PHYTOCHEMICAL QUANTIFICATION AND IN VITRO ANTIOXIDANT ACTIVITY OF THE LEAVES, STEM-BARK AND ROOT OF Nauclea latifolia

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Abstract: The phytochemical composition and anti-oxidant activities using Di-phenyl-1-picryl-hydrazyl (DPPH) Radical Scavenging assay, Ferric Reducing and Antioxidant Power (FRAP) and the Total Phenolic contents (TPC) of the extracts from the leaves, stem-bark and root of Nauclea latifolia. The phytochemical analysis revealed the presence of bioactive compounds in the entire plant samples. The leaves of Nauclea latifolia contained tannins 0.17 g/100g, alkaloid 2.29 g/100g, flavonoid 0.83 g/100g and saponin 0.56% saponin. The stem bark also contained 0.53, 2.58, 0.08 g/100g and 0.84% while the composition of the root are 0.13, 0.88, 0.32 g/100g and 0.72% tannins, alkaloids, flavonoids and saponin, respectively. The stem bark had the highest antioxidant potential; DPPH (30.60 mg/mL), FRAP (184.64 mg/GAE/g) and TPC (30.16 g/100g). Our findings provide evidence that the extract of Nauclea latifolia could be a potential source of natural antioxidants that may be used to combat stress related conditions. The phytochemical analysis supports the extensive use of the leaves bark and root of Nauclea latifolia in ethno-medicine in many parts of Africa. Overall, the result suggests that Nauclea latifolia could be a potential source of pharmacologically active natural product and/or for development of neutraceuticals.

Keywords: Antioxidants, Nauclea latifolia, neutraceuticals, phytochemicals, reactive oxygen species

Introduction
Free radicals, which belong to a group of reactive oxygen species (ROS), are produced through endogenous source, that is, the human body itself, and exogenous sources such as tobacco smoke, burning of fossil fuels and ozone (Krovánková et al., 2012). The imbalance between the production of ROS and the activity of the antioxidant defences is referred to as oxidative stress (Škrovánková et al., 2012). The inhibiting or protective effects of herbs or spices against the harmful consequences of oxidative stress are due to the presence of natural antioxidants in them (Khalaf et al., 2008). Antioxidant based drug formulations are used in the prevention and management of complex diseases which include atherosclerosis, stroke, diabetes, Alzheimer’s disease, and cancer (Khalaf et al., 2008; Devasagayam et al., 2004). Nauclea latifolia Smith (syn: Sarcocephalus latifolius (Sm.) Gruce) (family: Rubiaceae) is a straggling shrub or small tree of about 4 m high abundantly spread in all inter-tropical Africa. It normally produces interesting flowers, edible, but not appealing, large red fruits with long projecting stamens. Commonly used parts of Nauclea latifolia include the leaves, roots, stem, and fruits. The fruits serve as key source of food for the baboons, livestock, reptiles, birds, and man (Ayeleso et al., 2014; James et al., 2011; Faley and Akinwummi, 2016). Nkafamiya et al. (2006) pointed out that the fruits of Nauclea latifolia contain copper, iron, cobalt, calcium, magnesium, zinc, phosphorus, and vitamins (A, B1, B2, C, and E). In Cameroon, the roots are used to treat jaundice, yellow fever, rheumatism, abdominal pains and hepatitis and the bark to treat jaundice and loss of appetite (Ayeleso et al., 2014; Faley and Akinwummi, 2016). In Nigeria, the stem bark and roots of the plant are used against fever, jaundice, malaria, diarrhea, dysentery, hypertension and diabetes (Ayeleso et al., 2014, Faley and Akinwummi, 2016). Pharmacological studies on N. latifolia have shown antibacterial, antidiabetic and antiplasmodial activities (Donalisoet et al., 2013; Ayeleso et al., 2014). Previous phytochemical studies on N. latifolia have yielded a great number of indole alkaloids, triterpenes, steroids and saponins (Donalisoet et al., 2013). The present study was undertaken to test the ethanol/water (1:1) extract of N. latifolia leaves, stem bark and root for its antioxidant activity.

Materials and Methods
Sample collection
The various plant parts from N. latifolia were collected in the premises of the Ekiti State University Ado-Ekiti, Ekiti State, Nigeria. The plant was identified and authenticated at the herbarium of the Department of Plant Science, Ekiti State University Ado-Ekiti by Mr. Femi Omotayo.

Sample preparation
The leaves, root, and stem bark were washed with distilled water and air-dried at room temperature for 2 weeks. They were pulverized using a mechanical grinder. The powdered plant material was extracted with 50% ethanol-water; the extracts were filtered and evaporated to dryness with the aid of rotatory evaporator at 50°C. The concentrated extracts were stored in an air tight sample vials pending analysis.

Physicochemical estimation
Alkaloid content was quantified by the gravimetric method (Harborne, 1973); saponin, by combined solvent extraction (Obadoni and Ochuko, 2001) and flavonoids, as described by Böhm and Koçipai-Abayzan (1994) and the tannin content was quantified by the method described by Pearson, (1976).

Evaluation of in vitro antioxidant activity
Estimation of total phenolic compounds (TPC)
Total soluble phenolic content in each plant extract was determined using the Folin-Ciocalteu reagent (FCR) according to the method described by Ojong et al. (2016). Briefly, 0.1 mL of each concentration of plant extract was transferred to 100 mL Erlenmeyer flask then final volume was adjusted to 46 mL by addition of distilled water. After 3 min, 1 mL of FCR and 3 mL of Na2CO3 (2%) were added to this mixture. The mixture was then incubated for 2 h at room temperature (25°C) and the absorbance was measured at 760 nm. All the tests were performed in triplicate and the results averaged. The concentration of total phenolic compounds in each extract was estimated as milligram of gallic acid equivalent by linear interpolation of a gallic acid standard curve (Vinson et al., 1995).

Ferric reducing and antioxidant power (FRAP) assay
The total antioxidant potential of N. latifolia was determined using ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996; Ojong et al., 2016). FRAP reagent was freshly prepared and mixed in the proportion of 10:1:1 (v:v:v)
for solutions A:B:C, where A is 300 mmol/L sodium acetate trihydrate in glacial acetic acid buffer (pH 3.6); B is 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) (10 mM in 400 mM of HCl), and C is ferric chloride (20 mM). Gallic acid was used for a standard curve with all solutions. Each extract (75 μL) was transferred to a cuvette containing 2 mL of FRAP solution and after agitation, the absorbance was read after 12 min of incubation at a predetermined wavelength of 593 nm. The ferric reducing antioxidant power in each extract was determined as milligram of gallic acid equivalent by linear interpolation of a gallic acid standard curve.

**Di-phenyl-l-picryl-hydrazyl (DPPH) radical scavenging activity assay**

This spectrophotometric assay used the stable DPPH radical as the reagent to determine the DPPH scavenging activity using the method described by Nyaeta et al. (2009). 20 μL of the aqueous plant extract was introduced to 2 mL methanol solution of DPPH (0.3 mM) and incubated at 37°C in the dark for 30 min. The extract was replaced by methanol for the control and catechin for the standard. Absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. The percentage DPPH radical scavenging activity was calculated by comparing the results of the test with those of the control using the following equation:

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\text{Inhibition} \% = \left\{ \frac{[A \text{ blank} - A \text{ sample}]}{A \text{ blank}} \right\} \times 100
\]

**Results and Discussion**

Natural products derived from plant sources have assumed greater importance in recent days, due to the tremendous potential they offer in formulating new drugs which may protect humankind against many diseases (Balunas and Kinghorn, 2005; Khalid et al., 2013). The phytochemical and antioxidant contents of medicinal plants may contribute to protection against diseases (Saeed et al., 2012). Natural antioxidants have attracted a great deal of attention because of their health-promoting effects (Anwar et al., 2006). Oxidative stress occurs when the formation of bioactive oxidative products such as oxidizing agents, free radicals and reactive oxygen species, greatly overwhelms the capacity of the endogenous cellular antioxidant defense system, thus leading to potential damage of the cells and organs, and to the progression of degenerative diseases in humans (Schrader and Fahimi, 2006; Basu, 2010). Attention has been on antioxidants of natural origin due to their abilities to scavenge free radicals. Antioxidant capacity is associated with compounds that can protect a biological system against the damaging effect of ROS and reactive nitrogen species (RNS) (Ayalewo et al., 2014). The water : ethanol (50:50) extract obtained from different parts (leaf, stem bark and root) of *Nauclea latifolia*, showed variations in their phytochemicals and their antioxidant potential as presented in Figs. 1 to 4.

Fig. 1 presents the result for the quantitative phytochemical analysis of the leaves, bark and root of *N. latifolia*. The result showed the presence of alkaloid, flavonoid, tannin and saponin. The result indicated that the stem bark contained the highest amount of alkaloid (2.58 g/100g), while the root had the lowest amount of alkaloid (0.88 g/100g). Alkaloids have been reported to have analgesic effects (Omoyeni and Adeeye, 2009). The result further showed that the leaf extract was very rich in flavonoid (0.83 g/100g), while the stem bark had the highest content of tannin (0.53 g/100g) and saponin (0.84 %). Saponins are responsible for the haemolytic properties of plant parts. This confers to the plant the traditional medicinal function as a cholesterol binding agent (Coe and Abyazan, 1994). Saponin has a low value (0.56 %, leaves), 0.84% (stem bark) and 0.72% (root). The values are very low when compared with the reported values for *Nauclea latifolia* stem bark and root 12.01% and 5.57%, respectively by (Egbunget al., 2013). The wide difference observed may be due to seasonal variation, collection time and extraction methods. The value obtained for the leaf can also be compared to the reported value for *Nauclea latifolia* leaves 12.5% (Ezeet al., 2014). Saponin also has a relationship with sex hormones involved in controlling the onset of labour in women and subsequent release of milk called oxytocin (Okwu and Okwu, 2004).

Saponin assists in combating bacterial infections and counter fungus and viruses and have been shown to complement the effectiveness of some vaccines (Agoha, 1981; Osunwole, 1999). Therefore the leaves of *Nauclea latifolia* may prove to be useful in treating difficult fungal and yeast infections. This supports the use of the plant in treating sexually transmitted diseases such as gonorrhoea, syphilis and herpes in herbal medicine (Okwu and Okwu, 2004; Farquhar, 1996).

Flavonoids are biologically active phytochemicals whose functions include anti-inflammatory, antiallergic and anti-tumour agents. Some flavonoids e.g. isoflavones relieve hay fever, eczema, sinusitis and asthma, as well as reduce blood cholesterol and can prevent osteoporosis as well as ease menopause symptoms (Bohm and Kochiap-Abyazan, 1994). Flavonoids are free radical scavengers and are super antioxidant as phenolics which are water soluble and prevent oxidative cell damage and have strong anti-cancer properties (Salah et al., 1995; Paulet al., 2012). The flavonoid content of the different parts ranged between 0.08-0.83%. The presence of flavonoid in the leaves, bark and root of *Nauclealatifolia* supports its ethnomedicinal use. Flavonoids also show antimicrobial activities (Cushnie and Lamh, 2009). Tannins possess astringent properties and harden the healing of wounds and inflamed mucous membrane (Bohn and Kocipai-Abyazan, 1994). The tannin content of the stem bark (0.53 g/100g) and root (0.13 g/100g) of *Nauclea latifoliarepectively has a low value when compared with result reported by Egbunget al., 2013 for the bark (2.12%) and root (2.25%) of *Nauclea latifolia*. The tannin content support the use of the plant for treating wounds, various ulcers, haemorrhoids, frost bite and burn in herbal medicine because of antimicrobial effects of tannins (Akiyamaet al., 2011) as well as treating inflamed throats, mouth and as a veterinary intestinal astringents (Agoha, 1981).

The presence of these phytochemicals could contribute to the ethnomedicinal use of the plant to treat various ailments.
Phenolic compounds are found usually in both edible and non-edible plants with several biological effects which include antioxidant activity (Ayeleso et al., 2014). In this study, the total polyphenolic contents of the extracts of the leaves, stem bark and root were determined using the diluted Folin-Ciocalteu reagent. The total polyphenolic content was higher in the stem bark than the root and leaves with mean values of 30.16, 7.90 and 10.47 g/100g, respectively. Generally, the result showed that all the tested plant parts are rich in total polyphenols (Fig. 2).

The DPPH Radical Scavenging Activity in the extracts of the leaves, stem bark and root of Nauclea latifolia presented in Fig. 3. 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; Maizura et al., 2011). In this assay, purple colour of DPPH is reduced to \(\alpha,\alpha\)-diphenyl-\(\beta\)-picrylhydrazine (yellow coloured) when neutralized. The extent of the change in colour is proportional to the concentration and strength of the antioxidant (Saeed et al., 2012).

In this study, the three plant parts displayed antioxidant activity via free radical scavenging activities. The stem bark had the highest activity (30.59 mg/mL) while the root had the lowest activity (3.75 mg/mL) as shown in Fig. 3. The effect of antioxidants on DPPH could be due to their hydrogen donating ability (Gherraf et al., 2011).

The Ferric reducing antioxidant power in the ethanol extracts of the leaves and fruits of Nauclea latifolia were presented in Fig. 4. The extent at which the aqueous/ethanolic extract of the leaves and fruits of Nauclea latifolia could reduce ferric ions was carried out with FRAP assay. The action of electron donating antioxidants causes a change in the absorbance at 593 nm due to the formation of blue coloured Fe\(^{3+}\)-tripyridyltriazine (TPTZ) compound from the colourless oxidized Fe\(^{3+}\). The stem bark extract showed higher FRAP (184.64 mg/GAE/g sample) than the extract of the leaf and root (114.08 and 16.4184.64 mg/GAE/g sample) respectively as shown in Fig. 4.

**Conclusion**

Medicinal plants are cheaper than orthodox medicine which are not only very expensive but are also not readily available to the rural farmers (Atata et al., 2005). There is therefore a need for interest in the study of medicinal plants and the validation of herbs used in various localities in order to incorporate them into the healthcare system especially since more people are placing emphasis on natural products over synthetic drugs. This policy if pursued will assist to promote the spirit of plant conservation. From the results of the phytochemical analysis, we can conclude that the leaves, stem bark and root of Nauclea latifolia are important sources of important phytochemicals and phytonutrients which are in harmony with their use in ethnomedicine. The leaves, stem bark and root of the plant are also of significant antioxidant value and can be exploited in the development of nutraceuticals.

**References**


The Test of ethanol/water (1:1) extract of N. latifolia for its Antioxidant Activity


