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RESEARCH ARTICLE

Anticonvulsant studies on the isolated compounds from the leaves of *Scurrula parasitica* L (Loranthaceae)

Kamal Ja'afar Muhammad [a, b], Shajarahtunnur Jamil [a,*], Norazah Basar [a], Mohammed Garba Magaji [c]

[a] Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
[b] Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Garki, Abuja-Nigeria
[c] Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria

* *Corresponding author: shaja@kimia.fs.utm.my*

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Abstract

The leaves of *Scurrula parasitica* were effectively extracted by means of cold extraction method. Fractionation and purification of the *n*-hexane, ethyl acetate, and methanol crude extracts yielded eight compounds. These compounds were identified as quercetin (**1**), quercitrin (**2**), kaempferol 3-*O*-α-L-rhamnoside (**3**), (+)-catechin (**4**), lupeol (**5**), lupeol palmitate (**6**), β-sitosterol (**7**), and squalene (**8**). Compounds **1**, **4**, **5**, and **6** were investigated for anticonvulsant potentials using maximal electroshock test (MEST) in chicks and pentylenetetrazole-induced seizure test in mice while the effect of the compounds on motor coordination was investigated using beam walking assay. The compounds did not completely protect the mice against pentylenetetrazole-induced seizure, but increased the mean onset of myoclonic jerks and spasms in the animals. Quercetin (**1**) significantly ($p < 0.05$) increased the mean onset of spasm in the unprotected animals. The compounds also differentially protected the mice against mortality. Conversely, the compounds did not protect the chick against the MEST. Similarly, they did not significantly reduce the recovery time. In the beam walking assay, the increase in the number of foot slips observed in the study may be associated with the interaction of quercetin (**1**) and (+)-catechin (**4**) with the GABA system to produce clinical sedation. The findings of the present study suggest that the isolated compounds possess some mild anticonvulsant potential and may be beneficial in the management of petit mal epilepsy.

Keywords**:** *Quercetin, anticonvulsant, Scurrula parasitica, pentylenetetrazole, epilepsy*

INTRODUCTION

Epilepsy is a common and diverse brain condition characterized mainly by persistent and unpredictable disruptions of normal brain function [1]. The term seizures refer to a temporary change of behaviour due to irregular coordinated, and recurring burst firing of neuronal populations in the central nervous system (CNS). Another studies characterize seizures by frequent unpredictable impulsive convulsions caused by traumatic, metabolic, infectious, tumoral, or idiopathic (hereditary predisposition) conditions [2-3]. An incomplete seizure starts in a confined brain region; however, generalized seizures show prevalent participation of both hemispheres from the start.

Epilepsy is regularly managed, but not healed with treatment [3-4]. Numerous patient with epilepsy failed to have adequate control of their seizures despite the optimal use of available anti-epileptic drugs (AEDs) in the market. However, over 30% of individuals with epilepsy do not have seizures control even with the best available treatment, other patients do so at the expense of substantial toxic effects such as teratogenicity, hepatotoxicity, and adverse effects on cognition and behaviour [1,5]. Natural products originated from the marvel of biodiversity in which the interactions among creatures and their environment formulate the distinct complex chemical entities within the organisms that enrich their existence and competitiveness [6-7]. An estimated 25 % of medicinal drugs and 11 % of drugs considered fundamental by the WHO are originated from plants and many artificial drugs are obtained from plant-based precursor compounds [8]. The approximation available shows that, there are at least 250,000 species of plants in the world and of the obtainable statistics, about 150,000 of them are found in the tropics. In south east Asia alone, there are 35,000 species of which 8,000 are found in Malaysia. Until now, at least 654 species have been reported to possess medicinal values in the tropics. From this, a total of 1,230 species have been reported in Malaysia as medicinal plants which are used in traditional medicine [9-11]. Currently, many scientists are attempting to identify more plants which have medicinal values and the potential to be commercialized as herbal medicines. *Loranthaceae* is one of the plant family which are believed to have high medicinal values due to its wide use in many traditional medicine.

Generally called mistletoe, *Scurrula parasitica* L. is a member of the *Loranthaceae* family that is represented by 75 genera and 1000 species. The plant has several local uses and is widely distributed in Asia, Australia, and South America [12]. It is a semi-parasitic shrub with woody stem. The leaves are simple, stringy, and evergreen. Timbered suckers often regarded as adventitious roots connects to and infiltrate the branches of the host tree or shrub by a structure called the haustorium, through which they absorb water and nutrients from the host tree [13]. The leaves of genus *Scurrula* are used for the treatment of disease like cancer, malaria, and hypertension [14]. It also has common application in treating infusion for fatigue and cancer in Indonesia and Java [15]. Interestingly, the anticonvulsant activity of the ethyl acetate fraction of a mistletoe plant *Globimetula* *braunii* has been reported [16]. The two earlier investigations on the chemical constituents of *Scurrula parasitica* from China led to the isolation of several secondary metabolites [17-18]. Considering the importance of the therapeutic uses of these genera in the management of cancer, malaria, and hypertension, it is clear that a wider range of investigations on the biological activities are needed to be discovered for their pharmacological properties. Since the promising source of novel, safe, and active anticonvulsant substances appears to be plants, the present study therefore aimed at isolating the bioactive compounds from the leaves of *Scurrula parasitica* and test the compounds for anticonvulsants using pentylenetetrazole-induced seizure test, beam walking assay in mice, and maximal electroshock test in chicks (MEST).

MATERIAL AND METHODS

Collection and identification of plant material

The fresh leaves of *S. parasitica* parasite on *Pongamia pinnata* were collected from Universiti Teknologi Malaysia (UTM), Southern Malaysia (Latitude N 1º 33' 54.9", Longitude E 7º 103', 29.2") in August 2016. They were confirmed and authenticated at the Department of Landscape Architect, Faculty of Design and Architecture, Universiti Putra Malaysia, where the voucher specimens (No SK2800/17) for *S. parasitica* and (SK28001/17) for *P. pinnata* were compared with the existing specimens by Dr. Shamsul Khamis.

Extraction and isolation

The plant leaves were washed with clean water then air dried under the shade for several weeks. The dried leaves were then ground using laboratory grinder. The powdered plant material (1.4 kg) was extracted using cold extraction method using different polarity of solvents starting with *n*-hexane, EtOAc, and MeOH at room temperature for three days. The samples were concentrated on a rotary evaporator to obtain black gummy crude extracts of *n*-hexane, EtOAc, and MeOH. The fractionation and purification of *n*-hexane (SPPPH), ethyl acetate (SPPPE), and methanol (SPPPM) crude extracts were carried out using vacuum liquid chromatography (VLC), column chromatography (CC), preparative thin layer chromatography, and thin layer chromatography (TLC). Purification of SPPPE was conducted by repeated CC over SiO_2 with *n*-hexane: EtOAc as eluents afforded quercetin (**1**). The methanol extract, SPPPM was subjected to VLC (SiO_2 600 g, 10.0 cm × 10.0 cm) and eluted with $CHCl_3$:EtOAc:MeOH in stepwise gradient followed by CC over SiO_2 to afford quercitrin (**2**), kaempferol 3-*O*-α-L-rhamnoside (**3**), and (+)-catechin (**4**). The *n*-hexane extract was fractionated by VLC over SiO_2 (600 g, 10.0 cm × 10.0 cm) and eluted with *n*-hexane: $CHCl_3$: EtOAc in stepwise gradient followed by CC over SiO_2 with *n*-hexane: EtOAc as eluents to yield lupeol (**5**), lupeol palmitate (**6**), β-sitosterol (**7**), and squalene (**8**). The structures were determined using spectroscopic method including IR, NMR, MS, and comparison with reported data.

Experimental animals

Adult Swiss Albino mice of either sex (18-24 g) were used in this experiment. They were acquired from the Animal House Facility of the Department of Pharmacology and Therapeutics, ABU, Zaria. Day old white Rangers cockerels were obtained from the National Animal Production Institute (NAPRI), Shika, Kaduna, Nigeria. They were conserved at 23.0 ± 2.0 ℃, 12 h light and dark circle, fed with standard rodent feed, and water was provided *ad libitum*. They were kept in polypropylene cages throughout the study. All experimental protocol complied with the National institute of Health Guide for the Care and Use of Laboratory Animals (Publication No 5-23, revised 1985). All experimental protocols were approved by the Departmental Ethical Committee and an Ethical Approval Number DAC/W-OT/301-27 was obtained.

Maximal Electroshock Seizures Test (MEST) in chicks

The method adopted was consistent with that of Swinyard (1989) [19] and White (1995) [20]. The apparatus used was Ugobasile electroconvulsive machine (Model 7801), with corneal electrodes placed on the upper eyelids of the chicks. A shock duration, frequency, and pulse width were set and kept at 0.8 s, 100 pulses/sec and 0.8 m/s respectively. A current of 75 mA which produced tonic seizures in 95 % of the control chicks was used throughout the study. Groups of chicks (n=10) were administered with normal saline (10 mL/kg), isolated compound (30, 100, and 300 mg/kg) and phenytoin (20 mg/kg) thirty minutes prior to the administration of shock. An incident of tonic extension of the hind limbs was regarded as full convulsion and the recovery time was recorded in unprotected animals, while lack of tonic extension of the hind limbs was regarded as protection.

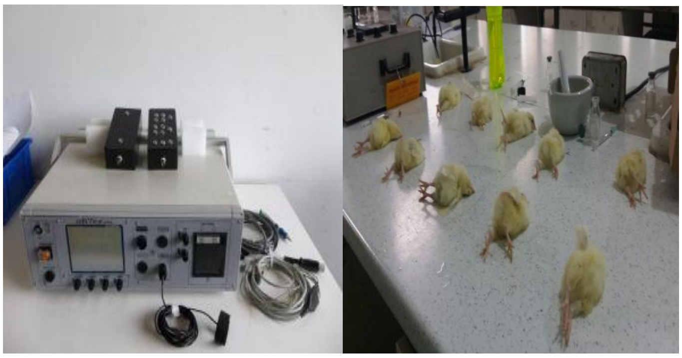

Fig. 1 Graphic picture of chicks after delivery of electroshock seizure.

Pentylenetetrazole induced seizure (PTZ) in mice

The PTZ (CD_{97}) test exploits a dose of pentylenetetrazole (85 mg/kg) to induce clonic convulsions that produces clonic seizures lasting for a period of at least five seconds in 95 % of the tested animals. Mice were divided into five groups (n = 6): the first group was pre-treated with normal saline (10 mL/kg); three groups of six mice each were given 300 mg/kg, 100 mg/kg, and 30 mg/kg body weight of the isolated compound, respectively; and the last group of six mice was pre-treated with sodium valproate (200 mg/kg) and served as positive control. After 30 minutes post-treatment, the convulsant was administered subcutaneously and the animals were individually monitored for the presence or absence of clonic spasm (of at least five seconds duration) to determine the compound ability to abolish the effect of pentylenetetrazol on seizures threshold [19]. The onset of twitching and myoclonic jerks were also recorded.

Fig. 2 Mice after seizure induced by sc-PTZ.

Beam walking assay (neuro toxicity)

This protocol was based on the method previously described [21]. Mice were tested for the ability to walk on a cylindrical beam. Before the trial, the mice were trained to walk across a horizontal beam (a meter long and 3 cm wide) elevated 30 cm above the table with the aid of two metallic supports. Three trials were performed for each mice, and were designed such that the mice would be aware of a goal box placed at the end of the beam. Trained mice were randomly divided into groups of six mice each. The animals were treated with normal saline (10 mL/kg), isolated compound (30 mg/kg, 100 mg/kg and 300 mg/kg), or diazepam (1 mg/kg). After 30 minutes posttreatments, mice were placed at one end of a cylindrical beam (80 cm long and 8 mm in diameter). The number of foot slips as well as time taken to complete the task (i.e time taken to poke into the entrance of the goal box) with a maximum time of 60 s allowed on the beam. For the mice fell, they were returned to the position where they fell from.

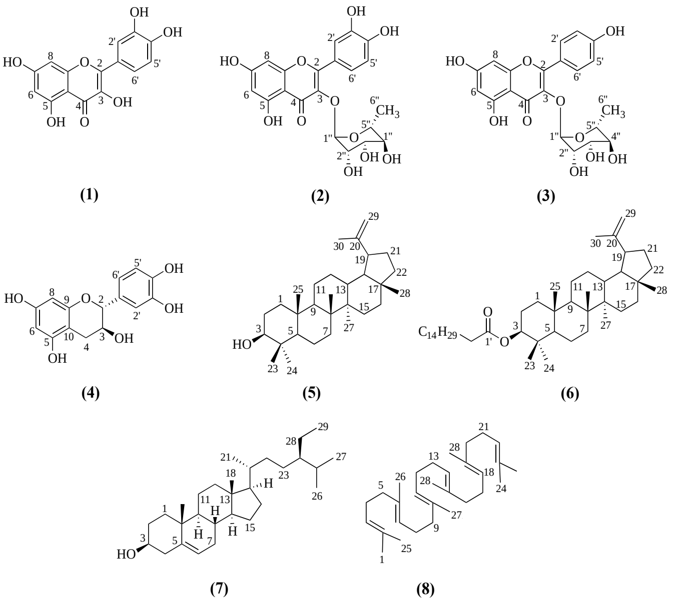

Fig. 3 Chemical structures of compounds **1-8** isolated from the leaves of *S. parasitica*.

RESULTS AND DISCUSSION

The main source of plant-based traditional medical practices arose from the investigative trial and error by man for centuries through palatability tests morbidity and mortality, while seeking nearby food for treatment of illnesses. Naturally occurring medicinal plants have been well acknowledged for their therapeutic uses for centuries. They have progressed and adapted over millions of years to endure insects, microbes, and weather to generate distinct, structurally varied secondary metabolites. The active principles of many drugs found in plants are composed of these secondary metabolites.

Table 1 Effect of compounds obtained from *Scurrula parasitica* against maximal electroshock in mice.

Compounds	Treatment mg/kg	Quantal protection	Mean time of recovery (Min)
Normal saline	10 ml/kg	0/10	3.94± 0.58
Quercetin	100	1/10	5.52± 0.91
	30	0/10	6.57± 0.36
	10	0/10	6.47± 1.41
(+)-Catechin	100	0/10	7.28± 0.87
	30	0/10	5.1± 0.67
	10	0/10	5.16± 0.48
Lupeol	100	0/10	8.17±0.80
	30	0/10	6.24±1.06
	10	0/10	6.53±1.24
Lupeol palmitate	100	0/10	6.52±0.99
	30	0/10	5.25±0.93
	10	0/10	6.44±0.52
Phenytoin 20	20	9/10	6.92± 0.00

Protection against seizure expressed as percentages, mean onset of seizure is expressed as mean ± SD, $p < 0.05$ (compared with normal saline treated control), n=10

The fractionation and purification of *n*-hexane (SPPPH: 27 g 1.87 %), ethyl acetate (SPPPE: 32 g, 2.20%), and methanol (SPPPM: 45 g, 3.10%) crude extracts led to the isolation of eight compounds which were identified as quercetin **1** (8 mg 0.13 % as yellow powder with R_f 0.56 in *n*-Hexane: EtOAc, 2:3, and m.p 300–302 ℃) [22]; quercitrin **2** (7 mg, 0.02 % as a yellow solid; R_f 0.62, $CHCl_3$: MeOH, 4.2:0.8, and m.p 176–178 ℃) [23]; kaempferol 3-*O*-α-L-rhamnoside **3** (7.5 mg, 0.02 % as a yellow powder; R_f 0.65, $CHCl_3$: MeOH, 4.2:0.8 and m.p 171–174 ℃) [24]; (+)-catechin **4** (9.2 mg, 0.02% as a pale brown powder with m.p. 174–176 ℃ and R_f value of 0.45 in *n*-hexane: EtOAc 1:4) [25]; lupeol **5** (28 mg, 0.52 % as a white powder; R_f 0.67 *n*-Hexane: EtOAc, 4:1 and m.p. 214–216 ℃) [26]; lupeol palmitate **6** (243 mg, 4.50 % as white waxy solid; R_f 0.23 *n*-Hexane:Et_2O, 4.6:0.4 and m.p 79–81 ℃) [27]; β-sitosterol **7** (174 mg, 3.22% as white crystalline needles; R_f 0.45 *n*-Hexane: EtOAc, 4.2:0.8 and m.p 132–134 ℃) [28]; and squalene **8** (151 mg, 0.34% as white waxy powder with R_f 0.78 *n*-Hexane: EtOAc, 4.9:0.1) [29]. These plants-derived secondary metabolites are flavonoids and triterpenes which lately have drawn attention because of their anticonvulsant effect. The effects of flavonoids on nervousness [30], depression [31], learning and memory processes [32-34], and nociception [35] have been reported. Also, betulinic acid obtained from extract of *Marcgraviaceae* showed anti-anxiety activity after oral and intraperitoneal administration in mice and rats [36].

Table 2 Effect of compounds obtained from *Scurrula parasitica* against pentylenetetrazole induce seizure.

Compounds	Treatment mg/kg	Quantal protection against seizure	Mean onset of myoclonic jerk (Min)	Mean onset of Seizure (Min)
Normal saline	10 mL/kg	0/6	2.17± 0.46	5.71± 1.05
Quercetin	100	0/6	5.14± 1.34*	13.55 ± 2.50*
	30	2/6	5.44 ± 0.93*	12.97 ±1.69*
	10	0/6	4.7± 0.89*	9.69± 1.79*
(+)-Catechin	100	2/6	4.17± 0.20*	10.85± 1.28*
	30	1/6	4.51± 1.41*	9.35± 1.02*
	10	1/6	5.02± 1.83*	9.37± 2.83*
Lupeol	100	0/6	5.09 ± 1.29*	9.34 ± 1.30*
	30	0/6	3.28 ± 0.99*	9.66± 2.20
	10	0/6	3.27 ± 0.61*	5.94± 1.26
Lupeol palmitate	100	1/6	4.15± 0.55*	4.59 ± 1.93
	30	2/6	3.94 ±0.87*	4.37 ± 1.06
	10	1/6	4.00 ± 0.87*	5.70 ± 0.89
Sodium valproate	200	6/6	4.14± 0.00*	21.21± 0.00

Data presented as Mean ± SD; $p < 0.05$ (Dunnet post hoc test for multiple comparison); n= 6.

The MEST test is believed to be a standard model for generalized tonic-clonic seizures that is exceptionally reproducible with reliable endpoints. The behavioral and electrographic seizures produced by MEST are reliable with the human disorder [19]. In the MEST test, all of the control animals exhibited seizure after the delivery of electroshock. The four compounds tested (except quercetin at 100 mg/kg body weight that gives 10% protection) did not protect the animals against tonic hind limbs extension in all the tested doses. However, mortality of the tested animals was not recorded for the entire compounds tested. The compounds that inhibit seizure spread (active in the MEST) are known to be sodium channel blockers such as carbamazepine, felbamate, phenytoin, and valproate or agents that block glutamatergic neurotransmission mediated by *N*-methyl-D-aspartate (NMDA) receptors [20; 37-38]. The absence of anticonvulsant activity in MEST (Table 1) indicates that, the tested compounds may not be suitable in the management of generalized tonic clonic and partial seizures. Meanwhile, pentylenetetrazole is the recognized chemoconvulsant used for evaluating antiepileptic drugs that triggers seizures by blocking the chloride channel coupled to the main $GABA_A$ receptor complex or shown to interact with GABA neurotransmitters. Thus, it has been used to identify compounds that raise the seizure threshold in brain [39-40]. Drugs such as diazepam and phenobarbitone that are considered as standard antiepileptic drugs that produces their effect by enhancing GABA mediated inhibition in the brain are protective against pentylenetetrazole-induced seizure. Ethosuximide can also block seizures induced by sc-PTZ by reducing t-type calcium currents [41].

Table 3 Effect of compounds obtained from *Scurrula parasitica* on motor coordination in mice.

Compounds	Treatment (mg/kg)	Number of foot slips	Time taken to complete the tasks (Secs)
Normal saline	10 mL/kg	0.33±0.21	19.04±0.55
Quercetin	100	4.15± 0.55*	18.60±0.34
	30	4.7± 0.89*	17.16±0.27
	10	5.14± 1.34*	18.01±0.16
(+)-Catechin	100	4.17± 0.20*	11.51±0.45
	30	4.51± 1.41*	15.17±0.43
	10	5.02± 1.83*	12.33±0.02
Lupeol	100	0.33±0.33	13.33±4.36
	30	0.33±0.21	17.33±6.12
	10	0.33±0.33	11.67±6.23
Diazepam	2	4.67±1.09*	60±0.00*

Data presented as Mean ± SD; $p < 0.001$ (Dunnet post hoc test for multiple comparison); n= 6

In the PTZ-induced seizure in mice, almost all of the control animals displayed myoclonic jerk, while some exhibited threshold seizure and loss of righting reflex with tonic forelimbs extension. However, all of the tested compounds prolong the onset of myoclonic jerking, spasm, seizure, and the time of death in the unprotected animals (Table 2). The abilities of all the tested compounds to delay the onset of myoclonic jerks, spasm, and/or time of death are also indicative of some mild to moderate protective effects. The mild anticonvulsant activity observed may also involve the augmentation of GABAergic neurotransmission, dopaminergic mechanism, inhibition of t-type calcium current, or blockade of glutamatergic neurotransmission mediated by NMDA receptors [42]. The functioning on the balance beam is a valuable measure of good coordination and balance, and can identify motor shortfalls due to age, central nervous system lesions, genetic, and pharmacological manipulations in young and older rodents in the beam walking assay (Table 3) [43-45]. In identifying motor coordination shortfalls stimulated by diazepam, the beam test was subtler than the rotarod. Stanley [21] indicated that only a 30 % $GABA_A$ receptor occupancy of diazepam was required to observed motor shortfalls on the beam compared to 70 % receptor occupancy for shortfalls on the rotarod. The foot slips and time taken to complete the task are very sensitive measures of determining benzodiazepine-like drugs induced motor coordination deficits and adequately predict clinical sedation involving GABAergic neurotransmission [21]. Previously, quercetin and catechin have been variously reported to enhance GABAergic neurotransmission and promote sleeps [46-47]. Therefore, the increase in the number of foot slips observed in the study may be associated with the interaction of these agents with the GABA system to produce clinical sedation. The anticonvulsant effect of quercetin and lupeol in animal models of seizure and their anticonvulsant properties were in accordance with previous investigations demonstrated in mice. Nieoczym *et al*. [48], have reported the weak and short term dose dependent anticonvulsant action of quercetin (400 mg/kg) against 6 Hz model psychomotor seizure induces by MEST and PTZ in mice. The compounds significantly increased the seizure threshold for 6 Hz induced seizure, but did not completely influence the seizure threshold in both MEST and PTZ tests in mice. Additionally, an observed anticonvulsant effect of quercetin at specific dose (50 mg/kg) against PTZ induced seizure was reported by Nassiril-Asl *et al*. [34]. The result showed an enhanced memory retrieval in the passive avoidance task and also increased oxidative stress in the kindled animals. It also attenuates seizure severity from the beginning of the kindling experiment by lowering the mean seizure stage and significantly increase the step through latency of the passive avoidance response compared to the control in the retention test. The observation that lupeol showed very weak to in active anticonvulsant activity was in conformity with the earlier published data by Ruta and colleagues in 2008 [49], that lupeol does not bound to $GABA_A$ receptor. No data was found on the anticonvulsant activity of lupeol palmitate. As such, these results have furthermore guided to a better understanding of its anticonvulsant activity.

CONCLUSION

The findings of the present study suggested that the isolated compounds possess some mild anticonvulsant potential which may be beneficial in petit mal epilepsy and further offered pharmacological basis for the ethnomedicinal use of the plants from this family in the management of convulsion and epilepsy. This is also the first report of the anticonvulsant effect of lupeol palmitate.

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