https://dx.doi.org/10.4314/jpb.v19i3.6
Vol. 19 no. 3, pp. 167-180 (September 2022)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Antiproliferative, antioxidant and antiplasmodial activities of the root bark of *Adenodolichos paniculatus* (Hua) Hutch (Fabaceae)

Olalekan S. OJERINDE[1,2*], Yongle DU[2], David G.I. KINGSTON[2], Ana L. VALENCIANO[4], Maria B. CASSERA[4], Atiene S. SAGAY[3], Taiwo E. ALEMIKA[1]

[1]Department of Pharmaceutical and Medicinal Chemistry; [3]Department of Obstetrics and Gynaecology; University of Jos, Jos. Nigeria. [2]Department of Chemistry and Virginia Tech Center for Drug Discovery, M/C 0212, Virginia Tech, Blacksburg. U.S.A. [4]Department of Biochemistry and Molecular Biology, and Center for Tropical and Emerging Global Diseases (CTEGD), University of Georgia; Athens, GA 30602, U.S.A.

Received 11th April 2022; Accepted 3rd August 2022

Abstract
Adenodolichos paniculatus Hua & Hutch (Fabaceae) is a plant whose roots are traditionally employed for the treatment of non-communicable diseases such as diabetes and cancer. The powdered root bark of *Adenodolichos paniculatus* were extracted with dichloromethane followed by 70% methanol to afford dichloromethane (DCM) and hydromethanolic (HME) extracts respectively. Thereafter, fresh powdered root was extracted with water via decoction method and lyophilized to afford aqueous extract (AQE). The extracts were then subjected to standard phytochemical studies, antiproliferative (A2780 ovarian cancer cell assay), antiplasmodial (Dd2 strain of *Plasmodium falciparum*), antioxidant (DPPH, FRAP and ABTS methods) and brine shrimp lethality assay studies. The DCM extract was found to possess high levels of total phenolics and flavonoids with notable potential antiproliferative (IC_{50} = 0.14 µg/ml), antiplasmodial (IC_{50} = 7.50 µg/ml) and cytotoxic (brine shrimp, IC_{50} = 0.547 µg/ml) activities. However, HME had significant antioxidant (DPPH, IC_{50} = 17.54 ± 0.03 µg/ml; ABTS, IC_{50} = 8.08 ± 0.05 µg/ml). Both HME and AQE were found to be inactive against the drug-resistant Dd2 strain of *Plasmodium falciparum* with an IC_{50} value > 100µg/mL. The study revealed the potential of *Adenodolichos paniculatus* as a promising antiproliferative agent and also corroborated the ethnomedical uses of the plant.

Keywords: *Adenodolichos paniculatus;* Fabaceae; Phytochemicals; A2780; *Plasmodium falciparum;* Antioxidant.

INTRODUCTION

Cancer is a global health burden as well as a leading cause of death worldwide [1]. Cancer cell penetrates and initiates the destruction of close tissues and organs that could result to death [2]. Among the non-communicable diseases, cancer still remains a great menace to the public health. Its impact on the economy of both developed and developing countries cannot be overemphasized. Several billions of dollars have been invested in prevention, diagnosis and treatment of cancer by various organisations and pharmaceutical companies [3]. Thus, cancer is still a major cause of death globally. According to WHO report, 27 million

*Correspondence. *E-mail*: milomuanya@unilag.edu.ng *Tel*: +234-8033295077. ISSN 0189-8442

new cases and 17.1 million deaths per year had been projected by 2050 [4]. Ovarian cancer remains one of the major causes of death among gynecological malignancies with difficulty in treatment [5]. Therefore, the management of ovarian cancer with chemotherapy has not been successful due to several challenges. These include repeated administration of chemotherapeutic anticancer agents which has resulted in multiple drug resistance, side effects such as nausea, bone marrow failure and lastly poor diagnosis at early stage [2,5]. However, the development of cancer has been associated with oxidative stress which occurs as a result of imbalance between the reactive oxygen species (ROS) in the cell of an organism and its antioxidant capacity. This causes the modification of DNA which results in its mutations, leading to the development of chronic diseases such as cancer, autoimmune disorders, aging, cardiovascular and neurodegenerative diseases [6,7]. The intake of food such as vegetables fruits, grains and supplements that contain antioxidants has shown to reduce the chances of developing these diseases [8]. Antioxidant substances such as polyphenols, terpenoids, alkaloids, pigments, glycosides and steroids present in natural products have shown to demonstrate some defense and protective mechanisms against oxidative stress [7,8]. These natural phytochemicals have demonstrated efficacy against different types of cancer by interrupting with the different stages of carcinogenesis, thereby preventing the development of cancer [9]. Meanwhile, over the five decades, scientists have crystallised their attentions on natural products searching for effective and potent anticancer substances with fewer side effects and better tolerance [10]. Therefore, plants remain to be a reservoir of highly pharmacological active phytochemicals for the treatment of various diseases [11].

Adenodolichos paniculatus (Hua) Hutch, locally known as Kwiwaa, wáákén wuta in Hausa or “fire bean” in English, is a shrub belonging to the family Fabaceae/Leguminosae and it widely distributed in Benin, Ghana, Northern Nigeria, Cameroon, Sudan, Guinea and Democrat Republic of Congo. Out of the 22 species of this genus widely distributed in tropical Africa, 15 species have been reported in Democratic Republic of Congo while *Adenodolichos paniculatus* is found only in Northern Nigeria. In folk medicine, the leaves are used for treating wounds, toothache, heartburn. The roots are used for the treatment of dysentery, liver problems while the stems are employed for the treatment of diarrhea and blennorrhoea [12]. The methanolic extract of leaf showed significant analgesic and anti-inflammatory activities [13]. Stigmasterol, β-sitosterol and nonanoic acid were isolated from the leaves [12,14] while some fatty acids were identified from the root with antibacterial activity [15].

However, there has been no report or previous work being published on the antioxidant and anticancer activities of the root bark of *Adenodolichos paniculatus*. Therefore, the objectives of this study were to carry out the antioxidant and anticancer activities of the root bark of *Adenodolichos paniculatus*.

EXPERIMENTAL METHODS

Plant collection and identification. The roots of *Adenodolichos paniculatus* were collected from mature shrubs in bushes around Binchi village, Bassa Local Government, Jos, Plateau, Nigeria between September and November, 2017. The plant was identified and authenticated by Mallam Umar S. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria with a voucher specimen number (ABU 3107) and likewise confirmed with the Herbarium Section of Forestry Research Institute of Nigeria (FRIN) with a voucher specimen FHI 0045485-0. The plant name was also checked against http://www.theplantlist.org. an extensive source of medicinal plants for

confirmation. Herbarium specimens of the plant was prepared and deposited at the Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria. The material was air-dried to a constant weight chopped to small pieces and ground to coarse powder. The dried powdered root bark was stored in cool, dry conditions.

Extraction. The dried root barks of *Adenodolichos paniculatus* (2.5 kg) were powdered and extracted twice with dichloromethane (DCM) (10L X 2) followed by 70% methanol (70% MeOH) (10L X 2) at room temperature for three and seven days respectively. Again, another powdered portions of the dried powdered roots (1.0 kg) was extracted by decoction, this time by boiling in water for 20 minutes. and then left standing for 24 hours. The decoction method is employed to simulate the method of application employed in traditional medical practice. The extracts (DCM and 70% MeOH) were filtered using whatman filter paper No. 1 and the filtrates were concentrated using rotary evaporator under reduced pressure to afford dichloromethane (DCM) and hydromethanolic (HME) extracts. Meanwhile, the aqueous extract was filtered using whatman filter paper No. 1 and the filtrate was lyophilised using FC-1-50 freeze dryer (Shanghai Bilon Instruments Ltd, China) to obtain lyophilised aqueous extract (AQE). The DCM, HME and AQE extracts were kept at –20^{o}C until further use.

Phytochemical screening. All the extracts were subjected to different phytochemical tests as described by Evans, 2009 [16] and Harborne, 1998 [17] to identify the following phytochemicals, Flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, steroids, glycosides and carbohydrates.

Determination of Total Phenolic Content (TPC). The total phenolic content of the various extracts of *Adenodolichos paniculatus* was evaluated by a colorimetric method utilizing Folin-Ciocalteu reagent according to the method previously reported [18]. The phenolic concentration of extract was evaluated from a gallic acid calibration curve. A volume equivalent to 500 µL aliquots of 10, 20, 30, 40, 50, and 60 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The tubes were vortexed for 10 s and allowed to stand for 2 hr at 25^{o}C. After incubation at 25 °C for 2 hr, absorbance of each reaction mixture was measured at 765 nm against reagent blank using the Shimadzu UV-Vis Spectrophotometer 1650 (Japan). A similar procedure was adopted for the extract as described above in the preparation of calibration curve. All determinations were performed in triplicate. The total phenolic content was expressed as mg gallic acid equivalents (mg GAE/g) of extract.

Determination of Total Flavonoid Content (TFC). The total flavonoid content of the extracts was measured by employing aluminium chloride colorimetric assay earlier reported [18]. An aliquot (1 mL) of extract (40 mg) or rutin standard solution with the following concentrations (10, 20, 40, 60, 80 & 100 µg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. To the flask, 300 µL of 5% $NaNO_2$ and 300 µL of 10% $AlCl_3$ were added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was brought to 10 mL by the addition of 2.4 mL H_2O. The solution was vortexed in order to mix the mixture thoroughly and the absorbance was measured at 510 nm against reagent blank using the UV-Vis Spectrophotometer 1650 Shimadzu, Japan. The total flavonoid contents of the extracts were expressed as rutin equivalents (mg RE)/g of extracts. All treatments were carried out in triplicate.

Antioxidant Assays

DPPH free radical scavenging activity. The antioxidant activity (free radical scavenging activity) of extract on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was

determined according to the method previously reported [18]. Twenty-five (25) mg of the extract was dissolved in methanol using 50 mL volumetric flask. The following concentrations of the extract were prepared 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95, and 0.98 µg/mL. All the solutions were prepared with methanol as solvent. A quantity (2 mL) of each prepared concentration was mixed with 4 mL of 50 µM DPPH solution in methanol. Experiment was done in triplicate. The mixture was vortexed for 10 s to homogenise the mixture and test tubes were incubated for 30 min at room temperature in the dark, after 30 min of incubation the absorbance was measured at 515 nm on a UV-Vis spectrophotometer (Shimadzu, UV-1620PC, Japan). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Gallic acid, vitamin C and rutin were used as standards with the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195 µM. Blank solution was prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH solution. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + extract/standard. Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of the mean of the antioxidant activity against concentration of the test extract (µg/mL).

Ferric Reducing Antioxidant Power (FRAP) assay. The ferric reducing property of the extracts was determined by using the assay earlier reported [19,20]. Briefly, 3.8mL of a freshly prepared solution of FRAP reagent {mixture solutions: 300mM acetate buffer, pH 3.6, 10 mM TPTZ in HCl (40 mM) and $FeCl_3$ (20 mM) at a ratio of 10:1:1 at 37 ^oC} was added to 200 µL of the extracts with different concentrations (0.98 – 500 µg/mL). The mixture was vortexed and incubated for 30 min at room temperature in the dark. After the incubation, the absorbance of the coloured product (ferrous tripyridyl triazine complex) was read at 595 nm using UV-Spectrophotometer (Shimadzu, Japan). Results are expressed in µMFe II/g of dry mass which were obtained from the standard curve prepared and EC_{50} was evaluated.

ABTS-free radical scavenging activity. The ABTS radical scavenging activity of the extracts was determined according to the method previously reported [21-23]. The ABTS assay assesses the total radical scavenging capacity based on the ability of an antioxidant to scavenge the stable ABTS radical ($ABTS^{\cdot+}$), which was produced by mixing 1.0 mL ABTS stock solution (7 mM) with 1.0 mL of potassium persulfate (2.45 mM) and allowing to stand in the dark at room temperature for 16-20 h before use. The solution was then diluted by mixing 1 mL $ABTS^{\cdot+}$ solution with 60 mL methanol to obtain an absorbance of 0.700 ± 0.000 units at 734 nm using the UV-spectrophotometer. $ABTS^{\cdot+}$ solution was freshly prepared for each assay. Plant extract (1 mL) at different concentrations (0.98 – 500 µg/mL) were allowed to react with 1 mL of the $ABTS^{\cdot+}$ solution. The mixture was incubated for 60 min at room temperature in the dark. After the incubation, the absorbance was taken at 734 nm using UV-spectrophotometer. The $ABTS^{\cdot+}$ scavenging capacity of the extract was compared with that of Vitamin C and percentage inhibition calculated as

$$\text{ABTS radical scavenging activity \%.} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance

of ABTS radical + extract/standard. ABTS radical scavenging activity of extracts was determined by IC_{50} value as stated above in DPPH assay.

Cytotoxicity assays

Brine shrimp test. Brine shrimp cytotoxicity assay is widely used in the bioassay for the bioactive compounds. The assay was carried out according to the principle and protocol previously reported [24,25]. The cytotoxicity screening of the extract was carried against a simple zoological organism, brine shrimp nauplii. *Artemia salina* leaches (brine shrimp eggs) was placed in a small tank containing 3.8% noniodized NaCl solution (sea water) for two days to hatch the shrimp and to be matured as nauplii. Forty (40.0) mg of the extract was dissolved in 2000 µL of DMSO (dimethyl sulfoxide). Then 100 µL of solution was taken into a test tube containing 5 mL of sea water and 10 shrimp nauplii. Thus, final concentration of the first test tube solution was 400 µg/mL. Then a series of solutions of varying concentrations was prepared from the stock solution by serial dilution. Thus, the concentrations of the obtained solution in each test tube were 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 µg/mL for 10 dilutions. One (1.0) mg of methotrexate (served as the positive control) was dissolved in distilled water to get an initial concentration of 100 µg/mL from which serial dilutions was made using sea water prepared to get 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1563 and 0.0781 µg/mL. The positive control solutions contained 10 living brine shrimp nauplii in 5 ml sea water. For negative control, 100 µl of DMSO was added to each of the premarked test tubes containing 5 ml of sea water and 10 shrimp nauplii. The vials were maintained under illumination. Survivors were counted after 24 h and the percentage mortality at each vial and control was determined using the equation:

% mortality = (no. of dead nauplii/ initial no. of live nauplli) x 100.

The concentration-mortality data was analyzed statistically by using probit analysis for the determination of LC_{50} values and linear regression for the extracts (25) and LC_{50} values less than 100 ppm (or 100 µg/mL) was considered significant and toxic.

In vitro antiproliferative assay against A2780 ovarian cancer cell line. The A2780 cell line is a drug-sensitive ovarian cancer cell line. The A2780 ovarian cancer cell line antiproliferative bioassay was performed at Virginia Polytechnic Institute and State University as previously reported [26,27]. Briefly, A2780 human ovarian cancer cell were grown to 95% confluency and harvested and resuspended in growth medium (RPMI-1640 supplemented with 10% fetal bovine serum and 2 mML-glutamine). Cells were counted using a hemacytometer and a solution containing 2.5 X 10^5 cells/mLwas prepared in growth media. Eleven columns of a 96 well microtiter plate was seeded with 199 µL of cell suspension per well, and the remaining column contained media only (100% inhibition control). The plate was incubated for 3 h at 37ºC/5% CO_2 to allow the cells to adhere to the wells. Following this incubation, extracts, prepared in DMSO, were added to the wells in an appropriate series of concentrations, 1 µL per well. One column of wells was left with no inhibitor (0% inhibition control), and four dilutions of paclitaxel was included as a positive control. The plate was incubated for 2 days at 37ºC/5% CO_2, then the media gently shaken from the wells and replaced with reaction media (supplemented growth medium containing 1% alamar Blue), and incubated for another 3 h. The level of alamar Blue converted to a fluorescent compound by living cells was then analyzed using a Cytofluor Series 4000 plate reader (Perseptive Biosystems) with an excitation wavelength of 530 nm, an emission wavelength of 590 nm, and gain of 45. The percent inhibition of cell growth was calculated using the 0% and 100% controls present on the plate, and an IC_{50} value

(concentration of cytotoxic agent which produces 50% inhibition) was calculated using a linear extrapolation of the data which lie either side of the 50% inhibition level. Samples were analyzed in triplicate on at least two separate occasions to produce a reliable IC_{50} value.

Antiplasmodial assay. The effect of each extract on parasitic growth of the P. falciparum Dd2 strain was measured in a 72 h growth assay in the presence of the drug as described previously [28]. Briefly, ring stage parasite cultures (100 µL per well, with 1% hematocrit and 1% parasitemia) were grown for 72 h in the presence of increasing concentrations of the extracts in a 5% CO_2, 5% O_2, and 90% N_2 gas mixture at 37 °C. After 72 h in culture, parasite growth was determined by DNA quantitation using SYBR Green I. The half-maximum inhibitory concentration (IC_{50}) calculation was performed with GraphPad Prism 6 software (GraphPad Software, Inc.).

Statistical analysis. All experiments were performed in triplicate and values were expressed as mean values with a standard error of mean (SEM). Statistical analysis was conducted using a one-way ANOVA. Nonlinear regression test was used to determine the IC_{50}. Correlations between the antioxidant activity and total phenolic and total flavonoid contents were examined using Pearson's correlation. Statistical analysis was performed using GraphPad Prism 6 software. Results were considered to be statistically significant at $P < 0.05$.

RESULTS

Extraction yields. After complete drying of the extracts, the extracts yields were calculated. It was observed that the extraction of *Adenodolichos paniculatus* with 70% MeOH resulted in the highest amount of total extractable compounds whereas the yield with dichloromethane was small in comparison to other solvents employed as shown on Table 1.

Phytochemical screening. The preliminary phytochemical screening of *Adenodolichos paniculatus* showed the presence of various secondary metabolites in all the three extracts, summarized on Table 2. The dichloromethane (DCM), hydromethanolic (HME) and aqueous (AQE) extracts showed the presence of flavonoids, terpenoids, steroids and cardiac glycosides. Saponins, tannins and anthraquinones are present only in HME and AQE while alkaloids are absent in all the extracts.

Determination of Total Phenolic and Flavonoid Contents. The TPC and TFC of extracts are summarized on Table 3. In this study, the total phenolic content (TPC) and total flavonoid content (TFC) in the extracts from *Adenodolichos paniculatus* were determined from the calibration curves of gallic acid ($y = 0.0069x + 0.0673$, $R^2 = 0.9947$) Fig. 1 and rutin ($y = 0.0008x + 0.0549$, $R^2 = 0.9905$), Fig. 2 respectively. Total phenolic contents of the extracts were in the range of 65.76 – 85.60 mg GAE/g of extract while flavonoid content ranged from 15.43 – 76.01 mg RE/g of extract. DCM extract had the highest phenolic (85.60 ± 3.15 mg GAE/g) and flavonoid contents (76.01 ± 0.79 mg RE/g). Hence, the potent antiproliferative activity of DCM may be due to the high levels of phenolic and flavonoid contents.

Determination of antioxidant properties

DPPH radical scavenging assay. The DPPH radical scavenging activities of the extracts were evaluated using IC_{50} as the parameter. The lower the IC_{50} (IC_{50} is the concentration of antioxidant required to scavenge 50% of DPPH radicals), the higher the antioxidant activity. As shown on Table 3, the three extracts showed significant DPPH radical scavenging ability. HME has the highest antioxidant activity (IC_{50} = 17.54 ± 0.03 µg/mL) while the lowest antioxidant activity was shown by the DCM (IC_{50} = 45.48 ± 1.50 µg/mL). The differences in the three extracts

could be due to the presence of different levels of phenolic compounds [29].

ABTS-free radical scavenging activity. As shown on Table 3, HME demonstrated significant antioxidant activity (IC_{50} = 8.80 ± 0.05 μg/mL) followed by the DCM (IC_{50} = 9.86 ± 0.01 μg/mL) whereas AQE had the lowest activity (IC_{50} = 23.99 ± 1.25 μg/mL). The activity was dose-dependent, increase in the concentration resulted in higher antioxidant activity.

Ferric Reducing Antioxidant Power (FRAP). As shown on Table 3, the data show that the extracts have the capability to reduce Fe^{3+} in a concentration-dependent manner. FRAP was calculated from calibration graph which was linear over the calibration range with R^2 value of 0.9905 (Fig. 3) whereas the EC_{50} was calculated from the absorbance. DCM demonstrated most ferric ion reducing antioxidant power with EC_{50} value of 153.10 ± 3.10 μg/mL. All the results were comparable to an Ascorbic acid standard that has EC_{50} value of 50.07 ± 2.43 μg/mL. The presence of high phenolic compounds also contributes to high FRAP activity.

Correlation between Total Phenolic Content, Total Flavonoid Content and antioxidant activities. The total phenolic and total flavonoid contents of the extracts were correlated with their antioxidant capacities to determine the strength of a linear association between antioxidant activity (DPPH, $ABTS^+$ radical scavenging activity and the ferric reducing antioxidant power) and total phenolic and flavonoid contents in all tested extracts, as summarized on Table 4. The results showed a strong correlation between total flavonoid contents and $ABTS^+$ radical scavenging activity (R^2 = 0.9452). In contrast, total phenolic contents had a low correlation with $ABTS^+$ radical scavenging activity (R^2 = 0.2976), this suggests that other classes of compound may be responsible for its activity. However, a positive correlation of the TFC and $ABTS^+$ radical scavenging activity suggests that flavonoid plays a major role in the $ABTS^+$ radical scavenging activity of the extracts. In addition, a positive correlation was observed between total phenolic content and DPPH and FRAP. This finding shows that polyphenolic compounds contribute immensely to the antioxidant activity of *Adenodolichos paniculatus* extracts.

Cytotoxic assays

Brine shrimp lethality test. All the extracts showed significant lethality against brine shrimp. The LC_{50} values were ranged from 0.547-0.762 μg/mL at 24 h (Table 5). The negative control, DMSO did not have any effect on brine shrimp lethality. DCM extract showed the most lethality against brine shrimp with the LC_{50} value of 0.547 μg/mL.

Antiproliferative assay. Based on the preliminary significant activity observed against the brine shrimp larvae, the *in vitro* antiproliferative of the three extracts was evaluated at different concentration against A2780 ovarian cancer cell line after 48 h treatment. Paclitaxel was used as positive control. Significant antiproliferative activity of the extracts was observed at IC_{50} values of 0.14, 11.0 and 20.0 μg/mL for DCM, HME and AQE respectively. The significant antiproliferative activity of DCM extract further corroborates the cytotoxic activity of the DCM extract against Brine shrimp (Table 5).

Table 1: Extraction yield of *Adenodolichos paniculatus* root bark extracts

Extract	Extraction yield (mg/g)	Yield (%)	Appearance
DCM	17.12	1.71	Light brown
HME	114.04	11.4	Dark brown
AQE	33.12	3.31	Dark brown

Table 2: Phytochemical screening of *Adenodolichos paniculatus* root bark extracts

Constituents	Test	Dichloromethane	70% MeOH	Aqueous
Alkaloids	Dragendorff's	-	-	-
Alkaloids	Wagner's	-	-	-
Anthraquinones	Bontrager's	-	+	+
Cardiac glycoside	Keller-Killiani	+	+	+
Steroids	Salkowski	+	+	+
Terpenoids	Liebermann-Burchard	+	+	+
Saponins	Froth	-	+	+
Tannins	Ferric chloride	-	+	+
Flavonoids	Lead acetate	+	+	+

+, Present; -, Absence

Table 3: Total Phenolic content, total flavonoid content and antioxidant activities of the extracts of *Adenodolichos paniculatus* root bark

Sample	Total Phenolic Content (mg GAE/g)[a]	Total Flavonoid Content (mg RE/g)[b]	IC_{50}(µg/mL) DPPH	IC_{50}(µg/mL) ABTS	EC_{50}(µg/mL) FRAP
DCM	85.60 ± 3.15	76.01 ± 0.79	45.48 ± 2.15*	9.86 ± 0.01*	153.10 ± 3.10*
HME	65.76 ± 0.75	15.43 ± 3.67	17.54 ± 0.03	8.08 ± 0.05	191.40 ± 2.67
AQE	84.80 ± 2.08	32.88 ± 1.41	42.69 ± 1.50*	23.99 ± 1.25	184.50 ± 3.29*
Ascorbic acid	ND	ND	2.87 ± 0.15	15.38 ± 0.45	50.07 ± 2.43
Rutin	ND	ND	27.72 ± 0.11	ND	ND
Gallic acid	ND	ND	4.57 ± 0.01	ND	ND

a = Total Phenolic Content was analysed as the gallic acid equivalent (GAE) mg/g of the extract; values are the average of triplicates; b = Total Flavonoid Content was analysed as the rutin equivalent (RE) mg/g of the extract; values are the average of triplicates; Each value represents the Mean ± SEM, n = 3, *significantly different from standard antioxidant at $P < 0.05$. ND Not Detected

Table 4: Pearson's correlation coefficients between phenolic contents (TPC, TFC) and antioxidant activity (DPPH, FRAP and ABTS) of three extracts of *Adenodolichos paniculatus* root barks

Factor[1)]	TPC	TFC	FRAP	DPPH	ABTS
TPC	1.000				
TFC	0.5174	1.000			
FRAP	0.9054*	0.2688	1.000		
DPPH	0.6481*	0.0637	0.8732*	1.000	
ABTS	0.2976	0.9452*	0.0951	0.0005	1.000

[1)] TPC: total phenolic content, TFC: total flavonoid content, FRAP: ferric reducing antioxidant powers, DPPH: DPPH radical scavenging activity, ABTS: ABTS radical scavenging activity, * correlation significant at 0.05 level (2 tailed).

Table 5: Antiproliferative, Brine Shrimp lethality and antiplasmodial activities of the extracts of *Adenodolichos paniculatus* root bark.

Sample	Antiproliferative (IC_{50}, µg/mL) A2780[b]	Antiplasmodial (IC_{50}, µg/mL) *P. falciparum* Dd2[e]	Cytotoxicity (LC_{50}, µg/mL) Brine shrimp
DCM	0.14	7.50	0.547 ± 0.001
HME	11.0	> 100	0.762 ± 0.011
AQE	20.0	> 100	0.678 ± 0.021
Paclitaxel[a]	0.013µM	NT	NT
Methotrexate[c]	NT	NT	0.038 ± 0.015
Artemisinin[d]	NT	6.73mM(0.0067µM)	NT

[a] Paclitaxel was used as positive control for antiproliferative; b A2780 ovarian cancer cell line used; c Methotrexate positive control for the (cytotoxic) Brine shrimp lethality test d Positive control for the antiplasmodial; e *P. falciparum Dd2* Malaria parasite used; NT = Not tested.

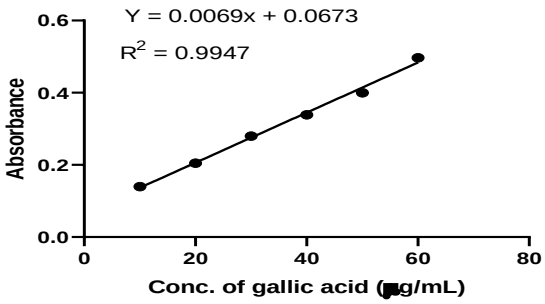

Figure 1. Calibration Curve of Gallic Acid (Each point represents the mean of three experiments)

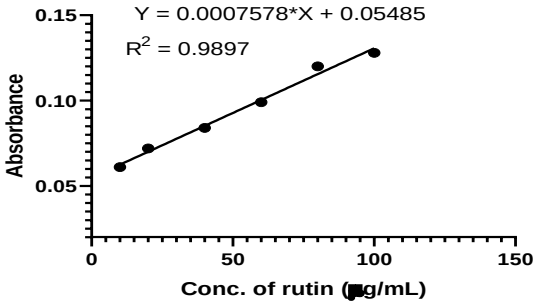

Figure 2. Calibration Curve of Rutin (Each point represents the mean of three experiments)

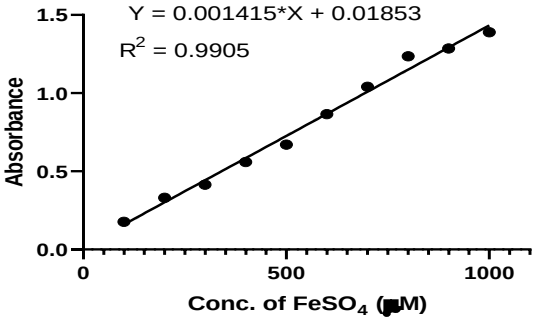

Figure 3. Calibration Curve of $FeSO_4$ (Each point represents the mean of three experiments)

In vitro antiplasmodial activity. As shown on Table 5, the *in vitro* antiplasmodial activity showed that the DCM extract had significant antiplasmodial activity against *Plasmodium falciparum* Dd2 strain with IC_{50} value of 7.50 µg/mL while the HME and AQE extracts had IC_{50} values > 100 µg/mL which shows that both HME and AQE extracts are inactive against *P. faciparum* Dd2 strain.

DISCUSSION

Medicinal plants still remain a major reservoir of pharmacological phyto-constituents for the treatment of various diseases. Fabaceae (Leguminosae) as a family is generally known for its various biological activities because of the presence of the potent secondary metabolites. They are majorly identified with flavonoids and other phenolic compounds with different biological activities. Phenolic compounds such as tannins and flavonoids among others are known for their strong antioxidant, antidiabetic, anticancer and anti-inflammatory activities [30]. The phytochemical analyses of the root bark extracts of *Adenodolichos paniculatus* revealed the presence of flavonoids,

terpenoids, anthraquinones, saponins, tannins and steroids. These phytochemicals are generally known for their medicinal importance. For instance, anthraquinones and their derivatives derived from plants are known to have laxative [31], anticancer [32], anti-inflammatory, antiarthritic, antifungal, antibacterial and antiviral [33]. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal activities [22]. Cardiac glycosides derived from plants are known to have anticancer properties especially ovary cancer and leukemia [34], they have been used to treat congestive heart failure and cardiac arrhythmia [22]. Terpenoids, according to research, they possess anticancer, antibacterial, antiviral, antihyperglycaemic, anti-inflammatory, antiparasitic and antioxidant activities [35]. Besides, flavonoids, one of the important phenolic compounds, have been known to possess anticholinesterase, anti-inflammatory, radical scavenging antioxidant, anticancer, antibacterial, antiviral activities among many others [36]. Saponins derived from plants are also known to have anticancer, antifungal, anthelmintic, antidiabetic, antibacterial, antiviral, anti-inflammatory and antioxidant activities among many others [37]. Other phytochemicals called tannins, commonly referred to as tannic acid, are water-soluble polyphenols that are available in many plant extracts. They are known to possess antimicrobial, antiviral, anticancer, antihelmintic and antioxidant activities [38]. These phytochemical compounds identified in the root bark extracts may be responsible for the biological activities demonstrated by *Adenodolichos paniculatus* and the reason for their use as a traditional medicine by the natives of North Central, Nigeria for the treatment of non-communicable diseases.

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [39]. The phenolic compounds from natural products contribute immensely to the antioxidative and other properties of plants and have received considerable attention due to their potential activities [39]. Phenolic compounds from plants are also known to be good natural antioxidant due to the presence of an aromatic benzene ring with substituted hydroxyl groups, including their functional derivatives. These are able to absorb free radicals and likewise chelate metal ions that could catalyze formation of reactive oxygen species which promotes lipid peroxidation [22]. Among polyphenols, flavonoids are of great importance because they help the human body to fight against diseases. The results show that the DCM possesses the highest phenolic and flavonoid contents followed by the AQE. This implies that the majority of the flavonoids from the root bark was extracted with the dichloromethane during the extraction. Furthermore, correlation between the antioxidant activity and total phenolic content and total flavonoid content were evaluated using Pearson's correlation and these results showed consistency with previous studies that a positive correlation existed between total phenolic, total flavonoid contents and antioxidant activities of extracts [40]. The high levels of phenolic and flavonoid in the extract may be responsible for the antiproliferative, Brine Shrimp lethality and antiplasmodial activity showed by the DCM. To the best our knowledge, this is the first report that described the total phenolic content and flavonoid content of the extracts of this plant.

Oxidative stress caused by free radicals has been implicated in many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [41]. Moreover, scientific studies have suggested that antioxidant compounds from natural sources, such as fruits and vegetables, have higher bioavailability and lower side effects

than the synthetic antioxidant agents [42]. However, antioxidants prevent free radicals from doing harm to human DNA, proteins, and cells by donating electrons to stabilize and neutralize the harmful effects of the free radicals [41].

The antioxidant ability and radical scavenging properties of plants are associated with its medicinal values. With respect to this, the antioxidant activity of *Adenodolichos paniculatus* root bark extracts was assessed for their free radical scavenging activity using DPPH radical scavenging assay, FRAP assay and ABTS assay methods.

The stable DPPH was reduced by all the extracts and thus changing the colour from purple to yellow to varying degree depending on the presence of antioxidant compounds. The degree of discolouration indicates the scavenging potential of the extract. HME has the highest radical scavenging activity followed by AQE. It is observed that the HME, extracted with 70% MeOH has the highest activity due to the polarity of solvent used. It has been reported that antioxidant activity of extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential. Aqueous methanol and 100% methanol have been reported to extract several active compounds from plant with significant activities [41]. So, the difference in the DPPH radical scavenging activity of the extracts was due to the extracting solvents used which significantly affected their radical scavenging potency. ABTS assay of the extracts also corroborated the earlier results from DPPH radical scavenging assay. In the ABTS test, both HME and DCM were potential candidates to control free radical formation. HME showed significant radical scavenging activity of the ABTS radicals. Furthermore, the reducing properties of extracts have been shown to exert their antioxidant action by donating a hydrogen atom to break the free radical chain [41]. The antioxidants present in the extracts (HME, DCM & AQE) also caused the reduction of Fe^{3+} - TPTZ complex to the ferrous form, and thus proved their reducing power. The ferric reducing power activity of the extracts was claimed to be due to the presence of polyphenols which serves as a significant indicator of its potential antioxidant activity [43]. The DCM showed a significant reducing property which makes the plant a good candidate as an antioxidant agent. Antioxidant activity of plant extracts has been known to play a significant role in the treatment of various diseases [44].

Brine shrimp lethality assay has been employed routinely in the primary screening of crude extracts for their potential cytotoxic effects against Brine shrimp, which also gives an indicative of possible toxicity of the test sample [45]. A number of novel anticancer agents have been isolated from plants using this bioassay and this happens because the bioassay has a good correlation with cytotoxic activity in some human solid tumour [46,47]. In the present study, the extracts showed significant cytotoxicity against the brine shrimp nauplii with respect to the control used, methotrexate (an anticancer agent). The presence of cytotoxic substances (anthraquinones, flavonoids, tannins, saponins and terpenoids) in the extracts may be responsible for such activity. Therefore, this significant lethality of the extracts of *Adenodolichos paniculatus* (LC_{50} values less than 100 μg/mL) to brine shrimp nauplii is an indicative of the presence of potent cytotoxic compounds which require further investigations.

Furthermore, in vitro antiproliferative activity of the extracts was evaluated using A2780 ovarian cancer cell line. DCM demonstrated the highest inhibition of the proliferation of the ovarian cancer cell when compared with standard used, paclitaxel, followed by the HME and AQE has the least activity. Antiproliferative activity has been

reported in the various genera of Fabaceae due to the presence of secondary metabolites such as alkaloids, flavonoids, triterpenoids and anthraquinones [48]. The biological potentials of this plant cannot be unconnected to the presence of these phytoconstituents in the extracts, especially in the DCM. To the best of our knowledge, this is the first report of in vitro antiproliferative activity described for this plant.

Malaria still remains one of the parasitic infectious diseases that has claimed lives from tropical and subtropical countries [49]. Several genera from Fabaceae family have been reported for their significant antiplasmodial activity due to the presence of phenolic compounds, anthraquinones and flavonoids [50-53]. In the present study, another genus from Fabaceae that showed promising antiplasmodial activity was *Adenodolichos paniculatus*. To the best of our knowledge, this is the first report of in vitro antiplasmodial activity described for this plant. Among the three extracts tested for in vitro antiplasmodial activity, HME and AQE were considered inactive (with $IC_{50} > 50$ μg/ml) while DCM showed a promising activity ($5 < IC_{50} \leq 10$ μg/ml) according to Bouzidi et al. [54] classification.

Conclusion. The preliminary study on the root bark extracts of *Adenodolichos paniculatus* revealed the biological potentials of the extracts, especially the DCM, for their antiproliferative, antioxidant and antiplasmodial activities. The phytochemicals present in dichloromethane of *Adenodolichos paniculatus* may be responsible for its antiproliferative, antioxidant and antiplasmodial activities. The high level of phenolic and flavonoid contents in DCM extract demonstrated its potential cytotoxic effect against the A2780 ovarian cancer cell by inhibiting its proliferation. These results also corroborated the ethnomedical uses of the roots of this plant for the treatment of non-communicable diseases. Further investigations of these phytochemicals in *Adenodolichos paniculatus* are recommended for their bioactivity guided fractionation, isolation and structural identifications, which may lead to new antiproliferative compounds with lower toxicity and high potency.

Acknowledgements

Research reported in this publication was supported by the Fogarty International Center (FIC); the NIH Common Fund, Office of Strategic Coordination, Office of the Director (OD/OSC/CF/NIH); Office of AIDS Research, Office of the Director (OAR/OD); National Institute of Neurological Disorders and Stroke, Office of the Director (NINDS/NIH); and the National Institute of Nursing Research (NINR/NIH) of the National Institutes of Health under Award Numbers D43TW010130. The content is solely the responsibility of the authors and does not necessarily represent the views of the National Institutes of Health.

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