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ANTICONVULSANT ACTIVITY OF METHANOL LEAF FRACTIONS OF *LAGGERA AURITA* LINN (ASTERACEAE) IN LABORATORY ANIMALS

***[1]Mustapha Ibrahim Gudaji, [2]Abdullahi Ibrahim Doma and [2]Sani Malami**

[1]Department of Psychiatry, Faculty of Clinical Sciences, Bayero University, Kano
[2]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University, Kano
Postcode:700233. P.M.B: 3452, Kano, Nigeria

*Corresponding authors' email: mgudaji@yahoo.com Phone: +2348037019253

ABSTRACT

The Plant *Laggera aurita* Linn, is a medicinal plant that is found in Africa and has been used in the treatment of many medical conditions. This study aimed to evaluate the anti-seizure effects of solvents fractions of methanol extract of *Laggera aurita* in chicks, mice and rats. Preliminary screening for phytochemicals and LD_{50} studies were carried out using standard protocols. The fractions were evaluated against electrically-induced seizures in chicks, Pentylenetetragudajizole (PTZ)-induced seizures in mice and PTZ-induced kindling in rats. Phytochemicals revealed were alkaloids, flavonoids, tannins, steroids, terpenoids and cardiac glycosides. Intraperitoneal (*I.p*) LD_{50} values were 1264 mg/kg and 1468 mg/kg for n-hexane, 2154 mg/kg and 2375 mg/kg for ethyl acetate and 3807 mg/kg and 3986 mg/kg for residual aqueous fractions in mice and chicks respectively. Maximal electroshock (MES) induced seizure model used on chicks that received graded doses of solvents fractions, did not protect them against seizures. N-hexane protected the mice against seizure at all doses, up to 50% at 200 mg/kg and 400 mg/kg, while the ethyl acetate and residual aqueous fractions confers no protection against seizures at all doses during the subcutaneous PTZ-induced seizure model tests. The n-hexane fraction produced significant protection against subcutaneous pentylenetetrazole (40mg/kg) induced kindling in rats at all the doses tested, while the ethyl acetate and residual aqueous fraction show no protection. The above findings suggest that the plant *Laggera aurita* possesses anticonvulsant activities which may have provided basis for its traditional use against epilepsy.

Keywords: Anticonvulsant, *Laggera aurita*, Pentylenetetrazole, Solvent fractions

INTRODUCTION

Worldwide, mental health disorders impact around 450 million individuals, of which 121 million are specifically affected by depression, and another sixty five million people from epilepsy (Mahendran *et al*., 2014)**.** Epilepsy as a word has it roots in ancient Greek which implies 'to seize', rightly describing this widespread and significant neurological condition (Hirtz *et al*., 2007). It occurs when abnormal electrical impulses in the brain network become uncontrollable, impacting the central nervous system (Danmalam *et al*., 2017). It is however, thought to be a prevalent and varied group of long-term neurological disorders marked by seizures (Chang and Lowenstein, 2003). It affects individuals of all ages, races, social backgrounds, genders, and geographic locations. Common causes include infections, trauma, metabolic disorders, or tumors, though it can also be idiopathic, occurring without any clear underlying cause aside from a potential genetic predisposition (Brodie *et al*., 2016).

Epilepsy encompasses a diverse range of chronic neurological disorders characterized by seizures, affecting around 100 million people worldwide at some point in their lives. While some cases may be idiopathic, many are caused by preventable and treatable factors such as infections, trauma, metabolic conditions, or tumors. Despite the challenges, early intervention and management can mitigate the significant physical, psychological, social, and economic consequences of epilepsy on individuals and families (Abubakar *et al*., 2016). Epilepsy is an enduring medical condition with a long history, impacting people of all ages across the globe. Notably, its occurrence varies between advanced and developing nations, with rates around 40-70 per 100,000 individuals in developed regions and higher rates of 80-140 out of 100,000 people in developing areas (Nazifi *et al*., 2017).

Although there have been considerable advances in epilepsy diagnosis and treatment globally, a significant treatment gap persists, leaving around eight million Africans with epilepsy without access to modern anti-epileptic drugs. The financial implications of epilepsy are substantial including high costs for medical care, loss of employment opportunities, and reduced household productivity, which vary according to the condition's severity, treatment outcomes, and time since diagnosis, affecting individuals, families, health care systems, and society as a whole (WHO, 2012; Yaro *et al*., 2015; Nazifi *et al*., 2017).

Epilepsy commonly affects individuals irrespective of their age, frequently without any identifiable cause. Worldwide, more than 65 million people live with epilepsy, with a disproportionate 80% residing in low and middle income countries (LMIC). Annually, 2.4 million new cases are diagnosed. Africa accounts for a significant proportion, with an estimated 10 million individuals living with epilepsy, and concerning 80% (8 million) not receiving proper treatment, as per WHO estimates (WHO, 2012).

Nigeria's epilepsy prevalence varies across communities, ranging from 15 to 37 per 1,000 individuals. The main contributing factors include meningitis, tumours, and traumatic brain injuries, especially those sustained in motor vehicle accidents. Alarmingly, Nigeria and East Africa have world's highest rates of road traffic accidents, resulting in a significant increase in post-traumatic epilepsy cases in the region (Nazifi *et al*., 2017).

The incidence of epilepsy fluctuates significantly across different age groups, with a notable pattern of high rates in early childhood, a decrease in early adulthood, and a subsequent surge in individuals over 65 years old.

Specifically, the incidence ranges from a high of 560 cases per 100,000 per year in infants to a low of 20 cases per 100,000 per year in individuals aged 15-30 years, highlighting a distinct bi-modal distribution (Maiha e*t al*., 2009; Nazifi *et al*., 2017).

The mortality rate for people with epilepsy under 35 years is dramatically higher, at 50 times than that of the general population, with most fatalities resulting from seizure- related injuries. The effects of epilepsy reach well beyond person suffering the ailment, causing significant social, educational, and economic challenges, including social exclusion, stigma, and disability. Learning difficulties often co-occur with epilepsy, especially in children. Moreover, the stigma associated with epilepsy can have a ripple effect, affecting not only the individual but also their loved ones (WHO, 2012). Although anticonvulsive medications are the cornerstone of epilepsy treatment, they fall short of providing a cure or preventing relapse. Furthermore, antiepileptic drugs (AEDs) are associated with a multitude of unwanted effects, such as tiredness, hypersensitivities, sleepiness, blood dyscrasias, teratogenic effects, mood changes, and brain issues, underscoring the need for innovative treatments that can better address the complexities of epilepsy (Yaro *et al*., 2015).

As defined by the World Health Organization (WHO), traditional medicine includes a wide array of health practices, knowledge, and beliefs that employ resources from plants, animals, and minerals sources, along with spiritual therapies, manual techniques, and exercises, to diagnose, treat, and prevent diseases while promoting general well-being. As the most accessible and affordable form of healthcare, traditional medicine plays a vital role in primary healthcare systems, particularly in tropical regions where conventional medicine is scarce and unaffordable. Notably, plant-based traditional remedies have been found to be a rich source of novel drugs, highlighting their significance in global healthcare (WHO, 2008).

MATERIALS AND METHODS

Authentication of collected plant material

The leaves of *Laggera aurita* were collected from Gandun Sarki, Gwale Local Government Area of Kano State in Nigeria, within December 2017. The plant was verified and authenticated by Bahauddeen at the Herbarium Unit of Biological Science Department, Bayero University, Kano, where voucher specimen (BUKHAN 0138) was deposited for future reference.

Machines and utensils

Electroconvulsive machine (Ugo Basile, model no. 7801), Mortar and Pestle, Animal cages, Electric oven, spatula, Stop watch, Syringes, Filter paper, Micropipette, water bath, weighing balance (Mettler model, Ohio, New York, USA).

Chemicals and drugs

Phenytoin, Pentylenetetrazole, Methanol, Ethyl acetate, N-hexane (Sigma Aldrich, St.Louis U.S.A.), Sodium Valproate (Sanofi St.Surrey, Canada), Tween 80 (Cole-Parmer Illinois U.S.A).

Laboratory animals

The study was carried out using adult Swiss Albino mice of both sexes (20-26 g), Wistar rats of both sexes (160-240g) sourced from the Animal House Unit of Department of Pharmacology, Bayero University, Kano, Nigeria. Day old Ranger cockerels (20 – 35 g), were however sourced from the National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State. The animals were kept on standard feed from Excel Feeds Plc (Kaduna, Nigeria) and had unrestricted access to food and water. They were housed in standard cages at room temperature and were allowed to acclimate to the laboratory environment for a minimum of five days before the experiments.

Preparation of crude extract

The plant leaves were rinsed with distilled water to remove any dirt and then dried in the shade. They were grounded into a fine powder using a mortar and pestle. A quantity of one kilogram of the powdered leaves was suspended in seven (7) liters of methanol, and the mixture was allowed to sit for 72 hours in large bottles with occasional shaking. After the extraction period, the crude methanol extract was filtered using Whatman No. 1 filter paper (1 mm mesh size) and concentrated in a water bath set at 45ºC until greenish-black residues formed. The percentage yields of both the crude methanol extract and the solvent fractions were calculated as the ratio of the weight (g) of the extract to the original weight (g) of the dried sample, using the following formula:

Percentage yield of extract = (measured weight of extract / total weight of sample) X 100

Separation of solvent fractions from the methanol extract

Solvents of varying polarity were used to subject the methanol crude extract of *Laggera aurita* leaf to liquid-liquid partitioning in order to separate it into different fractions. A volume of 400 ml of the reconstituted extract was placed in a separator funnel, and 400 ml of n-hexane and ethyl acetate were added sequentially in a 1:1 ratio, followed by vigorous shaking. Each solvent was allowed to stand for 30 minutes in the separator funnel until a clear separation line appeared, indicating the distinction between the supernatant and the sediment before desorption (Trease and Evans, 1983). This process was repeated three times to obtain sufficient quantities of each fraction. The n-hexane, ethyl acetate, and aqueous residue fractions were then concentrated using a water bath maintained at 45ºC. The concentrated fractions were stored in sealed containers and refrigerated at 2-4ºC for future use.

Initial identification of secondary metabolites

Qualitative plant constituents identification from the concentrated aqueous leaf extracts of *Laggera aurita* were carried out according to standard methods (Trease and Evans, 1983; Abdullahi *et al*., 2023).

In-vivo acute toxicity tests

Acute toxicity of aqueous extract of *Laggera aurita linn*; Nine (9) healthy Swiss albino mice of either sex, six (6) females and (3) males, weighing 20-26g were maintained under standard laboratory conditions and used for acute toxicity test according to Lorke's (1983) method.

The study was conducted in two phases. In the first phase, three groups, each consisting of three mice grouped together (two females and one male), were treated intraperitoneally (i.p.) with the residual aqueous fraction (RAF) of *Laggera aurita* at doses of 10, 100, and 1000 mg/kg body weight. The animals were monitored for signs of toxicity and mortality for 24 hours following treatment. In the second phase, four groups, each containing one mouse, were treated intraperitoneally with the RAF of *Laggera aurita* at four specific doses (200, 400, 800, and 1600 mg/kg), determined based on the results of the first phase. The LD_{50} value was then calculated as the square root of the product of the lowest dose that resulted in death and the highest non-lethal dose, representing the geometric mean of consecutive doses

associated with 0% and 100% survival rates. This same procedure was applied to the n-hexane (NHF) and ethyl acetate (EAF) fractions of the *Laggera aurita* extract.

Evaluation of anticonvulsant activity

The electrical shock-induced seizures method

This procedure that was previously described and carried out by Swinyard and Kupferberg (1985) and Browning (1992) was administered to determined anticonvulsant activity using chicks. Sixty (60) day old cockerels were randomly divided into 5 groups of 12 chicks each. The animals in group one were given normal saline (10 ml/kg) *i.p*, while second, third and fourth groups were given extracts in dose dependent fashion (100, 200, 400 mg/kg of n-hexane fraction, 150, 300, 600mg/kg of ethyl acetate fraction and 250, 500, 1000 mg/kg of residual aqueous fraction) intraperitoneally. The standard drug phenytoin was intraperitoneally administered to the animals in the last group at a dose of 20 mg/kg. After a period of half an hour, electrical shock was delivered in all the groups to induce seizures in the chicks using Ugo basile electroconvulsive machine (model 7801) by placing the corneal electrodes on the upper eyelid of the chick. Shock duration, frequency and pulse width were set and monitored at 90 mA, 1.0 sec, 200 Hz and 1.0 ms^{-1}respectively as presence and or absence of convulsive effects were observed.

The test method using Pentylenetetrazole for induction of seizures in mice

The experimental procedure followed the method of Swinyard *et al*., (1952). Thirty mice were randomly assigned to five groups, each containing six mice. The first group served as the negative control, where seizures were induced in animals without administering any extract or standard drugs; these mice received only normal saline via intraperitoneal injection (i.p.). Groups 2 to 4 were treated with graded doses of specific fractions: the n-hexane fraction (100, 200, and 400 mg/kg), the ethyl acetate fraction (150, 300, and 600 mg/kg), and the residual aqueous fraction (250, 500, and 1000 mg/kg). Group 5 served as the positive control, with mice treated with 200 mg/kg i.p. of sodium valproate.

After a 30-minute period, each mouse received a subcutaneous injection of freshly prepared pentylenetetrazole (PTZ) solution at a dose of 90 mg/kg to induce seizures. The animals were then monitored for 30 minutes for the onset and occurrence of seizures. A clonic spasm lasting at least 5 seconds was regarded as a seizure, while the absence of clonic spasm during the observation period was considered protective. The number of mice protected from seizures was recorded, and the anticonvulsant effect of each fraction was expressed as a percentage of protected mice in each group.

Pentylenentetrazole kindling induction model using rats

Following the methodology outlined by Gupta *et al*., (2001) and Dhir *et al*., (2007), a sub-convulsive dose of 40 mg/kg of PTZ was administered intraperitoneally every 48 hours over 20 days. Forty rats were assigned to five groups, each with eight animals. Group 1 served as the negative control, receiving only normal saline (1 mL/kg i.p.). Groups 2 through 4 were administered increasing doses of the experimental fractions: specifically, 100, 200, and 400 mg/kg for the n-hexane fraction; 150, 300, and 600 mg/kg for the ethyl acetate fraction; and 250, 500, and 1000 mg/kg for the residual aqueous fraction. As a positive control, Group 5 received 200 mg/kg of Sodium Valproate intraperitoneally. Thirty minutes after each treatment, all rats were given a subcutaneous injection of PTZ (40 mg/kg) to induce seizures. Seizure activity was observed for 20 minutes and classified appropriately (Racine, 1972; Wu *et al*., 2006).

Data Presentation and Analysis

Data were displayed in tables and charts as appropriate, with values expressed as Mean ± SEM. Statistical significance was assessed using one-way ANOVA, and two-way ANOVA was applied for kindling (across time and dose variables), followed by post-hoc analysis. A p-value of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

Yields of the leaf extract and fractions of *Laggera aurita*

Percentage yields of the crude methanol extract was 24.0 % w/w and solvent fractions accorded 7.0, 8.0 and 79.0 % w/w for the n-hexane, ethyl acetate and the residual aqueous fractions respectively.

Identified bio constituents of the leaf extract and fractions of *Laggera aurita*

Preliminary evaluation of the methanol leaf extract of *L. aurita* showed the presence of flavonoids, steroids/terpenoids, alkaloids, cardiac glycosides, tannins and cardiac glycosides as its bio constituents. N-hexane revealed the presence of steroids, alkaloids, flavonoids, and terpenoids, ethyl acetate revealed alkaloids, terpenoids, steroids, tannins and flavonoid and finally residual aqueous fractions showed cardiac glycosides, terpenoids and alkaloids.

Median lethal dose (LD_{50})

The intraperitoneal median lethal dose (LD_{50}) values of the fractions of *Laggera aurita* in mice, and chickens were found to be less than 3800mg/kg (Table 1).

Table 1: Median lethal dose of fractions of *Laggera aurita*

Fractions	Specie	Route	LD_{50} Values (mg/kg)
NHF	Mice	*IP*	1264
EAF	Mice	*IP*	2154
RAF	Mice	*IP*	3807
NHF	Chicks	*IP*	1468
EAF	Chicks	*IP*	2375
RAF	Chicks	*IP*	3986

NHF=N-hexane fraction EAF=Ethyl acetate fraction RAF=Residual aqueous fraction

Effect of n-hexane fraction of *Laggera aurita* on maximal electroshock induced seizure in chicks

The fraction offered no protection to the chicks against hind limb tonic extension (HLTE) induced by electrical shock and did not reduce the average recovery time of the chicks after electroshock treatment compared to the negative control group which only received normal saline before induction of seizures, as against those that were given extract or standard drugs. The standard anticonvulsant drug used phenytoin (20 mg/kg) protected all the chicks (100%) from hind limb tonic extension induced by maximal electroshock (Table 2).

Table 2: Effect of n-hexane fraction of *Laggera aurita* on maximal electroshock induced seizure in chicks

Treatment (mg/kg)	Mean Recovery time (min)	Quantal protection	%protection against seizure
N/S (10 ml/kg)	6.72±0.46	0/12	0.00
NHF (100)	11.25±1.45[a]	0/12	0.00
NHF (200)	8.33±1.08[a]	0/12	0.00
NHF (400)	10.33±1.18[b]	0/12	0.00
PHT (20)	0.00±0.00[b]	12/12	100.00

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with a = p< 0.05 and b = p< 0.01, level of significance respectively. n=12; N/S=Normal saline, PHT = Phenytoin, NHF= N-hexane fraction

Effect of ethyl acetate fraction of *Laggera aurita* on maximal electroshock induced seizure in chicks

The fraction offered no protection to the chicks against hind limb tonic extension (HLTE) induced by electrical shock and did not reduce the average recovery time of the chicks after electroshock treatment compared to the negative control group which only received normal saline before induction of seizures, as against those that were given extract or standard drugs. The standard anticonvulsant drug used phenytoin (20 mg/kg) protected all the chicks (100%) from hind limb tonic extension induced by the induced electrical shock (Table 3).

Table 3: Effect of ethyl acetate fraction of *Laggera aurita* on maximal electroshock induced seizure in chicks

Treatment (mg/kg)	Mean Recovery time (min)	Quantal protection	%protection against seizure
N/S (10 ml/kg)	3.08±0.85	0/12	0.00
EAF (150)	11.00±2.12[b]	0/12	0.00
EAF (300)	7.16±0.77[a]	0/12	0.00
EAF (600)	7.16±0.84[a]	0/12	0.00
PHT (20)	0.00±0.00[b]	12/12	100.00

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with a = p< 0.05 and b = p< 0.01, level of significance respectively. N/S=Normal saline, PHT = Phenytoin, EAF= Ethyl acetate fraction

Effect of residual aqoueus fraction of Laggera aurita on maximal electroshock induced seizure in chicks

The fraction offered no protection to the chicks against hind limb tonic extension (HLTE) induced by maximal electroshock and did not reduce the average recovery time of the chicks post induction of seizures compared to the negative control group which only received normal saline before induction of seizures, as against those that were given extract or standard drugs. The standard anticonvulsant drug used phenytoin (20 mg/kg) protected all the chicks (100%) from hind limb tonic extension induced by maximal electroshock (Table 4).

Table 4: Effect of residual aqueous fraction of *Laggera aurita* on maximal electroshock induced seizure in chicks

Treatment (mg/kg)	Mean Recovery time (min)	Quantal protection	%protection against seizure
N/S (10 ml/kg)	7.62±0.64	0/12	0.00
NHF (250)	9.75±1.05	0/12	0.00
NHF (500)	10.40±1.24[a]	0/12	0.00
NHF (1000)	12.16±1.70[b]	0/12	0.00
PHT (20)	0.00±0.00[b]	12/12	100.00

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with a = p< 0.05 and b = p< 0.01, level of significance respectively. N/S=Normal saline, PHT= Phenytoin, RAF= Residual aqueous fraction

Effect of n-hexane fraction of *Laggera aurita* on pentylenetetrazole-induced seizure in mice

The fraction protected the animals against pentylenetetrazole induced seizure. At 100mg/kg of the fraction, the percentage protection was found to be 33% which increases to 50% when the dose increases to 200mg/kg of the fraction, while increases of doses to 400mg/kg did not confer additional percentage of protection against seizure, the percentage protection at 400mg/kg of the fraction is still 50% same as that of 200 mg/kg. Valproic acid (200mg/kg) produced 100% protection against seizure and mortality as well. There was no statistically significant difference in the average onset of seizure between the control (Normal saline) group and extract at doses of 100, 200 and 400 mg/kg respectively at $p < 0.05$ (Table 5).

Table 5: Effect of n-hexane fraction of *Laggera aurita* on subcutaneous pentylenetetrazole-induced seizure in mice

Treatment (mg/kg)	Mean onset of seizure (min)	Quantal protection	%protection against seizure
N/S (10 ml/kg)	3.50±0.29	1/6	16.67
NHF (100)	6.00±2.05[a]	2/6	33.33
NHF (200)	7.00±3.06[a]	3/6	50.00
NHF (400)	8.60±3.26[b]	3/6	50.00
SV (200)	0.00±0.00[b]	6/6	100.00

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with a = p< 0.05 and b = p< 0.01, level of significance respectively. N/S=Normal saline, PHT= Phenytoin, RAF= Residual aqueous fraction

Effect of ethyl acetate fraction of *Laggera aurita* on pentylenetetrazole induced seizure in mice

The fraction shows less protection the animals against pentylenetetrazole induced seizure at all the three doses (150, 300 and 600 mg/kg) of the fraction, the percentage protection was found to be 16% which does not increase with the increase in dosage. Valproic acid (200 mg/kg) produced 100% protection against seizure and mortality as well. Statistical analysis did not produced significant difference in the average onset of seizure between the control (Normal saline) group and extract at doses of 150, 300 and 600 mg/kg respectively at $p < 0.05$ (Table 6).

Table 6: Effect of ethyl acetate fraction of *Laggera aurita* on subcutaneous pentylenetetrazole-induced seizure in mice

Treatment (mg/kg)	Mean onset of seizure (min)	Quantal protection	%protection against seizure
N/S (10 ml/kg)	3.55±0.39	1/6	16.67
EAF (150)	8.40±2.18[a]	1/6	16.67
EAF (300)	9.00±1.71[a]	1/6	16.67
EAF (600)	10.00±2.12[b]	1/6	16.67
PHT (20)	0.00±0.00[b]	6/6	100.00

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with a = p< 0.05 and b = p< 0.01, level of significance respectively. n=6; N/S=Normal saline, PHT = Phenytoin, EAF= Ethyl acetate fraction

Effect of residual aqueous fraction of *Laggera aurita* on pentylenetetrazole induced seizure in mice

The fraction shows protection against pentylenetetrazole induced seizure at all the three doses (150, 300 and 600 mg/kg) of the fraction, the percentage protection was found to be 33% which does not increase with the *increase* in dosage. Valproic acid (200 mg/kg) produced 100% protection against seizure and mortality as well. Statistical analysis did not produced significant difference in the average onset of seizures between the control (Normal saline) group and extract at doses of 150, 300 and 600 mg/kg respectively at $P < 0.05$ (Table 7).

Table 7: Effect of residual aqoueus fraction of *Laggera aurita* on subcutaneous Pentylenetetrazole-induced seizure in mice

Treatment (mg/kg)	Mean onset of seizure (min)	Quantal protection	%protection against seizure
N/S (10 ml/kg)	3.55±0.39	1/6	16.67
RAF (250)	5.67±0.71	0/6	0.00
RAF (500)	8.00±2.04[a]	2/6	33.33
RAF (1000)	8.50±2.53[a]	2/6	33.33
PHT (20)	0.00±0.00[b]	6/6	100.00

Values are mean± SEM, Data were analyzed using One-way ANOVA followed by Dunnett's post hoc test with a = p< 0.05 and b = p< 0.01, level of significance respectively. n=6; N/S=Normal saline, PHT = Phenytoin, RAF= Residual aqueous fraction

Effect of N-hexane fraction of *Laggera aurita* on pentylenetetrazole induced kindling in rats

The fraction at all doses tested reduced the kindling scores induced by sub convulsive dose (40 mg/kg) of pentylenetetrazole from day five (D5) today nine (D9). The reduction as recorded by seizure scoring model was generally statistically significant ($p<0.05$) throughout the treatment days (Figure 1).

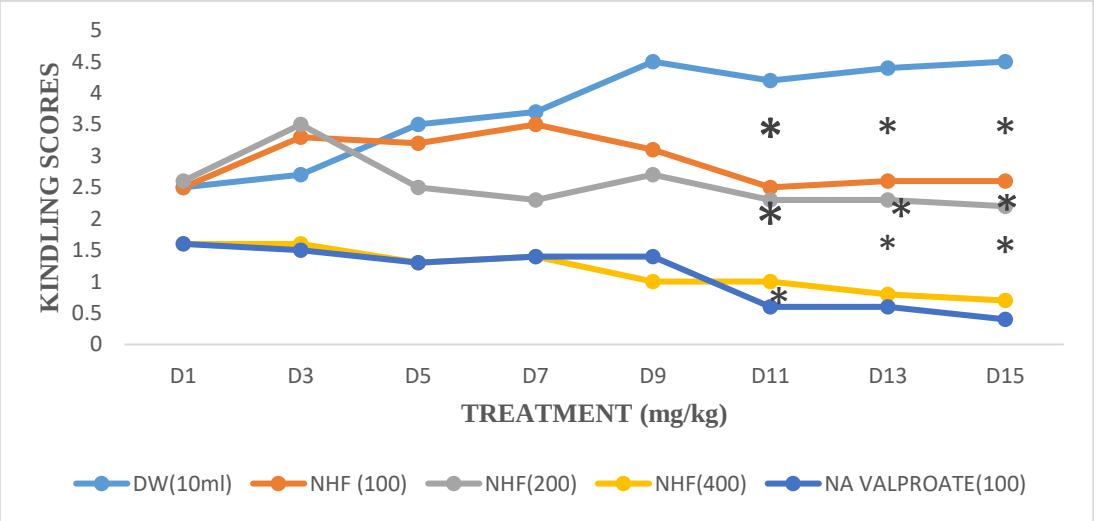

Figure 1: Effect of N-hexane fraction of *Laggera aurita* on pentylenetetrazole induced kindling in rats. Data presented as Mean ± SEM, n = 8; (Two-way ANOVA) followed by Dunnetts for multiple comparison; NHF=N-hexane Fraction, NA= Sodium

Effect of ethyl acetate fraction of *Laggera aurita* on pentylenetetrazole induced kindling in rats

The fraction at all doses did not reduce the kindling scores induced by sub- convulsive dose of subcutaneous pentylenetetrazole 40 mg/kg on all the days recorded whereas the sodium valproate reduces kindling (Figure 2).

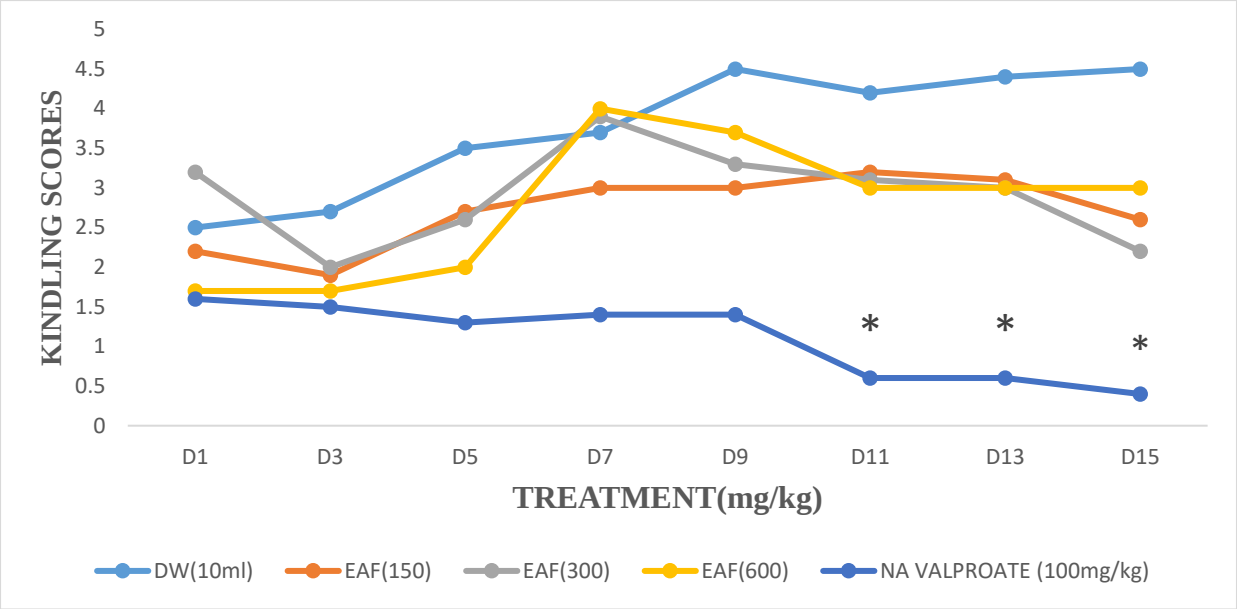

Figure 2: Effect of Ethyl acetate fraction of *Laggera aurita* on Pentylenetetrazole induced Kindling in Rats. Data presented as Mean ± SEM, n = 8; (Two-way ANOVA) followed by Dunnetts for multiple comparison; EAF=Ethyl Acetate Fraction, NA= Sodium

Effect of residual aqeous fraction of *Laggera aurita* on pentylenetetrazole induced kindling in rats

The fraction at all doses did not reduce the kindling scores induced by sub- convulsive dose of subcutaneous Pentylenetetrazole 40 mg/kg on all the days recorded whereas the sodium valproate reduces kindling (Figure 3).

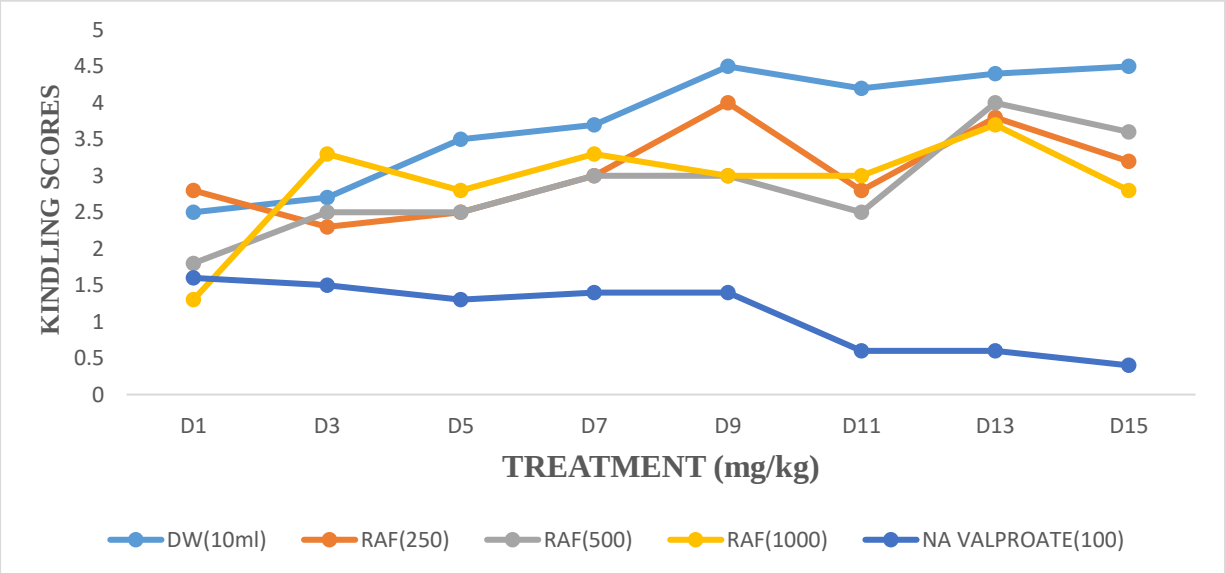

Figure 3: Effect of Residual aqueous fraction of *Laggera aurita* on pentylenetetrazole induced kindling in rats. Data presented as Mean ± SEM, n = 8; (Two-way ANOVA) followed by Dunnetts for multiple comparison; RAF=Residual aqeous Fraction, NA= Sodium

Discussion

Research by Abdulla *et al.*, (2013) established that *Laggera aurita* possesses triterpenes, flavonoids, saponin, cumarins, tannins and alkaloids as some of its active constituents. Through phytochemical screening, researchers can identify and characterize the diverse range of compounds in a plant that can be relevant in medical practice. This initial screening serves as a crucial step in understanding the plant's chemical profile, highlighting the presence of pharmacologically effective constituents that may be linked to its therapeutic effects (Shabbir *et al.*, 2013).

The initial phytochemical analysis of the methanol leaf extract and solvent fractions of *Laggera aurita* revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and cardiac glycosides, indicating the plants potential medicinal value. Although the phytochemical screening of *Laggera aurita* revealed a range of bioactive compounds, it is challenging to definitely attribute the observed anticonvulsant effect to a specific compound or group of compounds. Nevertheless, previous studies have shown that triterpenic steroids and saponins, which were detected in the screening, exhibit anticonvulsant properties in experimental seizure models, such as the MEST and ScPTZ, suggesting their potential contribution to the plant's anticonvulsant activity (Kasture *et al.*, 2002; Chaunhan *et al.*, 1988). The observed pharmacological activities of the solvent fractions may be attributed to the presence of these phytochemical constituents, suggesting a potential link between the plant's chemical composition and its biological effects especially as flavonoids have been reported to exhibit such action (Tourandokht *et al.*, 2010).

The electrical shock induced test is a well-established experiment which replicates the features of generalized tonic – clonic seizures, providing a valuable tool for evaluating the effectiveness of novel anticonvulsant therapies (Raza *et al*., 2001). Success in preventing tonic hind limb extention observed in the test indicates that an antiepileptic drug, such as phenytoin, carbamazepine, oxcarbazepine, or lamotrigine, is likely to be effective in treating generalized tonic-clonic and partial seizures (Browning, 1992; Ambawade *et al*., 2002). This is because these drugs have been shown to suppress convulsive activity at the initial centres, thereby demonstrating their potential as therapeutic agents for managing seizure disorders (Raza *et al*., 2001; Nazifi *et al*., 2017). Since the extract did not inhibit electroshock- induced seizure and failed to reduce the recovery time of convulsed chicks, indicates its unlikelihood to be an effective agent against generalized tonic-clonic seizures, which may limit its potential as an anticonvulsant.

Pentylenetetrazole (PTZ), a tetrazole derivative, serves as a prototype convulsant agent, and the subcutaneous PTZ seizure test is a widely used model for assessing a compound's ability to elevate the seizure threshold (DeDyn *et al*., 1992). The seizures induced by PTZ are clonic in nature, distinct from those characteristic of absence epilepsy. PTZ is thought to exert its convulsant effects by acting centrally as an antagonist at the GABA pathways, thereby disrupting ionic concentrations balance across the delicate neuronal membrane (Nagakannan *et al*., 2011; Tembe *et al*., 2017).

Research has shown that PTZ decreases GABAergic activity which is critical for stabilizing the excitatory and inhibitory neuronal impulses. GABA, the major inhibitory neurotransmitter, counteracts excitatory effects of glutamic acid, the primary excitatory neurotransmitter. Boosting GABA neurotransmission has been found to counteract seizures, while suppressing it can trigger seizure activity, underscoring the importance of preserving the balance between these two neurotransmitter systems (Rang *et al*., 2005; Hussein *et al*., 2017).

Agents capable of inhibiting PTZ-induced seizures are presumably thought to be effective in managing myoclonic seizures (McNamara, 2006). The anticonvulsant effects of drugs like exothuximide, which targets T-type calcium currents, and classic antiepileptics like diazepam and phenobarbitone, which potentiate GABA-mediated inhibition, have been demonstrated. Furthermore antiepileptic drugs effective in treating generalized petit-mal seizures, including phenobarbitone and benzodiazepines, have been found to elevate the PTZ-induced seizure threshold, highlighting their therapeutic utility (Rho and Saukar, 1999; Loscher *et al.*, 1991; Jain *et al*., 2011).

The anticonvulsant properties exhibited by some of the fractions (n-hexane and residual aqueous) suggest that the effect may have been exerted through one of two possible mechanisms: either by enhancing GABA neurotransmission, thereby promoting inhibitory effects centrally or by inhibiting glutermatergic neurotransmission mediated by N-methyl-D-aspartate (NMDA) receptors, thereby reducing excitatory activity centrally (Rogawski and Porter, 1990; Tembe *et al.*, 2017).

The kindling model is a well-established paradigm for studying epileptogenesis, following repeated exposure to subconvulsive doses of PTZ (Rivara *et al.*, 2012). This model has been shown to involve alterations in neurotransmitter systems, including a reduction in GABA receptor binding sites in the hippocampus, enhanced glutamate release, and increased nitric oxide levels (Riazi *et al*.,2006; Dhir *et al.*, 2007). Additionally, reduction in serotonin concentration centrally have been linked to deminish serotonin mediated neurotransmission in kindled animals, contributing to the development of epileptic seizures (Bazyan *et al.*, 2001; Kailash *et al.*, 2013; Zebrowska *et al.*, 2019).

Research identified and implicated the importance of calcium ion at the NMDA glutamate receptor in the process of epileptogenesis following kindling, making it one of the relevant points for the development of new antiepileptic medications like felbemate and topiramate, which seek to regulate its function and prevent the onset of seizures (Armijo *et al.*, 2000; Haggag *et al*., 2014). Activation of the chloride channel within the GABA A receptor complex is tied to the rapid hyperpolarization that characterizes the paroxysmal depolarizing shift, a pivotal mechanism underlying the kindling process that leads to increased seizure severity and epileptogenesis (Armijo *et al.*, 2000; Mazhar *et al*., 2016). N-hexane fraction of the methanol leaf extract of the plant showed antiepileptogenic effects by attenuating seizure severity across all tested doses, effectively blocking the seizures from reaching the full blown.

CONCLUSION

The n-hexane solvent fraction of *Laggera aurita* Linn demonstrated anticonvulsant properties and inhibited the progression of pentylenetetrazole induced kindling, suggesting that it possesses antiepileptogenic effects and may be a potential therapeutic agent for preventing epilepsy.

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