. *Nauclea latifolia* Sm. is a small tree commonly found in tropical Africa especially Nigeria, which grows as tall as 20 feet in height, forming canopies [3]. It is commonly known as pin cushion or African peach tree; In Nigeria, the Igbos call it “Ubuluinu”, “Mbom-mbong” in Efik, “Uche” by the Igede’s, “Egbesi” among the Yoruba’s and “Tafashiya” by the Hausas [5]. *N. latifolia* is used extensively in traditional medicine by the locals. Its infusions and decoctions from stems and roots are used mainly against malaria by traditional healers; as a chewing stick and as a remedy against stomach ache and tuberculosis [4]. It can be prepared together with other plants and applied against diarrhoea. Various researched pharmacological effect of *N. latifolia* include lowering blood pressure, anticonvulsant, antipyretic, analgesic, anxiolytic and sedative properties [6]. However, there is a dearth of information on the evaluation of its bioactive chemical contents, antioxidant properties and relationship between both properties. Hence this study evaluated these parameters.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents were purchased from Surechem Products and Sigma Aldrich. They were all of analytical grade.

Collection of Plant Samples, Identification and Extract Preparations

Healthy looking leaves of *Nauclea latifolia* were sourced from local farmers in Oyo State, Nigeria and identified by a botanist, Dr. J.O. Popoola from the Department of Biological Sciences, Covenant University, Ota, Nigeria. The leaves were cut into bits with knife and air dried to uniform size at room temperature for 10 days to remove all moisture. The samples were then ground into fine powder. The pulverised sample (800 g) was macerated in 80% ethanol (8 L) for 72 hrs. The filtrate obtained was concentrated using a rotary evaporator to yield a greenish-brown paste extract.

Qualitative Phytochemical Analysis

Test for tannins

To 1 mL of plant extract, 2 mL of 5% ferric chloride was added. Formation of greenish black indicated the presence of tannins.

Test for saponins

To 2 mL of plant extract, 2 mL of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicated the presence of saponins.

Test for flavonoids

5 mL of dilute ammonia solution was added to portions of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid. The appearance of yellow colouration indicated the presence of flavonoids.

Test for alkaloids

To 2 mL of plant extract, 2 mL of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green colour indicated the presence of alkaloids.

Test for anthocyanin and betacyanin

To 2 mL of plant extract, 1 mL of 2 N sodium hydroxide was added and heated for 5 minutes at 100 °C. Formation of yellow colour indicated the presence of betacyanin.

Test for quinones

To 1 mL of extract, 1 mL of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinones.

Test for glycosides

To 2 mL of plant extract, 3 mL of chloroform and 10% ammonia solution was added. Pink colour formation indicated the presence of glycosides.

Test for cardiac glycosides

To 0.5 mL of extract, 2 mL of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 mL of concentrated sulphuric acid. Brown ring formation at the interface indicated the presence of cardiac glycosides.

Test for terpenoids

To 0.5 mL of extract, 2 mL of chloroform was added and concentrated sulphuric acid was added carefully. Red-brown colour formation at the interface indicated the presence of terpenoids.

Test for triterpenoids

To 1.5 mL of extract, 1 mL of Liberman-Buchard reagent (0.5 mL of acetic anhydride + 0.5 mL of concentrated sulphuric acid) was added. Blue-green colour formation indicated the presence of triterpenoids.

Test for phenols

To 1 mL of the extract, 2 mL of distilled water followed by few drops of 10% ferric chloride was added. Formation of green colour indicated the presence of phenols.

Test for coumarins

To 1 mL of extract, 1 mL of 10% sodium hydroxide was added. Formation of yellow colour indicated the presence of coumarins.

Quantitative Phytochemical Analysis

Total Phenolic content

Total phenolic content (TPC) was determined according to the method of Sharma *et al.* [8]. Folin-ciocalteu reagent was added to 100 μ l of sample/standard in ratio 1:10. The solution was mixed and incubated at room temperature for 1 minute followed by the addition of 1.5 mL to 20% sodium carbonate. The final mixture was shaken and incubated for 90 minutes in the dark at room temperature. The absorbance was taken at 725 nm and the phenolic content was expressed as gallic acid equivalents GAE/g of sample.

Total flavonoid content

Total flavonoid content (TFC) was determined according to the method described by Sharma *et al.* [8]. In different test tubes, 0.5 mL extract, 2 mL of distilled water, followed by 0.15 mL of sodium nitrite (5% w/v) was added. After 5 min, 0.15 mL of aluminium trichloride (10 %) was added and incubated for 6 min. After incubation 2 mL of sodium hydroxide (4% w/v) was added. After 15 min of incubation reaction mixture turns to pink and absorbance was measured against blank *e.g.* distilled water at 510 nm. A natural flavonoid rutin was used as standard. The TFC was expressed in RE/g of sample.

β -carotene and Lycopene

The test for β -carotene and lycopene was carried out according to the method described by Sharma *et al.* [8]. The dried extract was vigorously shaken with 10 mL of acetone-hexane mixture (4:6) for 1 minute and filtered through Whatman N0.4 filter paper. The absorbance of filtrate was measured at 453, 505, 645 and 663 nm. The content of lycopene and β -carotene were calculated using equation (1) and (2), respectively. The values are expressed as μ g/g of extract.

$$\text{Lycopene (mg/100mL)} = -0.0458A_{663} + 0.372A_{505} + 0.0806A_{453} \quad (1)$$

$$\beta - \text{carotene (mg/100mL)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453} \quad (2)$$

Total Alkaloids

According to the methods of Senguttuvan *et al.* [7], 200 mL of 20% acetic acid was added to 5 g of sample and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to $\frac{1}{4}$ using water bath. To this sample, concentrated ammonium hydroxide was added drop wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The percentage of total alkaloids was calculated using equation (3).

$$\text{Total alkaloids (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100 \quad (3)$$

Antioxidant Assessment

2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity of NLE was carried out according to the method described by Sharma *et al.* [8]. 0.5 mL of DPPH was added to 0.5 mL aliquots of sample/standard in different concentrations. Control test tubes were loaded with 0.5 mL of dimethyl sulfoxide (DMSO) and 0.5 mL DPPH. After incubation at 37 °C for 30 min in dark, the absorbance was recorded at 517 nm. Ascorbic acid was used as a standard. The percentage scavenging by test sample at each concentration was calculated using the formula (4).

$$\text{Scavenged DPPH (\%)} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100 \quad (4)$$

Ferric reducing antioxidant power (FRAP)

The reducing power of NLE was determined according to the method described by Sharma *et al.* [8]. Briefly, 1 mL of sample was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The reaction mixture was incubated at 50 °C for 20 minutes. Then 2.5 mL of trichloroacetic acid (10%) was added and centrifuged for 10 minutes. An aliquot 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (0.1%). The absorbance of all solutions was measured at 700 nm and expressed as mg of ascorbic acid equivalent per g of powder (mg AAE/g powder).

Total antioxidant capacity (TAC)

TAC was determined according to the method described by Sharma *et al.* [8]. 0.3 mL of extract was combined with 3 mL reagent solution (0.6 M sulphuric acid, 28mm sodium phosphate and 4mM ammonium molybdate). The reaction mixture was capped and incubated at 95 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against blank (methanol 0.3 mL). Ascorbic acid was taken as the standard.

Statistical Analysis

Results were expressed as mean \pm standard deviation (SD) of replicate determinations. Statistical analysis of mean values was carried out by one-way analysis of variance (ANOVA) supplemented by Duncan multiple range test. Values were considered statistically significant at $p < 0.05$.

RESULTS

The qualitative phytochemical analysis of *N. latifolia* aqueous extracts as illustrated in table 1 revealed the presence of flavonoids, alkaloids, anthocyanins, betacyanins, phenols, saponins, terpenoids, cardiac glycosides and quinones; tannins, glycosides, triterpenoids and coumarins were not detected.

TABLE 1. Qualitative phytochemical analysis of *N. latifolia* ethanolic leaf extract.

Phytochemicals	<i>Nauclea latifolia</i>
Tannins	-ve
Saponins	+ve
Flavonoids	+ve
Alkaloids	+ve
Anthocyanin and betacyanins	+ve
Quinones	+ve
Glycosides	-ve
Cardiac glycosides	+ve
Terpenoids	+ve
Triterpenoids	-ve
Phenols	+ve
Coumarins	-ve

+ve=present; -ve=absent

When quantitatively analysed the total flavonoid, phenolic, lycopene, β -carotene, phenolics, flavonoid and alkaloid content were estimated to be 10.75 ± 0.17 mg RE/g, 58.08 ± 0.58 mg GAE/g, 0.038 ± 0.01 mg CAE/g, 0.120 ± 0.04 mg CAE/g and $0.32 \pm 0.08\%$ respectively as shown in table 2.

TABLE 2. Total flavonoid, phenolic, β - carotene, lycopene and alkaloid content of *N. latifolia* ethanolic leaf extract

Phytochemicals	<i>N. latifolia</i> Content
TFC (mg RE/g)	10.75 ± 0.17
TPC (mg GAE/g)	58.08 ± 0.58
β -carotene (mg CAE/g)	0.12 ± 0.04
Lycopene (mg CAE/g)	0.038 ± 0.01
Total Alkaloid (%)	0.32 ± 0.08

Data are mean \pm SD of triplicate determinations.

Figure 1 shows a dose-dependent increase in scavenging ability of DPPH radical for *N. latifolia* ethanolic leaf extract, butylated hydroxytoluene and ascorbic acid. *N. latifolia* ethanolic leaf extract scavenging ability was comparable with butylated hydroxytoluene while ascorbic acid exhibited the most scavenging ability. The IC_{50} of *N. latifolia* ethanolic leaf extract was 2.58 ± 0.08 mg/mL compared to 0.86 ± 0.02 mg/mL and $< 0.01 \pm 0.01$ mg/mL for butylated hydroxytoluene and ascorbic acid respectively as presented in table 3.

TABLE 3. IC_{50} of *N. latifolia* ethanolic leaf extract, butylated hydroxytoluene and ascorbic acid DPPH scavenging ability.

	IC_{50} (mg/mL)
<i>Nauclea latifolia</i>	2.58 ± 0.08^a
BHT	0.86 ± 0.02^b
AA	$<0.01 \pm 0.01^c$

Data are mean \pm SD of triplicate determinations. Values with different superscripts in a column are significantly different at $p < 0.05$.

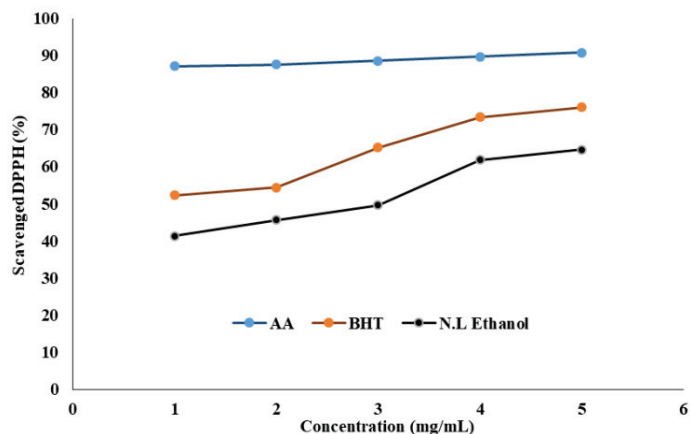


FIGURE 1. DPPH radical scavenging ability of butylated hydroxytoluene, ascorbic acid and *N. latifolia* ethanolic leaf extract

Figure 2 depicts the total antioxidant capacity and ferric reducing antioxidant power. A dose dependent increase was observed for both assay with 73.81 ± 2.27 mg AAE/g being the highest antioxidant capacity and 1314.45 ± 197.64 mg AAE/g being the highest reducing power recorded. The observed potent DPPH radical-scavenging, total antioxidant and ferric reducing capacity of the extract significantly corroborated the total phenolic content with a good correlation coefficient as illustrated in table 4.

TABLE 4. Result of correlation and regression analysis

Dependent Variable	Pearson Correlation			Linear Regression (Sig.)		
	DPPH	TAC	FRAP	DPPH	TAC	FRAP
TPC	0.925*	0.977**	0.981**	0.024	0.004	0.003

**Correlation is significant at 0.01 level (2-tailed).

*Correlation is significant at 0.05 level (2-tailed) at $p < 0.05$.

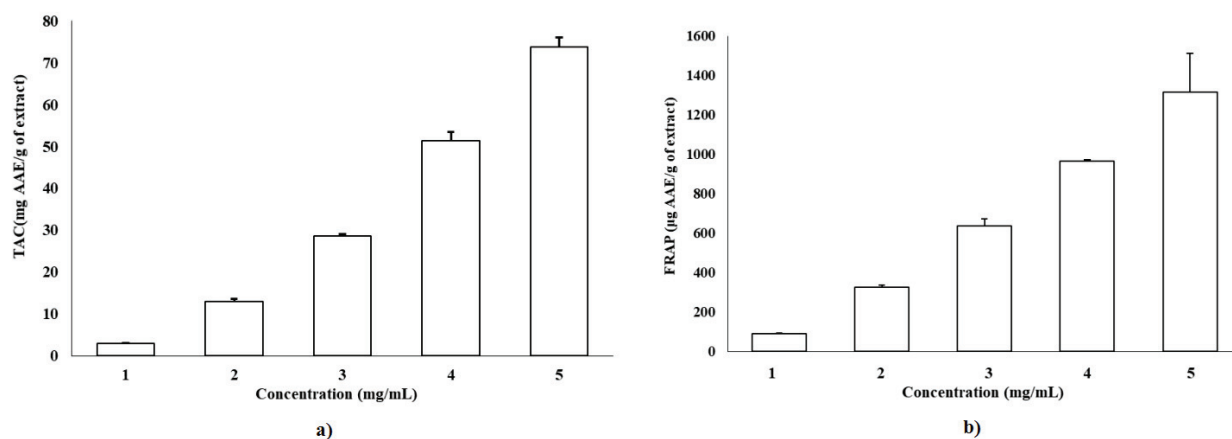


FIGURE 2. a) Total antioxidant capacity of *N. latifolia* ethanolic leaf extract, b) Ferric reducing antioxidant power of *N. latifolia* ethanolic leaf extract

DISCUSSION

The presence of flavonoids, alkaloids, anthocyanins, betacyanins, phenols, saponins, terpenoids, cardiac glycosides and quinones in *N. latifolia* ethanolic leaf extract is an indication that these secondary plant metabolites have a synergistic effect on the various pharmacological properties reported by Onyesom *et al.* [6]. These phytochemicals play a major role in preventing disease and promoting the general health status of individuals. They are also involved in scavenging free radicals, inhibiting carcinogen-activating enzymes and increasing the synthesis and activity of enzymes that detoxify carcinogens [16]. β -carotene and lycopene have been reported to be potentially active in reducing the risk of both breast and prostate tumours [13]. Strong antioxidant activity has been attributed to be the molecular mechanism underlying the association between carotenoids and cancer prevention. Phenolic compounds are known antioxidants, due to their ability to mop up free radicals and reactive oxygen species [11]. DPPH is a stable free radical which delocalises its spare electron to give a deep violet colour. In the presence of an antioxidant, a hydrogen atom is donated followed by loss of colour [12]. The results generated from this study demonstrated that *N. latifolia* ethanolic leaf extract possessed good free radical scavenging activity. This potential may be attributed to the appreciable amounts of phenolic and flavonoid content [10]. Donating electron to reduce ferric ion to ferrous ion and Mo (VI) to Mo (V) is an indication of reducing power. The reducing capacity of a compound may be directly translated as an indicator of antioxidant activity [14]. The strong reducing power of *N. latifolia* ethanolic leaf extract might be as a result of its phenolic content, as they are good electron donors [15]. The extract's antioxidant activity correlated positively with their phenolic contents as corroborated in a study by Awah *et al.* [17].

CONCLUSION

This study provides evidence that *N. latifolia* possesses significant antioxidant activities which can be attributed to the phenolic content and other bioactive compounds present in the extract. However, further *in vitro* and *in vivo* study is required to better understand the efficacy and mechanism of action of these bioactives present in these plant extracts to provide scientific proof for clinical employment of *N. latifolia* secondary metabolites in modern medicine.

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