



Phytochemical Analysis and Antidepressant Activity of *Ixora coccinea* Extracts in Experimental Models of Depression in Mice

Ixora coccinea Ekstrelerinin Fitokimyasal Analizi ve Sıçanlarda Depresyon Deney Modeli Üzerindeki Antidepresan Aktivitesi

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ABSTRACT

Objectives: The present study aims to assess the antidepressant activity of *Ixora coccinea* extracts in mice and phytochemical analysis of the active extract by GC-MS.

Materials and Methods: After oral administration of extracts, tail suspension test (TST), force swim tests (FST), and open field tests (OFT) were performed to assess the antidepressant activity. GC-MS analysis of methanol extract of *I. coccinea* was performed to ascertain the chemical constituents in the bioactive extract.

Results: The methanol extract of *I. coccinea* at dose of 100 and 200 mg/kg body weight, p.o. significantly reduced the total duration of immobility in the TST as well as FST ($p < 0.01$). *I. coccinea* extracts showed no significant changes in locomotor activity in OFT.

Conclusion: The methanol extract of *I. coccinea* possesses antidepressant-like properties in mice with no significant effect on locomotor activity in OFT.

Key words: Antidepressant activity, forced swim test, GC-MS, *Ixora coccinea*, open field test, tail suspension test

ÖZ

Amaç: Bu çalışmada, *Ixora coccinea* ekstrelerinin sıçanlardaki antidepresan etkisi araştırılmış ve GC-MS tekniği kullanılarak aktif ekstreinin fitokimyasal analizi yapılmıştır.

Gereç ve Yöntemler: Ekstrelerin oral yolla uygulanmasının ardından, antidepresan etkinin değerlendirilmesi için kuyruktan asma testi (KAT), zorunlu yüzme testi (ZYT) ve açık alan testi (AAT) kullanılmıştır. Biyoaktif ekstredeki fitokimyasal bileşenleri aydınlatmak amacıyla *I. coccinea* metanollü ekstresi üzerinde GC-MS analizi yapılmıştır.

Bulgular: *I. coccinea* metanollü ekstresi 100 ve 200 mg/kg dozda, oral yolla uygulandığında, KAT ve ZYT testlerinde toplam hareketsizlik süresini anlamlı bir şekilde azaltmıştır ($p < 0.01$). *I. coccinea* ekstreleri AAT'de lokomotor aktivite üzerinde anlamlı derecede bir etki göstermemiştir.

Sonuç: *I. coccinea* metanollü ekstresi sıçanlarda, AAT'de anlamlı derecede lokomotor aktiviteye neden olmadan antidepresan-benzeri etkiye sahiptir.

Anahtar kelimeler: Antidepresan aktivite, zorunlu yüzme testi, GC-MS, *Ixora coccinea*, açık alan testi, kuyruktan asma testi

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INTRODUCTION

Depression is a common illness. It was estimated that 350 million people are affected by this illness. Suicides can be the result of depression. It has been estimated that every year, approximately 1 million deaths occur due to depression. Depression is a heterogeneous disorder that often manifests with various symptoms at the psychological, behavioural, and physiologic levels.¹ Although treatment with commercially available antidepressant drugs is effective, a significant number of patients do not achieve continuous remission, despite intensive management, and only 60% of patients are responsive to currently available antidepressants.² The most common adverse effects of these antidepressants include agitation, nausea, headache, sleeplessness or drowsiness, and sexual problems. The impulsive clinical response to antidepressant drugs and high susceptibility to adverse effects are major clinical problems³ thus, novel therapeutic agents are still needed to treat depression. Herbal treatment is another effective alternative to treat depression. The search for novel therapeutic plants that mitigate depressive disorders has been extensively explored over the past decade.⁴ Thus, developing an effective and safe chemical compound that originates from traditional medicinal herbal remedies may provide a method to minimize adverse effects and to shorten the entire process and reduce the cost of drug discovery compared with conventional chemistry-based drug discovery.⁵

Ixora coccinea Linn. (*Rubiaceae*) is a bushy, rounded shrub found in the subtropical region of Florida. The plant is grown as ornamental plant in India. It is commonly known as Rangon (Bengali), flame of wood (English), Bandhaka (Sanskrit). The flowers contain cycloartenol esters⁶ and have cytotoxic, hepatoprotective,⁷ antitumor, antimicrobial activity,⁸ and wound healing activity.⁹ The leaves contain triterpene ixorene,¹⁰ ixorapeptide I, ixorapeptide II,¹¹ and quercitrin,¹² and have cardioprotective,¹³ antinociceptive,¹⁴ antioxidant,¹⁵ antidiarrhoeal,¹⁶ antiasthmatic,¹⁷ hypoglycaemic, and hypolipidemic activity,¹⁸ and the roots show antioxidant activity.¹⁹

From a literature review, it appears that *I. coccinea* was used in folk medicine to treat various ailments such as in inflammatory conditions including sprains, eczema, contusions, and boils. The aim of the present study was to evaluate the antidepressant activity of *I. coccinea* stem extracts and perform gas chromatography-mass spectrometry (GC-MS) analysis of the active extract of *I. coccinea*.

MATERIALS AND METHODS

Harvesting and authentication of plant material

The *I. coccinea* stems were collected from the Dhule District, M.S., India, identified by Dr. S.G. Kotwal, HOD, Dept. of Botany, K.T.H.M. College, Nashik authenticated by Dr. Rao P.S.N., Scientist, B.S.I., Pune. The herbarium of the plant specimen has been deposited at B.S.I. Pune, the voucher specimen No. ARS-1 reference no: BSI/WC/Tech/2006/667.

Chemicals and drugs

Chloroform and methanol were obtained from Merck Ltd. (Mumbai, India). Gum acacia was from Sd fine-chem, (Mumbai, India). All chemicals and solvents used in the study were of analytical grade. Normal saline solution, imipramine, and fluoxetine were purchased from pharmacy shop.

Extraction of plant material

The stems of *I. coccinea* were air dried in the shade avoiding exposure to direct sunlight and were then pulverized in a grinder. The stem powder (#60-80) material was successively extracted using chloroform and methanol with the continuous extraction method with the help of Soxhlet apparatus. After completion of extraction, the solvent was distilled out and the extract was dried through vacuum drying.

The extract suspension was prepared in 1% acacia solution by trituration. The fluoxetine or imipramine tablet powder equivalent was suspended in normal saline solution. All solutions were freshly prepared whenever required.

Animals and treatment

The animal experiments were performed in accordance with the guidelines for the care and use of laboratory animals, of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Animal Ethical Committee of S.M.B.T. College of Pharmacy, Dhamangaon, Nasik, (M.S.), India (Registration No.1329/ac/10/CPCSEA). Male albino mice (22-26 g and 3 to 4 months) were used for the study. All animals were maintained under controlled conditions of temperature (22±2°C) and illumination (12 h light-dark cycle), with free access to food and water. Groups of six animals were structured and in order to reduce the influence of day variation all assays were conducted from 11 to 15 h and all assays were performed in a special noise-free room with controlled illumination.

The mice were divided into six groups (n=6) and received the following oral doses for 7 days:

Group I: Vehicle treated group - physiologic saline solution,

Group II: Test - Suspension of chloroform extract of *I. coccinea* in 1% acacia solution (100 mg/kg body weight per day),

Group III: Test - Suspension of chloroform extract of *I. coccinea* in 1% acacia solution (200 mg/kg body weight per day),

Group IV: Test - Suspension of methanol extract of *I. coccinea* in 1% acacia solution (100 mg/kg body weight per day),

Group V: Test - Suspension of methanol extract of *I. coccinea* in 1% acacia solution (200 mg/kg body weight per day),

Group VI: Positive control - fluoxetine or imipramine (10 mg/kg body weight per day).

Acute toxicity studies

The acute oral toxicity of the extracts of *I. coccinea* was tested using the up and down procedure as per the Organization for Economic Cooperation and Development test guidelines. Animals were dosed, one at a time, at 24 h intervals. Depending on the outcome, the dose for the next animals was adjusted up.

For further doses, a dose progression factor of 3.2 was used. The next dose was administered according to the mortality of the animal. The dose was increased if the animal survived. After reaching 2000 mg/kg body weight dose, four additional animals were administered the same dose.²⁰

Assessment of antidepressant activity

Forced swim test (FST)

The FST was performed according to the method described by Porsolt et al.²¹ with a minor modification. Mice were individually forced to swim in an open cylindrical container (diameter 14 cm, height 20 cm), with a depth of 15 cm of water at 25±2°C. The experimental procedures were performed on days 4 and 7, 60 min after the administration of test components. Each mouse was judged to be immobile during 6 min. Immobility time in the FST was measured when the animals ceased struggling and remained motionless while floating in the water. The water in the containers was changed after each trial.²²

Tail suspension test (TST)

The TST was performed according to the method described by Rosa et al.²³ Mice were suspended 50 cm above the table with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility during a 6-min period was scored manually. Immobility time in TST was measured when animals showed no limb or body movements, hung passively and completely motionless, except for movements caused by respiration.²⁴

Open-field test (OFT)

The locomotor activity was assessed using an OFT according to the method described by Herrera-Ruiz et al.²⁵ in order to detect any link between locomotor activities and antidepressant activity of the *I. coccinea* extracts. The OFT was performed on mice that received treatments, which were used to determine immobility time in FST/TST 60 min before being observed in the open-field. Animals were placed individually in a box (30x30x15 cm), with the floor divided into 9 equal squares. After habituation to the arena for 5 min, the number of squares crossed with all paws, grooming, and rearing events were observed for 6 min. The box was cleaned with 10% ethanol after each trial.²⁶

Phytochemical investigation of active extracts using GC-MS

The GC-MS analysis of the methanol extract of *I. coccinea* was performed at SAIF Panjab University Chandigarh, India. The chemical composition of the extracts was determined using a Thermo Scientific TSQ 8000 GC-MS with a direct capillary interface fused with silica capillary column TG 5MS (30 m x 0.25 mm, 0.25 µm). The methanol extract of *I. coccinea* were injected with helium used as a carrier gas at constant rate 1 mL/min, in pulsed splitless mode. The solvent delay was 2 min and the injection size was 1 µL. The mass spectrophotometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV and scanning from m/z 50-500. The GC temperature program started at 60°C then elevated to 280°C at a rate of 10°C/min, with a 10 min hold at 280°C. The injector, ion source, and detector temperatures were set at

250°C, 230°C, and 280°C, respectively.^{27,28} The peaks separated in GC-MS were identified using National Institute of Standards and Technology mass spectral databases.

Statistical analysis

All experimental results are given as the mean ± standard error of the mean. To compare the test and control groups, One-way analysis of variance (ANOVA), followed by Dunnett's test was performed. A value of p<0.01 was considered to be significant.

RESULTS

Extraction

Fresh 250 g of stems of *I. coccinea* yielded 8 g (3.20%) and 18.55 g (7.42%) of chloroform extract and methanol extract respectively.

Acute toxicity studies

Chloroform and methanol extract of *I. coccinea* showed neither behavioural changes nor mortality with an oral dose of 2000 mg/kg.

Antidepressant activity

The effects of *I. coccinea* extracts on the immobility time in the forced swim test

The methanol extract of *I. coccinea* showed an antidepressant effect in the FST because it significantly reduced the immobility time compared with the vehicle treated group (184.00±4.76 sec.) (Figure 1). The immobility times of the methanol extract of *I. coccinea* and the chloroform extract of *I. coccinea* for doses of 100 and 200 mg/kg/day on the 7th day were found as 138.00±6.763 and 124.7±6.36 sec, and 172.70±6.259 and 160.00±7.849 sec, respectively. The chloroform extract of *I. coccinea* did not reduce immobility time significantly. The group treated with fluoxetine showed good activity (111.83±4.826 sec). No significant difference was observed in the immobility time of *I. coccinea* extracts on 4th day and the 7th day in the FST.

The effect of *I. coccinea* extracts on the immobility time in the TST

In the TST, the methanol extract of *I. coccinea* showed a significantly decreased immobility time compared with the vehicle-treated control group (180.00±6.23 sec) (Figure 2).

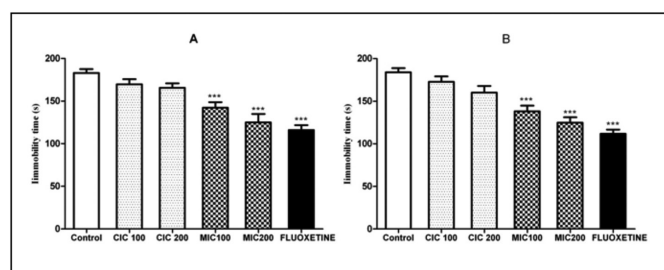


Figure 1. Effects of *I. coccinea* extracts (100 and 200 mg/kg) and fluoxetine (10 mg/kg) on the immobility time in the forced swim test on 4th day (A) and 7th day (B). The results are expressed as the mean ± standard error of the mean, n=6 in each group. ***p<0.001 VS the vehicle-treated control group. (CIC: Chloroform extract of *I. coccinea*, MIC: Methanol extract of *I. coccinea*)

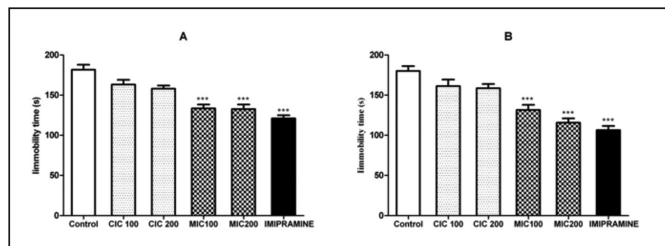


Figure 2. Effects of *I. coccinea* extracts (100 and 200 mg/kg) and imipramine (10 mg/kg) on immobility time in the tail suspension test on 4th day (A) and 7th day (B) in mice. The results are expressed as the mean \pm standard error of the mean, n=6 in each group. ***p<0.001 VS the vehicle-treated control group. (CIC: Chloroform extract of *I. coccinea*, MIC: Methanol extract of *I. coccinea*)

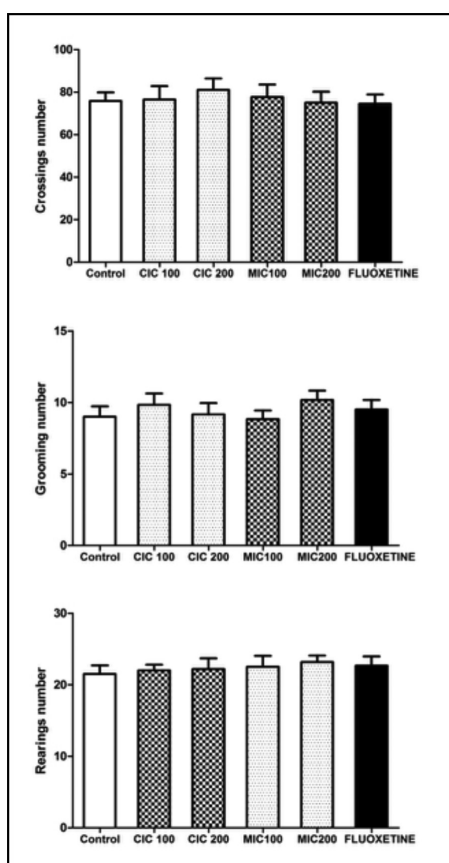


Figure 3. Effects of *I. coccinea* extracts (100 and 200 mg/kg) and fluoxetine (10 mg/kg) after 7th day administration on the number of crossings, and rearing and grooming activities in the open field test in mice. The results are expressed as the mean \pm standard error of the mean, n=6 in each group. (CIC: Chloroform extract of *I. coccinea*, MIC: Methanol extract of *I. coccinea*)

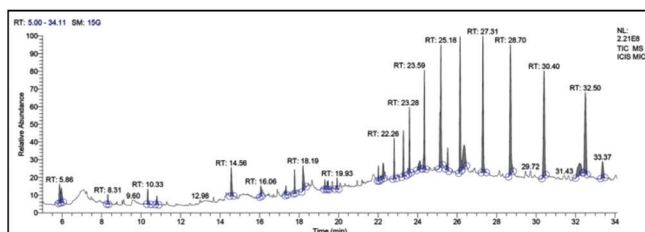


Figure 4. GC-MS chromatogram of methanol extract of *I. coccinea*

The mean immobility time of the methanol extract of *I. coccinea* treated group for 100 and 200 mg/kg dose was 131.50 ± 6.515 and 115.8 ± 5.78 sec, respectively. The chloroform extract of *I. coccinea* did not show a significant effect on immobility time (161.3 ± 8.044 and 158.50 ± 5.476 sec). Imipramine, a non-selective reuptake inhibitor, which was used as positive control, significantly decreased the immobility time during the test session (106.50 ± 5.156 sec). No significant difference was observed in the immobility time of *I. coccinea* extracts on the 4th and 7th days in the TST.

The effects of I. coccinea extracts in the open field test

No significant differences were observed in the number of squares crossed, and rearing and grooming activities between the vehicle-treated group and the *I. coccinea* extracts-treated group, as well as positive control group (Figure 3).

GC-MS analysis of pharmacologic active extract of *I. coccinea*

The results obtained from GC-MS analysis lead to the identification of the phytoconstituents present in the methanol extract of *I. coccinea*. The GC-MS spectra (Figure 4) indicated the presence of 2-Methoxy-4-vinylphenol, 3,4-Dimethoxy-6-methylpyrocatechol, 4-(3-hydroxy-1-propenyl)-2-methoxy-Phenol, methyl ester of Hexadecanoic acid, n-Hexadecanoic acid, methyl ester of 9-Octadecenoic acid (Z), Methyl stearidonate, Heneicosane, 16,17-Epoxyandrostane, Triacotane, Diisooctyl phthalate, Tetracosane, Stigmast-4-en-3-one, Squalene, and β -Sitosterol (Table 1).

DISCUSSION

Although *I. coccinea* has been used to treat nervous shock in traditional medicine, its specific neuropharmacologic activities have not yet been demonstrated. The FST and TST are the most common animal models used for screening antidepressant activity. In both tests, animals are placed in an inescapable situation and the decrease in immobility time indicates antidepressant-like activity.^{29,30} In the FST, mice are forced to swim in a restricted space from which they cannot escape and it assumes a characteristic behaviour of immobility. This behavior reflects a state of despair or lowered mood, which can be reduced by agents that are therapeutically active in human depression. The TST also induces a state of immobility in animals similar to that in the FST. Fluoxetine is a classic selective serotonin reuptake inhibitor (SSRI), it is bound at the primary site of pre-synaptic serotonin transporter with very high affinity, and it has higher serotonergic activity than other classic SSRIs.²⁴ Imipramine prevents reuptake of noradrenaline and serotonin resulting in their increased availability in the synapse, and therefore, an increase in adrenergic and serotonergic neurotransmission.³¹

Psychostimulants, convulsants, and anticholinergics are able to increase locomotor activity in the OFT and give a false positive result in the TST and FST.³² Agents that show a hyperkinesia effect also produce false positive effects in the TST and FST by reducing the immobility time.³³ Therefore, OFT was used to exclude these false effects that could be associated

Table 1. Compounds present in the methanol extract of *I. coccinea* using GC-MS analysis

Sr. no	Retention time	Name of compound	Mol. formula	Mol. weight
1.	10.80	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150.17
2.	14.56	3,4-Dimethoxy-6-methylpyrocatechol	C ₉ H ₁₂ O ₄	184.18
3.	16.06	4-(3-hydroxy-1-propenyl)-2-methoxy- Phenol	C ₁₀ H ₁₂ O ₃	180.20
4.	17.78	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45
5.	18.19	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
6.	19.47	9-Octadecenoic acid (Z), methyl ester	C ₁₉ H ₃₆ O ₂	296.48
7.	19.93	Methyl stearidonate	C ₁₉ H ₃₀ O ₂	290.42
8.	22.02	Heneicosane	C ₂₁ H ₄₄	296.57
9.	22.26	16,17- Epoxyandrostane	C ₁₉ H ₃₀ O	274.48
10.	22.82	Triacontane	C ₃₀ H ₆₂	422.81
11.	23.28	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390.55
12.	23.59	Tetracosane	C ₂₄ H ₅₀	338.65
13.	24.11	Stigmast-4-en-3-one	C ₂₉ H ₄₈ O	412.69
14.	25.53	Squalene	C ₃₀ H ₅₀	410.71
15.	33.37	β-Sitosterol	C ₂₉ H ₅₀ O	414.70

with psychostimulants, convulsants, and anticholinergics or hyperkinesia.² The main difference between antidepressants and psychostimulants is that antidepressants would not increase locomotor activity.³⁴ In addition, the finding suggested that the reduction of immobility time elicited by the methanol extract in the FST as well as in the TST was a specific result of its antidepressant mechanism. In the TST and FST, the methanol extract of *I. coccinea* decreased the immobility time, which was not due to any psychostimulant, anticholinergic or convulsant effect, or hyperkinesia activity.

The methanol extract of *I. coccinea* decreased immobility time, whereas the chloroform extract showed no effect in either the TST or the FST. The immobility in TST and FST, referred to as behavioral despair in animals, is believed to reproduce a condition similar to human depression.³⁵

In the present study, the methanol extract of *I. coccinea* was analyzed using GC-MS. To date, no reports exist on the GC-MS analysis of *I. coccinea* stems. From GC-MS analyses, bioactive extracts that show significant antidepressant activity contain fatty acid or esters such as methyl ester of hexadecanoic acid, n-hexadecanoic acid, methyl ester of 9-Octadecenoic acid (Z), Methyl stearidonate, Heneicosane, 16,17-Epoxyandrostane, Triacontane, Diisooctyl phthalate, Tetracosane; steroidal such as Stigmast-4-en-3-one, Squalene and β-Sitosterol; and phenolics such as 2-Methoxy-4-vinylphenol, 3,4-Dimethoxy-6-methylpyrocatechol, 4-(3-hydroxy-1-propenyl)-2-methoxy-Phenol. Phenolic compounds show good antidepressant activity;^{36,37} the methanol extract of *I. coccinea* showed prominent antidepressant activity due to these phytoconstituents.

CONCLUSION

The present study provides the first evidence that the methanol extract of *I. coccinea* has significant antidepressant activity in the TST and FST models of depression in mice. The antidepressant activity may be due to the presence of phenolic components. Further research is required to elucidate the mechanism of its action.

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Conflict of Interest: No conflict of interest was declared by the authors.

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