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ANTIOXIDANT CAPACITY AND DNA DAMAGE PROTECTIVE ACTIVITY OF THE ROOT EXTRACT OF *NAUCLEA LATIFOLIA*

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ABSTRACT

Damage to the genome caused by free-radicals is fundamental to the genesis of genetic diseases. Plants are rich in phytochemicals including tannin, saponin, flavanoids, alkaloids and phenolics, with a variety of metabolic activities, which includes antioxidant potential. *Nauclea latifolia* is used by traditional herbal medicine practitioners to treat many common illnesses including chronic diseases in Northwest Nigeria Phytochemical screening and antoxidant studies were carried out on the root extract of *Nauclea latifolia.* The phytochemical studies was done following standard procedures. Antioxidant activity of *Nauclea latifolia* extract was studied by mixing 0.03mM solution of DPPH radical (1, 1-diphenyl-2-picryl hydrazyl) with different concentrations (100, 50, 25, 10,5 and 2.5µgml^{-1}) of the extract. The DNA nicking assay using fenton's reagent was also carried out to ascertain the DNA damage protective activity of the *N. latifolia* root extract. This was done using supercoiled pBR322 plasmid DNA and different concentrations (100 µg/ml and 200 µg/ml) of the extract. The phytochemical studies revealed the presence of bioactive plant metabolites; tannins, flavonoids, alkaloids, saponins, anthraquinones, carbohydrates, phytosteroids and diterpene. Antiradical activity of the root extract of *N. latifolia* using DPPH (1, 1-diphenyl-2-picryl hydrazyll) showed that the root extract of *N. latifolia* inhibited the DPPH free radical dose dependently. The DNA nicking assay showed ability of the extract to protect the integrity of plasmid DNA pB322 from the deleterious effect of hydroxyl radical generated by fenton's reaction in same manner as standard antioxidant, l-ascobic acid. *Nauclea latifolia* extract demonstrated antioxidant activities, ability to scavenge-free radicals and protects the integrity of DNA.

KEYWORDS: Antioxidant activity; DNA nicking; *Nauclea latifolia*; Radicals.

INTRODUCTION

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS), such as nitric oxide, hydrogen peroxide, peroxynitrite, superoxide, and hydroxyl radical, are implicated in oxidative stress in cells, DNA damage,[1] cells aging,[2] neurodegenerative disease like Alzheimer's and Parkinson's[3] and cancer.[4] Antioxidant compounds can intervene through catalytic chelating metals or by direct radical scavenging (radical-chain breaking).[5,6,7] Damage to the genome is fundamental to the genesis of genetic diseases. Plants are rich in phytochemicals including tannin, saponin, flavanoids, alkaloids and phenolics, with a variety of metabolic activities, which includes antioxidant potential. Antioxidants protect from damage caused by ROS and concomitant lipid peroxidation, protein damage and DNA strand breaking.[8] These natural antioxidants are in high demand in bio-pharmaceuticals, nutraceuticals and food industries.[9] *Nauclea latifolia* (family:*Rubiaceae*) commonly known as African peach tree is a straggling shrub or small tree native to tropical Africa and Asia. The plant is known as *Tafashiyaa* in Hausa, also called Tuwo mbiri in Sokoto and Kebbi states of Nigeria. In south eastern part of Nigeria the plant is known as ubuluinu (Igbo) and opepe by the Yorubas in western Nigeria. Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus. The plant is also used in the treatment of ailments like malaria,[10,11,12,13] gastrointestinal tract disorders,[14], sleeping sickness,[15] prolong menstrual flow,[16] hypertension[10] and as chewing stick.[17] The antioxidant activities of the leaves and fruit of *N. latifolia* plant has been reported.[18] This study was therefore aimed at evaluating the antioxidant capacity and DNA damage protective activity of the root extract of *Nauclea latifolia.*

MATERIALS AND METHODS

Plant Material

The root of *Nauclea latifolia* was obtained from Mahuta village in Fakai Local Government Area of Kebbi state, Nigeria. The plant was identified by a Taxonomist, Malam A.M. Umaru, of the Hebarium, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto and voucher specimen, with number UDUH|ANS|0080 deposited at the herbarium of the University.

Preparation of Extract

The root of the plant was collected, washed under running tap water, air dried to constant weight and pulverized by pounding using pestle and mortar. Then the powdered material was extracted in distilled water by soaking and regularly shaking for 24hrs. Two hundred grams of the powdered plant material was macerated in 200ml of distilled water for 24hrs. The liquid filtrate obtained was concentrated at 40^0C in hot air oven.[19] The yield (weight/weight) of plant extract was recorded and stored at 4^oC in a refrigerator until required.[20]

Phytochemical Screening

The phytochemical study of the root of *Nauclea latifolia* was conducted using methods that has been outlined.[21,22,23,24] Standard procedures were followed to test for and identify the following phytochemical constituents of the plant; Alkaloids, Flavonoids, Glycosides, Tannins, Saponins and Steroids.

Antioxidant Studies

One hundred grammes of the pulverized *N.latifolia* root was macerated with 500ml 90% ethanol three times. Solvent was evaporated under reduced pressure in hot air oven at approximately 40°C. The dried extract obtained was dissolved in ethanol 90% to a final concentration of 1000µg ml^{-1} (*N.latifolia* sample stock solution), then the different concentrations of *N.latifolia* (200, 100, 50, 40, 30, 20, 10µg ml^{-1}) were prepared and used for the test.

Anti-Free radical activity test

The antiradical activity of the extract was estimated according to the procedure that has been described. (25) A 0.3mM solution of DPPH radical (1, 1-diphenyl-2-picryl hydrazyl) solution in 90% ethanol was prepared and then 1 ml of this solution was mixed with 2.5 ml of different concentrations (100, 50, 25, 10, 5 and 2.5µgml^{-1}) of the *N. latifolia* root extract (sample). The mixture was incubated for 30 minutes in dark place at room temperature, absorbance (A) was measured at 518 nm in a spectrophotometer.

The percentage of the radical scavenging activity (RSA) was calculated by the following equation:

$$RSA\% = [(A\ control - A\ sample)\ x\ 100]/A\ control$$

A control Ethanol 90% (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2.5 ml) was used as a negative control. L-ascorbic acid

solution freshly prepared (at the concentrations of 100, 50, 25, 10, 5, 2.5 μgml^{-1}) was used as a positive control. The IC_{50} value for extract (sample), defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the *Nauclea latifolia* root extract (μgml^{-1}) against the mean percentage of the radical scavenging activity.

DNA Nicking Assay of *Nauclea latifolia* root extract

The DNA nicking assay was performed using supercoiled pBR322 plasmid DNA.[26] The *N.latifolia* root extract (10µl) of different concentrations (100 µg/ml and 200 µg/ml) and pBR322 plasmid DNA (0.5 µg) were incubated for 10 min at room temperature followed by the addition of 10 µl Fenton's reagent (30 µl of 30 µM H_2O_2, 500 µl of 50 µM ascorbic acid, and 800 µl of 80 µM $Fecl_3$). The reaction mixture was incubated for 30 min at 37°C and analysed on 1 % agarose gel using ethidium bromide.[27]

RESULTS AND DISCUSSION

The phytochemical constituent, antioxidant effect and DNA damage protective activity of the aqueous root extract of the plant *Nauclea latifolia* was studied. The root extract of *Nauclea latifolia* yielded 4.5% after extraction of 2kg of the pulverized *Nauclea latifolia* root with water. The extract was brownish crystalline solid with bitter taste. The phytochemical studies carried out on the plant material revealed the presence of bioactive plant metabolites; tannins, flavonoids, alkaloids, saponins, anthraquinones, carbohydrates, phytosteroids and diterpene (Table 1), in relatively large amounts which are natural phytochemical compounds found in many plant-based foods.[28,29] These phytochemical compounds are said to act as defense against ultraviolet radiation, oxidants and pathogens.[30] Polyphenols possess some biological activities and beneficial properties such as antioxidant, anti-allergic, anti-inflammatory, anti-viral and anti-microbial, anti-proliferative, anti-mutagenic, anti-carcinogenic, free radical scavenging, regulation of cell cycle arrest, apoptosis and induction of antioxidant enzymes. Also modulation of some important cell signaling pathways such as nuclear factor kappa-B (NF-κB), activator protein-1 DNA binding (AP-1), extracellular signal-regulated protein kinase (ERK), phosphoinositide 3 (PI3) kinase/protein kinase B (Akt), mitogen-activated protein kinases (MAPK), and nuclear factor erythroid 2 related factor 2 (Nrf2) have also been reported for the polyphenols.[28,31] These active metabolites found in this plant material may possibly be responsible for some of the reported activities such as the antioxidant properties which make it potent in ameliorating chronic metabolic disorder such as diabetes mellitus.

Antioxidants have ability of arresting some lipid related symptoms of diabetes mellitus.[32,33] The antioxidant studies of the root extract of *Nauclea latifolia* focused on the DPPH radical scavenging activity of the root extract of *Nauclea latifolia*. Free radical scavenging effect of *Nauclea latifolia* root extract measured at different concentrations with L-ascorbic acid as standard is shown in table 2 and figure 1. Antiradical activity of the root extract of *N. latifolia* using DPPH (1, 1-diphenyl-2-picryl hydrazyll) showed that the root extract of *N. latifolia* inhibited the DPPH free radical dose dependently. The IC_{50} is 21.22µg and 3.6µg for *Nauclea latifolia* and L-ascorbic acid respectively. Therefore, the free radical scavenging properties of the *N. latifolia* root extract can be beneficial in preventing oxidative stress and probably related diabetic vascular complications. Furthermore, antioxidants which may act at different levels to inhibit the formation of reactive oxygen species (ROS), scavenge free radicals, or increase the antioxidants defense enzyme capabilities, might have contributed to the different medicinal properties of this plant as reported.[34] For example, hyperglycemia, an inevitable consequence of type 2 diabetes, is the source of most of the deleterious effects usually associated with diabetes mellitus. High blood glucose concentrations promote auto-oxidation of glucose to form free radicals. The generation of free radicals beyond the scavenging abilities of endogenous antioxidant defenses results in macro and microvascular dysfunction and polyneuropathy and reports has shown that antioxidants are effective in reducing diabetic complications.[35]

The DNA Nicking Assay was also carried out to ascertain the DNA damage protective activity of the *N.latifolia* root extract. The result of protective ability of *N.latifolia* root extract against fenton's reagent is shown in plate 1. The DNA nicking assay of the root extract of *N. latifolia* using supercoiled plasmid DNA PBr322 (Inspiralis, UK), showed evidence of protective effect of the extract on the plasmid DNA at both 100µg/ml and 200µg/ml against the destructive effect of fenton's reagent. The supercoiled plasmid DNA was destroyed by the fenton's reagent at the absence of the extract or l-ascobic acid (plate 1). The ability of *Nauclea latifolia* root extract to protect the DNA from the deleterious effect of Fenton's reagent, may indicate its ability to maintain the balance between the free radicals generated and antioxidants produced by the body reducing the development of degenerative diseases.[36,34] Furthermore, oxidative stress leads to protein, lipid, and DNA modifications that cause cellular dysfunction. The production of ROS and lipid peroxidation are increased in diabetic patients. These elevated ROS in diabetes can cause strand breaks in DNA and base modifications including oxidation of guanine residues to 8-hydroxy-2-deoxy-guanosine (8-

OHdG), an oxidized nucleoside of DNA.[37] In this study *Nauclea latifolia* root extract was able to protect the plasmid DNA from destruction and as such may prevent DNA damage in metabolic disorders such as diabetes mellitus.[38]

Table 1: Phytochemical Analysis of the Root of *Nauclea latifolia*.

Phytochemical Component	Qualitative	Quantitative (%w/w)
Alkaloids	+	3.2
Tannins	+++	13.6
Flavonoids	++	3.40
Saponins	+	1.80
Proteins	+	0.90
Carbohydrates	+	2.13
Anthraquinones	+	1.65
Terpeniods	+	0.8

+ indicates the degree of presence of various phytochemicals, ++ more presence than the former while +++ indicates highest presence of the phytochemicals

Table 2: Free Radical Scavenging activity of *Nauclea latifolia* root extract using 1,1,-diphenyl-2-picrylhydrazyl (DPPH) free radical.

Conc. Control (L-Ascorbic acid)µg/ml	Absorbance control Ascorbic acid	Conc.extract *N.latifolia* µg/ml	Absorbance *N.latifolia*	Inhibition % N.L	AS
200 µg/ml	0.884±0.001	200 µg/ml	0.132±0.001	85.07	100
150 µg/ml	0.724±0.002	150 µg/ml	0.130±0.0002	82.04	81.9
100 µg/ml	0.722±0.001	100 µg/ml	0.186±0.001	74.24	81.6
50 µg/ml	0.733±0.002	50 µg/ml	0.289±0.002	60.57	83.3
40 µg/ml	0.710±0.001	40 µg/ml	0.254±0.001	64.22	80.3
30 µg/ml	0.705±0.001	30 µg/ml	0.347±0.008	50.78	79.7
20 µg/ml	0.681±0.008	20 µg/ml	0.330±0.001	51.54	77.0
10 µg/ml	0.640±0.001	10 µg/ml	0.397±0.008	37.97	72.3
5 µg/ml	0.595±0.0002	5 µg/ml	0.430±0.001	27.73	67.3

Results expressed as mean absorbance ± SEM and inhibition %, n=3

N.L= *Nauclea latifolia*, AS= L-Ascorbic acid

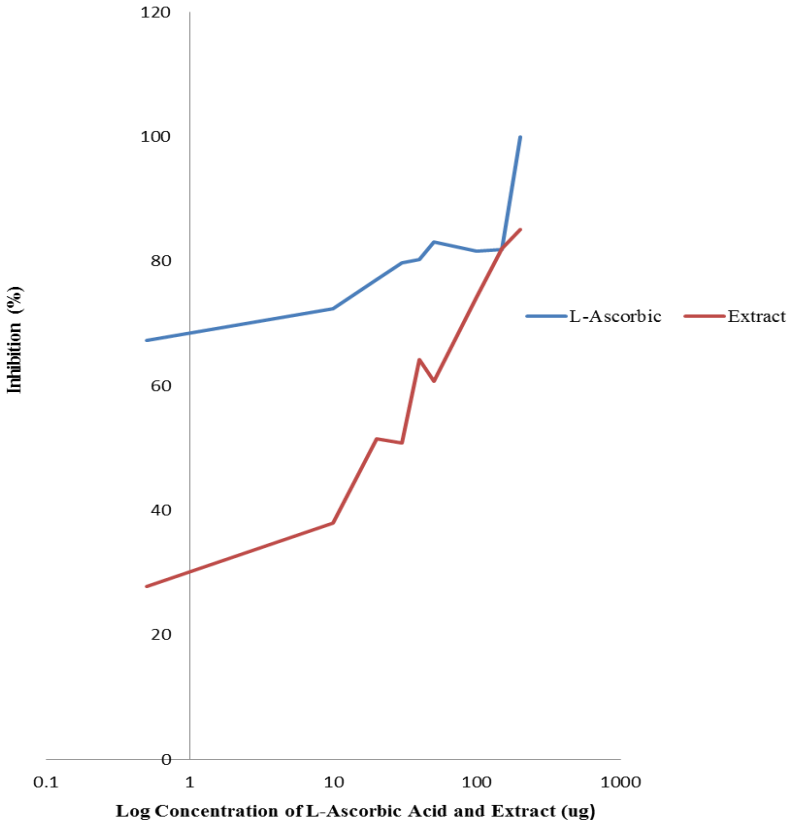

Figure 1:Percentage inhibition of DPPH by L-Ascorbic Acid and *Nauclea latifolia* Root Extract

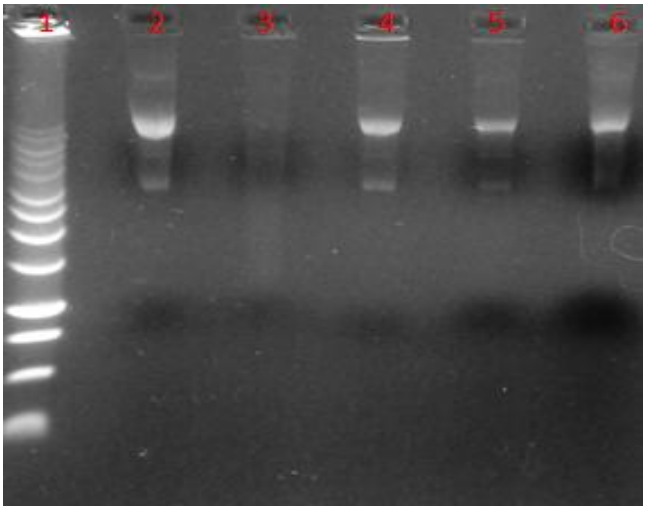

Plate 1 DNA Nicking assay of *N. latifolia* root extract using supercoiled plasmid DNA. Lane 1= DNA marker (1KB), Lane 2= PBr322(Super coiled Plasmid DNA) only, Lane 3= Supercoiled plasmid DNA + Fenton's Reagent,Lane 4= Supercoiled plasmid DNA + Fenton's Reagent + L-ascorbic acid, Lane 5= Supercoiled plasmid DNA + Fenton's Reagent+ N.latifolia (200ug/ml), Lane 6=Supercoiled plasmid DNA + Fenton's Reagent+ *N.latifolia* (100ug/ml). This work was done at the Centre for Advanced Medical Research and Training, UDUS, Nigeria.

CONCLUSION

This study demonstrated the antioxidant activities, and the ability of *Nauclea latifolia* root extract to scavenge-free radicals and protects the integrity of DNA. Conventional antioxidant testing techniques and DNA nicking assay are a powerful tool that can be used to measure the antioxidant and pro-oxidant effects of an extract on a biologically important component such as the DNA. The application of the DNA nicking method to *Nauclea latifolia* root extracts showed that *Nauclea latifolia* extract has antioxidant activity. Nonetheless, these results also agree with the classical chemical *in vitro* assay (DPPH) performed in this study, which showed that the *Nauclea latifolia* extract has antioxidant properties. It demonstrated the possible link between the capacity of the plant extract to protect against DNA damage. Root extract of *Nauclea latifolia* therefore has commensurable capacity to protect against DNA damage resulting from oxidative stress, which could have led to disease conditions such as neurodegenerative disease like Alzheimer's and Parkinson's and cancer.

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