

Protective effect of methanol leaf extract of *Sphaeranthus indicus* L. on an experimental model of epilepsy

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The present study was designed to investigate the protective effect of methanol extract of *S. indicus* L. leaves (SIME) on seizures using pentylenetetrazole (PTZ) and maximal electroshock (MES) models in mice. The methanol extract was obtained by maceration process of 500g leaves with methanol at room temperature for 72 hours. In MES model, the prevention of hind limb extension was considered as a protective action. In the PTZ induced seizure model, the presence or absence of clonic convulsion was considered as the endpoint. The extraction obtained were evaluated for antioxidant activity using free radical scavenging activity. The extract at a dose of 300 mg/kg b.w, p.o, resulted significant ($P < 0.0001$) antiepileptic activity by decreasing the duration of hind limb extension phase in MES model and delaying the onset of action in PTZ model with the control and other test groups. The SIME scavenged DPPH radical, by showing $IC_{50} = 25.68 \mu\text{g/mL}$ and exhibit significant result ($P < 0.0001$) at a dose of 300 mg/kg. The biochemical evaluation of SLME showed a significant increase (300 mg/kg) in the amount of GABA level brain against PTZ induced seizures in mice. The methanol extract of *S. indicus* L. leaves has antiepileptic activity, which might be because of flavonoids.

Keywords: Epilepsy, Flavonoids, GABA, Leaves, MES, Methanol extract, PTZ.

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Introduction

Epilepsy is one of the most common and well known serious neurological conditions¹. According to a recent survey, it has been found that around 70 million people to be diagnosed to have epilepsy worldwide and approximately about 90% are found in developing countries. Another data reveals that more than 12 million persons have been diagnosed with epilepsy in India, and may contribute to nearly about one-sixth of the global burden with an estimation of 1% considered as the prevalence of an epilepsy².

The aetiology of seizures can be considered either genetic or desultory, due to some severe damage or alterations in the cerebral region which may be connected to abnormalities in congenital cortical, traumas, shocks or neurocysticercosis. The depolarization disorders are the main cause for epilepsy, which are produced within the neural membrane, neuronal morphology and ionic medium. Such type of alterations can produce an imbalance in the inhibitory neurotransmitters such as inhibitory neurotransmissions i.e. gamma aminobutyric acid and

excitatory amino acids i.e. glutamate or aspartate and excitatory neurotransmitters.

Some of the other factors which are responsible for causing epilepsy is the oxidative stress which can damage the brain by producing free radicals. The production of free radicals is due to increase in the level of oxygen-rich (aerobic) metabolism, blood perfusion and an increase in the concentration of polyunsaturated fatty acids respectively, compared with different organs, having reduced antioxidant activity³.

In the present scenario, the herbal medications are gaining popularity because of their fewer side effects with better efficacy. Approximately 30% of the patients continue to have AEDs (antiepileptic drugs), which are mainly associated with side effects, chronic toxicity, drug interactions, drug-related toxicity. Therefore, to get overcome from all these side effects and toxicity of consuming AEDs, natural products are in their demandable concept with novel structures, and some advanced synergetic effects⁴.

Sphaeranthus indicus L., commonly known as *Mundi* (Hindi) and East Indian globe-thistle (English), belongs to the family Asteraceae or Compositae. It is

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a native of Chhindwara District, M.P. (India) and commonly found in the moist damp places of tropical zones of Garhwal Himalaya⁵⁻⁷. The herb is bitter and hot with a sharp sweet taste, used as a laxative, tonic, alternative; used in asthma, hemicranias, urinary discharges, dysentery, indigestion⁸.

However, no research has been carried out on the anticonvulsant activity along with the biochemical evaluation of *S. indicus* leaves. The objective of the present study was undertaken to assess the evaluation of the anticonvulsant activity of *S. indicus* leaves methanol extract against seizures using PTZ and MES models and also evaluating the oxidative stress by estimating the GABA level in mice brain.

Materials and Methods

Plant collection

The fresh leaves of *S. indicus* were collected in the month of July 2007 from the local areas of Hoshangabad, Madhya Pradesh (India). They were authenticated by Dr. R. S. Goudar, Department of Botany, R. L. S Institute, Belgaum. The plant specimen was deposited at R. L. S Institute with voucher number (RLI/Bot/07) for further reference.

Preparation of the extract

The fresh leaves of *S. indicus* were shade dried and reduced to a fine powder (#40 mesh size). Around 500 g of powdered plant material was subjected to maceration process with methanol at room temperature for 72 hours and was filtered. The extract was then concentrated and dried to yield 3.86% w/w.

Preliminary phytochemical evaluations

The extracts were subjected to preliminary phytochemical screening for various chemical constituents as per literature^{9,10}.

Pharmacological investigations

Animals

Albino mice of either sex weighing 22-25 g were obtained from K. L. E. S's College of Pharmacy, Belgaum. Animals were grouped into 6-10 animals per cage at a relative humidity of 45-55% and a temperature of 25±1°C. Animals had free access to the supply of food and water. The experimental protocol was approved (KLE/IEAC/2007/RN-12) by the Institutional Animals Ethical Committee, Belgaum.

Acute toxicity study

Acute toxicity study was assessed in mice by using an acute oral toxic class method of Organization of

Economic Co-operation and Development (OECD), 423 guidelines¹¹.

Drugs used

PTZ, Diazepam tablet I. P (Valium 5, Nicholas Priamal Ltd., India), Phenytoin tablet I.P (Eptoin, Acme formulation Pvt. Ltd.), Ascorbic acid (Sigma Chemical Co), 2,2-diphenyl-1,1-picrylhydrazyl (DPPH).

Evaluation of antioxidant activity

Free radical scavenging using DPPH

The free radical scavenging activity of methanol extract of the plant in different concentrations was carried out by using Brand-Williams *et al.* method¹². Different aliquots of test and standard (Ascorbic acid) were added to 3 mL of 6x10⁻⁵ mol/L of DPPH solution in methanol. The tubes were then shaken vigorously and were stored at room temperature for about 30 minutes in the dark place. The absorbance of the samples and standard were determined at 517nm by spectrophotometry. All readings were measured in triplicate and averaged. The percentage of DPPH scavenging activity was calculated using formula³:

% antioxidant activity =

$$\frac{(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}})}{\text{Absorbance}_{\text{control}}} \times 100$$

Assessment of anticonvulsant activity

Electrically-induced convulsion (MES)

Various phases of convulsion are produced during MES i.e., myoclonic, clonus, hind limb extension and post-convulsive stupor. An increase in the duration of clonus and hind limb extension was considered as a pro-convulsant activity. The prevention of hind limb extension was considered as a protective action of the drug.

Five groups of six mice of either sex were used. Group I served as control and received normal saline. Group II received the standard drug, Phenytoin (40 mg/kg i.p). Group III, IV and V were treated orally with methanol extract of *S. indicus* leaves at a dose of 50, 150, 300 mg/kg respectively. MES was induced in all mice by means of electroconvulsimeter as an external device, of 150 mA electric stimulation for 0.2 seconds was applied using ear clip electrodes after 60 minutes of administration of standard and test drugs respectively. The incidence and duration of extensor tonus were observed and noted. Complete abolition of hind limb tonic extension was considered as 100% protective action of the drug.

The herbal extract of *S. indicus*, which prevent the hind limb extension, was considered to possess the anticonvulsant activity.

Chemically-induced convulsion (PTZ)

Mice of either sex were randomly allotted into five different groups in which Group I served as control and received normal saline. Group II received Diazepam (1 mg/kg, i.p) and served as standard. Group III, IV and V were treated orally with methanol extract of *S. indicus* at a dose of 50, 150 and 200 mg/kg respectively.

Five groups of mice (n=6) were treated with oral administration of a standard drug and herbal extracts and were observed for 45 and 60 minutes, respectively. The animals were observed after 30 minutes of PTZ injections for onset, presence or absence of clonic convulsions. The mortality was also observed and noted¹³.

Biochemical parameter

Estimation of GABA level in mice brain by spectrophotometry

The isolated GABA from mice brain was transferred into homogenization tube containing 5 mL of 0.01 M hydrochloric acid. The homogenate brain was then again transferred to a bottle containing 8 mL of absolute alcohol (ice cold) and allowed to keep for 1 hour at 0 °C. The mixture was centrifuged at 16,000 rpm for 10 minutes. The supernatant was washed with 5 mL of 75% alcohol for three times. 1 mL of water and 2 mL of chloroform were added to the samples which were evaporated to dryness at 70 °C on the water bath. The samples were then centrifuged at 2000 rpm. The upper layer of the samples containing GABA was separated and applied as spot on Whatman filter paper. The ascending technique was developed in which n-butanol, acetic acid and water (25:6:30) was the mobile phase. The paper was air-dried and then sprayed with 0.5% ninhydrin solution prepared in 95% ethanol. The paper was then again allowed for drying at 90 °C for 1 hour. The blue colour spot was developed on paper and cut into a defined section. The cut paper section was heated with 2 mL of ninhydrin solution on a water bath for 5 minutes. Exactly 5 mL of distilled water was added to the solution and kept for 1 hour. About 2 mL of resultant supernatant was decanted and absorbance was carried out by measuring at 570 nm, using spectrophotometry. The GABA was used as a standard to extrapolate the absorbance of the samples³.

Statistical analysis

All data are presented as mean±SEM and analyzed by One-way ANOVA, followed by Dunnett's tests. The measured data were analyzed and expressed as standard error mean. The resultant value with $P < 0.01$ were considered statistically significant.

Results

Preliminary phytochemical evaluation

Preliminary phytochemical screening of extracts revealed the presence of carbohydrates, flavonoids, alkaloids, volatile oil, fats and oils, tannins and phenolic compounds.

Antioxidant activity

DPPH free radical scavenging

The antioxidant activity of four different concentrations was determined and shown in Table 1. The extract was significantly reduced DPPH at a concentration of 80 µg/mL (74.66%). The EC₅₀ of extract against DPPH scavenging free radical activity was found to be 25.68 µg/mL, calculated by using linear regression, $y = 0.4866x + 37.5$ ($R^2 = 0.9437$), as shown in Fig. 1.

Table 1 — Antioxidant activity of methanol extract of *S. indicus* L. Leaves

Concentration (µg/mL)	DPPH Scavenging activity (%)	
	Methanol extract of <i>S. indicus</i> L. leaves	Ascorbic acid
20	44.33±0.67	79.60±0.88
40	61.00±0.57	83.33±0.88
60	67.33±0.33	87.33±0.33
80	74.66±0.67	94.01±0.57

The mean value of n=3; ±SD; Standard deviation

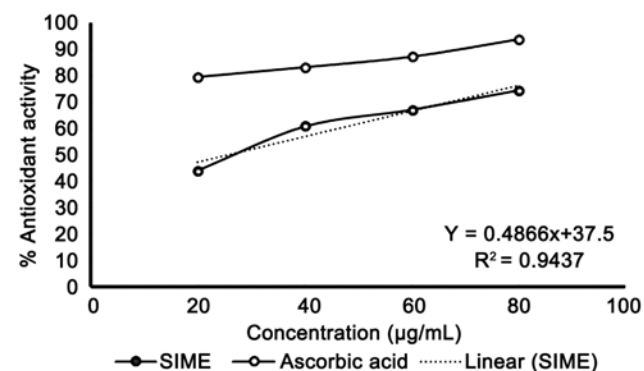


Fig. 1 — DPPH Scavenging free radical activity. SIME= *Sphaeranthus indicus* L. methanol extract, standard drug= Ascorbic acid.

Pharmacological investigations

Acute toxicity study

The extracts of *S. indicus* were found to be safe and there was no mortality up to a dose of 2000 mg/kg, p.o body weight in mice according to the OECD guidelines 423.

Electrically-induced convulsion (MES)

Fig. 2 illustrated the effect of *S. indicus* leaves methanol extract on maximal electroshock-induced convulsion in mice. Statistical analysis measured by One-way ANOVA revealed the significant difference between various phases of convulsion among groups (F (4, 40) = 5308; $P < 0.0001$), time (F (3, 40) = 41897; $P < 0.0001$) and an interaction (F (12, 40) = 1988; $P < 0.0001$) between group and convulsion phases as shown in Table 2. The data resulted from the anticonvulsant effect of methanol extract of *S.indicus* leaves showed decreased the duration of hind limb extension which was most significant ($P < 0.0001$) when compared to control.

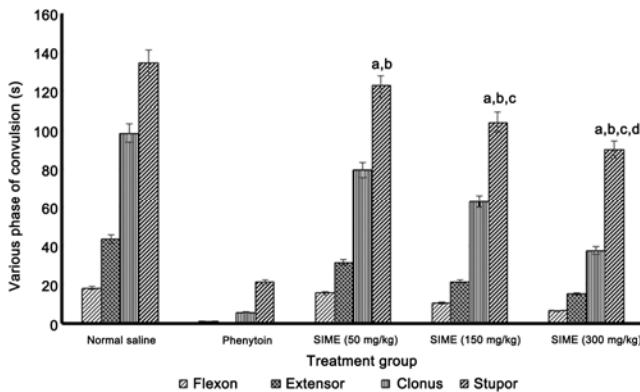


Fig. 2 — Maximal electroshock induced convulsion in mice. All values are mean±SEM (n=6). ^a $P < 0.0001$ compared to negative control (normal saline group), ^b $P < 0.0001$ compared to phenytoin, ^c $P < 0.0001$ compared to SIME (50 mg/kg), ^d $P < 0.0001$ compared to SIME (150 mg/kg) (measured by one-way ANOVA, followed by Dunnett’s multiple comparison test); SIME= *Sphaeranthus indicus* L. methanol extra.

Chemically-induced convulsion (PTZ)

Fig. 3-4 illustrated the effect of *S. indicus* leaves methanol extract on pentylenetetrazole-induced convulsion in mice and the mortality rate of mice during the experiment. The statistical analysis measured by One-way ANOVA revealed the significant difference in the duration of clonic seizures among groups (F (4, 8) = 371015; $P < 0.0001$) and time

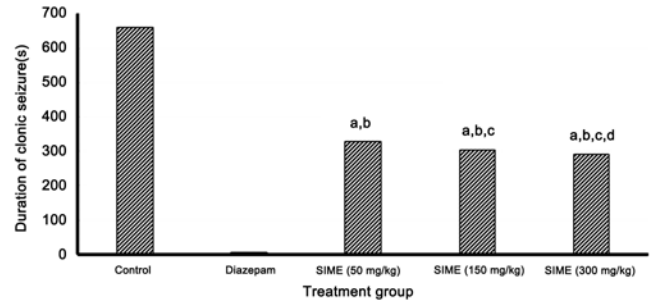


Fig. 3 — Duration of clonic seizure (s) during PTZ induced convulsion in mice. All values are mean±SEM (n=6). ^a $P < 0.0001$ compared to negative control, ^b $P < 0.0001$ compared to Diazepam (standard), ^c $P < 0.0001$ compared to SIME (50 mg/kg), ^d $P < 0.0001$ compared to SIME (150 mg/kg) (measured by one-way ANOVA, followed by Dunnett’s multiple comparison test); SIME= *Sphaeranthus indicus* L. methanol extract.

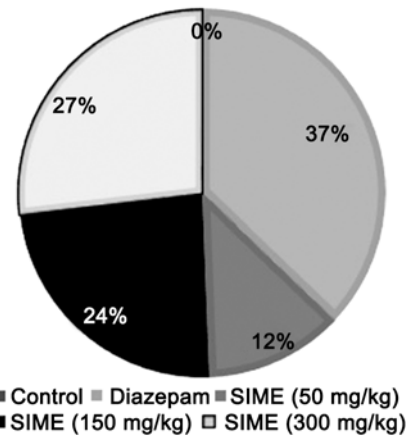


Fig. 4 — Mortality protection % during induced convulsion in mice.

Table 2 — Effect of *S. indicus* L. leaves methanol extract on maximal electroshock-induced in mice

Group	Treatment	Time (s) in various phase of convulsions				Recovery/Death
		Flexion	Extensor	Clonus	Stupor	
1	Normal Saline 1 mL/rat, p.o	18.16±0.22	43.59±0.30	98.63±0.30	134.30±0.91	R
2	Phenytoin 40 mg/kg, i.p	1.33±0.88	0.00±0.00	6.02±0.24	21.15±0.45	R
3	SIME (50 mg/kg)	15.55±0.23	31.50±0.27	79.63±0.32	122.89±0.59	R
4	SIME (150 mg/kg)	10.67±0.22	21.55±0.29	63.13±0.59	104.18±0.24	R
5	SIME (300 mg/kg)	6.63±0.19	15.25±0.30	37.52±0.29	90.04±0.09	R

n= 6; n= number of mice used in experiment (one-way ANOVA, followed by Dunnett’s multiple comparison test). Data represent mean±SEM. SEM= Standard error of mean, SIME= *Sphaeranthus indicus* L. methanol extract.

(F (2, 8) = 0.007371; $P = 0.9927$) in the occurrence of clonic seizures (Table 3).

Methanol extract exhibited significant anticonvulsant activity by delayed the onset, duration and frequency of seizure ($P < 0.0001$) at the dose of 300 mg/kg, p.o compared against negative control (normal saline) and other groups (SIME 50 mg/kg, SIME 150 mg/kg).

Biochemical evaluation in brain tissue

S. indicus L. showed an effective result for the dose of 300 mg/kg in brain tissue, which was similar to the effect with diazepam ($P < 0.01$; Dunnett's Test), as shown in Table 4.

Discussion

The term "Epilepsy" has been recognized by the Ayurveda system of medicine in India for about over twenty centuries¹⁴. Charaka described "Epilepsy is a disease characterized by derangement of the mind and memory. Therefore, victims' experiences disturbances in or loss of consciousness and undergo all kinds of ugly scenes"¹⁵.

The initiation phase of seizures is characterized by two concurrent events either (1) high-frequency bursts of action potentials or (2) by hyper-synchronization in an aggregate of neurons. Due to influx of extracellular calcium (Ca^{2+}), the bursting activity is occurred by

long-lasting depolarization of the neuronal membrane, which may lead to the opening of voltage-dependent sodium (Na^+) channels, the influx of Na^+ and generation of the repetitive action potential. These activities are followed by a hyperpolarization mechanism after which potential is mediated through gamma-aminobutyric acids (GABA) receptors or potassium (K^+) channels. The synchronized bursts from a sufficient number of neurons may lead to the production of spike discharge on the EEG^{16,17}.

The literature survey has revealed the presence of several phytochemical constituents, such as sesquiterpene lactone like 7 α -hydroxyeudesm-4-en-6,12-olide; sesquiterpene acid, flavonoid as 5-hydroxy-7-methoxy-6-C-glycosylflavone, essential oil, alkaloid as sphaeranthine¹⁸. The present work has also shown evidence of the presence of carbohydrates, flavonoids, alkaloids, volatile oil, fats and oils, tannins and phenolic compounds. The anticonvulsant property of the plant was investigated using GABAergic neurotransmission animal model i.e. MES and PTZ-induced convulsions. The mechanism of convulsion action of PTZ seems to be related to the abolition of the inhibitory functions of the GABA neurotransmitter. PTZ has a greater affinity towards the chloride ionophore of the postsynaptic GABA receptor complex, by which it antagonizes GABAergic function¹⁹. The present study showed that the methanol extract of *S. indicus* L. leaves was able to minimize the duration of various phases of seizures and showed significant anticonvulsant activity, using MES and PTZ models by either direct modification on GABA production in the brain. Although the mechanism of action of the extract is not clear, therefore the methanol leaves extract could be useful to treat the convulsions in the future.

S. indicus leaves showed antioxidant activity using DPPH free scavenging activity might be linked to the anticonvulsant effect. The experimental administration of ascorbic acid in animals can reduce the damage in the neurons and also scavenge the production of free radicals which are particularly enhanced during inflammation processes and neurodegenerative disorders³.

Table 3 — Effect of *S. indicus* L. leaves methanol extract on pentylenetetrazole-induced convulsion in mice

Group	Treatment	Duration of clonic seizure (s)	Mortality protection %
1	Normal saline (10 mL/kg)	659.58±0.30	0
2	Diazepam (1 mg/kg)	5.83±0.12	100
3	SIME (50 mg/kg)	328.63±0.31	33
4	SIME (150 mg/kg)	302.38±0.19	64
5	SIME (300 mg/kg)	291.20±0.58	72.33

n= 6; n= number of mice used in the experiment (measured by one way ANOVA, followed by Dunnett's multiple comparison test). Data represent mean±SEM. SEM= Standard error of mean, SLME= *Sphaeranthus indicus* L. methanol extract

Table 4 — Estimation of brain GABA on PTZ- induced seizure in mice

Variable	Groups (n=6)				
	Vehicle	Diazepam	PTZ-SI 50 mg/kg	PTZ-SI 150 mg/kg	PTZ-SI 300 mg/kg
GABA(ng/g of brain tissue)	22.01±1.42	86.00±1.78**	45.12±2.45	69.00±1.12*	77.01±1.5**

All values are mean±SEM (n=6); n=number of mice used. PTZ= pentylenetetrazole; * $P < 0.05$; ** $P < 0.01$; SI=*Sphaeranthus indicus* L.

The anticonvulsant activity of methanol extract of *S. indicus* leaves may be due to the combined effects of flavonoids, carbohydrates, alkaloids, volatile oils, fats and oils, tannins and phenolic compounds. But there is a probability that flavonoids may be most responsible chemical constituents for activity. Because as per literature, it has been found that flavonoids do possess the action on central nervous system²⁰. All these findings suggest that methanol extract of *S. indicus* L. may be beneficial in generalized tonic-clonic and absence seizures. Therefore, it can be concluded that the methanol extract does possess the anticonvulsant activity and antioxidant activity. However, this claim demands thorough investigation in other models for anticonvulsant activity and its specific mode of action.

Conclusion

The present research work has strongly revealed the importance of *Sphaeranthus indicus* L. as an optimistic herbal drug in convulsions and other forms of cognitive impairments. In future, all these interpretations will be considered as an important tool for study many more impacts such as neurocognitive effects of a crude drug

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Conflict of interest

The authors have no conflict of interest.

References

- Nassiri-Asl M, Shariati-Rad S and Zamansoltani F, Anticonvulsant effects of aerial parts of *Passiflora incarnate* extract in mice: Involvement of benzodiazepine and opioid receptors, *BMC Complement Altern Med*, 2007, **7**(1), 26.
- Amudhan S, Gururaj G and Satishchandra P, Epilepsy in India I: Epidemiology and public health, *Ann Indian Acad Neurol*, 2015, **18**(3), 263-277.
- Herrera-Calderon O, Santivi  nez-Acosta R, Pari-Olarte B, Enciso-Roca E, Montes V M C, *et al.*, Anticonvulsant effect of ethanolic extract of *Cyperus articulatus* L. leaves on pentylenetetrazole induced seizure in mice, *J Tradit Complement Med*, 2018, **8**(1), 95-99.
- Gupta Y K and Malhotra J, Antiepileptic drug therapy in the twenty first century, *Indian J Physiol Pharmacol*, 2000, **44**(1), 8-23.
- Arya V S, *Indian Medicinal Plants*, vol 5, (Orient Longman Limited), 1997, 180.
- Bhattacharjee S K, *Handbook of Medicinal Plants*, (Pointer Publisher), 1998, 328.
- Agrawal V S, *Drug Plants of India*, vol 2, (Kalyani Publishers), 1997, 656.
- Kirtikar K R and Basu B D, *Indian Medicinal Plants*, vol 2, (International Book Distributors), 1999, 1347.
- Kokate C K, *Practical Pharmacognosy*, (Vallabh Prakshan), 1986, 135-136.
- Khandelwal K R, *Practical Pharmacognosy*, (Nirali Prakashan), 2002, 157-159.
- OECD/OCDE, OECD Guidelines for the testing of chemicals, revised draft guidelines, Acute Oral Toxicity-Acute Toxic class methods, Revised Document October, 2000, 423.
- Brand-Williams W, Cuvelier M and Berset C, Use of a free radical method to evaluate antioxidant activity, *LWT - Food Sci Technol*, 1995, **28**(1), 25-30.
- Achliya G S, Wadodkar S G and Dorle A K, Evaluation of CNS activity of *Brahmi Ghrita*, *Indian J Pharmacol*, 2005, **37**(1), 33-36.
- Health, Myths and Facts about Epilepsy, in *The New Indian Express* (Indian Epilepsy Association, Bangalore), 2006 Nov, 21, 1 (col. 1,4,5).
- Health, in *The New Indian Express* (About Indain Limited), 2006 Nov, 21, 1 (col.6).
- Daniel H L, Seizures and Epilepsy, in *Harrison's principle of internal medicine*, edited by Kasper D, Braunwald E, Fauci A S and Hauser S L (The McGraw-Hill Companies, USA), 2005, 2357.
- Allen CM C and Lueck C J, Neurological Disease, in *Davidson's principles and practice of medicine*, edited by Haslett C, Chilvers E R, Boon N A and Colledge N R (Elsevier Science Ltd, UK), 1167.
- Shakila R, Review on *Sphaeranthus indicus* Linn. (Kottaikkarantai), *Pharmacognosy Review*, 2013, **7**(14), 157-169.
- Gupta Y K, Malhotra J, George B and Kulkarni S K, Methods and considerations for experimental evaluation of antiepileptic drugs, *Indian J Physiol Pharmacol*, 1999, **43**(1), 25-43.
- Du X M, Sun N Y, Takizawa N, Guo Y T and Shoyama Y, Sedative and anticonvulsant activities of goodyerin, a flavonol glycoside from *Goodyera schlechtendaliana*, *Phytother Res*, 2002, **16**(3), 261-263.