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ANTIEPILEPTIC ACTIVITY OF *VISCUM ARTICULATUM* BURM AND ITS ISOLATED BIOACTIVE COMPOUND IN EXPERIMENTALLY INDUCED CONVULSIONS IN RATS AND MICE

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ABSTRACT
The whole plant of *Viscum articulatum* Burm has been traditionally used in India for the treatment of neurological disorders including epilepsy. The study was planned to establish the antiepileptic potential of two fractions of the extracts of *Viscum articulatum* in experimental animals and to identify and characterize the antiepileptic active principles in the extracts. The chloroform and the methanolic extracts of *Viscum articulatum* and their isolated compounds were evaluated for their antiepileptic potential in Picrotoxin and N-methyl-D-aspartic acid (NMDA) induced convulsion models. Estimation of brain gamma-aminobutyric acid (GABA) was carried out to investigate the mechanism of action of the extracts and the isolated compounds. The extracts and one of the isolated compounds from the methanolic fraction, Syringaresinol, exhibited a dose-dependent protection against picrotoxin and NMDA induced convulsions in rats and mice respectively. However, the chloroform fraction did not prevent NMDA induced convulsions in mice. The findings of the experiments on various animal models clearly demonstrated that the extracts and one of the isolated compounds possess antiepileptic activity, possibly by enhancing inhibitory GABAminergic neurotransmission. The results indicate that the chloroform and the methanolic extracts of *Viscum articulatum* and one of the isolated compounds from the methanolic fraction, Syringaresinol, showed protection against picrotoxin and NMDA induced convulsions in rats and mice respectively.

KEYWORDS: *Viscum articulatum*, Picrotoxin, NMDA, GABA, Antiepileptic activity.

1. INTRODUCTION
Epilepsy is a chronic neurological disorder characterized by recurrent unprovoked seizures which are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain.[1] The incidence of unprovoked seizures in the general population is 57 to 63 per 100,000 persons and the incidence of epilepsy is 46 to 48 per 100,000.[2] Currently available synthetic antiepileptic drugs have the disadvantage of dose- related toxicity and adverse effects like reduced bone mineral density and teratogenic effects.[3] The aerial parts of *Viscum articulatum* belonging to the family, Viscaceae has been traditionally used in ulcers, blood diseases, epilepsy and as an aphrodisiac.[4] Two new phenolic glycosides, 1-O-benzyl-[5-O-benzoyl-β-D-apiofuranosyl(1-2)]-β-D-glucopyranoside and 4`-hydroxy-7,3`–dimethoxyflavan-5-O-β-D-glucopyranoside, together with nine flavanones 3-11, have been isolated from the dried whole plants of *Viscum articulatum*. A few isolated compounds showed weak anti-HIV-1 activity.[5]

Preliminary studies in our laboratory revealed that the methanolic extract of *Viscum articulatum* Burm was effective in preventing convulsions in rats in two animal models.[6] This finding suggested that the extract could be a potential natural source of anticonvulsant and could have a greater importance as therapeutic agent. In an effort to further scientifically validate and rationalize the antiepileptic potential of the whole plant of *Viscum articulatum*, the study was planned to identify and characterize the antiepileptic active principles in the extracts of *Viscum articulatum* and to establish the possible mechanism of action of antiepileptic activity in experimental animals.

2. MATERIALS AND METHODS
2.1. Plant material
The aerial parts of *Viscum articulatum* (Family-Viscaceae) was collected from Tirupati, Chittoor district of Andhra Pradesh, India. The plant was identified, and authenticated by Dr. K. Madhava Chetty,

Sri.Venkateswara University, Tirupati, Andra Pradesh, India.

2.2. Preparation of extracts

The aerial parts of *Viscum articulatum* was mechanically reduced to a coarse powder and subjected to hot continuous successive extraction in a Soxhlet apparatus with solvents in the increasing order of polarity using petroleum ether, chloroform and methanol under controlled temperature (50-60°C). Extracts were concentrated below 50°C and further drying was carried out using a rotary flash evaporator. The dried extracts were stored in a refrigerator for further use.

2.3. Drugs

All chemicals, drugs and solvents used in the study were of analytical grade and were obtained commercially.

2.4. Animals

Albino rats (Wistar strain) and albino mice of either sex weighing between 150-200 g and 25-30 g respectively were procured from an authorized dealer and acclimatized for seven days under laboratory conditions and fed with commercially available rat pellet diet. Water was allowed ad libitum under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC Approval No: DSCP/RG/RGUHS/IAEC/01/15-16 of Dayananda Sagar College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines.

2.5. Evaluation of Antiepileptic activity

2.5.1. Picrotoxin induced convulsion model in rats

Healthy albino rats of either sex weighing 180-220 g were divided into six groups of six animals each. Group 1 received the vehicle (10 ml/kg p.o of 1% w/v CMC) and served as the solvent control group. Group 2 to 5 received chloroform (CHVA) and methanolic extracts (MHVA) of Viscum articulatum at two different dose levels (150 mg/kg b.w. and 300 mg/kg b.w) orally. Group 6 received the standard drug, Phenobarbitone at 40 mg/kg i.p. The extracts and standard drug was administered for a period of seven days. Picrotoxin (7 mg/kg, i.p.) was injected 60 min after oral administration of the test drugs. Onset of seizures was taken as the parameter to assess the antiepileptic activity[7]. The same procedure was repeated using the isolated compounds as the test samples.

2.5.1.1. Estimation of Brain GABA

The rats were killed by decapitation at predetermined intervals after the administration of test drugs, Standard drug and vehicle followed by subjecting the animals to Picrotoxin induced convulsions. The brains were rapidly removed, blotted, weighed and taken into the ice cold 5 ml trichloro-acetic acid (10% w/v), homogenized and centrifuged at 10,000 rpm for 10 min at 0ºC. GABA was determined by the measurement of the formed fluorescent product resulting from the reaction of GABA with ninhydrin in an alkaline medium in the presence of glutamate. The GABA content in the brain was expressed in μg g^{-1} of the wet brain tissue.[8]

2.5.2. NMDA induced seizures in mice

Mice of either sex weighing 25-30 g were randomly allotted into various groups consisting of six animals in each group. The vehicle (10 ml/kg p.o. of 1% w/v CMC), Dizocilpine hydrogen maleate (0.05 mg/kg i.p.) and the different extracts at two different doses was administered to groups of animals one hour before NMDA (100 mg/kg i.p.) administration.[9] Mice was observed for turning behavior within 30 min which was characterized by two consecutive 360° cycles fulfilled by the same animal. Animals that do not exhibit turning behavior within the 30 min observation period were declared protected. The time of onset of this behavior in all animals was recorded.

2.6. Data Analysis

The data obtained in the results of the study were expressed as mean ± S.E.M. Statistical difference between means were determined by one way ANOVA followed by Dunnet's test. $P < 0.05$ was considered statistically significant in all cases.

2.7. Isolation and characterisation of compounds from the methanolic extract of *Viscum articulatum*

The methanolic extract was used for the isolation of compounds using column chromatography. Different ratios of mixture of solvents like Hexane: Chloroform to Chloroform: Methanol in the ratios of 0-100% was used to collect the fractions for the isolation of the pure compounds. From the collected fractions, 58 mg of Compound 1 was obtained in Chloroform: Methanol solvents at the ratio of 20:80 and 25 mg of Compound 2 were obtained in the same solvent system at the ratio of 35:65 respectively. Spectral analysis of the two purified compounds provided the structural interpretation

3. RESULTS

3.1. Picrotoxin induced convulsions in rats

The results of picrotoxin induced convulsion depicted in Table 1 and Fig.1 revealed that picrotoxin (7 mg/kg i.p.) elicited seizures in all animals treated with only the vehicle (1% w/v CMC) with an onset of tonic convulsions at 3 ± 0.25 min and produced 100% mortality. The chloroform fraction delayed the onset of tonic convulsions at 150 mg and 300 mg/kg b.w respectively (10 ± 0.73 and 12 ± 0.25 min) in comparison with the solvent control. The methanolic fraction also delayed the onset of tonic convulsions at 150 mg and 300 mg/kg b.w respectively (12 ± 0.25 and 13 ± 0.68 min) in a dose dependant manner, in comparison with the solvent control. Phenobarbitone (40 mg/kg b.w) significantly delayed the onset of seizures (16 ± 0.36 min) and provided 100% protection. The isolated compound, Syringaresesinol, significantly delayed the onset of tonic convulsions at 10 mg/kg and 20 mg/kg,, i.p. respectively in a dose dependent manner in comparison with the

solvent control. However, Hentriacontanol did not exhibit any significant antiepileptic activity in the doses used as depicted in Table 3 and Fig. 3.

Table 1: Effect of Chloroform and methanolic extract of *Viscum articulatum* on Picrotoxin induced convulsions in rats.

Treatment	Dose (mg/kg. *b.w*)	No. of animals Convulsed	Animals Protected	Latency of Tonic Convulsions (min)
Solvent control	10 ml	6/6	0	3 ± 0.25
Phenobarbitone	40	6/6	6	16 ±0.36***
CHVA	150	6/6	2	10 ± 0.73**
CHVA	300	6/6	5	12 ± 0.25**
MHVA	150	6/6	5	12 ± 0.25**
MHVA	300	6/6	6	13 ± 0.68**

Values are expressed in terms of mean ± S.E.M, ** p<0.01, ***p<0.001 vs control group - One way ANOVA followed by Dunnett's test

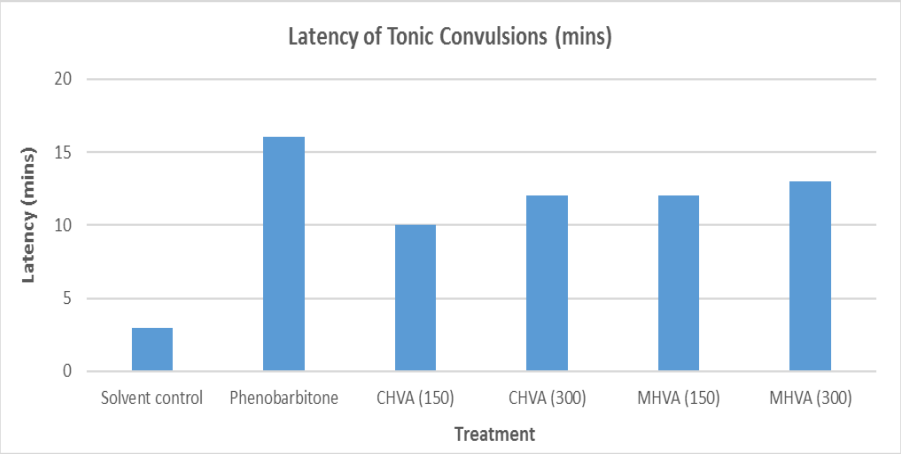


Fig. 1. Effect of Chloroform and methanolic extract of *Viscum articulatum* on Picrotoxin induced convulsions in rats.

3.1.1. Estimation of brain GABA

The brain GABA level was estimated in picrotoxin induced convulsions in rats. Pretreatment with the standard and the methanolic extracts showed significant increase in the brain GABA levels when compared to control (Table 4 and Fig.4).

Table 5 and Fig.5 to be included in the text

3.2. NMDA induced seizures in mice

Administration of NMDA at 100 mg/kg, *i.p.* showed significant turning behavior in mice. Pretreatment with standard (Dizocilpine hydrogen maleate) and methanolic extract at different doses significantly antagonized NMDA induced turning behavior in mice where as the mice treated with the chloroform fraction did not antagonize the turning behavior significantly when compared to control through Dunnett's multiple comparison test. The results are depicted in Table 2 and Fig. 2.

The isolated compound, Syringaresesinol, significantly antagonized the NMDA induced turning behavior in mice at 10 mg/kg and 20 mg/kg respectively in a dose dependent manner in comparison with the solvent control. However, Hentriacontanol did not exhibit any significant antiepileptic activity in the doses used as depicted in Table 5 and Fig. 5.

Table 2: Effect of Chloroform and methanolic extract of *Viscum articulatum* on NMDA-induced seizures in mice.

Treatment	Dose (mg/kg)	Onset of turning behavior (within 30 min)	% Protection
Solvent control	10 ml	7.35 ± 1.25	0
Dizocilpine hydrogen maleate	0.05	34.5 ± 2.5***	100
CHVA	150	6.75 ± 3.92ns	0
CHVA	300	8.55 ± 3.12 ns	0
MHVA	150	28.20 ± 3.60***	100
MHVA	300	30.5 ± 3.62***	100

Values are mean ± S.E.M, n=6, ns-not significant and ***p < 0.001 vs control - One way ANOVA followed by Dunnett's test.

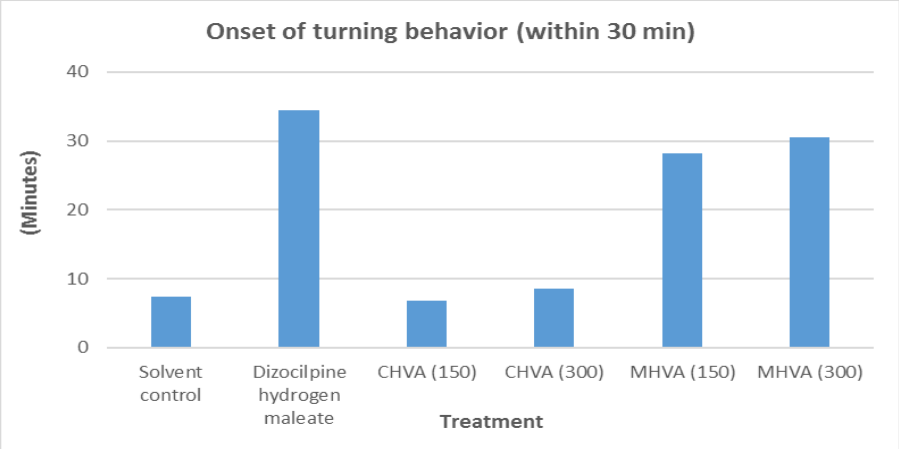


Fig. 2. Effect of Chloroform and methanolic extract of *Viscum articulatum* on NMDA-induced seizures in mice.

Table 3: Effect of Syringaresinol and Hentriacontanol on Picrotoxin induced convulsions in rats.

Treatment	Dose (mg/kg. *b.w. i.p*)	No. of animals Convulsed	Animals protected	Latency of Tonic Convulsions (min)
1% w/v CMC	10 ml	6/6	0	3 ± 0.25
Phenobarbitone	40	6/6	6	12 ±0.36***
Syringaresinol	10	6/6	4	8 ± 0.24*
Syringaresinol	20	6/6	4	10 ± 0.27*
Hentriacontanol	10	6/6	0	3 ± 0.22*
Hentriacontanol	20	6/6	0	3 ± 0.20*

Values are expressed in terms of mean ± S.E.M, *p<0.05, ***p<0.001 vs control group - One way ANOVA followed by Dunnett's test.

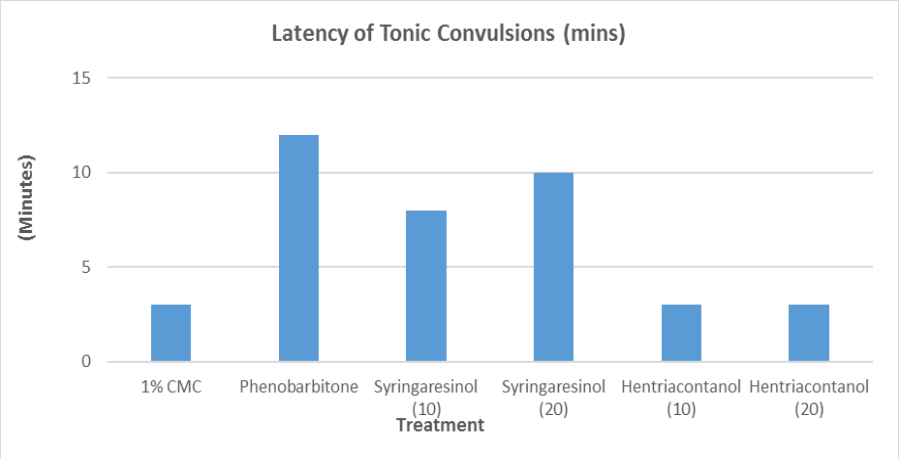


Fig. 3. Effect of Syringaresinol and Hentriacontanol on Picrotoxin induced convulsions in rats.

Table 4: Estimation of extracts and the isolated compounds on Brain GABA levels in Picrotoxin induced convulsions in rats.

Treatment	Dose (mg/kg)	Concentration of GABA (µg/g of wet tissue)
1% CMC	-	28.24 ± 1.61
Phenobarbitone	40	76.99 ± 1.44***
CHVA	150	28.75 ± 1.89ns
CHVA	300	32.04 ± 1.74ns
MHVA	150	62.37 ± 2.24**
MHVA	300	70.17 ± 2.42**
Syringaresinol	10	32.37 ± 2.24**
Syringaresinol	20	40.17 ± 2.42**

Values are mean ± S.E.M, n=6, ns-not significant, **p < 0.01, ***p < 0.001 vs control - One way ANOVA followed by Dunnett's test

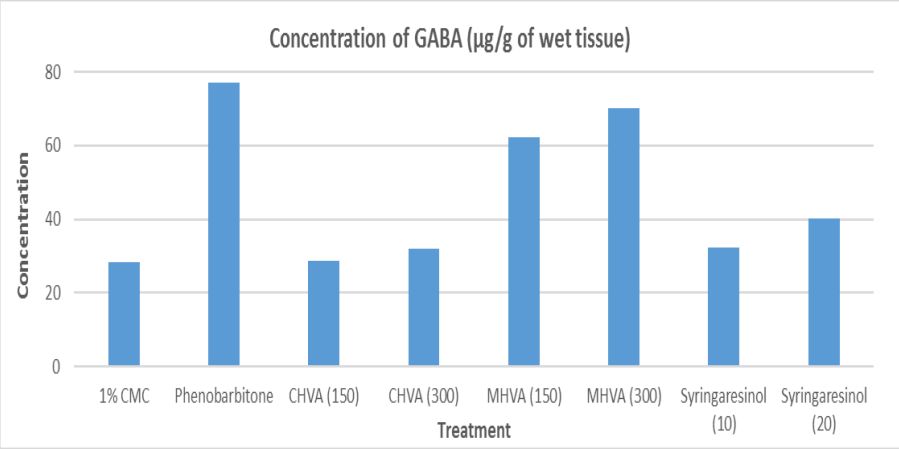


Fig. 4. Estimation of extracts and the isolated compounds on Brain GABA levels in Picrotoxin induced convulsions in rats.

Table 5: Effect of Syringaresinol and Hentriacontanol on NMDA induced seizures in mice.

Treatment	Dose (mg/kg)	Onset of turning behavior (within 30 min)	% Protection
Control (Vehicle)	-	7.35 ± 1.25	0
Dizocilpine hydrogen maleate	0.05	34.5 ± 2.5***	100
Syringaresinol	10	20.20 ± 2.20**	100
Syringaresinol	20	22.10 ± 1.25**	100
Hentriacontanol	10	6.75 ± 3.92ns	0
Hentriacontanol	20	8.55 ± 3.12 ns	0

Values are mean ± S.E.M, n=6, ns-not significant, **p < 0.01, ***p < 0.001 vs control - One way ANOVA followed by Dunnett's test

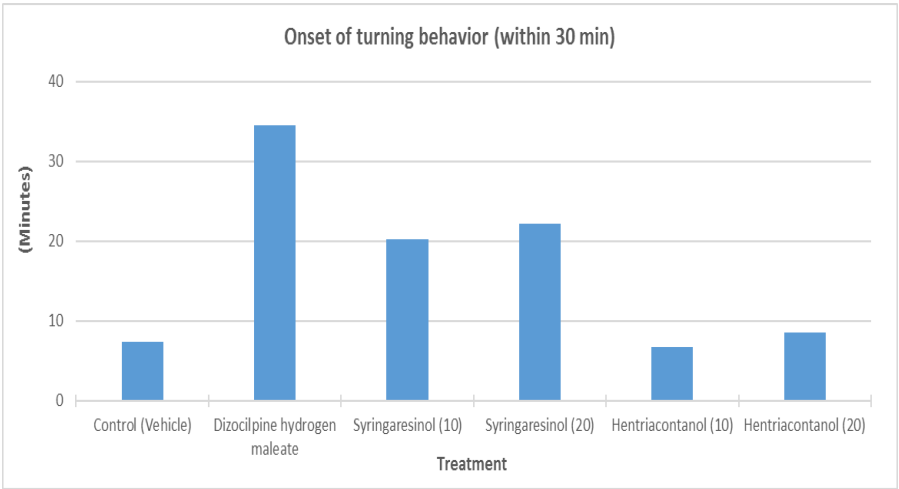


Fig. 5. Effect of Syringaresinol and Hentriacontanol on NMDA induced seizures in mice.

3.3. Isolation of Hentriacontanol and Syringaresinol from methanolic fraction of Viscum articulatum extract.

The two isolated bioactive compounds were simple in structure and were characterized using FTIR, LCMS, ^{1}H NMR and ^{13}C NMR. Compound 1 was obtained as a white semi solid powder. Comparison of its physical and spectral data with the published values confirmed the identity of the compound as Hentriacontanol, an aliphatic alcohol. Compound 2 was obtained as a pale yellow powder and identified as Syringaresinol, a lignan, as the spectral data was in agreement with the literature. The spectral data of the two compounds is as follows

Compound 1

Mass: EI-MS m/z 452.85g/mol

IR: 3344.05 (OH), 3.13.49, 2941.18, 2827.55, 1448.49, 1216.07, 1024.96, 756.38 (CH_2), 663.34

Proton ^{1}H NMR ($CDCl_3$): 0.96 (H-CH_3), 1.29(H) 1.15 1.48 (54H),1.23 (quint), 3.53 (t2H, -CH_2 -OH), 1.73(2H) 1.23 (quint,) 1.23 (2H,quint)

^{13}C NMR (CDCl$_3$): 14.06 (CH_3), 23.06 (CH_2), 26.83 (CH_2), 30.65 (CH_2), 31.89 (CH_2), 33.05 (CH_2), 63.07 (CH_2)

Compound 2

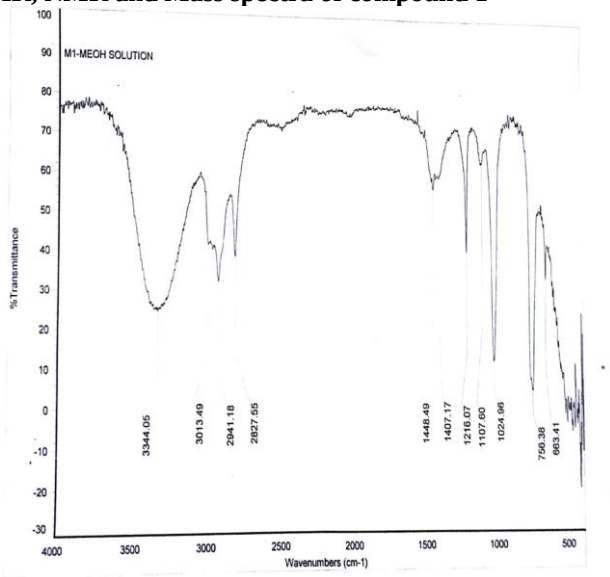
Mass: EI-MS m/z 418M+

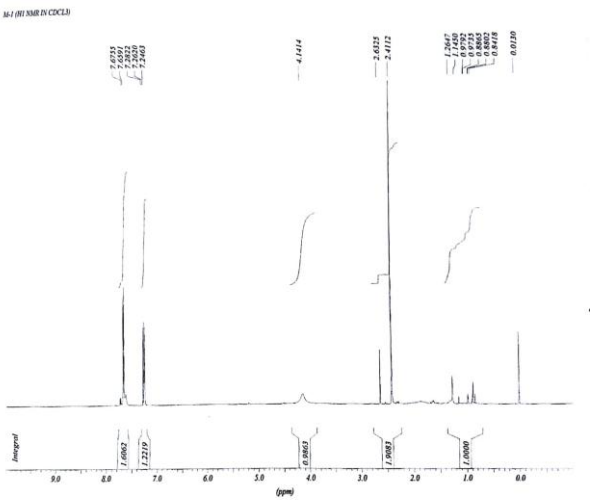
IR: 3409 (OH), 1624 (Phenyl Group), 1537 and 1524 (Phenyl Group), 1223 (C-O-C), 1050 (C-O-C), **^{13}C NMR (CDCl$_3$)**: 150 (C3' 5', 3", 5"), 134.6 (C-1'), 132.1 (C-4"), 102.7 (C-2, 6', 2", 6"), 86 (C-2, 6'), 72.2 (C-4, 8'), 55.8 (C-3', 3", 5" OCH), 54.6 (C-1, 5')

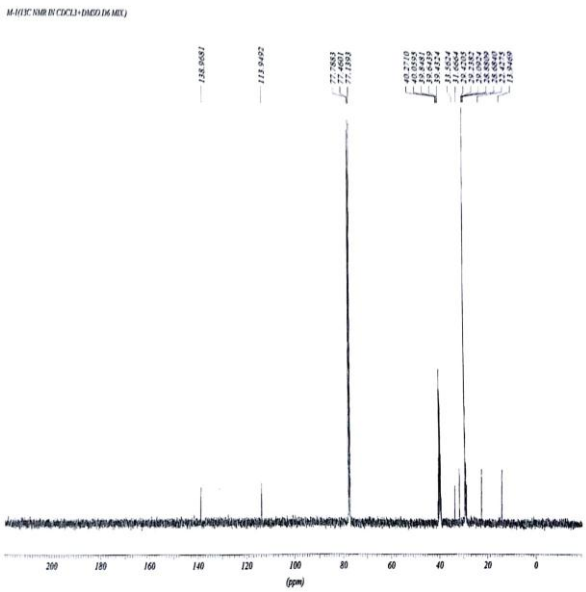
^{1}H NMR, (CDCl$_3$): 6.88 (4H, H_2', 6', 2", 6"), 5.52 (2H, C4, 4" OH), 4.52 (2H, H_2, 6'), 4.14 (2H, MH-4a), 3.85 (2H, M, H-4b, 8b), 3.81 (12H, S, C3', 5', 3", 5"-OCH_3), 3.23 (2H, M, H-1, 5)

Structure of isolated Compound 1-Hentriacontanol (H_3C-$(CH_2)29$-CH_2-OH)

IR, NMR and Mass spectra of compound 1







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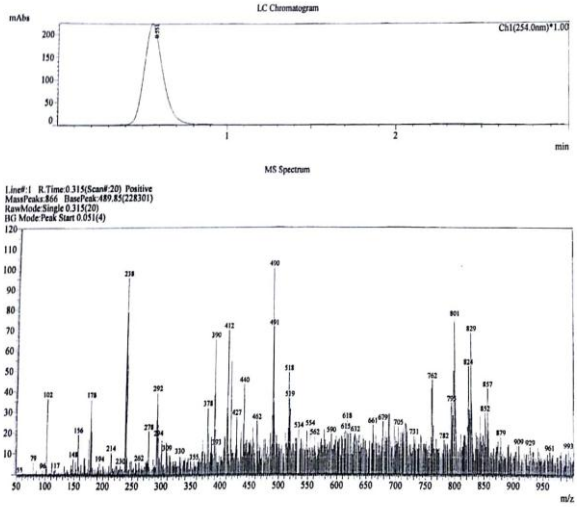
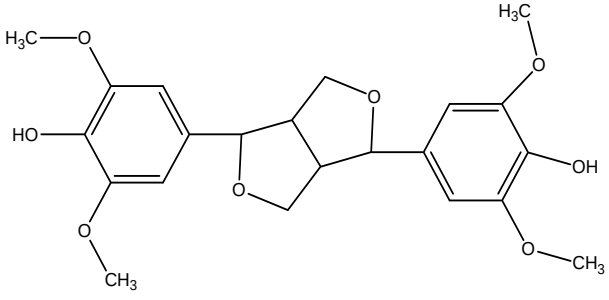
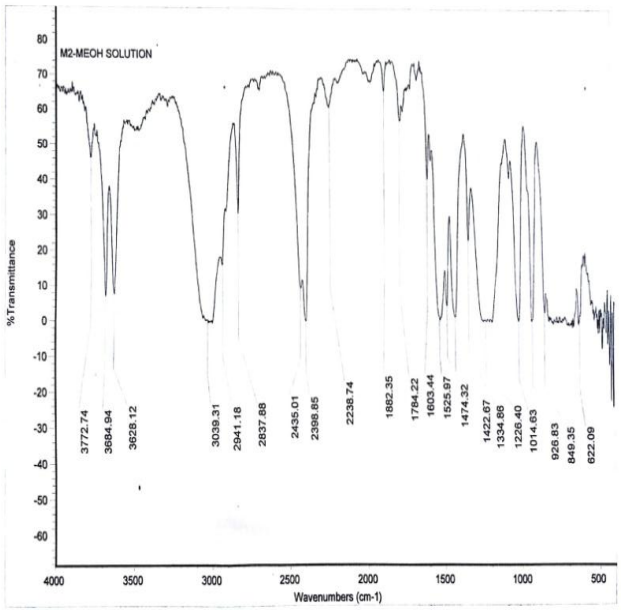
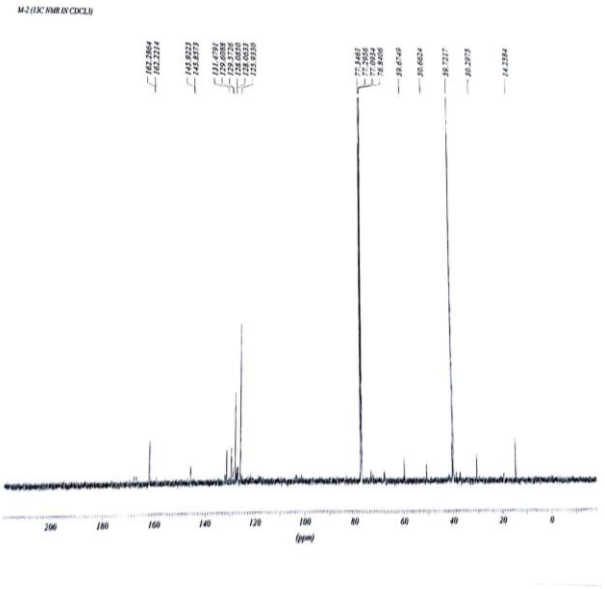


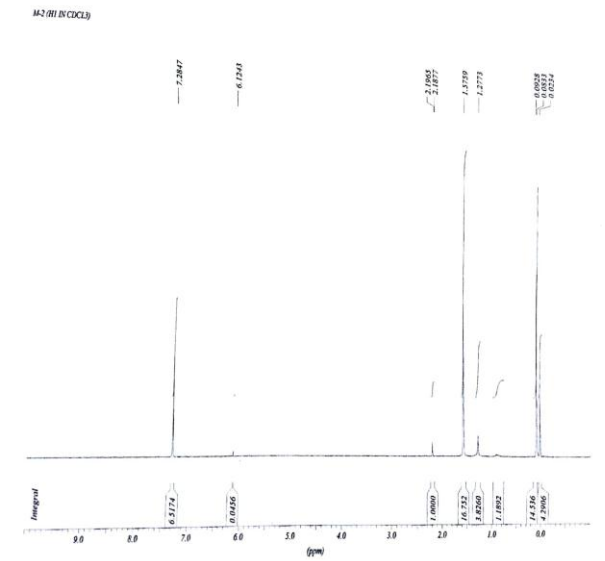
Structure of isolated Compound 2 – Syringaresinol



IR, NMR and Mass spectra of Compound 2







4. DISCUSSION

The conventional drugs used for different types of epilepsy are associated with many side effects such as neurotoxic effects, cognitive deficits and teratogenic effects, which decrease their clinical utility.[10] Recently, the search for novel natural compound from medicinal plants for various neurological disorders has progressed significantly owing to their less serious side effects and better tolerability.[11] Anticonvulsant studies with different extracts of *Viscum articulatum* and its isolated compound, Syringaresinol showed a significant protection in picrotoxin induced convulsion model in a dose dependent manner. GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion conductance through opening of the chloride ion channel.[12]

The findings of the present study, therefore, tend to suggest that the methanolic and the chloroform fractions of the extracts and the isolated bioactive compound from the methanolic extract inhibited Picrotoxin induced seizures possibly by interfering with GABA ergic neurotransmission. NMDA induced seizures in rats and mice have been studied as a model of refractory seizures and are inhibited by NMDA receptor antagonists like Dizocilpine hydrogen maleate.[13] In NMDA induced turning behaviour in mice the methanolic extract and the isolated compound Syringaresinol, protected the mice from turning behaviour in a dose dependent manner in comparison to the solvent control. However, the chloroform extract failed to protect the animals against NMDA induced seizures in mice at two different doses. The suppression of turning behaviour produced by the methanolic fraction of *Viscum articulatum* and the isolated compound from the same fraction suggests that the test samples might be inhibitory against glutaminergic excitatory responses at the NMDA receptor. However, Hentriacontanol did not show any significant suppression of turning behaviour indicating no antiepileptic activity.

5. CONCLUSION

The methanolic fractions of *Viscum articulatum* and one of the isolated compounds from the same fraction, Syringaresinol exhibited anticonvulsant potential in two models of epilepsy probably by a neuromodulatory effect. There are numerous molecular mechanisms through which drugs can block seizure spread and elevate seizure threshold. Hence it could be postulated that the isolated compound exerts its antiepileptic effect effects through multiple mechanisms involving GABAergic transmission. This finding suggests that the extract could be a potential natural source of anticonvulsant and could have a greater importance as therapeutic agent.

ACKNOWLEDGEMENT

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