Phytochemical and antioxidant studies on the stem bark of *Pseudocedrela kotschyi* (Meliaceae)

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Abstract

Pseudocedrela kotschyi commonly known as Dry-zone cedar and hard cedar-mahogany belongs to the family of Meliaceae. It is widely spread from east Senegal to western Ethiopia, Uganda and Nigeria. The stem bark of the plant has been used for the treatment of numerous diseases among different cultures around the globe. The study aimed at evaluating the phytochemical constituents and the antioxidant effects of *P. kotschyi* stem bark as claimed traditionally. Qualitative and quantitative Phytochemical screening were done according to standard methods. Antioxidant potentials of the *P. kotschyi* stem bark crude extracts of (Hexane, Ethyl acetate and Methanol) and isolated compound were estimated using Qualitative and quantitative 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays. Qualitative preliminary phytochemical screening of the extracts of *P. kotschyi* revealed the presence of phytochemicals such as carbohydrate, alkaloids, tannins, flavonoids, cardiac glycosides, saponins and steroids/ triterpenes and for qualitative screening, ethyl acetate extract had the highest quantity of phytochemicals tested as represented in table 4 in percentages. The antioxidant activity was observed qualitatively by the change of DPPH colour on TLC plates from purple to yellow. Quantitative antioxidant activity revealed the following IC_{50} values 324.22, 46.37, 91.77and 34.09 µg/mL for hexane, ethyl acetate, methanol and ascorbic acid, respectively. Ethyl acetate extract had the highest antioxidant effect. The effects of the extracts is dose dependent and statistically significant($p<0.05$). The studies established that *P. kotschyi* stem bark is rich in phytochemical constituents and was validated to have free radical scavenging effect.

Keywords: *Pseudocedrela kotschyi*, Antioxidant, Extracts, Screening, Phytochemical constituents.

Intoduction

Oxidative stress has been demonstrated to be involved in many diseases such as cardiovascular, rheumatoid arthritis, neurodegenerative diseases, alcoholic and non-alcoholic steatohepatitis, diabetes and

cancer (Marnett, 2000). Due to the constantly increasing incidence of these diseases, there is need for continuous research of natural products (e.g. *Pseudocedrela kotschyi*) in order to have new antioxidants that will be effective, affordable, accessible and with fewer side effects. Many plants and *P. kotschyi* inclusive have been used ethnomedically to treat diseases in which oxidative stress is implicated, validation of the folkloric claims of these plants will offer a scientific basis for the development of their bioactive constituents and could provide novel lead compounds or precursors.

Phytochemicals are majorly responsible for the effects of medicinal plants. It is necessary to carry out phytochemical studies in order to qualitatively and quantitatively screen phytochemicals and isolate them as pure compounds. Phytochemical studies will serve as a guide to researchers to know specific phytochemicals that are found in plants or families and responsible for specific pharmacological effects.

Pseudocedrela kotschyi (schweinf) Harms is a small deciduous tree that belongs to the family of Meliaceae. It is widely spread in the savanna zone from east Senegal to western Ethiopia, Uganda and Nigeria (Burkill, 1997). It is commonly called Dry-zone cedar and hard cedar-mahogany. Some of its local names are *Emi gbegi* in Yoruba's and *Tuna* in Hausa's. *Pseudocedrela kostschyi* is a deciduous and monoecious small tree that is up to (12–20) m tall; bole branchless of up to 7.5 m, straight, cylindrical and up to 70 cm in diameter. The bark surfaces are grey, longitudinally fissured, inner bark has reddish veins, crown oblong to pyramid-shaped and usually dense with young twigs being short-hairy (Burkill, 1997)

The ethno medical use of *Pseudocedrela kotschyi* against diseases among different cultures around the world justified its potential medicinal value. The root and stem bark are used for the treatment of fever, malaria, diarrhoea, worm infestation and oral infection (Okunade *et al.,* 2007; Tapsoba and Deschamps, 2006). *Pseudocedrela kotschyi* wood is also used as a chewing stick for dental cleaning in western Nigeria (Akande and Hayashi, 1998). The decoction of the leaf is used traditionally in the folk medicine in Nigeria for the treatment of a number of diseases and health conditions, including, fever, pains, diabetes and convulsion (Akuodor *et al.,* 2015; Akuodor *et al.,* 2013; Georgewill and Georgewill, 2009; Anuka *et al.,* 1999). The root has bitter taste and is used ethno botanically in the treatment of gastro-intestinal diseases, toothache and internal wounds (Okunade *et al.,* 2007; Kassim *et al.,* 2009; Akande and Hayashi, 1998).

Some of the chemical constituents of *P. kotschyi* reported includes limonoids, 7-desacetoxy-7- oxogedunin and pseudrelones A, B and C which displayed good antiprotozoal activity (Hay *et al.,* 2007). The limonoid 7-deacetoxy-7-hydroxy-gedunin isolated from *Pseudocedrela kotschyi*, showed anti-HIV activity. The leaves contain 3-0-rhamnosides of myricetin and quercetin, and 3-0-glucosides (or galactosides) of these aglycones (Asase *et al.,* 2008) and stigmasterol from hexane extract (Atinga *et al*. 2018).

Pharmacologically, *P. kotschyi* root extracts have been shown to inhibit the *in vitro* growth and development of the schizont stage of *Plasmodium falciparum (*Kassim *et al.,* 2009*)*. The leaves have been reported to have some antibacterial and antifungal activity (Ayo *et al.,* 2010). The aqueous stem bark extract was investigated to have antiulcer activity (Akah *et al.,* 2001).The present study aimed at screening the phytochemical constituents and antioxidant effects of *P. kotschyi* stem bark as claimed traditionally.

Materials and Methods

Chemicals, Reagents and Equipment

All chemicals and reagents used were of standard grades, and equipment were accurately calibrated.

Collection, Authentication and Preparation of Plant Material

Plant Collection and Identification

The stem bark of *P. kotschyi* was collected from Dajin Kudingi area, Samaru district of Sabon Gari Local Government, Kaduna State in Mach 2017. The plant was identified by Malam Namadi Sanusi at the Department of Botany, Ahmadu Bello University, Zaria-Nigeria with a voucher number 900243. The voucher specimen was deposited for future reference.

Preparation of plant sample

The Stem bark of *P. kotschyi* was appropriately cleaned, air dried and ground to coarse powder using grinding machine (3.5 KVA). The powder was stored in airtight containers for further use.

Extraction of Plant Materials

Stem bark powder of *P. kotschyi* (1.2 kg) was successively extracted in 2.5 litres each of hexane, ethyl acetate and methanol for 72 h using cold maceration and the filtrate was concentrated to dryness on a water bath at 100^0 C and stored in desiccator for further use.

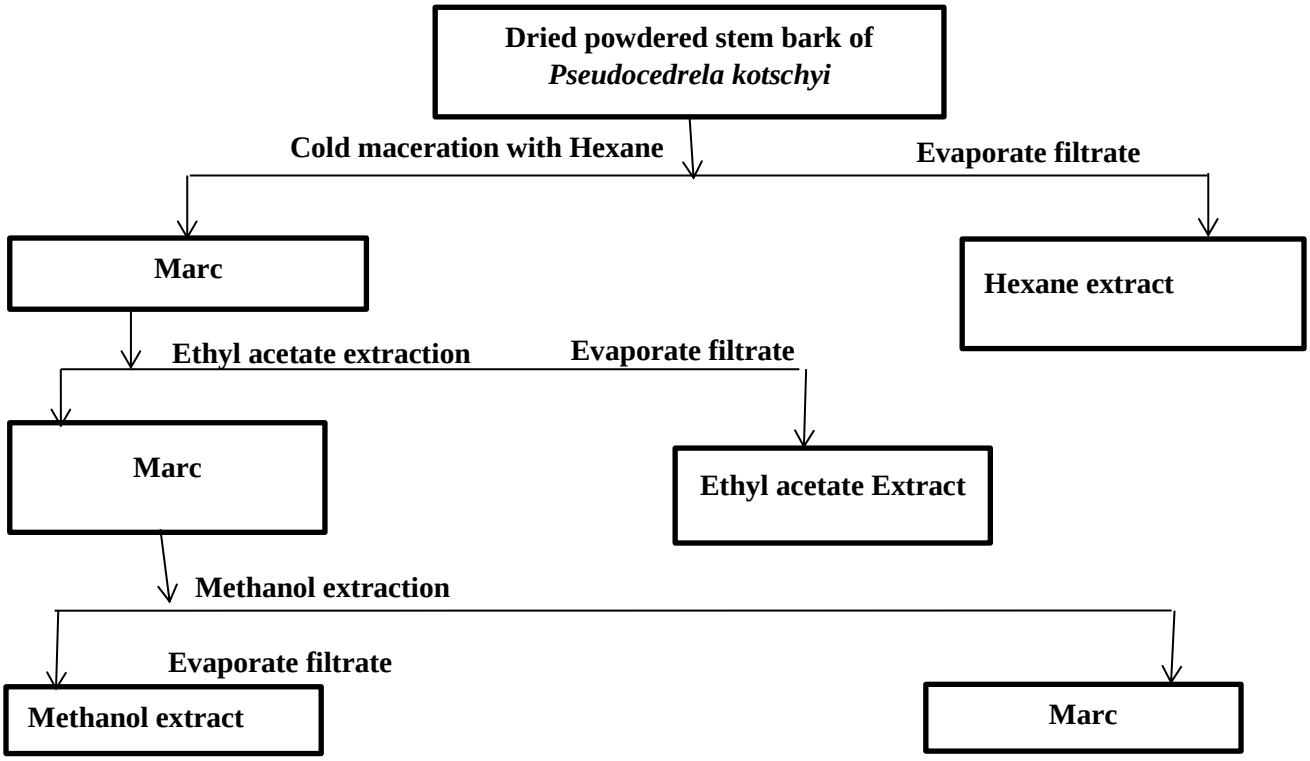


Figure 1: Schematic representation of successive extraction of *Pseudocedrela kotschyi* stem bark (Emmanuel, 2018)

Phytochemical Screening

Qualitative Phytochemical Screening

The n-hexane, ethyl acetate and methanol extracts were subjected to qualitative phytochemical screening using standard method as described by Evans (2009) and Sofowora (1993) to asctertain the presence of saponins , alkaloids, tannins, anthraquinone, Steroids/Triterpenes, flavonoids, carbohydrate, Cyanogenetic glycoside, Cardiac glycosides.

Quantitative Phytochemical Determination

Determination of Alkaloids: This was done according to the alkaline precipitation gravimetric method as described by Harborne, (1973). Three gram of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 h at 28^{o} C. It was later filtered via what man No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH_4OH until the alkaloid was precipitated. The precipitated alkaloid was received in a weighed filter paper and washed with 1% ammonia solution then dried in the oven at 800^{o} C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Determination of Flavonoids: This was carried out according to the procedure described by Harborne (1973). Five gram (5g) of the plant sample was boiled in 50 mL of 2M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered through whatman No 42 filter paper. A quantified volume of the extract was treated with equivalent volume of ethyl acetate starting in drop wise. The flavonoid precipitated was recovered by filtration using weighed filter paper. The weight difference gave the quantity of flavonoid in the sample.

Determination of Tannins: Tannin content was estimated by the Folis-Denis colorimetric method as described by Kirk and Sawyer (1998). Five gram of the sample was dispersed in 50 mL of distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28^{o} C and later filtered through what man filter paper No. 42 grade. Two millilitres of the extract was dispersed into 50 mL volumetric flask then 2 mL standard tannin solution (tannic acid) and 2 mL of distilled water were added in separate volumetric flasks to serve as standard. Reagent and 2.5ml of saturated Na_2C0_3 solution were added respectively to each of the flask and later. The content of each flask was made up to 50mls with distilled water and allowed to be incubated at 28^{o} C for 90 minutes. Their respective absorbance was measured in a spectrophotometer at 260 nm using the reagent blank to calibrate the instrument at zero.

Saponin determination: Saponin was determined using the method of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55° C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90° C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty milliliters of n-butanol was added to the

aqueous layer. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage.

Determination of Anthraquinone: The method described by Soladoye and Chukwuma, (2012) was adopted for the determination of anthraquinone. Fifty milligram of the fine powder sample was soaked in 50 mL of distilled water for 16 h. The suspension was heated in water bath at 70^{o} C for an hour. After the suspension was cooled, 50 mL of 50% methanol was added to it and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450 nm and compared with a standard solution containing 1mg/100 mL alizarin with the absorption-maximum 450 nm.

Determination of Cardiac glycosides: Cardiac glycoside content in the sample was quantified using Buljet's reagent as described by method of El-Olemy et al, 1994. One gram of the coarse powder of stem bark of *P. kotschyi* was soaked in 10 mL of 70% alcohol for 2 h and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95 mL aqueous picric acid + 5 mL 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

Determination of total phenols: The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 minutes. Five millilitres of the extract was pipetted into a 50 mL flask then 10 mL of distilled water was added. Two milliters of ammonium hydroxide solution and 5 mL of concentrated amylalcohol were also added. The samples were made up to mark level and left to stand for 30 min for colour development. This was measured at 505 nm.

Determination of total terpenoids: Two gram of *P. kotschyi* stem bark powder was weighed and soaked in 50 ml of 95% ethanol for 24 h in a conical flask. The extract was filtered and the filtrate extracted with petroleum ether (60-800C) was concentrated to dryness. The dried ether extract was treated as total terpenoids (Kwaji et al, 2015; Ladan et al., 2014).

Antioxidant Screening

Qualitative Antioxidant Screening

This was carried out on pre-coated thin layer chromatography (TLC) plate. The TLC profile of each of the extracts was ran in its best solvent system, the profiles obtained were sprayed with 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as detecting reagent. Colour of the plates were observed; a yellow spot or deep orange spot against a purple background suggested the presence of free radical scavenging compounds (Huang *et al.*, 2005; Adeloye *et al.*, 2007).

Quantitative Antioxidant Screening

The determination of DPPH radical scavenging activity of the 3 stem bark extracts of *P. kotschyi* was carried out according to the method described by Mensor *et al.*, (2001). Sample stock solution of each extract (1 mg/mL) was diluted with methanol into various concentrations 5, 10, 25, 50, 125 and 250 µg/ mL, respectively.

To each test tube, 2.5 mL sample solution of different concentrations of (5, 10, 25, 50, 125 and 250 µg/mL in methanol), 1mL of DPPH solution was added (0.3 mM in methanol)

and allowed to stand for 30 minutes at room temperature, the absorbance of the solution was then measured at 518 nm. The free radical scavenging activity of the extract was determined by comparing its absorbance with that of a blank solution (Methanol (1 .0 mL) plus extract solution (2.5 mL). The ability to scavenge the DPPH radical was calculated using the following equation;

$$AA\% = 100 - \frac{[(Abs\ sample - Abs\ blank) \times 100]}{Abs\ Control}$$

Methanol (1 .0 mL) plus extract solution (2.5 mL) was used as blank and DPPH 0.3 mM (1.0 mL) plus extract solution (2.5 mL) was used as sample. DPPH 0.3 mM (1 mL) plus methanol (2.5 mL) was used as negative control and Solution of ascorbic acid served as positive control.

Statistical Analysis

The results were expressed as mean ± standard errors of the mean (SEM) for all values. The Data obtained from antioxidant studies were further analyzed by SPSS (version 20) using one-way analysis of variance (ANOVA) and Dunnett's post hoc test at $p < 0.05$ significant level. Minimum inhibitory concentration (IC_{50}) values were obtained using regression equation between samples concentrations at 95% confidence level. Tables, figures and graphs were used to summarize some data obtained (Altman and Bland, 1983).

Results

Phytochemical screening of the *P. kotschyi* stem bark

Preliminary Phytochemical screening of the *P. kotschyi* stem bark

The qualitative phytochemical studies of the *P. kotschyi* stem bark extracts of n-Hexane, Ethyl acetate and Methanol revealed the presence of saponins, alkaloids, tannins, anthraquinone, Steroids/Triterpenes, flavonoids and carbohydrate.

Table 1: Preliminary Phytochemical Screening of the Extracts of *Pseudocedrela kotschyi*

Tests	Hexane	Ethyl acetate	Methanol
Saponins			
Frothing test	Absent	Present	Present
Haemolysis	Absent	Present	Present
Alkaloids			
Dragendorff's	Absent	Present	Present
Mayer's	Absent	Present	Present
Hager's (Picric acid)	Absent	Present	Present
Wagner's test	Absent	Present	Present
Carbohydrate			
Molish's	Absent	Present	Present
Fehling's	Absent	Present	Present
Steroids/Triterpenes			
Salkowski	Present	Present	Present
Lieberman-Burchard	Present	Present	Present
Flavonoids			
Sodium Hydroxide	Abesent	Present	Present

Tannins			
Ferric chloride	Absent	Present	Present
Lead sub acetate	Absent	Present	Present
Cardiac glycosides			
Keller- killiani	Present	Present	Present
Anthraquinones			
Borntrager	Absent	Absent	Absent
Modified Borntrager	Absent	Absent	Absent
Cyanogenetic glycoside	Absent	Absent	Absent

Quantification of Phytochemicals in the Extracts of *Pseudocedrela kotschyi* Stem Bark

Table 2: Percentage Quantity of Phytochemicals in the Extracts of *Pseudocedrela kotschyi* Stem Bark

Phytochemicals	Quantity of Phytochemicals in Extracts		
	Hexane (%)	**Ethyl acetate (%)**	**Methanol (%)**
Alkaloids	0.0042±0.0002	0.3512±0.0017	0.2926±0.0020
Tannins	0.0017±0.0001	0.7921±0.0041	0.6017±0.0037
Flavonoid	0.0024±0.0002	0.9524±0.0031	0.8609±0.0027
Cardiac glycosides	0.0321±0.0012	0.0153±0.0026	0.0361±0.0018
Terpenoid	0.6215±0.0026	0.0231±0.0043	0.0303±0.0030
Phenol	0.0032±0.0001	0.7523±0.0062	0.6031±0.0011
Anthraquinone	0.0045±0.0003	0.0274±0.0009	0.0125±0.0014
Saponin	0.0002±0.0000	0.0539±0.0037	0.0742±0.0027

Values (%) are means ±SD (Standard deviation) of three determinations

Antioxidant Properties

Qualitative Antioxidant Properties

The TLC profile of each of the extracts was developed in its best solvent system, and sprayed with 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) as detecting reagent. Yellow spots were observed against a purple background for each of the extract suggesting the presence of free radical scavenging compounds in them. The plates below show the effects of the extract against DPPH and their R_f values.

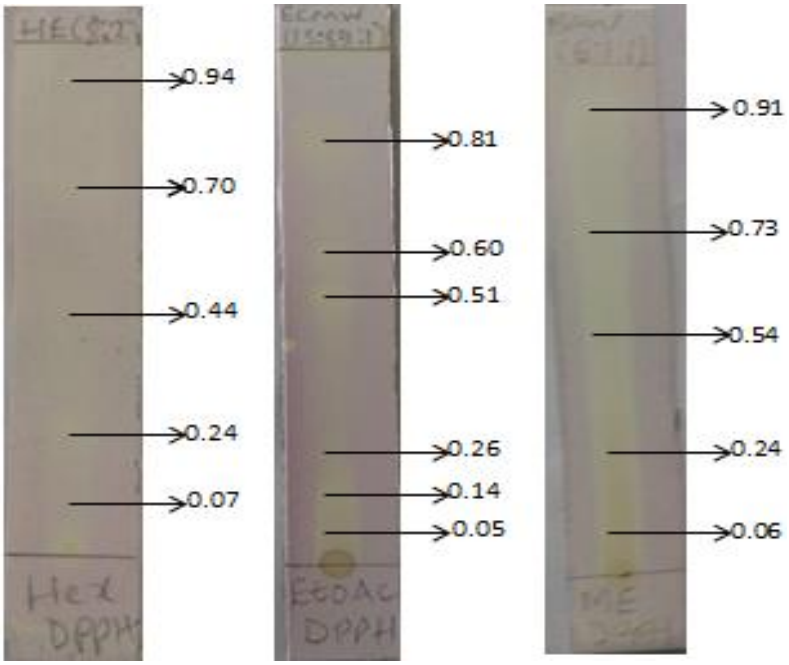


Plate I **Plate II** **Plate III**

Plate I: Chromatogram of Hexane extract on pre-coated silica gel plate developed in Hexane: Ethyl acetate (8:2) sprayed with DPPH, 5 spots were observed with their R_f values.

Plate II: Chromatogram of Ethyl acetate extract on pre-coated silica gel plate developed in Ethyl acetate: Chloroform: Methanol: Water (15:8:4:2) sprayed with DPPH, 6 spots were observed with their R_f values.

Plate III: Chromatogram of Methanol extract on pre-coated silica gel plate developed in Buthanol: Acetic acid: Water (6:1:1) sprayed with DPPH, 5 spots were observed with their R_f values.

Quantitative Antioxidant Properties

The antioxidant properties of the *P. kotschyi* stem bark was carried out on the three extracts and isolated compound to ascertain their free radical scavenging potentials on DPPH as compared with standard antioxidant. The effects of the test agents were found to be dose dependent.

Table 3 Percentage of antioxidant activity of different extracts in the *P. kotschyi* stem bark

Conc. (µg/mL)	Hexane (%)	Ethyl acetate (%)	Methanol (%)	Ascorbic Acid (%)
5	-40.90 ± 2.09	14.10 ± 1.48	-5.17 ± 1.50	19.12 ± 0.16
10	-20.74 ± 1.28	38.48 ± 1.31	19.09 ± 3.20	36.01 ± 0.57
25	-8.60 ± 0.51	52.66 ± 1 .62	41.36 ± 2.02	53.32 ± 1.19
50	3.64 ± 0.84	64.53 ± 0.99	54.84 ± 2.12	75.30 ± 1.10
125	17.49 ± 1.49	86.70 ± 2.08	72.46 ± 1.29	90.04 ± 1.03
250	25.35 ± 6.71	97.05 ± 1.32	90.19 ± 2.04	97.21 ± 1.21
IC_{50}	**324.22**	**46.37**	**91.77**	**34.09**

Values (%) are means ±SD (Standard deviation) of three determinations

Values are obtained from regression line with 95% confidence level

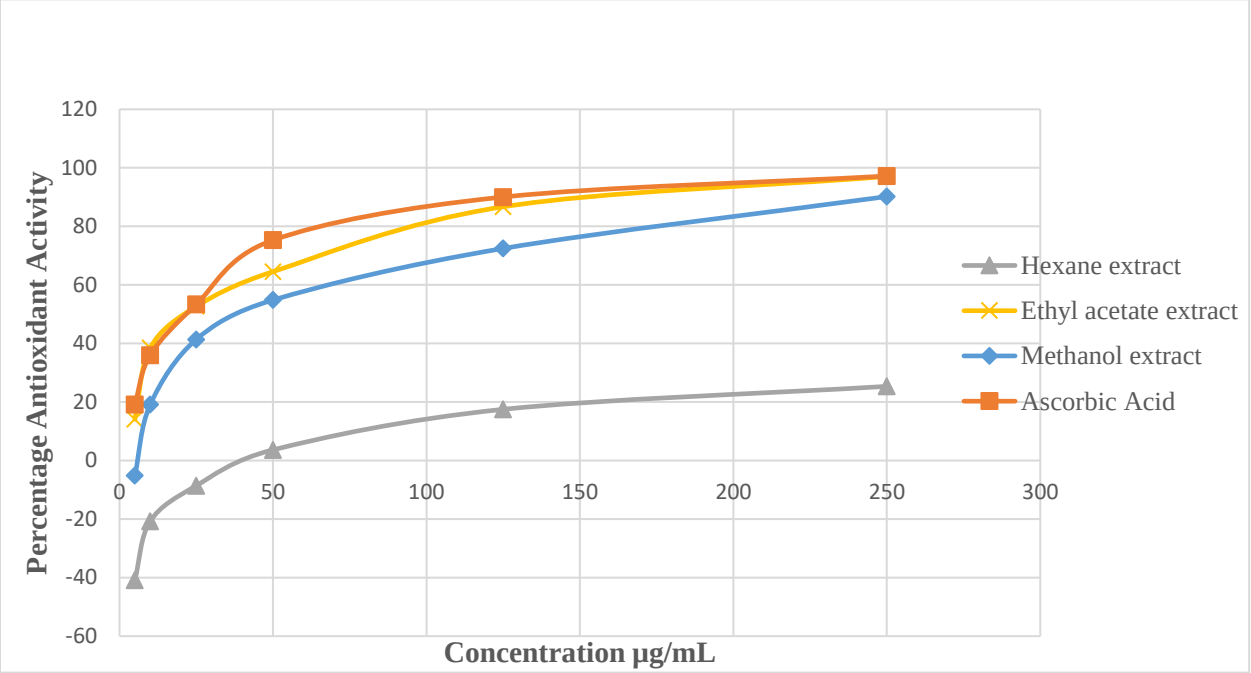


Figure 2.1: Antioxidant activity against concentration for Hexane, Ethyl acetate and Methanol extracts of *P. kotschyi* stem bark and Ascorbic Acid

All values are significantly different at $p<0.05$

Discussion

Preliminary phytochemical screening of the extracts of *P. kotschyi* revealed the presence of some phytochemicals namely carbohydrate, alkaloids, tannins, flavonoids, cardiac glycosides, saponins, steroids/ triterpenes (Table 1). Hexane extract only had steroids/triterpenes and cardiac glycosides (Table 1), this could be as a result of minimal composition of nonpolar phytochemical constituents in the plant and the low polarity of n-hexane extracts (Harborne, 2009). Steroids/triterpenes was found in all the extracts (n-hexane, ethyl acetate and methanol). Anthraquinone and Cyanogenetic glycoside were absent in all the extracts but Oyeleke *et al*., (2008) and Omonkhelin *et al*., (2009) reported that some members of Meliaceae family have anthraquinone and cardiac glycosides. Methanol and ethyl acetate extracts have similar composition of phytochemical constituents (i.e. alkaloids, cardiac glycosides, saponins, flavonoid, tannins and steroids/triterpenes) but in varied concentrations (Table 1) and this is understandable because the polarity index of methanol at 5.1 is only 0.6 greater than that of ethyl acetate at 4.4. However, the quantitative phytochemical study showed that ethyl acetate extract had the highest quantity of phytochemical constituents (Table 2).

For qualitative antioxidant properties, the TLC profile of each of the extracts (hexane, ethyl acetate and methanol) was established in its best solvent system and sprayed with 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) as detecting reagent. Yellow spots observed against a purple background on the TLC plates (I, II, III) for each of the extracts Hexane, Ethyl acetate and Methanol) suggested their capacity to bleach DPPH due to the presence of free radical scavenging compounds (tannins, flavonoid, phenols) in them. The effects exhibited by the extracts (hexane, ethyl acetate and methanol) against DPPH could serve as a good preliminary result for antioxidant potentials (Oke and Hamburger, 2002; Budzianowska and Budzianowska, 2006) and guide the monitoring of isolation of antioxidant compounds from crude extracts and fractions using preparative TLC or column chromatography (Ahmed, 2011). The outcome of this study is in accordance with the report of Sreenivasan *et al.,* 2007; Mensor *et al.,* 2001 and Matkowski *et al.* 2008.

The quantitative antioxidant study of the *P. kotschyi* stem bark was successfully carried out using DPPH assay for free radical scavenging potentials of the extracts. The results showed that hexane, ethyl acetate, methanol and ascorbic acid have IC_{50} values of 324.22, 46.37, 91.77 and 34.09 µg/mL respectively (Table 3). It can be observed that ethyl acetate and methanol extracts had the best antioxidant activities with the IC_{50} values of 46.37 and 91.77 µg/mL (Table 3) respectively but the antioxidant activity of ethyl acetate extract was marked because even the lowest dose (5µg/mL in methanol) was able to bleach DPPH. Hexane extract gave the lowest activity with the IC_{50} values of 324.22 µg/mL (Table 3) compared to those of other extracts and ascorbic acid (IC_{50} 46.37, 91.77, 34.04 µg/mL). The was no statistical significant difference at $p< 0.05$ between the activities of ascorbic acid and ethyl acetate extract and this could be seen in the graph in figure 2.1. There was statistical significant difference at $p< 0.05$ for the activities of ascorbic acid and other extracts as could also be seen in figure 2.1. The antioxidant activities of all the extracts and ascorbic acid were observed to be dose dependent (Table 3) as reported by Akinmoladun, (2007) and Mensor *et al.*,

(2001). The remarkable antioxidant activities exhibited by ethyl acetate and methanol extracts (Table 3) suggest that they could serve as potential free radicals scavenging agents and their activities is probably due to their phenolic, alkaloids, flavonoids, terpenoids, and tannins constituents and the presence of hydroxyl group attached to the aromatic ring of their molecules (Mensor *et al.*, 2001; Akinmoladun *et al.*, 2007; Sri-sudewi *et al*., 2014; and Cait *et al*., 2003). The facts mentioned above have proven the folkloric use of this plant in traditional medicine in Nigeria for the treatment of numerous diseases and health conditions such as malaria, fever, pains, diabetes and convulsion (Akuodor *et al.*, 2015; Akuodor *et al.*, 2013; Georgewill and Georgewill, 2009;)

The scavenging effects of different extracts of *P. kotschyi* on the DPPH radicals increased with increase polarity of solvent used for each extracts and observed to be dose dependent. The degree of the scavenging effect is summarized in the following order: Ethyl acetate > methanol > Hexane.

Conclusion

Phytochemical tests showed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, triterpenes and saponins in hexane, ethyl acetate and methanol extracts of *P. kotschyi* stem bark. Ethyl acetate extract had the highest quantity of phytochemical constituents. The extracts exhibited free radicals scavenging effects that were dose dependent. This supports the basis for the use of this plant traditionally for the treatment and managment different ailments including cancer, malaria.

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Conflict of Interest

The authors declare that there is no conflict of interest of any kind among them in this studies.

Ethical Consideration

This study did not involve animals and human subjects and as a result, ethical consideration was irrelevant.

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