African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 16 No.1; pp. 39-49 (2024)

Original Research Article

PHYTOCHEMICAL AND ANTICONVULSANT SCREENING OF THREE MEDICINAL PLANTS USED IN THE TREATMENT OF EPILEPSY IN THE SOUTH-WESTERN PART OF NIGERIA

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ABSTRACT

The burden of epilepsy in developing countries made medicinal plants like *Xylopia aethiopica* fruit; *Khaya grandifoliola*, *Alstonia boonei* etc an alternative source in epilepsy management in the south-western part of Nigeria. The aim of the study was to provide pharmacological rationale for the ethnomedicinal use of the plants in epilepsy management. The oral medial lethal dose of methanol stem bark extracts of *Alstonia boonei* (MEAB) and *Khaya grandifoliola* (MEKG) and methanol fruit extract of *Xylopia aethiopica* (MEXAF) were done in accordance with the Organization for Economic Cooperation Development guideline. Quantitative and qualitative phytochemical profiling of the extracts was done. Anticonvulsant screening was carried out on the extracts (doses: 75, 150 and 300 mg/kg) using the pentylenetetrazole (PTZ)-induced seizure and maximum electroshock tests (MEST). Results showed that the MEXAF has the highest amount of phytochemicals except for saponins in MEKG; and MEAB with the least amount (but higher alkaloid) than MEKG. The TLC showed different bands of spots of the extracts. In the PTZ test, MEXAF showed 100 % protection against mortality at 300 mg/kg; MEAB with 66.67 % protection at 75 mg/kg and MEKG 0 % protection. MEAB, MEKG and MEXAF nonsignificantly increased the onset of seizure and latency to death. In the MEST, MEXAF, MEKG and MEAB at 75 mg/kg protected 50, 33.3 and 16.67% of the animals against tonic hind limb extension respectively and nonsignificantly ($p>0.05$) decreased recovery time except MEXAF which significantly ($p<0.05$) decreased the recovery time at a dose of 75 mg/kg. It was concluded that the extracts possess anticonvulsant activities hence, the pharmacological credence for the ethnomedicinal use of these plants in treating epilepsy.

ARTICLE INFO

Received 28 October, 2023
Accepted 14 March, 2024
Published 26 March, 2024

KEYWORDS

Xylopia aethiopica
Alstonia boonei
Khaya grandifoliola
Anticonvulsant
Phytochemicals

INTRODUCTION

Epilepsy is a long-standing disease of the brain characterized by two or more seizures occurring at least 24 hours apart [1]. Seizures are short-lived signs and/or symptoms which results from abnormal excessive neuronal activity in the brain possibly manifesting into episodes of involuntary movement and sometimes impairment of consciousness [2]. The frequency of seizures occurrence may be one or less per year and to several

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https://doi.org/10.59493/ajopred/2024.1.5 ISSN: 0794-800X (print); 1596-2431 (online)

per day. While the duration may be as brief as attention arrest, muscle jerks to seizures that are severe and prolonged. Seizures may be focal, generalized and unknown depending on the onset [3] [4].

The burden of epilepsy is worldwide of which over 50 million people are affected. It is estimated that epilepsy affects the quality of life of over 13 million people and about 125,000 death and 5 million new cases of the disease diagnosed yearly worldwide [5,6] . The proportion of people with epilepsy in need of treatment at any given time ranges from 4 to 10 per 1000 people. The proportion of new cases of epilepsy varies among low- or middle- and high-income countries with an estimate of 139 and 49 per 100,000 respectively. About 80 % of epilepsy patients reside in low- and middle-income countries. This may results from endemic risk factors such as malaria, neurocysticercosis, road traffic accident, birth-related injuries; poor healthcare facilities and quality antiseizure medications, and other preventive healthcare programs [6]. Despite the reduction in epilepsy-related deaths witnessed between 1990 to 2016 globally, poor improvement was seen in developing countries with a number of African countries having continuous increase over the period [6,7].

Antiseizure drugs remain the mainstay in the treatment of epilepsy and when appropriately used, about 70% of people with epilepsy becomes seizure-free [8,9]. However, about 30 % of the people with epilepsy remain unresponsive to both the old and the newly developed antiseizure medications. Moreover, these drugs have untoward side effects and considerable drug interactions [8,9].

Medicinal plants are assortment of many bioactive compounds effective in the treatment of many diseases including epilepsy. They have attracted global importance even in developed world despite the availability of modern medicine and advanced healthcare delivery system [10]. The high incidence of untreated and uncontrolled seizures among people with epilepsy, poor healthcare facilities, inadequate access to quality antiseizure drugs and high rate of death due to epilepsy especially in resource-constraint nations have made herbal medicine as alternative to be frequently used in the management of epilepsy and potential sources of new drugs [11,12]. In Nigeria, *Alstonia boonei, Khaya grandifoliola*, *Xylopia aethiopica* etc are widely used medicinal plants among the people of South-western part of Nigeria in the treatment of epilepsy [13]. A decoction of varied parts of *Alstonia boonei* (stem bark); *Xylopia aethiopica* (fruits); *Khaya grandifoliola* (stem bark) is prepared and 200 mL taken three times daily, recommended for epilepsy patients [13]. This study therefore, aimed at establishing the pharmacological rationale for the use of these plants in epilepsy management.

MATERIALS AND METHODS

Drugs and Chemicals

Pentylenetetrazole, and methanol (Sigma Aldrich, Germany), phenobarbital (Biopharma India), phenytoin (Epanutin^{R}) (Pfizer USA), sodium valproate (Epilim^{R}) (Sanofi-aventis Spain), distilled water (Juhel Pharmaceutical Nigeria) and Methanol stem bark extracts of *Alstonia boonei, Khaya grandifoliola* and methanol fruit extract of *Xylopia aethiopica.*

Animals

Swiss albino mice (18-25 g), and cockerels (30-40 g) were procured from the Department of Pharmacology and Therapeutics' Animal House Facility, Ahmadu Bello University, Zaria. Mice were kept in makeshift cages made of propylene, exposed to natural light and the day cycle, given an unlimited access to water and standard laboratory animal feed. Mice were acclimatized in the laboratory for five days before experimentation. Institutional Animal Ethics Committee's Approval was obtained with the approval No: ABUCAUC/2022/009. Experiments were carried out between 9:00 and 18:00 hours of the day.

Collection of Plant and Extraction

The leaves, fruits and stem barks of *Xylopia aethiopica,* and *Khaya grandifoliola* were collected in a bush in Zaria, Kaduna State, Nigeria while the leaves and stem barks of *Alstonia boonei* were collected in the bush Ado-Ekiti, Ekiti State Nigeria. The freshly harvested plant materials were identified by a Botanist; Mallam Umar Gallah in the Botany Department, Kaduna State University (KASU). A voucher specimen was prepared with voucher numbers: KASU/BSH/664 (*Xylopia aethiopica*); KASU/BSH/2340 (*Khaya grandifoliola*); and KASU/BSH/6771 (*Alstonia bonnei*) and deposited in the Botany Department Herbarium Unit, Kaduna State University, Kaduna State.

The stem barks of *Khaya grandifoliola, Alstonia boonei* and fruits *of Xylopia aethiopica* were removed, washed and air-dried separately under the shade to achieve a constant weight after which they were separately reduced to coarse powder using mortar and pestle. The powdered materials; *A. bonnei* 2.5 kg; *K. grandifoliola* 1.8 kg; and *X. aethiopica* 325 g were extracted separately with 8, 3.5 and 1.5 L of 70 % methanol respectively by cold maceration and shaken occasionally for one week. The macerated mixtures were filtered using a mucilage cloth and allowed to sediment for 24 hours before decantation. The decanted filtrates were heated on a water bath at a temperature 40 ^{0}C to obtain a dried solid mass subsequently referred to methanol stem bark extracts of: *K. grandifoliola* (MEKG); *Alstonia boonei* (MEAB); and methanol fruit extract of *Xylopia aethiopica* (MEXAF) from which the doses to be administered were prepared just before the experiment. The yields of the extracts in percentage were calculated and thereafter preserved in a desiccator until needed for use.

Phytochemical Analysis

The extracts: MEAB, MEKG and MEXAF were screened for class of phytochemical constituents present according to the method described by [14].

Quantification of Phytochemical Constituents

Phenolics Determination

According to Folin-Ciocalteu method [15], the phenolic content was determined spectrophotometrically. In summary, separate test tubes were pipetted with the plant extracts (0.5 mL of 1 mg/mL); standard gallic acid (0.02-0.1 mg/mL), and the dissolving solvent serving as control. Two and half milliliters of 10% v/v Folin-Ciocalteu's reagent were added to this. The mixture was vortexed and the reaction was allowed to stand for about five minutes at room temperature. After that, the mixture was vortexed, 2 mL of 7.5 % anhydrous sodium carbonate was added, and it was then allowed 30 minutes incubation at 40 °C. As a blank, the control solution was employed. After incubation, the absorbance was measured at 765 nm using the ultraviolet-visible spectrophotometer. Three replicates of the experiment were conducted. Based on the graph equation ($y = 0.0004x – 0.00023$, R^2: 0.9789) of gallic acid standard/calibration, the phenol content was extrapolated. It was then expressed as gallic acid equivalent mg/g using the CV/m equation, where "m" refers to the mass of extract in grams, "V": volume used to dissolve the extract milliliters, and C: concentration as determined by the standard graph equation in µg/mL.

Flavonoid Determination

We employed the colorimetric assay for aluminum chloride, as reported in [16]. The methodology relies on measuring the yellow-orange hue that results from the reaction of flavonoid with $AlCl_3$. In summary, separate test tubes were filled with 0.5 mL aliquots of the extract mixtures (1 mg/mL), 0.2–1 mg/mL of the standard (quercetin), and the dissolution solvent (i.e the control). Two milliliters of distilled water was added to each test tube, 0.15 mL sodium nitrite (5%) was thereafter added to the mixtures and then allowed to stand for 6 minutes. After then, 0.15 mL of $AlCl_3$ (10%) was added, allowed to stand for an additional five minutes before adding 1 mL of 1 M NaOH. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 510 nm using the control solution after adding distilled water to make it up to 5 mL. Three replicates of the experiment were conducted. In the same way as previously mentioned in the phenolics above, the flavonoid content was determined using the equation, $y = 0.0034x + 0.0009$ form the calibration curve, $R^2 = 0.9963$, and expressed as mg/g of quercetin equivalent using the formula CV/m.

Tannin Content

The protocol outlined in reference [17] was accepted. Three milliliters of vanillin-methanol (4% w/v) and 1.5 mL HCl acid were added to 0.5 mL of the various solvent extracts (1 mg/mL). Various concentrations, 0.02-1 mg/mL of the standard tannic acid, and the control (dissolution solvent) were prepared. The mixture was then vortexed. For fifteen minutes, the solution was made to stand at room temperature. The absorbance of the solution was measured at 725 nm. Three replicates of the experiment were conducted. Tannic acid equivalent (CE)/g was calculated using the $y = 0.0003x - 0.0027$, calibration graph equation, $R^2 = 0.9960$, and then expressed as µg using the CV/m formula, as previously discussed in phenolics.

Alkaloid Content

The procedure outlined by Fazel et al. [18] was adopted in determining the total alkaloid content. After dissolving the plant extract (1 mg/mL) in 2 N HCl, the mixture was filtered. 0.1 N NaOH was used to bring the phosphate buffer solution's pH to neutral. Five milliliters of bromocresol green solution and 5 mL phosphate buffer were added to 1 mL of the solution, which had been transferred to a separating funnel. After giving the mixture a good shake, the complex that had formed was extracted using chloroform. Chloroform was used to dilute the extract to volume in a 10 mL volumetric flask. At 472 nm, the complex's absorbance in chloroform was measured. Every experiment was run three times, and the outcomes were averaged. The equation, $y = 0.0055x - 0.0163$ from the calibration graph, $R^2 = 0.9838$, was used to extrapolate the alkaloid content. The content was then expressed as µg/g atropine equivalent using the formula, CV/m, as previously discussed in phenolics.

Total Saponins

The procedure described by Makkar [19] was followed based on the colorimetric reaction of vanillin-sulphuric acid. Fifty microliters of plant extract was mixed with 250 µL of distilled water. Thereafter, 250 µL of vanillin reagent (i.e 800 mg vanillin per 10 mL of 99.5% ethanol) was added. Then 2.5 mL of sulphuric acid (72%) was added, then mixed, kept in a water bath at 60 °C for 10 min. This was then cooled in ice cold water, the absorbance read at 544 nm wavelength. The values were expressed as diosgenin equivalents expressed as mg/g extract derived from a standard curve.

Acute Toxicity Study

The guidelines for the limit test described by the Organization for Economic Cooperation and Development (OECD 425) [20] were used to estimate the oral median lethal dose (LD50) of MEAB. Briefly, 5 female nulliparous and non-pregnant mice housed individually in the single cages were used for the study. Each mouse was fasted for 4 hours and weighed before drug administration. A 5000 mg/kg dose of methanol stem bark extract of *A. boonei* was administered per oral to a mouse after which food was withheld for 2 hours and then observed within 48 hours for mortality. Thereafter, the remaining four mice were dosed as previously done for the first mouse and observed individually for at least half an hour in the first 24 hours with much focus at the initial 4 hours, then daily for 14 days. Observation of signs of toxicity made were changes in skin, fur, eyes, mucus membranes, somatomotor activity, tremor, diarrhoea, lethargy, sleep, respiratory, circulatory, behavioural pattern and coma. At the end, all the surviving animals were humanely sacrificed. The same procedure was repeated for MEKG and MEXAF at doses of 5000 and 2000 mg/kg respectively.

Thin Layer Chromatography (TLC)

The TLC profiling of MEXAF (0.1 g) solution made by dissolving in 10 mL of methanol was carried. The sample was applied on the TLC plates using a capillary tube and thereafter, developed in a developing chamber containing a mixture of solvents in different proportions: hexane: ethyl acetate (7:3); ethyl acetate: methanol (9:1); ethyl acetate: methanol: water (10:2:1); ethyl acetate (100 %) and ethyl acetate: chloroform: methanol: water (15:4:4:1). The developed plates were dried in a fume hood and photographs were taken by using an inbuilt 8-megapixel camera. Thereafter, the developed plates were sprayed with P-anisaldehyde for general test and heated in an oven and photographs were taken.

For specific tests, plates were further developed in solvent systems of ethyl acetate: chloroform: methanol: water (15:4:4:1); ethyl acetate: methanol: water (10:2:1); hexane: ethyl acetate (7:3) and each sprayed with specific reagents; Dragendorff for alkaloids, Bontrager for anthraquinones, aluminium chloride for flavonoids, ferric chloride for tannins and phenolics and Lieberman Burchard for steroids and triterpenes. These were then heated in an oven and observed for spots colour changes. The same procedure was repeated for MEAB and MEKG.

Anticonvulsant Screening

Pentylenetetrazole-induced Seizure Test

The procedure of Löscher & Schmidt, [21] was followed. Sixty-four animals were grouped into 11 of six mice each at random. Groups 1 and 2 were given 10 mL/kg of distilled water and 200 mg/kg of sodium valproate orally respectively. Groups: 3-5 received 75, 150, and 300 mg/kg of MEAB; 6-8 were treated with 75, 150 and 300 mg/kg of MEKG and; 9-11 were administered 75, 150 and 300 mg/kg of MEXAF respectively per oral. One hour after drug administration, PTZ (85 mg/kg,) was administered subcutaneously to all the groups of animals and observed for a period of 30 minutes for myoclonic jerk, tonic spasm and mortality. Animals were considered to abolish the effect of PTZ if no general clonus/tonic spasm or mortality occurred during the period of observation. Percentage protection against seizure was calculated using Eq. 1.

$$Percentage\ protection = \frac{N_p}{N_g} \times 100 \quad \text{.....Eq. 1}$$

N_p = number of animals protected against seizure
N_g = number of animals in the group

Maximum Electroshock Test (MEST)

The study was carried out based on Löscher & Schmidt, [21] method. Eleven groups of six cockerels each were created by randomly dividing the sixty-four animals. Groups 1 and 2 were given oral doses of 10 mL/kg of distilled water and 25 mg/kg of phenytoin respectively. Groups: 3-5 received MEAB (75, 150, and 300 mg/kg); 6-8 received MEKG (75, 150 and 300 mg/kg); and 9-11 received MEXAF (75, 150 and 300 mg/kg) per oral respectively. One hour after drug administration, animals received trans-auricular electroshock (85 mA; 50 Hz; 0.6 ms) through the eye-lid electrodes using a stimulator apparatus and then observed for tonic extension of the hind-limbs. The ability of the compound to prevent tonic extension of the hind limb and or reduced the recovery time from stupor is considered anticonvulsant activity [21]; [22]. The same procedure was repeated for MEKG and MEXAF. Percentage protection against tonic hind limb extension was calculated using Eq. 2.

$$Percentage\ protection = \frac{N_p}{N_g} \times 100 \quad \text{.....Eq. 2}$$

N_p = number of animals protected against tonic hind limb extension
N_g = number of animals in the group

Statistical Analysis

Means ± SEM were used to express the data. Statistical analysis of One-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test, was used with a p-value of less than 0.05 taken to be statistically significant.

RESULTS

Percentage Yield of Different Extracts

The percentage yield of the different extracts was 1.68; 11.95 and 24.51 for MEAB; MEKG and MEXAF respectively (Table 1).

Phytochemical Constituents of Different Plant Extracts

MEAB, MEKG and MEXAF contain flavonoids, cardiac glycosides, tannins, saponins and steroids. However, none of the extracts contain anthraquinones (Table 2).

Quantification of Phytochemical Constituents Present in Different Extracts

The quantity of the class of phytochemical constituents present in different extracts expressed in mg/g. MEXAF has the highest content of tannins, alkaloids, flavonoids and phenolics. Saponins was highest in MEKG with least quantity of alkaloids. The amount of alkaloid was higher MEAB than MEKG and MEAB contains the least quantity of flavonoids followed by MEKG (Table 3).

Thin Layer Chromatogram of Plant Extracts in Different Solvent System under UV at 366 nm

Chromatogram of extracts in different solvent system (Hexane: Ethyl acetate, 7:3; and 100 % ethyl acetate) under UV observation shows a number of spot bands. A compound from the chromatographic profile could be seen to be common to all the extracts but with different surface area and intensity. The ethyl acetate chromatogram eluted a greater number of bands than the hexane: ethyl acetate solvent system (Plate 1).

Thin Layer Chromatogram of Extracts in Different Solvent System After Para-anisaldehyde Spray

Chromatogram of extracts in different solvent system (Hexane: Ethyl acetate, 7:3; Ethyl acetate: Methanol, 9:1; Ethyl acetate: Methanol: Water, 10:2:1; 100 % Ethyl acetate; Ethyl acetate: Chloroform: Methanol: Water, 15:4:4:1) after spraying with P-anisaldehyde showing different colours of spots. The hexane: ethyl acetate extracted more compounds than other solvent systems. A green spot of compound was found to be unique to MEAB which was more conspicuous in all other solvent systems except hexane: ethyl acetate (Plate 2).

Table 1: Percentage Yield of Different Extracts

Extracts	Weight of Powder (g)	Weight of Extract (g)	Percentage Yield (%)
MEAB	2500	42.1	1.68
MEKG	1870	223.5	11.95
MEXAF	325	100	24.51

MEAB: Methanol Stem Bark Extract of Alstonia boonei; MEKG: Methanol Stem Bark Extract of Khaya grandifoliola; MEXA: Methanol Fruit Extract of Xylopia aethiopica

Table 2: Phytochemical Constituents of Different Plant Extracts

Phytochemicals	MEAB	MEKG	MEXAF
Phenolics	+	+	+
Flavonoids	+	+	+
Cardiac Glycosides	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Steroid and Triterpenes	+	+	+
Alkaloids	+	+	+
Anthraquinones	-	-	-

+: Present; -: Absent; MEAB: Methanol Stem Bark Extract of Alstonia boonei;
MEKG: Methanol Stem Bark Extract of Khaya grandifoliola; MEXAF: Methanol Fruit Extract of Xylopia aethiopica

Table 3: Phytochemical Quantification of Different Plant Extracts

Phytochemicals	MEAB	MEKG	MEXAF
Alkaloids (mg/g)	8.00	6.30	11.88
Phenolics (mg/g)	23.08	148.08	185.58
Flavonoids (mg/g)	3.89	7.77	29.24
Tannins (mg/g)	55.23	181.9	227.9
Saponins (mg/g)	9.73	30.422	15.48

MEAB: Methanol Stem Bark Extract of Alstonia boonei; MEKG: Methanol Stem Bark Extract of Khaya grandifoliola; MEXAF: Methanol Fruit Extract of Xylopia aethiopica

Thin Layer Chromatogram of Extracts in Different Solvent Systems after Specific Reagent Spray

Liebermann Burchard spray showed a number of spots with reddish brown and green colorations. The chromatogram of Drangendorff spray did not show any visible spot coloration. The chromatogram of ferric chloride spray shows black green colorations. In the chromatogram of Bontrager's spray, MEKG showed a pink coloration. The chromatogram of aluminum chloride spray showed a yellow colour of spots (Plate 3).

Acute Toxicity Study

The oral medal lethal dose of methanol stem bark extracts of *Alstonia boonei* and *Khaya grandifoliola* are estimated to be ≥ 5000 mg/kg while that of the methanol fruit extract of *Xylopia aethiopica* was estimated to be ≥ 2000 mg/kg.

Anticonvulsant Study

Effect of Plant Extracts on Pentylenetetrazole-Induced Seizure Properties in Mice

In the PTZ-induced seizure test, MEAB at all doses non-signifcantly ($p>0.05$) increased the onsets of seizure and death with a highest percentage protection of 66.67 against mortality

at 75 mg/kg. MEKG neither protected the animals against mortality nor significantly increased the onset of seizure and death. MEXAF protected 100 % of the animals against mortality at 300 mg/kg and 66.67 % protection at 75 and 150 mg/kg. However, there was a non-significant ($p>0.05$) increase in the mean onset of seizure and death only at 75 mg/kg (Table 4).

Effects of Different Plant Extracts against Maximal Electroshock-Induced Seizure in Chicks

MEAB protected 16.67 % of the animals against tonic hind limb extension only at 75 mg/kg but non-significantly ($p>0.05$) decreased the mean recovery time at all doses. MEKG non-significantly ($p>0.05$) reduced the recovery time at all doses

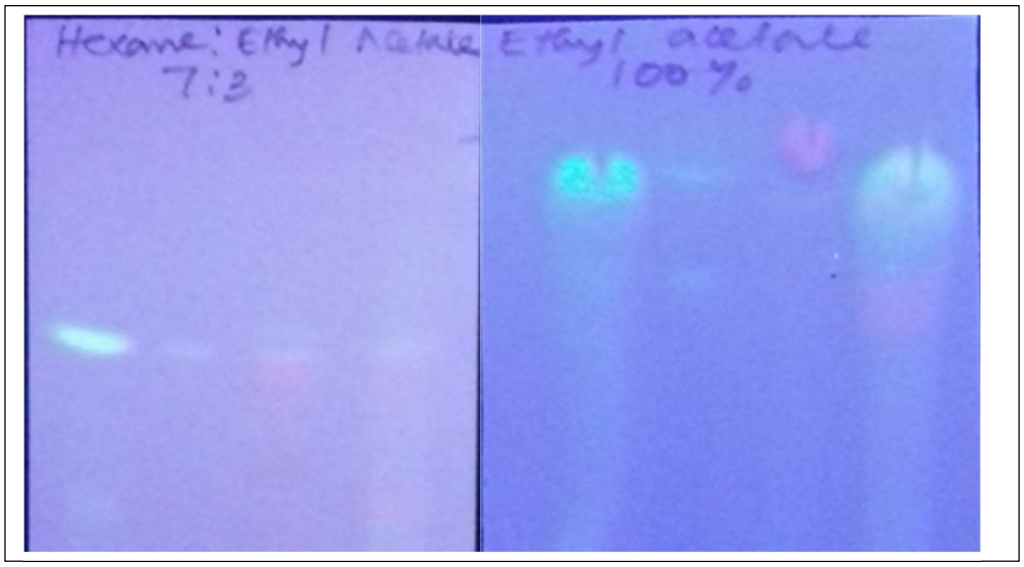

Plate 1: Thin Layer Chromatogram of Extracts in Different Solvent System under UV (366 nm) Observation

Mb: *MEAB: Methanol Stem Bark Extract of Alstonia boonei;* ***Mk:*** *MEKG: Methanol Stem Bark Extract of Khaya grandifoliola; Ms: Methanol Stem Extract of Xylopia aethiopica, Methanol Fruit Extract of Xylopia aethiopica*
Mf: *MEXAF: Methanol Fruit Extract of Xylopia aethiopica*

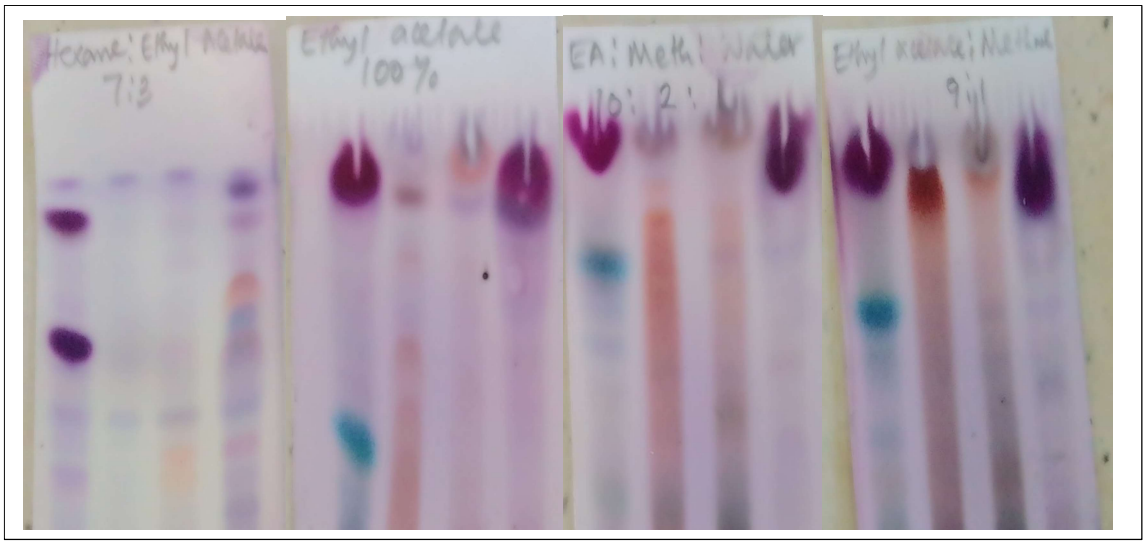

Plate 2: Thin Layer Chromatogram of Extracts in Different Solvent System After Para-anisaldehyde Spray

Mb: *MEAB: Methanol Stem Bark Extract of Alstonia boonei;* ***Mk:*** *MEKG: Methanol Stem Bark Extract of Khaya grandifoliola;* ***Ms****: Methanol Stem Extract of Xylopia aethiopica;* ***Mf:*** *MEXAF: Methanol Extract Fruit Extract of Xylopia aethiopica Fruit; EA: Ethyl acetate; Ch: Chloroform; Me: Methanol; Wa: Water*

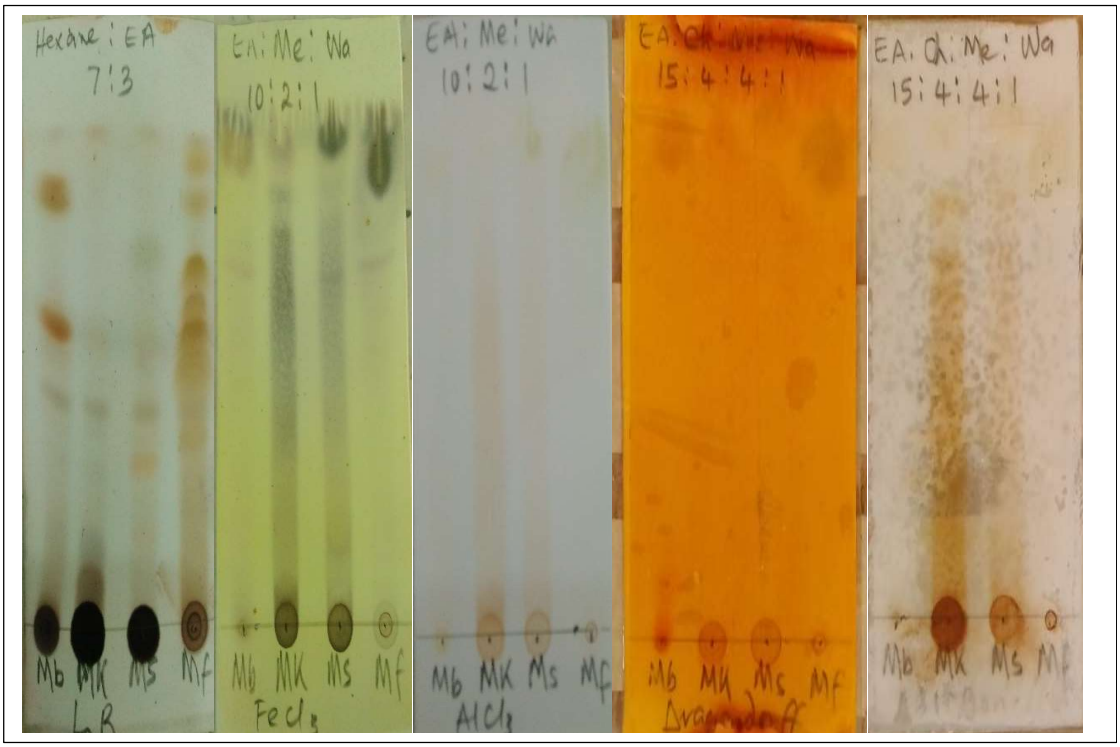

Plate 3: Thin Layer Chromatogram of Extracts in Different Solvent Systems after Specific Reagent Spray

Mb: *MEAB: Methanol Stem Bark Extract of Alstonia boonei;* ***Mk:*** *MEKG: Methanol Stem Bark Extract of Khaya grandifoliola;*
Ms: *Methanol Stem Extract of Xylopia aethiopica;* ***Mf:*** *MEXAF: Methanol Fruit Extract of Xylopia aethiopica;*
EA: Ethyl acetate; Ch: Chloroform; Me: Methanol; Wa: Water; $AlCl_3$: Aluminum Chloride; $FeCl_3$: Ferric Chloride

Table 4: Effect of Different Extracts on Pentylenetetrazole-Induced Seizure Properties in Mice

Treatment mg/kg	Mean Onset of Seizure (min)	Mean Onset of Death (min)	Percentage Protection Against Mortality
D/W 10 (mL/kg)	2.83±0.40	6.50±0.67	0
SV 200	6.17±0.48	-	100
MEAB 75	8.00±1.85	7.00±1.00	66.67
MEAB 150	5.67±1.31	15.25±0.63	33.33
MEAB 300	4.83±0.75	13.75±2.78	33.33
MEKG 75	6.33±1.58	9.00±2.52	0
MEKG 150	4.17±0.48	8.00±0.58	0
MEKG 300	3.00±0.37	7.50±0.99	0
MEXAF 75	8.17±2.41	22.00±8.00	66.67
MEXAF 150	4.56±0.34	13.00±3.00	66.67
MEXAF 300	4.00±0.52	-	100

Date presented as percentage protection against mortality, mean ± SEM for onset of seizure and death compared with D/W group Bonferroni post hoc test; n = 6; SV: Sodium Valproate; MEAB: Methanol Stem Bark Extract of Alstonia boonei; MEKG: Methanol Stem Bark Extract of Khaya grandifoliola; MEXAF: Methanol Fruit Extract of Xylopia aethiopica

Table 5: Effects of Different Extracts against Maximal Electroshock-Induced Seizure in Chicks

Treatment mg/kg	Percentage Protection Against THLE	Mean Recovery Time (min)
DW 10 (mL/kg)	0	9.83 ± 2.15
PNT 25	100	-------
MEAB 75	16.67	3.33 ± 1.09
MEAB 150	0	4.67 ± 1.02
MEAB 300	0	5.83 ± 0.65
MEKG 75	33.33	3.17 ± 1.04
MEKG 150	0	4.33 ± 1.05
MEKG 300	16.67	3.83 ± 1.04
MEXAF 75	50	2.17 ± 1,01*
MEXAF 150	16.67	5.50 ± 1.12
MEXAF 300	16.67	6.17 ± 0.98

*Data presented as % Protection against (THLE) tonic hind limb extension, mean ± SEM for recovery time, *p<0.01 compared with D/W group Bonferroni post hoc test; n = 6; PNT: Phenytoin; MEAB: Methanol Stem Bark Extract of Alstonia boonei; MEKG: Methanol Stem Bark Extract of Khaya grandifoliola; MEXAF: Methanol Fruit Extract of Xylopia aethiopica*

with 33.33 % protection against tonic hind limb extension only at 75 mg/kg. MEXAF at 75 mg/kg significantly *(p<0.01)* reduced the mean recovery time and protected 50 percent of the animals against tonic hind limb extension (Table 5).

DISCUSSION

Medicinal plants have enjoyed a wide patronage in the treatment of epilepsy most especially in rural and urban areas of low- and -middle come countries due to high incidence of epilepsy, poor healthcare facilities and access to quality antiseizure drugs [11,12]. Moreover, the failure of even the newer antiseizure drugs in controlling seizures in about 30 % people living with epilepsy have also made them attractive in search of better drugs for epilepsy treatment. Therefore, anticonvulsant screening of plants use in traditional system of medicine will not only establish pharmacological rationale for the ethnomedicinal uses but also serve as potential sources of newer and better antiseizure drugs.

The pentylenetetrazole-induced seizure and maximum electroshock test methods have remained a gold standard for screening potential anticonvulsant drugs [23]. Pentelentetrazole is a central nervous system stimulant and a convulsant which binds to the picrotoxin recognition site at the benzodiazepine-GABA-chloride ionophore receptor complex thereby noncompetitively inhibits the action of GABA on chloride conductance. This therefore reduces the inhibitory synaptic functions, thus increasing neuronal activity and subsequently causing generalized seizures in animals [24,25]. A single systemic injection of high dose PTZ induces acute seizure and mortality. Antiseizure drugs such as sodium valproate, phenobarbital, ethosuximide, retigabine and vigabatrin mitigate PTZ-induced seizure aggravation [26,27]. In our study using the high dose PTZ-induced seizure, the methanol stem bark extracts of *Alstonia boonei* and *Xylopia aethiopica* were able to increase the onset of seizure and prevent mortality and hence possess anticonvulsant activity against PTZ-induced seizure. We speculate from our study that, better anticonvulsant activity of MEAB may however reside at lower doses because the least tested doses of 75 mg/kg gave a better activity. This can also be possible because plant extract contains a large number of phytoconstituents with varying pharmacological activities whereby a convulsant and anticonvulsant compound can both be isolated from a plant extract. In contrast, the methanol fruit extract of *Xylopia aethiopica* extract, there was a better anticonvulsant activity as the dose increases at which maximum effect was observed at 300 mg/kg. However, MEKG did not show activity against PTZ-induced seizure at all tested doses due to inability to increase the onset of seizure and protection against mortality. Therefore, the ability of the methanol stem bark extract of *Alstonia boonei* and fruit extract of *Xylopia aethiopica* to mitigate PTZ-induced seizure may be due to the facilitation of GABA mediated chloride conductance that enhances the inhibitory synapses thus suppressing excessive neuronal hyperexcitation typical of seizure.

The maximal electroshock test is an experimental paradigm that induces acute epileptic seizures through an application of sufficient current capable of causing synchronous neural discharges in the brain [28,29]. It is an effective test capable of predicting drugs active against generalized seizures of the tonic–clonic (grand mal) type or preventing seizure spread through neural tissue [26,20,30]. Drugs such as carbamazepine, phenytoin effective in maximum electroshock test majorly act on Na^+ channels. However, a number of, and newly developed anticonvulsant drugs are effective in this model despite their interactions with other drug targets [31-33]. In the maximum electroshock test, the methanol stem bark extract of *Alstonia boonei* and *Khaya grandifoliola* and the fruit extract of *Xylopia aethiopica* at all dose showed anticonvulsant activities by being able to decrease the mean recovery time from stupor following tonic hind limb extension. However, there was much variation in the doses of the extracts in the

percentage protection against tonic hindlimb extension. The methanol fruit extract of *Xylopia aethiopica* showed highest anticonvulsant activity at 75 mg kg dose by being able to protect 50% of the animals against tonic hind limb extension. Similarly, anticonvulsant potential was not observed with MEKG and MEAB due to inability to protect at least 50 % of the chicks against tonic extension of the hind limb at all the doses. However, 33.33 and 16.67 % protection were observed at 75 mg/kg of MEAB and MEKG. These observed little protections against tonic extension of the hind limb in the MEST are promising anticonvulsant activities because no antiseizure drug is potentially effective against all forms of seizures. The moderate anticonvulsant activity of MEAB and MEXAF observed in the MEST tests imply possible interactions on the sodium ion channel blockade.

Methanol could be regarded as an efficient solvent of extraction for stem bark extract of *K. grandifoliola* and fruit extract of *X. aethiopica* but not for stem bark of *A. boonei* due to low percentage yield observed. The extraction yield is a measure of the solvent efficiency to extract specific components from the original material and gives an idea about the extractability of the plant [34]. Selecting the right solvent and extraction techniques is crucial for optimizing extract yield [35] and may also have an impact on pharmacological activity. Selecting the right solvent and extraction techniques is crucial for optimizing extract yield [35] and may also have an impact on pharmacological activity. Therefore, we speculate that the reduced anticonvulsant activities of MEKG and MEAB at all doses tested maybe due to poor extraction of bioactive compound in sufficient quantity responsible for anticonvulsant activity or abundant extraction of bioactive compound that may exert some level of proconvulsant activity. To further buttress our claim, anticonvulsant activity correlates well with higher alkaloid and lower saponins contents as observed with MEXAF and MEAB when comparing the quantification of the different extracts.

In the quantitative phytochemical analysis, MEXAF contain highest quantity of alkaloid, flavonoids, tannins and phenolics followed by MEKG (except for saponins content) then MEAB. In the qualitative phytochemical screening; flavonoids, alkaloids, saponins, tannins, cardiac glycosides were present in the extracts of which some were confirmed using the thin layer chromatography. These bioactive compounds have been known to possess many therapeutic effects either by acting singly or synergistically. Alkaloids, flavonoids, terpenoids, saponins, have been reported to have anticonvulsant activity [36]. Several types of alkaloids such as diterpenoid aconitum alkaloids, isoquinoline alkaloids, indole alkaloids, piperidine alkaloids, amide alkaloids, tetracyclic oxindole alkaloids etc have been reported to have anticonvulsant activity [36]. Plants contain a class of naturally occurring substances called flavonoids, which have a variety of pharmacologic qualities, including the ability to prevent seizures in several seizure models. [37-39]. Steroidal saponins have also be reported to have anticonvulsant activities [40]. Terpenoids such as citronellol, an acyclic monoterpene alcohol, (S)-(+)-carvone, α-terpineol have demonstrated anticonvulsant activity in PTZ- and picrotoxin-induced convulsions and MES-induced seizures [41]. The extracts are relatively safe based on oral median lethal dose estimated to be ≥ 2000 mg/kg.

CONCLUSION

Methanol stem bark extracts of *A. boonei, K. grandifoliola* and *X. aethiopica* fruit possess varying degree of anticonvulsant activities which correlates with high alkaloid and low saponins content. This therefore, provides the pharmacological credence for the use of these plants in the treatment of epilepsy in Nigeria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

Bukhari Mahmud: designed, carried out the Lab work, analysed data and drafted the manuscript. Sunday Abraham Musa: designed, supervised the work and proofread the manuscript. Mohammed Garba Magaji: designed, supervised the work and proofread the manuscript. Nuhu Mohammed Danjuma: designed, supervised the work and proofread the manuscript.

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