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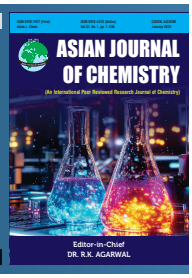

Isolation of Quercetin and Anticonvulsant Properties of Ethanol Extract Derived from Stem of *Tragia involucrata* Linn. (Euphorbiaceae)

N. SARASWATHY[1,*], A. SATISH[2], M. CHITRAVEL[2], S. KRISHNAVENI[2] and S.S. SYED ABUTHAHIR[3]

[1]Department of Science and Humanities, Parisutham Institute of Technology and Science, Thanjavur-613006, India
[2]Department of Chemistry, St. Joseph's College of Engineering and Technology, Thanjavur-614403, India
[3]PG and Research Department of Chemistry, Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli-620020, India

*Corresponding author: E-mail: saraswathyrajam@gmail.com

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Indian and Sri Lankan traditional medicines make the use of *Tragia involucrata* Linn., a plant belonging to the Euphorbiaceae family. This study aimed to isolate the bioactive flavonoid quercetin from the ethanolic extracts of stem of *T. involucrata* and evaluate its anticonvulsant effects. The ethanolic extracts from the plant underwent a thorough phytochemical analysis using column chromatography and TLC techniques. The isolated chemical was examined using mass spectrometry, UV, FT-IR, ^{1}H NMR, ^{13}C NMR, *etc*. The LD_{50} of the ethanolic extract was obtained using the acute toxicity assessments. Albino rats were subjected to convulsions induced by MES and lithium pilocarpine to evaluate the anticonvulsant effectiveness. The ethanolic extract of the stem diminishes the maximum electroshock (MES)-induced phases of flexion, hindlimb extension, clonus and stupor during convulsions. Lithium-pilocarpine convulsions were evaluated against a diazepam group and a control group and has shown anticonvulsant activity.

Keywords: Anticonvulsant activity, Ethanol extract, Lithium pilocarpine, Quercetin, *Tragia involucrata*.

INTRODUCTION

In both industrialized and developing nations, the demand for herbal medicines is on the rise because of a wide range of biological characteristics, an improved safety record and affordability across board assemblies [1,2]. Establishing the quality standards for herbal formulations, natural products and similar items is crucial in order to change the public's impression of subpar medicine production practices and create commodities that benefit society as a whole. Several diseases, including eczema, viral infections, cephalalgia, respiratory infections and hepatic disorders, have traditionally been treated using *Tragia involucrata*, a species of the Euphorbiaceae family [3]. *Tragia involucrata* Linn's pharmacognostic properties, phytochemical profile and physico-chemical characteristics are the focus of this investigation. The peculiar morphological traits of *Tragia involucrata*, a member of the Euphorbiaceae family, increase its adaptation to varied settings. An upright, often branching stem that is either glabrous or sparingly hairy characterizes this perennial herbaceous plant, which may reach a height of around one meter. Optimal photosynthesis and water retention in dry settings are made possible by the alternately oriented, oval to elliptic leaves that have a slightly serrated border and pronounced venation [4]. The ecological adaptability of *Tragia involucrata* is shown by the varied range of environments in which it normally thrives. These habitats include disturbed areas, meadows and rocky slopes [5]. Traditional methodologies and modern pharmacological research have come together in the study of *Tragia involucrata*. Many indigenous cultures have traditionally used *T. involucrata* for its purported therapeutic properties, treating anything from skin diseases to gastrointestinal issues [4].

Study of these ethnobotanical uses has recently shown that many plants with traditional medicinal uses, including *T. involucrata*, have pharmacological actions that have been validated by scientific investigations. The identified bioactive components in *T. involucrata*s, reinforcing its efficacy assertions. This herb has been traditionally utilized for the treatment of various conditions, including diabetes, epilepsy, inflammation, wounds, dermatitis, scabies and other skin issues [6-9]. The

benefits include alleviation of pain and treatment for bronchitis.

Drug development and the modern medical validation of traditional medicines are both greatly impacted by this endeavor. Epilepsy is seen as a longstanding disorder that was traditionally perceived as a spiritual affliction. More scientific investigation into the potential medicinal applications of *T. involucrata* is necessary, but the importance of preserving traditional traditions is underscored by the fact that indigenous knowledge and contemporary science are working together. This study aimed to isolate the bioactive flavonoid quercetin from the stem of *T. involucrata* and assess its anticonvulsant properties. A comprehensive phytochemical analysis was conducted on the ethanolic extract of the plant. After conducting column chromatography, TLC was employed to analyze the extracts. The isolated molecule was analyzed using FT-IR, ^{1}H NMR, ^{13}C NMR, UV, mass spectrometry and other techniques. The LD_{50} of the ethanolic extract was established through acute toxicity studies [10,11]. Albino rats were administered MES and lithium pilocarpine to induce convulsions for the purpose of evaluating the efficacy of anticonvulsants.

EXPERIMENTAL

Plant collection and identification: In July 2023, in the hamlet of Papanasam, coordinates: 10.9333ºN, 79.2833ºE, India, fresh *Tragia involucrata* (Euphorbiaceae) samples were collected from the roadside. Dr. John Britto, Director of the Rapinet Herbarium at St. Joseph's College in Thiruchirappalli, India, acquired the certificate of registration (2013/NS001) after identifying and authenticating the plant. After collecting the plant stems, they were dried beneath sunshades for later use.

A Perkin Elmer-Lambda35 spectrophotometer was used to acquire the UV spectra. A Perkin Elmer-Spectrum Rx1 infrared spectrophotometer was used to collect the FTIR spectra. A 5 mm ^{1}H and ^{13}C running at 500 MHz Bruker AV NMR apparatus was used to acquire the ^{1}H and ^{13}C NMR spectra. The Bruker 45X-GC-44 was used to record the mass spectra.

Plant extract: The stem of *Tragia involucrata* (1 kg) was air-dried and powdered, then exposed to extraction with ethanol in a soxhlet extractor for 18 h, maintaining a temperature below the boiling point. A rotary evaporator (Superfit - ROTAVAP, India) was used to concentrate the extract in a vacuum at 400. Eighty grams of residue were recovered.

Phytochemical screening: Initial phytochemical analysis was conducted employing established qualitative techniques. A screening test was conducted for alkaloids, coumarin, flavones, flavonoids, phenol, steroid and tannin, adhering to the methods outlined by Harborne [10].

Column chromatography: Increasingly polar solvents were employed to chromatograph 10 g of ethanol stem extract on a silica gel column (100-200 mesh). On a silica gel column (Merck, India), 100% hexane was used to elute the mixture. The following ratios of solvents were then introduced: 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and eventually 100% ethyl acetate. Terpseofluorometric analysis was performed on all fractions that were collected. Based on the TLC profile, the fractions that had similar R_f values were combined.

Animals: Animals approved for use in this study were male and female Albino rats weighing between 125 and 140 g obtained from Department of Pharmacy, Srikrupa Institute of Pharmaceutical Science, Siddipet, India. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) (SIPS/2017/IAEC/34/20) and followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

MES induced seizure test: An electro-convulsiometer (Techno Electronics, Lucknow) induces seizures in Albino rats by applying 150 mA to ocular electrodes (INCO, India) for 0.2 sec. There exist four primary categories of animals. Group I was part of the vehicle control group. In the trial, participants in Group II were administered 100 mg/kg of the plant extract, whereas those in Group III were given 200 mg/kg. Group IV is administered a reference standard of 25 mg/kg of phenytoin. It is advisable to administer the medication 1 h prior to the onset of convulsions. The subjects were monitored through different stages of MES seizures, including tonic flexion, extension, clonus, stupor and mortality. Antiepileptic efficiency of the extracts was assessed by removing or reducing extensor phase length. The mean and standard error were noted [12,13].

Induction of status epilepticus in albino rats using lithium pilocarpine: The Wistar rats, both male and female, were divided into four sets of six, with the first set acting as a control and the fourth set as standard. The first three groups received just the vehicle, whereas the fourth group was given 1 mg/kg of diazepam. A 12 h post-administration of 3 mg of lithium sulfate intravenously, the seizure-inducing agent pilocarpine (30 mg/kg i.p.) was delivered. Data on rearing and forelimb clonus were utilized to clarify the outcomes of the study investigating the effects of plant extract administered at intraperitoneal doses of 100 and 200 mg/kg. ANOVA was used to analyze the data comprehensively [14].

RESULTS AND DISCUSSION

Column chromatography: Column fractions with an R_f value of 0.46 that matched quercetin were obtained by mixing hexane and ethyl acetate in an 80:20 ratio in a TLC mobile phase solvent comprising chloroform and methanol (1:1). The fractions were subsequently combined and crystallized, resulting in a final yield of approximately 80 mg. The procedure was conducted multiple times utilizing a large quantity of samples until the required amount of quercetin was achieved.

Phytochemical screening: A comprehensive phytochemical analysis was conducted on the plant extracts obtained from ethanol. The alkaloids, flavonoids and tannins present in the extract could subsidise to the anticonvulsant properties of the *T. involucrata* plant and detailed in Table-1.

The ultraviolet spectrum indicates the existence of two separate bands. The detection of Band I at 372 nm and Band II at 255 nm indicates that *Tragia involucrata* is associated with a flavonoid (Fig. 1). Velu *et al.* [15] demonstrated that the methanol and hexane extracts of *T. involucrata* posses

TABLE-1
EXPLORING THE PHYTOCHEMICAL PROFILE OF THE ETHANOLIC EXTRACT FROM THE STEM OF *Trajia involucrate*

Tests for phytocontituents	Tests/Reagents	Inference
Alkaloids	Dragondorff's/Mayer's reagent	Positive
Coumarin	Sodium chloride test	Negative
Flavones	Shinoda's test	Positive
Flavonoids	Ammonia test	Positive
Phenol	Phenol reagent	Positive
Steroid	Libermann's test	Positive
Tannin	Lead acetate	Positive

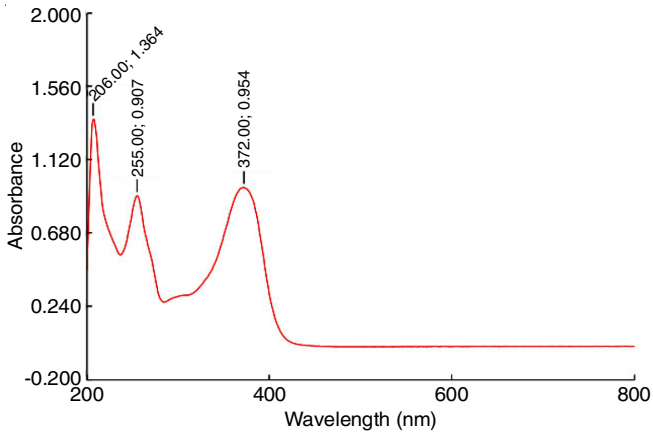

Fig. 1. The ultraviolet spectrum of a compound isolated from *Tragia involucrata* stem powder

variety of phytochemicals present in the extract, including phenols, terpenoids and steroids, have been associated with a reduced risk of cancer. Several studies indicates that numerous phytochemicals, including flavonoids, terpenes, alkaloids, lactones and coumarins, possess anticonvulsant properties [16,17].

The IR spectrum indicates that the absorption band at 3437 cm^{-1} confirmed the characteristic OH stretching vibration peak of phenol (Fig. 2). Absorptions at 2888 cm^{-1} correspond to the aliphatic CH_2 stretching, whereas 307 cm^{-1} is ascribed to aromatic –CH. The keto group is observed at 1729 cm^{-1}, whereas the aromatic -C=C stretching at 1601 cm^{-1}, ketones exhibit C-CO-C stretching and bending vibrations at 1127 cm^{-1}, while aryl ethers and phenols have C-O stretching vibrations at 1285 cm^{-1}. These findings are in the accordance with the results of Sathiyadevi & Subramanian [18].

The ^{1}H NMR displays a distinct peak that validates the structure of quercetin (Fig. 3a). A doublet at δ 6.18 ppm and 6.19 ppm indicates the presence of a proton on H-6. A doublet at δ 16.40 and 6.41 indicates the presence of a proton at H-8. The 5′-H proton is validated by the doublet at δ 6.87 and 6.89 ppm. A double-doublet at δ 7.53, 7.53, 7.54 and 7.55 ppm indicates the presence of a proton at H-6′. A doublet at δ 7.68 and 7.67 ppm indicates the presence of the 2′-H proton. In a similar manner, the presence of five-OH protons is validated by the peaks observed at δ 9.31, 9.37, 9.60, 10.7 and 12.5, corresponding to 3′-OH, 4′-OH, 3-OH, 6-OH and 5-OH, respectively [19]. The ^{13}C NMR analysis reveals peaks corresponding to 15 distinct carbons, ranging from 93.799 to 176.290 ppm. 136.18 verifies C-3 carbon, 176.29 verifies C-4 carbon, 161.17 verifies C-5 carbon, 164.33 verifies C-7, 98.2 verifies C-6, 93.7 verifies C-8, 156.5 verifies C-9, 103.46 verifies C-10, 145.5 verifies C-2 (Fig. 3b). This provides additional confirmation of the structure of quercetin [20].

The mass spectrum reveals a parent peak at *m/z* 303, thereby confirming the molecular weight of the flavonoid quercetin. Additional fragment peaks observed at $[M+H-H_2O-CO]^+$ = 256.32 and $[M+H-CO]^+$ = 274.3 further validate the structure of quercetin (Fig. 4). The isolated compound was confirmed to be quercetin through comparison with the reference substance and a mass spectral library system. The prior studies indicated a comparable peak, thereby validating that the isolated compound is identified as quercetin [21].

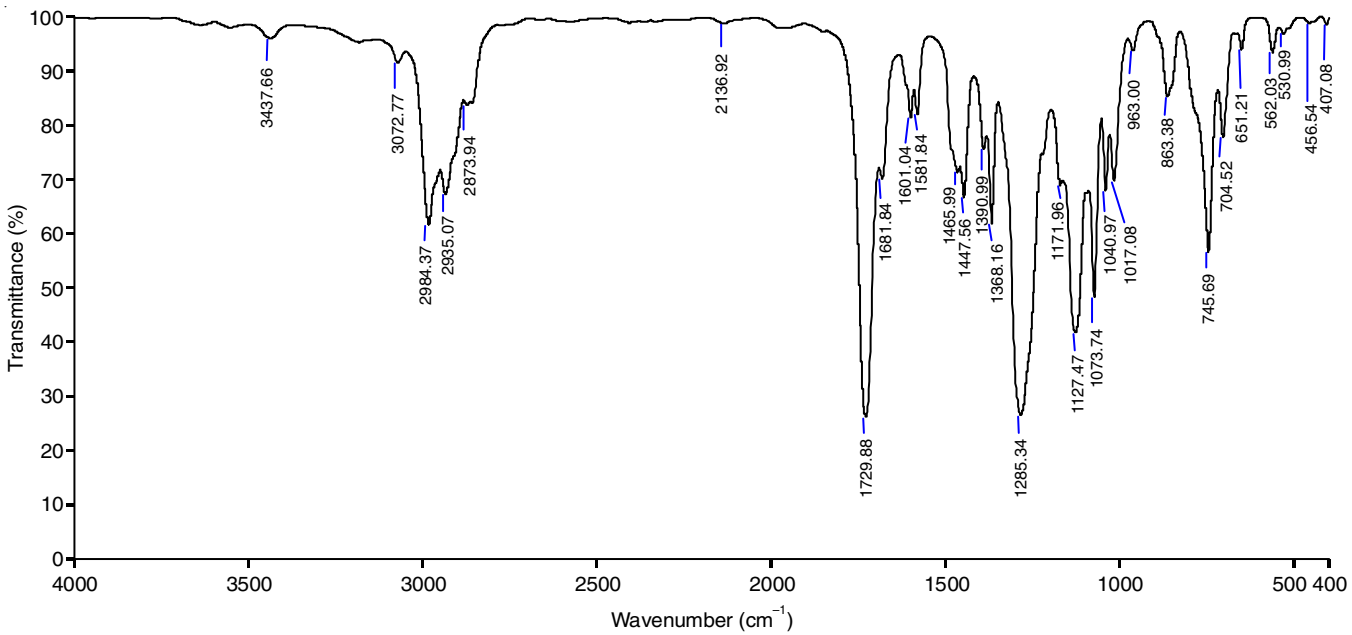

Fig. 2. FTIR image of compound isolated from *Tragia involucrata* stem powder

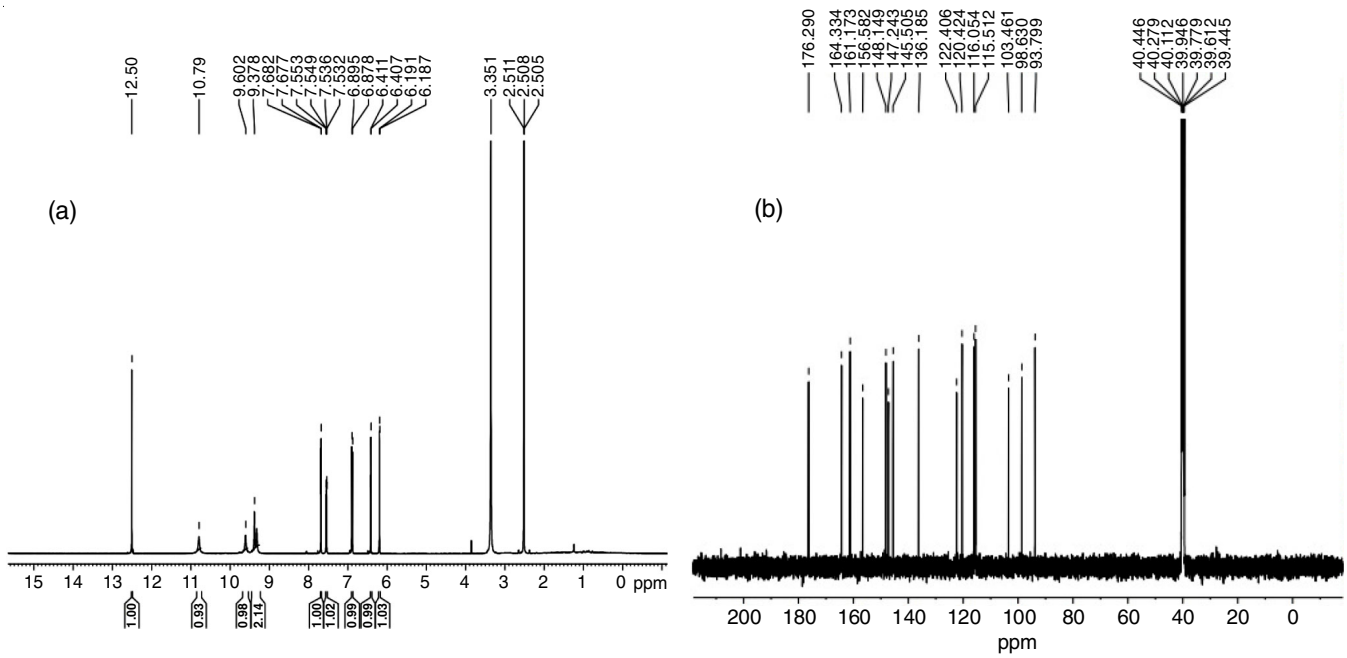

Fig. 3. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of isolated compound of *Tragia involucrata* stem powder

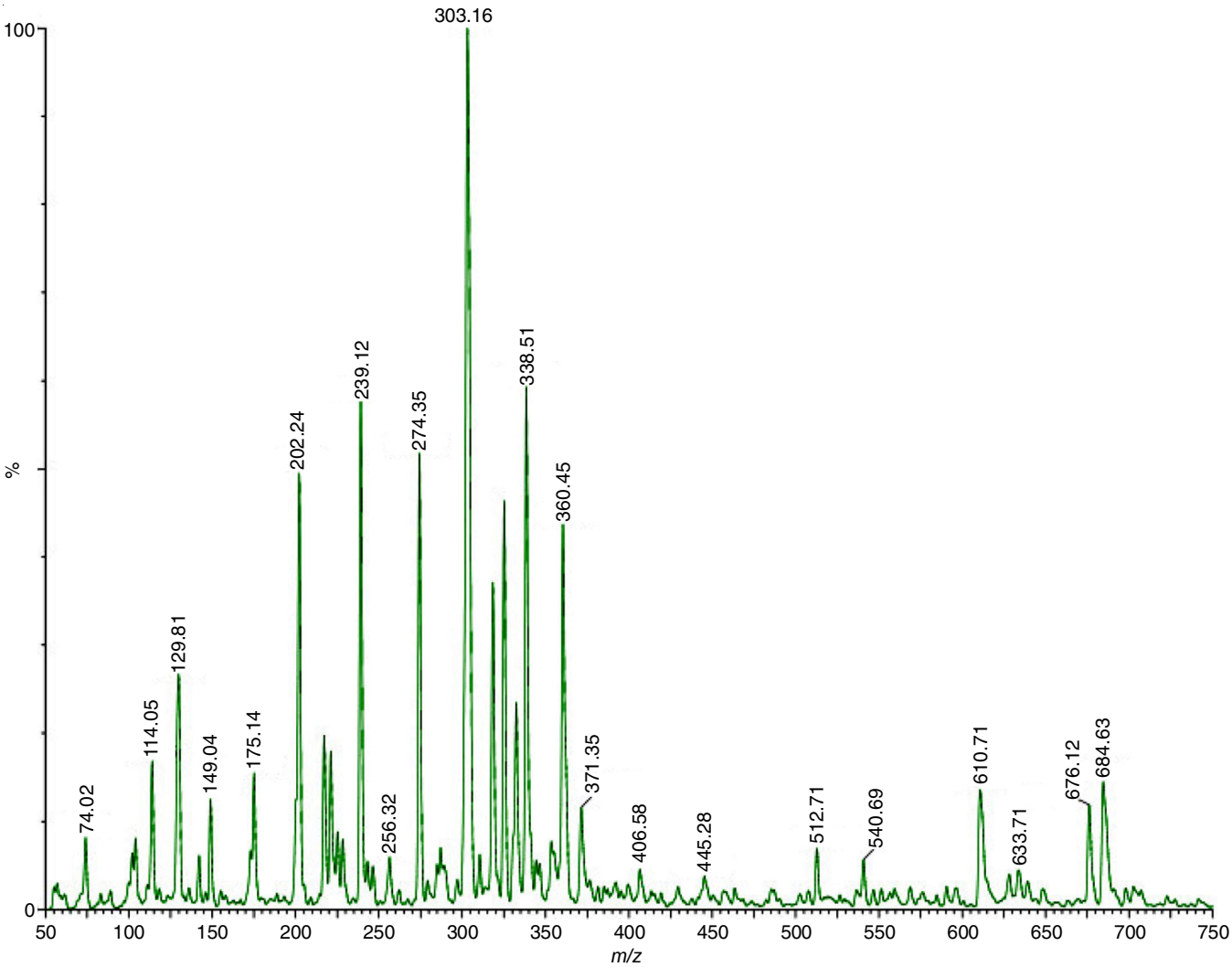

Fig. 4. Mass spectrum analysis of the isolated compound derived from the stem powder of *Tragia involucrata*

TABLE-2
IMPACT OF PLANT EXTRACT ON CONVULSIONS INDUCED BY MES IN ALBINO RATS

Experimental group	Dose (mg/kg) b.w.	Time (sec) of various phase of convulsion				Mortality (%)
		Flexion	Extension (HLTE)	Clonus	Stupor	
Control (CMC)	2.0 mL/kg (p.o)	16.9 3 ± 0.9	14.3 ± 1.0	20.7 ± 1.4	185.0 ± 5.0	0/6 (0.0%)
Trajia involucrata plant extract	100 (p.o)	9.50 ± 0.76*	9.50 ± 0.76*	12.5 ± 0.6	165.0 ± 5.4	0/6 (0.0%)
Trajia involucrata plant extract	200 (p.o)	7.50 ± 0.76*	9.50 ± 0.76	12.5 ± 0.6	165.0 ± 7.9	0/6 (0.0%)
Phenytoin	25 mg/kg (p.o)	4.00 ± 0.36*	0.0**	5.0 ± 0.01	21.70 ± 1.9	0/6 (0.0%)

Values are presented as Mean ± SEM: (n = 6). Statistical significance indicated at $*p < 0.05$, $**p < 0.01$ in comparison to control.

TABLE-3
EFFECT OF *T. involucrata* ETHANOLIC EXTRACT ON INDUCED STATUS EPILEPTICUS FROM LITHIUM-PILOCARPINE

Treatment	Dose	Latency to rearing with forelimb clonus (min) Mean ± SEM
Control	–	19.32 ± 0.25
Trajia involucrata plant extract	100 mg/kg, p.o.	39.77 ± 1.5
Trajia involucrata plant extract	200 mg/kg, p.o.	52.54 ± 0.93
Standard (diazepam)	2.0 mg/kg, i.p.	69.45* ± 5.5

Data are presented as Mean ± SEM: (n = 6). Significance at $*p < 0.01$ relative to control.

Maximal electroshock seizure test: The outcomes of the maximal electroshock seizure test, which was carried out on the ethanolic stem extract of *Tragia involucrata*, are shown in Table-2. During various stages of convulsions, the mortality rate is observed alongside the flexion, extension, clonus and stupor observations. At 100 mg/kg and 200 mg/kg dosages, the MES-induced convulsion was observed in flexion (9.50 ± 0.76 and 7.50 ± 0.76), extension (9.50 ± 0.76 and 9.50 ± 0.76), clonus (12.5 ± 0.6 and 12.5 ± 0.6) and stupor (165.0 ± 5.4 and 165.0 ± 7.9) phases, along with comparisons to the control group. The results shows that neither the plant extract nor the conventional phenytoin caused any deaths.

Test for seizures induced by lithium pilocarpine: Table-3 displays the findings of the plant extracts in albino rats that were given lithium pilocarpine to produce status epilepticus. The control group exhibited a rearing duration of 19.32 min with forelimb clonus. Group II at 100 mg/kg demonstrates a duration of 39.77 min, while Group III at 200 mg/kg exhibits a duration of 52.54 min. The standard diazepam shows a duration of 69.45 min. These findings indicate that the stem extract is more effective in managing convulsions compared to standard diazepam.

The use of acute toxicity experiments, the LD_{50} of the ethanolic extract of plant was determined. Concentrations that are higher than 2,000 mg/kg of body weight have been traced. For the objective of determining whether or not anticonvulsants are effective, MES and lithium pilocarpine were given to albino rats in order to cause convulsions (also known as seizures). The ethanolic extract of the stem of *T. involucrata*, when compared to phenytoin, results in a considerable reduction in the length of the phases of flexion, hindlimb extension, clonus and stupor that are generated by maximal electroshock (MES) during convulsions. Studies indicate that substances inhibiting glutamatergic receptors or voltage-gated Na^+ channels [22] could potentially avert seizures induced by MES. The observation that MES and lithium pilocarpine-induced seizures are inhibited indicates that the ethanol extract of *T. involucrata* may function through various mechanisms such as obstructing glutamatergic receptors or voltage-gated Na^+ channels, enhancing GABAergic neurotransmission or reducing T-type Ca^{2+} currents in the brain.

Conclusion

In conclusion, quercetin was successfully extracted from *Tragia involucrata* stems in this investigation. The isolated quercetin component was examined using mass spectrometry, FT-IR, NMR and UV techniques. The ethanolic extract of *T. involucrata* demonstrated a noteworthy anticonvulsant efficacy in rat models of MES and seizures produced by lithium pilocarpine. The results indicated that investigating the plant's safety, toxicity, efficacy, and therapeutic dosage could facilitate the development of a novel drug for convulsions in the near future.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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