



ISOLATION OF STEROID AND ANTICONVULSANT STUDIES ON ETHANOLIC EXTRACT OF THE BARK OF *CASSINE GLAUCA* (CELASTRACEA)

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ABSTRACT

Cassine glauca (Celastraceae) is widely prevailed in India and Srilanka, the plant parts are used in traditional medicine such as menstrual disorders, skin wounds and cuts, rheumatism and epilepsy. The aim of this study is to establish the phytochemical constituents present in the bark of *cassine glauca* and also to determine the anticonvulsant activity. The preliminary phytochemical screening of ethanolic extract revealed the presence of alkaloids, carbohydrate, steroid, Tannin, Phenol and Flavonoids. The phytoconstituents present in the ethanolic extract are separated using Hexane: Ethylacetate as solvent system by column chromatography. Silica gel is used as fixed phase. By this method one of the phytoconstituent is separated which led to the isolation of stigmasterol. The structure of stigmasterol compound is elucidated using chemical test and spectroscopic techniques. The LD₅₀ of ethanolic extract is found out by acute toxicity studies. It is found above 2000mg/kg body weight. Evaluation of anticonvulsant activity using MES and lithium pilocarpine induced convulsion in albino rats. The ethanolic extract of *cassine glauca* bark decreased the duration of Flexion, Hindlimb extension, clonus and stupor phase of MES induced convulsions as compared to control. Lithium-pilocarpine induced convulsions are compared with control and standard Diazepam. The results of the study reveal that ethanol extract of *cassine glauca* bark possess anticonvulsant effect.

KEY WORDS

Cassine glauca, ethanol extract, Stigmasterol, Anticonvulsant, MES, Lithium Pilocarpine.

INTRODUCTION

The plant *Cassine glauca* commonly known as karuvaili belongs to the family celastraceae. It is an evergreen tree, very common in foot hills to 1200m. In India and Srilanka it is also planted as an ornamental. A graceful moderate sized, deciduous tree, with grey or blackish bark opposite, elliptic, Coriaceous leaves and small yellowish-brown flowers in axillary. The bark exudes a copious water sap and cut. It is occasionally planted as an ornamental tree in gardens [1]. Roots and leaves of *cassine glauca* are used in treating cough with phylegm, swellings [2] and menstrual disorders [3]. The plant

paste is used on skin wounds and cuts [4] and bark powder is used in treating rheumatism [5].

Epilepsy is a chronic disorder of the central nervous system (CNS) of various etiologies. These are caused due to alteration in the body system that causes excessive discharge of the central neurons [6]. The problem with currently available antiepileptic drugs is i) They do not affect epileptogenesis. ii) They are associated with serious side effects, including teratogenicity, chronic toxicity iii) They have adverse effects on cognition and behavior [7]. In addition, even after the seizures have long been suppressed, continuous medication is necessary.

The aim of this study is to isolate and identify one of the phytochemical constituents present in the bark of *cassine glauca* and to determine the anticonvulsant activity in the albino rats experimental model using ethanolic extract of the plant barks.

MATERIALS AND METHODS

Collection and identification of plant material

Cassine glauca (Family: Celastraceae) was collected freshly on the road sides in July from papanasam, Tamilnadu, India. The plant was identified and authenticated by personal of Rapinet Herbarium, St. Joseph's college, Thiruchirappalli, Tamilnadu, India. The bark of the plants were collected and shade dried.

Preliminary Phytochemical screening

Preliminary phytochemical screening is carried out on the ethanolic extract of the plant according to standard chemical tests to detect the phytochemical constituents present [8,9].

Extraction and isolation of the compound

Air-dried and pulverized bark of *cassine glauca* (1 kg) is extracted with ethanol. The extract is filtered and the solvent evaporated under reduced pressure, 90 g of residue is obtained. This extract is subjected to chromatography over silica gel packed in n-hexane. Gradient elution is done using Hexane: Ethyl acetate solvent at different ratios are used. Repeated column chromatography with hexane: ethylacetate (80:20) yielded one of the phytoconstituent. The structures have been elucidated using spectral methods (IR, NMR and MASS).

Animals

Albino rats (125-140g) of either sex are selected for experimental study. All the studies conducted are approved by Institutional animal ethical committee, Department of pharmacy, Srikrupa institute of pharmaceutical science, Telungana, India.

MES Induced Seizure test

The seizures are induced by maximal electroshock in albino rats with the help of electro convulsimeter by passing current of 150mA for 0.2 second using corneal electrodes (INCO, India). The animals are divided in four groups. Group I served as vehicle control group. Group II and Group III served as test groups treated with the plant extract administered a dose of 100 and 200 mg/kg respectively. Group IV is received Phenyton (25mg/kg)

as a reference standard. The drugs are given one-hour prior induction of convulsions. The number of animals protected from tonic hind limb extension seizure and duration of observed tonic hind limb extension seizure is recorded in each dose group [10].

Lithium pilocarpine induced status-epilepticus in albino rats

Status epilepticus is induced by administration of pilocarpine (30mg/kg) 24h after lithium carbonate (3 mEq/kg). The effect of plant extract (100 and 200 mg/kg) is studied on the rearing with forelimb clonus by administered the plant extract 30 mins before injection of pilocarpine. Diazepam is used as a reference standard in a dose of 1mg/kg [11].

Results and Discussion

The preliminary phytochemical screening of ethanol extract of the bark of *cassine glauca* indicated the presence of alkaloid, carbohydrate, steroid, Tannin, Phenol and Flavonoids as shown in Table-1.

The isolated compound was identified by IR, MASS, H^1 and C^{13} NMR.

The NMR spectrum shows the values of the peaks are correlated with the earlier literature (Figure-1). The H^1 NMR spectrum of compound varied between 0.699 to 5.350. The NMR shows the presence of 48 protons and the important peaks at 3.51 of C-3 carbon attached proton and a peak at 5.34 of C-6 carbon attached proton. 0.821 correspond to C-27 attached proton, 0.806 related C-26 carbon attached proton and 0.841 related to C-29 proton. The above set peaks are correlates to the structure of the phyto-sterols of stigmasterol [12].

The C^{13} NMR of compound stigmasterol revealed 29 carbon atom signals (Figure-2). The peaks are related with earlier Literature 12.05 ppm confirms C-18 carbon, 12.24 ppm confirms C-27 carbon, 18.99 ppm confirms C-21 Carbon, 19.40 confirms C-19 carbon, 71.78 confirms C-3 carbon, 138.30 confirms C-22 carbon and 140.77 confirms C-5 Carbon [13]. IR values: Absorption bands are 3333.36 cm^{-1} that is characteristic of OH stretching (Figure-3). Absorptions at 2934.77 cm^{-1} and 2864.86 cm^{-1} are due to aliphatic C-H stretching's. Other absorption frequencies include 1668.18 cm^{-1} as a result C=C stretching and at 1457.58 cm^{-1} is a bending frequency for cyclic $(CH_2)_n$. The absorption frequency at 1055.86 cm^{-1} signifies cycloalkane. The above peaks confirm the group present as in the Stigmasterol [14].

Table 1: Phytochemical screening of cassine glauca bark ethanolic extract.

Tests for phyto constituents	Tests/ Reagents	Inference
Alkaloids	Dragondorff's / Mayer's reagent	+ve
Carbohydrate	Molisch test	+ ve
Coumarin	Sodium chloride test	- ve
Anthocyanin	H ₂ SO ₄ test	-ve
Flavonoids	Ammonia test	+ ve
Phenol	Phenol reagent	+ ve
Steroid	Libermann's test	+ ve
Tannin	Lead acetate	+ ve

Table 2. Anticonvulsant effect of Plant extract on the MES-induced convulsion in mice

Experimental group	Dose mg/kg b.w.	Time in seconds of various phase of convulsion				Mortality (%)
		Flexion	Extension (HLTE)	Clonus	Stupor	
Control (CMC)	2.0mL/kg (p.o)	16.9 3±0.9	14.3±1.0	20.7±1.4	185.0±5.0	0/6 (0.0%)
<i>Cassine glauca</i> Plant Extract	100 (p.o)	7.23±1.5*	8.06±0.61*	5.6±0.06	118.3±9.3	0/6 (0.0%)
<i>Cassine glauca</i> Plant Extract	200 (p.o)	4.42±0.39*	0.0**	6.9±0.03	39.40±2.4	0/6 (0.0%)
Phenytoin	25mg/kg (p.o)	4.00±0.36*	0.0**	5.0±0.01	21.70±1.9	0/6 (0.0%)

Data are expressed as Mean ±SEM: (n=6). Significance at *P<0.05, ** P<0.01 as compared to control

Table 3. Effect of *Cassine glauca* ethanolic extract on Lithium- Pilocarpine induced status epilepticus

Treatment	Dose	Latency to rearing with forelimb clonus (min) Mean ±SEM
Control	-----	19.32±0.25
<i>Cassine glauca</i> Plant Extract	100 mg/kg, p.o.	25.52±0.49
<i>Cassine glauca</i> Plant Extract	200 mg/kg, p.o.	32.81±0.92
Standard (diazepam)	2.0mg/kg, i.p.	69.45*±5.5

Data are expressed as Mean ±SEM: (n=6). Significance at * P<0.01 as compared to control

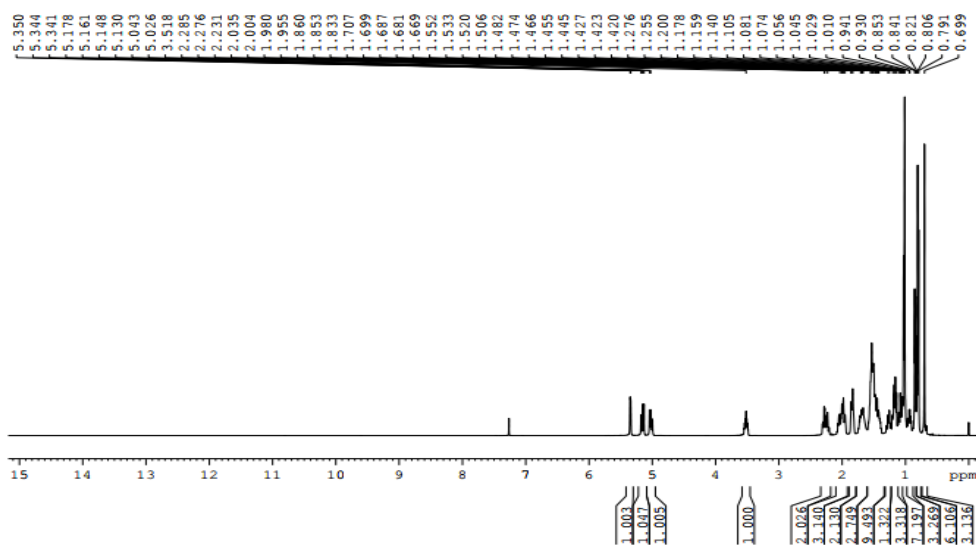


Figure-1: ^1H Nmr Spectrum of Isolated Compound of *Cassine Glauca* Bark Powder

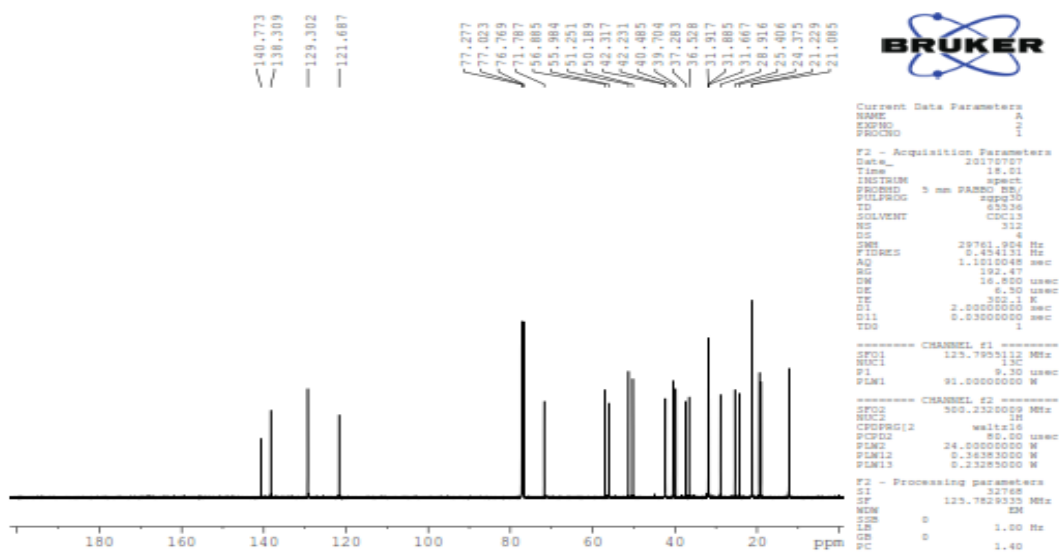


Figure-2: ^{13}C Nmr Spectrum of Isolated Compound of *Cassine Glauca* Bark Powder

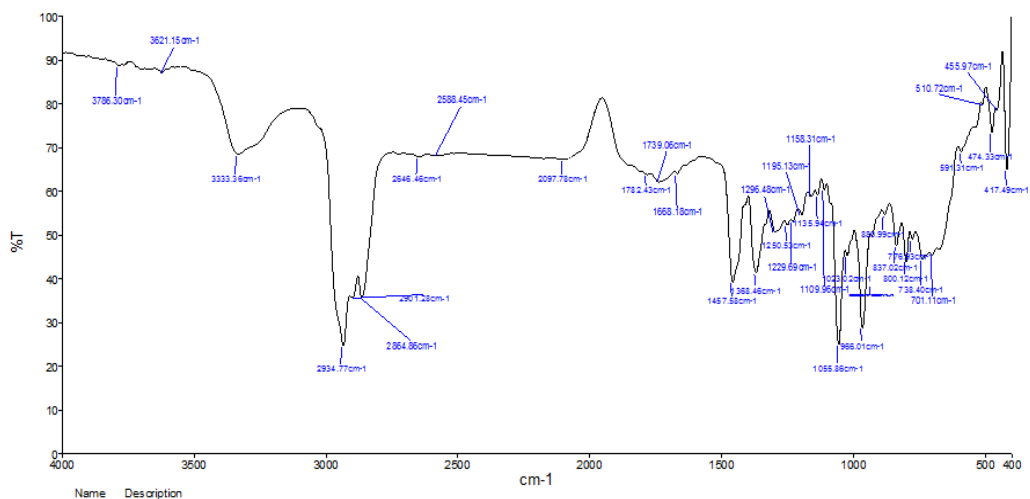


Figure-3: FTIR Spectrum of Isolated Compound of *Cassine Glauca* Bark Powder

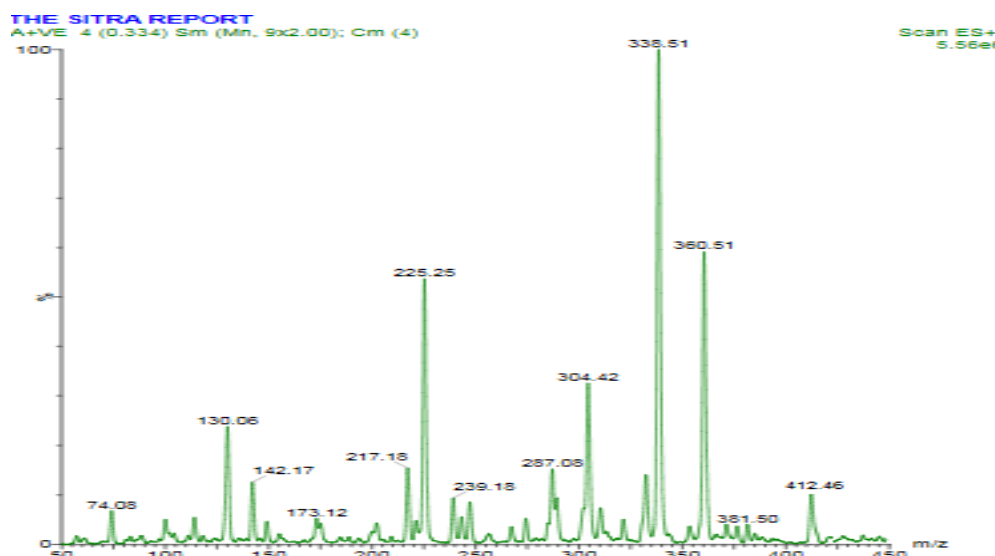


Figure-4: Mass Spectrum of Isolated Compound of *Cassine Glauca* Bark Powder

The mass spectrum of the sample is shown in figure 4 shows a peak at 412 m/e which confirms the molecular formula of stigmasterol ($C_{29}H_{48}O$). The peaks at 397 and 255 are due to fragmentation of (M-CH₃) and (M- side chain-ring D cleavage – H₂O) + which also confirms the structure of stigmasterol [15].

Maximal electroshock seizure test

In maximal electroshock seizure test it was observed that the ethanolic extract of cassine glauca bark is shown in the Table-2. The various phases of convulsion are shown in the tables. The flexion, extension, clonus and stupor for various phases of convulsions are seen and mortality percentage is also noted. The MES induced convulsion by flexion (7.23 ± 1.5 and 4.42 ± 0.39), Extension (14.3 ± 1.0 and 8.06 ± 0.61), clonus (5.6 ± 0.06 and 6.9 ± 0.03) stupor (118.3 ± 9.3 and 39.40 ± 2.4) phase are those for 100mg/kg and 200mg/kg doses and the results are compared with control. The both plant extract and standard phenytoin show mortality 0%.

Lithium pilocarpine induced seizures test

The effect of study plant extracts in albino rats on lithium pilocarpine induced status epilepticus are shown in Table-3. Group I control show latency to rearing with forelimb clonus 19.32 minutes. Group II *cassine glauca* 100mg/kg shows 25.52 mins and Group III *cassine glauca* 200mg/kg shows 32.81 mins and standard Diazepam shows 69.45 mins. The result show that the bark extract shows anticonvulsant but the values is less than standard Diazepam.

CONCLUSION

The results of the present study established the presence of biologically active phytochemicals in the bark extract. The data also suggested that the bark extract contain stigmasterol. Hence these findings help to validate the use the plant in the management of epilepsy. Thus, from the above discussions and facts the researcher suggests that if research is carried out in the plant on various dimensions like its safety, toxicity, efficacy and therapeutic dose fixation, the new drug for treating convulsions with scientific evaluation can be found in near future.

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