



ANXIOLYTIC ACTIVITY OF AQUEOUS AND ALCOHOLIC WHOLE-PLANT EXTRACTS OF GRANGEA MADERASPATANA L. IN MICE AND RATS

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ABSTRACT

Background: Grangea maderaspatana (L.) Poir. (Asteraceae), traditionally used as a stomachic, sedative and carminative, has been pharmacologically studied for its oestrogenic, antifertility, analgesic, anti-inflammatory, antiarthritic, cytotoxic, antioxidant, hepatoprotective and antimicrobial activity, but its anxiolytic potential has not been systematically evaluated. **Aim:** To evaluate the anxiolytic activity of aqueous (AQEWGM) and alcoholic (ALEWGM) whole-plant extracts of G. maderaspatana. **Materials and Methods:** AQEWGM and ALEWGM were prepared by cold maceration and screened for phytoconstituents. Acute oral toxicity was assessed in mice up to 2000 mg/kg (OECD 420), and three doses (100, 200 and 400 mg/kg, p.o.) were selected. Anxiolytic activity was evaluated using the staircase, elevated plus-maze, hole-board and mirror-chamber tests in mice and rats, with diazepam (2 mg/kg) as standard. **Results:** Both extracts were non-toxic up to 2000 mg/kg. AQEWGM and ALEWGM (100-400 mg/kg) significantly increased steps climbed and rearings in the staircase test; increased open-arm entries/time and reduced closed-arm entries/time in the elevated plus-maze without altering total arm entries; increased head-dips, time in head-dips and rearings while reducing latency to first head-dip and defecation in the hole-board test (with a comparatively weaker effect of ALEWGM on head-dipping itself); and reduced latency to enter while increasing entries into, and time spent in, the mirror chamber - effects broadly comparable to diazepam. AQEWGM was consistently more active than ALEWGM across all four models. Phytochemical screening showed carbohydrates, steroids/triterpenoids and flavonoids in both extracts, with saponins additionally present in ALEWGM. **Conclusion:** Whole-plant extracts of G. maderaspatana possess significant anxiolytic-like activity across four behavioural paradigms, plausibly related to their terpenoid, steroid, flavonoid and saponin content, supporting further mechanistic investigation of the plant as a source of anxiolytic leads.

KEYWORDS: Grangea maderaspatana; Anxiolytic activity; Elevated plus-maze; Hole-board test; Mirror-chamber test.

INTRODUCTION

Anxiety disorders are among the most prevalent categories of mental illness, characterised by excessive and unrealistic worry about everyday events that may be diffuse or directed at specific objects and situations.^[1] They carry a substantial burden in terms of reduced quality of life,^[2] impaired social and occupational functioning and high rates of comorbidity,^[3] and are highly prevalent in the general population,^[4,5] a burden confirmed across a broad meta-analytic literature.^[6]

Current pharmacotherapy for anxiety relies heavily on benzodiazepines and other GABAergic agents, which

remain effective but are limited on chronic use by sedation, tolerance, dependence and withdrawal;^[7] a substantial proportion of patients also respond inadequately to existing treatment,^[8] and diagnosis and management of anxiety disorders remain clinically challenging.^[9] This has sustained interest in plant-derived anxiolytics that might offer a more favourable safety profile.

Grangea maderaspatana (L.) Poir. (Asteraceae), known locally as Davana (Kannada) or Mustaru (Hindi), is a prostrate annual herb that grows as a common weed of rice fields, wetlands and riverbanks throughout India,

Pakistan, Bangladesh, Africa and Indochina.^[10] The plant is traditionally used as a stomachic, sedative and carminative, and its root is used as an appetiser, astringent, diuretic and anthelmintic, with reported traditional use in disorders of the chest and lungs, headache, paralysis, rheumatism and muscle pain.^[10-14] Phytochemical investigations have identified flavonoids, diterpenes, an eudesmanolide, steroids and clerodane derivatives in the plant,^[15-18] and pharmacological studies have reported oestrogenic, antifertility, analgesic, anti-inflammatory,¹⁹ antiarthritic, cytotoxic, antioxidant, hepatoprotective and antimicrobial activity for various extracts of the species. Despite this pharmacological breadth and its traditional use as a sedative, the anxiolytic potential of *G. maderaspatana* whole-plant extracts had not been systematically investigated.

The present study was therefore undertaken to evaluate the anxiolytic activity of aqueous (AQEWGM) and alcoholic (ALEWGM) whole-plant extracts of *G. maderaspatana* using the staircase, elevated plus-maze, hole-board and mirror-chamber models, and to relate this activity to the preliminary phytochemical profile of the two extracts.

MATERIALS AND METHODS

Collection and authentication of plant material

The whole plant of *G. maderaspatana* was collected from Raichur, Karnataka, India, in October 2019 and authenticated by Dr. Pramod Kumar, Department of Pharmacognosy, V. L. College of Pharmacy, Raichur.

Preparation of extracts

The shade-dried, coarsely powdered whole plant was extracted separately with ethanol and with water by cold maceration for 10 days at room temperature ($25 \pm 2^\circ\text{C}$); 10 ml of chloroform was added as a preservative during aqueous extraction. Each extract was filtered and concentrated on a water bath maintained below 50°C to yield the alcoholic extract (ALEWGM) and the aqueous extract (AQEWGM), which were stored in a desiccator until use.

ANIMALS

Albino mice (20-25 g) and albino rats (125-200 g) of either sex were procured from Shri Venkateshwara Enterprises, Bengaluru, and acclimatised for 7 days under standard husbandry conditions ($26 \pm 2^\circ\text{C}$, 45-55% relative humidity, 12:12 h light/dark cycle) with free access to standard pellet feed and water *ad libitum*. Animals were fasted overnight before dosing, with free access to water, and experiments were conducted during the light period. All procedures were approved by the Institutional Animal Ethics Committee of V. L. College of Pharmacy, Raichur (CPCSEA registration no. 557/PO/Re/S/02/CPCSEA, dated 27.11.2017) and conducted in strict compliance with CPCSEA guidelines.

Drugs and chemicals

Diazepam (Ranbaxy Laboratories Ltd., Mumbai) was used as the standard anxiolytic agent. A 2% w/v gum acacia suspension in distilled water served as the vehicle for the extracts and as the normal control.

Preliminary phytochemical screening

AQEWGM and ALEWGM were subjected to standard qualitative tests for the presence of alkaloids, carbohydrates, steroids/triterpenoids, saponins, tannins, flavonoids, proteins/amino acids, glycosides and fixed oils/fats.^[30]

Acute oral toxicity study

Acute oral toxicity of AQEWGM and ALEWGM was determined in albino mice according to OECD Guideline No. 420 (up-and-down procedure) up to a limit dose of 2000 mg/kg. Animals were observed for behavioural changes and mortality for 48 h. As no toxicity was seen at the limit dose, three doses – 1/20th (low, 100 mg/kg), 1/10th (medium, 200 mg/kg) and 1/5th (high, 400 mg/kg) of the 2000 mg/kg dose – were selected for the pharmacological studies.

Evaluation of anxiolytic activity

For each of the four models described below, animals were divided into eight groups of six ($n = 6$): normal control (2% gum acacia, *p.o.*), standard (diazepam 2 mg/kg, *p.o.*; *i.p.* in the hole-board test), and AQEWGM or ALEWGM at 100, 200 or 400 mg/kg (*p.o.*), dosed once daily for 7 consecutive days. Testing was carried out on day 8, 1 h after the respective treatment.

Staircase test:^[31] Mice were placed at the foot of a five-step staircase (steps 2.5 cm high, 10 cm wide and 7.5 cm deep) and the total number of steps climbed and total number of rearings were recorded over a 3 min test period.

Elevated plus-maze (EPM) test: 32 Mice were placed at the centre of a plus-shaped maze comprising two open arms and two enclosed arms (16×5 cm, walls 12 cm high on the closed arms) extending from a common central platform (5×5 cm), elevated 25 cm above the floor, facing an enclosed arm. The number of entries into, and time spent in, the open and closed arms, and the time spent on the central platform, were recorded over a 5 min test period.

Hole-board test:^[33] Rats were placed individually in a wooden chamber ($40 \times 40 \times 25$ cm) with 16 floor holes (3 cm diameter), elevated from the ground. Latency to the first head-dip, the total number and total duration of head-dips, the number of rearings and the number of faecal boli (defecation units) were recorded over a 5 min test period.

Mirror-chamber test:^[34] Mice were placed at a fixed corner of an apparatus comprising a mirrored cube (13 cm side, open on one face) positioned within an outer

wooden chamber (40 × 40 × 30.5 cm) so as to form a 5 cm corridor around the cube. Latency to enter the mirrored chamber, the number of entries, and the total time spent in the chamber were recorded over a 5 min test period.

Statistical analysis

Data are expressed as Mean ± SEM (n = 6). The *P* value < 0.05 was considered significant. The one-way ANOVA followed by Dunnett's multiple comparison test has been performed.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening (Table 1) showed that both AQEWGM and ALEWGM contained carbohydrates, steroids/triterpenoids and flavonoids; saponins were detected only in ALEWGM, while alkaloids, tannins, proteins/amino acids and fixed oils/fats were absent from both extracts.

Table 1: Preliminary phytochemical screening of AQEWGM and ALEWGM.

Phytoconstituent class	AQEWGM	ALEWGM
Alkaloids	–	–
Carbohydrates	+	+
Steroids and triterpenoids	+	+
Saponins	–	+
Tannins	–	–
Flavonoids	+	+
Glycosides	+ a	+ a
Proteins and amino acids	–	–
Fixed oils and fats	–	–

+ = present; – = absent. a Positive with Baljet's test; Legal's test negative for both extracts.

Acute oral toxicity

No mortality or overt behavioural toxicity was observed with either extract up to the limit dose of 2000 mg/kg, indicating a favourable acute safety margin for both AQEWGM and ALEWGM. Low (100 mg/kg), medium (200 mg/kg) and high (400 mg/kg) doses were accordingly selected for the pharmacological studies.

Anxiolytic activity

Staircase test: Both extracts produced a significant, dose-dependent increase in the number of steps climbed and number of rearings relative to control, comparable in direction to diazepam, with the exception of the low dose of ALEWGM, which did not significantly alter the number of rearings (Table 2).

Table 2: Effect of AQEWGM and ALEWGM on the staircase test in mice.

Treatment	Steps climbed (3 min)	No. of rearings (3 min)
Normal control (2% gum acacia)	7.66 ± 0.49	8.33 ± 0.33
Standard (Diazepam 2 mg/kg)	32.67 ± 0.88***	24.33 ± 0.49***
AQEWGM 100 mg/kg	16.00 ± 0.43***	15.00 ± 1.39***
AQEWGM 200 mg/kg	25.83 ± 0.58***	19.67 ± 0.42***
AQEWGM 400 mg/kg	29.33 ± 0.99***	22.00 ± 0.37***
ALEWGM 100 mg/kg	12.50 ± 0.58***	10.00 ± 0.37 ns
ALEWGM 200 mg/kg	19.00 ± 0.87***	14.67 ± 0.33***
ALEWGM 400 mg/kg	22.67 ± 0.67***	17.33 ± 0.42***

n = 6; values are mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001 vs. normal control (statistical test to be confirmed by authors, see Materials and Methods); ns = not significant.

Elevated plus-maze test: AQEWGM and ALEWGM at all three doses significantly increased the number of entries into, and time spent in, the open arms, and significantly decreased entries into and time spent in the closed arms, without altering the total number of arm

entries – indicating an anxiolytic rather than a non-specific locomotor effect (Table 3, Figure 1). A significant reduction in time spent on the central platform was seen with diazepam but not with either extract.

Table 3: Effect of AQEWGM and ALEWGM on the elevated plus-maze test in mice.

Treatment	Open-arm entries	Closed-arm entries	Open-arm time (s)	Closed-arm time (s)	Central platform (s)	Total entries
Normal control	6.83±0.48	16.33±0.76	36.67±1.17	161.83±1.85	98.50±1.91	24.17±0.87
Standard	15.00±0.73***	12.16±0.70***	127.00±1.80***	88.83±2.24***	95.33±1.54***	26.83±0.83

Treatment	Open-arm entries	Closed-arm entries	Open-arm time (s)	Closed-arm time (s)	Central platform (s)	Total entries
(Diazepam 2 mg/kg)						ns
AQEWGM 100 mg/kg	13.00±0.57***	9.33±0.55***	97.67±1.62***	48.66±2.01***	128.50±1.28***	22.33±0.71 ns
AQEWGM 200 mg/kg	14.50±0.76***	9.50±0.42***	115.20±3.62***	68.00±0.93**	136.83±2.15***	23.67±1.12 ns
AQEWGM 400 mg/kg	15.00±0.57***	9.83±0.60***	124.20±2.62***	69.50±1.28***	135.67±3.03***	26.17±1.12 ns
ALEWGM 100 mg/kg	12.83±0.60***	9.00±0.57***	95.67±1.02***	42.33±2.63***	136.33±3.93***	22.00±1.07 ns
ALEWGM 200 mg/kg	13.50±0.42***	9.33±0.33***	102.33±1.80***	58.33±1.80***	140.50±1.57***	22.50±0.43 ns
ALEWGM 400 mg/kg	13.67±0.71***	9.50±0.50***	104.00±1.46***	61.66±1.60***	138.00±1.81***	22.67±0.71 ns

n = 6; values are mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 vs. normal control (statistical test to be confirmed by authors, see Materials and Methods); ns = not significant.

Hole-board test: AQEWGM significantly increased the number and duration of head-dips at the medium and high doses, and significantly reduced the latency to first head-dip and increased rearings at all doses; the low dose was without effect on head-dips. ALEWGM did not

significantly alter the number or duration of head-dips at any dose tested, but significantly reduced latency to first head-dip and increased rearings at all doses. Both extracts significantly reduced the number of defecation units (Table 4).

Table 4: Effect of AQEWGM and ALEWGM on the hole-board test in rats.

Treatment	No. of head dips (5 min)	Time in head dips (s)	Latency of first head dip (s)	No. of rearings	No. of defecation units
Normal control	23.83±0.75	42.67±0.76	55.66±1.43	7.17±0.65	3.67±0.49
Standard (Diazepam 2 mg/kg)	36.67±1.56***	61.50±0.99***	11.00±0.58***	23.17±0.79***	0.50±0.34***
AQEWGM 100 mg/kg	24.83±1.01 ns	47.00±0.58*	37.50±0.92***	14.67±1.12***	1.83±0.31**
AQEWGM 200 mg/kg	28.17±0.54**	49.33±1.69***	23.00±0.58***	18.00±0.45***	1.50±0.22***
AQEWGM 400 mg/kg	31.83±0.65***	54.17±1.35***	15.00±1.03***	21.00±1.77***	0.67±0.33***
ALEWGM 100 mg/kg	24.00±0.73 ns	43.67±0.92 ns	35.50±2.58***	14.67±0.84***	1.83±0.31**
ALEWGM 200 mg/kg	25.50±0.67 ns	44.33±0.71 ns	26.17±1.25***	17.67±0.61***	1.33±0.21***
ALEWGM 400 mg/kg	27.00±0.37 ns	46.50±0.76 ns	24.17±0.98***	20.33±0.71***	0.83±0.31***

n = 6; values are mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 vs. normal control (statistical test to be confirmed by authors, see Materials and Methods); ns = not significant.

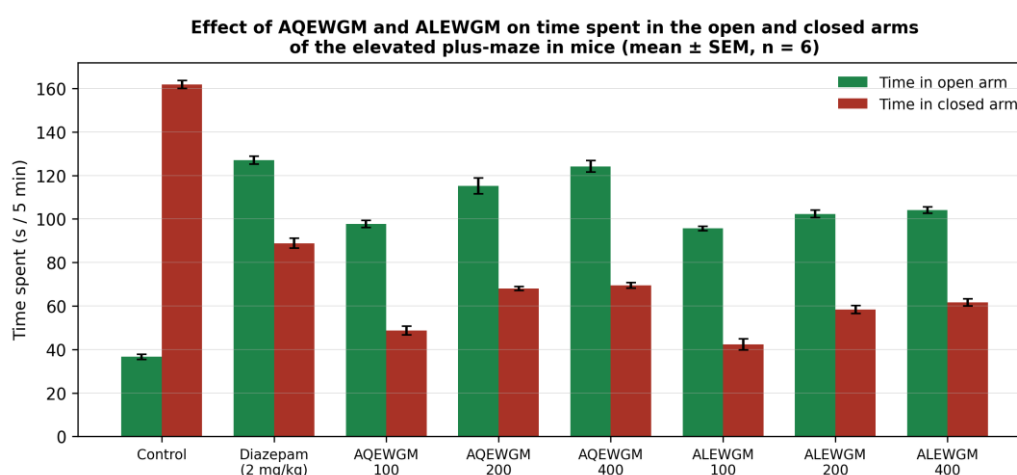
Mirror-chamber test: AQEWGM and ALEWGM significantly reduced the latency to enter the mirror chamber and significantly increased both the number of

entries into, and time spent in, the chamber at all three doses tested, in a manner comparable to diazepam (Table 5).

Table 5: Effect of AQEWGM and ALEWGM on the mirror-chamber test in mice.

Treatment	Latency to enter (s)	Time spent in chamber (s)	No. of entries
Normal control	142.00 ± 0.73	39.33 ± 0.76	1.67 ± 0.21
Standard (Diazepam 2 mg/kg)	2.80 ± 0.21***	138.00 ± 1.95***	8.50 ± 0.76***
AQEWGM 100 mg/kg	69.17 ± 1.47***	106.80 ± 0.65***	6.33 ± 0.67***
AQEWGM 200 mg/kg	54.00 ± 1.98***	112.00 ± 0.89***	6.83 ± 0.48***
AQEWGM 400 mg/kg	46.83 ± 0.95***	126.30 ± 0.88***	7.17 ± 0.48***
ALEWGM 100 mg/kg	98.00 ± 0.37***	96.00 ± 0.58***	4.00 ± 0.58*
ALEWGM 200 mg/kg	86.17 ± 0.95***	105.50 ± 0.76***	4.67 ± 0.49**
ALEWGM 400 mg/kg	79.00 ± 1.16***	117.80 ± 0.95***	5.17 ± 0.61***

n = 6; values are mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 vs. normal control (statistical test to be confirmed by authors, see Materials and Methods); ns = not significant.

**Figure 1: Effect of AQEWGM and ALEWGM on time spent in the open and closed arms of the elevated plus-maze in mice (mean ± SEM, n = 6).**

DISCUSSION

The staircase test is a simple, rapid screening model for anxiolytic activity in rodents, in which step-climbing reflects exploratory/locomotor activity while rearing is taken as an index of the anxiety state; a reduction in rearing together with maintained or increased step-climbing is generally interpreted as anxiolytic.^[31] In the present study, AQEWGM and ALEWGM increased both parameters relative to control, consistent with an anxiolytic-like action, though the profile diverged somewhat from the classical benzodiazepine pattern, in which rearing is typically reduced.

The elevated plus-maze exploits the approach-avoidance conflict generated when rodents are exposed to an elevated, open environment; anxiolytic agents increase the time spent and entries made into the open arms, while anxiogenic agents have the opposite effect, and the model is sensitive to compounds acting at the GABA-benzodiazepine complex as well as to non-benzodiazepine anxiolytics.^[32] The significant, dose-dependent increase in open-arm entries and time, together with reduced closed-arm entries and time and an

unchanged total number of entries, is consistent with a genuine anxiolytic effect of both extracts rather than a change in general locomotor activity.

In the hole-board test, head-dipping behaviour is considered sensitive to the emotional state of the animal, with an anxiolytic state generally reflected by an increase in head-dipping.^[33] AQEWGM increased head-dips, time spent head-dipping and rearings, and reduced latency to first head-dip and defecation – a full anxiolytic-type profile. ALEWGM reduced latency to first head-dip, increased rearings and reduced defecation, but did not significantly alter the number or duration of head-dips, suggesting a comparatively weaker effect of the alcoholic extract in this particular paradigm.

The mirror-chamber test relies on the approach-avoidance conflict generated by a novel stimulus – in this case the animal's own reflection – with anxiolytic agents reducing the latency to enter and increasing the number of entries and time spent in the chamber.^[34] Both extracts reduced latency to enter and increased entries and time

spent at all three doses tested, an effect comparable to diazepam.

Anxiolytic activity has been linked mechanistically to enhancement of GABAergic transmission and to interactions with noradrenergic, serotonergic and other CNS mediator systems.^[20-29] Given this multiplicity of potential targets, a single unifying mechanism cannot be assigned from behavioural data alone, and the present findings should be interpreted as evidence of anxiolytic-like activity rather than proof of a specific receptor mechanism.

Preliminary phytochemical screening identified carbohydrates, steroids/triterpenoids and flavonoids in both extracts, with saponins additionally present in ALEWGM. Terpenoids, steroids, flavonoids and saponins have each been individually associated with anxiolytic activity in other plants, and their co-occurrence in *G. maderaspatana* provides a plausible, though not yet mechanistically confirmed, phytochemical basis for the anxiolytic-like effects observed here. AQEWGM showed a consistently greater effect than ALEWGM across all four models, suggesting a relatively higher concentration or greater bioavailability of the active constituent(s) in the aqueous extract.

CONCLUSION

Aqueous (AQEWGM) and alcoholic (ALEWGM) whole-plant extracts of *Grangea maderaspatana* were non-toxic up to 2000 mg/kg in mice and produced significant, dose-dependent anxiolytic-like activity across the staircase, elevated plus-maze, hole-board and mirror-chamber models, with AQEWGM consistently more active than ALEWGM. These effects are plausibly attributable to the terpenoid, steroid, flavonoid and saponin content of the extracts, supporting further bioactivity-guided fractionation and mechanistic studies of *G. maderaspatana* as a source of anxiolytic leads.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Barlow DH. Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory. *Am Psychol.*, 2000; 55(11): 1247-63.
2. Mendlowicz MV, Stein MB. Quality of life in individuals with anxiety disorders. *Am J Psychiatry*, 2000; 157: 669-82.
3. Wittchen HU, Fehm L. Epidemiology, patterns of comorbidity and associated disabilities of social phobia. *Psychiatr Clin North Am.*, 2001; 24(4): 617-41.
4. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*, 2005; 62(6): 593-602.
5. Somers JM, Goldner EM, Wataich P, Hsu L. Prevalence and incidence studies of anxiety disorders: a systematic review of the literature. *Can J Psychiatry*, 2006; 51: 100-13.
6. Olatunji BO, Cisler JM, Tolin DF. Quality of life in anxiety disorders: a meta-analytic review. *Clin Psychol Rev.*, 2007; 27(5): 572-81.
7. Baldwin D, Anderson I, Nutt D. Evidence-based guideline for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. *J Psychopharmacol.* 2005; 19: 567-96.
8. Bystritsky A. Treatment-resistant anxiety disorders. *Mol Psychiatry*, 2006; 11(9): 805-19.
9. Charles I, Shelton DO. Diagnosis and management of anxiety disorders. *J Am Osteopath Assoc.* 2004; 104(3): 2-5.
10. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. 2nd ed. Delhi: Periodical Experts, 1933; 1336.
11. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants*. New Delhi: Council of Scientific and Industrial Research, 1956; 1: 127.
12. Anonymous. *The Wealth of India: Raw Materials*. New Delhi: Publications and Information Directorate, CSIR., 1992; 2: 580.
13. Bakshi DN, Pal DC. *A Lexicon of Indian Medicinal Plants*. 2nd ed. Naya Prakash, 2001; 285-6.
14. Pullaiah T. *Encyclopedia of World Medicinal Plants*. 2nd ed. New Delhi: Regency Publications, 2006; 1036.
15. Rojatk SR, Chiplunkar YG, Nagasampagi BA. A diterpene from *Cipadessa fruticosa* and *Grangea maderaspatana*. *Phytochemistry*, 1994; 37(4): 1213-4.
16. Lange GL, DeCicco CP. Constituents of *Grangea maderaspatana*: a new eudesmanolide. *J Nat Prod.*, 1989; 67(7): 596-7.
17. Iyer CSR, Iyer PR. Steroids from *Grangea maderaspatana*. *Phytochemistry*, 1978; 17(11): 2036-7.
18. Singh P, Jain S, Jakupovic J. Clerodane derivatives from *Grangea maderaspatana*. *Phytochemistry*. 1988; 27(5): 1537-9.
19. Suleyman H, Demirezer LO, Kuruuzum-Uz A, Banoglu ZN, Gocer F, et al. Anti-inflammatory effect of the aqueous extract from *Grangea maderaspatana* plant. *J Ethnopharmacol.*, 1999; 65: 141-8.

20. Millan MJ. The neurobiology and control of anxious states. *Prog Neurobiol.*, 2003; 70: 83-244.
21. Charles B, Nemeroff MD. The role of GABA in the pathophysiology and treatment of anxiety disorders. *Psychopharmacol Bull.*, 2003; 37(4): 1333-1463.
22. Ressler KJ, Nemeroff CB. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Int J Pharmacol.*, 2000; 12: 2-19.
23. Bremner JD, Krystal JH, Southwick SM, Charney D. Noradrenergic mechanisms in stress and anxiety: preclinical studies. *Synapse.* 1996; 23(1): 28-38.
24. Dunn AJ, Swiergiel AH, Palamourchouk V. Brain circuits involved in corticotropin-releasing factor–norepinephrine interactions during stress. *Ann N Y Acad Sci.* 2004; 1018: 25-34.
25. Dubovsky SL, Thomas M. Beyond specificity: effects of serotonin and serotonergic treatments on psychobiological dysfunction. *J Psychosom Res.*, 1995; 39: 429-44.
26. Lopez G, Artigas F, Adell A. Unraveling monoamine receptors involved in the action of typical and atypical antipsychotics on glutamatergic and serotonergic transmission in prefrontal cortex. *Curr Pharm Des.*, 2010; 16(5): 502-15.
27. Feldman S, Weidenfeld J. Involvement of endogenous glutamate in the stimulatory effect of norepinephrine and serotonin on the hypothalamo-pituitary-adrenocortical axis. *Neuroendocrinology / J Neurol.*, 2004; 79(1): 43-53.
28. Mathew JS, Rebecca BP, Dennis SC. Recent advances in the neurobiology of anxiety disorders: implications for novel therapeutics. *Am J Med Genet.*, 2008; 148: 89-98.
29. Ninan PT. The functional anatomy, neurochemistry and pharmacology of anxiety. *J Clin Psychiatry.* 1999; 60(22): 12-7.
30. Kokate CK. Textbook of Pharmacognosy. 3rd ed. New Delhi: Vallabh Prakashan, 2008; 13-7.
31. Paavo P, Marika V. Small platform stress increases exploratory activity of mice in the staircase test. *Prog Neuropsychopharmacol Biol Psychiatry*, 2001; 25: 1435-44.
32. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav.*, 1986; 24: 525-9.
33. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd ed. New Delhi: Vallabh Prakashan, 1999; 133-5.
34. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents. *Indian J Exp Biol.*, 1997; 35: 565-75.