International Journal of Phytopharmacology
Research Article

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

ANTIEPILEPTIC POTENTIAL AND MECHANISTIC EVALUATION OF PAVONIA PROCUMBENS: A STUDY ON FREE RADICAL SCAVENGING AND CHOLINESTERASE INHIBITION

Sreenathi B* Chaitanya D, Sushma B, Pavan Kumar N, Pravallika M, Bhavitha K

Department of Pharmaceutical Analysis, Jagan's College of Pharmacy, Nellore-524346

ABSTRACT

The World Health Organization (WHO) describes traditional medicine, including herbal medications, as therapeutic modalities that predate the creation and dissemination of modern medicine and continue to be utilized today, often for hundreds of years. In this research, Pavonia procumbens was selected to investigate its antiepileptic potential and related mechanisms. The plant leaves were dried, powdered, and extracted using ethanol to produce the ethanol extract of Pavonia procumbens (EEPP), which was stored for further use. Various assays were employed to determine the extract's efficacy in scavenging free radicals through distinct reaction mechanisms. Additionally, the study explored the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities using Ellman's method. Spectrophotometry-based assays utilizing specific substrates and a colorimetric method were conducted to measure AChE and BChE inhibition. The findings of this study contribute to understanding the therapeutic potential of Pavonia procumbens, particularly in its application as an antiepileptic agent, and support its relevance in traditional medicine practices.

Key words: : World Health Organization (WHO), Pavonia procumbens, acetylcholinesterase (AChE), Butyrylcholinesterase (BChE), Antiepileptic Potential, Free Radical Scavenging.

Corresponding Author:Sreenathi B - E-mail: sreenadhbikki1156@gmail.com

Access this article online

Quick Response code

Received:28.06.2024	**Revised**:15.07.2024	**Accepted**:18.07.2024

INTRODUCTION

Plants have served as the foundation for several traditional medical systems all throughout the world and continue to offer humans new treatments. The World Health Organization (WHO) describes traditional medicine (including herbal medications) as therapeutic modalities that were in use before the creation and dissemination of modern medicine and are still in use today, frequently for hundreds of years. Due to its effectiveness, safety, cultural acceptance, and less side effects, herbal medications are employed. Plant chemical components are regarded to be more compatible with the human body since they are a part of the physiological processes of living systems. The indigenous medical systems in India are Ayurveda, Siddha, Unani, and Folk Medicines. In Ayurveda, about 8,000 herbal remedies have been standardized. There are 67 medicinal plants listed in the Rigveda, 81 in the Yajurveda, 290 in the Atharvaveda, and 1100 and 1270 in the Charak and Sushruta Samhita, which are still used in traditional formulations. Medicinal plants play a key role in the development of potent therapeutic agents. Oxygen is an essential element for life, living systems. Oxidative properties of oxygen play an important role in diverse biological process.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) describes free radicals and other non-radical reactive derivatives. The reactivity of radicals is stronger than non-radical, although radicals are less stable. Free radicals generally involved in chain reactions, a series of reactions leads to regenerate a radical that can begin a new cycle of reaction. Anti-oxidants are substances capable to end up free radicals and prevent them from causing cell

damage. Free radicals are capable causing a wide number of health problems which include cancer, heart diseases, and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The endogenous defense system includes different enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reducates (GR), and non enzymatic defense system included vitamin E, vitamin C and reduced glutathione (GSH). Free radicals are continuously produced by the body via enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, cytochrome P_{450} system and oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria. Human body system is enriched with natural antioxidants and can prevent the onset of diseases and treat diseases caused due to free-radical mediated oxidative stress.

Epilepsy is a Neurological Condition that can produce brief disturbances called seizures in the brain's electrical function. The most common types of seizure in adults are, partial complex with secondarily generalized. Together representing about 80% of prevalent cases. In adolescents there are approximately equal numbers of partial and generalized seizures.

Materials and Methods

Collection of specimens

Fresh stems of Pavonia procumbens was collected local area in Nellore, Andhra Pradesh from during December. The plant powder is air dried for 7 days under shade and ground into a fine powder (M. Manoranjitham, *et al.,* 2015 & Gamble, J.S *et al.,*1935).

Phytochemical Screening

Materials

Petroleum ether, chloroform, methanol, distilled water, hydrochloric acid, sulphuric acid, acetic anhydride, sodium nitroprusside, pyridine, potassium hydroxide, phenolphthalein, ferric chloride, gelatin, sodium chloride, lead acetate, bromine, magnesium, benzene, silica gel, Mayer's reagent Dragendroff's reagent, Wagner's reagent, Hager's reagent, alcoholic α-Naphthol, Fehling's reagent, Benedict's reagent, Millon's reagent, Biuret reagent, Ninhydrin solution.

Extraction

About 1kg of the powdered stem material was successively extracted with solvents like petroleum ether, chloroform, Benzene and ethanol in a Soxhlet apparatus. The extracts were concentrated and traces of the solvent were completely removed under reduced pressure and stored in vacuum desiccators for further use. Aqueous extract was prepared by macerating the stem powder in double distilled water. The extract was concentrated in water bath and stored in desiccators.

Preliminary Phytochemical Screening (Henry, A.N *et al.,*1987 & Kokate C.K *et al.,*1990)

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents as per the standard procedures given by Kokate and Trease and Evans.

Detection of Alkaloids

Small portions of solvent-free extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

a. Mayer's test: Filtrates were treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate was indicates the presence of alkaloids.

b. Dragendroff's test: Filtrates were treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate was indicates the presence of alkaloids.

c. Wagner's test: Filtrates were treated with solution of iodine in potassium iodide (Wagner's reagent) and formation of brown precipitate was indicates the presence of alkaloids.

d. Hager's test: Filtrates were treated with a saturated solution of picric acid (Hager's reagent) and formation of yellow precipitate was indicates the presence of alkaloids.

Invitro Antioxidant Activity

The in vitro antioxidant activity of the Ethanol extract of Pavonia procumbens (EEPP), was carried out using the following 2 assay methods in accordance with previously reported procedures as below.

Superoxide radical scavenging activity

Superoxide radical scavenging activity was determined by the nitro blue tetrazolium (NBT) reduction method. In this assay, the non-enzymatic phenazine methosulfate/nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple colour formazan. The reaction mixture contained phosphate buffer (0.5 mL, 100mM, pH 7.4), 1.0 mL of NADH (0.4mM), 1.0 mL of NBT (0.156mM), 0.1 mL of PMS (0.06mM) and 3 mL of the EEPP and standard drug (Quercetin) of various concentrations (10- 50 µg/mL, in 90% ethanol). After incubation at 25 ºC for 1 h, the absorbance of the reaction mixture was measured at 560 nm against an appropriate blank to determine the quantity of formazan formed (Khandelwal, KR *et al.,* 2006 & VYA. Barku *et al.,* 2013).

Hydroxyl radical scavenging activity

Hydroxyl radicals were generated by the Fenton reaction using Fe3+/ascorbate/EDTA/H_2O_2 system. The hydroxyl radical generated in the system attacks

deoxyribose which eventually results in the formation of thiobarbituric acid (TBA, which reacting substance (TBARS) which was estimated. The reaction mixture contained 0.1 mL of 2-deoxy-2- ribose (10mM), 0.33mL of phosphate buffer (50mM, pH 7.4), 0.1 mL of FeCl3 (0.1 mM), 0.1 mlethylenediamine tetra-acetic acid (EDTA) (0.1mM), 0.1 mL of H_2O_2 (mM), 0.1 mL of ascorbic acid (1mM) and 1.0 mL of various concentrations (10-50 µg/mL) of the EEPP and standard (Quercetin). After incubation for 45 min at 37 ºC, 1.0 mL of 2.8% (v/v) TCA, and 1.0 mL of [thiobarbituric acid, TBA, 0.5% (v/v) in 0.025 mol/L NaOH solution containing 0.2% (w/v) of butylated hydroxyl anisole, BHA] were added in the reaction mixture, and the mixture was incubated at 95ºC for 15 min to develop the pink chromogen. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution (Biju John, *et al.*, 2014).

In all the two above methods, the percentage inhibition of scavenging activity was calculated using the following equation

Percent inhibition (%) = $(A_{control}-A_{test})/A_{control}\times 100$

Where,

$A_{control}$ is the absorbance of the control and

A_{test} represents the absorbance of a test substance

Evaluation of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibitory activity by Ellman's method

AChE and BChE activity was measured by using spectrophotometer based on Ellman's method (Sethi PD *et al.*,1996 & McCord, J.M. *et al.*, 1969). The AChE and BChE activity assay was carried out using acetylthiocholine iodide and butyrylthiocholine iodide as substrates, respectively, based on a colorimetric method, as described previously (Rajesh, V., *et al*., 2015 & Badami, S. *et al.*, 2007). 10 µL of EEPP solution in 0.2% DMSO, 79 µL of 20 mM sodium phosphate buffer (pH 7.6), and 1 µL enzyme preparation (with final concentrations: 0.087 unit/mL for AChE, or 0.035 unit/mL for BChE, and final concentrations: 1 to 500/1000 µM for compounds tested) were mixed and preincubated for 15 min. To the mixture, 10 µL substrate solution was added (final concentrations 1.5 mM for acetylthiocholine iodide, or 4 mM for butyrylthiocholine iodide) and incubated for 30 min. The reaction was stopped by adding 900 µL DTNB-phosphate-ethanol reagent. The absorption was read immediately at 412 nm on a microplate reader. The concentration of the test compound required to inhibit AChE or BChE activity by 50% (IC_{50}) was calculated using an enzyme inhibition dose response curve, with galanthamine as standard to draw the standard curves.

$$\% \textbf{ inhibition} = \textbf{100} - [(\frac{\textbf{Change of sample absorbance}}{\textbf{Blank absorbance}}) \times \textbf{100}]$$

STATISTICAL ANALYSIS

The values were considered as triplicate readings as per procedure and were represented as Mean and its standard error. The data was subjected to ANOVA one way analysis to find the difference in the samples.

RESULTS

The phytochemical analysis helps in formulating pharmacopoeial standards. The chief phytochemicals present in the different extracts of Pavonia were flavonoids, polyphenols, alkaloids, triterpenoids, steroids, tannins, carbohydrates, saponins and aminoacids.

In vitro antioxidant activity

Superoxide radical scavenging activity

EEPP showed superoxide radical scavenging activity in a concentration dependent manner. At the concentration of 50 µg/mL, EEPP exhibited 89.104±1.336% of scavenging activity, while, Quercetin showed 87.947±1.537% of scavenging activity at the same concentration.

Hydroxyl radical scavenging activity

The percentage inhibition of hydroxyl radicals was 89.363±1.667% for EEPP at the concentration of 50µg/mL. The inhibitory activity of Isolated molecule was comparable with that of the standard drug, Quercetin (90.529±1.665%) at the same concentration. The percentage scavenging activity of EEPP was statistically similar as compared to Quercetin (Figure 1.1).

Invitro acetylcholinesterase and Butyrylcholinesterase inhibition of EEPP in Ellman's method

Based on the table 4 showcasing the concentration-dependent effects of Pavonia leaf ethanol extract (EEPP) on acetylcholinesterase and butyrylcholinesterase inhibition, several significant trends are evident. The results demonstrate a dose-dependent relationship between EEPP concentration and inhibition of both acetylcholinesterase and butyrylcholinesterase activities. For acetylcholinesterase inhibition, the standard compound showed a gradual increase in inhibition percentage from 34.54% at 10 µg/ml to 95.66% at 500 µg/ml. Similarly, EEPP exhibited an escalating inhibition profile across the same concentration range. Notably, at higher concentrations (250 and 500 µg/ml), EEPP approached the inhibitory efficacy of the standard compound.

Regarding butyrylcholinesterase inhibition, a comparable trend emerged. The standard compound showcased an incremental rise in inhibition from 23.37% to 84.36%, while EEPP mirrored this pattern, displaying inhibition percentages climbing from 7.14% to 80.47%. EEPP demonstrated higher IC_{50} values compared to the standard for both acetylcholinesterase and

butyrylcholinesterase inhibition, indicating a relatively lower potency in inhibiting these enzymes. These findings suggest that EEPP exhibits notable concentration-dependent inhibitory effects on acetylcholinesterase and butyrylcholinesterase, albeit with higher concentrations required for comparable efficacy to the standard compound. Further investigations into the specific bioactive components within EEPP contributing to these inhibitory effects could offer valuable insights for potential therapeutic applications targeting cholinesterase-related conditions.

Table 1: Preliminary Phytochemical Analysis of various extracts of Pavonia procumbens

Sl.No.	Test	P.ether	Benzene	Chloroform	Methanol	Water
1.	Carbohydrates	-	-	-	+	+
2.	Alkaloids	-	+	-	+	+
3.	Glycosides	-	-	-	+	+
4.	Tannins	-	-	-	+	+
5.	Steroids	+	+	+	-	-
6.	Triterpenoids	+	+	+	+	-
7.	Volatile oils	-	-	-	-	-
8.	Fats and fixed oils	-	-	-	-	-
9.	Flavanoids	-	-	-	+	+
10.	Polyphenols	-	-	-	+	+
11.	Saponins	-	-	-	+	+
12.	Aminoacids	-	-	-	+	+
13.	Gums and mucilages	-	-	-	-	+

Table 2: Superoxide radical scavenging activity of EEPP

Concentration (µg/ml)	% inhibition by EEPP	% inhibition by Standard drug
10	39.211±3.106	28.678±1.714
20	54.529±4.186	44.997±1.932
30	62.836±2.896	63.294±1.737
40	78.772±1.308	74.135±1.313
50	89.104±1.336	87.947±1.537

Values were expressed as Mean±SEM (n=3)

Table 3: Hydroxyl radical scavenging activity of EEPP

Concentration (µg/ml)	% inhibition by EEPP	% inhibition by Standard drug
10	44.256±2.836	29.739±1.885
20	56.425±2.164	44.803±1.333
30	64.199±1.297	62.423±2.263
40	75.674±1.184	75.936±2.528
50	89.363±1.667	90.529±1.665

Values were expressed as Mean±SEM (n=3)

Table 4: Invitro acetylcholinesterase and Butyrylcholinesterase inhibitory activity of EEPP

S. No	Concentration (µg/ml)	% Inhibition			
		Acetylcholinesterase Inhibition		Butyrylcholinesterase Inhibition	
		Standard	EEPP	Standard	EEPP
1	10	34.54±1.39	14.47±1.82	23.37±2.21	7.14±0.53
2	50	53.36±1.33	34.75±2.48	44.14±2.74	21.55±1.38
3	100	69.94±1.18	48.15±2.75	61.65±2.76	34.47±2.75
4	250	85.87±2.94	72.29±3.88	74.18±1.85	58.88±2.18
5	500	95.66±2.48	89.64±3.31	84.36±2.45	80.47±2.98
IC_{50}		**61.54±2.25µg**	**131.18±2.45µg**	**60.87±2.21µg**	**130.87±2.47µg**

Values were expressed as Mean±SEM (n=3).

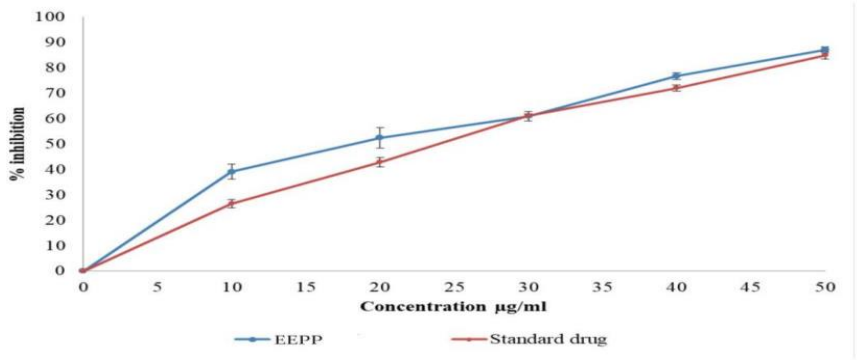

Figure 1.1: Superoxide radical scavenging activity. Values are mean ± SEM of 3 replicate experiments.

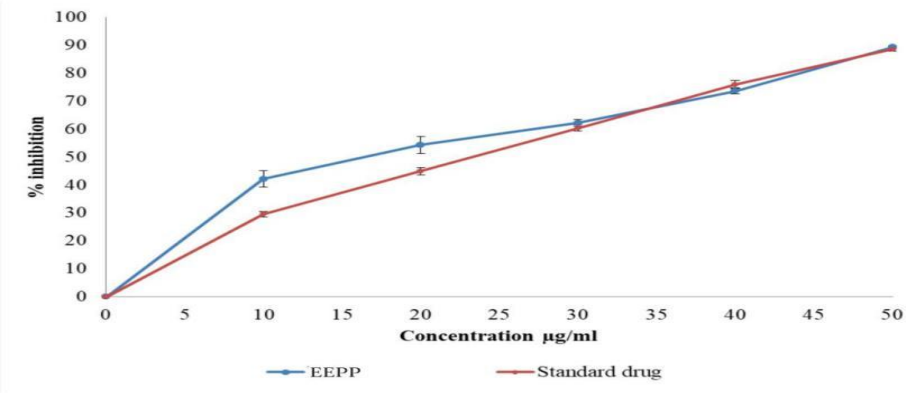

Figure 1.2: Hydroxyl radical scavenging activity. Values are mean ± SEM of 3 replicate experiments

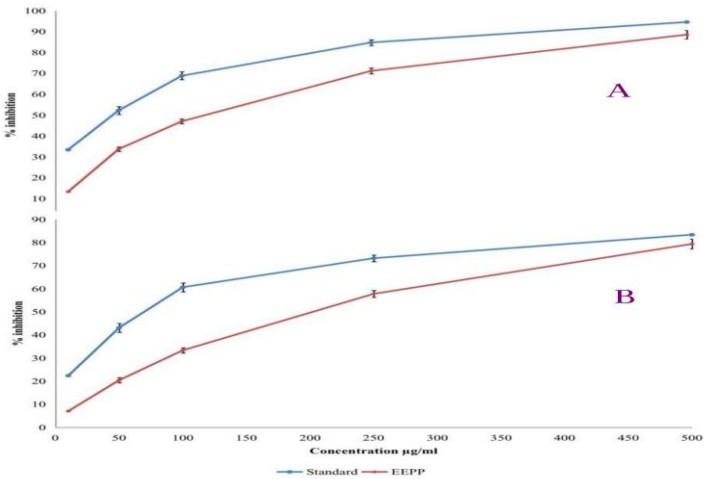

Figure 1.3 Invitro anti-epileptic activities

acetylcholinesterase inhibition activity B. butyrylcholinesterase inhibition activity of EEPP in Ellman's method

DISCUSSION

The relationship between epilepsy and behavioral disorders like anxiety and depression has been recognized in various studies (Maje IM, et al., 2012 &Verma S, et al., 2012). Patients with epilepsy often experience higher rates of emotional disorders compared to those with other chronic illnesses. However, the mechanisms underlying the development of anxiety in epilepsy remain poorly understood. Cholinergic dysfunction has been implicated in epilepsy (Gopalreddygari V, et al., 2010).Acetylcholinesterase (AChE) plays a crucial role in terminating cholinergic transmission by hydrolyzing acetylcholine (ACh) in the brain.

The concentration-dependent inhibition of AChE observed in the study aligns with the potential role of cholinergic dysfunction in epilepsy-related anxiety. Furthermore, the presence of Quercetin in Pavonia species, as mentioned in the literature, adds depth to the discussion, given Quercetin's neurobiological relevance (Hosseinzadeh H, et al., 2013).

Notably, butyrylcholinesterase, which also hydrolyzes ACh albeit less efficiently, emerges as a potential therapeutic target in epilepsy. Inhibition of butyrylcholinesterase has been associated with increased brain acetylcholine levels, potentially improving cognition. Future investigations into the precise mechanisms underlying the extract's actions on cholinesterases and its impact on cholinergic transmission in epilepsy-associated anxiety could offer valuable insights into potential therapeutic interventions.

This study sheds light on the potential therapeutic implications of Pavonia stem ethanol extract (EEPP) in the context of neurological conditions, specifically epilepsy-related anxiety. Additionally, the presence of Quercetin in Pavonia species further emphasizes the compound's relevance in neurological contexts. The findings suggest that EEPP exhibits inhibitory actions on key enzymes involved in cholinergic transmission, offering insights into its potential as a modulator of neurotransmitter dynamics, specifically acetylcholine, implicated in anxiety responses associated with epilepsy. The correlation between the extract's actions on cholinesterases and established links between cholinergic dysfunction and epilepsy-related anxiety opens avenues for potential therapeutic interventions. However, this work also presents areas for future exploration and improvement. One crucial aspect is the need for further elucidation of the specific neurobiological mechanisms underlying EEPP's actions on cholinesterases and its subsequent impact on cholinergic transmission in epilepsy-associated anxiety. Additionally, in vivo studies to validate these findings in animal models could provide more comprehensive insights into the extract's efficacy and safety profile.Furthermore, exploring the extract's broader neuroprotective effects and its impact on other behavioral manifestations associated with epilepsy could pave the way for a more holistic therapeutic approach.Continued investigations into EEPP's mechanisms and broader therapeutic effects could offer valuable insights for the development of novel adjunctive therapies for neurological disorders.

Conclusion

REFERENCES

1. M. Manoranjitham and S. Premalatha, Antibacterial activity and Phytochemical Screening of methanolic extract of Pavonia procumbens, *Int. J. Adv. Res. Biol. Sci*. 2(11), 2015, 256–259
2. Gamble, J.S. Flora of the presidency of Madras, Botanical Survey of Calcutta, India. 1935.
3. Henry, A.N; Kumari, G.R. and Chitra V. Flora of Tamilnadu India. Botanical Survey of India, Southern Circle, Coimbatore, India. 3, 1987, 258.
4. Kokate C.K; Purohit, A.P; Gokhale S.B., Practical Pharmacognosy", 1st ed, Nirali prahashan, pune.1990, 123.
5. Khandelwal, KR, Practical Pharmacognosy, 16th edn, *Nirali Prakashan, Pune*. 2006
6. VYA.Barku, Y Opoku-Boahen, E Owusu-Anash and EF Menash. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of Amaranthus spinosus. *Asian Journal of Plant Science and Research.* 3(1), 2013, 69-74.
7. Biju John, Sulaiman CT, Satheesh George and VRK Reddy. Total phenloics and flavonoids in selected medicinal plants from Kerala. *International Journal of Pharmacy and Pharmaceutical Sciences.* 6(1), 2014, 406-408.
8. Sethi PD. 1st ed. New Delhi: CBS Publishers; 1996. High Performance Thin Layer Chromatography Quantitative *Analysis of Pharmaceutical Formulation.*
9. McCord, J.M. and Fridovich, I., Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *Journal of Biological chemistry,* 244(22), 1969, 6049-6055.
10. Rajesh, V., Bharathi, P., Priya, M., Arumugam, P. and Jayaraman, P., Phytochemical potential of pavonia procumbens (Wall. ex wight & arn.) walp. with reference to antioxidant and antidiabetic activity. *International Journal of*

Pharmacognosy, 7, 2015, 793-801.

11. Badami, S. and Channabasavaraj, K.P. In Vitro. Antioxidant Activity of Thirteen Medicinal Plants of India's Western Ghats. *Pharmaceutical biology*, 45(5), 2007, 392-396.
12. Maje IM, Yaro AH, Magaji MG. Anticonulsant screening of the methanolic stem bark extract of Tamarindus indica linn in laboratory animals. *Nig J Pharm Sci*.11(2), 2012, 50-57.
13. Verma S, Sinha R, Kumar P, Amin P. Study of Convolvulus pluricaulis for antioxidant and anticonvulsant activity. *Central Nervous System Agents.* 12(1), 2012, 55-59.
14. Gopalreddygari V, Knchi SP. Evaluation of the anticonvulsant effect of Centella asiatica (gotu kola) in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission. *Epilepsy & Behavior* 17, 2010, 332-335.

Hosseinzadeh H, Ramezani M, Shafaei H. Anticonvulsant Effect of Berberis integerrima L. Root Extracts in Mice. *J Acupunct Meridian Stud.* 6(1), 2013, 12- 17.