

Radical Scavenging Activity, Brine Shrimp Lethality Assay and HPLC Analysis of Methanol Extracts of *Secamone afzelii* (Roem. & Schult.) K. Schum and *Ceiba pentandra* (Linn.) Gaertn.

T. ABU^{1,2 ABCD}, O.O. OGBOLE^{2* ACEF}, E. O. AJAIYEoba^{2 AF}

¹Bioresources Development Centre, Odi, National Biotechnology Development Agency, Abuja

²Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Medicinal plants produce several phytochemicals with strong free radical scavenging activities.

Objectives: To evaluate radical scavenging activity, brine shrimp toxicity and high-performance liquid chromatography (HPLC) analysis of the methanol extracts of *Secamone afzelii* (SA) leaves and *Ceiba pentandra* (CP) stem-bark.

Materials and methods: The leaves of SA and CP stem-bark were extracted with methanol and the total phenolic content (TPC) of the extracts was determined and expressed as gallic acid equivalents (GAE). The extracts were evaluated for their nitric oxide scavenging activity, antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, toxicity using brine shrimp lethality assay (BSLA) and phytochemical constituents using HPLC.

Results: The TPC in SA extract was 21.26±1.29 while that of CP extract was 95.31±8.71 mg GAE. The SA and CP extracts showed antioxidant activity by DPPH with IC₅₀ of 97.60 and 22.35 µg/mL as compared to ascorbic acid (1.4±0.34) and gallic acid (2.8±0.78). Similarly, the extracts showed nitric oxide scavenging activity of 22.50±1.3 and 5.40±0.3 µg/mL respectively compared to gallic acid with IC₅₀ value of 1.1±0.1 µg/mL. In BSLA, SA extract had LC₅₀ value of >1000 µg/mL while CP extract and Cyclophosphamide (standard) had LC₅₀ value of <1000 µg/mL. HPLC analysis suggested that 8-β-L-arabinofuranosyl-6-β-D-glucopyranosylapigenin, kaempferol-3-O-glucoside, kaempferol-3-O-(6"-malonyl-glucoside), kaempferol-3-O-rhamnoglucoside and palitantin were the major components in SA extract while the major components in CP extract were lateritin/beauvericin, procyanidin B2, pavetannin, (-)-catechin and genistein-8-C-glucoside.

Conclusion: The results demonstrate the free radical scavenging properties of SA and CP extracts which could be attributed to the presence of phenolic compounds.

Keywords: Radical scavenging, phytochemicals, phenolic compounds

INTRODUCTION

Most human diseases are associated with the accumulation of free radical such as Nitric oxide (NO), a highly reactive chemical species often produced in the human system by normal biological reactions and also by various exogenous factors. This reactive chemical species is implicated in degenerative or pathological processes when the human endogenous antioxidant defence system that protects against oxidative damage is overwhelmed (Dawson *et al.*, 1992; Huang *et al.*, 2005). Hence there is need to supplement antioxidant levels through intake of dietary or plant-derived antioxidants (Terao *et al.*, 1994; Houghton, 1995).

The plant kingdom has been the most significant source of drugs used for the treatment and prevention of many diseases, regardless of the current concern with chemical synthesis as a tool to discover and manufacture drugs. Moreover, at the beginning of 21st century, 11% of the 252 drugs considered as basic and essential by the World Health Organization were exclusively of flowering plant origins (Veeresham, 2012). Plant-based antioxidants have attracted special interest because they are non-toxic or less toxic (Grice, 1986; Wichi, 1988). There have been reports of strong synthetic antioxidants compounds but they have proven to be highly carcinogenic (Shimizu *et al.*, 2001) hence it has become necessary to search for antioxidants from natural sources with less toxic side effect.

Secamone afzelii (Asclepiadaceae) is a familiar creeping woody climber found on fences and trees. It is indigenous to tropical West and Central Africa. In Western African traditional medicine, *S. afzelii* is used for stomach problems, diabetes, colic, dysentery and kidney problems. The whole plant boiled with rice is used as purgative for children. The decoction of the

METHODOLOGY

Plant materials

The stem-bark of *Ceiba pentandra* was collected at Oke-Ado Molete at U.M.C premises and leaves of *Secamone afzelii* were collected at the University of Ibadan premises. The plants were identified and authenticated by Shasanya O.S at Forest Herbarium Ibadan (FHI), Jericho Ibadan and was appropriately compared by Mr. P. Agwu at the Department of Pharmacognosy Herbarium, University of Ibadan. The plants voucher numbers were also provided (FHI 109996 and 109995).

entire plant is prescribed for cough, catarrhal conditions and as galactagogue. For the treatment of gonorrhoea, the whole plant is crushed with fresh palm nuts and oil (Gill, 1992). According to Houghton *et al.* (1996), *S. afzelii* extracts have been shown to exhibit anti-inflammatory properties. The latex cells usually contain a latex rich in triterpenes. Other constituents include cyanogenetic glycosides, saponins, tannins and cyclitols (Evans, 2002). The leaves are rich in α -tocopherol (one of the forms of vitamin E), a compound with established antioxidant properties (Mansah *et al.*, 2004; Houghton *et al.*, 2005).

Ceiba pentandra of the family Malvaceae is a very large, deciduous tree up to 60 m tall. In many countries in Africa, the stem bark of *C. pentandra* is in the management of diarrhoea, localized oedemas, wash sores, furuncles, leprosy macules, relieve stomach complaints, hernia, blennorrhoea, heart-trouble, asthma, gargles for gingivitis and sometimes toothache (Burkill, 1985). A bark-decoction is given to children suffering from rickets; bark sap is given to sterile women to promote conception by reason of the fecundity of the seed in Ivory Coast (Burkill, 2000). Its pharmacological properties include antidiarrheal, antimicrobial, wound-healing and anti-inflammatory activity (Noreen *et al.*, 1998; Nwachukwu *et al.*, 2008; Sule *et al.*, 2010). The following compounds have been isolated from the bark of this plant; vavain 3'-O-B-D glucoside, and its aglycone, vavain; flavan-3-ol, (+)-catechin (Ylva *et al.*, 1998), pentandrin and pentandrin glucoside and beta-sitosterol and 3-beta-D-glucopyranoside (Ngounou *et al.*, 2000).

Literature review reveals a dearth of information on radical scavenging activity and toxicity assessment using brine shrimp on these medicinal plants. Hence, we have carried out an evaluation of their radical scavenging activity, toxicity assessment and HPLC analysis.

Plant extraction

The two plants collected were dried under shade and ground into powder. 200 grams of each powdered plant sample were then subjected to extraction by maceration using methanol for 72 hours. The extracts were filtered and solvent removed using rotary evaporator at under a reduced temperature of 40°C. The plants extracts were stored in the refrigerator at 4°C till needed for analysis.

Determination of total phenolic content

The concentration of phenolic in plant extracts was determined using spectrophotometric method (Singleton *et al.*, 1999). Methanol solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanol solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.0 mL of 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 mL methanol. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at $\lambda_{max} = 765$ nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolic was measured (mg/mL) from the calibration line; then the content of phenolic in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

Determination of DPPH radical scavenging activity

The free radical scavenging ability of the extracts was determined with DPPH radical and the bleaching method of Silva *et al.* (2006). Briefly, 100 μ L of blank/standards/plant sample dilutions followed by 150 μ L of DPPH (3 g dissolved in 60 mL of ethanol) were pipetted into 96-well microplates and incubated for 30 minutes. In methanol or aqueous solution, it generated stable free radicals by the delocalization of the free electrons; which in turn produced a deep purple colored solution. Absorbance values of these concentrations were calculated at 517 nm in Spectramax Gemini XS microplate reader and the decreasing value of DPPH at 517 nm is directly proportional to the radical scavenging activity (Brand-Williams *et al.*, 1995). Percentage inhibition of DPPH free radical (I%) was calculated by using the following equation: $I\% = ((\text{Absorbance of blank} - \text{Absorbance of sample}) / (\text{Absorbance of blank})) \times 100$. 50% of inhibition (IC₅₀) of extract concentration was calculated from the graph; where the percentage of inhibition (I%) was plotted against extract concentration.

Nitric oxide scavenging assay

Plant extracts were dissolved in distilled water for this quantification. Sodium Nitroprusside (40 mM) in

standard phosphate buffer saline (20 mM, pH 7.4) was added to different concentrations (25-800 μ g/mL) of the extracts in tubes and were incubated at 29°C for 3 hours. Control experiment without the test compounds but with equivalent amounts of buffer was conducted in an identical manner. After 3 hours, equal volumes of supernatant from the incubated samples and freshly prepared griess reagent were transferred into a 96 micro-well plate. It was incubated for 10-15 minutes and the absorbance of the colour developed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylenediamine hydrochloride was observed at 550nm on spectrophotometer. The same procedure was with gallic acid which was used as reference standard in comparison with the test agents. A 1:50 dilution of 10 mM of NaNO₂ was also used for the calibration curve. Percentage inhibition ability of the plant extracts respect to the negative control was calculated by the following formula; % inhibition = (Average of test)/(Average of control) \times 100

Brine shrimp lethality assay (BSLA)

The experiment was carried out using the method described by Mclaughlin, (1991). Brine shrimp eggs were obtained from the Department of Pharmacognosy, University of Ibadan. Briefly, *Artemia salina* cysts (brine shrimp eggs 0.1 g) were allowed to hatch in natural sea water, containing 3.8 g/L salt, obtained from Bar beach, Ikoyi, Lagos. The larvae (nauplii) were placed in sea water for 48 hours at 25°C under constant aeration and illumination to ensure survival and maturity before use. Stock solutions (10 mg/mL) of plant extracts were made and diluted serially in clean test tubes of 10 mL volume to obtain five final concentrations (1000–1 μ g/mL). Ten nauplii were collected with the aid of a pipette and added to the serially diluted test solutions. Tests were carried out in triplicate. The negative control consisted of ten nauplii per tube in sea water without plant extract while Cyclophosphamide was used as the positive control. After the 24 hour incubation at 25 °C, a magnifying lens was used to count the number of dead larvae and the percentage mortality was calculated. Larvae were considered dead only if they did not move for few seconds after pricking with sharp object during observation. The 50% lethal concentration (LC₅₀ value) and the standard error mean (SEM) were calculated using a non-linear regression curve contained in the Graph pad prism statistical software.

High performance liquid chromatography (HPLC) analysis

The extracts were subjected to qualitative HPLC analysis to identify the type of metabolites present. A reverse-phase HPLC system (Thermo-scientific NX 5 μ M C-18 column [250 \times 4.6 mm]) was operated under isocratic conditions. The column temperature was set at 25 °C. A variable wavelength detector was set at 235 nm. 0.1% trifluoroacetic acid in nanopurewater (solvent A) and 0.1% trifluoroacetic acid in methanol (solvent B) was used as the eluent. For the analysis,

RESULTS

Determination of total phenolic content

The phytochemicals present in plants have the potential for preventing chronic diseases. The multiple properties of these phytochemicals have made them more attractive, as they can modulate various aspects of diseases (Csepregi *et al.*, 2016). The plant extracts used for this study showed varying total phenolic content (Table 1). The gallic acid equivalents (GAE) of the extracts are 21.26 \pm 1.29 mg/g for *S. afzelii* leaves and 95.31 \pm 8.71 mg/g for *C. pentandra* stem-bark. As potent antioxidants, phenolics from previous studies have demonstrated its significance in the protection against human diseases.

Determination of DPPH radical scavenging activity

The electron donation ability of natural products can be measured by DPPH purple coloured solution been bleached (Nunes *et al.*, 2012). The method is based on scavenging DPPH through the solution of a radical species or antioxidant that discolourises the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants (Krishnaiah *et al.*, 2011). Hence, a large decrease in the absorbance of the reaction mixture which indicates significant free radical scavenging activity was demonstrated in the methanol extracts of *S. afzelii* leaves with inhibitory concentration at 50% (IC₅₀) value of 97.60 μ g/mL and *Ceiba pentandra* stem bark with IC₅₀ value of 22.35 μ g/mL, as compared with the

the mobile phase composed of 70% of A and 30% B at 0 min, then linear gradient to 100% of B over 40 min and held at that composition for 10 minutes at a flowrate of 1 mL/min. The auto-sampler injected 20 μ L of each sample and all peaks were detected by ultraviolet (UV) photodiode array detector. To determine the nature of compounds present in the extracts, the peaks generated in the chromatogram of each extract were compared to the compounds collection contained in the HPLC library.

standard drugs with IC₅₀ values of 1.4 \pm 0.34 (Ascorbic acid) and 2.8 \pm 0.78 (Gallic acid) (Table 1). The copious amount of phenolic content in the plant extracts may be responsible for the antioxidant activity as demonstrated in various studies. According to Mensah *et al.* (2004), methanol extract of *S. afzelii* stem demonstrated antioxidant activity using DPPH assay and the active compound identified was α -tocopherol, a phenolic compound.

Nitric oxide (NO) scavenging assay

The methanol extracts of *S. afzelii* leaves with IC₅₀ value of 22.50 \pm 1.3 μ g/mL and *C. pentandra* stem-bark with IC₅₀ value of 5.40 \pm 0.3 μ g/mL, including the standard (Gallic acid) with IC₅₀ value of 1.1 \pm 0.1 μ g/mL (table 1), have demonstrated a significant decrease in the nitric oxide radical due to their scavenging ability. Nitric oxide is an oxidant as it reacts with reactive oxygen species (ROS). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Nabavi *et al.*, 2008a,b). Nitric oxide is known to be a ubiquitous free-radical moiety, which is distributed in tissues or organ systems and is supposed to have a vital role in neuromodulation or as a neurotransmitter in the central nervous system (Gulati *et al.*, 2006).

Table 1: Total Phenolic Content, DPPH scavenging and Nitric Oxide scavenging activities of methanol extracts of the investigated medicinal plants

Plant Extracts/ Drugs	TPC (GAE mg/g of extract)	DPPH, IC ₅₀ (μ g/mL)	NO, IC ₅₀ (μ g/mL)
<i>S. afzelii</i> leaves	21.26 \pm 1.29	97.60	22.5 \pm 1.3

<i>C.pentandra</i> stem-bark	95.31 ± 8.71	22.35	5.4±0.3
Ascorbic Acid (Standard I)	ND	1.4 ± 0.34	ND
Gallic Acid (Standard II)	ND	2.8 ± 0.78	1.1±0.1

ND: Not Determined; Standard drug and extracts express as IC₅₀ ± standard error of mean (SEM)

Brine shrimp lethality assay (BSLA)

Brine Shrimp bioassay is considered as a rapid preliminary screening for the presence of bioactivity compounds and also used to determine the toxicity of plant extracts (Ogbole *et al.*, 2017). According to Meyer *et al.* (1982), extracts derived from natural products which have LC₅₀ ≤ 1000 µg/mL are known to possess toxic effects. The results obtained showed that the activity of the plant extracts was concentration-dependent. *S. afzelii* leaves extract had LC₅₀ value above 1000 µg/mL indicating that they are non-toxic to brine shrimp larvae. On the contrary, the *C. pentandra* stem-bark extract and cyclophosphamide

had LC₅₀ value below µg/mL which is an indication that they are toxic to brine shrimps' larvae. The BSLA result is summarized in table 2. However, plants found to be toxic to brine shrimp are likely to be good candidate for anti-cancer research (Ramachandran *et al.*, 2011), hence, *C. pentandra* could likely be a good candidate for anti-cancer research due to the numerous phenolic compounds present. Kumar *et al.* (2016) had previously evaluated the *in vitro* cytotoxicity and *in vivo* antitumor activity of *C. pentandra* bark extracts and were shown to be effective on MCF-7 and B16F10 cancer cell lines.

Table 2: Brine shrimp lethality activities of the plants extracts

Plant	LC ₅₀ (µg/mL)	Toxicity Effect
<i>S. afzelii</i> leaves	>1000	Non-toxic
<i>C. pentandra</i> stem-bark	<1000	Toxic
Cyclophosphamide (Standard)	<1000	Toxic

HPLC analysis of the investigated plant extracts

The HPLC analysis of methanol extract of *S. afzelii* leaves showed 11 distinct peaks which revealed the presence of 8-β-L-arabinofuranosyl-6-β-D-glucopyranosylapigenin, kaempferol-3-O-glucoside, kaempferol-3-O-(6"-malonyl-glucoside), kaempferol-3-O-rhamnoglucoside and palitantin, respectively as the major compounds, as observed in the chromatogram and UV spectra (Fig. 1 and 2). Figure 2 show the UV spectral assignment of major peaks of the methanol extract of *S. afzelii* leaves. Kaempferol-3-O-glucoside also known as astragalins is a phenolic compound that has been isolated from *Phytolacca*

americana and *Phegopteris connectilis*. This compound demonstrated anti-inflammatory and analgesic properties (Klaus-Peter, 1999; Parveen *et al.*, 2007). Kaempferol-3-O-(6"-malonyl-glucoside) and Kaempferol-3-O-rhamnoglucoside are members of the class of compounds known as flavonoid-3-O-glycosides. Kaempferol-3-O-(6"-malonyl-glucoside) was found in endive and lettuce and demonstrated anti-amyloidogenic properties while Kaempferol-3-O-rhamnoglucoside, also known as nicotiflorin was found in ginkgo nuts and tea. This makes these compounds potential biomarkers for the consumption of these food products (Nakayama *et al.*, 1978;

Parinda *et al.*, 2009). Palitantin is an antifungal and antiprotozoal compound used in biochemical research which was first isolated from *Penicillium palitauis*. It demonstrated moderate antimycobacterial property (Fuska *et al.*, 1970). The HPLC profile of methanol extract of *C. pentandra* stem bark showed 22 distinct peaks. Peak 1, 2, 4, 8 and 14 revealed the presence of lateritin/beauvericin, procyanidin B2, pavetannin, (-)-catechin and genistein-8-C-glucoside, respectively, were the major compounds present, as observed in the chromatogram and UV spectra (Figs. 3 and 4). Figure 4 show the UV spectral assignment of the major peaks of the methanol extract of *C. pentandra* stem-bark. Lateritin, a morphine-2,5-dione (depsipeptide) was confirmed to be structurally equivalent to a monomer of beauvericin. Beauvericin is a trimeric lactone of lateritin. Lateritin has demonstrated to exhibit inhibitory effect on cholesterol acyltransferase isolated from *Gibberella lateritium* and beauvericin have shown to exhibit cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome C release (Hasumi *et al.*, 1993; Wang and Xu, 2012). Procyanidins, including procyanidin B2 are oligomers

of flavonoids which are mostly found in fruits and food products have attracted increasing attention in the fields of nutrition and medicine due to their potential health benefits. They have shown to demonstrate anti-inflammatory, anti-arthritis and anti-allergic properties, prevention of skin aging and the ability to scavenge reactive oxygen and nitrogen species (Jorge *et al.*, 1991; Zhao *et al.*, 1999). Catechin which was also identified in the methanol extract of *C. pentandra* stem-bark is a class of flavonoids and natural antioxidants with variety of antioxidant properties. Catechin also has the ability to suppress human cell proliferation and induces apoptosis (natural cell death) of breast cancer cells by facilitating cell cycle arrest (Zhao *et al.*, 1999). The most commonly observed isomer of catechin, (+)-catechin version has been identified in *C. pentandra* in previous studies (Noreen *et al.*, 1998; Reygaert, 2018), which is different from the stereoisomer [(-)-catechin] identified in this study. Genistein 8-C-glucoside, an isoflavone have shown to demonstrate antioxidant and cytotoxicity properties (Antosiak *et al.*, 2007).

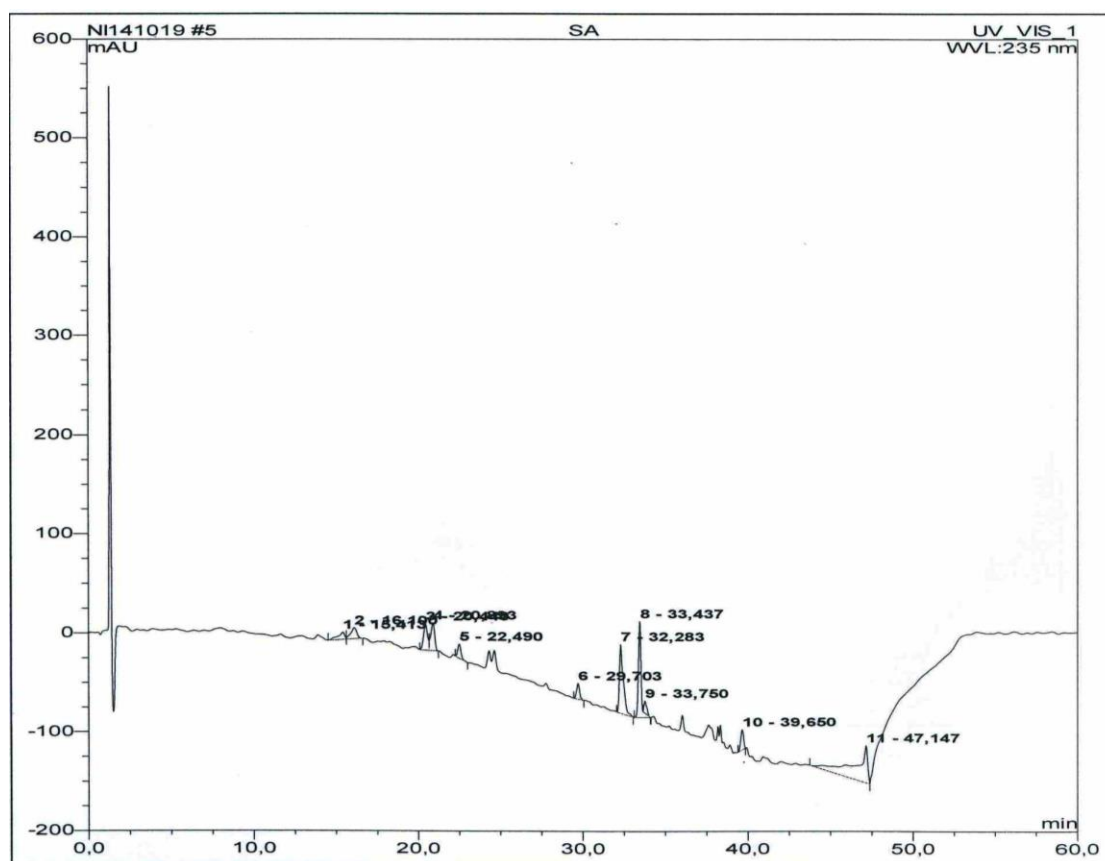


Figure 1: Reverse Phase-HPLC Quantitative Chromatogram of the methanol extract of *S. afzelii* leaves

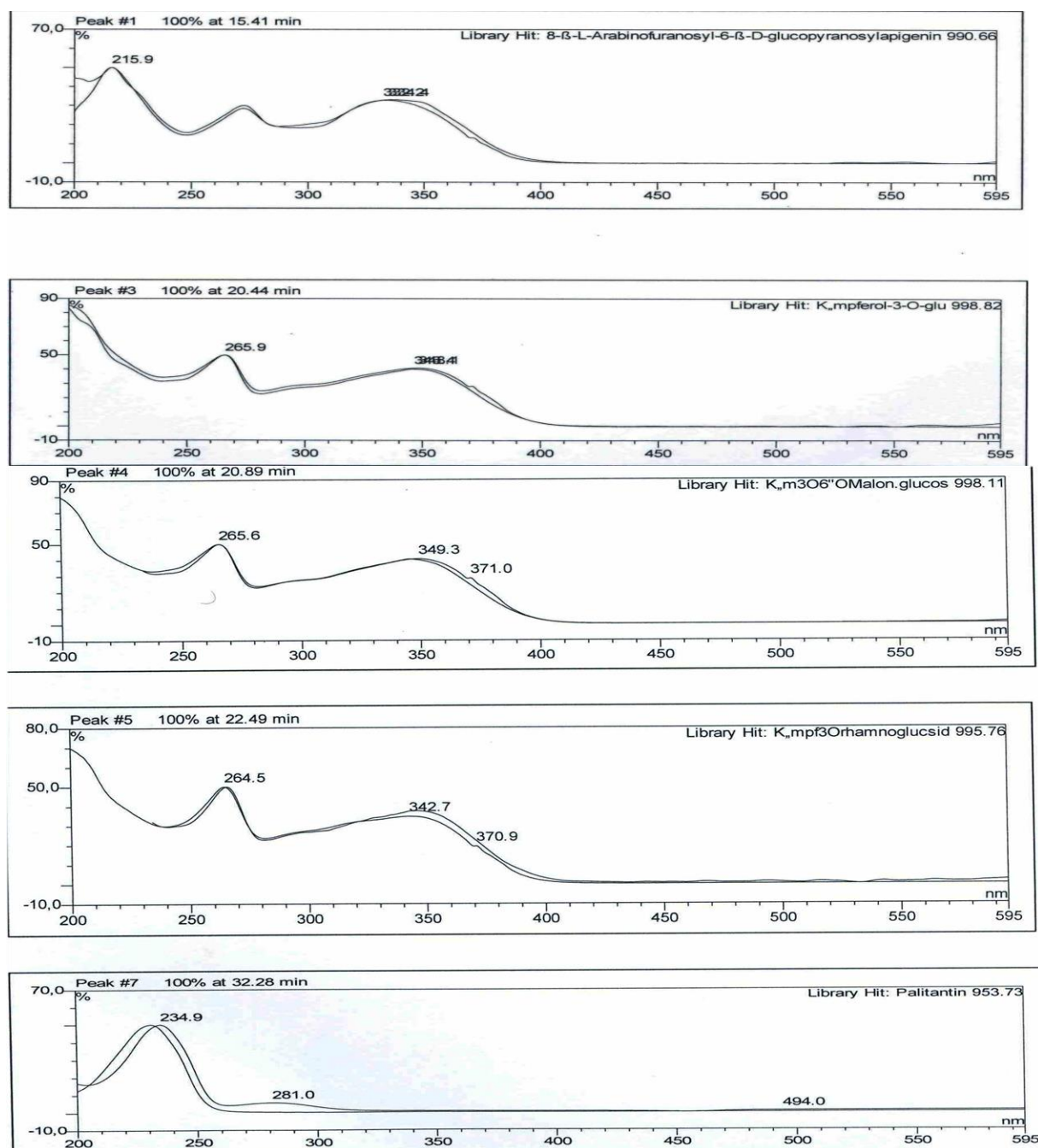


Figure 2: UV spectra assignment of major peaks of the methanol extract of *S. afzelii* leaves

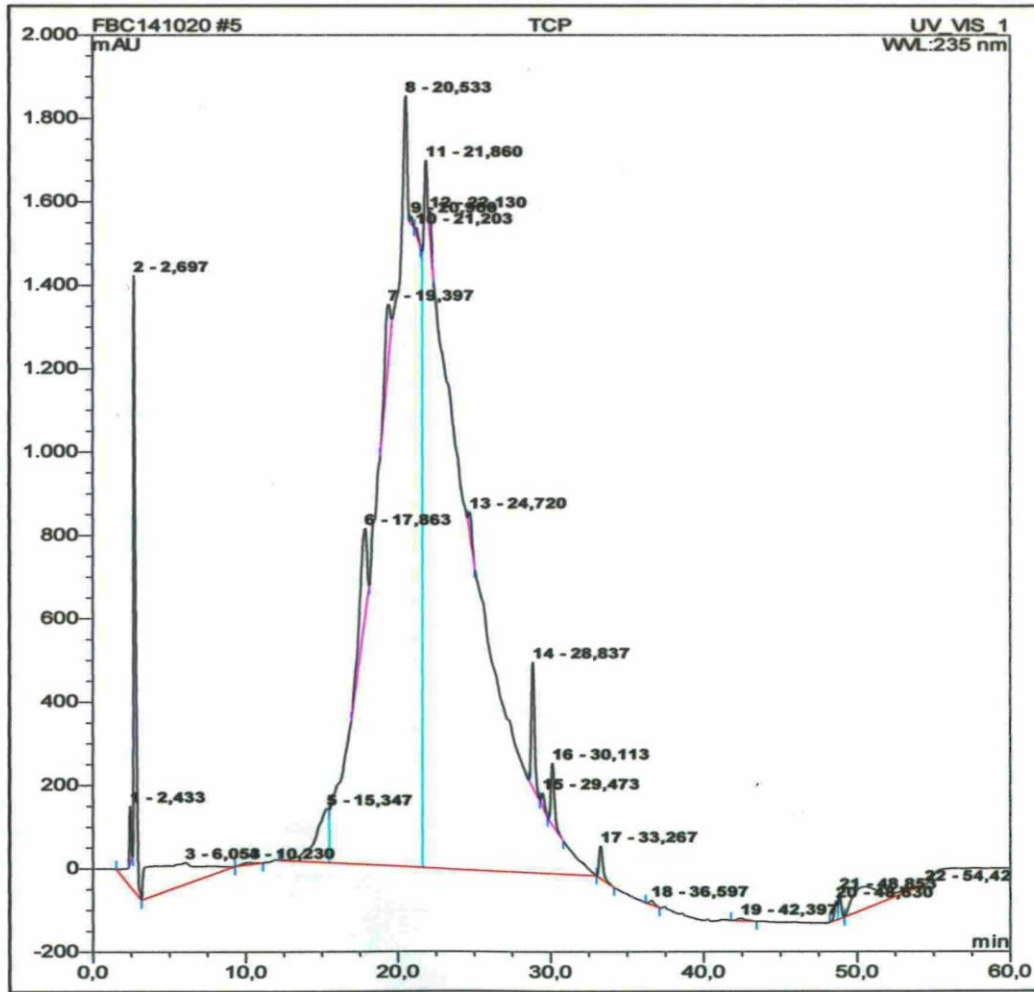


Figure 3: Reverse Phase-HPLC Quantitative Chromatogram of the methanol extract of *C. pentandra* stem-bark

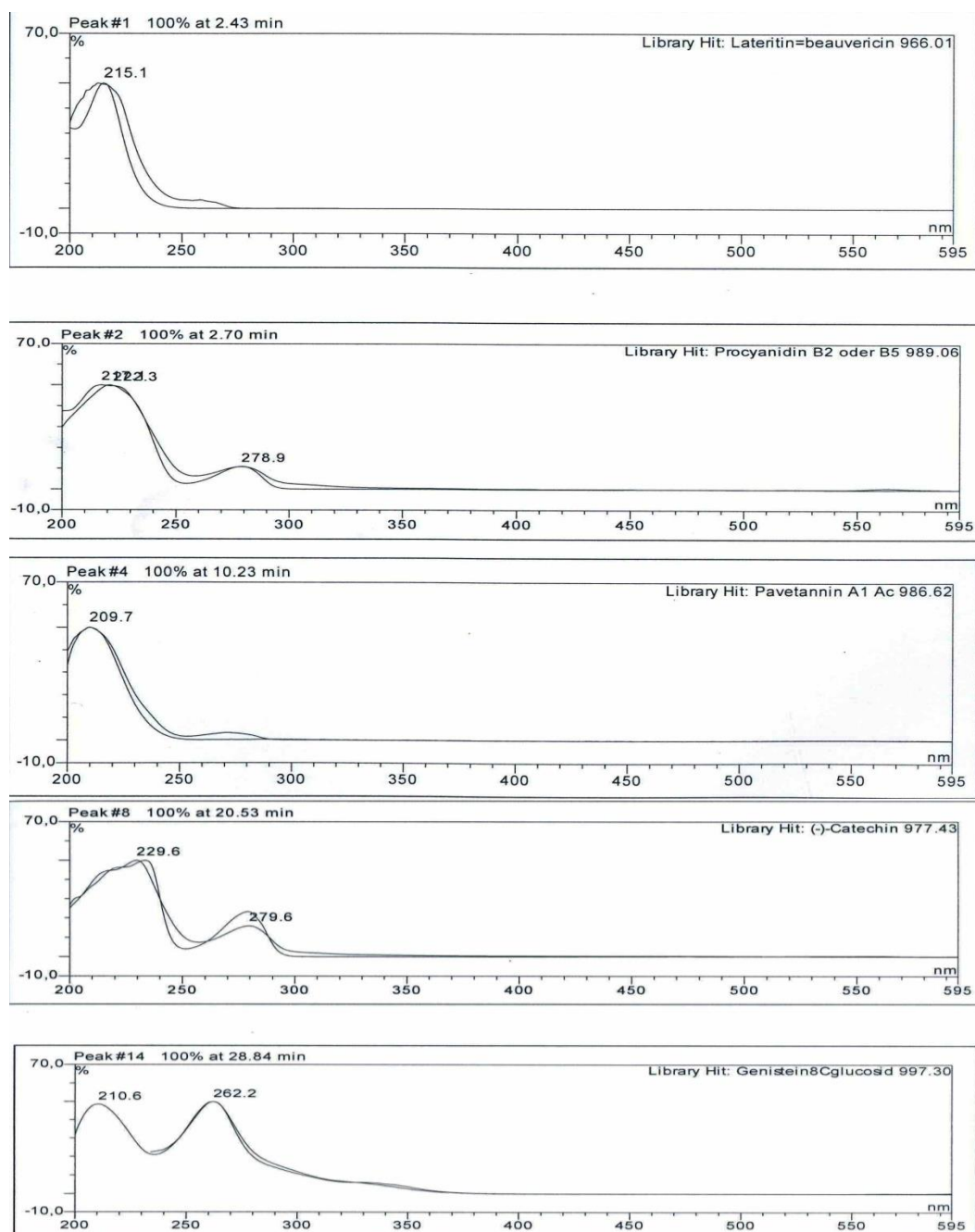


Figure 4: UV spectra assignment of major peaks of the methanol extract of *C. pentandra* stem bark

CONCLUSION

In conclusion, the methanol extracts of *C. pentandra* stem bark and *S. afzelii* leaves demonstrated antioxidant activity by scavenging the DPPH and nitric oxide free radicals. The major compounds present in the plant extracts were identified which can be very helpful in the search for new drugs for various diseases associated with free radical generation. Five

compounds each which are mostly phenolics were identified in the both plant extracts. From literature, some of these compounds have demonstrated various biological and pharmacological effects such as antioxidant, cytotoxicity, antimicrobial, anti-inflammatory and anti-arthritic effects respectively. Some of the compounds identified such as 8- β -L-

arabinofuranosyl-6-β-D-glucopyranosylapigenin, Kaempferol-3-O-(6"-malonyl-glucoside), Palitantin and Lateritin are yet to be fully explored. Therefore, there is need for further research on the medicinal plants as this would serve the purpose of established

benchmarks for future plant research. Moreover, this may be the first time some of these compounds are identified in *C. pentandra* stem bark and *S. afzelii* leaves.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the Department of Pharmaceutical Biology and Biotechnology, Heinrich Heine Universität Dusseldorf, Germany for providing the equipment and materials for the HPLC analysis.

We also acknowledge the staff of the Forest herbarium Ibadan (FHI) and Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI) for the identification of plants.

REFERENCES

- Antosiak, A., Milowska, K., Maczynska, K., Rozalska S. and Gabryelak, T. (2007). Cytotoxic activity of genistein-8-C-glucoside from *Lupinus luteus* L. and genistein against human SK-OV-3 ovarian carcinoma cell line. *Med Chem Res.*, 26(1):64-73. Doi: 10.1007/s00044-016-1725-5.
- Brand-Williams, W., Cuvelier, M. AndBerset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1):25-30.
- Burkill, H.M. (1985). The useful plants of west tropical Africa. Vol. 1.
- Burkill, H.M. (2000). The useful plants of West Tropical Africa II Botanical Gardens, Kew, 1:481.
- Csepregi, K., Neugart, S., Schreiner, M. AndHideg, E. (2016). Comparative Evaluation of total antioxidant capacities of plant polyphenols. *Molecules*, 21, 208.
- Dawson, T.M., Dawson, V.L.and Snyder, S.H. (1992). A novel neuronal messenger molecule in brain: The free radical, nitric oxide. *Annu. Neural.*, 32:297-311.
- Evans, W.C. (2002). Trease and Evans Pharmacognosy. WB Saunders Ltd, London, pp 32-547.
- Fuska, J., et al. (1970). The effect of palitantin, a metabolite of *Penicillium frequentans* on *Leishmania brasiliensis*. *Folia Microbiol. (Praha)*, 15, 111.
- Gill, L.S. (1992). Ethnomedicinal uses of plants in Nigeria. University of Benin Press, Benin city, Nigeria, p 103.
- Grice, H.C. (1986). Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. *Food Chem. Toxicol.*, 24:1127-30.
- Gulati, K., Ray, A., Masood, A. and Vijayan, V.K. (2006). Involvement of nitric oxide (NO) in the regulation of stress susceptibility and adaptation in rats. *Indian J Exp Biol*,44:809-815.
- Hasumi, K., Shinohara, C., Iwanaga, T. and Endo, A. (1993). Lateritin, a new inhibitor of acyl-CoA:cholesterol acyltransferase produced by *Gibberella lateritium* IFO 7188. *J Antibiot.*, 46:1782-1787.
- Houghton, P. (1995). The role of plants in traditional medicine and current therapy. *J Alter CompleMed.*, 1:131-143.
- Houghton, P.J.,Woldermariam, T.Z., O'Shea, S. and Thyagarajan, S.P.(1996). Two securinega-type alkaloids from *Phyllanthus amarus*. *Phytochemistry*, 43:715-717.
- Houghton, P.J., Hylands, P.J., Mensah, A.Y., Hensel, A. and Peters, A.M. (2005). In vitro tests and ethnopharmacological investigations: wound healing as an example. *Journal of Ethnopharmacology*, 100(1-2): 100-107.
- Huang, D.H., Chen, C., Lin, C. and Lin, Y. (2005). Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatic* Forsk.) constituents. *Botanical Bulletin of Academic Science*, 46:99-106.
- Jorge, M.R., Jacques, R., Veronique, C., Annie, C. and Michel, M. (1991). Procyanidin dimers and trimers from grape seeds. *Phytochemistry*, 30(4):1259-64.
- Klaus-Peter, A. (1999). Phenolic constituents of the fern *Phegopterisconnectilis*. *Phytochemistry*, 52(5):929-934.Doi:10.1016/S0031-9422(99)00326-X.
- Krishnaiah, D., Sarbatly, R. AndNithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing*, 89(3):217-233.
- Kumar, R., Kumar, N., Ramalingayya, G.V., Setty, M.M. and Pai, K.S.R. (2016). Evaluation of *Ceiba pentandra* (L) Gaertner bark extracts for *in vitro* cytotoxicity on cancer cells and *in vivo* antitumor activity in solid and liquid tumor models. *Cytotechnology*, 68(5):1909-1923.
- Mclaughlin, J.L. (1991). Crown gall tumours on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractionation. *Methods in plant Biochemistry*, 6:1–32.

- Mensah, A.Y., Houghton, P.J., Akyirem, G.N.A., Fleischer, T.C., Mensah, M.L.K., Sarpong, K. and Adosraku, R., John Wiley & Sons Ltd. (2004). Evaluation of the antioxidant and free radical scavenging properties of *Secamoneafzelii* Rhoem. *Phytotherapy Research*, 18, 1031-1032.
- Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Med*, 45(5):31-34.
- Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F. and Jafari, M. (2008b). Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* Trautv and *Froripia subpinnata*. *Pharmacologyonline*, 3:19-25.
- Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Hamidinia, A. and Bekhradnia, A.R. 2008a. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline*, 2:560-567.
- Nakayama, M., Horie, T., Tsukayama, M., Masumura, M. and Hayashi, S. (1978). Isolation of Kaempferol-3-O-rhamnoglucoside, a flavonoid glycoside from *Zephranthes candida*. *Zeitschrift fur Naturforschung C.*, 33(7-8):587-8. Doi:10.1515/znc-1978-7-822.
- Ngounou, F.N., Meli, A.L., Lontsi, D., Sonndengam, B.L., Atta-Ur-Rahman., Choudhary, M.I., Malik, S. and Akhtar, F. (2000). New isoflavones from *Ceiba pentandra*. *Phytochemistry*, 54(1):107-110.
- Noreen, Y., el-Seedi, H., Perera, P. and Bohlin, L. (1998). Two new isoflavones from *C. pentandra* and their effect on cyclooxygenase catalysed prostaglandin biosynthesis. *J. Nat. Prod.*, 61, 8-12.
- Nunes, R., Anastacio, A. and Carvalho, I.S. (2012). Antioxidant and free radical scavenging activities of different plant parts from two *Erica* species. *Journal of food quality*, 35(5):307-314. Doi.org/10.1111/j.1745-4557.2012.00459.x.
- Nwachukwu, I.N., Allison, L.N., Chinakwe, E.C. and Nwadiaro, P. (2008). Studies on the effects of *Cymbopogon citratus*, *Ceiba pentandra* and *Loranthus bengwelensis* extracts on species of dermatophytes. *The Journal of American Science*, 4(4):58-67.
- Ogbole, O.O., Segun, P.A. and Adeniji, A. J. (2017). 'In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts', pp. 1–10. doi: 10.1186/s12906-017-2005-8.
- Parinda, K., Yuki, N., Tetsuhiro, N., Kenji, K., Kunihiko, T., Masatoshi, I., Shigeharu, H., Hachiro, S. and Kaeko, K. (2009). Identification of an antiamyloidogenic substance from mulberry leaves. *Neuroreport*, 20(13):1214-1218.
- Parveen, Z., Deng, Y., Saeed, M.K., Dai, R., Ahamad, W. and Yu, Y.H. (2007). Anti-inflammatory and Analgesic activities of *Thesium chinense* Turcz extracts and its major flavonoids, Kaempferol and Kaempferol-3-O-glucoside. *Yakugaku Zasshi*, 127(8):1275-79.
- Ramachandran, S., Vamsikrishna, M., Gowthami, K., Heera, B. and Dhanaraju, M. (2011). Assessment of cytotoxic activity of *Agave cantula* using brine shrimp (*Artemia salina*) lethality bioassay. *Asian J Sci. Res.*, 4(1):90–4.
- Reygaert, W.C. (2018). Green Tea Catechins: Their use in treating and preventing infectious diseases. doi.org/10.1155/2018/9105261.
- Shimizu, K., Kondo, R., Sakai, K., Takeda, N., Nagahata, T. and Oniki, T. (2001). Novel vitamin E derivative with 4-substituted resorcinol moiety has both antioxidant and tyrosinase inhibitory properties. *Lipids*, 36: 1321-1326.
- Silva, E.M., Souza, J.N.S., Rogez, H., Rees, J.F. and Larondella, Y. (2006). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry*, 101:1012-18.
- Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299:152-178.
- Sule, M.I., Njingaa, N.S., Musa, A.M., Magaji, M.G. and Abdullahi. (2010). Phytochemical and anti-diarrhoeal studies of the stem bark of *Ceiba pentandra*. *Nigerian Journal of Pharmaceut. Sci.*, 8(1):143-148.
- Terao, J., Piscula, M. and Yao, M.C. (1994). Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospholipid bilayers. *Archives of Biochemistry and Biophysics*, 308:278-284.
- Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *J. Adv Pharm Technol Res.*; 3:200-1.
- Wang, Q. and Xu, L. (2012). Beauvericin, a bioactive compound produced by Fungi: a short review. *Molecules*, 17(3):2367-77. Doi: 10.3390/molecules17032367.

- Wichi, H.P. (1988). Enhanced tumor development by butylated hydroxyanisole (BHA) from the perspective of effect on the fore stomach and oesophageal squamous epithelium. *Food Chem. Toxicol.*, 26:717-723.
- Zhao, J., Wang, J., Chen, Y. and Agarwal, R. (1999). Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3'-gallate as the most effective antioxidant constituent. *Carcinogenesis*, 20(9):1737-45. Doi:10.1093/carcin/20.9.1737.
- Ylva, N., Hesham, E., Premila, P. and Lars, B. (1998). Two new isoflavones from *Ceiba pentandra* and their effect on cyclooxygenase-catalyzed prostaglandin biosynthesis. *Journal of Natural Products*, 61(1):8-12.

*Address for correspondence: Omonike O. Ogbole

Conflict of Interest: None declared

Department of Pharmacognosy,
University of Ibadan,
Ibadan, Nigeria
Telephone: +234-8056434577
E-mails: nikeoa@yahoo.com

Received: 21 June, 2021

Accepted: 01 December, 2021