EVALUATION OF ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITIES OF *MAJORANA HORTENSIS*

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Summary

At present, anxiety and depression are the most frequent psychiatric conditions commonly found. The search for novel pharmacotherapy from herbal plants for psychiatric illnesses has significantly progressed. The present study was performed to evaluate the anxiolytic and antidepressant like activities of the *Majorana hortensis* extract. The alcohol extract of this plant at various doses ranging from 100, 200 and 300 mg/kg were administered once in a day for 7 days and Diazepam (2mg/kg, P.O) was used as a positive control. The anxiolytic and antidepressant activities were performed after both single and repetitive treatment for 7 days using elevated plus maze and forced swimming tests respectively. The results showed that the extract decreased immobility time with the increase swimming time. However, no changes in number of open arm entries and time spent in open arm were observed. These results suggested the anti-depression activity of the plant extract. Therefore, *Majorana hortensis* may be served as a potential resource for natural psychotherapeutic agent against depression.

Keywords: Anti-anxiety, anti-depressant, *Majorana hortensis*, elevated plus maze, forced swimming test.

Introduction

According to the World Health report [1], approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 [2]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models[3]. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes [4]. Anxiety may interfere with intelligence, psychomotor function and memory [5]. The benzodiazepines are considered the drug of choice in the

treatment of anxiety. Unfortunately, there are several side effects [6]. Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous [7]. The complexity of daily life in modern society frequently leads to varying degree of anxiety and depression. Mood, depression and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries [8].

These considerations implicate the search for new anxiolytic and antidepressant agents that have a fast onset of action present with less side effects and a wider safety margin. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases [9].

Various plants are being used in complementary and alternative medicines for management of anxiety. *Origanum* species have been particularly attributed with mood enhancing properties by aroma therapists. Volatile oils isolated from whole plant *Majorana hortensis* are often used in the treatment of anxiety [10]. A review of literature revealed that *Majorana hortensis* is highly reputed plant, and has been widely employed in herbal medicine and aromatherapy [11] but no significant work has been carried out on the anxiolytic effects and anti-depressant activity of the plant extracts. So, the present study was designed to evaluate the anti anxiety activity and anti depressant of ethanol extract of *Majorana hortensis.*

Materials and methods

Plant material: The fresh whole plant of Majorana hortensis were collected from East Godavari District of Andhra Pradesh and authenticated by Botanist Dr. S.V.Ganagaraju, Head of the Department, Goluguri Bapiraju Degree College, Anaparthi, Andhrapradesh. The voucher specimen was preserved in the department of Pharmacology laboratory of Bharathi College of Pharmacy for future reference. The plant was processed, powdered coarsely and coarse plant materials were used for extraction.

Preparation of extracts: Whole plant *Majorana hortensis* were dried in shade and powdered. The powdered whole plant (100g) were subjected to successive soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether (60 – 80 °C), chloroform and ethanol. Before each extraction the powdered material was dried in hot air-oven below 50 °C. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath.

Extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material. The yield of the extract petroleum ether (60-80 °C), chloroform and ethanol was 1.97%, 3.61%, 4.12%, w/w respectively.

Preliminary phytochemical group test: The preliminary phytochemical group test of the ethanol extract of whole plant *Majorana hortensis* was performed by the standard methods [12].

Test for Alkaloids: Small quantity of the methanol extract of whole plant *Majorana hortensis* Linn was treated with few drops of diluted hydrochloric acid, filtered and di- vided into four portions. The first portion was treated with Mayer's reagent and formation of yellowish buff colored precipitate indicated positive test for alkaloids. The second portion treated with Dragendroff's reagent and the development of orange brown precipitate shows the presence of alkaloids. The third portion was treated with Wagner's reagent and the formation of reddish brown precipitate suggested the presence of alkaloids. Final portion was treated with Hager's reagent and the development of yellowish precipitate demonstrated the positive presence of alkaloids.

Test for amino acids: Small amount of the methanol extract of whole plant *Majorana hortensis* was dissolved in a few milliliters of distilled water and treated with Ninhydrin at the pH range of 4 to 8. The formation of purple color suggested the presence of amino acids.

Test for flavonoids: Small quantity of the ethanol extract of whole plant *Majorana hortensis* was dissolved in ethanol and was hydrolyzed with 10% sulphuric acid and cooled. Next, it was extracted with diethyl ether and divided into three portions in three separate test tubes. One ml of diluted sodium carbonate solution, 1ml of 0.1M sodium hydroxide solution and 1ml of diluted ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

Test for steroids and triterpenoids: The presence of steroids and triterpenoids in ethanol extract of dried whole plant *Majorana hortensis* was confirmed through Libermann-Burchard reaction, by dissolving 10 mg of methanol extract of dried whole plant *Majorana hortensis* in 1 ml of chloroform and 1 ml of acetic anhydride and add 1-2 ml of concentrated sulphuric acid slowly. A reddish violet ring at the junction of the two layers confirmed the presence of triterpenoids and steroids. Salkowski test was utilized to confirm the presence of steroids. Concentrated sulphuric acid was added to the chloroform solution of methanol extract of whole plnat *Majorana hortensis*, appearance of reddish-blue color in the chloroform layer and green fluorescence in acid layer, suggested the presence of steroids.

Test for reducing sugar: Aqueous solution of methanol extract of whole plant *Majorana hortensis* was prepared by dissolving sufficient quantity of methanol extract of whole plant *Majorana hortensis* in minimum amount of distilled water. The aqueous solution of extract was filtered and divided into several portions. Equal volume of Benedict's reagent was mixed with same portion of the aqueous extract in a test tube and heated for few minutes. Formation of brick red precipitate confirmed the presence of reducing sugars. Equal volume Fehling's solution was added to the aqueous solution of extract in a test tube and heated for few minutes. Development of brick red color demonstrated the presence of reducing sugars.

Test for Tannins: The aqueous solution of extract was treated separately with 10% aqueous potassium dichromate solu- tion, 5% ferric chloride solution and 10% aqueous lead acetate solution. Development of yellowish brown precipitate, greenish black color and yellow color precipitate, respectively, demonstrated the presence of tannins.

Tests for Saponins: Small quantity of ethanol extract of whole plant *Majorana hortensis* was dissolved in minimum amount of distilled water and shaken in a graduated cylinder for 15 minutes. Formation of stable foam suggested the presence of saponins.

Preliminary phytochemical group tests for the ethanol extract of whole plant *Majorana hortensis*

Sl.no.	Phytoconstituents	Ethanol extract of whole plant *Majorana hortensis.*
1	Alkaloids	+
2	Steroids	+
3	Triterpenoids	+
4	Amino Acids	+
5	Flavonoids	+
6	Reducing Sugar	+
7	Tannins	+
8	Saponins	+

Animals: Swiss albino male mice weighing 20-25g and albino Wister rats of either sex weighing 160-180g each were housed in standard metal cages at room temperature. They were provided with food and water ad libitum. The rats were allowed a one-week acclimatization period before the experimental sessions.

Anxiolytic activity

Elevated plus maze test: Elevated plus maze test was performed in five groups of six male Wistar rats, after 30 minutes of oral administration of 5 ml/kg 2% CMC (vehicle control); 100, 200 and 300 mg/ kg of ethanol extract of *M. hortensis* to the group I-V respectively and intraperitoneal injection of 2 mg/kg of diazepam (drug control) to the III group of animals. Elevated plus maze test consists of a plus shaped maze, elevated 45 cm above ground level. It has two open (10 X 50 X 40 cm) arms. The test rat was placed in the central square area (10 X 10 cm) of the plus maze and time spends by the animals in open arm during a 5 min observation period was noted. Data for vehicle control, diazepam and different doses of the methanol extract (100, 200 and 300 mg kg/1) of *M. hortensis* leaves treated groups were compared [13].

Treatments: Animals were divided into five (I-V) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (2%), in a dose of 0.25ml. Group II was a positive control and was given standard drug, diazepam (2mg/kg, orally), suspended in the vehicle. Group III-V were treated as test groups and were given ethanol extract of whole plant *M. hortensis* at different doses of 100, 200 and 300mg/kg respectively. All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test.

Antidepressant activity

Forced swimming test: The FST is the most widely used pharmacological in vivo model for assessing antidepressant activity [14]. 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. 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**.**

Treatments: Animals were divided into five (I-V) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (2%), in a dose of 0.25ml. Group II was a positive control and was given standard drug, imipramine (20mg/kg, orally), suspended in the vehicle. Group III-V were treated as test groups and were ethanol extract of whole plant of *M. hortensis* at different doses of 50, 20 and 10 mg/kg respectively. All the test solutions, standard drug and control were administered orally 30 minutes prior to experiment.

Statistical Analysis:
The data were expressed as mean ± standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's 't'-test, p values less than 0.001 were considered as significance.

Results

Phytochemical Screening:
The preliminary phytochemical analysis of *M. hortensis* showed that the plant contains Alkaloids Steroids, Triterpenoids, Amino Acids, Flavonoids, Reducing Sugar, Tannins, Saponins, but alkaloid, sterol, protein, phenols, are absent.

Elevated Plus Maze Model:
The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard Diazepam(Fig.1)

Fig. 1 Elevated Plus Maze Model

Treatment	Dose(mg/kg)	Number of entries in open arm for 5 min	Time spent in open arm for 5 min (Sec)
Control	0	1.6 ± 0.24	29.6 ± 3.18
Diazepam	2	6.2 ± 0.31	21.1 ± 13.8
M. hortensis	100	3.2 ± 0.24	24 ± 3.67
M. hortensis	200	4.5 ± 0.60**	55 ± 11.5**
M. hortensis	300	5.5 ± 0.60***	98 ± 11.5***

Values are expressed as mean ± SEM (n = 6);
***P<0.001, **P<0.01 when compared to control diazepam;

Anti depressant activity:
The possible antidepressant effect of *M. hortensis* after intraperitoneal administration was studied in the forced swimming test. In this test (Fig. 2), animals treated with three doses of *M. hortensis* (50, 20 and 10 mg/kg, i.p) showed decreases in their immobility times, which was significant ($p<0.001$) when compared with control. Similarly, animals treated with imipramine (35 mg/kg), as expected, showed a significant decrease in the immobility time ($p<0.001$).

Fig. 2 Anti depressant activity

Group no.	Drug treatment	*Dose mg/kg*	Immobility period, mean ±S.E.M [n=6]
I	Control	NaCl (5 ml/kg)	180 sec
II	Imipramine HCl (30 mg/kg)	30	**125 sec.
III	Ethanol extract	50	***160 sec.
IV	Ethanol extract	20	**145 sec.
V	Ethanol extract	10	**130 sec.

Values are expressed as mean ± SEM (n = 6);
***P<0.001, **P<0.01 when compared to control diazepam;

Discussion

The incidence of anxiety and depression in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Despite the widely popular use of *Majorana hortensis* for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects.

In this work, it was demonstrated that the administration of different doses of the ethanol extract of *M. hortensis* in mice was able to induce antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of anti depressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants [15]. In the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [16]. Results showed that the administration of the *M. hortensis* produced a diminution of immobility time (a posture thought to reflect a state of ‘‘behavior despair’’ in which animals have given up the hope to escape) of mice exposed to the forced swimming. In the present study, ethanol extract (50, 20and 10 mg/kg, p.o)

administered to mice, produced significant antidepressant-like effect in FST and their efficacies

were found to be comparable to imipramine (30 mg/kg, po). The effects produced by *M. hortensis* upon the open field test demonstrated that these products do not modify the spontaneous locomotor activity of mice, which indicates that the plant extract exerts antidepressant effects without modifying significantly this parameter. Therefore, it is probable that these effects are not related to the stimulation of general motor activity [17]. It has been established that the shortening of immobility time in the forced swimming and the tail

suspension tests depends mainly on the enhancement of central 5-HT and catecholamine

neurotransmission [18]. Early evidence of a role for noradrenaline in depression came from the discovery that drugs, either causing or alleviating depression, acted to alter the noradrenaline metabolism. Furthermore, depletion studies carried out in treated and untreated patients indicated a role for serotonin and noradrenaline in depression [19]. Harmaline alkaloids present in *M. hortensis* act as reversible monoamine oxidase inhibitors and in common with other beta

carboline binds to 5-hydroxy tryptamine (HT) receptors [20]. MAO regulates the metabolic

degradation of catecholamines, serotonin and other endogenous amines in central nervous system. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. Also the flavonoids components of M. Hortesis might be interacting with adrenergic and serotonergic systems in mediating the antidepressant effects of M. hortensis.

Conclusion

From the above observations we can conclude that ethanol extract of *M. hortensis* pocesses anxiolytic activity & Anti depressant at both the dose level which is comparable with the standards. However further studies are required to know the exact mechanism of action of *M. hortensis* as anxiolytic.

Acknowledgements

The author is thankful to, Bharathi College of pharmacy, Bharathinagara, Karnataka, India for providing necessary facilities throughout this work.

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