



EVALUATION OF ANTICONVULSANT ACTIVITY OF CALLISTEMON CITRINUS (CURTIS) SKEELS (MYRTACEAE) VOLATILE OIL

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ABSTRACT

The use of medicinal plants is on the rise due to the increase of various diseases and shortcomings of orthodox medicine. For many ailments including convulsion, conventional medicine has not been able to find a lasting solution. This study was directed towards assessing the ethnomedicinal use of *Callistemon citrinus* leaves in the management of convulsion. The volatile oil of the leaves was extracted and an acute toxicity test was carried out following Lorke's description. Maximal electroshock (MES), strychnine and pentylenetetrazol anticonvulsant methods were used. Separate groups of albino mice were given 200, 400 and 800 mg/kg doses of the volatile oil. Drug solutions; 30 mg/kg phenobarbitone for MES and 2 mg/kg diazepam for strychnine and pentylenetetrazol models were administered as a positive control. The start of tonic leg extension, duration and percentage mortality was recorded. Doses of 200 and 400 mg/kg significantly ($P < 0.05$) inhibited seizure in the mice with scores of 40 % each in the MES model. There was a dose-dependent reduction in the duration of seizures with 68.47, 70.27 and 81.08 % reductions in the pentylenetetrazol model. No significant coverage was given in the strychnine model. *C. citrinus* oil protected the mice against pentylenetetrazol and maximal electroshock-induced convulsion hence could contribute to the medical treatment of epilepsy.

Keywords: Anticonvulsant, *Callistemon citrinus*, Pentylenetetrazol, Strychnine, Maximal electroshock

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INTRODUCTION

Plants have been used as food and medicine from the beginning of time. They are priceless assets, and the fact that they are readily available and accessible is an added advantage. There has been a lot of studies on plant phytochemicals, which could lead to new therapeutic options for diseases of the central nervous system (CNS), such as epilepsy and convulsive disorders. Epilepsy affects 1-2 percent of the global population, with 80 percent of those affected living in under-developed nations. Aside from the health ramifications, there are also social, cultural, and economic implications (Newton and Garcia, 2012). In low-income countries, the deficiency and high cost of anticonvulsant medications poses a serious problem. There is therefore a need to explore other available treatment options with better effects like improved efficacy against drug-resistant seizures, low cost as well as reduced or no unpleasant effects.

Some medicinal plants have demonstrated ability as new, safe treatment alternatives (Kakooza-Mwesige, 2015). Many species of aromatic plants have been used ethnomedicinally as sedative and antiepileptic agents but there is still lack of controlled experimental reports on therapeutic use (Sahu *et al.*, 2012). Essential oils have been used in places with rich cultures like India, Brazil, China and the Middle East in managing convulsive disorders (Debas *et al.*, 2006). They have also been researched and documented for their sedative, anxiolytic and neuroprotective activities (De Sousa *et al.*, 2015; Abuhamdah *et al.*, 2015). Several compounds have been found in these oils which have pharmacological inputs on central nervous system targets which are implicated in epilepsy.

Some plants from the family Myrtaceae have been studied and found to have anticonvulsant activity. The volatile oil of *Syzygium aromaticum* (Myrtaceae) has been reported to possess anticonvulsant activity with 100 % survival rate in the Maximal Electroshock model (Pourgholami *et al.*, 1999). Daily treatment with eugenol, the major component of *S. aromaticum* also reduced the duration of seizure as well as the resultant mortality (Joushi and Salmani, 2017).

Eucalyptus urophylla (Myrtaceae) leaves oil reportedly increased the percentage of mice protected against pentylenetetrazole induced convulsion (Teixeira *et al.*, 2008).

Callistemon citrinus belonging to Myrtaceae family has similar constituents and in view of this, this study was aimed at evaluating the anticonvulsant activity of its volatile oil. It is found growing mostly in tropical Asia, Queensland, New South Wales, South America and Australia, (Oyededeji *et al.*, 2009). Its leaves are evergreen, aromatic, alternate, lanceolate with entire margin and anomocytic stomata. Stems are grey in colour and it grows to about 5-8 m in height (Spencer *et al.*, 1991).

The hot water infusion of *C. citrinus* leaves is used ethnomedicinally for the alleviation of cough, bronchitis, rheumatism and tuberculosis. It's also used as an insecticide, for urinary incontinence and cleansing genitourinary tract. (Sutar, 2014; Tabuti *et al.*, 2010).

Reported pharmacological activities for the genus include antimicrobial, (Oyededeji *et al.*, 2009) antithrombin (Chistokhodova *et al.*, 2002), repellent, and *in-vitro* antihelminthic activity (Srivastava, 2001) as well as larvicidal and pupicidal activities.

Considering the anticonvulsant activity of some plants in the family Myrtaceae as well as the bioactivity of *C. citrinus*, this study was aimed at evaluating its anticonvulsant potential.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF THE PLANT

C. citrinus leaves were obtained from Benin City, Edo state, in September, 2019. Botanical identification was carried out by Dr Henry Akinnibosun, in the Plant biology and Biotechnology Department, University of Benin, Benin City, Edo State. Voucher specimens were deposited in the herbarium under the code number: UBH-C467.

EXTRACTION OF VOLATILE OIL

The fresh leaves of *C. citrinus* were separated from the stem, weighed (500 mg) and extracted using a Clavenger-type apparatus by hydrodistillation. The essential oil was collected in a glass vial and stored in the refrigerator at 4°C until time of use.

SOURCE AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Mature albino mice of both sexes weighing between 15g-35g were procured from pharmacology department and housed in plastic cages while being fed dry rodent pellet feeds (grower) and water for 2 weeks for the purpose of acclimatization. Ethical approval was sought from the Ethics committee, faculty of pharmacy, with ethical approval number EC/FP/021/08. Experimental procedures were done following the Institute for Laboratory Animal Research Guidelines for the Care and Use of laboratory Animals.

ACUTE TOXICITY TEST

Oral median lethal dose (LD₅₀) of *C. citrinus* volatile oil was analyzed by Lorkes method (1983). In the first phase, three groups of three mice each received oral doses of 10, 100 and 1000 mg/kg doses of the volatile oil respectively. Three groups of one mouse each were administered oral doses of 1600, 2900 and 5000 mg/kg of the volatile oil respectively in the second phase.

In the two phases, the animals were observed for signs of diarrhea, tremor, writhing and mortality within a 24 hr period. At the end of the test, the LD₅₀ of the volatile oil was determined.

ANTICONVULSANT STUDIES

MAXIMAL ELECTROSHOCK MODEL

Twenty-five mice were weighed and divided into five subsets comprising 5 mice each. Control group received 0.2 ml of water. The essential oil of *C. citrinus* was given at doses of 200, 400, and 800 mg/kg orally to the second, third and fourth group respectively. Phenobarbitone given intraperitoneally at dose of 30mg/kg to the fifth group served as reference anticonvulsant drug. Tonic convulsions of hind limb extremities of mice were triggered 1 hr later using electrical current (50 Amp, 100 Hz, and 0.2 seconds) via ear clip electrodes. The number of animals protected from convulsion were noted (Loscher *et al.*, 1991).

PENTYLENETETRAZOL INDUCED CONVULSION

Another twenty-five mice were weighed and randomly divided into five groups, comprising five mice each. Group 1 served as the control group and each mouse received 0.2 ml of water orally. Groups 2, 3 and 4 received increasing oral doses of the volatile oil; 200, 400 and 800 mg/kg body weight. Group 5 served as the standard and was

administered 2 mg/kg dose of diazepam intraperitoneally. Convulsion was induced after 1 hr by giving each animal 70 mg/kg doses of pentylenetetrazol intraperitoneally.

The onset of CNS stimulation, onset of tonic-clonic seizures, duration of convulsion and protection against mortality were noted (Merit and Putman, 1938).

STRYCHNINE INDUCED SEIZURE MODEL

The procedure described by Bogdanov *et al.* in 1997 was followed here. Twenty-five mice were weighed and allotted into five groups, comprising five mice each. Group 1 served as the control group, each receiving 0.2 ml of water. Groups 2, 3 and 4 received 200, 400, and 800 mg/kg of *C. citrinus* volatile oil respectively. The standard, diazepam was administered to group five intraperitoneally at 2 mg/kg. After 30 minutes, all mice received 2 mg/kg doses of strychnine.

Specific changes in the animals such as time for onset of CNS stimulation, onset of seizures, duration of seizures as well as protection against mortality were documented.

STATISTICAL ANALYSIS

Data were documented as mean \pm standard error of mean (S.E.M). Statistical analysis was done using one way analysis of variance followed by Dunnet's post hoc test (Graph pad Prism version 7, San Diego, USA). $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

With much focus on evidence-based medicine and the availability of modern laboratory technologies, renewed attention has been generated in research on herbal products so as to identify effective and safe antiepileptic compounds (Kumar and Khanum, 2012).

Oral doses of 10, 100 and 1000 mg/kg of the volatile oil of the leaves of *C. citrinus* produced no toxic effect or mortality in the mice (Table 1) which was evidenced by a lack of change in animal behavior as food and water intake were normal during phase one of the acute toxicity study. In the second phase, doses of 1600 mg/kg produced no mortality while mortality was observed with 2900 and 5000 mg/kg doses (Table 1). Investigations from this study revealed that the volatile oil of the leaves *C. citrinus* was without any fatal effect at doses up to 2154mg/kg and was fairly non-toxic which is within the standard range of 500–5000 mg/kg body weight (Sharif, 2015)

Table 1: Acute toxicity testing of *C. citrinus* volatile oil in phase 1 and 2

Dose (mg/kg)	No of deaths/No of animals	Percentage mortality (%)
Phase 1		
10	0/3	0
100	0/3	0
1000	0/3	0
Phase 2		
1600	0/1	0
2900	1/1	100
5000	1/1	100

Phase 1: n=3, phase 2: n=1

At doses of 200 and 400 mg/kg, the volatile oil offered 40% protection against maximal electroshock-induced convulsion while 800 mg/kg offered no protection. It could thus be said that the anticonvulsant effect of the volatile oil was not dose-dependent (Table 2). Partial seizures as well as generalized tonic clonic seizures are predicted to be effectively managed with drugs which provide protection against maximal electroshock-induced seizures (Merit and Putman, 1938). Drugs like phenytoin and phenobarbitone which inhibit voltage-dependent sodium channel can be used to prevent convulsion induced by maximal electroshock. The oil protected the mice against maximal electroshock induced seizure which demonstrates that it might be acting by inhibiting voltage dependent sodium channels and subsequently be effective against generalised tonic-clonic seizures.

Table 2: Effect of volatile oil of *C. citrinus* on maximal electroshock induced convulsion in mice

Treatment groups	No of mice protected against convulsion (n=5)	Percentage of mice protected against convulsion (%)
Control (water)	0	0
200 mg/kg	2	40
400 mg/kg	2	40
800 mg/kg	0	0
Phenobarbitone (30 mg/kg)	5	100

The oil delayed the onset of seizures and reduced seizure duration significantly compared to the control ($P < 0.05$). Dose-dependent reductions in the seizure duration and mortality were observed, with 800 mg/kg having the highest reductions of 81.08 % and 80 % respectively (Table 3). Diazepam completely protected the mice against convulsion while no protection was offered to the group given water as 100 % mortality was recorded. Pentylentetrazole is a drug that can stimulate the central nervous system with resulting epileptogenic effects and is commonly used to identify extracts or compounds that can prevent or reduce seizures (Koutroumanidou *et al.*, 2013). PTZ induces convulsion by antagonizing Gama-aminobutyric acid (GABA) receptor chloride ion channel complex (Corda *et al.*, 1990). The opening of the chloride channel by GABA agonistic activities causes major inhibitions of the central nervous system which presents as sleep or sedation and ultimately, inhibition of convulsion (Kaila *et al.*, 2014). It is therefore likely that the anticonvulsant activity of the oil could be mediated via GABAergic mechanisms.

Table 3: Effect of the volatile oil of *C. citrinus* on Pentylentetrazole induced convulsion in mice

Treatment groups	Onset of CNS Stimulation (secs)	Onset of seizures (secs)	Duration of seizures (secs)	% Reduction in duration of seizures (%)	Protection against mortality (%)
Control	110.00 ± 17.69	144.40 ± 13.62	44.80 ± 4.04	-	0
200 mg/kg	65.00.80 ± 4.77	266.80 ± 10.03	14.00 ± 2.03**	68.47	0
400 mg/kg	127.80 ± 44.89	228.00 ± 93.72	13.20 ± 4.18**	70.27	20 *
800 mg/kg	80.80 ± 9.46	157.40 ± 86.77	8.40 ± 3.86***	81.08	80 ***
Diazepam (2 mg/kg)	-	-	-	100	100 ***

The results are expressed as mean ± SEM and percentage, n=5 mice/group, * indicates significance compared to negative control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Strychnine produces a convulsant effect as a non-competitive blocker of the inhibitory neurotransmitter glycine. In the Strychnine model, increasing doses of the volatile oil resulted in delayed onset of seizures and reduced percentage mortality ($P < 0.05$) compared to the control, but no full protection was offered. This reveals the oils ability to interact with the glycine-mediated inhibitory pathway. Diazepam significantly reduced seizure duration compared to other treatment groups (Table 4).

Though the delay in onset of CNS stimulation observed in both pentylentetrazol and strychnine models was not statistically significant, it indicates that the plant oil may have centrally mediated actions.

Table 4: Effect of volatile oil of *C. citrinus* on strychnine induced convulsion in mice

Treatment groups	Onset of CNS stimulation (secs)	Onset of seizures (secs)	Duration of seizures (secs)	Protection against mortality (%)
Control	128.00 ± 5.93	140.40 ± 1.72	14.60 ± 1.29	0
200 mg/kg	218.80 ± 13.17	332.20 ± 74.11*	23.40 ± 4.47	0
400 mg/kg	210.00±22.78	299.20 ± 36.33*	47.20±16.18	20*
800 mg/kg	190.00 ±32.57	254.20 ±37.81	31.20 ± 8.56	60**
Diazepam (2 mg/kg)	105.00 ± 9.343	196.00 ± 11.56	6.20 ± 0.58	0

Values are expressed as mean ± SEM, n=5, *P < 0.05, **P < 0.01 compared to negative control.

C. citrinus oil in this study produced 40 % protection in the MES protocol which is lower than that reported for *S. aromaticum* (Myrtaceae) volatile oil where 100 % protection against mortality was recorded. On the other hand, *C. citrinus* oil effectively reduced seizure duration and percentage mortality in a dose-dependent manner in the PTZ model but *S. aromaticum* oil was observed to be ineffective in preventing convulsion in this model (Pourgholami *et al.*, 1999). This variation in activity may have been due to differences in the quality and quantity of the components of the oils.

Generally, activities of essential oils are linked to the main constituents of the oils. (Bakkali *et al.*, 2006). The essential oil components of *C. citrinus* are many with 1, 8-cineole (58 %) and α -pinene (24.1 %) being the most abundant (Gupta *et al.*, 2008). *Tetrapleura tetraptera* pod volatile oil, is composed chiefly of 1, 8-cineole and protected 78% of animals from seizures induced by leptazol (Nwaiwu and Akah, 1986). Raza *et al.* in 2008 also reported that minimal neurological deficit induced by *Nigella sativa* seed may be due partly to its α -pinene content. The oil of *Angelica archangelica*, has been reported to contain 3-carene, α -pinene and limonene, and it decreased the duration of tonic convulsions as well as the recovery time after seizure in pentylenetetrazole mice (Pathak *et al.*, 2010).The presence of 1, 8-cineole and α -pinene in *C. citrinus* oil may therefore be responsible for the observed anticonvulsant activity in this study.

CONCLUSION

In conclusion, the volatile oil of *C. citrinus* demonstrated anticonvulsant activity which was more significant in the Pentylenetetrazole (PTZ) model and can be potentially useful in the management of myoclonic and absence seizures. Further study is needed to confirm the exact mechanism of action of this volatile oil.

ACKNOWLEDGEMENT

We thank Mr. Kingsley Ugwu from Pharmacognosy department, University of Benin for the collection of the plant. Special thanks also go to the laboratory staff of Pharmacology department for making the necessary equipment available.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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