In Vitro and in Vivo Antioxidant Effect of the Stem Bark Extract of *Pseudocedrela kotschyi* in Pentylenetetrazole-kindled Rats.

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ABSTRACT

This study evaluates the antioxidant and anti-kindling effects of the methanol stem bark extract of *Pseudocedrela kotschyi*, which is commonly used in folk medicine to treat dysentery, malaria, toothache and rheumatism. To investigate the anticonvulsant activity of the plant, Pentylenetetrazole induced kindling model was employed using fifty rats divided into five groups (n=10). In-vitro antioxidant activity of methanol, n -hexane, chloroform, n-butanol and residual aqueous fractions was evaluated using the DPPH radical scavenging activity method. The total phenolic and total flavonoid contents of the extract and fractions were evaluated. The serum and brain homogenates were investigated for malonyldialdehyde, reduced glutathione, superoxide dismutase and catalase enzyme activity.

The extract at doses of100 and 200 mg/kg significantly ($p< 0.05$ and $p< 0.01$) protected the rats against PTZ induced kindling (stage 5 convulsion). Lipid peroxidation was significantly inhibited in the brain homogenates at all doses tested (50,100 and 200 mg/kg).Similarly the extract at the test doses increased significantly ($p<0.05$ –$p<0.001$) the activities of reduced glutathione, superoxide dismutase and catalase enzyme in both serum and brain homogenates.

In conclusion the methanol extract of *P. kotschyi* stem bark extract may contain bioactive principles with anti-kindling and antioxidant Properties. This may also provide an explanation for its traditional use in the management of epilepsy.

KEYWORDS: Antioxidant, anti-kindling, *Pseudocedrela kotschyi* and Rats.

INTRODUCTION

An antioxidant is a chemical, substance or enzyme that has the ability to scavenge free radical species. One major role of antioxidants in the cell is to prevent damage due to reactive oxygen species (ROS), which includes hydrogen peroxide (H_2O_2), superoxide anion (O_2^{-}) and the hydroxyl radical (OH^-). In the body, antioxidants are of two types: simple molecules such as ascorbic acid (vitamin c), α tocopherol (vitamin E), carotenoids and flavonoids prevents propagation of free radical reactions in all cell membranes and enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GP_X) and catalase (CAT) which scavenge free radicals before they start an oxidation chain. (Milvia, 2013).

In epileptic attacks, oxidative stress occurs, free radicals are produced and membrane lipid peroxidation happens, all of which cause tissue damage (Ilhan *et al*., 2005). The generated oxidative stress in the brain is a common mechanism of cellular damage in many acute neurological attacks, such as seizure activity and diseases like Alzheimer's disease (Oliver *et al*., 1990). Also, membrane lipids are full of unsaturated fatty acids such as arachidonic acid and most of them are susceptible to lipid peroxidation process which leads to the destruction of the membrane and hindering its functions (Kim *et al*, 2000; Mandegary *et al*., 2004). Kindling model of epilepsy is produced by repeated administration of an initially sub-convulsive electrical or chemical stimulus that results in an increase in seizure activity, culminating in a generalized seizure (Kupfeberg, 2001).The Pentylenetetrazole induced kindling model has been employed in investigating plants with potential antiepileptogenic activity (Gupta *et al*., 2003;Rahmati*et al*., 2013).

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Currently available antiepileptic drugs are effective in managing epilepsy in most patients, though 10% of patients are still refractory despite treatment (Rang *et al*., 2011). Among the currently available antiepileptic drugs only few have been reported to have antiepileptogenic activity, they include the benzodiazepines, phenobarbitone, valproic acid and Leviteracetam (Silver *et al*., 1991; Bolanos *et al*., 1998; Loscher*et al*., 2000).In this study we investigated the antioxidant activity of the stem bark extract of *Pseudocedrela kotschyi* in PTZ- kindled rats. Though the preliminary anticonvulsant activity of the plant has been reported, there is a need to investigate its antiepileptogenic activity (Anuka *et al*., 1999).

MATERIALS AND METHOD

Plant Material

The stem bark of *Pseudocedrela kotschyi* was collected in the month of July 2013 from Zuru local government area, Kebbi state, Nigeria. It was identified by Mr. U.S Gallah of the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, and a voucher specimen (900243) was deposited for future reference.

Preparation of the extract

The stem bark was chopped, cleaned, air dried for 12 days and milled into a coarse powder using a pestle and mortar. Five hundred grams (500 g) of the coarse powder was exhaustively extracted with 5 L of 95% methanol in 500 ml aliquots using a Soxhlet extractor for 12 h daily for three days. The extract was concentrated using Büchi RE121 rotary evaporator (Büchi Labor technik AG, Switzerland) and subsequently dried in a Hetovac VR-1 freeze dryer (Heto Lab. Equipment AS, Denmark).

Phytochemical screening

The extract was screened for the presence of saponins, flavonoids, alkaloids, anthraquinones, tannins, reducing sugars, terpenoids and cardiac glycosides using standard protocols (Sofoworah, 1993; Trease and Evans, 1999).

Animals

Wistar rats of both sexes weighing between 140 and 200g were obtained from the animal house facility of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria . The animals were acclimatized to laboratory conditions for seven days prior to the experiments. During acclimatization, 5 rats were housed per polycarbonate cage, with free access to normal diet (48% carbohydrate, 23% crude protein, 3% crude fat, 8% crude ash, 5% crude fibre and 13% moisture) and tap water *ad libitum*. The rats were maintained on Excel Feeds. All experimental protocols were approved by the University animal ethics committee.

Acute toxicity studies

According to the OECD guideline 423 for acute oral toxicity studies, ten animals of equal numbers of male and female rats were used and each received a single oral-dose of 2000 mg/kg. The rats were fasted overnight and administered with a single oral dose of 2000 mg/kg of the extract one at a time (OECD, 2002). Each rat was observed for signs of toxicity and mortality every 30 minutes for the first four hours and then two hourly for forty eight hours. Each animal was further observed daily for fourteen days.

Drug Administration

All drugs and reagents, unless stated otherwise, were purchased from Sigma-Aldrich (Sigma-aldrich, St. Louis U.S.A). The freeze-dried methanol extract of *Pseudocedrela kotschyi* was diluted in saline to desired concentrations and administered orally (p.o.) by gastric gavage in a volume of 10 ml/kg. *P. kotschyi* extract was administered at a dose of 50,100 and 200 mg/kg p.o, 1 hour before administration of 35 mg/kg of pentylenetetrazole .

In- vitro antioxidant studies

DPPH Scavenging activity

The method described by Shimada (1992) was employed; 96-well microplate method was used to measure the scavenging activity towards the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity. DPPH solution (0.04%w/v) was prepared and kept in the dark. To a 96-well flat bottom plate were added 100 µl of extract and 200 µl of DPPH solution. Samples were prepared in triplicates for each concentration used, and at least eight different concentrations were used. The plate was shaken to ensure thorough mixing before being covered and placed in the dark at room temperature. After 30 minutes incubation, the plate was read in Power X340 microplate reader (Bio-Tek instruments USA) at the wavelength of 517 nm. The control contained 100 µl methanol and 200 µl DPPH (0.1 mM) solution. The standards used in this assay were quercetin and BHT. The free radical scavenging activity was calculated using the formula:

Free radical scavenging activity (%) = $(A_c-A_s)/ A_c$ x 100

Where A_c is absorbance of control, and A_s absorbance of samples.

Total Phenolic count

The total phenolic content of the stem bark extract was determined by the Folin-Ciocalteu method with some modification (Singleton and Rossi, 1965).12.5 µl of extract and standard (gallic acid, in HPLC grade

methanol) was added to 50 µl of distilled water and 12.5 µl of Folin-Ciocalteu reagent. After 3min of incubation at room temperature, 125 µl f 20% sodium carbonate was added and mixed. After 30 min of incubation at room temperature, absorbance of the mixture was measured at 765nm using a Power X340 microplate reader (Bio-Tek instruments USA). Gallic acid was used to produce standard calibration curve. The total phenolic content of the sample was expressed as Gallic acid equivalents (GAE), which reflected the phenolic content as amount of Gallic acid in sample. All samples were analyzed in triplicate.

Total flavonoid content

Colourimetric aluminum chloride method with some modifications was used for the determination of the total flavonoid content (Nabavi *et al*., 2009). 50 µl of extract in HPLC grade methanol was separately added to 5 µl of 10% aluminium Chloride, 5 µl of 1M Sodium acetate and 140 ml distilled water, and left at room temperature for 40min.The absorbance of the reaction mixture was measured at 415 nm using Power X340 microplate reader (Bio-Tek instruments USA).The blank consisted of 50 µl of HPLC grade methanol and 150 µl distilled water. Rutin was used to produce standard curve. Total flavonoid content of the samples was expressed as rutin equivalents which reflected the flavonoid content as amount of rutin in sample. All samples were analyzed in triplicates.

Pentylenetetrazole induced kindling

Fifty rats of both sexes were divided into five groups (n=10), groups i was the negative control while groups ii-v were treated with sub-convulsive dose of pentylenetetrazole (PTZ, 35 mg/kg, i.p) every second day (48 hourly) until full kindling was achieved in the saline treated group (seizure score of 4-5).Groups iii-v were treated with 50, 100 and 200 mg/kg p.o of *Pseudocedrela kotschyi* one hour before PTZ administration .During treatment, each rat was placed in the testing chamber (a transparent plastic cage) for observation and recording of behavior for thirty minutes. The PTZ injection was stopped when the animals showed adequate kindling, i.e. seizure score of 4 or 5 on three consecutive injections (Fischer and Kittner, 1998). Seizure scores after 30 minutes each were defined as follows: Stage 0: no response, Stage 1: ear and facial twitching, Stage 2: myoclonic body jerks, Stage 3: myoclonic body jerks, rearing, Stage 4: generalized clonic convulsions turning over onto one side position and Stage 5: turning over onto the back, generalized tonic-clonic seizures (or death within 30 minutes) (Racine, 1972, Ozlem and Okan, 2015).

In vivo Antioxidant studies

Lipid Peroxidation Assay

Lipid peroxidation was estimated by the spectrophotometric measurement of malondialdehyde (MDA) levels by using the thiobarbituric acid reactive substances method previously described by (Ohkawa *et al*. (1979). MDA level was determined twenty four hours after the 35 mg/kg PTZ challenge, the kindled rats were sacrificed by decapitation and whole brain was removed out and homogenized (100 mg/ ml) in ice-cold 0.1 M phosphate buffer (pH 7.4) .One hundred and fifty microliter of the supernatant was diluted to 500 µl double deionized water. 250 µl of 1.34% thiobarbituric acid were added to all the tubes, followed by addition of equal volume of 40% trichloroacetic acid. The mixture was shaken and incubated for 30 minutes in a hot boiling water bath with a temperature > 90^0c .Tubes were allowed to cool to room temperature and the intensity of the pink-colored complex formed was measured at 532 nm in a spectrophotometer using 0 concentration as blank. The Absorbance decreases with increasing ability to inhibit lipid peroxidation.

Calculation: Conc in µmol/L = Absorbance/1.56 x 100

Measurement of lipid per oxidation level in serum

Twenty four hours after the 35 mg /kg PTZ challenge, blood was collected from cornea by retro – orbital puncture for the estimation of serum malondialdehyde (MDA) prior to decapitation of the animals. After blood collection the blood samples were left to coagulate at room temperature for 1 hour and serum was separated by centrifugation at 2000 rpm for 10 minutes. The extent of lipid peroxidation was quantified by measuring the thiobarbituric acid reactive substances (TBARS)–malondialdehyde produced during peroxidation of lipids. The same procedure described above was used for the estimation of MDA in the rat sera.

Determination of Reduced glutathione in the brain and serum of Kindled rats

The method described by (Patterson and Lazarow, 1955) was employed. The principle is based on the fact that Glutathione reacts with an excess of alloxan to produce a substance with an absorption peak at 350 nm. One millilitre of the supernatant and 7.0 ml of water was mixed; 2.0 ml of 25% metaphosphoric acid was added and centrifuged. To 1 ml of this supernatant, 2 ml of phosphate buffer (pH-8.4), 0.5 ml of Ellman's reagent [5'5-dithiobis (2-nitrobenzoic acid)] and 0.4 ml of double distilled water were added. The absorbance was measured at 412 nm against blank which contained only 2 ml of phosphate buffer solution and 0.5 ml of Ellman's reagent (DTNB).Absorbance was read at 305nm

Enzymatic assay for catalase in the brain and serum of PTZ Kindled rats

The method of (Beers and Sizer, 1952) was employed. The principle involves:

$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

The disappearance of peroxide is followed spectrophotometrically at 240nm. One unit is equal to one µmole of hydrogen peroxide decomposed per minute under specified conditions of 25^0c.Two tubes were labelled as test and blank, Reagent A was pipetted into the tube containing 2.9 ml of blank while Reagent B pipetted into the second tube containing 2.9 ml of the test material. They were equilibrated to 25^0C; the absorbance was monitored at A_{240} until constant and was recorded using spectrophotometer. Reagent C (catalase solution) was added and mixed immediately by inversion and the time required for the A_{240} to decrease from 0.45 to 0.40 absorbance units was recorded.

Constituents of the Reagents:

Reagent A- 50 mM potassiumbuffer, pH 7.0 at 25^0C

Reagent B- 0.036% w/w Hydrogen peroxide solution (H_2O_2)

Reagent C- Catalase solution (brain homogenate/serum)

Enzymatic assay of superoxide dismutase in the brain and serum of PTZ kindled rats

The method of Zou et al., 1986 was adopted; Six tubes were arranged in rows of 3s, to the first row, 0.10ml of buffer, 0.83 ml of distilled water and 0.05 ml of sample (serum or brain homogenate) were pipetted respectively. To the second row 0.15ml, 0.83 and nil samples were pipetted respectively. The test tubes were incubated at 25^0c for 10min, and then transferred into a cuvette and 0.02ml pyrogallol was added.

The content was mixed thoroughly by inversion and the increase in absorbance was measured at 430 nm using the maximum linear rate for both test and blank.

Calculation:

$$\text{The \% inhibition} = \frac{(X-Y) \times 100}{Z}$$

X= Δ A420nm/minute of blank

Y= Δ A420nm/minute of sample

Z= Δ A420nm/minute of blank

$$\text{The number of units of SOD in the assay is thus} = \frac{\text{\% inhibition}}{(100 - \text{Inhibition})}$$

STATISTICAL ANALYSIS

Results were expressed as the Mean ± Standard Error of Mean (SEM) and Percentages. Statistical analysis of data was done using Graph Pad Prism statistical software (version 6.0). Statistical comparison between groups was made using analysis of variance (ANOVA) and Kruskal Wallis test where necessary. When a statistically significant difference was obtained, a post hoc Dunnets / Dunns test was performed for multiple comparisons. Values of $p< 0.05$ were considered significant.

RESULTS

Phytochemical Constituents and yield of *Pseudocedrela kotschyi*

Phytochemical screening of the methanol extract of the plant revealed the presence of tannins, saponins, flavonoids, steroids and carbohydrates, whereas anthraquinones and alkaloids were absent.

Acute Toxicity Studies of *Pseudocedrela kotschyi*

A Limit dose of 2000 mg/kg produced no noticeable changes in the general behavior of the treated rats and no death was recorded, therefore the LD_{50} was greater than 2000mg/kg.

Effect of *P. kotschyi* on PTZ induced kindling in rats

In the extract treated group *P. kotschyi* at doses of 100 and 200 mg/kg (figure 1) significantly $p < 0.05$) prevented the development of kindled states in rats and none of the animals in these treatment groups achieved a seizure score 5.

Effect of *P. kotschyi* extract on antioxidant enzymes in the serum of PTZ kindled rats

Table 1 and 2 Show the effect of the plant extract on the activities of antioxidant enzymes in the serum of control and extract treated rats. There was a marked increase in the percentage inhibition of superoxide dismutase, catalase and the level of GSH in the serum of the extract treated rats when compared with PTZ kindled groups. There was an insignificant reduction in the level of Malonyldialdehyde which is a product of lipid peroxidation in the extract treated groups compared compare to the PTZ treated rats. Similarly the result in Table 3 indicated a significant inhibition of SOD, increase in the levels of catalase and GSH, followed by a significantly decreased level of MDA.

In vitro Antioxidant effect of *P. kotschyi* crude methanol extract and fractions

In- vitro antioxidant screening of the stem bark extract of *P. kotschyi* showed that the methanol stem bark extract of *P. kotschyi* had an EC_{50} of 0.01 mg/ml, total flavonoid count of 0.90 % and total phenolic count of 33.36 %.

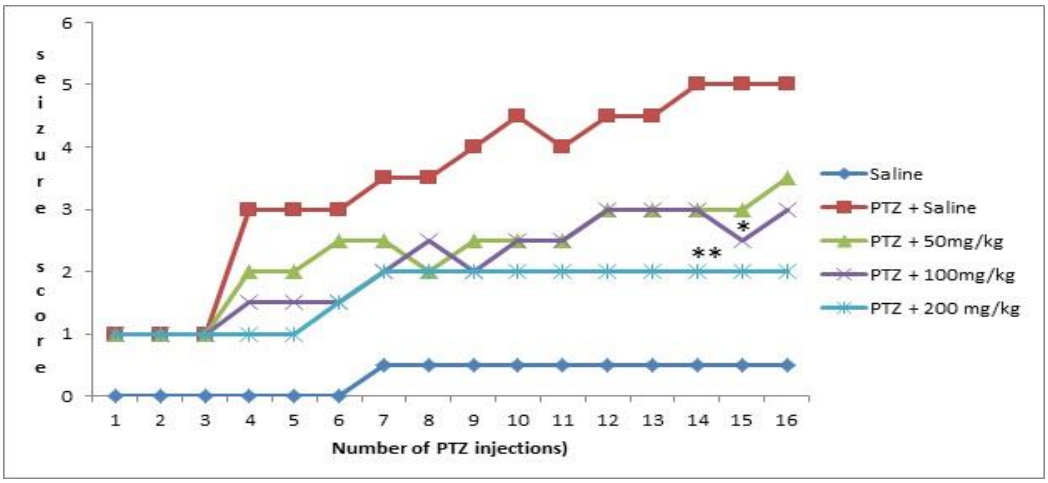

Figure 1: Effect of different doses of methanol extract of *Pseudocedrel kotschyi* (P.k) on the development of seizure in PTZ-kindled rats. P.k was injected 1 h prior to PTZ challenge. On the ordinate: seizure score (median). *p<0.05, **p < 0.01; n=10.

Table 1: Effect of methanol extract of *P. kotschyi* on lipid peroxidation, antioxidant enzymes and GSH in the serum of PTZ kindled.

Treatment (mg/kg)	MDA (µ mol/L)	GSH (mg/dL)	SOD (%)	CAT(units/ml)
CONTROL	1.72 ± 0.27	44.42 ± 2.19	95.33 ± 13.48	103.64 ± 8.85
PTZ + NS	2.92 ± 0.65	12.2 ± 1.84	78.83 ± 4.86	88.06 ± 9.60
PTZ + P.k 50	2.73 ± 0.85	18.23 ± 3.31	93.87 ± 1.25*	71.3 ± 6.83
PTZ + P.k 100	2.37 ± 0.74	22.8 ± 1.18*	87.55 ± 2.41	80.18 ± 9.92
PTZ + P.k 200	2.53 ± 0.68	25.95± 2.93**	95.61 ± 1.42*	113.37 ± 15.43

Data are expressed as mean ± SEM (n=10); P.k = *Pseudocedrela kotschyi*. *P < 0.05 and ** P < 0.01 statistically significant compared to the PTZ+ NS group.

Table 2: Effect of Methanol Extract of *P. kotschyi*on Lipid Peroxidation, Antioxidant Enzymes and GSH in the brain of PTZ Kindled Rats.

Treatment (mg/kg)	MDA (µmol/L)	GSH (mg/dL)	SOD (%)	CAT(units/ml)
CONTROL	10.5 ±1.89	51.68 ± 6.85	97.87 ± 2.29	307.2 ± 36.43
PTZ + NS	28.05 ± 2.34	24.05 ± 4.04	72.3 ± 2.10	235.14 ± 39.92
PTZ + P.k 50	18.70 ± 4.89	35.33 ± 2.58	91.2 ± 2.36**	223.5 ± 29.83
PTZ+P.k100	9.58 ± 2.42**	41.47 ± 3.62**	87.95 ± 2.62*	330.51 ± 42.19
PTZ+P.k 200	9.88 ± 2.17**	46.28 ± 5.44**	96.67 ± 2.26**	631.3 ± 57.58***

Data are expressed as mean ± SEM (n=10); P.k = *Pseudocedrela kotschyi*. *P < 0.05, ** P < 0.01 and ***P<0.001; statistically significant compared to the PTZ+ NS group.

DISCUSSION

In this study we evaluated the antioxidant effects of the aqueous extract of *P. kotschyi* on pentylenetetrazole-induced kindled rats. It was observed that 48 hourly administrations of the extract to rats protected against development of kindled states, further evaluation of brain and serum from the rats supported the antioxidant potential of the plant. Antiepileptogenesis is a pharmacological phenomenon that describes the prevention of the biological processes that lead to chronic epilepsies (Avanzini and Franceschetti 2003). The kindling

model is useful and reliable in determining anticonvulsant activity and development of epileptogenesis, it also offer several advantages in the screening of potential anticonvulsant drugs, studying brain excitability and for developing AEDs (Pavlova et al.,2004; Omoniyi and Olufunmilayo, 2013).

Although direct mechanism of PTZ action is not known, literature records reveal that it causes alterations in GABAergic systems, Glutamergic systems and antioxidant defense systems (Ozlem and Okan, 2015).PTZ-induced kindling is an acknowledged experimental model of human epilepsy and useful for the study of seizure mechanisms (Mason and Cooper, 1972). In this study the PTZ- induced kindling model was employed to evaluate the anti-epileptogenic potential of *P. kotschyi*, the extract significantly ($p<0.05$ and $p<0.01$) suppressed the development of epileptogenesis in the highest doses tested (100 and 200 mg/kg). The ability of the extract to suppress epileptogenesis may be due to one of the several mechanisms of abolition of PTZ action mentioned earlier or due to its ability to inhibit oxidative stress which causes free radical generation and lipid peroxidation. This assumption was supported by the findings that in the PTZ-kindled animal, oxidative stress is possibly one of the factors that are involved in the pathophysiology of epilepsy (Rahmati *et al*., 2013).

Epilepsy is accompanied by reversible convulsion which induces production of reactive oxygen species (ROS) in the brain (Rahmati *et al*., 2013). Reactive oxygen species are chemically reactive molecules containing oxygen. Examples include peroxides, superoxide, hydroxyl radical, and singlet oxygen. In a biological context, ROS are formed as a natural by-product of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. Since it is supposed that free radicals mediate convulsion development, nowadays searching for antiepileptic drugs with antioxidant and neuro-protective effects are of interest. Free radicals are involved in pathogenesis of many neurological diseases such as epilepsy. Free radicals have been implicated in a number of seizure models including PTZ kindling, and some antioxidants have been shown to be effective in these seizure models (Patrick, 2011).

The important effect of free radical is lipid peroxidation at polyunsaturated sites on biological membranes and tissue injury which leads to cell membrane destruction and dysfunction (Gupta, 1999.Normally biological effects of free radicals in the body are controlled by a lot of antioxidants and via antioxidant enzymes like SOD (Sudha *et al*.,2001; Ilhan *et al*., 2006). Free radical produced act on seizure via inactivation of glutamine synthesis that result in the enhancement of L-glutamate brain level (Alabadi et al., 1999; Haliwell and Gutteridge 1991). In the present study *P. kotschyi* methanol stem bark extract was able to protect the rats against generalized tonic clonic seizures (GTCS) induced by PTZ, additionally there was a significantly ($p<0.01$)reduced level of MDA in the brain of the rats treated with 100 and 200mgkg of the extract, indicating protection against lipid peroxidation. The concentrations of GSH and catalase were significantly ($p<0.001$) increased followed by a significantly increased percentage inhibition of SOD ($p<0.01$) in the extract treated groups (100 and 200 mg/kg). Furthermore none of the rats attained seizure score of 5.0 (GTCS). It is therefore safe to suggest that the extract may be exploiting the antioxidant mechanisms as its anti-epileptogenic activity.

In the in-vitro antioxidant studies *P. kotschyi* showed significant antioxidant activity in the DPPH, there was an increase in the TPC and TFC. The free radical scavenging activities of extracts depends on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components (Shimada *et al*., 1992; Fukumoto and Mazza, 2000). DPPH assay is based on the ability of 2, 2-diphenyl-1-picrylhydrazyl, a stable free radical to decolorize from purple to yellow color in presence of antioxidants at a maximum wavelength of 517 nm. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analyzed extract (Guo *et al*., 2007; Molyneux, 2004). In this study the antioxidant compounds present in the methanol extract, fractions and standard neutralized the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH, thereby changing the color from purple to the yellow colored stable diamagnetic molecule diphenylpicrylhydrazine. *P. kotschyi* stem bark extract and fractions showed significant increase in the total phenolic and flavonoid counts. The n-butanol portion contains the highest amount of phenolic compounds and flavonoids. Flavonoids are the most common group of polyphenolic compounds found ubiquitously in plants. Flavonoids are potent antioxidant and their ability to act as antioxidants

depends upon their molecular structure, the position of hydroxyl groups and other features in the chemical structure which are important for their antioxidant and free radical scavenging activities (Kumar *et al*., 2013; Kumar and Pandey 2013).

The results of the present study revealed that the *P. kotschyi* extract possesses significant antioxidant activity, and the antioxidant activity of the stem bark extract of *P. kotschyi* may be due to its content of phenolic compounds and flavonoids in addition to its ability to decolorize DPPH. Antioxidant activities of flavonoids from *P. kotschyi* have been previously reported. Similarly antioxidant activity of different parts of the plant have been documented; they include, the leaf ethanol extract (Essiet *et al*., 2016), nephroprotective activity of the root extract of the plant against oxidative stress, (Ojewale *et al*., 2014). The ability of *P. kotschyi* to reduce the maximal effect in PTZ kindled rats may be attributed to the presence of antioxidant compounds in the plants demonstrated by its ability to inhibit lipid peroxidation and scavenge free radicals. This probability is buttressed by the results of the in vitro and in vivo experiments. Thus, it is plausible to say that *P. kotschyi* reduced the progression of epileptogenesis through antioxidant mechanisms.

CONCLUSION

This study has shown that the methanol extract of *P. kotschyi* stem bark contains bioactive principles with anti-kindlling and antioxidant properties. The result has lent credence to the traditional claims that the plant is used for the management of epilepsy. Further research needs to be carried out to isolate the bioactive compounds responsible for the observed pharmacological effects.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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