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### Research Article

## Anticonvulsant Activity of *Helianthus tuberosus* Against Maximal Electroshock Induced Convulsions in Rats

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### ABSTRACT

Epilepsy is an episodic brain dysfunction featured by recurring erratic spontaneous seizures followed by cognitive, social, neurobiological, and psychological consequences. Conventional anti-epileptic drugs are associated with several untoward effects, and hence long-term treatment compliance is a major problem in the management of epilepsy. Herbal drugs have shown promising efficacy as potent anticonvulsants in the past few years. In light of this, the anticonvulsant effect of alcoholic extract of leaves of *Helianthus tuberosus* (AHT) against maximal electroshock (MES) induced convulsions was investigated. In the present investigation, an indigenous plant, *H. tuberosus* was studied for its protective effect against maximal electroshock (MES) induced convulsions in Wistar albino rats. The rats were pre-treated with different doses (100, 200, 400 mg/kg) of alcoholic extract of leaves of *H. tuberosus* for 14 days, and then, they were subjected to maximal electroshock seizures (40 mA for 0.2 seconds) treatment. Alcoholic extract of leaves of *H. tuberosus* at the dose of 400 mg/kg significantly reduced the duration of hind limb extension and the protection of rats against maximal electroshock-induced seizures. The reference standards phenytoin (20 mg/kg) provided complete protection. Thus, the present study revealed an anticonvulsant effect of *H. tuberosus* against maximal electroshock-induced convulsions in rats.

### INTRODUCTION

Epilepsy is a chronic disorder that causes unprovoked, recurrent seizures. A seizure is a sudden rush of electrical activity in the brain.<sup>[1]</sup> It is usually caused by abnormal electrical discharge of neurons in the brain due to several etiological factors such as cerebral damage, neurocysticercosis, traumas, congenital cortical abnormality, cerebral tumour or infections, and an imbalance between excitatory and inhibitory neurotransmitters of brain.<sup>[2]</sup> More than 80 million people have epilepsy globally, with 80% of them being from developing countries. The incidence of epilepsy is 40–70 per 100000 per year in developed countries; it is much higher in developing countries. Its prevalence rate ranges from 5 to 10 per 1000 population affecting all the age groups, especially young people in first two decades of life and elderly with higher chances of mortality.<sup>[3,4]</sup> Epileptic seizures can be treated with several categories of

anti-epileptic drugs such as barbiturates, benzodiazepines, phenytoin, ethosuccinimide, carbamazepine, gabapentin, levetiracetam etc with more than one mechanism of action.<sup>[5]</sup> These anti-epileptic drugs are associated with a wide range of dose-dependent side effects, chronic toxicity, and teratogenicity. More than 35% of the patients continue to suffer from epileptic seizures despite being on anti-epileptic drugs, emphasizing the need for alternative medicines devoid of such adverse effects.<sup>[6]</sup> Medicinal plants have been frequently used to treat epilepsy in traditional medicine, wherein they have exhibited their efficacy as promising anticonvulsant medicines and have been put forward as invaluable sources of new anti-epileptic drugs.<sup>[7]</sup> Hence a need arises for novel anticonvulsant drugs with improved efficacy and reduced adverse effects and drug interactions, unlike the conventional anti-epileptic drugs available.<sup>[8]</sup> *H. tuberosus* L., is one of such herbal medicines can be effective against epilepsy.<sup>[9]</sup> *H. tuberosus*, commonly known

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as Jerusalem artichoke, is a traditional medicinal plant belonging to the sunflower plant family. Its therapeutic potential has been well documented in traditional, complementary, and alternative medicine for more than a century. Several pharmacological investigations have revealed that *H. tuberosus* possesses anticancer, antioxidant, antifungal, anti-diabetic,  $\alpha$ -Glucosidase inhibitory properties and is used as a functional food.<sup>[10]</sup> To the best of our knowledge *H. tuberosus*, has been traditionally claimed to possess anticonvulsant and nervine tonic properties but hasn't yet been explored scientifically.<sup>[7,9]</sup> In light of this, the investigation of the anticonvulsant effect of alcoholic extract of leaves of *H. tuberosus* (AHT) against maximal electroshock (MES) induced convulsions was carried out.

## MATERIAL AND METHODS

### Plant Material Collection and Authentication

Fresh leaves of *H. tuberosus* were collected from the area of Marathwada region of Maharashtra, India. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India, Pune, India. (Voucher specimen no. BSI/WC/Tech./2020/225). The plant materials were shade dried until all the water molecules evaporated, and plants became well dried for grinding. After drying, the plant materials were ground well using a mechanical blender into a fine powder and transferred into airtight containers with proper labeling for future use.

### Preparation of Extract

*H. tuberosus* leaves powder was subjected to extraction by using different types of solvents, i.e., pet. Ether, chloroform, ethanol, and methanol by continuous soxhlet extraction method. A rotary vacuum evaporator removed the solvents; the remaining mass of extract was concentrated and dried.<sup>[10,11]</sup> The extracts were stored in a desiccator for further phytochemical screening. Alcoholic extract of leaves of *H. tuberosus* was used for further study.

### Preliminary Phytochemical Screening

The alcoholic extract of leaves of *H. tuberosus* was analyzed for phytochemical constituents such as terpenoids, alkaloids, quinones, flavonoids, saponins, steroids, and phenolic compounds using the standard qualitative phytochemical methods.<sup>[12,13]</sup>

### High-Performance Thin Layer Chromatography (HPTLC) Studies of Extract Instrumentation

HP-TLC system of CAMAG, Muttenz, Switzerland, Anchrom Enterprises (I) Pvt. Ltd, Mumbai, consisting of sample applicator (Linomat 5), Twin trough chamber with lid {10×10 cm, CAMAG, Muttenz, Switzerland}, UV cabinet (Aetron, Mumbai) with dual-wavelength (254/366 nm) and the HPTLC photo-documentation (Aetron, Mumbai) was used for study.<sup>[14]</sup> FT-IR, NMR was recorded at the Department of Chemistry, North Maharashtra University,

Jalgaon LC-MS was carried out at Venture Centre, Pune. IR spectra were recorded using KBr on "JASCO FT-IR 460 plus" by DRIFT method. <sup>1</sup>H-NMR spectra was recorded in CDCl<sub>3</sub> solution on "FTNMR VARIAN MERCURY YH-300" using tetramethyl silane (TMS) as internal standard. Purity checking and Mass Spectra recording was carried by liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) with accurate mass measurements up to four decimals. It was recorded on Agilent LC-MS Q-TOF (6200 series TOF/6500 series) (5301 Stevens Creek Blvd, Santa Clara, CA 95051, United States) equipped with a dual AJS ESI with improved sensitivity [AJS-ESI: Agilent Jet Stream Electrospray Ionizer] and Q-TOF B.05.01 software version.

### Chromatographic Conditions

The sample was spotted in the form of bands of the width of 6 mm with a 100  $\mu$ L sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminum plate 60 F<sub>254</sub> (5 cm × 10 cm) with 250  $\mu$ m thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/second were employed.

The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Ethyl Acetate: Methanol (8: 2 v/v) as mobile phase. The optimized chamber saturation time for the mobile phase was 15 minutes. The length of the chromatogram run was 8 cm and the development time was approximately 15 minutes. TLC plates were dried in a current of air with the help of a hair drier.<sup>[15]</sup>

### Sample Preparation

10 mg of alcoholic extract of leaves of *H. tuberosus* was dissolved in 100 mL of methanol. 10  $\mu$ L volume of clear supernatant sample was applied on the TLC plate.<sup>[16]</sup>

### Calculation of R<sub>f</sub> Values

Plate was observed in the daylight, under UV light (254 and 366 nm). The following formula calculated retention factor (R<sub>f</sub>).<sup>[17]</sup>

$$R_f = \frac{A}{B}$$

A = distance between the point of application and central point of the spot of material being examined.

B = distance between the point of application and the mobile phase front.

### Drugs and Chemicals

All the chemicals and drugs were purchased from standard vendors from the local market.

### Animals

Swiss male albino mice (18–22 g) and Wistar albino rats of either sex (150–200 g) were used. They were maintained at 25 ± 2 °C and relative humidity of 45 to 55% and under



standard environmental conditions (12 hours light: 12 hours dark cycle). The animals had free access to food. All the experiments were carried out between 12:00–16:00 hours. The animals were shifted from animal house to the laboratory one hour before the start of the experiment. The respective apparatus was cleaned with damp cloth wherever necessary to avoid possible bias due to other trials left by previous animals.

### Ethical Clearance

Institutional Animal Ethical of D. Y. Patil College of Pharmacy, Akurdi, Pune approved the protocol (Approval No.-DYPCOP/IAEC/2021/05).

### Preliminary Acute Toxicity Test

Healthy adult male Swiss albino mice (18–22 g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic cooperation and development (OECD-2000). The mice were observed continuously for 2 hours for behavioral and autonomic profiles and for any sign of toxicity or mortality up to seven days.<sup>[18]</sup>

### General Pharmacological Observation

Behavioral effects of AHT (100, 200 and 400 mg/kg) were assessed by the method described by Irwin *et al.* (1968). The mice were divided into six groups (n = 6) and treated with AHT extracts at 30, 100 and 300 mg/kg doses. The animals were then placed in an observation cage and observed after 30 minutes of administration up to 2 hours for behavioral changes. The observation parameters consisted of body position, locomotion, rearing, respiration, righting reflex, and lacrimation. The observation parameters consisted of body position, alertness, reactivity to touch stimuli, righting reflex and lacrimation.<sup>[19]</sup>

### Evaluation of Anticonvulsant Activity using MES induced Convulsions

48 rats (220–250 gm) of either sex were randomly divided in 08 groups, each consisting of 06 rats, and were treated with respective doses of AHT for 14 days. On 14<sup>th</sup> day, one hour after administering the respective treatment, all rats were subjected to maximal electroshock shock (40 mA for 0.2 seconds) using an electro-convulsimeter. The duration of hind limb extension and percentage of rats protected (i.e., abolishment of hind limb extension before 10 seconds) was recorded and compared with control rats. Phenytoin (20 mg/kg p.o.) was used as a reference standard.<sup>[20,21]</sup>

### Statistical Analysis

The results were reported as mean  $\pm$  SEM. Differences between group means were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test to assess the significance of differences between individual groups.  $p > 0.05$  was considered insignificant.

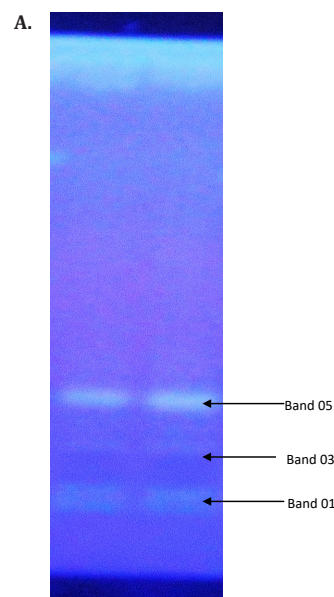
## RESULTS

### Preliminary Phytochemical Screening:

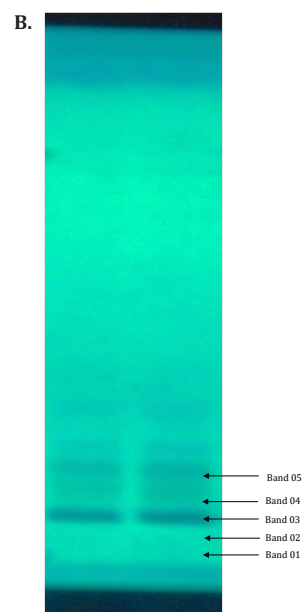
The results of preliminary phytochemical screening hydro alcoholic extract of seeds of *H. tuberosus* revealed the presence of saponins, alkaloids, tannins, steroids, glycosides.

### HP-TLC Studies of Extract

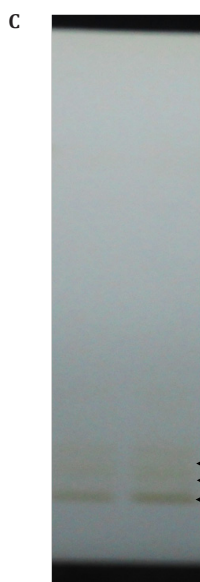
The Band 5 at Rf Value 0.37 was scratched and subjected to structure elucidation (Figs. 1-7, Table 1 and 2).



**Fig. 1:** Alcoholic extract of leaves of *H. tuberosus* at 366 nm, volume applied 10 mL



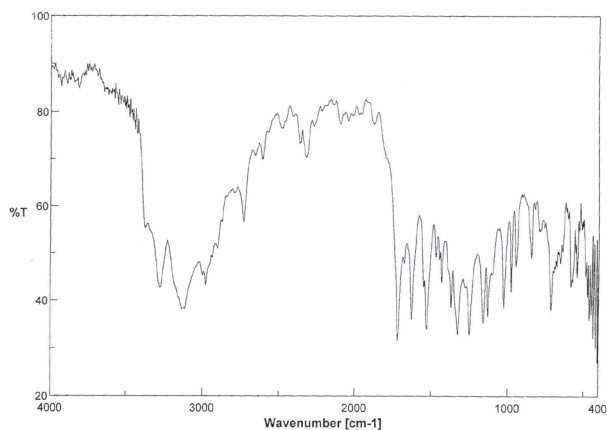
**Fig. 2:** Alcoholic extract of leaves of *H. tuberosus* at 254 nm, volume applied 10 mL. The Band 5 at Rf Value 0.37 was scratched and subjected to structure elucidation



**Fig. 3:** Alcoholic extract of leaves of *H. tuberosus* at visible light, volume applied 10 mL.

The spot at Rf Value 0.37 was scratched, extracted with methanol and evaporated to dryness (The process required semi preparative TLC to achieve a sufficient amount) for further analysis by IR, NMR and Mass Spectrometry

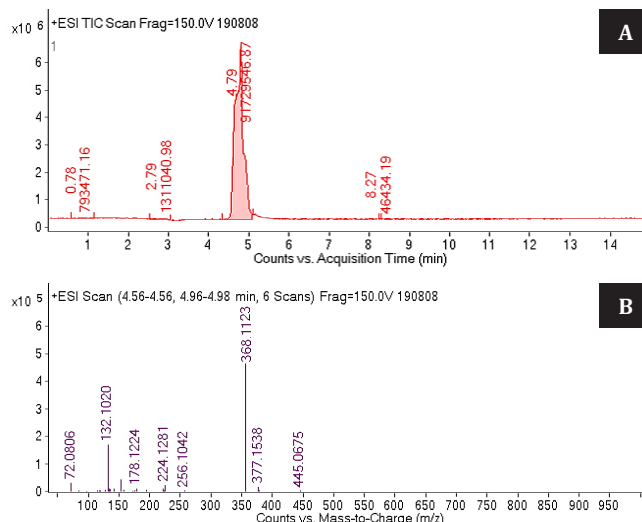
#### Spot at Rf Value – 0.37



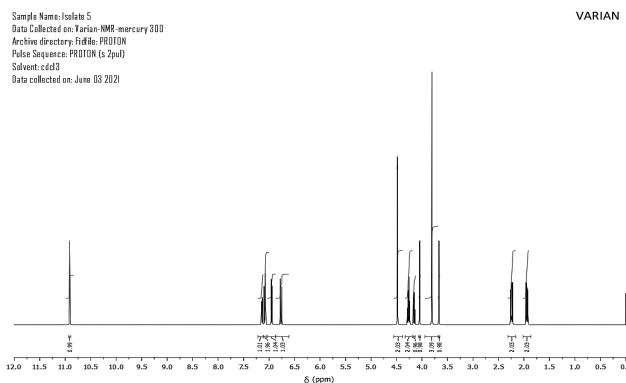
**Fig. 4:** FT-IR Spectrum of Spot at Rf – 0.37

**Table 1:** FT-IR spectral data for compound at Rf-0.37

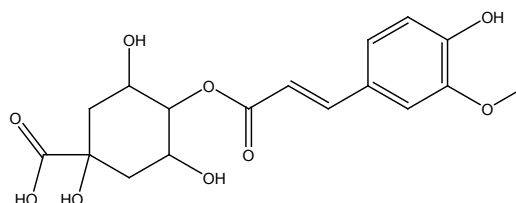
S. No.	Part of molecule	Vibration	General range (cm <sup>-1</sup> )	Frequency (cm <sup>-1</sup> )
1	Ar Ring	a) C=C stretch b) C-H stretch c) C-H bend d) Overtone	1500–1650 3000–3100 740–762 1700–2000	1528 3081 713 1700–2000
2	-COOH	O-H stretch	2500–3500	2600–3400
3	-COOH-	C=O stretch	1650–1750	1714
4	CH <sub>2</sub> -(Aliphatic)	a) C-H stretch b) C-H bend	2850–3000 1300–1470	2978 1430
5	-C=C- bond	a) C=C stretch	1600–1680	1612



**Fig. 5:** LC-MS A) chromatograph and B) Spectrum of compound at Rf-0.37



**Fig. 6:** NMR Spectrum of compound at Rf-0.37



**Fig. 7:** 4-((E)-3-(4-hydroxy-3-methoxyphenyl) acryloyloxy)-1,3,5-trihydroxycyclohexanecarboxylic acid

Probable Structure of the Isolated Compound is as in Fig. 7.

#### Preliminary Acute Toxicity Test

Oral administration of alcoholic extract of leaves of *H. tuberosus* (AHT) did not produce any toxic effect in mice, and no mortality was observed up to 2000 mg/kg, thus being safe.

#### General Pharmacological Observation

Mice orally treated with the AHT extract (100, 200 and 400 mg/kg) and subjected to the general observations did not differ in their behavior and other parameters determined during the observation periods. They were



**Table 2:** Interpretation of NMR spectrum

Sr. No.	$\delta$	No of Protons	Multiplicity	Type
1	1.944	2 H	D	2 CH <sub>2</sub> Protons of the cyclohexane ring
2	2.242	2 H	D	2 CH <sub>2</sub> Protons of the cyclohexane ring
3	3.802	3 H	S	3 Protons of CH <sub>3</sub>
3	3.673, 4.086, 4.458	4 H	S	O-H Protons
4	4.152 and 4.264	3 H	M	3 protons (1 H on each carbon) of cyclohexane ring
5	6.672 - 7.046	5 H	M	Protons of aromatic ring and attached to C=C part
6	10.858	1 H	S	COOH proton

**Table 3:** Effect of alcoholic extract of *H. tuberosus* (AHT) leaves against MES induced convulsions

Treatment	Duration of hind limb extension (Mean $\pm$ SEM)	Mice protected/ Mice used	% Protection
Vehicle	20.59 $\pm$ 0.65	0/6	0
AHT100	20.36 $\pm$ 0.55	0/6	0
AHT200	19.70 $\pm$ 0.72	6/6	0
AHT400	17.51 $\pm$ 0.52*	2/6	33.33
Phenytoin 20	8.64 $\pm$ 0.40**	6/6	100

alert with normal grooming, touch response and pain response. Alertness, limb tone, and grip strength were normal, and the animals did not show staggering gait or contractions.

### Evaluation of anticonvulsant activity using MES induced convulsions

The AHT 400 mg/kg has shown a significant reduction in the duration of hind limb extension compared to vehicle-treated control rats. The reference standard phenytoin was most effective in this regard. However, as far as the prescribed duration of hind limb extensor phase to label as protection is concerned, then AHT 400 mg/kg and phenytoin 20 mg/kg are effective (Table 3).

## DISCUSSION

Epilepsy is characterized by recurrent seizures, defined as the manifestation of disordered and paroxysmal neuronal discharges in the brain. There are several conventional anticonvulsant drugs available for the control and treatment of epilepsy in epileptic patients.<sup>[22,23]</sup> However, most of these synthetic drugs have been documented to possess many toxic effects.<sup>[24]</sup> Therefore, it is essential to approach the development of safe, effective, and cheap anticonvulsant agents from plants and other natural resources. Research in the context of applying traditional and herbal medicines to treat several ailments, including epilepsy, has been increasing in the past few decades.<sup>[25,26]</sup> In light of this, the preclinical screening of anticonvulsant activity of AHT extract was carried out in Wistar albino rats against MES-induced seizures.

The phytoconstituents in herbal medicines play an important role in the execution of their pharmacological effects.<sup>[13]</sup> Hence the AHT extract was subjected to preliminary phytochemical analysis, and the results revealed the presence of the various phytoconstituents such as saponins, alkaloids, tannins, steroids, glycosides. The preliminary phytochemical analysis does not give us an idea about the specific constituent for the anticonvulsant activity. Hence taking into consideration the revealed preliminary phytoconstituents, the extract was further subjected to HPTLC analysis.<sup>[15-17]</sup> The results of the HPTLC analysis revealed the presence of phytoconstituents, namely 4-((E)-3-(4-hydroxy-3-methoxyphenyl)acryloyloxy)-1,3,5-trihydroxycyclohexanecarboxylic acid.

Before evaluating the pharmacological activity of any drug, it has to be screened for its toxicity. In this context, the extract was subjected to acute oral toxicity testing.<sup>[18,19]</sup> The results revealed that the extract was safe up to 2000 mg/kg dose without any difference in their behavior and other parameters. The anticonvulsant activity of the extract was determined by using MES induced seizure model.

The MES seizure test is a widely applicable preclinical model of epilepsy because drugs that are effective against tonic hind limb extension induced by electroshock generally have proven to be effective against partial and tonic-clonic seizures in human beings.<sup>[27]</sup> In the present study, AHT extract significantly ( $P < 0.05$ ) reduced the duration of hind limb extension and showed potential anticonvulsant activity at a dose of 400 mg/kg. The results were following the previous studies carried out.<sup>[4,5]</sup> It has often been stated that an anti-epileptic drug that blocks maximal electroshock-induced tonic extension acts by blocking the spread of seizure, which may be attributed to the anticonvulsant mechanism of the extract.<sup>[28]</sup> Also, the presence of 4-((E)-3-(4-hydroxy-3-methoxyphenyl)acryloyloxy)-1,3,5-trihydroxycyclohexanecarboxylic acid phytoconstituents may be attributed to the anticonvulsant activity of the extract.

## CONCLUSION

In the present investigation, AHT extract showed significant prevention of MES-induced seizures, suggesting the putative

mechanisms of action, which might be due to different phytochemicals in these extracts interacting simultaneously. The exact mechanisms and their contributions to the anticonvulsant property of these extracts will be better understood after detailed phytochemical and biochemical analysis, which is underway.

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