EVALUATION OF ANTIEPILEPTIC POTENTIAL OF HIBISCUS SABDARIFFA LINN

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Summary

Epilepsy is one of the most common neurological disorder affecting approximately 1% of the population worldwide. Each year about 181,000 people are diagnosed with epilepsy amongst which 80% of patients have good seizure control if they strictly adhere to the prescribed treatment regimen. Though the side effects associated with the prolonged treatment i.e. dizziness, double vision, stomach upset, lethargy etc. can not be ignored. Thus to avoid side effects many herbal medicines are being used in the form of herbal extracts, herbal formulations or as an isolated compound.

Many documented evidences on the use of *Hibiscus sabdariffa* Linn. as diuretic, anti-diabetic, antihypertensive, antioxidant, and antibacterial are reported. The plant is also claimed to be useful in epilepsy. However no scientific data confirming the antiepileptic activity of the plant is available. Therefore the aim of the present research work was to evaluate the antiepileptic potential of *Hibiscus sabdariffa* Linn.using Pentylenetetrazole (PTZ) induced and Strychnine induced Seizures in Albino Swiss mice.

Methanolic extract and its varius fractions i.e.n-hexane,ethyl acetate and methanolic fractions were screened for antiepileptic activity.

Methanolic Extract (200mg/kg) demonstrated a marked dose dependent protection (*P<0.0001) from epileptic seizures induced by PTZ (90 mg/kg. i.p) followed by a

Methanolic Fraction (200mg/kg) (*P<0.001) as compared to a moderate response against Strychnine (3 mg/kg i.p) induced seizures in mice.

Thus it can be concluded that methanolic extract of *Hibiscus sabdariffa* exhibits significant anti-epileptic activity by modulating the duration and frequency of opening the GABA mediated chloride channels leading to protection from epileptic seizures.

Keywords: *Hibiscus sabdariffa*, Delphinidin-3-glucoxyloside, Antiepileptic.

Introduction

Hibiscus sabdariffa (family Malvaceae) consists of large number of cultivated species. This is an annual erect, bushy, herbaceous sub-shrub growing to 8 ft (2.4 m) in height bearing auxiliary white flowers with a reddish centre at the base of the staminal column and fleshy and bright red fruits[1]. The red anthocyanin pigments present in their calyces are used as food coloring agents[2]. In Ayurvedic literature of India, different parts of this plant have been recommended for various ailments like hypertension, pyrexia and liver disorders. It is traditionally used as antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, sedative, stomachic and tonic. It is also reported to be used in treatment of Paralysis, Epilepsy, Convulsions, and Spasm.

The phytochemical review highlights presence of vitamins like riboflavin, niacin, ascorbic acid etc. Other constituents like β – carotene, anisaldehyde, arachidic acid, citric acid, malic acid, tartaric acid glycinebetaine, trigonelline, anthocyanins, cyanidin-3-rutinoside, delphinidin, delphinidin-3-glucoxyloside, hibiscicus acid, delphinidin-3-sambubioside and cyanidin-3-sambubioside are also reported.[3-5]

Most of the CNS disorders like Epilepsy, Insomnia, and pain are treated by modern system of medicine. Although the effect of medicine is immediate there are number of associated side effects which are alarming when used for long duration of time.

Thus to avoid these side effects like dizziness, double vision and stomach upset, lethargy that are associated with the prolonged anti-epileptic medication, many herbal drugs like *Taxus Wallichiana*[10], *Nardostachys Jatamans*[6-9], *Scutellaria baicalensi*[10], *Cestrum Nocturnum, Bacopa Monnieri*[11-14] and *Hibiscus sabdariffa* Linn are being used in the form of extracts, formulations or as an isolated compounds.

Thus the plant *Hibiscus sabdariffa* owing to its natural abundance and therapeutic importance deserves additional evaluation with respect to its anti-epileptic potential.

Materials and Methods

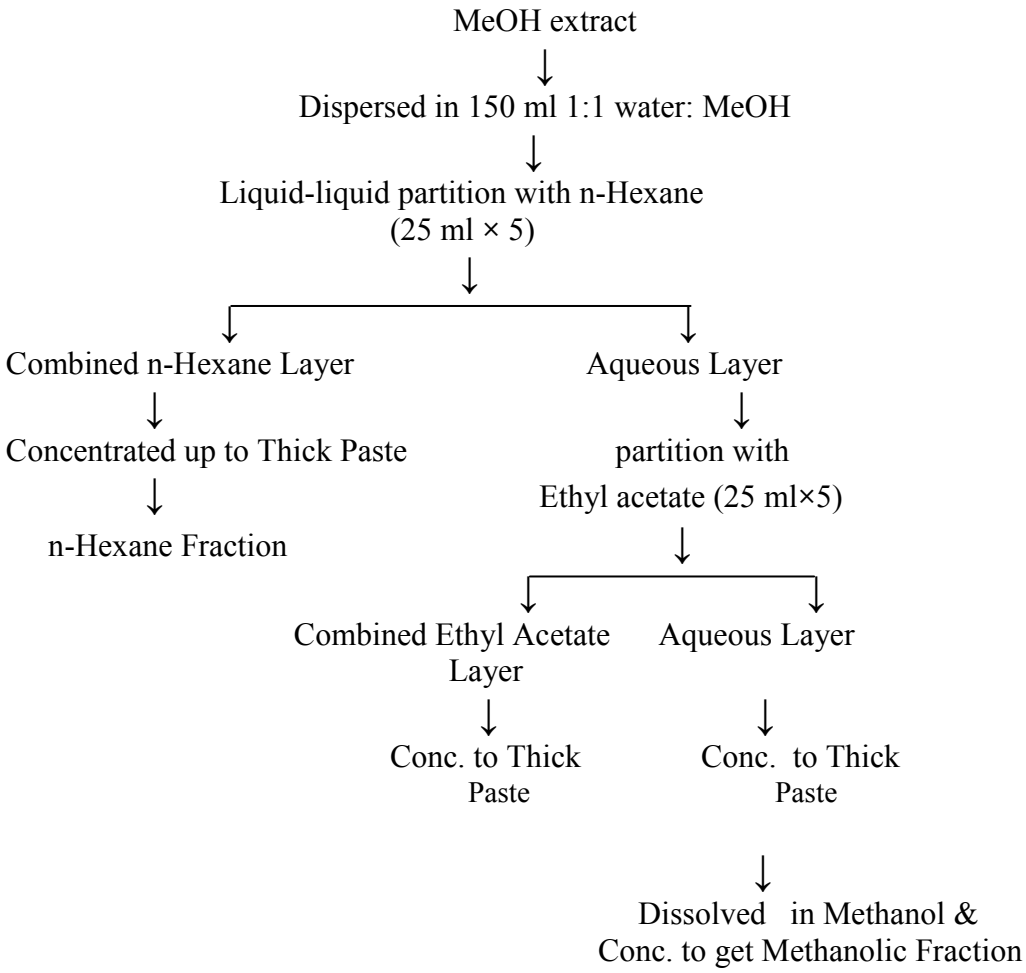
Collection and Authentification of Plant material:

Mature and fresh leaves of *Hibiscus sabdariffa* L. were collected from Latur, Maharashtra, India, in November-January and dried at room temperature in shade away from sunlight. The plant was authenticated by Mr. P.G. Diwakar, Deputy Director Botanical survey of India, Pune (VRK-IBSI/WRC/Tech/2010/1031).

Extraction[15]

The dried leaves were gently crushed by hand and subjected to extraction in a soxhlet extractor.

Dried coarse powder of leaves of *Hibiscus sabdariffa* L.
↓
Soxhlet extraction with Methanol
↓
Dry Methanolic extract.
↓
Fractionation of Methanolic Extract
↓



All the extracts i.e. Methanolic Extract (HSME), Methanolic Fraction (HSMF), n-Hexane Fraction (HSHF) and Ethyl Acetate Fraction (HSEAF) were recovered dried and used for the biological screening.

Animals:

Albino Swiss mice weighing around 25-40 gm were used. The animals were provided with standard laboratory food and tap water *ad libitum* and maintained at natural day night cycle. All the experiments were conducted in an isolated and noiseless condition.

Chemicals:

Diazepam (Ranbaxy Pvt Ltd, Mumbai B.No.: 68492473), Pentylenetetrazole (Research Lab Fine Chem Industry, Mumbai B.No.: 45790702), Strychnine (Research Lab Fine Chem Industry, Mumbai B.No.: 60750611) were used in the present investigation.

Acute Toxicity study[16]

Toxicity studies were conducted as per internationally accepted protocol drawn under OECD guidelines in Swiss albino mice at a dose level up to 5000mg/kg. Mice were fasted for overnight and maintained with water and *ad libitum* .The mice were separated into different groups (n=6) and were orally fed with increasing doses (175, 550,1750, 5000 mg/kg body weight) of methanol extract of *Hibiscus sabdariffa* Linn. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr, and 24 hr to detect changes in the autonomic or behavioral response and also for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma and then monitored for any mortality for the 14 days.

Antiepileptic Activity: [17]

Anti-epileptic activity by Pentylenetetrazole (PTZ) induced Seizures

The mice were divided into eight groups of six animals each. Group I was treated with vehicle (Saline) and Group II with Diazepam at a dose of 1mg/kg i.p. Group III, IV, V, VI, VII, and VIII were administered with Methanolic Extract (HSME), Methanolic Fractions (HSMF), n-Hexane Fraction (HSHF), Ethyl Acetate Fraction (HSEAF) at doses 100 and 200 mg/kg respectively i.p. All the drug treatment was given thirty minutes before the injection of pentylenetetrazole (90 mg/kg. i.p). Each animal was placed in individual plastic cage and observed for forty five minutes .The onset of general clonus was recorded and tonic-clonic convulsions were studied.

Anti-epileptic activity by Strychnine induced Seizures

For strychnine induced convulsions model, same procedure as mentioned in Pentylenetetrazole induced convulsions protocol was adopted using Strychnine (3 mg/kg i.p).

Results

Anti-epileptic activity by Pentylenetetrazole (PTZ) induced Seizures:

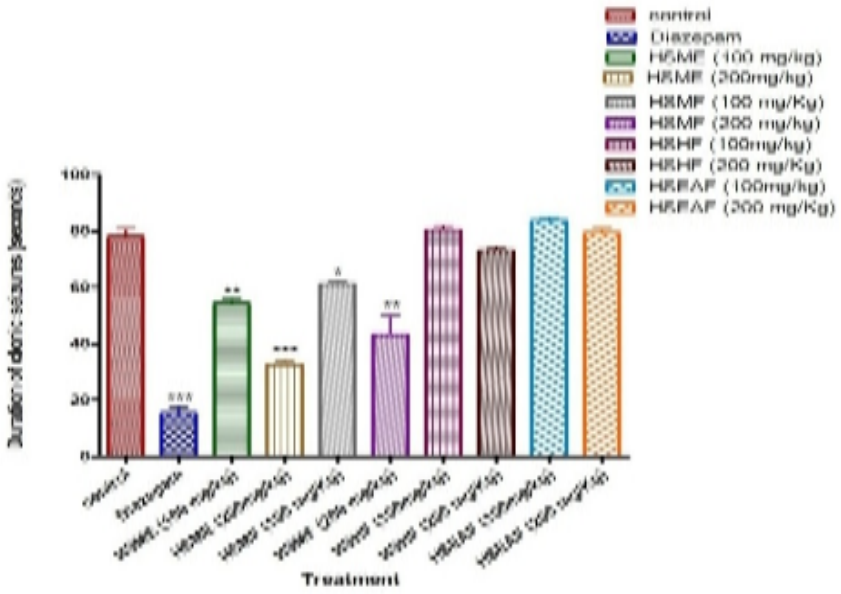
The onset of general clonus, tonic clonic convulsions were recorded (Table No.1-4, Fig. 1-2)

Table No.1: Duration of Clonic Seizures of Methanolic Extract and Methanolic Fraction using Pentylenetetrazole:

Duration of Clonic Seizures					
Control	**Diazepam**	**HSME (100 mg/kg)**	**HSME (200mg/kg)**	**HSMF (100 mg/Kg)**	**HSMF (200 mg/kg)**
78	23	50	29	59	50
65	11	56	35	63	50
79	12	58	32	61	50
79	19	52	33	64	50
82	17	59	36	58	50
85	10	51	31	62	50

Table No.2: Duration of Clonic Seizures of n -Hexane Fraction and Ethyl Acetate Fraction using Pentylenetetrazole:

Duration of Clonic Seizures			
HSHF (100mg/kg)	**HSHF (200 mg/Kg)**	**HSEAF (100mg/kg)**	**HSEAF (200 mg/Kg)**
78	75	82	78
85	70	83	80
83	71	85	82
80	72	88	85
81	72	83	76
75	78	81	75



Data was analyzed using one way ANOVA followed by Dunnet's test (*P<0.05, **P<0.001, ***P<0.0001)

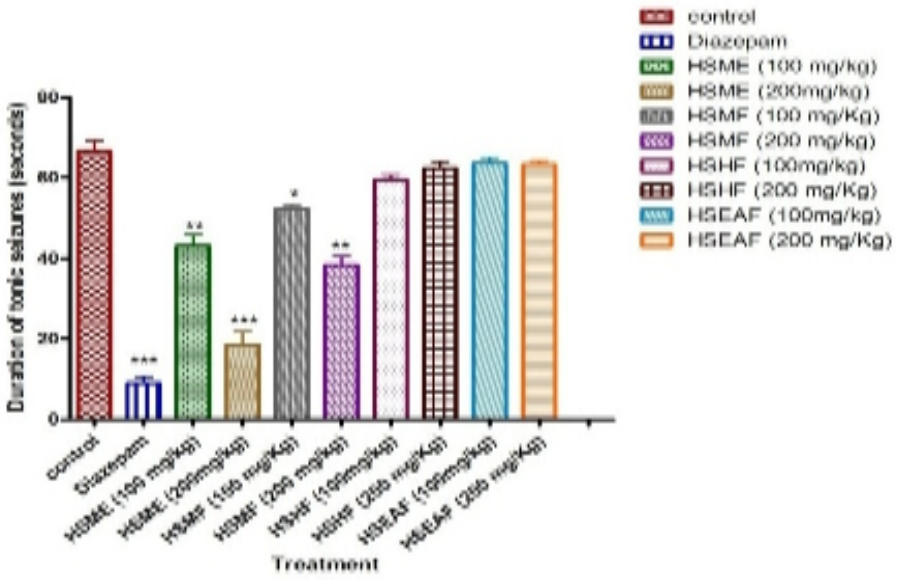
Figure 1: Duration of Clonic Seizures of *Hibiscus sabdariffa* Linn for Methanolic Extract (HSME), Methanolic Fraction (HSMF), n -Hexane Fraction (HSHF), Ethyl Acetate Fraction (HSEAF) in Pentylenetetrazole induced model.

Table No.3: Duration of Tonic Seizures of Methanolic Extract and Methanolic Fraction using Pentylenetetrazole:

Duration of Tonic Seizures					
Control	**Diazepam**	**HSME (100 mg/kg)**	**HSME (200mg/kg)**	**HSMF (100 mg/Kg)**	**HSMF (200 mg/kg)**
67	13	45	21	49	30
76	8	54	14	55	35
71	12	42	32	54	40
59	11	38	12	51	38
66	8	45	10	53	42
61	2	35	22	52	45

Table No.4: Duration of Tonic Seizures of n -Hexane Fraction and Ethyl Acetate Fraction using Pentylenetetrazole:

Duration of Tonic Seizures			
HSHF (100mg/kg)	**HSHF (200 mg/Kg)**	**HSEAF (100mg/kg)**	**HSEAF (200 mg/Kg)**
64	67	68	60
60	68	65	65
61	62	66	66
55	59	63	64
59	58	59	63
58	60	61	62



Data was analyzed using one way ANOVA followed by Dunnet's test (*P<0.05, **P<0.001, ***P<0.0001)

Figure 2: Duration of Tonic Seizures of *Hibiscus sabdariffa* Linn for Methanolic Extract (HSME), Methanolic Fraction (HSMF), n-Hexane Fraction (HSHF), Ethyl Acetate Fraction (HSEAF) in Pentylenetetrazole induced model

Anti-epileptic activity by Strychnine induced Seizures:

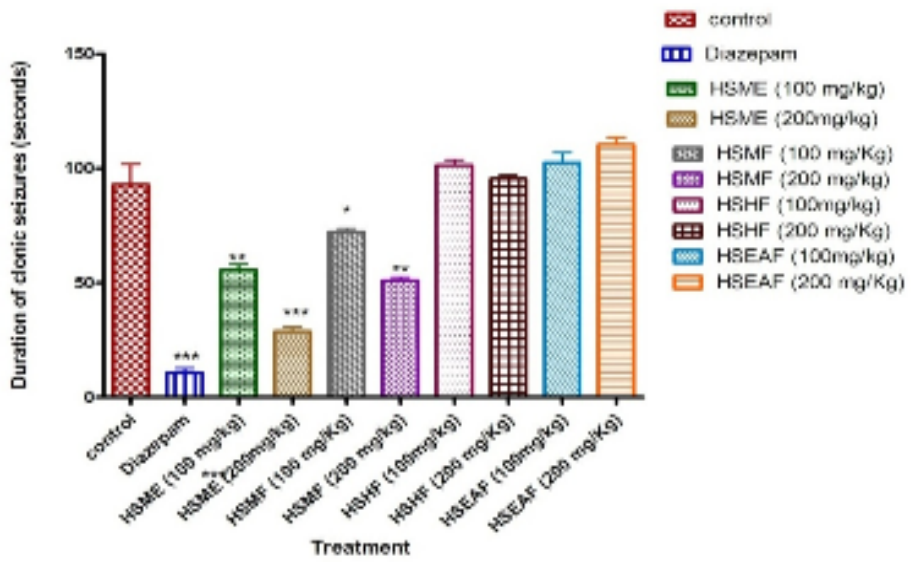
The onset of general clonus was recorded and tonic clonic convulsions are shown in Table No.5-8 and in Figure 3-4.

Table No. 5: Duration of Clonic Seizures of Methanolic Extract and Methanolic Fraction using Strychnine:

Duration of Clonic Seizures					
Control	**Diazepam**	**HSME (100 mg/kg)**	**HSME (200mg/kg)**	**HSMF (100 mg/Kg)**	**HSMF (200 mg/kg)**
101	16	59	23	67	54
112	7	52	25	76	47
78	16	65	27	75	48
67	7	56	29	73	49
121	6	48	32	72	53
78	12	53	36	71	55

Table No.6: Duration of Clonic Seizures of n-Hexane Fraction and Ethyl Acetate Fraction using Strychnine:

Duration of Clonic Seizures			
HSHF (100mg/kg)	**HSHF (200 mg/Kg)**	**HSEAF (100mg/kg)**	**HSEAF (200 mg/Kg)**
109	92	102	106
97	93	118	102
95	94	112	103
99	95	95	114
101	96	95	118
107	102	93	119



Data was analyzed using one way ANOVA followed by Dunnet's test (*P<0.05, **P<0.001, ***P<0.0001)

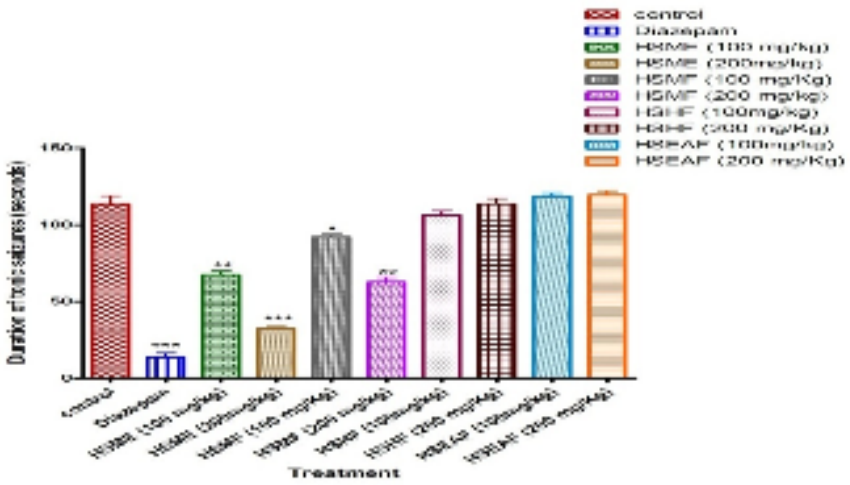
Figure 3: Duration of clonic Seizures of *Hibiscus sabdariffa* Linn for Methanolic Extract (HSME), Methanolic Fraction (HSMF), n -Hexane Fraction (HSHF), Ethyl Acetate Fraction (HSEAF) in Strychnine induced model.

Table No.7: Duration of Tonic Seizures of Methanolic Extract and Methanolic Fraction using Strychnine:

Duration of Tonic Seizures					
Control	**Diazepam**	**HSME (100 mg/kg)**	**HSME (200mg/kg)**	**HSMF (100 mg/Kg)**	**HSMF (200 mg/kg)**
118	15	78	34	86	55
121	18	70	35	90	65
112	21	65	29	93	76
100	16	66	28	95	58
98	9	60	32	99	60
132	3	65	36	89	62

Table No.8: Duration of Tonic Seizures of n -Hexane Fraction and Ethyl Acetate Fraction using Strychnine:

Duration of Tonic Seizures			
HSHF (100mg/kg)	**HSHF (200 mg/Kg)**	**HSEAF (100mg/kg)**	**HSEAF (200 mg/Kg)**
98	98	110	110
99	110	115	115
102	116	120	120
110	118	125	127
113	119	122	126
115	121	119	119



Data was analyzed using one way ANOVA followed by Dunnet's test (*P<0.05, **P<0.001, ***P<0.0001)

Figure 4: Duration of Tonic Seizures of *Hibiscus sabdariffa* Linn for Methanolic Extract (HSME), Methanolic Fractions (HSMF), n –Hexane Fraction (HSHF), Ethyl Acetate Fraction (HSEAF) in Strychnine induced model.

Discussion

Epileptic seizures due to PTZ are induced due to down regulation of GABA mediated chloride channel as PTZ is an antagonist for this receptor complex. In the present investigation HSME (200mg/kg) demonstrated a significant protection from PTZ induced seizures (*P<0.0001) whereas HSMF (200mg/kg) exhibited comparatively mild protective effect (*P<0.001). Other fractions i.e HSHF and HSEAF did not show any significant results. Hence it could be deduced that HSME and HSMF may be modulating the duration and frequency of opening the GABA mediated chloride channels leading to protection from epileptic seizures (Table 1-4, Fig.1, 2).

Strychnine is an inhibitor of Glycine synthesis which is an inhibitory neurotransmitter involved in inhibition of excitatory process like seizures. Both HSME and HSMF in a dose of 200mg/kg each exhibited a dose dependent protection from Strychnine induced seizures in mice stating that the drugs HSME and HSMF may be influencing the synthesis of Glycine at the neuronal synaptic vesicles.However the reduction in number of seizures was less as compared to that for PTZ induced seizures. (*P<0.0001,*P<0.001), (Table 5-8, Fig. 3, 4).

The observations provide scientific credence to the theory that drugs HSME and HSMF modulate GABA mediated chloride channel and Glycine synthesis.

Acknowledgments

We would like to acknowledge Principal Mrs. Kiran Bhise, Allana College of Pharmacy, for making us avail the facilities of this college.

References

1. Ashutosh Kar. Pharmacognosy and Pharmacotechnology, New Age, International Publishers, New Delhi, 2003 ISBN: 81-224-1501-6, 20.Hou *et. al.,* Biochem Biophysics, 440 (1), 101 – 109, 2005.
2. *Dafallah*, A. A., Al-Mustafa, Z.: Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa.Amer. J. Chinese Med.* 24: 263 – 269, 1996.

3. Farombi, E. O., & Fakoya, A. Free radical scavenging and antigenotoxic Activities of natural phenolic compounds in dried flowers of Hibiscus Sabdariffa L. Molecular Nutrition and Food Research, 49, 1118–1120, 2005.
4. Ali M. B., Salih W. M., Mohamed A. H., Homeida A. M., *J. Ethnopharmacol.*, 31, 249 - 257 1991.
5. Kamei, H. *et. al.,* Suppression of tumor cell growth by Anthocyanins in vitro. Cancer Investigation, 13, 586–590, 2003.
6. Farjon, A. *World Checklist and Bibliography of Conifers*. Royal Botanic Gardens, Kew ISBN 1-900347-54-7, 1998.
7. Hui KM, MS Wang HY, *et. al*., "Anxiolytic effect of wogonin, a benzodiazepine receptor ligand isolated from Scutellaria baicalensis Georgi". *Biochem. Pharmacol.* 64 (9): 1415–24, 2002.
8. Dalby, Andrew, *Dangerous Tastes: The story of Spices*, London: British Museum Press, ISBN 0714127205 (US ISBN 0-520-22789-1) 83 – 88, 2000.
9. Dalby, Andrew, "Spikenard" in Alan Davidson, *The Oxford Companion to Food*, 2nd ed. by Tom Jaine (Oxford: Oxford University Press, ISBN 0-19-280681-5), 2006.
10. Hui KM, MS Wang HY, *et. al*., "Anxiolytic effect of wogonin, a benzodiazepine receptor ligand isolated from Scutellaria baicalensis Georgi". *Biochem. Pharmacol.* 64 (9): 1415–24, 2002.
11. Warrier, P K; V P K Nambiar, C Ramankutty, V.P.K. & Ramankutty, R Vasudevan Nair *Indian Medicinal Plants: A Compendium of 500 Specie*s. Orient Blackswan. pp. 238, 1996.
12. Stough C, Downey LA, Lloyd J *et al*. "Examining the nootropic effects of a special extract of Bacopa Monniera on human cognitive functioning: 90 day double-blind placebo-controlled randomized trial." *Phytother Res*. 22: 1629-1634, 2008.
13. Dhanasekaran M, Tharakan B, Holcomb LA *et al*. "Neuroprotective mechanisms of ayurvedic antidementia botanical Bacopa monniera." *Phytother Res*. 21:965-969, 2007.
14. Pravina K, Ravindra KR, Goudar KS *et al*.. Safety evaluation of Baco Mind in healthy volunteers: a phase I study." *Phytomedicine*. 14:301-308, 2007.
15. Mukherjee, P.K, *Quality Control of Herbal Drugs* (1st edition: 133, 176, 177, 189, 193, 380, 384, and 492). An approach to Evaluation of Botanicals. Business Horizons Publication, New Delhi, 2002
16. OECD. Guidelines for the testing of chemicals. 223:2009.p.1-24.
17. Pharmacologyonline3:263-27,2008, Madhavan *et al*.