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Cytotoxic effects of Nigerian Ethnomedicinal Plant Extracts on Three Cancer Cell Lines and their Antioxidant Properties

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Abstract

Several chemotherapeutic agents have been associated with cancer treatment but with numerous adverse effects. In this study, the cytotoxic and antioxidant effects of methanol extracts of ten selected plants from Nigerian ethnomedicine were assessed as a probable source of natural products for the treatment of cancer. The phenolics and flavonoid contents, and *in vitro* antioxidant assays (DPPH, nitric oxide, total antioxidant capacity, ferric reducing antioxidant power, metal chelating, lipid peroxidation) on the plant extracts were evaluated. *In vitro* cytotoxic effects of the extracts on Brine Shrimps, Rhabdomyosarcoma (RD), Breast (MCF-7), and Cervical (HeLa) cancer cell lines were also evaluated. The plant extracts demonstrated a broad spectrum of antioxidant properties. All the extracts exhibited moderate toxicity on brine shrimp. *Pacrilima nitida* exhibited the highest inhibition on the growth of RD (CC_{50}=17.97 µg/mL) and MCF-7 (CC_{50}=38.79 µg/mL) cell lines while *Cocos nucifera* exhibited the highest inhibition on the growth of HeLa (CC_{50}=24.16 µg/mL). These plants exhibited a relatively cytotoxic and antioxidant effect and thus could contribute to anticancer drug discovery and many other disease conditions due to their antioxidant properties.

Keywords: Antioxidant, Breast, Cervical, Cytotoxic, Medicinal Plants, Rhabdomyosarcoma

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1. Introduction

It is impossible to overstate the importance of free radicals in disease initiation. Most of these free radicals, including hydroxyl, lipid peroxide, nitric oxide, and hydrogen peroxide radicals react with cell components and damage them, leading to several degenerative or pathological conditions like cancer and many other diseases (1, 2).

With approximately 10 million deaths in 2020, cancer is globally the major cause of death (3). A complicated, poorly understood chemistry of genetic and environmental factors triggers it. Currently available cancer therapies, including surgery, radiation, and chemotherapy, are expensive (4). Furthermore, despite the high cost of development, novel synthetic chemotherapeutic agents developed in the last decade that are currently in clinical use have failed to meet expectations (5). According to Bansal and Malhotra (6), these synthetic agents that are chemotherapeutic are often associated with toxic undesirable effects on normal cells. As a result, novel, effective, and cost-effective anticancer agents with minimal damaging side effects on normal cells are constantly in

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demand. This necessitated sourcing new cytotoxic agents from medicinal plants.

The plant kingdom is extremely crucial in human life. In many nations around the world, they are a common cancer treatment alternative (7). Certain agents including vinblastine, vincristine, irinotecan, topotecan, camptothecin, taxol, and podophyllotoxin obtained from diverse plants have been shown to exhibit anticancer properties (8). A variety of compounds with potent antioxidant properties have been discovered in plants. These antioxidants may help to prevent and treat cancer and other disorders by shielding cells from free radical damage (9). According to several studies, the presence of antioxidants in medicinal plants may be responsible for their anticancer efficacy (10). Furthermore, as compared to modern (allopathic) medications, medicinal plants maybe more readily available, less expensive, and have more appropriate safety profile (9). Therefore, the cytotoxic effect of methanol extracts and their antioxidant potentials from southwestern ethnomedicinal plants were assessed in this study.

2. Materials and methods

2.1. Collection of plants and extraction

The selected plants were collected in their natural environment (Table 1). Mr. P. Agwu of the Pharmacognosy Herbarium, University of Ibadan (DPHUI), Mr. D.P.O Esimekhuei of the University of Ibadan Herbarium (UIH), and Mr. T.K. Odewo of the Forest Herbarium Ibadan (FHI) identified and validated the plants by suitable voucher specimens. Relevant websites including (www. theplantlist.org) and (www. worldfloraonline.org) were also used to validate the plant names further. The parts of the plants collected were dried under a shade and ground into a coarse powder at room temperature. Maceration was carried out on 200 g each of plant material in methanol for 72 hours and was stirred occasionally. Filtration was carried out and the extracts were dried using a rotary evaporator. After the dryness of the extracts, they were then stored at 4 °C till analysis.

2.2. Total phenolic content

The phenolics present in the extracts were

Table 1. Plant species analysed for antioxidant and cytotoxic activities.

Sample code	Plant	Family	Local name	Part used	Voucher number	Place of collection
AAL	*Allophylus africanus* P. Beauv.	Sapindaceae	Akanro	Leaves	FHI 109809	UI Botanical Garden, Ibadan, Oyo state
BNL	*Baphia nitida* G. Lodd.	Leguminosae	Irosun	Leaves	FHI 106544	Isore, Ipara-Remo L.G.A, Ogun state
CAB	*Chrysophyllum albidum* G. Don	Sapotaceae	Agbalumo	Stem bark	FHI 108856	FRIN, Ibadan, Oyo state
CNH	*Cocos nucifera* Linn.	Arecaceae	Agbon	Husk fibre	FHI 107825	Orogun, Ibadan, Oyo state
CPL	*Combretum paniculatum* Vent.	Combretaceae	Ogan	Leaves	UIH 23152	Amina way, UI Ibadan
CVS	*Cola verticillata* (Thonn.) Stapf ex A. Chev.	Sterculiaceae	Obi Olooyo	Seed	UIH 23136	Obale, Akure, Ondo state
MSI	*Musa sapientum* Linn.	Musaceae	Ogede	Inflorescence	FHI 110120	Mama Anat, UI, Ibadan, Oyo state
PNL	*Pacrilima nitida* (Stapf) T. Durand & H. Durand	Apocynaceae	Abeere	Leaves	FHI 108794	Isore, Ipara-Remo L.G.A, Ogun state
PPL	*Parinari polyandra* Benth.	Chrysobalanaceae	Aboidefin	Leaves	DPHUI 231	Iseyin, Oyo state
VCW	*Vernonia cinerea* (L.) Less	Asteraceae	Ewe-Oghan	Whole	FHI 109827	Orogun, Ibadan, Oyo state

quantified by folin ciocalteu's phenolic oxidizing reagent as reported by Singleton *et al*. (11). 0.1 mL from the stock solution in distilled water (0.9 mL) was thoroughly mixed with the phenolic reagent (0.2 mL) and 7% Na_2CO_3 (w/w) solution (1 mL) after 5 minutes was added up which was built up to 2.5 mL. It was then incubated at 30 °C for 1½ hrs. At 750 nm, the absorbance was quantified in contrast to the negative control (1 mL of distilled water with no extract). The standard used to quantify the extract's Gallic Acid equivalent (GAE) was Gallic Acid at 0.2-1.0 mg/mL after generating a calibration curve.

2.3. Total flavonoid content

In contrast to the sample extracts, standard quercetin with concentrations (0.2 to 1 mg/mL) was employed. The aluminum chloride colorimetric test method described by Miliauskas *et al.* (12), was used. 0.1 mL extracts/standard in distilled water (0.4 mL) was mixed with sodium nitrite (5%) (0.1 mL). Five minutes later, 10% aluminum chloride (0.1 mL) and sodium hydroxide (0.2 mL) were added up, then top up with distilled water to 2.5 mL. A blank was used to compare the absorbance at 510 nm. The plant's total flavonoid level was determined as mg Quercetin equivalent/gram of crude extract.

2.4. Antioxidant Assays

2.4.1. DPPH assay

The extracts' ability to free radical scavenging was evaluated with DPPH and the bleaching method (13). Briefly, 100 µL of blank/standards/plant sample dilutions followed by 150 µL of DPPH (3 g dissolved in 60 mL of methanol) were pipetted into 96-well microplates and incubated for 30 minutes. It formed stable free radicals in solutions of aqueous or methanol by delocalizing free electrons, resulting in a purple solution. The values of absorbance for the concentrations (5-0.15625 mg/mL) were calculated at 517 nm in the Spectramax Gemini XS microplate reader. When the value of DPPH at 517 nm reduces, the radical scavenging activity increases (14). Percentage inhibition (I%) = ((Blank absorbance – Sample absorbance)/(Blank absorbance)) ×100. Fifty percent inhibition (IC_{50}) of concentration of extracts was computed from the graph; where the I% was plotted in contrast to the concentrations of the extracts.

2.4.2. Nitric oxide scavenging assay

For this quantification, the procedures of Panda *et al*. (15), with minor modifications were used. Different concentrations (25-800 µg/mL) of extracts in tubes were incubated at 29 ℃ for 3 hours with sodium nitroprusside (40 mM) in phosphate buffer saline (20 mM, pH 7.4). The control experiment was carried out in the same way, but without the test agents and with the same amount of buffer. After 3 hours, 96 micro-well plates were filled with equal amounts of supernatant from the incubated samples and freshly made Griess reagent. The color obtained in the nitrite and sulphanilamide diazotization process with naphthyl ethylenediamine hydrochloride successive coupling was seen after being incubated for 15 minutes. The absorbance was read at 550 nm on a spectrophotometer. The same approach was utilized to compare the test agents to a standard, Ascorbic Acid. The calibration curve was also made using a 1:50 dilution of 10 mM $NaNO_2$. Percentage inhibition of the extracts with respect to the negative control was computed by the formula; % inhibition = (Average of test agents)/ (Average of control) ×100

2.4.3. Total antioxidant capacity assay

Molybdenum (VI) reduction to Molybdenum (V) by the crude extracts/standard, followed by the production of a green phosphate/Molybdenum (V) complex at an acidic pH, is the basis for this approach (16). A solution of sodium phosphate (28 mM), sulphuric acid (0.6 M), and ammonium molybdate (4 mM) were prepared and 1 mL was pipetted into the extracts (0.1 mL) as well as the standard, ascorbic acid solution (20-100 µg/ml). The reacting mixture tubes were incubated for 90 minutes at 95 °C. The absorbance of the reacting mixture was quantified at 695 nm in contrast to a blank containing only distilled water. The antioxidant functions of the extracts were expressed as an ascorbic acid equivalent.

2.4.4. Ferric reducing antioxidant power (FRAP) assay

The concept behind this technique is to use electron-donating agents to reduce to a blue ferrous-coloured state from a colourless ferric-tripyridyltriazine complex in the presence of antioxidants (17). A reagent mixture of acetate buffer (300 mmol/L; pH 3.6), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (10 mmol/L), and $FeCl3.6H_2O$ (20 mmol/L) in a proportion of 10:1:1 was prepared and 1 mL was added to an aliquot (50 µL) of the extract (0.1 mg/mL) and Ascorbic acid solution (50 µL) with concentrations (20-100 µg/mL) and then mixed thoroughly for 10 minutes. The absorbance was quantified at 593 nm in contrast to a blank that contains reagent and distilled water (50 µL). All measurements were carried out at 30 °C, with no sunlight directed to the samples. The equivalent concentration (EC) of antioxidants that produces a ferric reducing capacity comparable to that of the ascorbic acid (standard) was used to calculate the reducing power.

2.4.5. Metal chelating ability assay

This was carried out by the procedures of Singh and Rajini (18) with few adjustments. 20 times dilution was done on 5 mM ferrozine solutions and 2 mM $FeCl_2.4H_2O$. 1 mL of an aliquot of various concentrations (5-0.15625 mg/mL) of the extract was combined with 1 mL of $FeCl_2.4H_2O$. The reaction began by adding 1 mL of ferrozine after 5 minutes of incubation. The combination was vigorously shaken, and the solution absorbance was quantified spectrophotometrically at 562 nm after another 10 minutes of incubation. The percentage inhibition of the production of ferrozine–Fe^{+2} complexes was estimated as % chelating effect = [(Ac-As)/Ac]× 100

Where Ac = Absorbance of control, As = Absorbance of samples tested.

2.4.6. Anti-lipid peroxidation assay

According to the method of Ohkowa *et al*. (19), the total quantity of products of lipid peroxidation contained in the extracts was evaluated by the thiobarbituric acid (TBA) approach, and this quantifies the malondialdehyde (MDA) reactive products. 0.5 mL of phosphate buffer (0.1 M, pH 8.0) and 24% TCA (0.5 mL) were added to 0.5 mL of samples. At room temperature, it was incubated for 10 minutes, and afterward, centrifugation of the mixture was done for 20 minutes at 2000 rpm. In the following, 0.33% TBARS (0.25 mL) in 20% acetic acid was added to the supernatant (1 mL), and the combination was heated at 95 °C for 60 minutes. As soon as it was cooled, the reacting product (pink color) was quantified at 532 nm (MDA Extinction Coefficient: ε532 =1.53x 10^5 $M^{-1}cm^{-1}$).

2.5. Brine shrimp lethality assay (BSLA)

This assay detects the presence of bioactive molecules in natural products. The experiment was carried out in accordance with Mclaughlin (20). Briefly, 0.1 g of brine shrimp eggs were hatched in natural seawater, which is equivalent to 3.8 g/L of saltwater. To ensure survival and maturity, the nauplii (larvae) were placed in seawater for 48 hours at 25 °C with steady exposure to air and lights. The plant extracts were prepared and diluted serially to attain 1000 to 1 µg/mL concentrations. 10 nauplii were pipetted out and added to the test solutions that were serially diluted. The test was performed three times. The negative control (a tube containing ten nauplii in seawater with no extract) and the positive control (cyclophosphamide [CTX]) were used as comparisons. After a 24-hour incubation period at 25 °C, the number of deaths was counted, and the percentage of mortality was computed. GraphPad Prism statistical software was used to obtain the 50 percent lethal concentration value (LC_{50}) and the standard error means (SEM).

2.6. MTT assay to determine the extracts' effect on cell proliferation

2.6.1. Cell culture

RD, MCF-7, and HeLa cells were gotten from WHO reference polio laboratory, University College Hospital, Ibadan, Nigeria. Eagle's minimum essential medium (EMEM) supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 units/mL), streptomycin (100 mg/mL), L-glutamine (2 mM), 0.07% NaHCO3, and 1% non-essential amino acids and vitamin solution were used for the cell culture. The cultures were sustained in a humidified condition with 5% CO_2 at 37 °C and split every two weeks.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of the selected medicinal plant methanol extracts.

Plant	TPC (GAE mg/mL)[a]	TFC (QUE mg/mL)[a]
Allophylus africanus	744.67±23.44	162.73±2.37
Baphia nitida	876.08±17.02	276.26±2.89
Chrysophyllum albidum	1097.54±11.67	216.45±1.23
Cocos nucifera	755.07±7.31	84.68±1.40
Combretum paniculatum	671.61±9.17	189.16±11.14
Cola verticillata	838.79±20.84	402.18±3.58
Musa sapientum	653.09±20.84	209.01±3.15
Pacrilima nitida	772.58±16.77	353.06±3.68
Parinari polyandra	743.15±24.37	95.11±3.33
Vernonia cinerea	659.59±7.92	48.95±0.18
Control	503.68±1.83	18.79±0.46

[a]:Each value in the table was obtained by calculating the average of three determinations ±standard error of mean (S.E.M).

2.6.2. Cytotoxicity assay

Using the methods described by Mosmann (21), the ability of the cells cleaving to MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Sigma, Chem), by the action of mitochondrial enzyme succinate dehydrogenase, was evaluated. The monolayers of the cells grown in 96 well-microtitre plates reached confluency in 24 hours. Pre-solubilization of each crude extract in dimethyl-sulphoxide (DMSO) at 37 °C and 10-fold dilutions were serially done to give concentrations of 1000 to 0.01 µg/mL. Incubation of cells with varying extracts concentration was then done at 37 °C in a CO_2 environment, CTX (positive control), and negative control (growth medium alone) in triplicate for 72 hours. Furthermore, the viability of the cell was evaluated microscopically for the cytopathic effect (CPE) if it is present or absent. When the 72 hours of treatment elapsed, the supernatants were taken out from the wells, and MTT solution (25 µL, 2 mg dissolved in 1 mL of PBS) was applied per well. To dissolve the formazan crystals, the plates were incubated at 37 °C for 2 hours before DMSO (75 µL) was applied per well. The microtiter plates were agitated for 15 min, and the optical density was quantified by a multi-well spectrophotometer (Multiskan, Thermo Fisher Scientific, Waltham, MA) at 492 nm. The extract concentration required to reduce cell viability by half was identified as the 50% cytotoxic concentration (CC_{50}). A non-linear regression curve included in the GraphPad Prism software was used to compute the CC_{50} value.

2.7. Statistical Analysis

All experiments were carried out in triplicates and data were presented as mean±standard error of the mean (SEM). Data were analysed using GraphPad Prism (6.0) and One-way ANOVA was used to determine the level of statistical significance and P values <0.05 were considered significant.

3. Results

3.1. Total Phenolic and Flavonoid content

A significant amount of phenolics and flavonoids were found in the 10 plant crude extracts (Table 2). Methanol extract of *C. verticillata* seeds recorded the highest flavonoid content (402.18±3.58 GAE mg/mL) while the values of phenolic content were the highest in methanol extract of *C. albidum* bark (1097.54±11.67 QUE mg/mL).

3.2. Antioxidant assays

The inhibitory concentration at fifty percent (IC_{50}) values on the effect of methanol extracts on DPPH scavenging activity showed that *C. verticillata* (IC_{50}=0.08±0.04 mg/mL) had the highest activity as compared to the standard, Ascorbic Acid (IC_{50}=0.079±0.007 mg/mL) as shown in Fig-

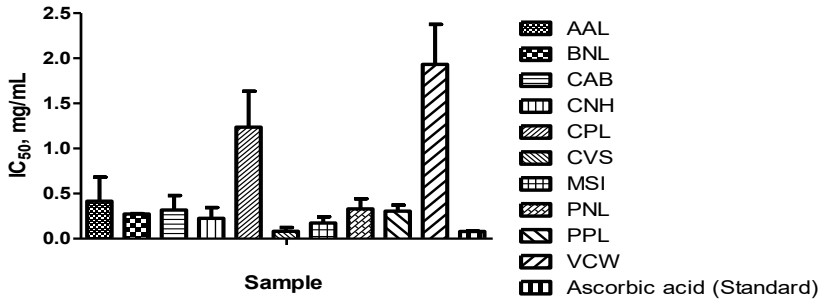

Figure 1. IC_{50} values of DPPH assay for free radical scavenging activity of methanol plant extracts.

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The selected medicinal plant extracts demonstrated a broad spectrum of nitric oxide scavenging properties (Figure 2). The methanol extract of *P. nitida* leaves (IC_{50}=29.91±0.51 µg/mL), *C. nucifera* (IC_{50}=34.52±0.78 µg/mL), and *C. albidum* (IC_{50}=38.77±0.67 µg/mL) demonstrated the most significant decrease in the nitric oxide radical contrasted to the standard, Ascorbic acid (IC_{50}=44.03±0.41 µg/mL).

Plant extracts reduced metal chelating ability in the range of 0.130±074-1.100±104 mg/mL in the metal chelating assay (Figure 3).

The reduction capacity of the extracts ranged between 46.57±0.16-103.48±1.78 mg AAE/g (Table 3) where *C. albidum* extract exhibited the highest total antioxidant capacity (TAC) with 103.48±1.78 mg AAE/g as compared to the control with the lowest TAC of 43.17±2.79 mg AAE/g.

As shown in Table 3, the highest Fe^{2+} quenching potential was reported in *C. albidum* extract with 920.19±6.74 mg AAE/g while *Musa sapientum* extract gave the least ferric reducing antioxidant power with a value of 434.08±79.65 mg AAE/g.

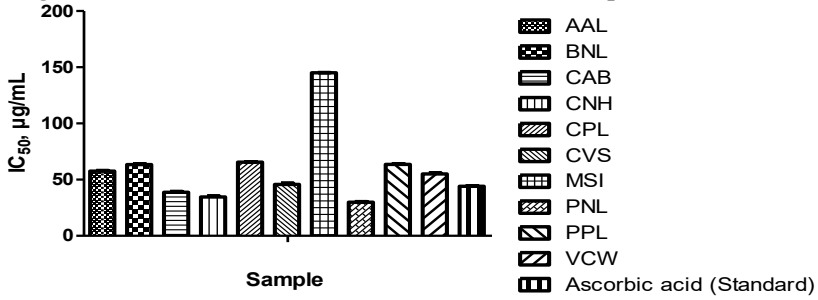

Figure 2. IC_{50} values of nitric oxide assay for free radical scavenging activity of methanol plant extracts.

The plant extracts showed a broad spectrum of lipid peroxidation inhibition, with a drop in MDA levels in the range of 0.2250-0.3010±003 mg/mL (Table 3).

3.3. Brine shrimp lethality effect

Table 4 shows that all of the ten plant extracts had variable levels of cytotoxicity as com-

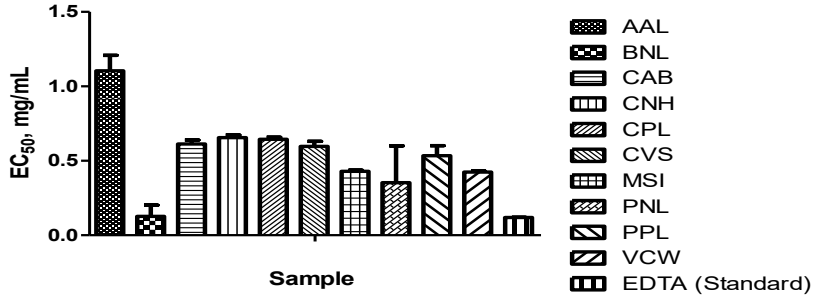

Figure 3. EC_{50} values of nitric oxide assay for free radical scavenging activity of methanol plant extracts.

Table 3. Antioxidant properties of the selected medicinal plant extracts

Plant	TAC (mg AAE/g)	FRAP (mg AAE/g)	Total MDA (mg/mL)
Allophyllus africanus	68.98±1.19	703.94±20.4	0.232±0.013
Baphia nitida	78.72±0.35	540.39±2.24	0.255±0.004
Chrysophyllum albidum	103.48±1.78	920.19±6.74	0.310±0.003
Cocos nucifera	71.10±2.03	713.27±11.92	0.293±0.019
Combretum paniculatum	67.66±2.29	516.15±13.13	0.253±0.014
Cola verticillata	64.63±0.92	818.39±21.68	0.269±0.007
Musa sapientum	46.57±0.16	434.08±79.65	0.255±0.004
Pacrilima nitida	60.87±1.31	721.11±14.86	0.273±0.012
Parinari polyandra	82.08±1.84	907.53±48.04	0.267±0.017
Vernonia cinerea	77.75±0.95	548.98±6.00	0.225±0.003
Control	43.17±2.79	241.17±5.6	0.161±0.002

[a]TAC: Total antioxidant capacity; FRAP: Ferric reducing antioxidant power; MDA: Malondialdehyde; [b]Each value in the table was obtained by calculating the average of three determinations ±standard error of mean (S.E.M).

pared with the standard CTX. The LC_{50} values of all the extracts were less than 1000 µg/mL, signifying the existence of bioactive molecules. When compared to the standard, CTX ((LC_{50}= 63.82 µg/mL), the *C. nucifera* husk-fibre extract exhibited the maximum cytotoxicity on the nauplii, with a lethal value of 107.3 µg/mL.

3.4. MTT assay

The result obtained showed that *P. nitida* extract had the highest cytotoxicity (CC_{50}=17.97 µg/mL) followed by *C. nucifera* extract (CC_{50}=59.05 µg/mL) on RD cell line as compared to CTX (standard) with CC_{50}=0.97 µg/mL. Similarly, the extract of *P. nitida* had the highest cytotoxicity (CC_{50}=38.79 µg/mL) followed by the extract of *C. nucifera* (CC_{50}=97.36 µg/mL) on MCF-7 cell line as contrasted to CTX (CC_{50}=4.01 µg/mL). On HeLa cell line, extract of *C. nucifera* had the highest cytotoxic activity (CC_{50}=24.16 µg/mL) followed by extract of *C. albidum* (CC_{50}=27.76 µg/mL) as compared to CTX

Table 4. Brine shrimp's lethal activity and *in vitro* cytotoxicity of methanol extract of selected plants on RD, MCF-7, and HeLa cells.

Plants	BSLA, LC50	MTT Assay, CC50 (µg/mL, n = 3)		
	(µg/mL, n=3)	RD	MCF-7	HeLa
Allophylus africanus	230.4	67.80	> 100	90.64
Baphia nitida	210.4	> 100	> 100	79.21
Chrysophyllum albidum	122.5#	77.71	98.03	27.76#
Cocos nucifera	107.3#	59.05	97.36	24.16#
Combretum paniculatum	183.7	> 100	> 100	92.22
Cola verticillata	127.3#	> 100	> 100	30.97
Musa sapientum	135.7	> 100	> 100	89.88
Pacrilima nitida	105.1#	17.97#	38.79#	28.20#
Parinari polyandra	131.6	> 100	> 100	> 100
Vernonia cinerea	136.5	> 100	> 100	> 100
Cyclophosphamide[*]	63.82	0.97	4.01	3.63

[a]RD- Human Rhabdomyosarcoma; MCF-7– Human breast cancer cells; HeLa- Cervical cancer cells. [b]MTT method, with the cells incubated with the crude extracts for 72 h. [*]Cyclophosphamide was used as the positive control. [c].#activity not statistically different significantly when compared with positive control Cyclophosphamide.

(CC_{50}=3.63 µg/mL) as presented in Table 4.

4. Discussion

4.1. Total phenolic and flavonoid content

It has been recognized that phenolics and flavonoids are the major groups of phytochemicals that exhibited most of the antioxidant properties in plants and they are found to have many other biological properties such as anticancer, antiarthritic, antiulcer, antimicrobial, antiangiogenic (22, 23). The significant amount of phenolics and flavonoids provide high medicinal activities on the extracts of the plants studied (Table 2).

4.2. Antioxidant assays

Oxidative stress plays an important role in mediating free radicals which have been linked with diseases such as atherosclerosis and cardiovascular complications, tumors (24). The addition of plants with high antioxidant properties would be of great need to scavenge free radicals when the primary defense systems of the body are weighed down by oxidative stress. The various antioxidant assays used in this study showed that the selected medicinal plant extracts demonstrated a broad spectrum of free radical scavenging activities. An assay method is not adequate for the assessment of the antioxidant potential of exogenous/endogenous molecules. Moreover, various assays differ in terms of the principle of the assay and experimental states (25). For instance, some assays use producers of organic radicals e.g. DPPH, while some utilize metal ions for oxidation e.g. FRAP. Atina *et al*. (26) also reported the need to quantify the antioxidant activity of samples using various assays in comparison because different methods did not give the same results.

DPPH radical scavenging assay revealed varying potent antioxidant effects of the plant extracts (Figure 1). Predicting antioxidant activities by DPPH assay has been greatly used since it requires a quite short time for evaluation. This indicated a connection between its activity and the level of the total flavonoid. The reason for antioxidants' action on DPPH could be attributed to their ability to donate hydrogen. Phenolics and flavonoids scavenge the DPPH radicals by their ability to donate hydrogen (27).

Nitric oxide is an oxidant as it reacts with reactive oxygen species (ROS) which is linked with cancer, inflammation, and other pathological states (28). From the results in Figure 2, the nitric oxide inhibitory effects of *P. nitida*, *C. nucifera*, and *C. albidum* were higher which is in correlation with the result of cytotoxic studies as shown in Table 4.

Metal ions in excess can cause a variety of problems in the body. The ability of plant extract to chelate iron (II) is significant since it has been implied that transition metal ions are greatly involved in oxidative damage in neurological illnesses including Parkinson's and Alzheimer's (29). It is proposed that plant extracts' iron (II) chelating properties are due to endogenous chelating molecules such as polyphenolic molecules which were evidenced in the result shown in Figure 3.

The high total antioxidant capacity of *C. albidum* may perhaps be due to the abundance of phenolic content in *C. albidum* as presented in Table 2. According to new research, flavonoids and related polyphenols play a significant role in medicinal plants' ability to reduce phosphomolybdate levels. Because of its hydrogen and electron-donating ability, the phosphomolybdenum test identifies antioxidants e.g., ascorbic acid, α-tocopherol, carotenoids, certain aromatic amines, cysteine, and phenolics (16).

FRAP assesses the extracts' reducing capability according to the methods of Benzie and Strain (17). As shown in Table 3, the highest Fe^{2+} quenching potential was reported in *C. albidum* extract with 920.19±6.74 mg AAE/g. The extract revealed a substantial link between total phenolic content and the FRAP, which supports the findings of Benzie and Szeto (30).

Oxidative degradation of lipids that involves the process of taking up electrons from lipids in cell membranes by the free radicals which result in cell damage is termed "Lipid Peroxidation" (31). The extracts' ability to inhibit the generation of thiobarbituric acid (TBA) *in vitro* in the lipid system is used in this study and the results of the study are shown in Table 3. Lipid peroxidation produces a variety of aldehyde compounds in biological systems, with MDA being the most important derivative. Lipid peroxidation's protective

effects differ which is dependent on the plant part, the quality, and the number of phytochemical components detected in the extracts (32), which could be the reason the extracts demonstrated different levels of MDA.

4.3. Brine shrimp lethality effect

Table 4 shows that all of the ten plant extracts had variable levels of cytotoxicity as compared with the standard (CTX). It serves as a pilot screen that may be followed up with a bioassay that is more detailed especially when the isolation of active molecules is carried out. Plants that have been discovered to be poisonous to brine shrimp are possible candidates for anti-cancer research (33).

4.4. MTT assay

This assay assesses the viability of cells and is sensitive, quantitative, and reproducible. In a variety of cell lines, the extent of formazan generated is related to the cell number directly (34). The American National Cancer Institute (NCI) defines crude extracts that are cytotoxic as a CC_{50}<30 µg/mL in a preliminary assessment after a 72-hour exposure interval (35). *C. nucifera* husk-fibre contains polyphenols, especially catechins which have been shown as its major constituents (36), and this could be connected to the cytotoxic activities of the extract. Similarly, molecules such as epicatechin, epigallocatechin, and procyanidin B5 have been isolated from *C. albidum* stem bark which could also be connected to its cytotoxic activity in this study (37). *P. nitida* seeds have been demonstrated to exhibit cytotoxic activities which could be the presence of the abundance of alkaloids (38), and this could also be found in the leaves which may be responsible for its cytotoxic activity in this investigation.

5. Conclusion

A range of analytical methods was used to ascertain the total phenolic and flavonoid levels in 10 crude extracts. The plant extracts were found to have a wide range of antioxidant and free radical scavenging properties in the various antioxidant assays employed in this investigation. There was a link between the antioxidant activities of some plant extracts and their total phenolic and flavonoid levels. However, some investigations have reported a poor direct link between antioxidant potentials and total phenolic and flavonoid levels with no definite justification. Three out of the ten extracts investigated in this study (*P. nitida*, *C. albidum*, and *C. nucifera*) showed the most significant cytotoxic activity. This lends credibility to the ethnopharmacological approach to select specific plant species for the bio-discovery of natural products. As a result, more research is needed to corroborate this, as well as to uncover novel natural molecules that are antioxidant and cytotoxic substances of interest. Because some of these therapeutic plants have yet to be investigated, studies evaluating their antioxidant and cytotoxic properties in an *in vivo* system should be conducted.

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Conflict of Interest

None declared.

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