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ANTICONVULSANT POTENTIAL OF METHANOL STEM BARK EXTRACT OF *MILICIA EXCELSA* (MORACEAE) IN MICE

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ABSTRACT

Milicia excelsa stem bark is used as an anticonvulsant in Nigerian folkloric medicine but is yet to be scientifically evaluated. Hence, this study assessed the anticonvulsant property of the crude methanol stem bark extract of *Milicia excelsa* (MSB) in mice. The anticonvulsant potential of the extract was assessed using Picrotoxin-, Pentelynetetrazole-, Strychnine-, and Isoniazid-induced convulsion models in mice. The phytochemicals present in the extract were assayed using spectrophotometer while the different functional groups in the extract were determined using Fourier Transform-infra red (FT-IR) analyzes. The extract showed significant ($p \leq 0.05$) prolongation of the onset of tonic convulsion and death latencies with 50% protection at different doses in all the anticonvulsant models used. The total alkaloid in the extract was estimated to be 72.7 ± 20.7 mg atropine equivalent per gram of MSB, the tannin content to be 70.2 ± 3.6 mg gallic acid equivalent per gram of MSB while total flavonoid was estimated to be 32.7 ± 5.1 mg quercetin equivalents per gram of MSB. The FT-IR analysis confirmed the presence of functional groups in alkaloid. This study concluded that extract may have anticonvulsant property which may be attributed to the abundance of alkaloid, either in synergy or additive to other phytochemicals present in *Milicia excelsa* stem bark extract.

Keywords: Alkaloids, anticonvulsant, Fourier transform-infra red analysis, *Milicia excelsa*, tannins

INTRODUCTION

Epilepsy is adjudged as the second most prevalent and severe neurological aberrations after stroke (Sander and Shorvon, 1996; Hema *et al.*, 2009), which accounts for one in hundred (1%) of the global burden of diseases (Vyawahare *et al.*, 2007). Seizure occurs as a consequence of a sudden disparity between the excitatory and inhibitory neurotransmission which subsequently favours excitations in the network of cortical neurons in the brain (Cakmak, 2006). Seizure may also be precipitated by the excessive/overproduction of acetylcholine, norepinephrine, and

serotonin in the brain (Fathima *et al.*, 2015). γ-aminobutyric acid (GABA) has been implicated in convulsion induced by picrotoxin, isoniazid, and pentylenetetrazole (Ishola *et al.*, 2013) and glycine antagonism on glycine receptors in seizures induced by strychnine in mice (Ishola *et al.*, 2013).

Many epileptic patients do not experience seizure alleviation experience coupled with the undesirable effects associated with the optimal use of the currently available antiepileptic medications (Vyawahare *et al.*, 2007; Pandeya *et al.*, 2009), have hitherto warranted the search for affordable, tolerable, novel and effective drugs from botanicals, since numerous botanicals have been reviewed for their anticonvulsant potentials (Quintans-Junior *et al.*, 2008; Kumar *et al.*, 2012). Therefore, their extracts can be essential source of safer and better tolerated drugs for the treatment of epilepsy (Quintans-Junior *et al.*, 2008; Kumar *et al.*, 2012).

Milicia excelsa otherwise known as *Chlorophora excelsa* plant is a large deciduous tree which may grow up to 50 m high and found naturally in the humid forestry areas of the West Africa region (Agyeman *et al.*, 2009).

In Nigerian folkloric medicine, the stem bark is used to treat insanity (Sonibare *et al.*, 2008), convulsion (Wahab, 2015) and insomnia (Ariwaodo *et al.*, 2012) among other folkloric uses. Pharmacologically, the anti-inflammatory and sedative-hypnotic effects of the *Milicia excelsa* stem bark have earlier been reported (Olajide *et al.*, 2005; Akinpelu *et al.*, 2020a). Similarly, the leaf has been reported to possess anticonvulsant, antipsychotic, antihypoxic, antistress, anxiolytic, antiamnesic and cognitive enhancing effects (Akinpelu *et al.*, 2018a; Akinpelu *et al.*, 2018b; Akinpelu *et al.*, 2019a; Akinpelu *et al.*, 2019b; Akinpelu *et al.*, 2020b). The antidiarrheal and aphrodisiac activities of the root bark extract of *Milicia excelsa* have also been reported (Adebayo *et al.*, 2019; Gbolade *et al.*, 2022).

The study was designed to assess the anticonvulsant potentials of methanol stem bark extract of *Milicia excelsa* (MSB) in mice and the inherent phytocompounds that could be responsible for its pharmacological effects. This is in furtherance of the earlier ethonobotanical report of the stem bark being used in combating convulsion in folkloric medicine (Wahab, 2015).

Figure 1. A. The medicinal plant *Milicia excelsa* (Moraceae) and B. the stem barks used to prepare the plant extract

MATERIALS AND METHODS

Plant material and extraction

Milicia excelsa (Welw) C.C Berg stem bark was identified collected in January 2021 and authenticated by Mr. G. A. Ademoriyo of the Department of Botany, Faculty of Sciences, Obafemi Awolowo University (OAU), Ile-Ife. The collection was done within OAU, Ile-Ife campus. The herbarium number IFE-17482 was thereafter obtained. The stem bark of *Milicia excelsa* was cut into pieces and subsequently air dried at room temperature for two weeks. The dried stem barks were mechanically ground into fine powder and 0.5 kg of the fine powder was cold macerated with 2.5 liters of absolute methanol for 72 h. The filtrate was concentrated *in vacuo* set at 40^{o}C to produce 4 g (0.8%) yield of the crude extract.

Drugs

Strychnine, Picrotoxin, Pentylenetetrazol and Tween 20 were purchased from Sigma Chemicals Co, St. Louis, Missouri, U.S.A; Phenobarbitone from May and Baker, Lagos, Nigeria; Isoniazid from Mancleods Pharmaceuticals Ltd., India, Diazepam (Roche, Basel, Switzerland) and normal saline (N/S) (Unique Pharmaceutical Limited, Lagos, Nigeria). All the drugs were prepared fresh on each day of the experiment.

Laboratory animals

Adult male mice (18-25 g) were gotten from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife. They were allowed to acclimatize to the laboratory conditions under 12 hr day/12 hr dark cycle for 14 days. The mice were fed with standard animal pellets and had free access to clean drinkable water. Experimental procedures were carried out in conformity with the National Institute of Health (NIH, 1985). They were fasted overnight prior to the commencement of the behavioural assays.

Spectrophotometric phytochemical estimation of total alkaloid in the extract

The total alkaloid in MSB was quantified as earlier described (Adebayo *et al.*, 2019). Briefly, the absorbance of the extract and atropine standard solutions (20, 40, 80 and 100 µg/ml) were measured against reagent blank at 470 nm using UV/Visible spectrophotometer. The total alkaloid was estimated in mg of atropine equivalent (AE) per gram of MSB.

Spectrophotometric phytochemical estimation of total flavonoid in the extract

The total flavonoid in MSB was quantified as earlier described using aluminium chloride colorimetric assay (Adebayo *et al.*, 2019). Briefly, the absorbance of the extract and quercetin standard solutions (20, 40, 80 and 100 µg/ml) were measured against reagent blank at 510 nm using UV/Visible spectrophotometer. The total flavonoid content was estimated in mg of quercetin equivalent (QUE) per gram of MSB.

Spectrophotometric phytochemical estimation of tannin content in the extract

The tannin content in MSB was quantified using Folin-Ciocalteu method as earlier described (Adebayo *et al.*, 2019). Briefly, the absorbance of the MSB and gallic acid standard solutions (20, 40, 80 and 100 µg/ml) were measured against the blank at 725 nm using UV/Visible spectrophotometer. The tannin content of MSB was estimated in mg of gallic acid equivalent (GAE) per gram of MSB.

General experimental protocols

Adult male albino mice were grouped into 5 experimental groups (n=6). Group-I received vehicle (2% Tween 20 in N/S, 10 mL/kg, *p.o*). Group II-IV received oral MSB (125, 250 or 500 mg/kg), while Group-V

received intraperitoneal injection (i.p.) of standard anticonvulsant drug (positive control). The doses of the extract were selected based on the median lethal dose of ≥ 5000 mg/kg of MSB in earlier literature (Akinpelu *et al.,* 2020a).

Picrotoxin (PTX)-induced convulsions

One hour after oral administration of the vehicle (group 1) or MSB (125, 250 and 500 mg/kg in groups 2-4) or 30 minutes following i.p. injection of diazepam (1 mg/kg) in group 5, mice in all groups were intraperitoneally injected PTX (10 mg/kg). Mouse was singly monitored for 30 minutes immediately after the i.p. injection of PTX for the latency to the tonic-clonic convulsion and death time. Mouse that survived after the PTX injection beyond 30 minutes was adjudged protected (Velluci and Webster, 1984).

Pentylenetetrazole (PTZ)-induced convulsions

One hour after oral administration of the vehicle (group 1) or MSB (125, 250 and 500 mg/kg in groups 2-4) or 30 minutes following i.p. injection of diazepam (1 mg/kg) in group 5, mice in all groups were intraperitoneally injected PTZ (85 mg/kg). Mouse was singly monitored for 30 minutes immediately after the i.p. injection of PTZ for the latency to the tonic-clonic convulsion and death time. Mouse that survived after the PTZ injection beyond 30 minutes was adjudged protected (Swinyard *et al.,* 1989).

Isoniazid (INH)-induced convulsion

One hour after oral administered of the vehicle (group 1) or MSB (125, 250 and 500 mg/kg in groups 2-4) or 30 minutes following i.p. injection of diazepam (5 mg/kg) in group 5, mice in all groups were intraperitoneally injected INH (250 mg/kg). Mouse was singly monitored for 30 minutes immediately after the i.p. injection of INH for the latency to the clonic, tonic seizures and death time. Mouse that survived after the INH injection beyond 60 minutes was adjudged protected (Chindo *et al.,* 2014).

Strychnine (SCN)-induced convulsion

One hour after oral administration of the vehicle (group 1) or MSB (125, 250 and 500 mg/kg in groups 2-4) or 30 minutes following i.p. injection of Phenobarbitone (5 mg/kg) in group 5, mice in all groups were intraperitoneally injected SCN (4 mg/kg). Mouse was singly monitored for 30 minutes immediately after the i.p. injection of SCN for the latency to the tonic-clonic convulsion and death time. Mouse that survived after the SCN injection beyond 60 minutes was adjudged protected and percentage protection calculated (Swinyard *et al.,* 1989).

Statistical analysis

Results expressed as mean ± S.E.M (n=6). The significance difference between the treatment groups and control group were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test using GraphPad InStat® Biostatistics software (GraphPad Software, Inc., La Jolla, USA). The significance level was set at $p \leq 0.05$ compared to the control group.

RESULTS

Spectrophotometric quantitative phytochemical estimations of methanol extract of *Milicia excelsa* stem bark

Phytochemical quantification of the phytoconstituents in MSB showed the presence of total alkaloid to be 72.74 ± 20.71 mg of atropine equivalent per gram of MSB; total flavonoids of 32.68 ± 5.13 mg of quercetin equivalent per gram of MSB and tannin as 70.17 ± 3.60 mg of garlic acid equivalent per gram of MSB [Table 1].

Table 1: Phytochemical Estimation of Methanol Stem Bark Extract of *Milicia excelsa*

Phytoconstituents	Quantities
Total Alkaloid	72.7 ± 20.7 mg atropine equivalent per gram of MSB
Tannin	70.2 ± 3.6 mg gallic acid equivalent per gram of MSB
Total flavonoid	32.7 ± 5.1 mg quercetin equivalents per gram of MSB

Values denote ± SD (n=3).

Fourier transform infrared spectra of methanol extract of *Milicia excelsa* stem bark.

The FT-IR spectra of MSB showed the absorption band at 3336.0 cm^{-1} which indicates the presence of hydroxyl group (3400 – 3200 cm^{-1}), at 2972.9 cm^{-1} indicates C-H group (2975 – 2855cm^{-1}), at 1650 cm^{-1} revealed C=O group (1690 – 1640 cm^{-1}), the band at 1379.1 cm^{-1} indicates OH bend of phenols or tertiary alcohol (1410 – 1310 cm^{-1}), the absorption band at 1045.5 and 1085.5 indicate the existence of C-O-C functional group (1150 -911 cm^{-1}) while at 879.7 cm^{-1} indicates the occurrence of C-C group (800 – 1000 cm^{-1}) [Figure 1].

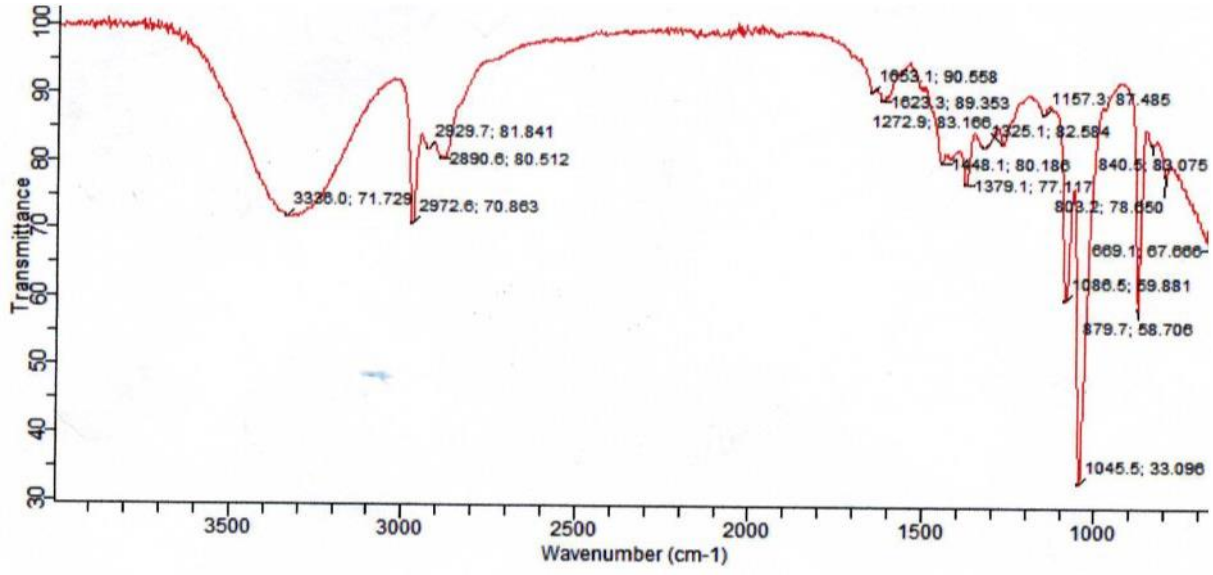

Figure 1: Fourier transform-infrared Spectrum of methanol stem bark extract of *Milicia excelsa*

Effect of methanol extract of *Milicia excelsa* stem bark on picrotoxin-induced convulsion

There was significant ($p<0.05$) delay in the latency to tonic convulsion at 125, 250 and 500 mg/kg, while the death time was only significantly ($p<0.05$) elongated at 250 and 500 mg/kg compared to the control. Diazepam (1 mg/kg) significantly ($p<0.05$) delayed the latencies to clonic and tonic convulsions and death time [Figure 2]. The MSB at 250 and 500 mg/kg protected 50% of the mice in each respective group while diazepam (1 mg/kg) protected 100% in the positive control group. However, MSB at 125 mg/kg offered no protection in picrotoxin-induced convulsion [Table 2].

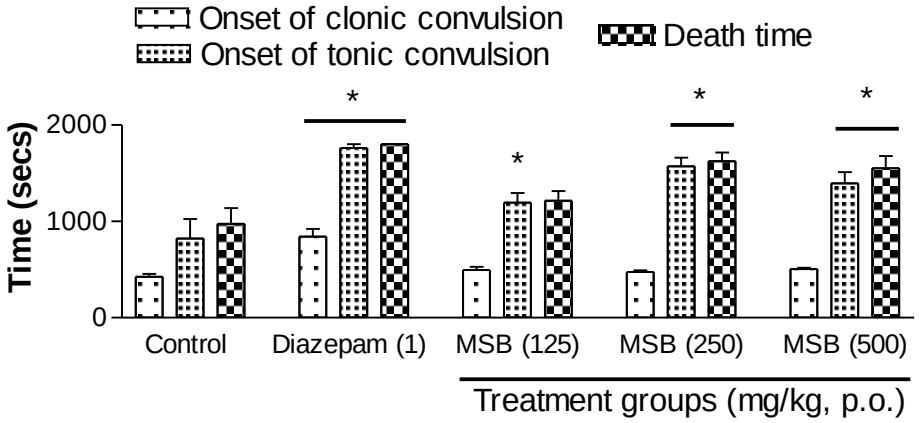

Figure 2: Effect of methanol stem bark extract of *Milicia excelsa* (MSB) on convulsions induced by picrotoxin in mice.
Each bar stands for Mean ± SEM (n=6), one-way ANOVA; Dunnett's post hoc test. *p<0.05 compared to control.

Table 2: Protective effect of methanol stem bark extract of *Milicia excelsa* in picrotoxin-induced convulsion

Treatment groups	Quantal protection	% protection
Control (10 mL/kg) + PTX	0/6	0
DZP (1 mg/kg) + PTX	6/6	100
MSB (125 mg/kg) + PTX	0/6	0
MSB (250 mg/kg) + PTX	3/6	50
MSB (500 mg/kg) + PTX	3/6	50

DZP; diazepam, PTX; picrotoxin, MSB; methanol stem bark extract of *Milicia excelsa*

Effect of Methanol Extract of *Milicia excelsa* Stem Bark on Pentylenetetrazole-induced Convulsion

There was significant (p<0.05) prolongation in the latency to tonic convulsion and death time at 250 mg/kg. Diazepam also significantly (p<0.05) extended the onset of clonic and tonic convulsions as well as the death time compared to the control (Figure 3). The MSB at 250 and 500 mg/kg offered 50% protection respectively while diazepam (1 mg/kg) protected 100% in the positive control group [Table 3].

Effect of Methanol Extract of *Milicia excelsa* Stem Bark on Convulsion Induced by Strychnine

The MSB (125, 250 and 500 mg/kg) and phenobarbitone (30 mg/kg) significantly (p<0.05) extended the onset of clonic, tonic convulsions and death time compared to the control (Figure 4). The MSB protected 50, 33.3 and 50% of the experimental mice at 125, 250 and 500 mg/kg respectively while Phenobarbitone (30 mg/kg) offered 50% protection in convulsion induced by SCN [Table 4].

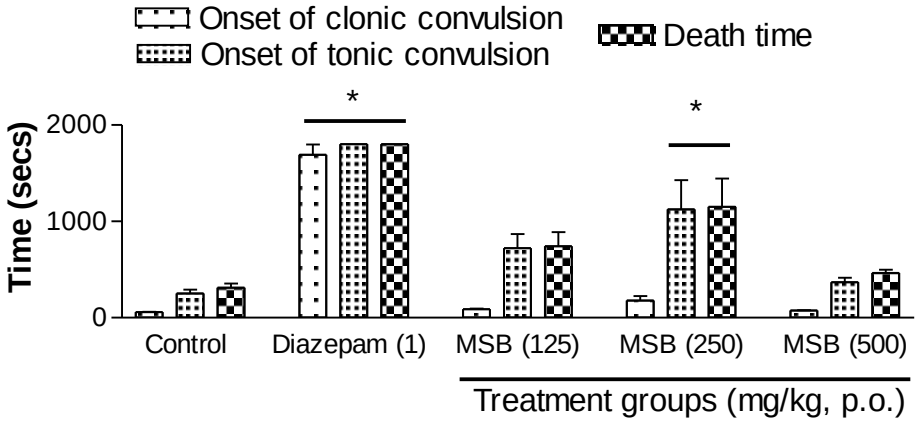

Figure 3: Effect of methanol stem bark extract of *Milicia excelsa* (MSB) on convulsions induced by pentylenetetrazole in mice

Each bar stands for Mean ± SEM (n=6), one-way ANOVA; Dunnett's post hoc test. *p<0.05 compared to control.

Table 3: Protective Effect of Methanol Stem Bark Extract of *Milicia excelsa* in Pentylenetetrazole-induced Convulsion

Treatment	Quantal protection	Percentage Protection
Control (10 mL/kg) + PTZ	0/6	0
DZP (1 mg/kg) + PTZ	6/6	100
MSB (125 mg/kg) + PTZ	0/6	0
MSB (250 mg/kg) + PTZ	3/6	50
MSB (500 mg/kg) + PTZ	3/6	50

DZP; diazepam, PTZ; pentylenetetrazole, MSB; methanol stem bark extract of *Milicia excelsa*

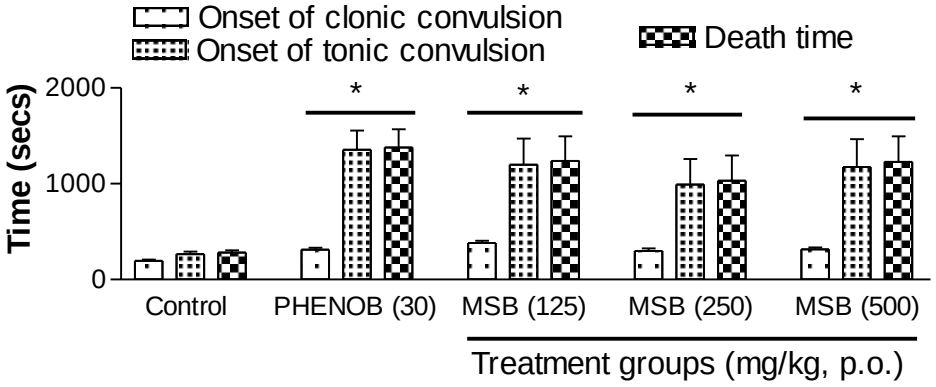

Fig. 4: Effect of methanol stem bark extract of *Milicia excelsa* (MSB) on convulsions induced by strychnine.

Each bar stands for Mean ± SEM (n=6), one-way ANOVA; Dunnett's post hoc test. *p<0.05 compared to control.

Table 4: Protective Effect of Methanol Stem Bark Extract of *Milicia excelsa* in Strychnine-induced Convulsion

Treatment	Quantal Protection	Percentage Protection
Control (10 mL/kg) + SCN	0/6	0
Phenobarbitone (30 mg/kg) + SCN	3/6	50
MSB (125 mg/kg) + SCN	3/6	50
MSB (250 mg/kg) + SCN	2/6	33.3
MSB (500 mg/kg) + SCN	3/6	50

SCN; strychnine, MSB; methanol stem bark extract of *Milicia excelsa*

Effect of methanol extract of *Milicia excelsa* stem bark on convulsion induced by isoniazid

The MSB at 500 mg/kg and diazepam (5 mg/kg) significantly ($p<0.05$) extended the onset of clonic, tonic convulsions and death time compared to the control while the onset of tonic convulsion was only significantly ($p<0.05$) extended at 250 mg/kg [Figure 5]. The MSB (500 mg/kg) and diazepam (5 mg/kg) protected 50 and 100% of the experimental mice respectively in convulsion induced by INH in mice [Table 5].

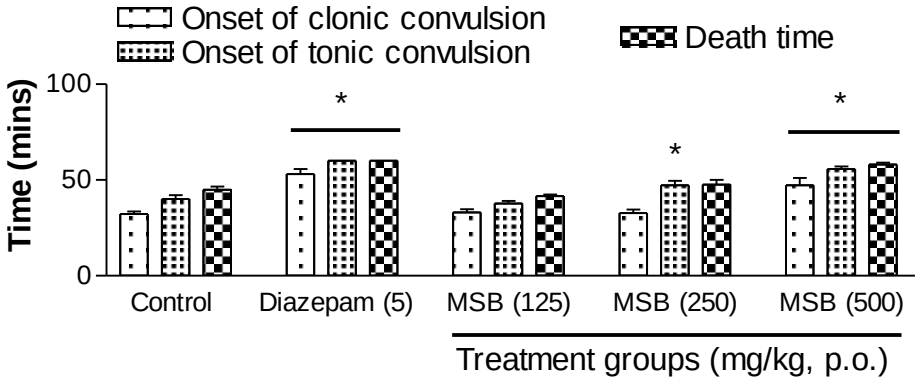

Figure 5: Effect of Methanol Stem Bark Extract of *Milicia excelsa* (MSB) on Convulsion induced by Isoniazid

Each bar stands for Mean ± SEM (n=6), one-way ANOVA; Dunnett's post hoc. *$p<0.05$ compared to control.

DISCUSSION

Epilepsy remains a medical unmet condition not only because many epileptic patients remain refractory to the existing pharmacological interventions (Bialer, 1997; Perruca *et al.*, 2007), but also, their adverse effects remain to be fully circumvented (Gates, 2000). Hence, the search for newer antiepileptic drug is pertinent and imperative. This study therefore, reported the anticonvulsant potential of methanol stem bark extract of *Milicia excelsa* using mouse models of convulsions widely used to screen medicinal plants for anticonvulsant activities (Daanaa *et al.*, 2018), thus providing scientific basis for its traditional use.

Table 5: Protective effect of methanol stem bark extract of *Milicia excelsa* in isoniazid-induced convulsion

Treatment	Quantal Protection	Percentage Protection
Control (10 mL/kg) + INH	0/6	0
DZP (5 mg/kg) + INH	6/6	100
MSB (125 mg/kg) + INH	0/6	0
MSB (250 mg/kg) + INH	0/6	0
MSB (500 mg/kg) + INH	3/6	50

DZP; diazepam, INH; isoniazid, MSB; methanol stem bark extract of *Milicia excelsa*

Previous scientific evaluation has reported the oral acute toxicity of *M. excelsa* stem bark to be ≥ 5g/kg in mice (Areola *et al.*, 2015). From the previous report of the oral acute toxicity of the extract, lower oral doses of 125, 250 and 500 mg/kg were used in the evaluation of the anticonvulsant potential of MSB reported in this study. The observed behavioural protection offered by MSB as well as the elongation of the latencies to tonic convulsion and death in convulsion induced by picrotoxin is indicative of an anticonvulsant potential of MSB. The observed anticonvulsant potential therefore, may be mediated via the boosting of the chloride currents through picrotoxin–sensitive chloride channels (Smith *et al.*, 2001). This behavioural finding agrees with many medicinal plants such as *Passiflora incarnata* and *Crinum glaucum*, known for their anticonvulsant potentials in convulsion elicited by picrotoxin in mice (Ishola *et al.*, 2013; Nassiri-Asl *et al.*, 2007).

Pentylenetetrazole, a GABA antagonist induces convulsion via the blockade of convulsion by blocking the γ-aminobutyric acid ($GABA_A$) receptor-Chloride channel complex and subsequently results in the inhibition of GABA-dependent activity. The prolongation in the latency to tonic convulsion and death time, as well as protection offered by MSB in this model showed that MSB may possess anticonvulsant bioactive ingredient(s). The MSB may therefore, be acting either to boost the affinity for GABA or prolong the duration of opening of the GABA gated channels (Corda *et al.*, 1990). This finding is in concordance with the anticonvulsant effects of *Crinum glaucum* against the chemoconvulsant property of PTZ in mice (Ishola *et al.*, 2013).

The anticonvulsant potential demonstrated by MSB against the chemoconvulsant property of SCN is suggestive of an anticonvulsant potential which may be acting via the facilitation of the inhibitory action on the glycinergic neurotransmission pathway in the spinal cord. This finding agrees with the previous reports of the beneficial effect of *Passiflora edulis* and *Hybanthus enneaspermus* plant extracts in mouse models of strychnine-induced convulsion (Ngo *et al.*, 2004; Hemalatha *et al.*, 2003).

The observed behaviuoral protection offered by MSB in convulsion induced by isoniazid is implicative of an anticonvulsant potential probably acting via facilitation of either GABA synthesis by stimulating the L-glutamate or hindering the GABA breakdown by GABA transaminase (Bassavaraj *et al.*, 2011). This behavioural finding agrees with the report of a medicinal agent with protective effect against the chemoconvulsant effect of isoniazid in mice (Bassavaraj *et al.*, 2011).

The spectrophotometric quantitatification of the phytoconstituents in MSB revealed the presence of reported therapeutic anticonvulsant phytocompounds such as alkaloids (Quintans-Júnior *et al.,* 2007; Bhutada *et al.,* 2010), flavonoids and polyphenolic substances (Lu and Foo, 2001) and tannins (Anaka *et al.,* 2014) in earlier studies of the anticonvulsant potentials of the medicinal plants. Therefore, the anticonvulsant potential of MSB may be ascribed at least to the occurrence of these phytochemicals in MSB.

The functional groups analyses using FT-IR assists in the prediction of the overall physicochemical properties of plant extracts as well as their structure-activity relationships (Poojary *et al.,* 2015). The FT-IR spectral of MSB showed hydroxyl functionality which has been reported to be an integral part of most of the phenolic phytocompounds such as flavonoids and tannins (Poojary *et al.,* 2015) and some alkaloidal extracts from medicinal plants (Shami, 2016). Therefore, the anticonvulsant potentials of MSB may at least in part be attributed to the presence of the hydroxyl functional group. It has earlier been demonstrated that the alkaloidal extracts from medicinal plants have O-H, N-H, C=O and C-H functional groups (Schulz and Baranska, 2007), since MSB contained some of these functional groups as shown by the FT-IR spectral of MSB. It will therefore, not be out of place to at least in part corroborate the presence of alkaloids in MSB found as evident from the phytochemical assay of MSB.

CONCLUSION

In conclusion, this study suggested that MSB may possess anticonvulsant property. This study further suggested that the additive and/or synergistic effects of alkaloids, phenol and other phytocompounds in MSB may be responsible for the observed anticonvulsant effect of MSB.

REFERENCES

Adebayo, M.A., Adedokun, O.A., Akinpelu, L.A. and Okafor P.O. (2019). Evaluation of antidiarrheal activity of methanol root bark extract of *Milicia excelsa* (Welw) C.C Berg (Moraceae) in rats. *Drug Research*, 69(8): 439-444.

Adebayo, M.A., Akinpelu, L.A., Okwuofu, E.O., Ibia, D.E., Lawson-Jack, A.F. and Igbe I. (2020). Anticonvulsant, antiamnesic and anxiolytic activities of methanol leaf extract of *Bambusa vulgaris* (Poaceae) in mice. *Journal of African Association Physiological Sciences*, 8(2): 149-157.

Agyeman, V.K., Ofori, D.A., Cobbinahgh, J.R. and Wagner, M.R. (2009). Influence of *Phytolyma lata* (Homoptera psyllidae) on seed growth of *Milicia excelsa*. *Ghana Journal of Forestry*, 25: 29-39.

Akinpelu, L. A., Aiyelero, O. M., Olayiwola, G. (2019a). Ethanol leaf extract of *Milicia excelsa* mitigates anxiety and depressive-like behaviours induced by acute restraint stress in mice. *GSC Biological and Pharmaceutical Sciences*, 6(2): 30-39.

Akinpelu, L.A., Adebayo, M.A., Aiyelero, O.M., Fajana, A., Isede, N.R., Akinbiyi, I.A. and Sani A.I. (2020a). Acute toxicity and sedative-hypnotic effects of ethanol stem bark extract and fractions of *Milicia excelsa* (Moraceae) in mice. *Advances in Pharmacy and Pharmacology*, 8(2): 11-18.

Akinpelu, L.A., Akanmu, M.A., Obuotor, E.M. (2018a). Mechanism of anticonvulsant effects of ethanol leaf extract and fractions of *Milicia excelsa* (Moraceae) in mice. *British Journal of Pharmaceutical Research International*, 23(4): 1-11.

Akinpelu, L.A., Olawuni, I.J., Ogundepo, G.E., Olayiwola, G. and Fajana, A. (2020b). Phytochemical estimations and antihypoxic effect of ethanol leaf extract of *Milicia excelsa* (Moraceae) in mice. *GSC Biological and Pharmaceutical Sciences*, 10(2): 24-29.

Akinpelu, L.A., Adebayo, M.A., Fajana, A., Adeniyi-Akee, M.A., Ubogu, S.E. and Aminu, N.S. (2019b). Phytochemical analyses, anxiolytic and anti-amnesic effect of methanol stem bark extract of *Vitex doniana* (Sweet) in mice. *Nigerian Journal of Natural Products and Medicine*, 23: 104-111.

Akinpelu, L.A., Akanmu, M.A. and Obuotor, E.M. (2018b). Antipsychotic effects of ethanol leaf extract and fractions of *Milicia excelsa* (*Moraceae*) in mice. *British Journal of Pharmaceutical Research International* 22(6): 1-10.

Anaka, O.N., Ozolua, R.I., Ikpefan, E.O., Diana, O. and Ogieva, D.O. (2014). Anticonvulsant activity of the aqueous extract of *Allium cepa* L. (Amaryllidaceae) in rats and mice. *Journal of Pharmacy and Bioresources*, 11(1): 1-7.

Areola, J.O., Babalola, O.O., Ilesanmi, O.R., O.O. and Oyedapo O.O. (2015). Toxicity studies of the ethanolic stem bark extract of *Milicia excelsa* (Welw.) C. C. Berg in wistar rats. *American Journal of Biochemistry*, 5(6): 131-137.

Ariwaodo, J.O., Chukwuma, E.C. and Adeniji, K.A. (2012). Some medicinal plant species of Asamagbe Stream Bank Vegetation, Forestry Research Institute of Nigeria, Ibadan. *Ethnobotany Reasearch and Application*, 10: 541-549.

Basavaraj, P., Shivakumar, B., Shivakumar, H. and Manjunath, V.J. (2011). Evaluation of anticonvulsant activity of *Tabernaemontana divaricata* (Linn) R. BR. flower extract. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(3): 310-315.

Bhutada, P., Mundhada, Y., Bansod, K., Dixit, P., Umathe, S. and Mundhada, D. (2010). Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice. *Epilepsy and Behaviour*, 18(3): 207-10.

Bialer, M. (1997). New antiepileptic drugs that are second generation to existing antiepileptic drugs. Expert Opinion on Investigational Drugs, 15(6):637-47.

Cakmak, Y.O. (2006). Epilepsy, electroacupuncture and the nucleus of the solitary tract. *Acupuncture Medicine*, 24: 164-168.

Chindo, B.A., Ya'u, J., Danjuma, N.M., Okhale, S.E., Gamaniel, K.S. and Becker, A. (2014). Behavioral and anticonvulsant effects of the standardized extract of *Ficus platyphylla* stem bark. *Journal of Ethnopharmacology*, 154: 351–360

Corda, M.G., Giorgi, O., Longoni, B., Orlandi, M. and Biggio, G. (1990). Decrease in the function of γ-aminobutyric acid coupled chloride channel produced by repeated administration of pentylenetetrazole in rats. *Journal of Neuroscience*, 55: 1216-1221.

Daanaa, S., Abotsi, W.K.M., Boakye-Gyasi, E. and Woode, E. (2018). Anticonvulsant effect of the hydroethanolic leaf extract of *Psydrax subcordata* (DC.) Bridson in murine models. *Journal of Ethnopharmacology*, 213: 384-394

Fathima, S.N, Vasudevamurthy, S. and Rajkumar, N. (2015). A review on phytoextracts with antiepileptic property. *Journal of Pharmaceutical Science and Research*, 7(11): 994-1003

Gates, J.R. (2000). Side effect profiles and behavioral consequences of antiepileptic medications. *Epilepsy and Behaviour*, 3: 153-159.

Gbolade, A.A., Ojieho, E.S., Adebayo. M.A. and Igwe, S.C. (2022). The potential of *Milicia excelsa* (Welw.) C. C. Berg (Moraceae) root bark methanol extract and fractions in the treatment of male infertility in rats. *African Journal of Pharmaceutical Research and Development*, 10(1): 43-50.

Hema, B., Bhupendra, S., Mohamed Saleem, T.S. and Gauthaman, K. (2009). Anticonvulsant effect of *Drosera burmannii* Vahl. *International Journal of Applied Research in Natural Product*, 2(3): 1-4.

Hemalatha, S., Wahi, A.K., Singh, P.N. and Chansouria, J.P.N. (2003). Anticonvulsant and free radical scavenging activity of *Hybanthus enneaspermus*: a preliminary screening. *Indian Journal of Traditional Knowledge*, 2(4): 383-388.

Ishola, I.O., Olayemi, S.O. and Idowu, A.R. (2013). Anticonvulsant, anxiolytic and hypnotic effects of aqueous bulb extract of *Crinum glaucum* A. Chev (Amaryllidaceae): role of GABAergic and nitrergic systems. *Pakistan Journal of Biological Sciences*, 6: 701-710.

Kumar, S., Reecha, M., Gundeep, B., Anupam, J. and Anupam, S. (2012). Plants and plant products with potential anticonvulsant activity – a review. *Pharmacognosy Communications*, 2(1): 3-99.

Lu, Y. and Foo, L.Y. (2001). Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chemistry*, 75:197-202.

Nassiri-Asl, M., Shariati-Rad, S. and Zamansoltani, F. (2007). Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: involvement of

benzodiazepine and opioid receptors. *BMC Complementary and Alternative Medicine*, 7: 26.

Ngo, B.E., Benoite, E.N., Ekoundi, C., Dong, C., Mbomo, R.E.A., Rakotonirina, S.V. and Rakotonirina A. (2004). Sedative and anticonvulsant properties of *Passiflora edulis* dried leaves decoction in mice. *African Journal of Traditional Complementary and Alternative Medicine*, 1: 63-71.

Olajide, O.A., Kolawole, O.T., Fagbohun, T.R. and Ajayi, F.F. (2005). Evaluation of the anti-inflammatory properties of *Chlorophora excelsa* stem bark extract. *Pharmaceutical Biology*, 43(9): 746-748.

Pandeya, S.N., Kumar, R. and Pathak, A.K. (2009). Natural Anticonvulsants: A review. *Research Journal of Pharmacy and Technology*, 2(4): 670-679.

Perucca E, French J, Bialer M. (2007). Development of new antiepileptic drugs: Challenges, incentives, and recent advances. *Lancet Neurology*, 6(9):793-804.

Poojary, M.M., Vishnumurthy, K.A. and Adhikari, A.V. (2015). Extraction characterization and biological studies of phytochemicals from *Mammea suriga*. *Journal of Pharmaceutical Analysis*, 5(3): 182–189.

Quintans-Junior, L.J., Almeida, J.R.G.S., Lima, J.T., Nunes, X.P., Siqueira, J.S., Oliveira, L.E. G., Almeida, R.N., Athayde-Filho, P.F. and Barbosa-Filho, J.M. (2008). Plants with anticonvulsant properties - a review. *Brazilan Journal of Pharmacognosy*, 18: 798-819.

Quintans-Júnior, L.J., Silva, D.A., Siqueira, J.S., de Souza, M.V., Almeida, R.N. and Silva-Júnior, R.G.C. (2007). Anticonvulsant properties of the total alkaloid fraction of *Rauvolfia ligustrina* Roem. et Schult. in male mice. *Revista. Brasileira. Farmacognosy*, 17(2): 176-180.

Sander, J.W. and Shorvon, S.D. (1996). Epidemiology of epilepsy: The size of the problem. *Journal of Neurology, Neurosurgery and Psychiatry*, 61: 433–443.

Schulz, H. and Baranska, M. (2007). Identification and quantification of valuable plant substances by IR and raman spectroscopy. *Vibrational Spectroscopy***,** 43: 13-25.

Shami, A.M.M. (2016). Isolation and identification of alkaloids extracted from Local Plants in Malaysia. *Annals of Chromatography Separation Techniques*, 2(1): 1016.

Smith, I. S., Thopson, S. J. K., Sargent, J. B. and Heal, J. D. (2001). BTS 72664 – a novel CNS drug with potential anticonvulsant, neuroprotective and antimigraine properties. *CNS Drug Review*, 7: 146-171.

Sonibare, M.A., Soladoye, M.O. and Subuloye, T.O. (2008). Ethnobotanical Survey of Anti-Psychotic Plants in Lagos and Ogun States of Nigeria. *European Journal of Scientific Research*, 19(4): 634-644.

Swinyard, E.A., Woodhead, J.H., White, H.S. and Franklin, M.R. (1989). Experimental selection, qualification and evaluation of anticonvulsants. In: Levy R, Mattson R, Meldrum BS, Penry JK, Dreifuss FE. (Eds), Antiepileptic Drugs, 3rd ed. Raven press New York, 85-102

Vellucci, S.V. and Webster, R.A. (1984). Antagonism of caffeine-induced seizures in mice by Ro15-1788. *European Journal of Pharmacology*, 97(3- 4):289-293.

Vyawahare, N.S., Khandelwal, A.R., Batra, V.R. and Nikam, A.P. (2007). Herbal anticonvulsants. *Journal of Herbal Medicine and Toxicology*, 1: 9-14.

Wahab, O.M. (2015). Ethnomedicinal antiepileptic plants used in parts of Oyo and Osun States, Nigeria. *Botany Research International*, 8 (4): 77-81.