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In-vitro Antioxidant Properties of Nauclea Latifolia Unriped Fruits

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Abstract

The study aimed to investigate the in-vitro antioxidant properties of methanol and N-hexane crude extracts of Nauclea latifolia unriped fruits. Qualitative and quantitative phytochemical analysis of methanol and N-hexane crude extracts of Nauclea latifolia unriped fruits were determined spectrophotometrically in duplicates and their antioxidant activities, examined in-vitro using 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2) and ferric reducing antioxidant power assay (FRAP), all were done according to standard laboratory practices. Statistical analysis were carried out by one-way analysis of variance (ANOVA) supplemented by Duncan multiple range test using SPSS version 20. Results obtained revealed, the presence of phenol, flavonoids, tannins and alkaloids in both extracts, however, methanol extract revealed a higher concentration of tannin (0.994 ± 0.070 mg/ml) TAE when compared with N-hexane extract with a value of 0.263 ± 0.028 (mg/ml) TAE. Also, N-hexane extract revealed a higher percentage (15%) of alkaloid when compared to methanol extract (10%). The percentage inhibition of oxidation in both extracts decreased down the column with respect to decrease in concentration of the extracts. Methanol extracts exhibited higher percentage inhibition on DPPH (84.3%) at 100 mg/ml, N-hexane exhibited a higher inhibition of 88.8% for H_2O_2 at 100 mg/ml. Methanol and N-hexane extracts revealed a higher percentage inhibition of 87.6% and 88.0% respectively at 10 mg/ml for FRAP. The absorbance recorded also correlated with the level of percentage inhibitions of methanol and N-hexane crude extracts of Nauclea latifolia unripe fruits. The study thus revealed methanol and N-hexane extracts of unriped fruits of Nauclea latifolia possesses in-vitro antioxidant properties at the highest concentrations tested, and may be used in management of oxidative stress related diseases.

Keywords: Nauclea latifolia; 2,2-diphenyl-1-picrylhydrazyl (DPPH); Hydrogen peroxide (H_2O_2); Ferric reducing antioxidant power assay (FRAP)

Background

Oxidative stress is defined as a disturbance in the equilibrium between free radicals (FR), reactive oxygen species (ROS) and the endogenous defense mechanisms (McCord, 2000). Free radicals are produced as part of normal cellular function [1]. They may also be produced by endogenous and environmental sources. Endogenous sources include mitochondrial leak, respiratory burst, enzyme reactions and auto-oxidant reactions. Environmental sources include cigarette smoke, pollutants (such as ozone and nitrogen dioxide), ultraviolet light, ionizing radiation, and xenobiotics. The most important free radicals produced are the oxygen derivatives such as the superoxide radical ($O2•^-$), and the hydroxyl free radical (OH•) (Young and Woodside, 2001). Other examples of free radicals include nitric oxide (NO•), nitrogen dioxide (NO2•), peroxyl (ROO•), alkoxyl radicals (RO•) and lipid peroxyl (LOO•). These are highly reactive species, capable of damaging biologically relevant macromolecules such as deoxyribonucleic acid (DNA), proteins, carbohydrates and lipids in the nucleus and membranes of cells (Young and Woodside, 2001).

The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by reactive oxygen species (ROS) [2] and reactive nitrogen species (RNS) thereby enhancing the immune defense and lowering the risk of cancer and other degenerative diseases (Chatterjee et al., 2007). 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 (Khalaf et al., 2008). However, other natural and alternative plant sources known for antioxidant activities also exist.

Nauclea latifolia Smith (family: Rubiaceae) is a straggling, evergreen, multistemmed shrub or small tree native to tropical Africa and Asia. It normally produces interesting flowers, edible, but not appealing, large red ball fruits with long projecting stamens (James and Ugbede, 2011). Commonly used parts of Nauclea latifolia include the leaves, roots, stem, and fruits.

Phytochemical screening of the ethanolic extract of the fruits revealed the presence of alkaloids, tannins, saponins, phytates, flavonoids and cyanogenic glycosides, while proximate analysis revealed that the fruits are rich in proteins, fiber, carbohydrates, moisture, dry matter, vitamins (A, B1, B2, C, and E). The fruits were also shown to contain essential minerals such as copper, iron, cobalt, calcium, magnesium, zinc and phosphorus (Agyare et al., 2006). Isolation and characterization of some of the active constituents of the ripe and unripe fruits of the plants led to isolation of some phthalates (Eze and Obinwa, 2014) and fatty acid esters (Fadipe, 2014). Several other studies have confirmed the health potentials of Nauclea latifolia. Some of its medicinal effects include antimalarial (Benoit et al., 1998), antidiabetic (Gidado et al., 2005), antihypertensive (Nworgu et al., 2008), antipyretic and antinociceptive (Taiwe et al., 2011), and anti-inflammatory and analgesic activities (Goji et al., 2010). It is also known

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to have a strong antibacterial property (Okiei et al., 2011). This study is therefore aimed to investigate the antioxidant properties of methanolic and N-hexane crudes extracts of unripe fruits of Nauclea latifolia.

Materials and Methods

Study design

50g each of the powdered sample were accurately weighed using a Digital scale (Techne 7) and were macerated in 300 ml (0.3L) of each of the methanol (99.9%) and N-hexane (85%) respectively for 48hrs with intermittent shaking. The mixture was filtered using whatman's filter paper No 1 to obtain a solution devoid of solids, for methanol and N-hexane respectively. The crude extracts were further concentrated in a water bath set at 60°C for 72 hrs.

Subjects

Healthy and fresh unriped fruits of Nauclea latifolia were collected from Yandev, Gboko Local Government Area of Benue State, in the month of February, 2021 and were identified and authenticated in the Department of Botany, University of Agriculture Makurdi. The sliced unriped fruits were shade dried at room temperature for two weeks before being pulverized into smooth powder with an electric grinder. The resulting powder was stored in a polythene bag pending extraction [3].

Determination of percentage yield of methanolic and N-hexane extracts of Nauclea latifolia

Percentage yield of the extract were calculated as follows.

$$\text{Yield (\%)} = \frac{(W1 \times 100)}{W2}$$

Where,

W1 = Weight of extract after evaporation of solvent.

W2 = Dry weight of the sample before extraction.

Preliminary Phytochemical Screening

Test for Phenol

A few quantity of the sample (0.5 g) was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of phenols (Trease and Evans, 2002).

Test for Flavonoid

A few quantity of the sample (0.5 g) was dissolved in water and filtered. To this 2 ml of the filtrate, few drops 10% ferric chloride solution was added to produce a green-blue or violet colouration. A change in colour from green-blue or violet on addition of dilute ferric chloride was an indication of the presence of flavonoids (Trease and Evans, 2002).

Test for Tannin

Sample (0.5 g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The occurrence of a blue-black, green or blue-green precipitate indicated the presence of tannins (Trease and Evans, 2002).

Test for Alkaloid

A few quantity of the sample (0.5 g) was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate was an indication for the presence of alkaloids (Sofowora, 1993).

Quantitative Phytochemical Screening of Methanolic and N-hexane Crude Extract of Nauclea latifolia

Total phenolic content

The total phenolic content was evaluated by adopting a modified spectrophotometric method described by Singleton and Rossi (1965) with slight modifications.1g of both methanolic and N-hexane extracts were dissolved in 10ml methanol and N-hexane respectively [4].

0.5 ml of the filtrate (methanol and N-hexane) were added into separate test tube, followed by 0.5 ml of 1:10 dilution of Folin Ciocalteu reagent. After few minutes, 0.5 ml of 15% Na2CO3 solution was added and the solution was made up to 4 ml with distil water. The reaction mixture was allowed to stand for 20 minutes. The absorbance of the mixture was read at 760 nm and tannic acid was used as standard. Phenolics concentration was calculated using the formula, Absorbance of sample⁄Absorbance of standard×concentration of standard, where the concentration of standard = 0.1 mg/ml.

Total flavonoid content

This was determined spectrophotometrically using the method described by Jia et al., (1999) with some modifications. 1 g of both methanolic and N-hexane extracts were dissolved in 10ml methanol and N-hexane respectively.

1ml of the filtrate (methanol and N-hexane) were measured into separate test tube, and 75 µL of 5% $NaNO_3$ solution was added to each test tube. After five minutes, 150 µL of 10% Al_2Cl_3 was added followed by 500 µL of 10% NaOH and 275 µL of H_2O (distill water) after six minutes. The mixture was read using the ultra violet spectrometer at 510 nm and quercetin was used as standard. Flavonoid concentration was calculated using the formula, Absorbance of sample⁄Absorbance of standard×concentration of standard, where the concentration of standard = 0.1 mg/ml.

Total tannin content

Quantity of tannins was determined by using the spectrophotometer method (Adeyemi et al., 2014). 1g of both methanolic and N-hexane extracts were dissolved in 10ml methanol and N-hexane respectively.

1ml of the filtered sample (methanol and N-hexane) were pipetted out into separate test tubes, and mixed with 800µL of 0.1M $FeCl_3$ in 0.1M HCl and 800 µL K4Fe(CN)6.3H_2O. The absorbance was measured with a spectrophotometer at 395 nm wavelength within 10 minutes. Tannic acid was used as standard. Tannin concentration was calculated using the formula, Absorbance of sample⁄Absorbance of standard×concentration of standard, where the concentration of standard = 0.1 mg/ml. concentration was calculated using the formula, Absorbance of sample⁄Absorbance of standard×concentration of standard, where the concentration of standard = 0.1 mg/ml [5].

Total alkaloid content

The total alkaloid content was evaluated by adopting the method described by Adeyemi et al., (2014).

1g of both methanolic and N-hexane extracts were dissolved in 10ml methanol and N-hexane respectively. 2ml of the filtered sample (methanol and N-hexane) were pipetted out into separate beaker/ conical flask and were titrated with concentrated ammonial until precipitation was completed. The whole solution was allowed to settle down for five minutes.

Two filter papers were weighed and samples were filtered. The residues obtained were collected and washed with concentrated NH3. The filter papers were labelled and allow to dry. The residues were alkaloid, which were then dried and weighed. The differences in masses were recorded.

The percentage of total alkaloids was calculated using equation

Total alkaloids (%) = Weight of residue ×100

Weight of sample taken

Antioxidant activities of methanolic and N-hexane crude extracts of Nauclea latifolia

Scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH)

The ability to scavenge the stable free radical DPPH or antioxidant activity was determined using the DPPH free radical scavenging method (Hatano et al, 1988; Gulcin et al, 2002 and Mutee, 2010). 0.21g of 2, 2-diphenyl-1-picryhydrazyl radical (DPPH), a stable radical was dissolved in methanol (100 ml). 2 g of each extracts were dissolved separately in 20 ml 99.9% methanol and 85% N-hexane respectively. To 3.0 ml of the methanolic solutions of DPPH was added 0.5 ml (500 µl) of each of the extracts with concentration 100mg/ml. The same procedure was repeated for the extracts concentrations (50 mg/ml and 25 mg/ml) obtained from two fold serial dilution. The mixture was incubated in the dark for 10 minutes upon which the decrease in absorption at 517 nm of DPPH was measured. The actual decrease in absorption was measured against that of the control and the percentage inhibition was also calculated. The same experiment was carried out on butylated hydroxylanisole (BHA), α-tocopherol and ascorbic acid which are known standards antioxidant. All test and analysis were run in duplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

%RSA or % inhibition = (Abscontrol - Abssample) x100

Abscontrol

Where Abssample is the absorbance of the sample solution, Abscontrol is the absorbance of control. Absorbance of DPPH =2.115.

Scavenging effect on hydrogen peroxide (H_2O_2)

The antioxidant activities of methanolic and N-hexane crude extract of Nauclea latifolia scavenging effect on hydrogen peroxide were determined according to Oloyede and Farombi, 2010 method. A solution of 2 mM hydrogen peroxide was prepared in phosphate buffered-saline (PBS) pH 7.4. 2g of each extracts were dissolved separately in 20ml 99.9% methanol and 85% N-hexane respectively. To 3.0 ml of the H_2O_2 solution was added 0.5 ml of each of the extracts with concentration 100 mg/ml. The same procedure was repeated for the extracts concentrations (50 mg/ml and 25 mg/ml) obtained from two fold serial dilution. Decrease in absorbance of H_2O_2 at 285nm was determined spectrophotometrically 10 minutes later against a blank solution containing the reagents in distil water without the test sample. All tests were run in duplicates and averaged. The same experiment was carried out on Butylated hydroxyanisole (BHA), ascorbic acid and α-tocopherol which are known antioxidant standards. Absorbance of H_2O_2 = 1.030.

Antioxidant activity by ferric thiocyanate method

The antioxidant activities of methanolic and N-hexane extracts of Nauclea latifolia were determined by ferric thiocynate method (Mackie and McCartney, 1989). 2g of each extracts were dissolved separately in 20ml 99.9% methanol and 85% N-hexane respectively. A mixture of 2 ml of sample in 2ml 99.5% ethanol, 4 ml phosphate buffer (pH 7.0) and 2 ml of water was placed in a vial with a screw cap and placed in an oven at 600C in the dark for 10 minutes. To 0.1 ml (100µl) of this sample solution with concentration 10 mg/ml, 10 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocynate was added. After the addition of 0.1 ml of 2 x 10-2 M ferrous chloride in 3% hydrochloric acid to the reaction mixture, the absorbance of the red colour which developed was measured in 3 minutes at 500nm. This procedure was repeated for 5 mg/ml and 2.5 mg/ml of extracts concentrations obtained from two fold serial dilution. The control and standards were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of Butylated hydroxyanisole (BHA), ascorbic acid and α-tocopherol (reference compounds) (Oloyede et al., 2010) [6]. The inhibition of lipid peroxidation in percentage was calculated using this equation: % Inhibition = 1 - (A1/A2) X 100

Where A1 was the absorbance of the test sample and A2 was the absorbance of control reaction. Absorbance of FRAP = 2.766.

Data Presentation and Statistical Analyses

Results were expressed as mean ± standard deviation (SD) of replicate determinations. Statistical analyses of mean values were carried out by one-way analysis of variance (ANOVA) supplemented by Duncan multiple range test using SPSS version 20. Values were considered statistically significant at $p \leq 0.05$.

Results

Determination of percentage yield of methanolic and n-hexane extracts of unripe fruits of Nauclea latifolia

The determination of percentage yield of methanolic and N-hexane extracts of unripe fruits of Nauclea latifolia revealed that, N-hexane extracts had a higher yield (33.22%) as compare to that of methanolic extracts (28.78%) (Table 1).

Preliminary phytochemical screening

The preliminary phytochemical analysis of methanolic and N-hexane extracts of Nauclea latifolia unripe fruits as illustrated in table 2 revealed the presence of all the phytochemicals that were screened for, which include; phenols, flavonoids, tannins and alkaloids. Tannins were found to be present in very high concentration (+++) in the methanolic extracts of Nauclea latifolia unripe fruits, while the N-hexane extracts contained minimal concentration (+) of tannin and phenols.

Phenols and alkaloids contained minimal concentration (+) in the methanolic extracts of N. latifolia unripe fruits.Flavonoids contained moderate concentration for both extracts.

Quantitative phytochemical screening of methanolic and N-hexane crude extracts of nauclea latifolia unripe fruits

The quantitative phytochemical composition of methanolic and N-hexane extracts of Nauclea latifolia unripe fruits revealed that the phytochemicals were in different concentration.

Tannins were the most abundant (0.994 ± 0.070) in the methanolic extracts, while having a lower concentration (0.263 ± 0.028) in the N-hexane extracts.

Flavonoids concentrations were high in both extracts (0.659 ± 0.001, 0.649 ± 0.110) for methanolic and N-hexane extracts respectively.

Phenol concentrations in both extracts were almost the same, having the least concentration (0.230 ± 0.014, 0.226 ± 0.002) for methanolic and N-hexane extracts respectively.Alkaloids were expressed in percentage with a moderate concentration (15%) in N-hexane extracts and a lower concentration (10%) in methanolic extracts (Table 1).

W 1 = weight of extract after evaporation of solvent.

W = Dry weight of the sample before extraction.

% = percentage yeld.

(Table 2)

Key: + = minimally present

++ = moderately present

+++ = highly present

(Table 3)

Results are expressed as means + S.D; n = 2.

Key: QE = quercetin equivalence.

TAE = Tannic acid equivalence.

S.D = standard deviation.

Percentage inhibition of methanolic and n-hexane crude extracts of Nauclea latifolia unripe fruits

Percentage inhibition of methanolic extracts, N-hexane extracts and standards on DPPH

The percentage inhibition of methanol and N-hexane extracts of unripe fruits of nauclea latifolia along with antioxidant standards on 2,2-diphenyl-1-picrylhydrazyl (DPPH) revealed, methanolic extract with higher inhibition when compared to N-hexane extract. The inhibition decreases down the column with respect to decrease in the concentration of extracts (84.3%, 100 mg/ml; 84.1%, 50 mg/ml and 67.4, 25 mg/ml), [7] except for the N-hexane extracts as the inhibition tends to increase with respect to decrease in concentration of extracts (52.4%, 100 mg/ml; 61.5%, 50 mg/ml and 68.9%, 25 mg/ml) (Table 5).

Percentage inhibition of methanolic extracts, N-hexane extracts and standards on Hydrogen peroxide (H_2O_2)

The percentage inhibition for both extracts and antioxidant standards were found decreasing down the column in a concentration-dependent manner.

N-hexane extracts had higher inhibitory action on H_2O_2 (88.6%, 100 mg/ml; 75.0, 50 mg/ml and 60.5, 25 mg/ml) when compared with the corresponding methanolic extracts and standards.

When the inhibition of methanolic extracts were compared with that of the standards, result revealed that, standards provides higher inhibitory action on H_2O_2 than the corresponding methanolics extracts (Table 6).

Percentage inhibition of methanolic extracts, N-hexane extracts and standards on Ferric reducing antioxidant power assay (FRAP)

Methanolic extracts of Nauclea latifolia unripe fruits had excellent inhibitory action (87.6%, 100 mg/ml; 85.6%, 50 mg/ml and 83.0%, 25mg/ml) on FRAP than N-hexane extracts and the corresponding standards as illustrated in table 7. The percentage inhibition of extracts (methanol and N-hexane) and standards decreases down the column with respect to decrease in concentration of extracts and standards (Tables 4-6).

Scavenging effect of Nauclea latifolia extracts (methanol and n-hexane)

Scavenging effect of Nauclea latifolia extracts (methanolic and n-hexane) on 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The absorbance measurement of methanolic extracts, n-hexane extracts, α-tocopherol, ascorbic acid and BHA as illustrated by table8 revealed, methanolic extracts had strong scavenging activity on DPPH (0.332 ± 0.001, 0.336 ± 0.004 and 0.690 ± 0.014) than the extracts of N-hexane and standards. There was no significant ($p \leq 0.05$) increase when the absorbance at 100 mg/ml was compared with that of 50 mg/ml. While the absorbance of [8] methanolic extracts and standards decreases down the column with decreased concentration, the absorbance of N-hexane extracts tends to increase with decreased concentration. Values were considered statistically significant at $p \leq 0.05$.

Scavenging effect of Nauclea latifolia extracts (methanolic and n-hexane) on Hydrogen peroxide (H_2O_2)

The absorbance measurement of methanolic extracts, n-hexane extracts, α-tocopherol, ascorbic acid and BHA showed that, N-hexane extracts had strong scavenging activity on H_2O_2 (0.117 ± 0.002, 0.258 ± 0.035 and 0.407 ± 0.006) than the extracts of methanol, alha-tocopherol and BHA. No significant ($p \leq 0.05$) difference was observed when absorbance at 100 mg/ml was compared with 50 mg/ml concentration, but when compared with the 25 mg/ml concentration, there was a significant ($p \leq 0.05$) decrease. Ascorbic acid possessed the strongest scavenging activity on H_2O_2 (0.128 ± 0.016, 0.248 ± 0.014 and 0.250 ± 0.048) (Table 9).

Table 1: Percentage yield of methanolic and N-haxane extracts of *Nauclea latifolia.*

Extracts	W_2 (g)	W_1 (g)	% Yield
Methanol	50	14.39	28.78
N-hexane	50	16.61	33.22

Table 2: Preliminary phytochemical screening of methanolic and N-hexane crude extracts of *Nauclea latifolia.*

Phytochemicals	Methanolic extracts	N-hexane extracts
Phenols	+	+
Flavonoids	++	++
Tannins	+++	+
Alkaloids	+	++

Table 3: Quantitative phytochemical screening of methanolic and N-hexane crude extracts of *Nauclea latifolia* unripe fruits.

Phytochemicals	Methanolic extracts	N-hexane extracts
Phenols (mg/ml)TAE	0.230 ± 0.014	0.226± 0.002
Flavonoids (mg/ml) QE	0.659 ± 0.001	0.649 ± 0.110
Tannins (mg/ml) TAE	0.994 ± 0.070	0.263± 0.028
Alkaloids (%)	10	15

Table 4: Percentage inhibition of *Nauclea latifolia* extracts (methanolic and N-hexane), alpha-tocopherol,ascorbic acid and BHA on 2,2-diphenyl-1-picryl hydrazyl (DPPH).

Concentration of extracts (mg/ml)	Methanolic extracts (%)	N-hexane extracts (%)	Alpha-tocopherol (%)	Ascorbic acid (%)	BHA (%)
100	84.3	52.4	97.7	90	97.9
50	84.1	61.5	97.4	75.3	91.6
25	67.4	68.9	53.6	40.6	89.1

Table 5: Percentage inhibition of *Nauclea latifolia* extracts (methanolic and N-hexane), alpha-tocopherol, ascorbic acid and BHA on hydrogen peroxide (H_2O_2).

Concentration of extracts (mg/ml)	Methanolic extracts (%)	N-hexane extracts (%)	Alpha-tocopherol (%)	Ascorbic acid (%)	BHA (%)
100	65.8	88.6	82.3	87.6	85.4
50	55.1	75	81.6	75.9	76
25	52.7	60.5	36.4	75.7	32.2

Table 6: Percentage inhibition of *Nauclea latifolia* extracts (methanolic and N-hexane), alpha-tocopherol, ascorbic acid and BHA on ferric reducing antioxidant power assay (FRAP).

Concentration of extracts (mg/ml)	Methanolic extracts (%)	N-hexane extracts (%)	Alpha-tocopherol (%)	Ascorbic acid (%)	BHA (%)
10	87.6	88	51.4	96.9	63.8
5	85.6	68.9	48.6	69.9	61
2.5	83	64.9	46.6	48	58

Table 7: Absorbance of the Scavenging effect of *Nauclea latifolia* extracts (methanolic and N-hexane) and standards on 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Concentration of extracts (mg/ml)	Methanolic extracts (%)	N-hexane extracts (%)	Alpha-tocopherol (%)	Ascorbic acid (%)	BHA (%)
100	0.332 ± 0.001^{a}	1.006 ± 0.003^{c}	0.048 ± 0.016^{a}	0.212 ± 0.011^{a}	0.044 ± 0.008^{a}
50	0.336 ± 0.004^{a}	0.815 ± 0.026^{b}	0.055 ± 0.068^{a}	0.522 ± 0.001^{b}	0.177 ± 0.005^{b}
25	0.690 ± 0.014^{b}	0.658 ± 0.016^{a}	0.982 ± 0.141^{b}	1.256 ± 0.000^{c}	0.231 ± 0.043^{b}

Scavenging effect of Nauclea latifolia extracts (methanol and n-hexane) on ferric reducing antioxidant power assay (FRAP)

The absorbance measurement of methanolic extracts, n-hexane extracts, and standards revealed, methanolic extracts scavenging activity on FRAP (0.352 ± 0.001, 0.398 ± 0.034 and 0.471 ± 0.004) was significantly higher ($p\leq0.05$) than that of N-hexane extracts and standards (Table 10).

(Table 7)

Absorbance of the Scavenging effect of Nauclea latifolia extracts (methanolic and N-hexane) and standards on 2,2-diphenyl-1-picrylhydrazyl (DPPH) [9].

Values are expressed as mean ± S.D; n=2.Values with different superscript down the column are considered statistically significant $p\leq0.05$.

(Table 8)

Values are expressed as mean ± S.D; n=2. Values with different superscript down the column are considered statistically significant $p\leq0.05$

(Table 9)

Values are expressed as mean ± S.D; n=2.Values with different superscript down the column are considered statistically significant $p\leq0.05$.

Discussion

Essential source of new chemical substances with potential therapeutic effects is thought to be obtained from medicinal plants (Eisner, 1990; Goji et al., 2010). The antioxidant contents of medicinal plants may contribute to protection against diseases (Saeed et al., 2012). Natural antioxidants have attracted a great deal of public and scientific attention because of their health promoting effects (Anwar et al., 2006). An imbalance between the production of reactive oxygen species (ROS) and the activity of the antioxidant defences leads to oxidative stress (Krovankova et al., 2012). In the pathology of several human diseases such as atherosclerosis, inflammation, cancer, rheumatoid arthritis, and neurodegenerative diseases like Alzheimer's disease and multiple sclerosis, ROS have been implicated (Krovankova et al., 2012). Attention is on antioxidant agents of natural origin due to their abilities to scavenge free radicals (Saeed et al., 2012; Osawa et al., 1990). Antioxidant capacity is associated with compounds that can protect a biological system against the damaging effect of ROS and reactive nitrogen species (RNS) (Karadag et al., 2009) [10].

The presence of phenols, flavonoids, alkaloids and tannins in the unriped fruits of Nauclea latifolia methanolic and N-hexane extracts is an indication that these secondary plant metabolites have a synergistic effect on the various pharmacological properties reported by Onyesom et al., (2015).

Quantitative phytochemical analysis revealed, total flavonoid content were higher in both methanol (0.659 ± 0.001) and N-hexane (0.649 ± 0.110) extracts. Total phenol and tannin contents were significantly higher ($p\leq0.05$) in methanol extracts than the N-hexane extracts.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is used as a stable free radical to determine the antioxidant activity of natural compounds and the scavenging of stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants (Suhaj, 2006; Ozturk et al., 2007 and Maizura et al., 2011). In this assay, the deep violet colour of DPPH is reduced to α,α-diphenyl-β-picrylhydrazine (yellow coloured) when neutralised. The extent of the change in colour is proportional to the concentration and strength of the antioxidants (Saeed et al., 2012). The results generated from this study demonstrated that the unriped fruits of Nauclea latifolia methanolic and N-hexane

Table 8: Absorbance of the Scavenging effect of *Nauclea latifolia* extracts (methanolic and N-hexane) and standards on hydrogen peroxide (H_2O_2).

Concentration of extracts (mg/ml)	Methanolic extracts (%)	N-hexane extracts (%)	Alpha-tocopherol (%)	Ascorbic acid (%)	BHA (%)
100	0.352±0.001[a]	0.117±0.002 [a]	0.182±0.033 [a]	0.128±0.016[a]	0.150 ± 0.071 [a]
50	0.462±0.002[b]	0.258±0.035 [b]	2.251±0.054 [c]	0.248±0.014[b]	2.307 ± 0.037 [c]

Table 9: Absorbance of the Scavenging effect of *Nauclea latifolia* extracts (methanolic and N-hexane) and standards on ferric reducing antioxidant power assay (FRAP).

Concentration of extracts (mg/ml)	Methanolic extracts (%)	N-hexane extracts (%)	Alpha-tocopherol (%)	Ascorbic acid (%)	BHA (%)
10	0.342 ± 0.014 [a]	0.332 ± 0.001 [a]	1.343 ± 0.009[a]	0.085± 0.010 [a]	1.000 ± 0.105 [a]
5	0.398 ± 0.034 [a]	0.860± 0.037 [ab]	1.422 ± 0.001[ab]	0.833±0.970 [a]	1.080 ± 0.014 [a]
2.5	0.471 ± 0.004 [b]	0.971 ± 0.305 [c]	1.477 ± 0.071[b]	1.439± 0.001 [a]	1.163 ± 0.035 [a]

extracts possessed good free radical scavenging activity. This potential may be attributed to the appreciable amounts of phenolic and flavonoid content of both extracts. According to Kumar and Pandey, (2013), Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. The B ring hydroxyl configuration is the most significant determinant of scavenging of ROS and RNS because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical (Cao et al., 1997). The structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of a 3', 4'-dihydroxy, i.e., a o-dihydroxy group (3', 4'-catechol structure) in the B ring, possessing electron donating properties and being a radical target. In general, the radical-scavenging activity of flavonoids depends on the molecular structure and the substitution pattern of hydroxyl groups, i.e., on the availability of phenolic hydrogens and on the possibility of stabilization of the resulting phenoxyl radicals via hydrogen bonding or by expanded electron delocalization (Rice-Evans et al., 1996) [11].

Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; (2) scavenging ROS; and (3) up regulation or protection of antioxidant defenses (Halliwell and Gutteridge, 1998; [12] Mishra et al., 2013). Flavonoid action involves most of the mechanisms mentioned above. Some of the effects mediated by them may be the combined result of radical scavenging activity and the interaction with enzyme functions. Flavonoids inhibit the enzymes involved in ROS generation, that is, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADH oxidase, and so forth (Brown et al., 1998). This has translated to the high percentage inhibition obtained as well [13].

N-hexane extracts possessed high scavenging activity on H_2O_2 than methanolic extracts. This is because, H_2O_2 partially oxidizes N-hexane (Jean and Serge, 1998) due to chemical inertness of alkanes. when the absorbance of the three concentrations were compared, absorbance decreased significantly ($p \leq 0.05$), in a concentration-dependent manner [14].

Donating electron to reduce ferric ion to ferrous ion is an indication of reducing power. The reducing capacity of a compound may be directly translated as an indicator of antioxidant activity (Navghare and Dhawale, 2017). The strong reducing power of Nauclea latifolia methanolic and N-hexane extracts might be as a result of its phenolic content, as they are good electron donors (Pereira and Maraschin, 2015). Both extracts possessed antioxidant activity on FRAP. However, it has been observed that the methanolic extracts exhibited strong activity with the increase in polarity, indicating that highly polar organic compounds may play important role in the antioxidant activities (Ganiyat et al., 2014) [15]. The antioxidant activity exhibited by extracts was greater than the reference compounds alpha tocoherol, however, the antioxidant activity possessed by ascorbic acid surpassed that of extracts and other standards (Alpha tocoherol and BHA).

Conclusion

This study provides evidence that Nauclea latifolia unriped fruits possessed significant antioxidant activities in a concentration dependent manner on (DPPH, H_2O_2, FRAP) which can be attributed to the phenolics, flavonoid, tannin, alkaloid and other bioactive compounds present in the extracts.

Recommendation

Further in-vitro and in-vivo study is required to better understand the efficacy and mechanisms of action of these bioactives compounds present in this plant extracts to provide scientific proof for clinical employment of Nauclea latifolia secondary metabolites in modern medicine.

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Declarations

This study was approved from the University of Agriculture Makurdi Benue State Nigeria, due to its contribution to knowledge and literacy presentation.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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