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Phytochemical Evaluation, Acute Toxicity, and Antiepileptic Efficacies of Leaf and Root Extract of *Boswellia dalzielii* Hutch. (Burseraceae)

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ABSTRACT

Boswellia dalzielii Hutch (Burseraceae) is a popular medicinal plant in Northeastern Nigerian ethnomedicine, known for its ability to treat epilepsy. This research aimed to scientifically evaluate the safety and antiepileptic use of aqueous extracts from the leaf and stem bark. The pulverized leaf and root bark were extracted using distilled water through the reflux method and were screened for phytochemicals. Lorke's method was used to calculate the lethal dose (LD_{50}), and the antiepileptic effects were assessed in rats using pentylenetetrazole and strychnine-induced seizure models. One-way analysis of variance was used to evaluate the result. Preliminary screening of phytocompounds of the aqueous extracts from *Boswellia dalzielli* leaves and root bark identified saponins, tannins, flavonoids, and terpenoids in both extracts, with alkaloids found only in the leaf extract. Acute toxicity tests, administered orally and intraperitoneally, showed an LD50 value of ≥ 5000 mg/kg, indicating that the extracts are safe for medicinal use. 250 and 500 mg/kg doses of aqueous leaf and root extracts conferred protection of 60 and 80%: 40 and 60% respectively against pentylenetetrazole-induced convulsion as well as protected 40 and 80; 20% and 60% of rats against strychnine-induced convulsion for both the leaf and root bark extracts respectively, when compared to sodium valproate (20 mg/kg). This standard drug conferred 100% protection. The aqueous leaf and root extracts of *Boswellia dalzielii* are revealed in this study to have antiepileptic efficacy, at the doses used, although the leaf is more potent than the root bark aqueous extract. Thus, this study has provided scientific justification for their medicinal use in the treatment of epilepsy.

Keywords: Convulsion; *Boswellia dalzielii*; Leaf; Root; Pentylenetetrazole; Strychnine; Aqueous Extract

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INTRODUCTION

For thousands of years, nature has been the source of medicine in the maintenance of human health. Coarse, decoctions, or portions and herbal drinks of plant materials in different forms have been used by people for healing over the past centuries. Studies have led researchers to dig deep into the medicinal plants used by the locals to ascertain the mechanism of action, improve the preparations applied, and find a standard remedy [1]. Epilepsy is one of the most common severe neurological diseases that impacts everyone worldwide of all ages around the world. It is expressed by periodic seizures and is caused by many factors. In most cases, there is a hidden genetic cause, but some recurring causes of epilepsy include brain damage, birth defects, head trauma, stroke, and neurological infections. In approximately 50% of epilepsy cases, a definite underlying cause can be identified [2]. presently, available anticonvulsant drugs are synthetic molecules causing adverse effects, including weight gain, liver toxicity, birth defects, and withdrawal [1]. The treatment of disease through the administration of available antiepileptic drugs is indicative, as these drugs restrain seizures but fail to treat the underlying pathological process in the brain [3]. Every year, 2.4 million people worldwide are diagnosed with an epileptic condition [4]. This has obligated the search for new drugs and compounds within the plant kingdom in the treatment of several neurological disorders, including epilepsy [5].

Boswellia dalzielii is a tree of the Savanna Forest, identifiable by its papery, raggedly peeling bark. The northern parts of the Ivory Coast have the Nigeria Dwarf palm tree, which is also seen to scatter. It finds planting as a village stockade on the volcanic peak massif of northern Nigeria. As a live fence, it brings (*Ba-samu*) and prevents (*Hannu*) bad luck. Hence, the Hausa names for the Nigerian Dwarf palm tree have been adopted for the tree [6]. The bark contains whitish scented exudate, which is burnt alone or with other fragrant resins to fumigate the clothes and the room to drive out flies and mosquitoes. In Northern Nigeria, the bark is cooked to obtain a large quantity of a wash for fever, rheumatism, and ulcers. The solution is taken orally for gastrointestinal troubles (stomachic). [7]. This study sought to validate a scientific basis for the application of aqueous leaf and root-bark extract of *Boswellia dalzielii* in the therapy and regulation of epilepsy by the residents of Northern Nigeria to find a lead plant that could serve as a potential anti-epileptic treatment. The expected data generated from this study could serve as a reference point on which other related studies could be built upon in the future, which may facilitate the integration of the plant for use in modern medicine.

MATERIALS AND METHODS

Plant Collection

Fresh leaves and root bark of *Boswellia dalzielii* were obtained from Chibok in the Chibok LGA of Borno State. The plants were evaluated and confirmed by Professor S.S. Sanusi, a Taxonomist at the Faculty of Pharmacy, University of Maiduguri, Nigeria. A voucher with the specimen number 012A was assigned to the plant and stored for prospective applications.

Preparation of the Plant Extracts

The leaf and the root plant material were dried at ambient temperature for 14 days and subsequently milled into coarse powder with a mortar and pestle. The pulverized leaf and root bark (500g) were extracted using 5000 mL distilled water by the hydro distillation method for six (6) hours. The mixtures were sieved utilizing Whatman No. 1 filter paper and concentrated rotary evaporator set at 40^{o} °C. The leaf and root extracts were encoded BDL and BDR, respectively, and stored in a desiccator until needed for use.

Phytochemical Screening

Preliminary screening of secondary metabolites on both leaf and root bark extracts were carried out according to the standard phytochemical methods described by Evans [8] as adopted by Yakubu *et al.* [10]. The extracts were screened for the presence of classical phytochemicals which

include: alkaloids, tannins, flavonoids, saponins, anthraquinones, terpenoids, cardiac glycosides and carbohydrates.

Animals

The animal house of the Department of Biochemistry, University of Maiduguri, was used to house 80 – 200g Male and female Wistar strain rats for the research. In a well-ventilated cage, standard laboratory feeds (wheat offal) were provided, and water was available ad libitum.

Drugs and Drug Solutions

To keep the drug solution stable, fresh solutions were made every day experiment. They were kept in airtight, amber containers in the refrigerator until use. PTZ and strychnine were purchased from Sigma Chemical Co. (St. Louis, USA). Sodium Valproate (Fawdon Manufacturing Centre, Newcastle-upon-Tyne, UK).

Drug Administration Routes

Both extracts were administered intraperitoneally, pentylenetetrazol, strychnine and Sodium valproate were administered subcutaneously.

Pharmacological Studies

Acute toxicity study

The acute toxicity (LD_{50}) of both aqueous extracts of *Boswellia dalzielii* was evaluated as per the methodology described by Lorke [10] and adopted by Yakubu *et al.* [11].

In this research, the oral and intraperitoneal routes were used. Phase one involved nine rats, divided into three sets of three for every route. The doses of aqueous extracts given in the phase are 10, 100, and 1000 mg/kg body weight, monitored for 24 hours for mortality by intraperitoneally and oral route. During phase II, for each route, 3 animals were grouped for each group. As per phase I, the aqueous extracts were given at the specified doses. The rats were kept under observation for the first critical hours for evidence of toxicity and mortality, and 7 days thereafter, daily. The formula below was applied to determine the LD50:

$$LD_{50} = \sqrt{a \times b}$$

Where

a = the least dose that killed a rat

b = the highest doses that did not kill a rat

PTZ – Induced Seizure in Rats

The method of Medugu *et al.* [12] was used. 30 rats were separated into six (6) groups of each having five (5). Using the aqueous leaf and root extract, group one was pretreated with normal saline (10 ml/kg oral), whereas 250mg/kg and 500mg/kg of leaf and root bark extract were administered to group two, three & four, and five groups, respectively. Sodium valproate (20mg/kg) was used to treat the sixth (6^{th}) group intraperitoneally. After thirty minutes, a convulsive dose of PTZ (65 mg/k) was administered subcutaneously to rats in all the groups and they were observed for 30 minutes. The extract's ability to stop the effect of the PTZ is seen when there's an absence of clonic spasm for at least five seconds.

Strychnine-Induced Seizure in Rats

The procedure used is as explained by Lehmann *et al.* [13] as adopted by Medugu *et al.* [12]. In brief, strychnine convulsion was administered in rats via subcutaneous injection of 100mg/kg of strychnine nitrate. 30 minutes before the strychnine administration, four (4) groups of 5 rats each were administered with the aqueous leaf and root extract of *Boswellia dalzielii* intraperitoneally. Sodium valproate (200mg/kg) was given to the sixth (6^{th}) group intraperitoneally, which acted as the positive control, whereas group I was administered 10 ml/kg normal saline as the negative control. Tonic extensor jerks of the hind limbs were observed, followed by death within half an hour. The absence of tonic limb extension was taken as an indication of the anticonvulsant potential of *Boswellia dalzielli* against strychnine-induced seizure.

Statistical Analysis

Statistical significance of the pharmacological findings was evaluated using one-way ANOVA, then Tukey-Kramer's test. A p-value that is less than 0.05 is considered significant.

RESULTS

Phytochemical Constituents of Leaf and Root Bark Aqueous Extracts of *Boswellia Dalzielii*

Table 1 shows the phytocompounds of water-based extracts derived from the leaf and root bark of *Boswellia dalzielii*. The study indicates tannins, flavonoids, terpenes, saponins, and steroids in the leaf and root bark extracts.

Effect of Aqueous Leaf and Root Extracts of *Boswellia dalzielii* on Pentylenetetrazole-Induced Wistar Rats

The water-based extracts derived from the leaf and root bark of *Boswellia dalzielii* exhibit antiepileptic effects in the rat model of pentylenetetrazole-induced epilepsy. The onset of spasm, convulsion, and death was significantly delayed in the groups treated with BDL and BDR extracts in comparison to the group pretreated with normal saline. 500 mg/kg, which is the higher dose, shows a substantial increase in survival (80%) compared to the group pretreated with normal saline (0%). *Boswellia dalziellii* root at 250 mg/kg and 500 mg/kg also demonstrates significant antiepileptic effects, with improved survival rates (40% and 60%, respectively). Sodium Valproate, a known antiepileptic drug, shows a 100% survival rate. These findings suggest that *Boswellia dalzielii* extracts, particularly at higher doses, have potential antiepileptic properties. Further studies may explore the underlying mechanisms and safety profiles of these extracts for potential therapeutic applications.

Effects of Aqueous Extracts from Leaf and Root Extracts of *B. dalzielii* on Strychnine-Induced Wistar Rats

250 milligrams per kilogram of leaf extract showed a notable decrease in the onset of spasm, epileptic episode, and death in comparison to the control. 500 milligrams per kilogram of the leaf extract showed significant effects on convulsion, death, and a significant increase in survival compared to the control. The root bark extract at both 250 milligrams per kilogram and 500 milligrams per kilogram revealed significant effects on all parameters compared to the control. Sodium valproate, the reference drug, showed a significant increase in survival, indicating its efficacy.

Table 1: Phytochemical Constituents of Leaf and Root Bark Aqueous Extracts of *Boswellia dalzielii*

S/N	Phytochemical Test	Inference	
		Leaf	Root
1	Carbohydrates	+	+
2	Tannins	+	+
3	Phlobatannins	-	-
4	Cardiac glycosides	+	-
5	Flavonoids	+	+
6	Terpenes	+	+
8	Saponins	+	+
9	Soluble starch	-	-
10	Alkaloids	+	-
11	Steroidal nucleus	+	+

Acute Toxicity Study

Table 3 presents the result of the intraperitoneal acute toxicity effect of *Boswellia dalzielii* aqueous leaf and root bark. At the doses of 10, 100, 1000, 2900, and 5000mg/kg of both leaf and root extract that were administered intraperitoneally, no mortality was recorded. Thus, the LD_{50} was estimated to be ≥5000mg/kg.

Table 3: Oral and Intraperitoneal Acute Toxicity Effect of *Boswellia dalzielii* Aqueous Leaf and Roots Bark Extracts

Phase	Dose (mg/kg)	No. of Rat	Mortality Rate			
			Leaf		Root	
			Oral route	IP route	Oral route	IP route
I	10	3	0/3	0/3	0/3	0/3
	100	3	0/3	0/3	0/3	0/3
	1000	3	0/3	0/3	0/3	0/3
II						
	1600	1	0/1	0/1	0/1	0/1
	2900	1	0/1	0/1	0/1	0/1
	5000	1	0/1	0/1	0/1	0/1

Table 4: Effect of *Boswellia dalzielii* Aqueous Leaf and Root Bark Extracts on Pentylenetetrazole-induced Epilepsy in Rats

Extract pretreatment (mg/kg	Onset of Spasm (min) Mean±SEM	Onset of convulsion (min) Mean±SEM	onset of death (min) Mean±SEM	Quantal death	Survival (%)
Control + 100 mg/kg of PTZ	14.44±0.40	7.32±0.90	16.42±0.23	0/5	0
BDL 250 mg/kg + 100 mg/kg of PTZ	11.60±1.10*	12.95±0.25*	20.92±0.25*	3/5	60
BDL 500 mg/kg + 100 mg/kg of PTZ	14.08±0.14*	17.14±0.31*	36.82±0.02*	4/5	80
BDR 250 mg/kg + 100 mg/kg of PTZ	10.00±1.05*	11.30±0.88*	13.20±1.25*	2/5	40
BDR 500 mg/kg + 100 mg/kg of PTZ	12.60±1.10*	14.95±0.25*	19.42±0.45*	3/5	60
Sodium Valproate (200mg/kg)	14.10±0.42	20.20±0.20	-	5/5	100

Keys: BDL: *Boswellia dalzielii* Leaf Extract; BDR: *Boswellia dalzielii* Root Bark Extract

Results are presented as Mean ± SEM; n=5. Data with the same superscript are statistically significant ($p < 0.05$).

Table 5: Effect of Aqueous Leaf and Root Extract on Strychnine-Induced Epilepsy in Rats

Extract pretreatment (mg/kg	Onset of Spasm (min) Mean ± SEM	Onset of Convulsion (min) Mean ± SEM	Onset of death (min) Mean ± SEM	Quantal death	Survival (%)
Control + 100 mg/kg of STY	13.54±0.60	8.32±2.00	18.58±0.20	1/5	20
BDL 250 mg/kg + 100 mg/kg of STY	11.24±0.60*	15.20±0.37*	21.60±0.21*	2/5	40
BDL 500 mg/kg + 100 mg/kg of STY	14.30±0.16*	21.70±0.70*	27.02±0.39*	4/5	80
BDR 250 mg/kg + 100 mg/kg of STY	07.24±0.60*	12.00±0.10*	15.70±0.30*	2/5	20
BDR 500 mg/kg + 100 mg/kg of STY	09.20±0.16*	11.50±0.70*	17.12±0.20*	3/5	60
Sodium Valproate (200mg/kg)	14.10±0.42	20.20±0.20	-	5/5	100

Keys: BDL: *Boswellia dalzielii* Leaf Extract; BDR: *Boswellia dalzielii* Root Bark Extract
Results are presented as Mean ± (SEM); n=5. Data with the same superscript are significant ($p < 0.05$).

DISCUSSION

The secondary metabolites and other plant ingredients are primarily responsible for the physiological effects shown in cell and animal research employing these plants [14]. Phytochemical components, particularly flavonoids, saponins, and tannins, were thought to be beneficial in a variety of central nervous system (CNS) illnesses [15]. The possibility of these metabolites having CNS activity similar to anticonvulsant effects was indicated by their existence as components of the plant.

Accoside A and bacoside B (constituents of saponin) have been proposed to possess anticonvulsant properties in animal models among these phytochemicals [16]. Phytochemicals and antiepileptic study by Yakubu *et al.* [11] showed that *Boswellia dalzielli* is effective against Maximal electrically induced seizure, where saponin was present in higher concentration. Thus, it is likely that the convulsion-inhibiting property of *Boswellia dalzielli* extracts may be attributed to the activity of saponins and flavonoids, among other compounds demonstrated to be in the extract.

The cute toxicity studies indicated that the aqueous leaf and root bark extract of *Boswellia dalzielii* is considered safe when delivered via the oral route at a dose up to 5000 mg/kg, following the OECD guideline for the limit test. During the initial hours after extract administration, animals treated with the extract exhibited behavioral changes, including reduced movement and heightened sensitivity to external stimuli.

The induction of convulsions by PTZ in mice has been extensively studied in the past and is commonly employed to assess herbal drugs, as it reflects various aspects of human epileptiform convulsions [17]. Pentylenetetrazole is a widely used seizure-inducing agent, and the convulsion-inhibiting property in the subcutaneous PTZ test is used to detect compounds that make seizures less likely by raising the brain's seizure threshold [18]. In the investigation of the effect of *Boswellia dalzielii* aqueous leaf and root bark extracts on pentylenetetrazol (PTZ)-induced epilepsy in rats, a comparison between the root bark (BDR) and leaf (BDL) extracts reveals distinct patterns of efficacy, emphasizing the superior impact of the root extract in mitigating PTZ-induced seizures. Notably, at a dose of 250 milligrams per kilogram, the root bark extract exhibits a significantly faster onset of spasms, convulsions, and death compared to the leaf extract. This trend persists at the elevated dose of 500 milligrams per kilograms, further emphasizing

the root extract's potency. These findings indicate that *BDZ* leaf and root, at respective dosages, can raise the seizure barrier. In line with the phytochemical result, which showed the presence of flavonoid in significant concentration, it has been reported that across a range of GABA_A receptors, hispidulin, a compound of flavonoid, can enhance the activity of a receptor without directly activating it (Singh *et al.*, 1988). The seizure-inducing effect may result from interference with GABA neurotransmission, specifically by suppressing GABA activity at GABAA receptors [19]. (White *et al.*, 1998). Pentylenetetrazole induces convulsive attacks through GABAergic mechanisms of inhibition [20]. The improvement and disruption of the transfer of GABA will lessen and intensify seizure activity, in that order [20]. Seizure-suppressing agents such as ethosuximide, valproic acid, phenobarbitone, and benzodiazepine are potent in the treatment of non-focal seizures, including absence and myoclonic types. They show dose-dependent inhibition of many convulsion types induced by PTZ [21] (Westmoreland et al., 1994). A key mechanism of action for seizure-suppressing agents (e.g, ETX and VPA) at the cellular level is the inhibition of low-voltage calcium currents in the thalamus neurons [22].

Strychnine was documented to have a clear mechanism of action, acting as a competitive antagonist against glycine, which acts by blocking its inhibitory effect, leading to convulsions [23]. Examining the efficacy of *Boswellia dalzielii* leaf (BDL) and root bark (BDR) extracts on strychnine-evoked seizures in Wistar rats, the results reveal distinct patterns of efficacy. In comparison to the control group receiving only 100 mg/kg of strychnine, the leaf extract at 250 mg/kg demonstrates a significant reduction in the onset of spasms, convulsions, and death. Strychnine does block chloride conductance prompted by glycine, thereby reducing the amplitude of inhibitory postsynaptic capacities [24]. The leaf extract at 500 mg/kg exhibits pronounced effects on convulsion and death, accompanied by a considerable increase in survival. On the other hand, the root bark extract at both 250 and 500 milligrams per kilogram doses consistently outperforms the leaf extract. Notably, the root extract induces a more rapid onset of responses, significantly decreasing the time to the onset of spasms, convulsions, and death, indicating a robust antiepileptic effect. Furthermore, a direct comparison between the leaf and root extracts reveals that the root extract consistently elicits a faster response across all measured parameters. At both 250 mg/kg and 500 mg/kg doses, the root extract demonstrates a significantly quicker onset of spasms, convulsions, and death when compared to the leaf extract. Glycine was revealed to be the core inhibitory transmitter in the spinal cord and brainstem of higher vertebrates [25].

CONCLUSION

This study on the extracts from *Boswellia dalzielii*, notably those from the leaves and the roots, showed significant antiepileptic activity and possesses a safe acute dose regimen. Both extracts had antiepileptic potential against PTZ-induced epilepsy; however, the BDR extract was more effective. Both extracts prevented convulsions in epilepsy generated by strychnine; however, at different doses, the root extract consistently outperformed the leaf extract. These findings highlight the need for more research into the therapeutic potential of *Boswellia dalzielii* in neurology and provide important insights into herbal therapies for epilepsy.

Disclaimer

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation, but for the advancement of knowledge. Also, the research was not funded by the producing company; rather, it was funded by the personal efforts of the authors.

Consent

It is not applicable.

Ethical approval

All experiments were conducted per the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No.80-23) as revised in 1996.

Competing interests

Authors have declared that no competing interests exist.

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