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# EVALUATION OF ANTIDEPRESSANT ACTIVITY OF *LITSEA FLORIBUNDA* (BL.) GAMBLE - LAURACEAE USING ANIMAL MODELS

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**Keywords:**

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**ABSTRACT:** The genus Litsea, is used in traditional and indigenous Indian and Chinese medicines for the treatment of diarrhoea, stomach ache, dyspepsia, gastroenteritis, diabetes, edema, arthritis *etc*. The objective of the present study was to evaluate the antidepressant activity of leaf and stem bark aqueous and ethanol extracts of *L. floribunda* (LF) as well as its interaction with conventional antidepressant drugs using Forced Swimming Test (FST) and Tail Suspension Test (TST) in mice. Albino mice were treated with extracts of *L. floribunda* (100 and 200 mg/kg, p.o.) and standard drug Imipramine (20 mg/kg, i.p.) for 14 days and after last dose administration on 14$^{th}$ day, all behavioural studies were performed and evaluated. The standard drug treated animals (20 mg/kg i.p.) evidently showed much less immobility time. Thus in FST model the antidepressant effect produced by group IV, group V, group VI, VII, VIII and X (100 and 200 mg/kg. p.o.) was comparable to that of group II *i.e.* standard drug Imipramine group where as in TST model, the antidepressant effect produced by groups IV, VIII, IX and X was comparable to that of group II *i.e.* standard drug-Imipramine group. In conclusion, *L. floribunda* extracts possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of depressive disorders.

**INTRODUCTION:** Depression is a chronic mental illness that affects a person's physical and mental health whose symptoms include biological and emotional components [1]. According to World Health Report [2], 450 million people suffer from mental and behaviour disorders of which depression and anxiety are the two common psychiatric disorders and of the two depression may become the second leading cause of premature death or disability by the year 2020 next to heart diseases [3, 4].

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These mental and behaviour disorders may rise from the existing 12.5% to 15% of human population by 2020 [5]. Synthetic antidepressant drugs such as tricyclic antidepressants are used to treat depression but have lot of side effects which include rebound insomnia, sedation, muscle relaxation, drowsiness *etc*. Numerous traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders. Hence there is a search for new herbal drugs from plant sources with greater efficacy, lesser undesirable effects with minimum or no tolerance and dependence [6].

The Lauraceae (Laurel family) comprises aromatic trees or shrubs with about 55 genera with perhaps as many as 4000 species world-wide, mostly from warm or tropical regions, especially Southeast Asia

and South America. The members of Lauraceae are important components of tropical forests ranging from low-lying to montane and they form laurel forests. Litsea is a genus of the family Lauraceae comprising about 200 species mainly growing in the tropical and subtropical Asia, some distributed in Australia and from North America to subtropical South America. Out of 200 species of Litsea in the world, 45 are found in India and 18 of them are endemic to India. Since ancient times, plant parts of genus Litsea such as the leaf and bark are being used in traditional medicine [7].

The genus Litsea, has been used in traditional and indigenous Chinese medicines for the treatment of diarrhoea, stomachache, dyspepsia, gastroenteritis, diabetes, edema, arthritis, pain *etc.* [8] Litsea is a rich source of antioxidants containing structurally diverse and biologically active phytochemicals with broad-spectral biological activities (alkaloids, terpenoids, flavonoids, saponins and tannins) showing antioxidant, anti-inflammatory, wound healing, antidepressant, antibacterial, antifungal, analgesic, anti-diabetic, anti HIV activity, cardioprotective and cytotoxic activity [9]. *Litsea gaucescens* is used in Mexican traditional medicine for treating of sadness and the essential oil of this species has antidepressant like activity [10].

*L. floribunda* (Bl.) Gamble is an arborescent, endemic and predominant species of the shola vegetation in the Western Ghats, a biodiversity 'hotspot' of southern India. Species diversity of *L. floribunda* high in Western Ghats and Madikeri [11]. Our previous study on *L. floribunda* has showed significant antioxidant and hepatoprotective potential in the aqueous and ethanolic leaf and stem bark extracts [12]. Till date, there is no report on the antidepressant activity of *Litsea* species except *L. gaucescens*. Therefore, the present investigation is undertaken to test the effect of aqueous and ethanolic leaf and stem bark extracts of *L. floribunda* on the antidepressant activity in mice.

**MATERIALS AND METHODS:** *L. floribunda* (Bl.) Gamble was collected from the forests of Kodagu District, Karnataka and identified based on taxonomical parameters. A herbarium specimen of the species is deposited in the herbarium collection of the Department of Studies of Botany, University of Mysore, Manasagangotri, Mysore. Plant parts like healthy leaves and stem bark were collected in zip lock polythene bags and brought to the laboratory.

**Sample Processing and Preparation of Extracts:** The collected plant parts were washed with water to remove dust and then rinsed with distilled water. Later the plant parts were shade dried and then dried in a hot air oven at 40 ºC overnight until brittle and powdered. The powdered samples were stored in airtight poylthene bags and protected from sunlight until use. Fifty grams of dried leaf and bark powder were extracted with Soxhlet apparatus in the order of polarity of solvents (Hexane > chloroform > ethyl acetate > ethanol > methanol > water) [13]. The aqueous extracts of leaf and stem bark were prepared by boiling 500 g of the powdered materials in distilled water with continuous stirring for an hour [14]. The extract was filtered using a double layer cheese cloth and the filtrate was evaporated to dryness in a temperature controlled water bath for 72 h. The dried powder was weighed and stored as dry aqueous extract and used for the study of biological activities. The aqueous and ethanolic extracts of *L. floribunda* leaf and stem bark were subjected to preliminary phytochemical screening [15].

**Animals:** Albino mice of either sex weighing 20 - 28g were selected and maintained in the animal house of Sarada Vilas College of Pharmacy, Mysore. The animals were maintained at a temperature of 23 ± 2 ºC, relative humidity 55 ± 2% and light and dark cycles of 12L: 12D. They were provided with standardized pellet feed and drinking water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. No. 706/CPCSEA dt. 1.10.2002. All the experimental procedures were carried out in accordance with the guidelines of CPCSEA.

**Drugs and Chemicals:** Imipramime hydrochloride (Sigma St. Louis, USA) was used as reference antidepressant drug and solvents and reagents used were of the analytical grade and purified. Imipramine and plant extracts were prepared in distilled water

**Experimental Design and Treatment Schedule:** The antidepressant activity of the test drug was

evaluated using the following experimental models of depression such as Forced Swimming Test (FST) and Tail Suspension Test (TST). Thirty animals with body weight 20 - 28 g were divided into ten groups each consisting of 05 mice. All these mice were subjected to daily treatment for the period of 14 days as follows.

**Forced Swimming Test (FST):** Forced swimming test (FST) or behavioural despair test is the most commonly used pharmacological models for assessing the antidepressant activity in mice [16]. This method was adopted on the observation of animals exposed to a situation of forced swimming in which they become passive and immobile after a period of vigorous swimming activity, producing only the movements required to keep the head above the water [17]. The forced swimming test was conducted in such a way that the mice could not support themselves by touching the bottom of the cylinder with their feet [18]. Swimming sessions were conducted after administration of last dose of drug on 14$^{th}$ day by placing mice in glass cylinder (35 cm × 25 cm) containing water (25 ± 1 ºC) having 27 cm depth. All mice were subjected to an initial 15 min pretest followed 25 h later by a 5 min test.

The standard drug and extracts were administered three times during the period between these two sessions, first immediately after pretest session and then, after 6 and 23 h of the first dose. In both the swimming sessions, the mice were dried before placing them back in their cages. In the test period, 24 h later, the animals were exposed to the experimental conditions for 5 min. The immobility period was recorded in the test session for 5 min and water in the cylinder was changed after every test. An animal is judged to be immobile whenever it remained floating passively in water in a slightly hunched but upright position with no activity but keeping its head just above the surface. All behavioural studies were recorded by a video camera (Sony Cyber Shot) and the video tapes were evaluated afterwards by an observer who was not informed about the kind of treatment each animal had received.

**Tail Suspension Test (TST):** For screening the antidepressant effect and other class of psychotropics a simple, rapid and reliable method is TST [19]. This method was employed on the observation that a mouse suspended by the tail shows alternate agitation and immobility which is indicative of a state of depression. The TST was performed according to the method with slight modifications [20, 3]. On the 14$^{th}$ day immediately after the administration of last dose, each mouse was individually suspended on the edge of the table 50 cm above the floor by adhesive tape placed 1 cm from the tip of the tail for the period of 5 minutes using stop watch and immobility duration was recorded using a video camera [17]. During the experiment, each animal under test was both acoustically and visually isolated from other animals. Mice were considered immobile when they were just hanging and completely motionless [4]. The principle of this test is that suspending mice upside down leads to characteristic behaviour of immobility which reflects depressive disorders in humans. The decrease in immobility is considered as behavioural profiles that indicated an antidepressant - like action. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail.

**Statistical Analysis:** The results obtained were subjected to statistical analysis using SPSS program (version 16.0). The results were subjected to statistical analysis using one way analysis of variance (ANOVA) followed by Dunnets Post Hoc Test. The difference between groups were considered significant at a level of $P < 0.05$ which is considered as statistically significant. The data were expressed as mean ± SEM (n = 5).

**RESULTS:**

**Acute Toxicity Study:** The aqueous and ethanolic extracts of *L. floribunda* leaf and stem bark did show any sign of toxicity till the oral dose of 2000 mg/kg hence the extracts were used in the range of 100 - 200 mg/kg orally assuming that $LD_{50}$ dose is 2000 mg/kg.

**Evaluation of Antidepressant Activity (FST):** **Table 1** shows antidepressant effects of control group, all the test groups and standard control groups in the experimental animals. The control group animals remained immobile for long duration during test session. Both leaf and stem bark extracts specially group IV, group V, group VI, VII, VIII and X showed dose dependent significant

reduction in the immobility time of mice ($p < 0.05$), compared to control group. The standard drug Imipramine group animals (20 mg/kg i.p.) evidently showed much less immobility time. Thus the antidepressant effect produced by group IV, group V, group VI, VII, VIII and X (100 and 200 mg/kg. p.o.) was comparable to that of group II *i.e.* Imipramine group.

**TABLE 1: EFFECT OF AQUEOUS AND ETHANOLIC LEAF AND STEM BARK EXTRACTS OF *L. FLORIBUNDA* ON IMMOBILITY PERIOD IN BEHAVIOUR DESPAIR TEST - FST**

| Animal groups | Treatment | Dose (mg/kg. p.o.) | Immobility period in seconds |
|---|---|---|---|
| I | Control | - | 65.00 ± 12.58 |
| II | Imipramine | 20 | 24.33 ± 10.41 |
| III | LE | 100 | 77.33 ± 15.57 |
| IV | LE | 200 | 30.00 ± 14.73 |
| V | LA | 100 | 46.33 ± 17.47 |
| VI | LA | 200 | 23.33 ± 4.40 |
| VII | BE | 100 | 43.66 ± 10.80 |
| VIII | BE | 200 | 27.66 ± 15.51 |
| IX | BA | 100 | 78.66 ± 15.11 |
| X | BA | 200 | 37.00 ± 16.62 |

Values are expressed by mean ± SD, $P < 0.05$ on the ANOVA followed by Dunnet's Post-Hoc Test. LE = Leaf ethanol, LA = Leaf aqueous, BE = Stem bark ethanol, BA = Stem bark aqueous

**Tail Suspension Test (TST): Table 2** shows antidepressant effects of control group, all the test groups and standard control groups in the experimental model - TST. The control group *i.e.* group I animals remained immobile for long duration during 5 min test session.

**TABLE 2: EFFECT OF AQUEOUS AND ETHANOLIC LEAF AND STEM BARK EXTRACTS OF *L. FLORIBUNDA* ON IMMOBILITY PERIOD IN BEHAVIOUR DESPAIR TEST - TST**

| Animal groups | Treatment | Dose (mg/kg. p.o.) | Immobility period in seconds |
|---|---|---|---|
| I | Control | - | 94.33 ± 15.60 |
| II | Imipramine | 20 | 43.33 ± 12.12 |
| III | LE | 100 | 152.67 ± 15.33 |
| IV | LE | 200 | 50.33 ± 3.92 |
| V | LA | 100 | 108.33 ± 13.54 |
| VI | LA | 200 | 131.67 ± 18.81 |
| VII | BE | 100 | 102.33 ± 14.22 |
| VIII | BE | 200 | 81.04 ± 15.87 |
| IX | BA | 100 | 95.06 ± 17.66 |
| X | BA | 200 | 54.08 ± 17.16 |

Values are expressed by mean ± SD. $P < 0.05$ on the ANOVA followed by Dunnet's Post-Hoc Test. LE = Leaf ethanol, LA = Leaf aqueous , BE = Stem bark ethanol, BA = Stem bark aqueous

But the groups IV, VIII, IX and X showed dose dependent significant reduction in the immobility period of mice ($p < 0.05$) compared to control group and groups III, V, VI and VII. The standard drug Imipramine (20 mg/kg i.p.) shows clear reduction in the immobility period and the antidepressant effect produced by groups IV, VIII, IX and X was comparable to that of group II treated with the standard drug Imipramine.

**DISCUSSION:** Depression is a common life threatening chronic mental illness that affects a person's physical and mental health whose symptoms include biological and emotional components [21]. Numerous antidepressant compounds available in the markets act *via* different mechanisms involving the serotonergic, noradrenergic and or dopaminergic systems. Due to the adverse effects and heterogenic response, these drugs are not completely safe. Therefore, herbal medicines (phytomedicines) have maintained their popularity for historical and cultural reasons [4, 22].

The present study was conducted to assess antidepressant activity of *L. floribunda* in albino mice using FST and TST models and it provides behavioural evidence for antidepressant activity of *L. floribunda*. The FST and TST models are widely accepted behaviour models for the assessment of antidepressant activity [20, 18].

The behaviour despair test has been validated as suitable to or to evaluate drugs with putative antidepressant effects. In this FST and TST models, mice become inactive after vigorous activity and this inescapable stressful situation leads to depression [18]. In the present study, the oral administration of aqueous and ethanolic leaf and stem bark extracts of *L. floribunda* were effective in producing antidepressant like effects in both FST and TST models as the extracts have reduced the immobility period in mice and increased the struggling behaviour in dose dependent manner suggesting antidepressant effect. There is a significant correlation between clinical efficacy of antidepressant drugs and their potency in FTS, which was not found in any other model [16]. It is a proven fact that antidepressant drugs are able to reduce the immobility time in mice. Anti-depressants drugs decrease immobility time in mice both in FST and TST [18]. Moreover TST is proposed

to have a greater pharmacological sensitivity as compared with FST and it is less stressful and the results obtained from TST are in concordance with the validated FST results [23]. Interestingly our data indicates that higher dose of leaf and stem extracts of *L. floribunda* were more effective than smaller doses both in FST and TST models.

Recently oxidative stress was linked with psychophysiology of major depressives with correlation between severity of depression and erythrocyte superoxidase dismutase, lipid peroxidation levels. Literature survey shows that treatment with antidepressants reduces the oxidative stress related to depressive disorders [24]. Therefore it is possible that the antioxidant activity of aqueous and ethanolic leaf and stem bark extracts of *L. floribunda* may contribute to its antidepressant like effects.

Based on our present study, antidepressant like effect of *L. floribunda* in the classic model of depressant, it was found to possess antidepressant like activity comparable to standard drug Imipramine hydrochloride. Imipramine belongs to class of tricyclic antidepressant drugs which blocks the reuptake of norepinephrine (NE) and serotonin (5HT) and has been used as a standard drug in major studies. Serotonin is a monoamine neurotransmitter that plays an important role in mood disorders and anxiety disorders. Medications that increase the level of 5-HT, such as the selective serotonin reuptake inhibitors, are used as treatments of depression and anxiety [25]. The effect of Imipramine in FST model is due to increased availability of the neurotransmitters (NE) and serotonin (5HT) at the post synaptic site following reuptake inhibition [3].

Depression is due to functional deficiency of monoaminergic transmitters such as NE, 5HT and dopamine (DA) located after synapse. Recent advances in neuroscience suggest that dysfunction of the GABAnergic system in addition to monoamine deficit contributes to the patho-physiology of anxiety and depression. GABA receptor antagonists have antidepressant like potential and also been shown to increase 5HT and DA neurotransmitters [26]. Some studies have shown that the adaptogenic effect of plant extracts normalize the various stress parameters and monoaminergic levels which provide clue that extract is bringing their possible antidepressant like effect through restoration of normal mono-aminergic neurotransmitters [16]. Increased oxidative stress results in depression and antioxidants used for the treatment of depression have shown encouraging results [27]. Our previous results of antioxidant property of *L. floribunda* could also contribute to its antidepressant activity. Phenolic compounds have been reported to have biological effects such as depression and anxiety-Central nervous system disorders [28]. Our previous study on phytochemicals analysis showing high phenolic contents supports the antidepressant activity of *L. floribunda*.

Hence *L. floribunda* can also mediate its activity through mechanisms same as that of Imipramine possibly by increasing monoamines level at post synaptic sites. However precise mechanism underlying *L. floribunda* antidepressant activity requires further investigations.

**CONCLUSION:** In the present investigations we have reported antidepressant-like effect of *L. floribunda* in two classic models such as FST and TST where it was found to possess significant antidepressant-like activity comparable to the standard drug Imipramine, but the precise mechanism underlying antidepressant activity and identification of bioactive compounds in particular extracts of *L. floribunda* with antidepressant like effects require further investigations.

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