ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

Anti Stress Activity (*in-vivo*) of Forskolin Isolated from *Coleus forskohlii*

Neha Tiwari [a*], Ashutosh Mishra [a], Ganesh Bhatt [b], Anil Chaudhary [c]
[a] A. N.D. College of Pharmacy Babhnan, Gonda, Uttar Pradesh, India.
[b] S.G.R.R. Dehradun, Uttarakhand, India.
[c] Deputy Manager, Cadila Pharmaceuticals LTD, Ahmedabad, Gujarat, India

Article info

Article History:
Received 16 September 2014
Accepted 28 October 2014

Keywords:
Traditional herbal remedies, Spectroscopic authentication, Elevated plus maze, Forced swimming test, Anti- stress therapeutics

Abstract

Coleus forskohlii (Labatiae) is a plant widely used as food and medicine worldwide but isolation of active compound (Forsklin) from hydroalcoholic extract and antistress activity of Forskolin is not reported. Dried and powder rhizome of *Coleus forskohlii* (Labatiae) was used to isolate the chemical constituent using phase column chromatography and TLC purification. Elevated plus maze (EPM) model and forced swimming test (FST) were performed to assess antistress activity on albino mice. (Doses of the isolated chemical constituents: 25, 50 and 100 mg/kg orally) The structure of the isolated compound was determined by FTIR, ^{1}H NMR and Mass spectroscopy. The results of elevated plus maze (EPM) model and forced swimming test (FST) revealed that isolated compound having antistress activity. This is the first report of antistress activity of Forskolin and isolation by column chromatography separation, and TLC purification. The result also revealed that Forskolin is a novel compound for the treatment of neurobiological disorder (stress). A scientific investigation of traditional herbal remedies (Forskolin) for stress may provide valuable leads for the development of alternative drugs and therapeutic strategies.

1. INTRODUCTION

Empirical use of medicine derived from plants has been widely disseminated since ancient times to treat a wide range of diseases. In the last decades, the interest in alternative therapies has raised markedly in a worldwide shape[1]. Drugs obtained from natural plant source are emerging as adjuvant / alternative therapies in the treatment of psychiatric disorders[2]. Medicinal plants playing significant role in the search of novel pharmacotherapy to treat psychiatric illnesses[3]. Plants have always been an exemplary source of drugs and many of the available drugs have been derived indirectly or directly from them[4].

Coleus forskohlii (Labatiae) is an important has been used as folk medicine in India[5]. It is the only source for Forskolin among the plant kingdom[6]. *Coleus forskohlii* plant have reputed medicinal uses, which includes antidepressant, antiaggregant, anticancer, antidiuretic, antiglaucomic, antimetastatic, antispasmodic, bronchodilator, bronchospasmolytic, cAMP-genic, cardiotonic, gastrostimulant, gluconeogenic, glycogenolytic, hypotensive, immunosuppressant, lipolytic, myorelaxant, neurogenic, pancreatostimulant, positive inotropic, secretagogue, sialagogue, thyrotropic and vasodilator[7]. Forskolin has become commercially available as a drug for treating heart disease in Japan[8]. Forskolin (7β-Acetoxy-8, 13-epoxy-1α, 6 β, 9 α-trihydroxy-labd-14-ene-11-one) a labdane diterpene compound is the active principle compound[9].

Stress is a common phenomenon that is experienced by every individual. When stress becomes extreme, it is harmful for the body and hence needs to be treated. Stress is involved in the pathogenesis of a variety of diseases including hypertension, peptic ulcer, immunodepression, reproductive dysfunction and behavior disorder[10]. Evidence reveled that stress impairs learning and memory and encounter several disorders including anxiety and depression[11]. Stress mapping or screening of biologically active constituents of natural origin, mainly from plant kingdom[12].

Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. Drugs like benzodiazepines, certain CNS stimulants such as amphetamines and caffeine as well as some anabolic steroids are routinely used by people to combat stress. The incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs[13]. Alternative are clearly needed because of the inability of the current therapies to manage condition of disease[14]. The first drugs used to treat pathologic condition of the CNS were based on natural resources[15]. People from different area of world using herbal medicine to alleviate affective disorders[16]. In Mexico, several medicinal plants are used to alleviate insomnia, depressed mood and anxiety[17]. The herbal formulations claimed to enhance physical endurance, mental functions and non-specific resistance of the body have been termed as adaptogen[13]. Various plants are being used in complementary and alternative medicines for management of stress[18]. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body. Herbal medicines are known to act synergistically in combination[13].

The objective of this work is isolation of forskolin by column chromatography separation and TLC purification of fractions from hydroalcoholic extract and development of indigenous botanical resources as in-expensive source for standardized crude antistress drug. Antistress activity was assessed by elevated plus maze test and forced swimming test.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant material

The rhizomes of *Coleus forskohlii* was collected from the High Altitude Plant Physiology Research Centre (HAPPRC), Srinagar, Uttarakhand, India in the month of March 2011 and deposited in National Botanical Research Institute, Lucknow, India for taxonomical authentication. The rhizomes were air dried for 20 days and crushed into coarse powder with a grinder and passed through 40-mesh sieve. They were stored in a well closed container separately.

Corresponding Author:
Neha Tiwari,
A. N.D. College of Pharmacy Babhnan, Gonda,
Uttar Pradesh, India.
Mobile no.: +91-9978943280 Fax: +91-05546-256894
Email: neha_ethano@rediffmail.com

2.2 Animals

Swiss albino mice (20-25 g) were bought from the Animal House of Siddhartha Institute of pharmacy, Dehradun, Uttarakhand, India. The animal room was maintained on a 12-h light and dark cycle with a constant temperature and humidity. Standard pellet food and tap water were available ad libitum. All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the Department of Pharmacology of the Siddhartha Institute of Pharmacy, Dehradun, Uttarakhand, India. The experiments were conducted in a sound proof laboratory. All the experimental procedures and protocols (IAEC / CPCSEA Proposal number 03) used in the study were reviewed by the Institutional Animal Ethics Committee.

2.3 Extraction and isolation of compound from rhizomes of *Coleus forskohlii*

The air dried and powder rhizome (2 kg) of *Coleus forskohlii* was extracted with ethanol: water (60:40, v/v) under reflux at room temperature. After exhaustive extraction the extracts were concentrated under reduce pressure (Burton et al., 2010) to give dark brown syrup of approximately 783 g (39.15% based on the dry weight of whole plants). The extract was suspended in n-hexane–MeOH–H2O (19:19:2, v/v/v), giving n-hexane (5.05 g) and MeOH–H_2O fractions. The latter fraction, after evaporation of the solvent, was partitioned in EtOAc–H_2O (1:1, v/v), yielding EtOAc (3.37 g) and H_2O fractions. The H_2O fraction was extracted with n-butanol (n- BuOH), which yielded n-BuOH (0.15 g) and residual H_2O fractions (0.03 g). The EtOAc-soluble fraction was subjected to silica gel column chromatography 60-G (500 g Merck)[19] and successively eluted with a stepwise gradient of n-hexane–EtOAc (9:1→0:1, v/v) which gave three major fractions, fractions A' (338.2 mg), B'(393.0 mg), and C'(152.7 mg), as arranged with the increasing order of polarity. Column fractions were analyzed by TLC on silica gel 60-G (0.2 mm thick)[19] and fractions with a similar TLC pattern were pooled and concentrated. The fraction A' (4 g) was further subjected for column chromatography on silica gel eluted with $CHCl_3$-acetone (1:0→0:1, v/v) yielded single compound (175 mg) confirmed by Rf value of TLC (n-hexane:ethyl acetate/ 6:4, v/v)[6].

2.4 Spectroscopic authentication

Spectral analysis FTIR, ^{1}H NMR and Mass of isolated compound was performed at Sophisticated Analytical Instrument Facility, Central Drug Research institute, Lucknow, India to authenticate the functional group, molecular weight and molecular formula. FTIR spectra were recorded on Perkin Elmer Spectrum RX1 using alcohol. ^{1}H NMR (400 MH_z) spectra were recorded on Bruker Advance 400 in $CDCl_3$ with tetramethylsilane as internal standard. The FAB mass spectra were recorded on a Jeol SX102/Da-600 mass spectrophotometer/Data System using Argon/Xenon 6 kv, 10 mA0 as the FAB gas. The accelerating voltage was 10 kV.

2.5 Experimental groups

The experimental groups of mice were divided in to five groups. Group I was control and was given normal saline in a dose of 10 ml/kg, p.o. Group II was a positive control treated with standard drug, Diazepam (2 mg/kg, i.p.), suspended in the vehicle. Group III-V was treated as test groups and was given isolated Forskolin at different dose. All the test solutions, standard drug and control were administered orally 30 minutes prior to experiment.

2.6 Assessment of antistress activity

2.6.1 Elevated plus maze test

Antistress activity was evaluated using the elevated plus maze model. The elevated plus maze consisted of two open arms (50 cm x 10 cm) crossed with two closed arms (50 cm x 10 cm x 40 cm). The arms were connected together with a central square (10 cm x 10 cm). The apparatus was elevated to the height of 70 cm in a dimly illuminated room. Mice were divided into groups of five, and received the compounds Forskolin at different doses viz .25, 50 and 100 mg/kg, saline (10 ml/kg, p.o.) as control and diazepam (2 mg/kg i.p.) was used as standard drug. One hour post administration, each mouse was placed individually at the center of the elevated maze. The time duration of the stay in open arm was noted[16].

2.6.2 Forced swimming test

The FST is the most widely used pharmacological *in vivo* model for assessing antistress activity. The swimming test includes two exposures to a water tank, spaced 1 day apart. For these experiments, the tank sizes were 22 cm in diameter and 40 cm in height. The tank had a rounded lid and contained 20 cm, high fresh water at 25 °C. Mice were divided into groups of five, and received the compounds Forskolin at different doses viz .25, 50 and 100 mg/kg, saline (10 ml/kg, p.o.) as control and diazepam (2 mg/kg i.p.) was used as standard drug. During the first exposure, mice not yet treated were placed in the tank and left there for 15 min. During the second exposure (test session), 30 min after the treatment, mice were placed in the tank and left there for 5 min during which their immobility time was observed. A mouse was considered immobile when it remained floating in the water, without struggling, making only very slight movements necessary to keep its head above the water[16].

2.7 Statistical analysis

All data are expressed as the mean ± S.E.M and were obtained from four distinct experiments. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's test. The significant difference was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Extraction and isolation

The compound was extracted with ethanol: water (60:40) at room temperature. The quantity of extract obtained after concentration under reduce pressure was approximately 783 g (39.15% based on the dry weight of whole plant). The physical appearance of concentrated extract was dark brown syrupy. Single compound was isolated from fraction A confirmed by TLC. The TLC examination confirmed that it was a single compound (Figure 1). The Rf value of isolated compound was found to be 0.48[6].

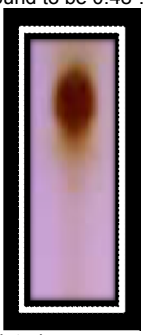

Figure 1: TLC of isolated compound from *Coleus forskohlii* rhizomes (Forskolin)

3.2 Structure elucidation of isolated compound from rhizomes of *Coleus forskohlii*

The FTIR spectra of isolated compound showed absorption peaks located at 1035.71, 1383.73 and 1624.72 in the region 500–4000cm^{-1}. The peaks corresponding to presence of fatty acids, carbonyl groups, flavanones and amide band of proteins (Figure 2)[20].

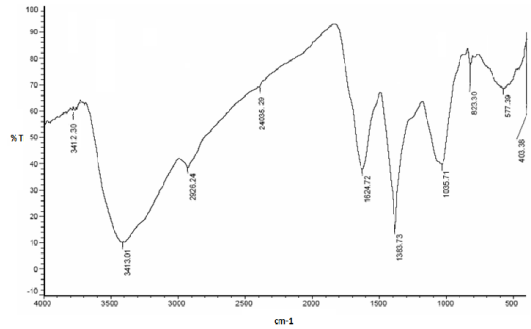

Figure 2: FTIR spectra of Forskolin

^{1}H NMR spectra show triplet at δ 3.30 ppm was assigned to 8 protons of fused aromatic compound.^{1}H NMR displayed 6 protons

of terminal methyl at δ 1.44 ppm. Chemical shift of the 2 protons adjacent phenol group appeared at δ 2.34 ppm (Figure 3)[20].

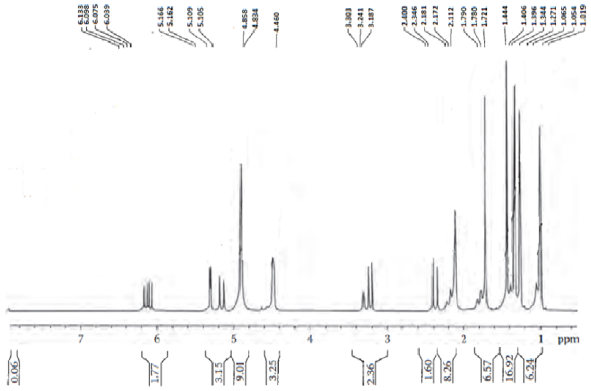

Figure 3: H-NMR spectra of Forskolin

Forskolin with a mass-to-charge-ratio of compound m/z 409(M^+), 437 (M^+ – Na) and 313 (MH^+ – $2H_2O$ – AcOH) (Figure 4).[20]

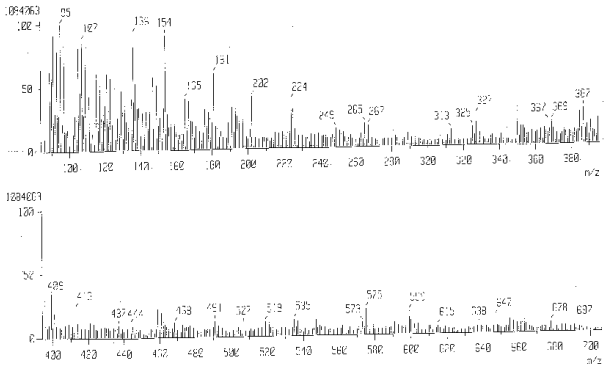

Figure 4: Mass spectra of Forskolin

From the spectral data the structure of the isolated compound was elucidated as Forskolin with molecular formula ($C_{22}H_{34}O_7$) (Figure 5).

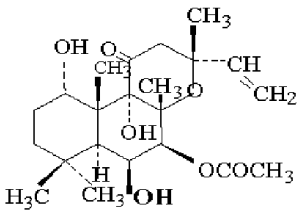

Figure 5: Chemical structure of Forskolin

3.3 Elevated plus maze test

Mice received the standard diazepam showed significant ($p < 0.05$) time spent 311.5 ± 5.36 sec in open arm at dose of 2 mg/kg i.p. (Figure 6). Time spent by mice in open arm treated with Forskolin was 119.0 ± 1.93, 135.3 ± 2.27 and 152.5 ± 1.87 second at the dose of 25, 50 and 100 mg/kg respectively. While the time spent in open arm by control group was 78.5 ± 2.96 second (Table 1).

In the closed arm entry (Figure 7) the time spent by mice treated with Forskolin was ($p < 0.05$) 591.3 ± 10.33, 562.8 ± 6.81and 546.8 ± 40second at the dose of 25, 50 and 100 mg/kg respectively. In other hand time spent by control group was 613.2 ± 5.91 second while time spent by standard group was 512.7 ± 2.88 second (Table 2).

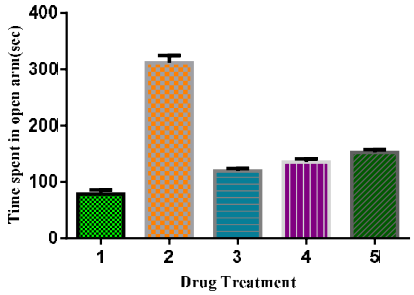

Figure 6: Time spent (sec) in open arm of elevated plus maze model (Bar 1: Control; Bar 2: Standard 2mg/kg; Bar 3: Forskolin 25mg/kg; Bar 4: Forskolin 50 mg/kg; Bar 5: Forskolin 100mg/kg).Value represent mean ± S.E.M, $p < 0.05$, n=6

Table 1: Effect of isolated compound Forskolin in elevated plus maze model (open arm) (n=6)

Time spent (s)				
Control	Standard Diazepam (mg/kg)	Forskolin (mg/kg)		
	2	25	50	100
Open arm	Open arm	Open arm	Open arm	Open arm
78.5 ± 2.96	311.5 ± 5.36*	119.0 ± 1.93	135.3 ± 2.27*	152.5±1.87*

Note: The observations are mean ± S.E.M and data were analyzed using graph prism pad as statistical unit.
** p < 0.05 (ANOVA followed by Dunnett's test, n = number of mice, p.o.: per oral*

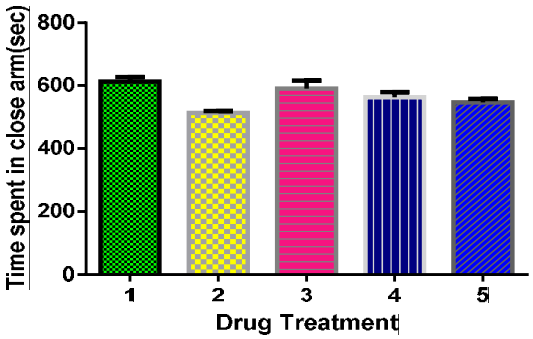

Figure 7: Time spent (sec) in close arm of elevated plus maze model (Bar 1: Control; Bar 2: Standard 2mg/kg; Bar 3: Forskolin 25mg/kg; Bar 4: Forskolin 50 mg/kg; Bar 5: Forskolin 100mg/kg).Value represent mean ± S.E.M, $p < 0.05$, n=6

Table 2: Effect of isolated compound Forskolin in elevated plus maze model (closed arm) (n=6)

Time spent (s)				
Control	Standard Diazepam (mg/kg)	Forskolin (mg/kg)		
	2	25	50	100
Close arm	Close arm	Close arm	Close arm	Close arm
613.2 ± 5.91	512.7 ± 2.88*	591.3 ± 10.33	562.8 ± 6.81	546.854 ± 40*

Note: The observations are mean ± S.E.M and data were analyzed using graph prism pad as statistical unit.
** p < 0.05 (ANOVA followed by Dunnett's test), n = number of mice, p.o.: per oral*

3.4 Forced Swimming Test

The control animals remained immobile for most of the time during the test session and immobility time of control group was 184.1 ± 0.22 second. Immobility time (Figure 8) of mice treated with

Forskolin was (p < 0.05) 184.7 ± 0.19, 194.9 ± 0.48 and 198.7 ± 0.60 second at the dose of 25, 50 and 100 mg/kg respectively. While mice treated with standard diazepam was 520.8 ± 0.21 second at the dose of 2 mg/kg (Table 3).

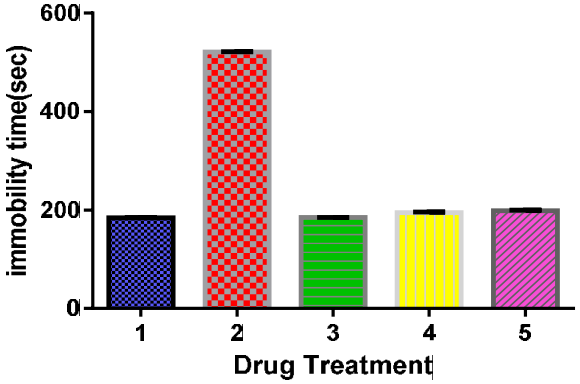

Figure 8: Immobility time (sec) in forced swimming test (Bar 1: Control; Bar 2: Standard 2mg/kg; Bar 3: Forskolin 25mg/kg; Bar 4: Forskolin 50 mg/kg; Bar 5: Forskolin 100mg/kg).Value represent mean ± S.E.M, p < 0.05, n=6

Table 3: Effect of isolated compound Forskolin in Forced swimming test (n=6)

Immobility Time (s)				
Control	**Standard Diazepam (mg/kg)**	**Forskolin (mg/kg)**		
	2	**25**	**50**	**100**
184.1 ± 0.22	520.8 ± 0.2*	184.7 ± 0.19	194.9 ± 0.48	198.7 ± 0.60*

Note: The observations are mean ± S.E.M and data were analyzed using graph prism pad as statistical unit.
n = number of mice, p.o.: per oral

In the EPM, besides decreased open arm exploration, Forskolin exhibited significant (p < 0.05) decreased closed arm entries and increased time spent in the open arm of Swiss albino mice. This suggests the highest anti stress effect was exhibited by Forskolin at a dose of 100 mg/kg. Moreover the compound showing dose dependent activity. All these results suggested that the extract is having anti stress activity.

In the swimming endurance test, the mice were forced to swim in a restricted space from which they cannot escape. This induces a characteristic behavior of immobility. It has been well-demonstrated that drugs with antistress activity increase swimming endurance.[21] Results of the swimming endurance test indicate clearly that the Forskolin was the most active constituent that reducing the immobility time significantly (p < 0.05) at a dose of 100 mg/kg. In the FST all the doses administered were able to reduce immobility time and simultaneously to enhance swimming.

4. CONCLUSION

The present study investigated the pultative behavioral effects of Forskolin. In conclusion, Forskolin has been used as an anti-stress remedy in folk medicine. Forskolin was successfully isolated from hydroalcoholic extract of rhizome using column chromatography, TLC purification. Spectral analysis result also revealed that isolated compound is Forskolin. Results of both model revealed that Forskolin having higher antistress activity at the dose of 100 mg/kg which discover the antistress activity of Forskolin. This potent compound from *Coleus forskohlii* may have potential for future development of anti- stress therapeutics. The result also revealed that Forskolin is a novel compound for the treatment of neurobiological disorder (stress).

REFERENCES

1. Dalsenter PS, Muller JC, et al "*In vivo* and *in vitro* reproductive toxicology" J Ethnopharmacol, 2009, 121: 229-233.
2. Kienzle S, Horn S. Herbal medicines for neurological diseases, Curr Opin Investig Drugs, 2002, 3: 763-767.
3. Zhang ZJ. Effects of herbal extracts and constituents in animal models of psychiatric disorders, life Sci, 2004, 75: 1659–1699.
4. Patil R, Ahirwar B, Ahirwar D. Current status of Indian medicinal plants with antidiabetic potential: a review, Asian Pac J Trop Biomed, 2011, 2: S291-S298.
5. Metzger H, Linder E. The positive inotropic-acting Forskolin, a potent adenylatecyclase activator, Drug Res, 1981, 31: 1248-1250.
6. Ugur C. The Strategies for Production of Forskolin vis-a-vis Protection Against Soil Borne Diseases of the Potential Herb *Coleus forskohlii* Briq, European J Med Plants, 2011, 1: 1-9.
7. Barkat AK, Naveed A, et al "Botanical Description of *Coleus forskohlii*: A Review" J. Med. Plants Resmb 2012, 6: 4832-4835.
8. Zeigler FE, Jaynes BH, Saindane MT. A synthetic route to forskolin, J Am Chem Soc, 1987, 109: 811-816.
9. Shah V, Bhat SV, et al "The occurrence of forskolin in Labiatae" Planta Med 1980, 39: 183-185.
10. Asad M, Singh AK, Dhamanigi SS. Anti stress activity of hydroalcoholic extract of *Eugenia caryophyllus*(clove) buds, Indian J Pharmacol, 2009, 41: 28-31.
11. Shankarnarayana BS, Srikumar BN, et al "*Euphorbia hirta* reverses chronic stress-induced anxiety and mediates its action through the $GABA_A$ receptor benzodiazepine receptor-CL^- channel complex" J Neural Transm, 2008, 115: 35-42.
12. Panossian A, Wikman G, Wagner H. Plant adaptogens III. Earlier and more recent aspects and concepts on their mode of action, Phytomedicine, 1999, 4: 287-300.
13. Desai SK, Desai SM, et al "Antistress activity of *Boerhaavia diffusa* root extract and a polyherbal formulation containing *Boerhaavia diffusa* using cold restraint stress model, Int J Pharm and Pharm Sci, 2011, 3: 130-132.
14. Marles RJ, Farnswort NR. Antidiabetic plants and their active constituents, Phytomedicine, 1995, 2: 137-189.
15. Gomes NGM, Campos MG, et al "Plants with neurobiological activity as potential targets for drug discovery" Prog Neuro-Psychopharmacol Boil Psychi, 2009, 33: 1372-1389.
16. Mora S, Millan R, et al "The hydroalcoholic extract of *Salvia elegans* induces anxiolytic and antidepressant like effects in rats" J Ethnopharmacol, 2009, 106: 76-81.
17. Zolla C. Traditional medicine in Latin America with particular reference to Mexico, J Ethnopharmacol, 1980, 2: 37-41.
18. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activities of different extracts from *Citrus paradisi* var. *Duncan,* Asia Pac J Chem Eng, 2010, 3: 98-100.
19. Burton G, Dansey MV, et al "Synthasis and $GABA_A$ receptor activity of A-homo analogues of neuroactive steroids, Eur J Med Chem, 2010, 45: 3063-3069.
20. Pavia DL, Lampman GM, Kriz GS. Introduction to spectroscopy. 3rd edition, Thomson brooks Ltd, United State, 2001, 40-44.
21. Varsha G, Bharatkumar P, Nikunj P. Antistress activity of *Argyreia speciosa* roots in experimental animals, J Ayurveda Integr Med, 2011, 3: 129-136.