

## Preliminary investigation of *Nauclea latifolia* ripe fruits for antioxidant and antidiabetic activities

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### Abstract

Fruits of *Nauclea latifolia* (Family Rubiaceae) have been used as food and medicinal plants. The ethnomedicinal reports indicated that it can be traditionally used for the treatment of dysentery, diarrhea, diabetes, malaria etc. The aim of this work was to evaluate the antioxidant,  $\alpha$ -amylase and  $\alpha$ -glucosidase activities of methanol extract of *N. latifolia* fruits at varying concentrations (20-100  $\mu\text{g/ml}$ ) using standard methods. The results of the DPPH and nitric oxide free radical scavenging capacity showed  $\text{IC}_{50}$  values 92.0 $\mu\text{g/ml}$  and 30.0 $\mu\text{g/ml}$  respectively indicating a good inhibitory capacity but lesser when compared to the standard, ascorbic acid which are < 10.0 $\mu\text{g/ml}$  and < 20.0 $\mu\text{g/ml}$  respectively. The analysis shows total phenolic contents of 147.9 $\pm$ 3.35mg/g and ferric reducing antioxidant potential of 1604.1 $\pm$ 9.20mg/g supporting the fact that phenolics are highly potent antioxidants. Based on the results obtained from  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition, the  $\text{IC}_{50}$  > 100 $\mu\text{g/ml}$  showing a lesser inhibitory activity when compared to the standard Acarbose with  $\text{IC}_{50}$  values of 50.1 $\mu\text{g/ml}$  and 44.0 $\mu\text{g/ml}$  respectively. The results clearly indicate that the methanol extract of *N. latifolia* has a moderate free radical scavenging activity resulting from various interaction between different components of the plant. It can be concluded that the fruits may provide natural source of bioactive compounds which is beneficial to human health and can be used as basis of folkloric remedies for diabetes.

**Keywords:** Amylase, Antioxidants, Diabetes, Glucosidase, *Nauclea latifolia*

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## INTRODUCTION

Medicinal plants of tropical sub-Saharan Africa have been broadly used by indigenous people for traditional remedies. It also provides an important therapeutic option for a large part of the communities (Moyo *et al.*, 2015, Haudecoeur *et al.*, 2018). Chemically reactive species containing oxygen, such as hydroxyl radical, singlet oxygen, peroxides are produced as a natural by-product of the normal metabolism of oxygen. Free radicals, belonging to a group of reactive oxygen species, are produced through endogenous source (human body) and exogenous sources such as tobacco smoke, burning of fossil fuels and ozone. Some of their biological roles include: the regulation of normal metabolic process and immune function including cell growth, energy production and synthesis of nucleic acids, hormones and proteins (Sen *et al.*, 2010). The imbalance between the produc-

tion of reactive oxygen species and the activity of the antioxidant defenses is referred to as oxidative stress (Krovánová *et al.*, 2012).

Oxidative stress has been known to pose serious health challenges whenever there is disparity between their production and the ability of the biological system to neutralize them (Hadi *et al.*, 2007). The interest in natural antioxidants has significantly increased; currently, there has been global awareness towards the use and exploration of antioxidants from natural origin.. These biologically active compounds have been reported to neutralize free radicals or decompose formation of peroxides. Diabetes is an endocrinological disorder arising from insulin deficiency. The disease is caused by the inability of pancreas to produce insulin or inability of the body metabolic system to properly use the insulin produced. Diabetes is a predominant disease among the citizens of both developed and developing countries. The disease

results in high urine production, thirst and blurred vision, lethargy and changes in energy metabolism (Patel *et al.*, 2012).

The search for medicinal plants for the treatment of diabetes is on the increase since it could provide prospects for the development of novel agents for the treatment of the disease (Abdul *et al.*, 2014).

*Nauclea latifolia* plant is from 'Rubiaceae' family. It is a shrub or small tree, native to tropical Africa. The plant is made of fruits, which has a lot of brownish seeds embedded within it and surrounded by a pink, edible and sweet-sour pulp. The fruits are usually red and fleshy when ripe, resembling hard strawberry and yellow when unripe (Iwu, 1993; Fadipe *et al.*, 2015). The plant has been used locally in the treatment of malaria, hypertension, worm infestation, bacteria and virus infections, and as a purgative (Fadipe *et al.*, 2015; Haudecoeur *et al.*, 2018). In Nigeria, *N. latifolia* fruits are occasionally used in the treatment of piles and dysentery (Fadipe *et al.*, 2015).

The purpose of the study was to assess the antioxidant and antidiabetic effect of ripe *N. latifolia* fruit extract. This study was prompted by the claim of some traditional health practitioners in the South-western part of Nigeria that the fruits of *N. latifolia* are effective remedies for the management and or control of diabetes.

## MATERIALS AND METHODS

The ripe fruits of *N. latifolia* were collected from Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria and was authenticated at the Department of Plant Science and Biotechnology. Folin-Ciocalteu reagent, iodine reagent, sodium nitroprusside, naphthylethylenediamine dihydrochloride, Griess reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), para-nitrophenylglucopyranoside, gallic acid and methanol were all supplied as analytical grade reagents and were used without any further purification.

**Sample extraction:** Disease free fruits were chopped into small pieces, sun dried for 3 h to reduce moisture content and later air-dried at room temperature for two weeks. The sample was ground into smaller particles using mortar and further pulverized using a mechanical grinder. About 1.5kg of the crude plant was extracted with methanol for 24 h, the mixture filtered and the filtrate was transferred into a rotatory evaporator and concentrated to dryness at 50 °C to obtain 44.5g of the extract which was then stored in an air tight sample vial pending further analysis.

**Evaluation of ferric reducing antioxidant potential (FRAP-Assay):** The FRAP assay, which is based on the ability of antioxidants to reduce  $Fe^{3+}$  to  $Fe^{2+}$  in the presence of 2,4,6-tri(2-pyridyl)s-triazine (TPTZ) to form an intense blue  $Fe^{2+}$ -TPTZ complex with an absorption maxima at 593

nm. The reaction is pH-dependent with an optimum pH of 3.6. The absorbance decrease is proportional to the antioxidant content (Benzie and Strain, 1996). In this assay, 0.2 ml of the extract was added to 3.8 ml of FRAP reagent prepared from the mixture of 300 mM sodium acetate buffer at pH 3.6, 10.0 mM TPTZ solution and 20.0 mM  $FeCl_3 \cdot 6H_2O$  solution. The reaction mixture was incubated at 37°C for 30 min and the absorbance increase at 593 nm was measured. Different concentrations of  $FeSO_4$  solution was used for calibration. The antioxidant capacity was calculated based on the ability of the plant samples to reduce ferric ions from the linear calibration curve and expressed as mmol  $FeSO_4$  equivalents per gram of sample. Ascorbic acid (Vitamin C) was used as a positive control.

### Determination of total phenolic content (TPC):

The total phenolic content of the methanol extract was determined using a adapted form of the Folin Ciocalteu reagent (FCR) method (Liu *et al.*, 2006). 1 mg of methanolic extract was dissolved in 10ml methanol (100ppm). 500 $\mu$ l (triplicates) were withdrawn into test tubes and 0.5ml of Folin Ciocalteu reagent together with 10ml of 7% sodium carbonate were added and vortex mixed. All the test tubes were wrapped with dark colored paper and the absorbance of the resulting blue color was measured at 765nm after 1 h. Quantitative measurements were performed, based on a standard calibration curve of five points ranging from 0 - 40 ppm of gallic acid in methanol. Methanol was used to prepare the blank, while the total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g crude extracts.

**Determination of DPPH radical assay:** This assay was carried using the method of Shirwaikar *et al.* (2006). This method depends on the reduction of purple DPPH to a yellow coloured diphenyl picrylhydrazine. The remaining DPPH with the maximum absorption at 517 nm was measured. About 2 ml of various concentrations (20-100  $\mu$ g/ml) of the extract were added to 2 ml solution of 0.1 mM DPPH. An equal amount of methanol and DPPH served as control, while the absorbance of the samples were measured after 20 min of incubation at 37 °C in the dark at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = \left[ \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \right] \times 100 \quad \dots(1)$$

### Evaluation of nitric oxide scavenging activity:

The nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH relate with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent (Green *et al.*, 1982).

About 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer was added to 2.0 ml of extract

and reference compound in different concentrations (20 - 100 µg/ml). The subsequent solutions were then incubated at 25 °C for 1 hr. Methanol was used as blank in the reaction. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent containing 1% sulphanilamide and 0.1% naphthylethylene diamine dihydrochloride in 2% H<sub>3</sub>PO<sub>3</sub> was added and absorbance of the chromophore formed was measured at 540 nm. The percent inhibition of the nitrite oxide generated was determined by comparing the absorbance values of control and test preparations using equation 1 as above.

**Determination of α-glucosidase inhibition:** The percent inhibitory effect of *N. latifolia* extracts on α-glucosidase activity was determined according to the chromogenic method described by Kim *et al.*, 2005. About 20-100 µg/ml of the different concentrations of *N. latifolia* fruits extract were pre-incubated with 5 units of α-glucosidase for 15 min, thereafter, 3 mM Para nitrophenylglucopyranoside (PNPG) dissolved in 20 mM phosphate buffer at pH 6.9 was added to start the reaction. The reaction mixture was further incubated at 37° C for 20 min and stopped by addition of 2 ml of 0.1 M sodium carbonate. The α-glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from PNPG at 400 nm. The tests were done in triplicates and the mean absorption was used to calculate percentage α-glucosidase inhibition which was calculated according to equation 1.

**Determination of α-amylase inhibition:** The percent inhibition of α-amylase activity was conducted using the starch-iodine method reported by Xiao *et al.* (2006). The total assay mixture comprising 120 µl of 0.02M sodium phosphate buffer (containing 6 mM sodium chloride, pH 6.9), 1.5 ml of salivary amylase and plant extracts (20-100) µg/ml were incubated at 37°C for 10 min. 1% (w/v) soluble starch was then added to each reaction mixture and were incubated at 37 °C for 15 min. 60 µl of 1 M HCl was added to stop the enzymatic reaction, followed by the addition of 300 µl of iodine reagent (5 mM each of I<sub>2</sub> and KI). The colour change was detected and the absorbance was measured at 620 nm while the percentage inhibition was calculated using equation 1 above.

## RESULTS AND DISCUSSION

**Antioxidant activity of *N. latifolia* fruit extract:** Antioxidants can be described as compounds with the ability to delay autoxidation either via the inhibition of free radical formation or interruption of free radical propagation. Their mode of action could be through one or more of many mechanisms which includes: scavenging of peroxidation initiating species, reduction of local O<sub>3</sub> concentrations, halting the autoxidative chain reaction, quenching of radical species and metal ion chela-

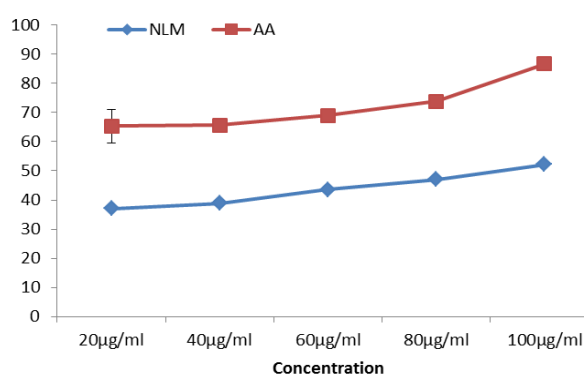
**Table 1.** FRAP and TPC contents of methanol extract of *N. latifolia* fruit (mean±SEM).

FRAP (AAE mg/100g)	TPC (GAE mg/100g)
1604.1± 9.20	147.9±3.35

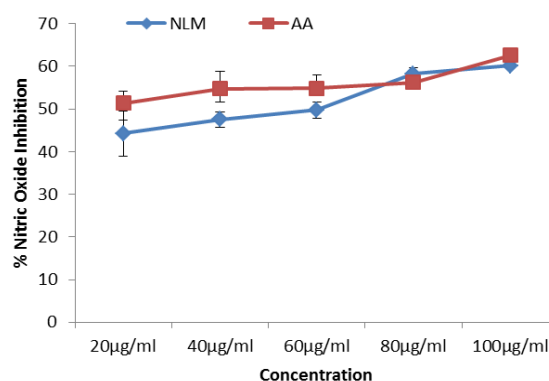
tion to prevent reactive specie generation or lipid decomposition (Brewer, 2011). The antioxidant activity of a specific antioxidant could be influenced by its hydro-/lipophilicity and structure (Koleva *et al.*, 2002). The antioxidant activities of *N. latifolia* fruits extract was evaluated using DPPH radical scavenging assay, nitric oxide scavenging activity, total phenolic content and FRAP assays.

The ability of antioxidants to reduce ferric ion (Fe<sup>3+</sup>) to ferrous ion (Fe<sup>2+</sup>) due to the donation of hydrogen atom which interrupts the free radical chain was explored in the FRAP assay. The reduction reaction led to the formation of blue coloured ferrous tripyridyltriazine complex (Fe<sup>2+</sup>-TPTZ), which is then determined spectrophotometrically by measuring absorption at 593 nm (Chung *et al.*, 2002). The FRAP value for methanol extract of *N. latifolia* fruits (Table 1) expressed in terms of the Ascorbic acid equivalent was found to be 1604.1± 9.20 AAE mg/100g. The FRAP assay confirms the presence of compounds that donate electrons to free radicals, which terminate the free radical chain reaction by transforming them into more stable substances (Labiad *et al.*, 2017). The *N. latifolia* fruits extract showed greater ferric reducing capability compared to hydroethanol, dichloromethane, ethyl acetate and hexane extracts of *Thymus satureioides* with FRAP values of 233.29, 153.45, 123.00 and 97.81 mg equivalent of ascorbic acid/g of extract respectively (Labiad *et al.*, 2017). When compared to FRAP analysis of aqueous extracts of *N. latifolia*, the methanol extract showed far greater activity (1604.1 mg AEE/100g ≈ 91.02 µmol AEE/g) compared to 12.23 µmol AEE/g (Ayeleso *et al.*, 2014). This corroborated the assertion that methanol is highly efficient in the extraction of antioxidants from plant materials (Esmaeli *et al.*, 2015).

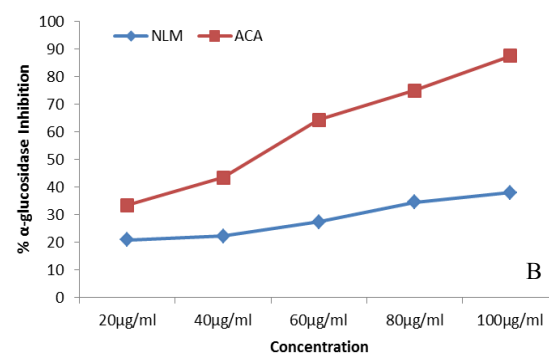
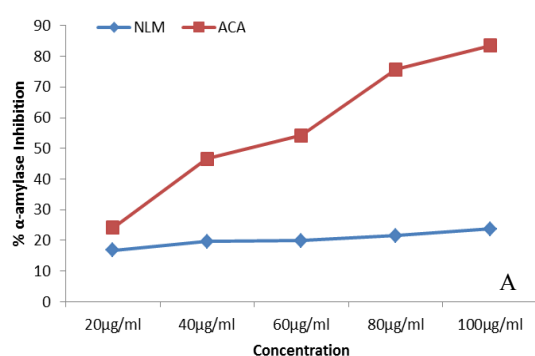
The total phenol content of the fruit extract of *N. latifolia* was 147.9±3.35 GAE mg/100g expressed as gallic acid equivalent (Table 1). Phenolic compounds contributes to antioxidative activities due to their capacity to donate the hydrogen atoms from their hydroxyl groups, it is thus generally inferred that the antioxidative activity of a plant extract could be directly correlated to its phenol content (Mahdi-Pour *et al.*, 2012). However, reports have emerged, that high phenolic content may not always result in high antioxidant activity (Agbor *et al.*, 2005) This is seen in the case of DPPH scavenging activity of *N. latifolia* fruits extract, which is relatively low despite the high phenolic content of the extract. For polyphenols, their antioxidant ac-



**Fig. 1.** DPPH scavenging activity of *N. latifolia* fruits (NLM) with Ascorbic acid (AA), (mean±SEM) of triplicates.



**Fig. 2.** Nitric oxide radical scavenging activities of *N. latifolia* fruits and Ascorbic acid (AA). Values are expressed as mean ± SEM, n=3.



**Fig. 3.** Percentage inhibition of  $\alpha$ -amylase (A) and  $\alpha$ -glucosidase (B) enzymes by methanolic extract of *N. latifolia* fruits. Acarbose was used as the reference (values are expressed as mean±SEM), n=3.

tivity is greatly determined by their chemical structure and electron donation/reception capability, resulting in delocalized unpaired electrons in the aromatic structure (Saha and Verma, 2014).

The DPPH radical assay is usually employed in determining the ability of a compound to scavenge free radicals or act as hydrogen donor (Singh *et al.*, 2012). The DPPH scavenging activity of an antioxidant is independent on the polarity of the compound but mainly dependent on the structure of the antioxidant. The  $IC_{50}$  value was found to be 92.0  $\mu$ g/ml, while that of Ascorbic acid (standard) was <10.0  $\mu$ g/ml (Fig. 1). Though the lower the  $IC_{50}$  value the better the antioxidant property of a plant material, the *N. latifolia* fruits extract showed moderate antioxidant activity for DPPH. The *N. latifolia* fruits extract showed a better antioxidant activity than *Origanum onites* and *O. vulgare* with  $IC_{50}$  of 395.75 and 335.0  $\mu$ g/ml respectively (Ozkan and Ozcan, 2016). In the study by Ayeleso *et al.* (2014), the DPPH radical activity of aqueous extracts of *N. latifolia* fruits and leaves showed an  $IC_{50}$  value of 120 and 20.64 mg/ml respectively. This indicated that the methanol extract of the plant showed far greater radical scavenging activity compared to the aqueous extract of the plant.

The nitric oxide radical quenching activity of the methanol extract of *N. latifolia* fruits was evaluated and compared with Ascorbic acid as the standard. The nitric oxide scavenging activity was determined by the ability of the extract to prevent nitrite formation by competing for oxygen, with the nitrogen oxide present in the reaction system. The extract exhibited a concentration dependent quenching activity, the maximum percentage inhibition obtained from the extract was 60.1% at the highest concentration of 100  $\mu$ g/ml with  $IC_{50}$  value of 30  $\mu$ g/ml (Fig. 2). The Ascorbic acid standard however showed a similar concentration dependent trend with a maximum inhibition percentage of 62.5% at the highest concentration of 100  $\mu$ g/ml with  $IC_{50}$  value < 20.0  $\mu$ g/ml. Nitric oxide plays important role in numerous biological processes like vasodilation, antimicrobial and antitumor activities by acting as effector molecules. It is also an active pleotropic mediator for physiological process like neuronal signaling, cell mediated toxicity regulation and platelet aggregation inhibition. It however forms peroxynitrite, a potential cytotoxic molecule, by reacting with superoxide anion under pathological conditions (Kumar and Kumar, 2009).

**$\alpha$ -glucosidase and  $\alpha$ -amylase activity of *N. latifolia* fruits:** The breakdown of starch into oligo-

saccharides and disaccharides through hydrolysis is mainly carried out in the body by  $\alpha$ -amylase. The oligosaccharides and disaccharides produced are subsequently hydrolysed by  $\alpha$ -glucosidase into monosaccharide which is then absorbed into the body through the small intestines resulting in increased postprandial glucose levels (Ranilla *et al.*, 2010).

The inhibitory activities of the methanol extract of *N. latifolia* fruits against  $\alpha$ -amylase and  $\alpha$ -glucosidase were evaluated using Acarbose as the control. Figure (3A), shows the inhibitory activity of the extract against  $\alpha$ -amylase. This indicates a direct proportionality with the concentration, however with a relatively low activity compared to the Acarbose reference sample. At the maximum concentration of 100  $\mu$ g/ml explored in this research, the percentage inhibition achieved by the plant extract was 23.74 % compared to 83.45 % attained by the reference standard. For its activity against  $\alpha$ -glucosidase (Fig. 3B), a similar trend was observed for the methanol extract with the percentage inhibition achieved at 100  $\mu$ g/ml being 38.08% while Acarbose showed a percentage inhibition of 87.56 at 100  $\mu$ g/ml. Though the extracts showed moderate inhibition activity, a high concentration of the extract will be required to achieve significant inhibition as 50% inhibitory activity was not achieved by the extract even at 100  $\mu$ g/ml compared to the standard.

The few  $\alpha$ -glucosidase inhibitors that are commercially available contain sugar components and require tedious multistep synthetic procedures. Their use is also usually associated with severe gastrointestinal side effects. Thus, active extracts like those of *N. latifolia* fruits can effectively replace  $\alpha$ -glucosidase inhibitors like acarbose and voglibose, outwitting their side effects.

## Conclusion

This study has shown that the methanol extract of *N. latifolia* fruit possessed good antioxidant activities as indicated by DPPH, FRAP, TPC and nitric oxide assays. It was, however, observed that with the high phenol content of the extract (147.9 $\pm$ 3.35 GAEmg/100g), the antioxidant activity (1604.1 $\pm$ 9.20 AAE mg/100g) of the extract should be significantly higher ( $P < 0.05$ ) with the general belief that high phenol content correlates with high antioxidant activity. The inhibitory activity of extract to  $\alpha$ -glucosidase and  $\alpha$ -amylase was slightly low, but the extract at high concentration could be used in order to avoid the side effects of synthetic inhibitors. With increasing incidence of diabetes in the urban and rural population throughout the world, there is a dire need for the development of indigenous and inexpensive drugs from natural origin for its treatment. This research has provided medicinal justification for the use of *N. latifolia*

fruits in the management, control and or treatment of diabetes. Summarily, the high antioxidant values and the moderate inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes substantiate the basis of using the plant extract as folkloric remedies for diabetes.

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