Trop J Nat Prod Res, May 2022; 6(5):806-810 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Total antioxidant and Anti-tyrosinase Activities of Methanol Extract of Ripe *Nauclea latifolia* Fruits and its Chromatographic Fractions

Olubunmi A. Akinwunmi,[1]* Olugbenga K. Popooola[1], Sarah O. Nwozo[3], Abimbola D. Olanipekun[2] and Francis J. Faleye

[1]*Department of Chemistry, Ekiti State University, Ado-Ekiti, Ekiti Sate, Nigeria*
[2] *Department of Chemical Sciences, Bamidele Olumilua University of Education, Science and Technology, Ikere -Ekiti, Ekiti State, Nigeria*
[3] *Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria*
[4] *Department of Industrial Chemistry, Ekiti State University, Ado-Ekiti, Ekiti Sate, Nigeria*

ARTICLE INFO

Article history:
Received 08 April 2021
Revised 12 May 2022
Accepted 19 May 2022
Published online xxxxxxxxxxx

ABSTRACT

Nauclea latifolia smith (family: *Rubiaceae*) is a valued medicinal plant widely distributed in the West and Central Africa with notable therapeutic values supporting its usage in the treatment of various ailments. This research focused on the investigation of the methanol extract of the plants' fruits for phytochemical content, antioxidant and anti-tyrosinase inhibition activities in an *in vitro* system. The phytochemical content of the plant and its chromatographic fractions were investigated using total phenolics, flavanol and flavonol, while the total antioxidant capacities were determined using a series of assays such as: ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC) and inhibition of iron-II induced lipid peroxidation (LPO). The plant demonstrated skin pigmentation inhibitory activity with an IC_{50} value of 127.3µg/mL. Some notable activities displayed by the chromatographic fractions from the plant extract include: NL-VII had the highest total phenolics (53340.5µmol GAE/g) and flavonol (84275.9µmol CE/g) contents. This same fraction also showed the best antioxidant activity using TEAC (12054µmol TE/g), and ORAC (19348.8µmol TE/g) assays. Similarly, NL-VIII had the best lipid peroxidation activity with IC_{50} value of 49.8µg/mL and tyrosinase enzyme inhibition (TYR): IC_{50} 124.4µg/mL. The methanol extract of the plant and some of its chromatographic fractions demonstrated varying degree of chemical composition, total antioxidant capacities and skin depigmentation activity. This is an indication that the plant may be useful in the management of oxidative stress related diseases.

Keywords: *Nauclea latifolia*, Phytochemicals, Antioxidants, Anti-tyrosinase, Skin pigmentation, Oxidative stress.

Introduction

Plants possess phytochemicals which are biologically active compounds that provide health benefits for humans.[1]Medicinal plants containing such compounds could be useful for the prevention and treatment of human ailments including diabetes, high blood pressure, oxidative stress, degenerative diseases, skin hyperpigmentation, and cancer.[2-3]Antioxidants interfere with oxidative processes by reacting with free radicals, chelating catalytic metal ions and also by acting as oxygen scavengers. Consequently, they are of great importance as therapeutic agents in preventing or slowing the progression of reactive oxygen species and associated oxidative stress - related degenerative diseases.[4] Several medicinal plants have been reportedly used as potential bio-resource for the treatment of oxidative stress- related degenerative diseases.[5-9] Skin pigmentation is a broad term that reflects an increased dispersion of melanin; the pigment that is responsible for the colour of human skin, hair, and eyes. The enzyme tyrosinase plays a key role in melanin biosynthesis. The melanin pigment is produced inside the melanosomes from the amino acid L-tyrosine and then converted by the enzyme tyrosinase to dopaquinone.

*Corresponding author. E mail: nikxyglo@yahoo.com
Tel: +2348163327327

Citation: Akinwunmi OA, Popooola OK, Nwozo SO, Olanipekun AD, Faleye FJ. Total antioxidant and Anti-tyrosinase Activities of Methanol Extract of Ripe *Nauclea latifolia* Fruits and its Chromatographic Fractions. Trop J Nat Prod Res. 2022; 6(5):806-810.

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The composition of the mixed melanins in many different ways forms many colour variations of the skin, hair, and eyes.[10] Skin hyperpigmentation is characterized by increased production and accumulation of melanin in the body, which can result in serious skin diseases or disorders like post inflammatory, hyperpigmentation, solar lentigo, and melisma. This occurs widely in the human population, and it is also considered to cause psychological disturbances.[11] Skin hyperpigmentation has received attention worldwide and various medicinal plants, which include *Achillea ptarmica*, *Allium* spp., *Astragalus* spp., Citrus *medica* L, *Cucumis sativus*, *Ficus carica*, have been explored and found applicable for the treatment of this disorder.[12] Following the ongoing exploration of this notable plant (*Nauclea latifolia*) embarked upon by our research group, we had previously reviewed most of the scientific information related to the chemistry, biology, and traditional history of this plant. [9] Nevertheless, other background information on the importance of *N. latifolia* includes its consideration as a valuable medicinal plant endemic to the savannah woodlands of West and Central Africa. The plant is generally known as African peach and has been reportedly used in East and West African sub-regions for the treatment of various disease like malaria, hypertension, prolonged menstrual flow, cough, gonorrhoea, stomach disorders, dysentery, ulcers, liver ailments, leprosy, and sleeping sickness. [13-14] *Nauclea latifolia* fruits have been reported for its antioxidant and anti-diabetic activities.[9] The purpose of this paper is to further evaluate the total antioxidant and anti-tyrosinase activities of the methanol extract of ripe *Nauclea latifolia* fruits and fractions obtained from open column chromatographic fractionation. This research will further provide medicinal justifications for the use of *N. latifolia* fruits in the management and treatment of oxidative stress - related degenerative diseases.

Materials and Methods

Preparation of Plant Extracts

N. latifolia ripe fruits were collected from the Ekiti State University campus, Ado-Ekiti, Ekiti State, Nigeria in February, 2019. The fruits were authenticated at the Department of Plant Science and Biotechnology by Mr. Femi Omotayo, where a voucher specimen with number UHAE2022050 was deposited. The chopped plant material was air dried at room temperature in the laboratory for 2 weeks before it was ground. 550 g of powdered plant material was soaked with methanol in an aspirator bottle for 72 hours. The mixture was then filtered using Whatman filter paper (10 - 500 mm). The filtrate obtained was concentrated using rotary evaporator (R-110, Buchi, Switzerland) at 35^{o}C under reduced pressure. The brown solid material obtained from *N. latifolia* fruits methanol extract was coded NL with a weight of 32.7 g (5.95% yield).

Preliminary phytochemical screening of plant extract

1 mg of the crude extract (NL) was dissolved in methanol (1:1 v/v) to make a solution (1 mg/mL) for TLC-screening. The samples were spotted 1 cm above solvent level on a 7x6 cm TLC-plate using a commercial silica PF_{254} plate coated on aluminium foil as the stationary phase. A solvent system solution containing 5mL of DCM - methanol (9:1, v/v) was used as the mobile phase. The development of the plate was done in a chromatographic tank, while visualization of bands on the chromatogram was accomplished by the use of vanillin-sulphuric acid spray reagent.

Column chromatographic fractionation of crude extract

The column chromatography was performed using silica gel 60H (0.040-0.063 mm particle size, Merck, South Africa) as stationary phase supported by a glass column. A portion of NL (30 g) was dissolved in methanol and pre-adsorbed onto silica in a petri dish. The pre-adsorbed material was applied to a silica column (16.5 X 24 cm) and eluted using a gradient of dichloromethane (DCM): methanol in the order of increasing polarity. Twenty - six (26) fractions were collected during the process using a flow rate of 8.5 mL per minute. The collected fractions (NL 1-26) were concentrated under reduced pressure (rotary evaporator) at 45^{0}C and developed on TLC with the following solvent system: DCM – methanol (9:1). Fractions of the same TLC characteristics were bulked together and sprayed with vanillin sulphuric acid to yield 8 main fractions coded NLI - VIII.

The Phytochemical composition of N. latifolia fruits

Determination of total polyphenol

The total polyphenol content in NL and its column fractions were determined according to the method described by Waterhouse,[15] with a few modifications using Folin-Ciocalteau's phenol reagent with gallic acid as the standard. Briefly, in 1.5mL Eppendorf tubes, five-fold diluted samples in 10% methanol were made. Also, appropriate concentrations of standards were made in accordance with the standard operating procedure (SOP). 25µL of diluted Folin-Ciocalteau reagent was placed in a 96-well plate and incubated at room temperature for 5 minutes. 100µL of 7.5 % Na_2CO_3 was added to the wells and left to react at room temperature for 2 hours, after which the absorbance of the mixture was measured using a microplate ELIZA reader (Thermo Electron Corporation, MA, USA) at 765 nm.

Determination of flavonoids

Total flavonoid content was determined by a colorimetric method using catechin as a standard.[16] In this assay, a five-fold diluted sample in 10% methanol was made in 1.5 mL Eppendorf tubes, followed by suitable dilutions of the catechin standard. In a 96-well plate, 50 µL diluted samples or standards were pipetted in triplicate, followed by 42µL of distilled water and 60 µL of sodium nitrite ($NaNO_2$). The mixture was left at room temperature for 10 minutes. Subsequently, 60 µL of aluminum chloride ($AlCl_3$) was added to each well, and then mixed yet again. After 5 minutes of incubation at room temperature, 60 µL of Sodium hydroxide (NaOH) was added to each well, mixed and the absorbance read using microplate ELIZA reader at 510 nm and compared to the standard (Thermo Electron Corporation, MA, USA).

Determination of flavonols

This method is based on the use of quercetin as the standard for measuring flavonol at 360 nm.[17] Briefly, a five-fold diluted sample in 10 % methanol was provided in 1.5mL Eppendorf tubes, followed by suitable quercetin standard dilutions to obtain appropriate SOP concentrations. 12.5 µL of diluted samples or standards was pipetted in triplicates in a 96-well assay plates. This was followed by addition of 237.5 µL of 2 % HCl to each well. The plates were left at room temperature 30 minutes before measuring absorbance using a microplate ELIZA reader (Thermo Electron Corporation, MA, USA).

Determination of flavanol

In this analytical method, 4-dimethylaminocinnamaldehyde (DMACA) reacts with flavanols to form a characteristics light blue colour measured at 640 nm.[18] Briefly, a five-fold diluted sample in 10 % methanol was measured in 1.5 mL Eppendorf tubes, accompanied by proper dilutions of catechin standard to obtain appropriate concentrations based on SOP. 50 µL of diluted sample or standards were pipetted in triplicates in a 96-well assay plate. This was followed by the addition of 250 µL of DMACA to all the wells. The plate was incubated for 30 minutes at room temperature and the absorbance read on a microplate ELIZA reader (Thermo Electron Corporation, MA, USA).

Total antioxidant capacities of N. latifolia fruit extract and fractions

The methanol crude extract NL alongside the combined column fractions NL (I-VIII) were subjected to biological assays. In all assays, epigallocatechin gallate (EGCG), was used as positive control in the same concentrations as the samples. Generally, samples were dissolved in dimethyl sulfoxide (DMSO) to make 1 mg/mL stock solution when necessary; these were further diluted with appropriate buffers depending on the type of assay and in accordance with the range of the standard calibration curve required. Further details on methodology are given below.

Oxygen radical absorbance capacity (ORAC) assay

ORAC was measured according to the method of Cao and Prior [19] with some modifications. Trolox (6hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), with increasing concentrations (0, 83, 167, 250, 333 and 417 µM), was used for standard calibration curve. The trolox standard series were diluted in 75 mM, pH 7.4 phosphate buffer previously prepared by mixing 18mL of 75 mM sodium di-hydrogen orthophosphate-1-hydrate ($NaH_2PO_4H_2O$) with 82mL of 75 mM di-sodium hydrogen orthophosphate dihydrate ($Na_2HPO_42H_2O$). EGCG was used as positive control. Trolox standards and samples (12 µL) were added in triplicates to designated wells in a black 96-well plate. Fluorescein stock solution was previously prepared by dissolving fluorescein sodium salt ($C_{20}H_{10}Na_2O_5$) (0.0225 g) in phosphate buffer (50 mL). This solution was diluted by adding fluorescein stock solution (10 µL) to phosphate buffer (2 mL), and then further diluted (240µL) in phosphate buffer (15 mL). 138µL of fluorescein stock solution diluted in phosphate buffer was added to each well. 50µL of peroxyl radical AAPH (2,2'-azobis(2-mehtyl-propionamidine) dihydrochloride), dissolved in phosphate buffer to a concentration of 25 mg/mL, was also added to each well. The final volume of the wells was 200 µL. The decay in fluorescence was measured with a fluorescence detector at 486 nm excitation and 538 nm emission wavelengths. The ORAC value, which is the net protection area under the fluorescein curve in the presence of an antioxidant is calculated by dividing the sample curve-area by the trolox curve-area. An ORAC unit is equivalent to the net protection of 1 µM trolox.

Ferric-ion reducing antioxidant power (FRAP) assay

FRAP was measured according to the method of Badarinath *et al*.[20] The acetate buffer was earlier prepared by dissolving 1.627 g sodium acetate in 16mL of glacial acetic acid in a 1 L media bottle. Distilled

water was added to the mixture to make 1 L of 300 mM acetate buffer at pH 3.6. 40 mM HCl was also previously prepared by adding 1.46 mL concentrated HCl (32 % HCl) to a 1 L media bottle, followed by dilution with distilled water to make 1 L. TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) was prepared by dissolving 9.3 mg of TPTZ in 3 mL of 40 mM HCl in a 15 mL conical tube to a 10 mM solution. Iron (III) chloride hexahydrate was prepared by dissolving 54 mg $FeCl_3$•$6H_2O$ in 10 mL distilled water in a 15 mL conical tube, to a 20 mM solution. L-ascorbic acid was prepared by dissolving 8.8 mg of ascorbic acid in 50 mL distilled water in a 50 mL screw cap tube. L-ascorbic acid was used as the standard with the calibration curve at concentrations of 0, 50, 100, 200, 500 and 1000 µL/mL. Epigallocatechin gallate (EGCG) was used as a positive control. FRAP reagent was prepared by adding 30 mL of acetate buffer, 3mL of TPTZ solution, 3 mL of $FeCl_3$ solution, and 6.6 mL of distilled water in a 50mL screw cap tube. 10 µL of the samples, ascorbic acid standards and EGCG control were added to designated wells, while 300 µL of the FRAP reagent was added to each well using a multichannel pipette. The plate was incubated in the dark at room temperature for 30 minutes before measuring the absorbance at 593 nm.

Trolox equivalent antioxidant capacity (TEAC) assay

TEAC was measured by the method of Badarinath *et al*.[20] 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt was prepared by dissolving 19.4 mg of ABTS in 5 mL of distilled water in a 15 mL screw cap tube, to make 7 mM solution. A 140 mM solution of potassium-peroxodisulphate was prepared by dissolving 189 mg $K_2S_2O_8$ in distilled water (5 mL) in a 15mL screw cap tube. The ABTS mix was then prepared by adding 88 µL of the previously prepared potassium-peroxdisulphate solution to 5 mL of the ABTS solution in a 15mL screw cap tube. The ABTS mix was then stored in a dark room at 4°C for 24 hours. The ABTS mix was diluted with ethanol to read an absorbance of 2 (±0.1) on the plate reader. Trolox (6-Hydro-2,5,7,8-tetramethylchroman-2-carboxylic acid) was previously prepared by dissolving 12.5 mg Trolox in 50 mL ethanol in a 50 mL screw cap tube, to make 1.0 mM solution. Trolox at concentrations of 0, 50, 100, 150, 250, and 500µL/mL was used as the standard calibration curve. EGCG was used as positive control. 25 µL of trolox standards, samples and EGCG as positive control were added in triplicates to designated wells in a clear well plate. 275 µL of the ABTS mix was added to each of the well using a multichannel pipette. The plate was incubated in the dark at room temperature for 30 minutes before being read. The samples were measured at 734 nm.

Fe (II)-induced microsomal lipid peroxidation (LPO) assay

LPO was measured according to the method of Snijman *et al*. [21] with some modifications. Trichloroacetic acid (TCA)-Ethylenediaminetetraacetic acid (EDTA)solution was prepared by dissolving 10 g of TCA and 29.2 mg of EDTA in 100 mL of distilled water. Butylated hydroxytoluene (BHT) solution was made by dissolving 0.8 g of BHT in 10 mL ethanol. TCA reagent (10% TCA, BHT & 1 mM EDTA) was made by adding 0.125 mL of the BHT solution to 100 mL TCA-EDTA solution. TBA (0.67% TBA) solution was prepared by dissolving 0.67 g of TBA in 100mL distilled water. A 2.5 mM of $FeSO_4$ solution was prepared by dissolving 139 mg of $FeSO_4 \cdot 7H_20$ in 200mL distilled water. Potassium phosphate buffer supplemented with 1.15% KCl to 0.01M (pH 7.4) was previously prepared by dissolving 1.150 g KCl, 1.742 g K_2HPO_4 and 1.370 g K_2HPO_4 in 100mL distilled water. Reagent blank (negative control) contained KCl-buffer only. EGCG and gallic acid were used as positive controls. 50 µL of samples, controls and blank were incubated with 300 µL microsomes at 37°C for 30 minutes in a shaking water bath. Samples were then added to 100 µL $FeSO_4$-solution and KCl-buffer followed by incubation at 37°C for 1 hour in a shaking water bath. After the incubation 1mL of TCA reagent was added to each tube and further vortexed and centrifuged at 2000 rpm for 15 minutes. 1mL of the supernatant was removed after centrifugation and added to new test tubes in which 1mL 0.67% TBA solution had been added. The samples were further vortexed and heated for 20 minutes at 90°C in a water-bath. The reagent blank, positive controls and samples were added in triplicates to designated wells in a clear well plate and measured at 532 nm. The percentage inhibition of the TBARS formation relative to the positive control can be calculated using:

The percentage inhibition = $[(A\ control - A\ sample) \div A\ control] \times 100$

Tyrosinase enzyme assay

This assay was done using a spectrophotometric method earlier described by Popoola *et al*. [22] Sodium phosphate buffer was made by mixing 50 mM monosodium phosphate (NaH_2PO_4) solution (5.999 g in 1 litre distilled H_2O) with 50 mM disodium phosphate (Na_2HPO_4) solution (7.098 g in 1 litre dH_2O) to pH 6.5. Reagent blank (negative control) contained sodium phosphate buffer only while Kojic acid (KJA) was used as positive control. In a 96-well plate, 70 µL of each sample working solution was combined with 30 µL of tyrosinase (200 Units/mL in sodium phosphate buffer) in triplicates. In parallel to these wells, samples were added in triplicates with buffer instead of enzyme. After incubation at room temperature for 5 minutes, 110µL of substrate (2 mM L-Tyrosine) was added to each well. The mixture was further left for 30 minutes after which the enzyme activity was determined by measuring the absorbance at 490 nm, while the % tyrosinase inhibition was calculated as:

$$Tyrosinase\ inhibition\ (\%) = [(A - B) - (C - D)] \div (A - B) \times 100$$

A = Absorbance of control with enzyme;
B=Absorbance of the control without enzyme;
C = Absorbance of sample with enzyme;
D = Absorbance of sample without enzyme.

Statistical Analysis

The results were expressed as the mean and standard deviation (SD) of three independent determinations. $P<0.05$ was considered as statistically significant. GraphPad Prism software was used to analyze the data, including one-way ANOVA and Bonferroni post hoc test.

Results and Discussion

The results of the preliminary phytochemical screening of the total extract using 10 % DCM-Methanol as the solvent system and vanillin sulphuric acid as a spray reagent for identification of the class of organic compounds indicated the presence of polyphenolic compounds by the appearance of yellow colour (TLC plates not shown).

The main column fractions NL (1-26) were combined according to their TLC characteristics to yield eight (8) combined fractions coded using roman numerals NL (I-VIII) and the results were summarized in Table 1. The TLC-profiles of collected fractions revealed different classes of polyphenols by their brown, yellow, and pink bands. The crude methanol extract (NL) displayed high content of phenolics 71546.4µmol GAE/g as well as high flavonol 426038.2 µmol CE/g.

Table 1: Group of fractions from the main column

Combined fractions	Code	Weight (mg)
1 – 6	NL-I	1,604.25
7 – 9	NL-II	1,029.10
10 – 14	NL-III	900.00
15	NL-IV	2,136.21
16 – 17	NL-V	1,906.71
18	NL-VI	1,333.09
19 – 21	NL-VII	1,672.28
22 – 26	NL-VIII	2,963.13

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The polar fractions obtained from the column eluted with solvent system DCM: Methanol 80:20 and 50:50 also displayed high flavonol content as NL-VII (84275.9) and NL-VIII (38358.3) µmol CE/g (Table 2). Flavanol was not detected in the methanol crude extract and all the column fractions.

Plants with high phenolic contents are known to possess natural antioxidant activity. The high phenolic content could be due to the presence of flavanols or flavonols, which are major constituents of biologically active medicinal plants. Our results agreed with the findings from the report of the qualitative phytochemical screening of the whole fruits of NL documented by Oyedeji-Amusa and Ashafa; [23] Iheagwam *et al*. [24] The presence of flavonols was reported in moderate concentration in the ripe fruit. As a result, the high levels of flavonol detected have been linked to a variety of pharmacological effects, including antioxidant, anti-diabetic, neuroprotective, antimicrobial and anti-inflammatory properties.[24-25] Table 3 presents the results of the total antioxidant capacities of the crude extract and the combined fractions.

Oxidative stress is a condition which contributes to the progression of several pathophysiological diseases in humans, including malignant melanoma, Parkinson's disease, Alzheimer's disease, cancers, heart and blood vessel disorders, arteriosclerosis, heart failure and heart attack. Accumulation of reactive oxygen species in skin cells can induce skin injury, hyperpigmentation, skin aging and oxidative stress. Antioxidants from plants might be an alternative strategy to combat ROS - induced skin aging and hyperpigmentation[26] Further, flavonols, which are active components of the fruit may be isolated from fractions NL-VII and NL-VIII of the crude in further analytical work.

The methanol extracts of NL fruits and the column fractions, especially NLV and VII, have high antioxidant activity, which could be due to the high phenolic content. These extracts have higher antioxidant potentials than the aqueous and hydro-ethanol extract reported by Ayeleso *et al*.[27]

Table 2: Quantitative chemical compositions of NL and NLI-VIII

Sample	Total phenolics (µmol GAE⁄g)	Flavanol (µmol QAE⁄g)	Flavonol (µmol CE⁄g)
NL	71546.4 ± 5.63	Nd	426038.2 ± 5.32
NL-I	440.1 ± 2.96	Nd	Nd
NL-II	2062.5 ± 4.74	Nd	574.1 ± 4.95
NL-III	1533.5 ± 2.18	Nd	3818.9 ± 6.62
NL-IV	2256.4 ± 5.23	Nd	11475.1 ± 6.54
NL-V	1658.6 ± 13.43	Nd	2945.7 ± 11.67
NL-VI	102.3 ± 8.13	Nd	10951.8 ±14.42
NL-VII	362.4 ± 3.49	Nd	84275.9 ± 6.51
NL-VIII	53340.8 ± 6.79	Nd	38358.3 ± 9.71

GAE: Gallic acid equivalent; QE: Quercetin equivalent; CE: Catechin equivalent;
Nd: not detected; NL: *N. latifolia* fruits methanol crude extract

Table 3: Total antioxidant capacities of NL and NLI-VIII

Sample Number	FRAP (µ mol AAE⁄ g)	TEAC (µ mol TE⁄g)	ORAC (µmol TE⁄g)
NL	138.52 ± 10.36	8246.79 ±2.35	153.93 ± 16.07
NL-I	2175.53 ±5.52	Nd	772.14 ± 4.63
NL-II	165.58 ± 3.81	Nd	370.60 ± 8.95
NL-III	2084.51 ±3.84	Nd	8110.71 ± 0.88
NL-IV	1592.04 ±5.65	7019.24± 5.62	9229.76 ± 4.06

Table 4: Fe (II)-Induced microsomal lipid peroxidation of NL and NLI-VIII

Sample	IC_{50} (µg/mL)
NL	50.88
NL-I	>100
NL-II	>100
NL-III	84.25
NL-IV	78.63
NL-V	52.38
NL-VI	62.13
NL-VII	55.33
NL-VIII	49.75
EGCG	36.25

nd: not detected; P < 0.05; EGCG: Epigallo catechin gallate

Table 5: Skin pigmentation inhibitory activity of NL and NLI-VIII

Sample	IC_{50} (µg/mL)
NL	127.25
NL-I	>250
NL-II	>250
NL-III	>250
NL-IV	>250
NL-V	>250
NL-VI	>250
NL-VII	233.13
NL-VIII	124.44
KJA	12.01

KJA: Kojic acid; nd: not detected P<0.05

The leaves and some active components isolated from the leaves have been reported in previous studies to exhibit high antioxidant capacities.[28] NL fruits have effective inhibition against LPO, which may be due to their ability to scavenge hydroxyl radicals. The leaves and stem bark of NL are known to possess LPO inhibitory activities.[29] LPO inhibitory activity in plants will minimize quality deterioration and, in turn, improve the antioxidant efficacy.[30] The tyrosinase enzyme assay revealed that NL and column fraction VIII showed mild tyrosinase inhibition with IC_{50} values of 127.25 and 124.44 µg/mL respectively (Table 5). The tyrosinase inhibition of column fractions NL (I-VI) could not be detected at the cut - off concentration of 250 µg/mL. The leaves of NL from previous study also exhibited mild tyrosinase inhibition.[29]

FRAP and TEAC measure electron transfer and acidic and neutral pH, respectively. In Table 3, column fraction NL-VII demonstrated a high TEAC value (12054.06µmol TE⁄g) as the methanol crude extract (8246.79µmol TE⁄g). TEAC was not detected in fraction NL-VIII but it showed a high FRAP value of 11883.56 µmol AAE⁄ g in acidic medium. This result demonstrated the effect of pH on the samples tested. Fractions NL- (V – VII) had high ORAC values for hydrogen ion transfer (16753.57; 17146.43, and 19348.81µmol TE⁄g, respectively). The results of LPO assay are presented in Table 4. The LPO assay shows that NL-VIII had the highest inhibitory effect on lipid peroxidation, with an IC_{50} value of 49.75 µg/mL. NL crude extract, NL-V and VII exhibited significant inhibitory effects on lipid peroxidation with IC_{50} values of (50.88; 52.38 and 55.33 µg/mL respectively) when compared to the other samples, and also with similar activity to the positive control EGCG (36.25 µg/mL). The high

percentage inhibition indicates that these fractions contain constituents with strong inhibitory effect on lipid peroxidation through scavenging of free radicals.

Conclusion

The results demonstrated that the biological activity of the fractions of *N. latifolia fruits* was significant; it can therefore be assumed that the fruits can efficiently contribute to the total antioxidant and anti-tyrosinase activities of the medicinal plant. The fruits may also be used as an alternative means of treating skin hyperpigmentation. Further work is required for the isolation, identification and biological characterization of the bioactive compounds in the fruits.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Hasler CM and Blumberg JB. Symposium on phytochemicals: Biochemistry and Physiology. J Nutri. 1999; 129:756S-757S.
2. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev. 2012; 70:257-265.
3. Du L, Shen Y, Zhang X, Prinyawiwatkul W, Xu Z. Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food Chem. 2014; 153:279-284.
4. Tepe B and Sokmen A. Screening of the antioxidative properties and total phenolic contents of three endemic Tanacetum subspecies from Turkish flora. Biores Technol. 2007; 98:3076-3079.
5. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). Molecules. 2010; 15:4324-4333.
6. Abo KA, Lawal IO, Ogunkanmi A. Evaluation of extracts of Triclisia suboardata Oliv and Heinsia crinita (Afz) G. Taylor for antimicrobial activity against some clinical bacterial isolates and fungi. Afr J Pharm Pharmacol. 2011; 5:125-131.
7. Ben IO, Woode E, Abotsi WKM, Boakye-Gyasi E. Preliminary phytochemical screening and in vitro antioxidant properties of Trichilia monadelpha (Thonn.) J. J. de Wilde (Meliaceae). J Med Bio Sci. 2013; 2:6-15.
8. Keta JN, Obaroh I, Shinkafi SA. Phytochemical screening and histological studies of *Cassia occidentalis* leaves extracts on the liver and kindey of albino rats in Katsina. J Nat Appl Sci. 2016; 5:152-162.
9. Akinwunmi OA, Olalekan OC, Adefemi S. Preliminary investigation of *Nauclea latifolia* ripe fruits for antioxidant and antidiabetic activities. J Nat Appl Sci. 2019; 11:718-723.
10. Smit N, Vicanova J, Pavel S. The hunt for natural skin whitenin agents. Intern J Molecul Sci. 2009; 10:5326-5349.
11. Adhikari A, Devkota H, Takano A, Masuda K, Nakane T, Basnet P, Skalko-Basnet N. Screening of Nepalese crude drugs traditionally used to treat hyperpigmentation: in vitro tyrosinase inhibition. Int J Cosmet Sci. 2008; 30:353-360.
12. Fahimi SH, Ghafari S, Fahimi Sh, Sahranavard S. Plants used to treat hyperpigmentation in Iranian traditional medicine: a review. Res J Pharmacogn. 2017; 4:71-85.
13. Balogun ME, Besong EE, Obu DC, Obu MS, Djobissie SF. *Nauclea latifolia*: A Medicinal, Economic and Pharmacological Review. Int J Plant Res. 2016; 6:34-52.
14. Busari MB, Hamzah RU, Muhammad HL, Yusuf RS, Madaki FM, Adeniyi JO, Ibrahim YO, Berinyuy EB. Phenolic rich-extracts from *Nauclea latifolia* fruit restored Lead acetate-induced liver and kidney damaged in Wistar rats. Clin Phytosci. 2021; 7:1-10.
15. Waterhouse AL. "Determination of total phenolics," in current protocols in food analytical chemistry, R. E. Wrolstad, Ed., units I, John Wiley & Sons Publishers, New York, NY, USA; 2003. I1.1.1–I1.1.8p.
16. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64:555-559.
17. Delcour JA and de Varebeke JD. A new colorimetric assay for flavonoids in pilsner beers. J Inst Brew. 1985; 91:37-40.
18. Treutter D. Chemical reaction detection of catechins and proanthocyanidins with 4-dimethylaminocinnamaldehyde. J Chromatogr A. 1989; 467:185-193.
19. Cao G and Prior RL. Measurement of oxygen radical absorbance capacity in biological samples. Meth Enzymol. 1999; 34:50-62.
20. Badarinath AV, Mallikarjuna Rao K, Madhu Sudhana Chetty C, Ramkanth STVSR, Rajan, TVS, Gnanaprakash K. A review on in-vitro antioxidant methods: Comparisions, correlations and considerations. Int J PharmTech Res. 2010; 2:1276-1285.
21. Snijman PW, Joubert E, Ferreira D, Li XC Ding Y, Green IR, Tyrrell RM. Ultraviolet radiation and free radical damage to skin. Biochem Soc Symp. 1995; 61:47-53.
22. Popoola OK, Marnewick JL, Rautenbach F, Ameer F, Iwuoha EI, Hussein AA. Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from *Helichrysum teretifolium*. Molecules. 2015; 20:7143-7155.
23. Oyedeji-Amusa MO and Ashafa AOT. Medicinal properties of whole fruit extracts of *Nauclea latifolia* Smith.: Antimicrobial, antioxidant and hypoglycemic assessments. S Afr J Bot. 2019; 121:105–113.
24. Iheagwam NF, Israel EN, Kayode KO, DeCampos OC, Ogunlana OO, Chinedu ON. *Nauclea latifolia* Sm. leaf extracts extenuates free radicals, inflammation and diabetes-linked enzymes. Oxid Med Cell Longev. 2020; Article ID 5612486, 13 pages.
25. Balogun ME, Besong EE, Obu DC, Obu MSU, Djobissie SFA. A medicinal, *Nauclea latifolia*: Econ Pharmacol Rev. 2016; 6:34-52.
26. Chatatikun M, Supjaroen P, Promlat P, Chantarangkul C, Warannuntakul S, Nawaat J, Tangpong J and Chiabchalard A. Antioxidant and tyrosinase inhibitory properties of an aqueous extract of Garcinia atroviridis Griff. Ex.T.Anderson Fruit Pericarps. Pharmacogn J. 2020; 12:71-78.
27. Ayeleso AO, Oguntibeju OO, Brooks NL. *In vitro* study on the antioxidant potentials of the leaves and fruits of *Nauclea latifolia*. Sci World J. 2014, Article ID 437081, 8 pages.
28. Ajayi OS, Aderogba MA, Akinkunmi EO, Obuotor EM, Majinda RRT. Bioactive compounds from Nauclea latifolia leaf extracts. J King Saud Univ Eng Sci. 2020; 32:2419-2425.
29. Adaramoye OA and Azeeze FA. Evaluation of antioxidant and acteylcholinesterase-inhibitory properties of methanol extracts of Nauclea latifolia, Cymbopogon citratus and Cocos nucifera: an *in vitro* study. Br J Med Med Res. 2014; 4:2156–2170.
30. Ilhami G. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012; 86:345-391.