

Phytochemical Screening and Anticonvulsant Activity of the Residual Aqueous Fraction of *Tapinanthus globiferus* Growing on *Ficus glums*

Abubakar K¹, Adebisi IM¹, Ugwah-Oguejiofor JC¹, Idris GO¹, Idris B² and Mshelia HE³

Abstract

Tapinanthus globiferus (Loranthaceae) is a plant used in traditional medicine for the treatment of rheumatism, syphilis, fever and epilepsy. In this study, the acute toxicity study revealed an oral LD₅₀ of ≥ 5000 mg/kg. The anticonvulsant activity of the residual aqueous fraction was investigated at doses of 250, 500, and 1000 mg/kg using the Pentylenetetrazole (PTZ) induced seizure and maximal electroshock seizure models in mice and chicks. In the PTZ induced seizure the fraction offered 62.5% protection against seizure and prolonged the onset of seizure from 54.23 ± 6.12 s to 290.86 ± 14.43 s. The plant decreased the minimum recovery time (MRT) after hind limb tonic extension (HTLE) from 213.4 ± 19.12 s to 136 ± 16.1 s in the 500 mg/kg group.

The CNS depressant effect was also studied using the Diazepam induce sleep model. The fraction used dose dependently decreased the onset of diazepam induced sleep 160.03 ± 19.24 s to 28.00 ± 7.18 s, with a marked increase in duration of sleep 39.00 ± 12.53 min to 198.17 ± 27.28 min in the 1000 mg/kg treated group.

The data obtained from this study suggest that the residual aqueous portion of the dried whole plant of *Tapinanthus globiferus* may possess bioactive compounds with anticonvulsant effect.

Keywords: *Tapinanthus globiferus*; Pentylenetetrazole; Seizure; GABA; Protection

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Introduction

Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need [1]. Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity [2] and many such plants need to be scientifically investigated.

Tapinanthus globiferus is mistletoe of the family *Loranthaceae*. It is woody with shrubs, usually aerial hemiparasites on other seed plants, often spreading along host by runners (epicortical roots), more rarely terrestrial root-parasitic shrubs or trees, nodes not articulated, glabrous or hairy, hairs often stellate or verticillate. *Tapinanthus globiferus* is locally known as mistletoe (English), *Kauchin* (Hausa), *afomo* (Yoruba), and *Osisi/Okwuma osa* (Igbo)

in Nigeria, and belongs to the family of *Loranthaceae* [3]. In Saudi Arabia, *Tapinanthus globiferus* is known as *Hadhal* [4].

Mistletoes of the *Loranthaceae* and *Viscaceae*, are widely used in various cultures in almost every continent to treat various ailments including hypertension, cancer, and diabetes, or used as a diuretic agent [3-5]. Other uses include cure for bone fracture and body pain [6]. *T. globiferus* is one of the mistletoes commonly consumed by the people of Akwa Ibom State for the treatment of hypertension, ulcers, epilepsy, diabetics, weakness of vision, and for promoting muscular relaxation before delivery [7].

Epilepsy is one of the oldest disease conditions known to mankind [8] and still the most common neurological condition affecting individuals of all ages. The incidence of epilepsy in developed countries is approximately 40 to 70 per 100 000 people in the

- 1 Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto, Nigeria
- 2 Department of Pharmacology, University Sains, Malaysia
- 3 Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Corresponding author: Abubakar K

✉ kabirsultan2002@gmail.com

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Tel: 234-8035863780.

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general population, while that of developing countries, are 80 to 140 per 100,000 [8]. The mainstay of epilepsy management is the use of antiepileptic drugs which have proved to be effective in reducing the onset and severity of attacks. Therapy is symptomatic in that available drugs inhibit seizure but neither effective prophylaxis nor cure is available [9]. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy [10,11].

Personal communication with traditional healers in Sokoto state, North western Nigeria provides enough evidence for the use of *T. globiferus* in the management of convulsive disorders. Literature search on the plant reveals the previous studies conducted which includes antibacterial effect [12] antioxidant effect [13] and antihypertensive studies via reduction of LDL and triglycerides.

Methodology

Plant collection and identification

Fresh whole plant *T. globiferus* growing on *Ficus glumosa* was collected from Zamfara State, Nigeria on 18th June, 2015. The plant was identified, authenticated and given a voucher number (UDUH/ANS/0076) by Mallam Lawali Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. Samples were kept for further reference. The fresh plants were then air dried at room temperature and size reduced into powder using pestle and mortar.

Preparation and fractionation of the extract

The method described by Ref. [14] modified by Ref. [15] was followed; is mentioned in **Figure 1**.

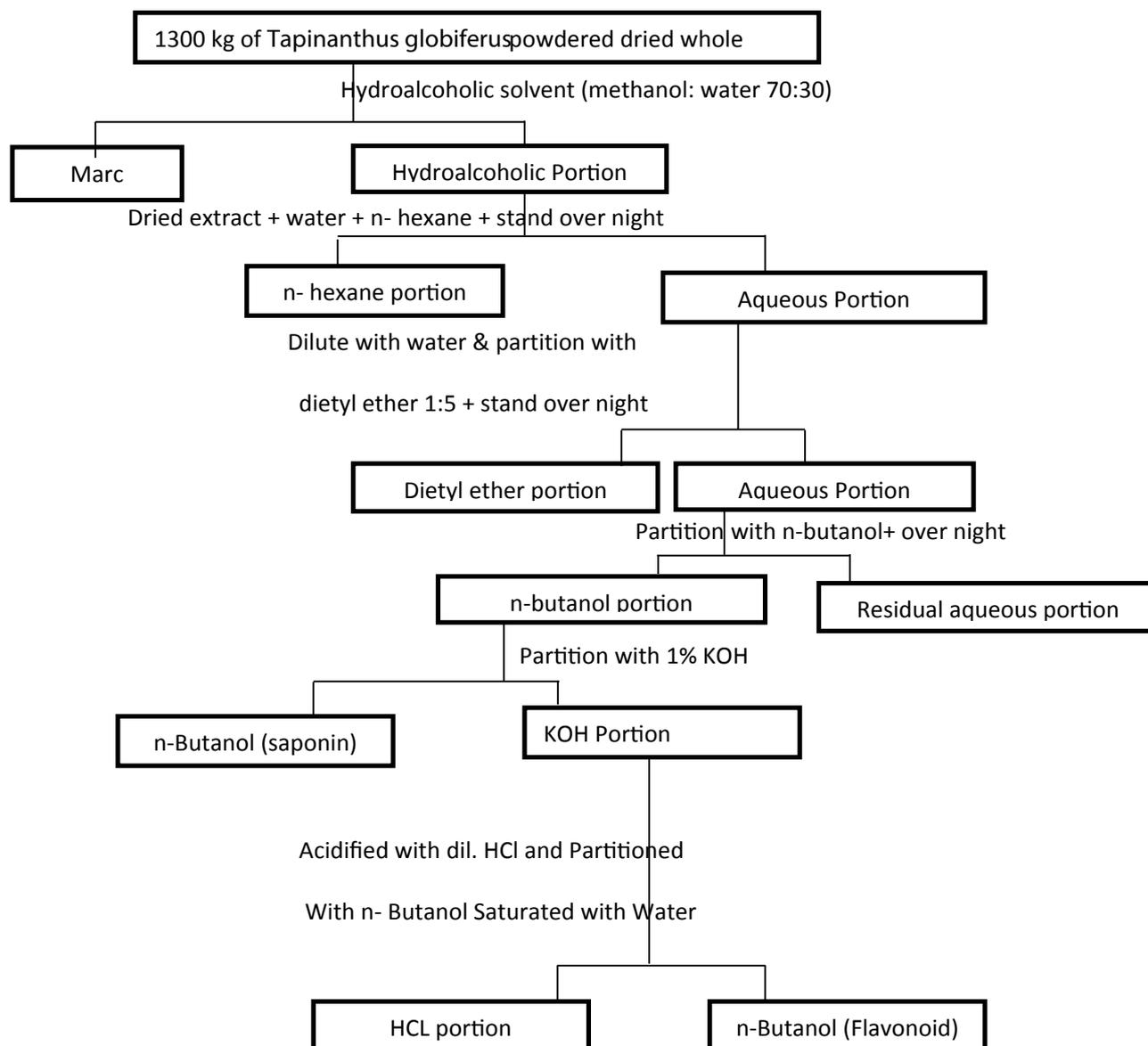


Figure 1 Schematic Chart for Fractionation of Flavonoids, Saponin from *Tapinanthus globiferus* powdered whole plant, from Ref. [14].

Experimental animals

Mice: Adult Mice of both sexes weighing between 24 g and 32 g were obtained from the Animal house facility of the Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria. The animals were maintained in a well-ventilated room, under ambient laboratory conditions of temperature and humidity and were fed on commercial feed and water except when fasting was required in the course of study. All experiment protocols were approved by the University animal ethics committee.

Chicks: Two day old chicks were bought from Ojuanu Agricultural enterprise No. 32 Sultan Atiku road Sokoto, Sokoto State prior to the experiment and were allowed to acclimatize to the laboratory environment before the practical.

Phytochemical screening

The dried residual aqueous extract of the whole plant of *Tapinanthus globiferus* was subjected to phytochemical screening tests for the detection of various constituents [16].

Acute toxicity studies

Acute toxicity study was determined using Ref. [17] via oral route in mice and chicks.

Diazepam-induced sleep in two day old chicks: The chicks were divided into four groups of ten chicks each. Group 1-3 received 250, 500, 1000 mg/kg of *T. globiferus* residual aqueous fraction respectively via intra-peritoneal route; group 4 received distilled water which served as control, in equivalent volume of the extract. Thirty minutes post administration all the groups were administered diazepam 10 mg/kg i.p. The onset and duration of sleep was observed and recorded with the chicks placed in individual cages. Loss of righting reflex [18] touching the floor with the beak and leaning on the wall of the cage for support was considered as the criterion for sleep, while the interval between the loss and the recovery of straightening was regarded as the duration of sleep [19].

Pentylentetrazole (Ptz) induced seizure in mice: The method described by Ref. [19] was employed for this study. 40 mice were divided into five groups each containing 8 mice each. The first group received diazepam 30 mg/kg body weight i.p, groups 2-5 received 250, 500, 1000 mg/kg body weight respectively of the extract orally, 0.2 mls distilled water i.p was given to the fifth group which served as the negative control. Clonic seizures were induced in the mice by the i.p. injection of 70 mg/kg of PTZ to all the groups after 1 hour. The onset of seizures in non-protected mice was also recorded.

Maximum electroshock test (Mest): The method described by Ref. [20,21] was used in this study. Two day old cockerels were used; they were randomly divided into five groups of ten animals each. The first group was administered distilled water 10 ml/kg i.p, while the second, third and fourth group received 250, 500, 1000 mg/kg ip of the extract respectively. The fifth group received phenytoin 20 mg/kg i.p, thirty minutes after drug treatment maximum electroshock was administered to the chicks to induce seizure in the chicks using Ugo Basile electroconvulsive machine (model 57800-001) with corneal electrode placed on the upper eyelids of the chicks after being moistened with normal saline.

The shock duration, frequency and pulse width were set and maintained at 0.6 s, 100 pulse/second and 0.6 m/s respectively. A current of 80mA was used throughout. Seizures were manifested as hind limb tonic extension (HLTE) in the chicks [22]. The ability of the extract to prevent this feature or shorten the recovery from HLTE was considered an indication of anticonvulsant activity [21,22].

Results

Percentage yield

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of powdered drug}} \times 100$$

$$77/1300 \times 100\% = 5.9\% \text{ w/w}$$

Acute toxicity studies

Acute toxicity studies: The oral LD₅₀ of the extract was found to be greater than 5000 mg/kg in both chicks and mice. From the results in **Table 1**, the residual aqueous fraction of *T. globiferus* contains carbohydrates, tannins, saponins, flavonoid, proteins, glycosides and steroids with the absence of anthraquinones and triterpenes.

Effect of *T. globiferus* on PTZ induced seizure

The result on **Table 2**; indicated that the residual aqueous portion of *T. g* 250 and 500 mg/kg significantly prolong the latency to PTZ induced seizure. Also 1000 mg/kg of the extract protected 62.5% of the mice against PTZ seizure. The anticonvulsant drug diazepam 5 mg/kg provided 100% protection against seizure and mortality.

Effect of *T. globiferus* residual aqueous fraction on maximal electroshock seizure

The result of the MES test indicated that the extract offered no protection against seizure and there was 100% death of all the chicks. Though there was a significant decrease in the mean recovery time, none of the chicks survived beyond 60 minutes after induction of seizure (**Table 3**).

Table 1 Phytochemical composition of the Residual aqueous fraction of *T. globiferus*.

Constituents	Result
Carbohydrates	+
Tannins	+
Alkaloids	+
Saponins	+
Flavonoid	+
Anthraquinones	-
Proteins	+
Glycosides	+
Steroids	+
Triterpenes	-

Where, +: Present; -: Absent

Discussion

The phytochemical screening of the *Tapinanthus globiferus* revealed the presence of constituents such as Saponins, Tannins, Glycosides, Protein, Steroids and Flavonoids. Pharmacological effects of plant phytochemicals have been previously documented. Alkaloids have been reported to function as amoebicides, expectorant, anaesthetic, analgesic and anti-helminthic [23]. Saponins have been said to be beneficial in lowering blood cholesterol [7,24]. Flavonoids also referred to as nature's biological response modifiers, have been found to have the ability to modify the body's reaction to allergens, viruses and carcinogens [25]. Anticonvulsant effect of saponins and flavonoids has been reported by Ref. [26-29].

Acute toxicity studies of the plant gave an LD₅₀ that can be classified as non-toxic. According to Dietrich Lorke 1 mg/kg is considered highly toxic, 10 mg as toxic, 100 mg/kg as moderately toxic, 1000 mg/kg as slightly toxic and nontoxic above 5000 mg/kg [17]. The highest dose used in this study was 1000 mg which represented 20% of the highest dose tolerated by the animals.

The potentiating of the sleep time suggests the presence of sedative properties in the extract of the plant [30,31] (**Figure 2**). The sedative properties could be related to the presence of some components in the fraction activating the benzodiazepine, barbiturate and/or GABA receptors in the GABAA receptor complex [32]. Diazepam act by binding to the GABAA receptor complex. Diazepam potentiates GABA-mediated inhibition via increase in the affinity of this inhibitory neurotransmitter to its recognition sites within the GABAA receptor complex, by increasing the opening frequency of the chloride ion channel which leads to the enhancement of influx of chloride anions into the neuron and subsequent hyperpolarisation [33].

Tapinanthus globiferus residual aqueous fraction at a dose of 250-500 mg/kg prolonged the onset of seizure. At a dose of 1000 mg/kg it offered 62.5% protection against myoclonic seizures induced by PTZ. The anticonvulsant drug (diazepam 5 mg/kg)

prevented the mice against seizure. The PTZ test is known to identify anticonvulsant drugs effective against myoclonic and absence seizure and may elicit seizures by inhibiting gabaergic mechanisms [34]. Standard antiepileptic drugs, Diazepam and Phenobarbiton are believed to produce their effects by enhancing GABA mediated inhibition in the brain [32]. From this study it can be said that the plant may be of benefit in human myoclonic and absence seizure.

The MES test is the most frequently used animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal" [35]. Electroshock seizures are characterized by tonic extension of the hind limb (HLTE), [15]. Antiepileptic drugs that antagonize MES-induced tonic extension are known to act by blocking seizure spread [36]. Moreover, drugs that inhibit voltage-dependent Na⁺ channels, such as phenytoin can prevent MES-induced tonic extension [36]. In this study maximal electroshock produced seizures in all the animals used but the extract decreased the duration of tonic clonic seizure. The ability of *T. globiferus* to decrease the duration of seizure in the MES test is indicative of its activity against generalized tonic clonic seizures [37,38].

The result of this study suggest that the residual aqueous fraction of *Tapinanthus globiferus* growing on *Ficus glumosa* possess bioactive principles with CNS depressant activity and; significant anticonvulsant activity in mice and chick; this supports the Ethno medical use of the plant in the treatment of epilepsy.

Recommendations

Further studies should be carried on the plant to isolate the compound(s) responsible for the anticonvulsant activity. Studies should also be carried out using other models of epilepsy to identify the possible mechanism of action. Pharmacological screening should be done generally on Mistletoes because they are used traditionally for treatment of many disease conditions.

Table 2 Result of the effect of the residual aqueous fraction of *T. globiferus* against PTZ induced seizure.

Treatment (mg/kg)	Onset of seizure (sec)	Quantal protection	% protection	% mortality
D/W	54.23 ± 6.12	0/8	0	87.5
250	280.11 ± 12.09*	0/8	0	100
500	290.86 ± 14.43*	2/8	25	37.5
1000	62.33 ± 6.60	5/8	62.5	0
Diazepam	0	8/8	100	0

Values are expressed as Mean ± SEM, n=8, *Statistically significant P<0.05 as compared with the negative control (one way ANOVA followed by Dunnett's test).

Table 3 Result of the effect of *T. globiferus* on maximal electroshocks seizure.

Treatment (mg/kg)	Mean recovery time (sec)	Quantal protection	% protection	% Mortality
D/W 10	213.4 ± 19.1	0/8	0	0
<i>T. g</i> 250	139 ± 16.9**	0/8	0	0
<i>T. g</i> 500	136 ± 16.1**	0/8	0	0
<i>T. g</i> 1000	102 ± 13.7**	0/8	0	0
Ph 20	68.5 ± 3.4***	8/8	100	0

Values are expressed as Mean ± SEM, n=10, *Statistically significant p<0.05, **p<0.01, ***p<0.001 as compared with the negative control. D/W=Distilled water, pH=Phenytoin.

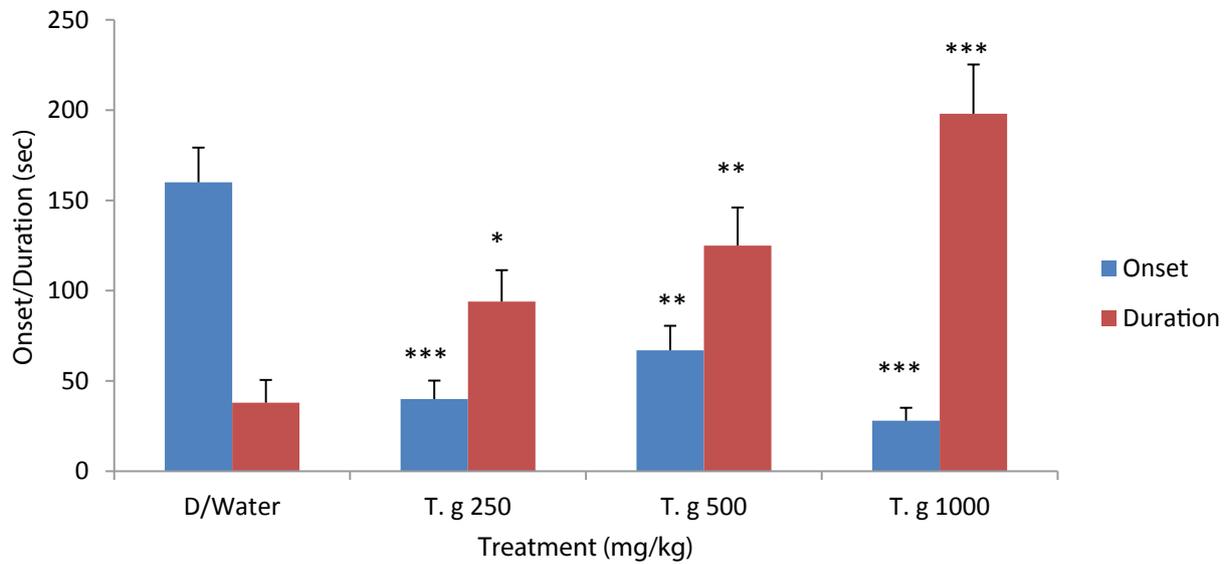


Figure 2 Sleep potentiating properties of *T. globiferus* in diazepam induced sleep model; Results are expressed as mean \pm SEM, n=8, *= $p < 0.05$ (one way ANOVA followed by Dunnett's test).

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