ANTI-OXIDANT AND WATER HOLDING CAPACITY OF *Nauclea latifolia* FRUIT

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Abstract: Fruits are considered in dietary guidance because of their high concentrations of dietary fibre, vitamins, minerals, (especially electrolytes) and more phytochemicals, especially antioxidant. Antioxidant is “any substance that when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate. The aim of this study is to determine the antioxidant and water holding capacity of *Nauclea latifolia* fruit. The phytochemical constituents and antioxidant properties of *N. atifolia* fruit were investigated. The fruit were extracted in ethanol. Qualitative phytochemicals analysis was determined. The antioxidant activities were examined in vitro using 2, 2-diphenyl-1-picrylhydrazyl radical, total phenol, ferric reducing antioxidant power assays and ferrious ion chelating. And the water holding capacity was examined. Phytochemical screening confirmed the presence of glycosides, flavonoids, tannin, alkaloids and saponins were present while Terpenoid and steroid were absent. *N. latifolia* ethanol fruit extract demonstrated effective antioxidant activity against 2, 2-diphenyl-1-picrylhydrazyl with an IC_{50} of 44.0 mg/ml. and it was shown that DPPH has antioxidant activity with a value of 75.62±0.001 mg/ml Total phenol, ferric reducing antioxidant power and ferrius ion chelating ability inhibition activity of the extract were 59.00±0.005, 57.39±0.001 and 78.61±0.001 mg/ml with IC_{50} 21.0, 80, 0.22 mg/ml, respectively. Excellent positive correlations between the DPPH and FIC of the extract were observed. The water holding capacity was found to be 41.53±0.02%. Hence the fruit of *N. latifolia*is of therapeutic value and may be exploited for its rich antioxidant components.

Keywords: Antioxidant, *Nauclea latifolia*, fruit, inhibition, oxidative stress

Introduction

“Fruits and vegetables are considered in dietary guidance because of their high concentrations of dietary fiber, vitamins, minerals, (especially electrolytes) and more phytochemicals, especially antioxidant” (Slavin and Lloyd, 2012). Various reviews were associated with low intake of fruits and vegetables with chronic diseases such as cardiovascular diseases, blood pressure, hypercholesterolemia, and osteoporosis, many cancers, chronic obstructive pulmonary diseases, respiratory problems as well as mental health (Williamson, 1996; Adebawo *et al.,* 2006; Celik and Topcu, 2006; Park *et al.,* 2011; Payne *et al.,* 2012). Despite an increasing focus on the health benefits of fruits and vegetables, their consumption is below the recommended intake (of at least 400 g per day which is five fruit per day) among adults (Schneider *et al.,* 2007). Therefore, considering how nutritional related health problems have risen drastically globally, it is critical that formal nutritional education aiming to increase knowledge and fruits and vegetables intake be given priority in health education programs and health promotion. This study provides an insight into the importance of fruits and vegetables as well as the benefits and progress of nutrition education in improving intake of both nutritious fruits and vegetables (Anderson *et al.,* 2010).

Sufficient intake of fruit and vegetables (fruit and vegetable) has been related epidemiologically with reduced risk of many non-communicable diseases such as Diabetes, chronic lung disease, mental illness, and lung disease. Currently, much interest are focused on the vital role of antioxidants which impart bright colour to Food and Vegetables (F and V) and act as scavengers cleaning up free radicals before they cause detrimental health effects (Kaur and Kapoor, 200l. An increased consumption of carotenoid-rich food and vegetables F&V maintains the cholesterol level in blood since they reduce oxidative damage and cause an increase in Low density lipoprotein (LDL) oxidation resistance (Southon, 2000).

Antioxidants are believed to play a very important role in the body defense system against ROS (Boxin *et al.,* 2002), Vivek and Surendra (2006). In another term antioxidant is “any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Halliwell and Gutteridge, 1995). Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. Regular consumption of anti-oxidative vegetables and fruits has been recognized as reducing the risk of chronic diseases (Dembinska-Kiec *et al*., 2008).

Nauclea latifolia smith (family: Rubiaceae) is a spreading, evergreen, multi-stemmed shrub or small tree native totropical Africa and Asia (Gidado *et al*., 2005). *N. latifolia* is a valuable medicinal plant that is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. It is known as African peach and may be used for traditional medicinal practices of the East and West African sub-regions of continental Africa (Dalziel, 1957) where various extracts of the plant are used for the therapeutic management of malaria (Gamaniel *et al*., 1995); hypertension (Akubue and Mittal, 1982); prolonged menstrual flow (Elujoba, 1995); cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailments (Traore *et al*., 2000).

In many African countries, the plant is commonly used as a remedy for diarrhea, pain, dental caries, septic mouth, and diabetes (Gidado *et al*., 2005). Other uses include treatment of malaria, leprosy, debility, hypertension, gastrointestinal disorders, prolonged menstrual flow and sleeping sickness (Kerharo, 1974; Elujoba, 1995).

In Nigerian traditional medicine, the stem bark, and roots of the plant are used against fever, jaundice, malaria, diarrhoea, dysentery, hypertension and diabetes (Okwori *et al*., 2008). The fruits are sometimes used in the treatment of piles and dysentery (Reitman *et al*., 1957). In addition, the plant is used in the treatment of sleeping sickness and prolonged menstrual flow (Elujoba, 1995). The plant is known as ‘Africa cinchona’ or ‘Africa quinine’ because of its reported anti-malarial

activity (Abbiw, 1990). In Northern Nigeria, a cold infusion of the stem bark is taken as a diuretic and anti-helmintic (Ademola *et al*., 2007). The Fulanis in Nigeria use the leaf extract to regularly deworm animals (Adebowale *et al*., 1993). In Kano (Nigeria), the plant is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeni & Hussain, 1991). The roots and major ingredients of *N. latifolia* are used in treatment of respiratory illnesses such as tuberculosis, asthma, bronchitis, cough and cold in Niger State (Abdullahi*et al*., 2007). In Hong, Adamawa State, concoctions, infusions, and decoctions from stem bark and roots are used against jaundice, fever, stomach ache, and dysentery (Maitera *et al*., 2011). In Benue State, decoction from the leaf is used to treat fever, filariasis, and chicken pox while the stem bark is used to treat infertility (Abbiw, 1990).

Materials and Methods

Sample collection and identification

The fruits sample of *Nauclea latifolia* was collected from Mubi Main market Adamawa State due to its proximity and accessibility and was identified by the staff of the Department of Botany Adamawa State University Mubi.

Sample preparation

30 gram of the edible portion of *Nauclea latifolia* was cut and washed under running tap and rinsed with distilled water to remove all contamination and it was shade dried under room temperature. The dried fruit was crushed and homogenized using a pestle and mortar. The homogenized sample was transferred into a 100 ml volumetric flask and 50% ethanol was added up to the mark. The mixture was shaken manually for 15 min and filtered under suction. In situations where the filtrate appeared to be very cloudy, the filtrate was centrifuged to obtain a clear supernatant liquid, which was subsequently used for the various analyses and the extracts were stored at −20°C until use. The freshly cut fruit was sealed in a plastic bag and stored at 4°C for 4–5 days after which it was treated with 50% ethanol in the same way as before the determination of Total phenol content (TPC) and Ascorbic acid content (Suntornsuk *et al.,* 2002; Singleton and Rossi, 1965).

Qualitative screening of phytochemical in Nauclea latifolia fruit

Qualitative phytochemical screening was carried out according to the standard method described by Shobana and Vidhya (2016). Preliminary chemical tests of methanol leaf extracts of *N. latofolia* to identify the presence of various phytochemical constituents.

Determination of antioxidant activity of N. latifolia fruit

Antioxidant contents

Total phenol content (TPC)

Total phenolic content (TPC) was determined according to the method described by Sharma *et al*. (2016) Folin-ciocalteu reagent was added to 100 μl of sample/standard in ratio 1:10. The solution was mixed and incubated at room temperature for1 minute followed by the addition of 1.5 mL to 20% sodium carbonate. The final mixture was shaken and incubated for 30 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.

Antioxidant activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

DPPH radical scavenging activity of *Nauclea latofolia* (NLE) was carried out according to the method described by Sharma *et al*. (2016) 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;

$$\%\ of scavenging\ activity.\left(ABS - ABC\frac{}{ABC}\right) * 100$$

ABS is the absorbance of the sample and the ABC of that of control. Experiments were done in triplicate

Ferric reducing antioxidant power (FRAP)

The reducing power of *Nauclea latofolia* (NLE) was determined according to the method described by Sharma *et al*. (2016). 1mL 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 ^{o}Cfor 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).

$$\%\ of FRAP.\left(ABS - ABC\frac{}{ABC}\right) * 100$$

ABS is the absorbance of the sample and the ABC of that of control. Experiments were done in triplicate

Ferrous ion chelating activity

The ferrous ion chelating (FIC) activity was measured by the decrease in absorbance at562 nm of the iron (II)-ferrozine complex (Carter, 1971; Dinis *et al.,* 1994). One milliliter of 0.125 mm $FeSO_4$, was added to 1.0 ml sample (with different dilutions), followed by 1.0 ml of 0.3125 mm ferrozine. The mixture was allowed to equilibrate for 10 min before measuring the absorbance. Sample solutions with appropriate dilutions will be used as blanks as the fruit extracts may also absorb at this wavelength. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the formula:

$$Chelatingeffect\ \% = \left(ABS - ABC\frac{}{ABC}\right) * 100$$

ABS is the absorbance of the sample and the ABC of that of control. Experiments were done in triplicate.

Determination of water holding capacity of Nauclea latifolia

Particle size: This was measured using the scattered laser light principle by means of a Malvern 260^{0}C Droplet and Particle Size Analyzer. Particle size of *N. latifolia* fruit components was determined by using sieves with various mesh sizes (Dural *et al.,* 1993).

Void space between particles: This was calculated from the *N. latifolia* fruit densities (cm^{3}/g) as measured by an Auto Pycnometer (Micrometrics Auto Pycnometer 1320, America).

Void space within particles: This was determined by using a Flowsorb Rapid Surface Area Analyzer as cm^{3}/g (Cummings *et al.,* 1980).

Modified centrifugation method for Water holding capacity: The centrifugation device was assembled in the following manner: the disc E was pushed inside the polyvinyl chloride tube C. A Whatman filter paper was used to filter *Nauclea latifolia.* Type PM 10 and 30 with nominal molecular cut-off of 10,000 and 30,000, respectively, was laid on top of the disc c followed by an O ring to secure the disc e and filter paper in position to prevent liquid passing around the filter. Tube f was placed inside tube C to rest on the O ring and tube g was made of two parts to prevent any movement during screwing of tube G. The assembled tubes designated as inner tubes were stoppered with a cork wrapped in aluminum foil to prevent evaporation or absorption of moisture after centrifugation. The inner tube was placed inside polythene tube A with a fitted support B. The centrifugation column system (capacity 7 ml) was fitted into small head (No.2/c75) of a refrigerated centrifuge (Damon B-20A. Sydney). The centrifuge head accommodated eight of the centrifuge columns. WHC no of

N. latifolia fruit was determined by the modified centrifugation method using a centrifugal force of 3000 g to determine the water content after incubation with excess water for 16 hr at 37°C (Chen *et al.,* 1984). The WHC was calculated according to the following equation:

$$\frac{"Wsw - Wds - Wwd * 100"}{"Wds"}$$

Where: Wsw is the weight of wet sample, Wds is the weight of dried sample and Wwd is the weight of water retained by the disc

Statistical analysis

Results was presented as mean ± standard error of mean (SEM). The student t-test was used to determine the significant difference between two groups. Confidence interval of 95% were taken as statistically significant. Data was analyzed using SPSS version 17 software.

Results and Discussion

Table 1 shows the result for the qualitative screening of phytochemical in *Nauclea latifolia* fruit, where the presence of the following phytochemicals were observed; glycosides, flavonoids, tannin, alkaloids and saponins are present while Terpenoid and steroid are absents. Table 2 shows the result for the % inhibition of DPPH free radical scavenging assay of standard and *N. latifolia* fruit, where the fruit extract has more ability to scavenge free radicals than the standard at all concentrations. The highest % inhibition of the extract is 73.62 ± 0.001 at the concentration of 100 mg/ml.

Table 1: Qualitative phytochemical screening of *Nauclea latifolia* fruit

Sample	Ethanol extract of *N. latifolia* fruit
Tannins	+
Alkaloids	+
Flavonoids	+
Saponins	+
Terpenoids	–
Steroid	–
Glycosides	+

+ means present; – means absent

Table 2: Percentage inhibition of DPPH free radical scavenging assay of *Nauclea latifolia* fruit

Conc. (mg/ml)	Inhibition of STD (%)	Inhibition of Extract (%)
20	24.08 ± 0.002[a]	37.13 ± 0.003[b]
40	45.85 ± 0.005[b]	44.10 ± 0.001[b]
60	53.53 ± 0.002[c]	63.32 ± 0.014[d]
80	58.53 ± 0.002[d]	71.73 ± 0.001[e]
100	62.19 ± 0.005[e]	73.62 ± 0.001[f]
IC_{50}		44.10 mg/ml

Values with different superscript across the column are statistically different P<0.05 while values with the same superscript are significantly the same

Table 3: Total phenol percentage inhibition of (FRAP) free radical scavenging activity of *Nauclea latifolia* fruit and the standard

Conc. (mg/ml)	Inhibition of STD (%)	Inhibition of Extract (%)
20	32.33 ± 0.004[a]	48.35 ± 0.009[b]
40	34.60 ± 0.006[b]	48.90 ± 0.001[c]
60	36.18 ± 0.001[c]	53.37 ± 0.008[d]
80	43.50 ± 0.001[d]	55.38 ± 0.001[e]
100	46.06 ± 0.002[e]	57.39 ± 0.002[f]
IC_{50}		21.0 mg/ml

Values found across the column with different superscript are statistically different P<0.05 while with the same superscript are statistically the same

Table 3 shows the result for the % inhibition of Free Radical Scavenging activity (FRAP) free radical scavenging assay of *N. latifolia* fruit and the standard. According to the result obtained, the fruit extract has the highest % inhibition of FRAP free radical scavenging (57.39 ± 0.002) than the standard.

Table 4 shows the result for the % of free radical scavenging activity of *Nauclea latifolia* fruit and standard drug. At the concentrations used in this study in (mg/ml), the fruit extract seemed to have more capacity to scavenge free radicals with the IC_{50} of 80 mg/m more than the standard. Table 5 shows the result for ferrous chelating ability of standard drug (diclofenac) and the fruit extract of *N. latifolia* fruit. The extract shows more ferrous chelating ability at the concentrations used in this study than the standard. The highest ferrous chelating % of the fruit extract is 78.61 ± 0.001% at 0.5 mg/ml concentration.

Table 4: Percentage of free radical scavenging activity of *Nauclea latifolia* fruit

Conc. (mg/ml)	STD (%)	Extract (%)
20	19.21 ± 0.015[a]	34.25 ± 0.001[b]
40	20.20 ± 0.016[b]	38.01 ± 0.009[c]
60	23.20 ± 0.020[c]	45.02 ± 0.000[d]
80	31.01 ± 0.015[d]	50.23 ± 0.001[e]
100	34.14 ± 0.003[e]	59.00 ± 0.005[f]
IC_{50}		80 mg/ml

Values found across the column with different superscript are statistically different P<0.05 while with the same superscript are statistically the same

Table 5: Ferrous chelating ability of *Nauclea latifolia* fruit

Conc. (mg/ml)	Inhibition of STD (%)	Inhibition of Extract (%)
0.1	27.76 ± 0.002[a]	38.05 ± 0.001[b]
0.2	26.15 ± 0.001[b]	49.53 ± 0.003[c]
0.3	28.12 ± 0.012[c]	55.02 ± 0.001[d]
0.4	30.07 ± 0.001[d]	66.88 ± 0.002[e]
0.5	34.00 ± 0.001[e]	78.61 ± 0.001[f]
IC_{50}		0.22 mg/ml

Values found across the column with different superscript are statistically different P<0.05 while with the same superscript are statistically thesame

Table 6: Water holding capacity of the fruit extract of *Nauclea latifolia*

Sample	Water holding capacity
Nauclea latifolia	41.53 ± 0.02

Table 6 shows the result for the water holding capacity of the fruit extract of *Nauclea latifolia.* The result 41.13 ± 0.02 shows that the fruit extract of *N. latifolia* has the ability to hold water.

Phytochemical constituents of Nauclea latifolia fruit

Bitterness of plants is due to the presence of alkaloids, mainly glycoalkaloids. Glycoalkaloids and saponins are known to exhibit antimicrobial activities and protect plant from microbial pathogen (Sezkowki *et al.,* 1988). The presence of flavonoids in the fruits and their leaves is effective and could serve as antioxidant agents (Bagchi *et al.,* 1999). Phytochemical screening of *N. latifolia* fruit as was shown in Table 1, revealed the presence of flavonoids, saponins, tannins, alkaloids and glycosides while terpenoids and steroids were absence, the presence of these polyphenolic compounds might have contributed immensely to the biochemical activity of the plant extract. Terpenoid exhibit various important pharmacological activities like anti-

inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Alkaloids are used as anesthetic agents and are found in medicinal plant (Abosi *et al.,* 2003). Tannin compounds are widely distributed in many species of plants, where they play a role of protection from predators and might help in regulating plant growth (Katie *et al.,* 2003).

Antioxidant activity of Nauclea latifolia fruit

Essential source of new chemical substances with potential therapeutic effects is thought to be obtained from medicinal plants (Goji *et al.,* 2010; Re *et al.,* 1999; Fainsworth *et al.,* 1989). The antioxidant contents of medicinal plants may contribute to protection against diseases (Eisner *et al.,* 1990). 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 this study, Antioxidant assay was done by Checking the DPPH, FRAP and Ferrous reducing capacity of the plant extract.

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 (Ozturk *et al.,* 2007; Suhaj, 2006). From the results obtained in Table 2 in this study which was 73.62 ± 0.001, 100 mg/ml, the plant extract was effective in scavenging free radicals and could serve a potent anti-cancerousagent.In addition, the plant extract was effective than the standard which had the inhibition rate of 62.19 ± 0.005 at 100 mg/ml. IC_{50} of 44.10 mg/ml was obtained from the activity of the plant extract and this might also add to the knowledge that the plant could be effective biochemically in curbing oxidative reactions.

The extent at which the ethanolic extract of the fruits of *Nauclea latifolia* could reduce ferric ions was done with FRAP assay. This assay is made possible by low molecular weight antioxidants of hydrophilic and/or hydrophobic nature (Gherrat *et al.,* 2011). FRAP assay has been used to compare antioxidant activity in plants and mammals (Priyanka*et al.,* 2013; Niemeyer *et al.,* 2003). The action of electron donating antioxidants causes a change in the absorbance at 593 nm due to the formation of blue colored Fe+2 tripyridyltriazine (TPTZ) compound from the colorless oxidized Fe^{3+} form (Gupta *et al.,* 2009; Li *et al*., 2006) . According to the results obtained in Table 3, the ethanol extract of the fruits shows moderate FRAP with the value 57.39 ± 0.002 at 100 mg/ml and IC_{50} of 21.0 mg/ml compared to the standard with the activity of 46.06 ± 0.002 at 100 mg/ml.

Diverse groups of phenolics, being the widest spread secondary metabolite in plant kingdom, have received much attention as potential natural antioxidant in terms of their ability to act as free radical scavengers and metal chelator (Cao *et al*., 1997). It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Apak *et al*., 2007).Thermal treatments increased the bioavailability of polyphenols most likely as a result of a weakening of the plant biomass allowing for greater bioavailability of polyphenols contained inside the cell walls (Ferracane *et al*., 2008). The phenolic contents are highest in the outer layers of some fruits and these are extremely exposed to the water. As a consequence total phenolics which are usually stored in fruits in the pectin or cellulose networks can be released during aquathermal processing, as heat can break the supramolecular structures, liberating the phenolics, especially the glycosides, which react better with the Folin–Ciocalteau reagent (Bunea *et al*., 2008). Hence it was clear that the phenolic content of the fruit was to some little degree. From the results obtained in Table 4, which were 59.00 ± 0.005 at 100 mg/ml with IC_{50} of 80 mg/ml; it may be said that the fruits has some ability to reduce ferric ions. The reducing capacity of a compound may as well serve as a significant indicator of its potential antioxidant activity (Apak *et al*., 2005). The activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Good linear relationship exists between the reducing power and TPC, suggesting the positive influence of the phenolics antioxidant properties of the fruit. The extracts may possibly consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

The extract in addition did show ferrous chelating ability at the concentration of 0.5 mg/ml with the activity of 78.61 ± 0.001 mg/ml more active than the standard with the activity of 34.00 ± 0.001 mg/ml at 0.5 mg/ml used in this study than the standard. This activity might have been resulted from the presence of phenolic compounds which are agents that aid anti-oxidation which was already stated. According to the result obtained from Table 6 which was 41.53±0.02 mg/ml, the water holding capacity analysis of the fruit extract of *N. latifolia* revealed that there might be much amount of water present in the fruits which may contribute to the nutritive value of the fruit and the weight. Water holding capacity of fruit can be used to ascertain the shelf life of fruits.

Conclusion

In conclusion, the results obtained from this study revealed that the ethanol extract of the fruits of *Nauclea latifoli*a have significant antioxidant potentials with the fruit demonstrating stronger activities to the phenolic content and other bioactive compounds present in the extract. The medicinal properties of this plant could be due to its antioxidant potentials as evident from this present work.

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Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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