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N-Hexane and Butanol Fractions of Methanol Leaf Extracts of *Cleistopholis Staudtii* Displayed Better Anticonvulsant Effects in Swiss White Mice

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ABSTRACT

Background: Patients use anticonvulsant drugs to manage and handle disorders that are associated with seizures such as epilepsy. One of such drugs is Phenobabirone which is relatively expensive. These drugs however, have made patients to continue to experience inadequate seizure control even while using them. There is need to investigate cheaper sources of anticonvulsant agents. The leaves of Cleistopholis staudtii has been used traditionally to handle convulsant cases and could in future shows therapeutic relevance and use. .

Objectives: This research investigated the Anticonvulsant effects of methanol leaf extracts and fractions of *Cleistopholis staudtii* on swiss white mice. It also compared the anticonvulsant effect of these fractions of the *Cleistopholis staudtii* leaves with those of Phenobabirone.

Methodology: Fifty Swiss white mice (18-32g) were divided into ten (10) groups (n=3)as they were given orally; 10ml/kg 5% tween 80 (Control Group), , 200mg/kg crude extract (Group 3), 400mg/kg crude extract (Group 4), 200mg/kg N-hexane fraction (group 5), 400mg/kg N-hexane fraction (Group 6), 200mg/kg Ethylacetate fraction (Group 7), 400mg/kg Ethylacetate fraction (Group 8), 200mg/kg Butanol fraction (group 9), 400mg/kg Butanol fraction (Group 10) while Group 2 (Positive control) was given 60mg/kg Phenobarbitone intraperitoneally . The groups had free access to water. These treatment were for seven(7) days after which they were injected with 200mg/kg Isoniazide and observations were made in regards to convulsion.

Results: The control group had convulsions at 34.7±6.07 minutes, while phenobarbitone delayed seizures to 90±0.0 minutes. The crude extract delayed convulsions to 47.3±14.91 minutes at 200 mg/kg and 65±21.93 minutes at 400 mg/kg. The N-Hexane fraction had a similar delay effect to phenobarbitone at both dosages, while the Ethyl Acetate and Butanol fractions had variable results. Mortality rates were 100% in the control group, reduced to 33% with phenobarbitone. The crude extract caused 100% mortality at both doses, and the N-Hexane, Ethyl Acetate, and Butanol fractions had 67% mortality at 400 mg/kg and 100% at lower doses. Phenobarbitone was the most effective, with 0.67±0.09 convulsions, compared to the crude extract with 6.7±1.50 convulsions at 200 mg/kg and 8.7±1.50 at 400 mg/kg. The N-Hexane fraction had 8.7±3.42 convulsions at 200 mg/kg and 10.3±1.70 at 400 mg/kg, while the Ethyl Acetate and Butanol fractions resulted in higher convulsion counts, especially at lower doses.

Conclusion: The Research showed that the Butanol Fraction of methanol leaf extracts of Cleistopholis staudtii exhibited better anticonvulsant effects in Swiss white mice.

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Keywords: *Cleistopholis staudtii,* anticonvulsant, Butanol Fraction, *phenobarbitone, seizures*

INTRODUCTION

Anticonvulsant medications, also known as antiepileptic drugs (AEDs), are used for the management and treatment of epilepsy together with other disorders associated with seizures. These drugs work by modulating electrical activity in the brain targeted at controlling, preventing or reducing seizures and their frequencies. One of such drugs is Phenobarbitone [36]. Though these drugs have improved patients's lives [40], they are linked with a variety of adverse effects such as cognitive impairment, mood disturbances, and systemic toxicity [42]. These adverse effects vary with the complicated multiple drug regimens and administrations [25]. These adverse effects pose challenges that affects the general wellbeing of the patients [9] and could proved stigmatic to them. These outcomes are refractory to conventional treatments [12]. *Cleistopholis staudtii*, a plant indigenous to tropical Africa, belonging to the Annonaceae family [10], has long been recognized in traditional medicine for its purported anticonvulsant properties ([26], [31]), also known as "African chewing gum" or "nete" in various African languages, it is used by traditionalists for treating a spectrum of ailments, including epilepsy and convulsions [31]. It is readily available in Nigeria. However, scientific evidence supporting its efficacy and safety in the context of epilepsy management remains scarce, particularly concerning the methanol leaves extract and its fractions. This research also bridged the gap between empirical and traditional knowledge of the plant highlighting the potential of ethnomedicine in discovering new pharmacological leads

MATERIALS AND METHODS

Plant Collection and Method of Extraction Cleistophilis staudii leaf

The research was conducted in the School Of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Nigeria and authenticated. Fresh leaves of African White Cheesewood were collected from Agulu in Anaocha Local Government Area of Anambra state and they were identified and authenticated. They were identified by Mrs. Amaka of the department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Agulu campus. The voucher number is PCG/483/A/021.

400g of the pulverized plant sample was mascerated in 1000ml of methanol over a period of 48 hours with intermittent shaking. The mixture was sieved using proclain cloth. It was further filtered using No. 1 whatman filter paper. The filtrate was concentrated using rotary evaporator over a reduced temperature and pressure. It was further concentrated using water bath at 50^{o}c. The crude extract was stored in a refrigerator for use. Active plants were screened qualitatively for phytochemicals using various methods ([44], [45], [46]).

Method of Fractionation of the Plant extract

Liquid- Liquid fractionation method was adopted in this study [47]. 200ml of 100mg/ml of the crude extract was reconstituted with methanol and was poured in a separating funnel. It was mixed with 100ml of distilled water. 400ml of 99% N-hexane was poured and the mixture was shaked rigorously, releasing pressure at intervals. The mixture was allowed to stand for 30 min for proper separation. After separating, N-hexane fraction was collected in a clean beaker using separating funnel. The residue was poured back to the separating funnels and 400ml of ethylacetate was poured in there and was shaked rigorously, allowed to seperate for 30mins. It was collected in a clean beaker when butanol fraction was the last to be collected following the process as in n-hexane and ethylacetate fractions.

Experimental Design and Anticonvulsant Procedure

Anticonvulsant activity of methanol leaves extract of Cleistopholis staudtii was studied using a model that involves the use of isoniazid with slight modifications [48]. A total of thirty adult swiss white mice were used. They were grouped into ten (10) groups of three (3) mice. They were acclimatized for two weeks (21 days) and monitored closely for signs of convulsion and inflammation. The animals were given treatment for 7 days as follows; Group 1 received 10ml/kg 5% tween 80 orally (Control Group), Group 2 received 60mg/kg

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Phenobarbitone intraperitoneally (Positive Control group), Group 3 received 200mg/kg crude extract orally, Group 4 received 400mg/kg crude extract orally, Group 5 received 200mg/kg N-hexane fraction orally, Group 6 received 400mg/kg N-hexane fractions orally, Group 7 received 200mg/kg Ethylacetate fraction orally, Group 8 received 400mg/kg Ethylacetate fraction orally, Group 9 received 200mg/kg Butanol fraction orally, Group 10 received 400mg/kg Butanol fraction orally.On the last day of treatment, 45mins post treatment, 200mg/kg Isoniazide was injected into the animals subcutaneosly. The animals were observed for the onset of convulsion, incidence of convulsion (number of convulsion) and death was recorded. The results were presented as mean ± SEM (standard error of mean) and analyzed using one way ANOVA followed by Dunnett post hoc test for multiple comparisons. A difference was considered significant at $p< 0.05$ between the tests and control Groups as well as among test Groups for measured value. Level of significance was calculated by One Way Analysis of Variance (ANOVA).

RESULTS OF THE STUDY

The Acute Toxicity (LD_{50}) of Methanol Leaf Extracts of Cleistopholis Staudtii

Table 1: Acute Toxicity Test (LD_{50}) of Methanol Leaf Extract

Phase	Dose	Death
1	10mg/kg	0/3
	100mg/kg	0/3
	1000mg/kg	0/3
2	2000mg/kg	0/1
	3000mg/kg	0/1
	4000mg/kg	0/1
	5000mg/kg	0/1

Thus LD_{50}> 5000mg/kg

The table above showed the acute toxicity test (**LD_{50}**) indicating that was employed and the result showed that the oral administration of Methanol leaf extracts and fractions of *Cleistopholis Staudtii* has a lethal dose above 5000mgkg^{-1} .

Table 4.2 Phytochemical Components of the crude extracts and Fractions of *Cleistopholis Staudtii*

	Phytochemical Constituents					
Chemical Extracts	**Saponins**	**Tannins**	**Flavaloids**	**Alkaloids**	**Cardiac Glycoside**	**Steriods**
Methanol Crude extract	++	+++	++	+	+	++
N-Hexane Fractions	++	+	+	+	+	+++
Ethyl acetate Fractions	+++	+++	++	+	+	++
Butanol Fractions	+++	+++	++	++	+	+++

Key: +… little amount, ++… moderate amount and +++… high amount.

The table provides a detailed overview of the concentration of various phytochemical constituents in different extracts. Overall, Ethyl Acetate and Butanol Fractions are most effective for extracting high levels of Saponins, Tannins, and Steroids, while the N-Hexane Fractions excel at extracting steroids. The Crude Extract, although it offers moderate levels of a range of phytochemicals, does not achieve the high concentrations seen in the specialized fractions. This information can guide the selection of solvents for targeted extraction of specific phytochemicals.

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Table: 4.3: Effects of Crude extracts and Fractions of Cleistopholis Staudtii on *onset of Convulsio,* the Mortality rate after Convulsion and Frequency of Convulsion.

Variables	**Onset of Convulsion (min)**	**% Death**	**Frequency of convulsion**
10ml/kg 5% tween 80	34.7 ± 6.07^{a}	100	31.3 ± 6.03^{a}
60mg/kg phenobarbitone	90 ± 0.0^{b}	33	0.67 ± 0.09^{b}
200mg/kg crude extract	47.3 ± 14.91^{b}	100	6.7 ± 1.50^{c}
400mg/kg crude extract	65 ± 21.93^{c}	100	8.7 ± 1.50^{c}
200mg/kg N-Hexane fraction	90 ± 0.0^{b}	67	8.7 ± 3.42^{c}
400mg/kg N-Hexane fraction	90.3 ± 1.82^{b}	67	10.3 ± 1.70^{c}
200mg/kg Ethylacetate fraction	78.7 ± 3.26^{b}	100	21.3 ± 3.01^{d}
400mg/kg Ethylacetate fraction	82 ± 6.91^{b}	67	7.3 ± 1.82^{c}
200mg/kg Butanol fraction	77 ± 1.44^{b}	100	11.3 ± 1.67^{c}
400mg/kg Butanol fraction	87.3 ± 6.84^{b}	67	6.7 ± 2.21^{c}

Values are presented as Mean±Standard Deviation (n=5); and the mesns with different letter superscripts are significantlt different (P=0.05) from paired across the table.

Effects of Crude extracts and Fractions of Cleistopholis Staudtii on the onset of Convulsion

The above table shows that the control Group, treated with 10ml/kg of 5% Tween 80, had convulsions at 34.7 minutes, showing no delay in onset of convulsion. Phenobarbitone (60mg/kg) significantly delayed convulsions to 90 minutes, highlighting its strong anticonvulsant effect. Crude extracts of Cleistopholis staudtii had mixed results: the 200mg/kg dose delayed convulsions to 47.3 minutes, and the 400mg/kg dose extended this to 65 minutes, indicating some efficacy with variability. Among the fractions, the N-Hexane fraction was most effective, delaying convulsions to around 90 minutes at both 200mg/kg and 400mg/kg doses, closely matching Phenobarbitone. The Ethylacetate fraction delayed convulsions to 78.7 minutes at 200mg/kg and 82 minutes at 400mg/kg, showing moderate variability. The Butanol fraction delayed convulsions to 77 minutes at 200mg/kg and 87.3 minutes at 400mg/kg, with noticeable variability at the higher dose. Phenobarbitone was the most effective at delaying convulsions, with the N-Hexane fraction being the closest in efficacy. The crude extract and other fractions exhibited varying degrees of effectiveness and higher variability.

Effects of Crude extracts and Fractions of Cleistopholis Staudtii on the Mortality rate after Convulsion

The study on mortality rates following convulsions revealed that the control Group (10ml/kg of 5% Tween 80) had 100% mortality, indicating no protective effect. Phenobarbitone at 60mg/kg significantly lowered mortality to 33%, demonstrating its effectiveness.The crude extract of Cleistopholis staudtii showed 100% mortality at both 200mg/kg and 400mg/kg doses, suggesting high toxicity or poor protective ability. Among the fractions, the N-Hexane fraction provided partial protection with 67% mortality at both doses. The Ethylacetate fraction had 100% mortality at 200mg/kg but reduced to 67% at 400mg/kg, showing some potential for protection at higher doses. The Butanol fraction also had 100% mortality at 200mg/kg and 67% at 400mg/kg, indicating toxicity with slight improvement at the higher dose.

Overall, Phenobarbitone was effective in reducing mortality, whereas the crude extract and its fractions generally showed high mortality rates, with only some fractions demonstrating improved outcomes at higher doses**.**

Comparison on the Efficacy of Crude Extract, Fractions and Phenobarbitone Reducing Convulsion

In the comparison of treatments for convulsions, Phenobarbitone (60mg/kg) was the most effective, resulting

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in only 0.67 convulsions with minimal variability, demonstrating its strong anticonvulsant properties. The crude extract of Cleistopholis staudtii showed limited efficacy, with 6.7 convulsions at 200mg/kg and 8.7 convulsions at 400mg/kg, indicating it was less effective than Phenobarbitone.

Among the fractions, the N-Hexane fraction showed moderate efficacy, with 8.7 convulsions at 200mg/kg and 10.3 at 400mg/kg, providing some anticonvulsant activity but still less effective than Phenobarbitone. The Ethylacetate fraction was less effective, with 21.3 convulsions at 200mg/kg and 7.3 at 400mg/kg, failing to reach the efficacy of Phenobarbitone. The Butanol fraction had mixed results, with 11.3 convulsions at 200mg/kg and 6.7 at 400mg/kg, showing some improvement at the higher dose but still falling short compared to Phenobarbitone.

Overall, Phenobarbitone was the most effective in reducing convulsions. The crude extract and its fractions varied in efficacy. The N-Hexane and Butanol fractions showed some potential but remained less significantly effective than Phenobarbitone, while the Ethylacetate fraction was the significantly least effective, especially at the lower dose (200mg/kg)

DISCUSSION

The phytochemical analysis of the sample indicates that the leaf is rich in saponins, flavonoids, cardiac glycosides steroids and alkaloids. The different levels of the phytochemicals in each of the fractions has shown to greatly impact their effects on convulsion especially for the Butanol fraction. The acute toxicity test results showed that the leave of the plant are safe for oral consumption up to a dose of 5000 mg/kg body weight. Additionally, research on *Cleistopholis staudtii* and other medicinal plants has generally found them to be non-toxic at standard dosages [2].

The result showed that Phenobarbitone is a highly effective anticonvulsant drug as it has done in other studies for various treatments [36] suggesting its high efficacy as an anticonvulsant agent. Among the fractions of the crude extract, the N-Hexane fraction was the most effective in delay of convulsion, matching those of Phenobarbitone. This could be due to its higher concentration of steroids. The results on mortality rate showed that the crud extract of the plant leaf showed a 100% mortality rate as it is consistent with previous works [7] as against the N-hexane Fraction which showed a 67% 67% mortality even at higher concentration. A previous research indicated that it could due to the higher concentration of steroid [3]. Phenobarbitone demonstrated th strong protective efficacy with mortality rate of 33% as also reported in another study [36]. The crude extracts of Cleistopholis staudtii showed variable results with frequency of convulsion with the butanol fraction showing the most promising efficiency in frequency (possibly due to its alkaloid content), followed by the N-hexane fractions though they were not as effective as the standard drug, Phenobarbitone.

CONCLUSION AND RECOMENDATIONS

In conclusion, this research study has highlighted the varying anticonvulsant effects of the crude extract and different fractions of *Cleistopholis staudtii*. The results showed that the N-hexane fraction exhibited a better overall anticonvulsant effect when compared to other fractions of the plant's leaf. While the butanol fraction is better at reducing the frequency of convulsion. These two fractions tend to exhibit these functions due to their high concentration of steroids and alkaloids respectively. Further research should experimenting on the isolated the steroids and alkaloids of the plant's leaves. this include detailed isolation, characterization, and mechanistic studies conducted to fully understand their therapeutic potentials.This will help to better understand the underlying mechanism as phytochemicals tend to be relatively specific in their mechanism of actions.

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