Phytochemical analysis and antidepressant activity of *Ixora coccinea* extracts in experimental models of depression in mice

Abstract

Objectives: Present study deals to access the antidepressant activity of *Ixora coccinea* extracts in mice and phytochemical analysis of active extract by GC-MS.

Materials and Methods: After oral administration of extracts, tail suspension test (TST), force swim tests (FST) and open field test (OFT) were performed to assess the antidepressant activity. GC-MS analysis of methanol extract of *Ixora coccinea* was performed to find out the chemical constituents in 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 not showed any significant changes in locomotor activity in OFT.

Conclusion: The methanol extract of *I. coccinea* possesses antidepressant-like properties in mice without any significant effect on locomotor activity in OFT.

Key words

Antidepressant activity; forced swim test; GC-MS; *Ixora coccinea*; open field test; tail suspension test

1. Introduction

The depression is a common illness, it was estimated that 350 million people are affected from this illness. Suicides can be result of the depression. It has been estimated that every year, approximately 1 million deaths occurs due to depression. Depression is a heterogeneous disorder that often manifests with various symptoms at the psychological, behavioural and physiological 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 side effects of these antidepressants includes 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 problems 3 thus, novel therapeutic agent are still needed to treat depression. Herbal treatment is another effective alternative to treat depression. The search for novel therapeutic plants that mitigate depressive disorder 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 methodology to minimize adverse side 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 subtropical region of Florida. Plant is grown as ornamental plant in India. It is commonly known as Rangon (Bengali), flame of wood (English), Bandhaka (Sanscrit) . Flowers contains cycloartenol esters[6] and have cytotoxic, hepatoprotective[7], antitumor, antimicrobial activity[8] and wound healing activity[9]. Leaves contains 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 hypolipidaemic activity [18] and roots shows antioxidant activity [19].

From literature review, it reveals that, *I. coccinea* was used in folk medicine to treat various ailments like in inflammatory conditions like sprains, eczema, contusions and boils. The aim of present study was to evaluate antidepressant activity of *I. coccinea* stems extracts and GC-MS analysis of active extract of *I. coccinea*.

2. Materials and methods

2.1. Harvesting and authentication of plant material

The *I. coccinea* stems were collected from 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.

2.2. 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 Fluxetine was purchased from Pharmacy shop.

2.3. Extraction of Plant material

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

The suspension of extract 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.

2.4. Animals and treatment

All experiments were conducted in accordance with guidelines for the care and use of laboratory animals of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental procedure. Male albino mice (22–26g and 3 to 4 month old), were used for 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 to six groups (n=6) and received following dose for 7 days orally

Group-I: Vehicle treated group - physiological saline solution

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

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

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

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

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

2.5. Acute toxicity studies

Acute oral toxicity of the extracts of *I. coccinea* was carried out by the up and down procedure (UDP) as per OECD (Organization for Economic Cooperation and Development) test guidelines. Animal was dosed, one at a time, at 24 h intervals. Depending on outcome the dose for the next animals was adjusted up. For further doses, a dose progression factor 3.2 was used. The next dose was administered according to mortality of the animal. The dose was increased if the animal survived. After reaching dose, four additional animals were administered the same dose[20].

2.6. Assessment of antidepressant activity

2.6.1. Forced swim test (FST)

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

2.6.2. Tail suspension test (TST)

The TST was performed according to the method described by Rosa et al. 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 was scored manually during 6 min. Immobility time in TST was measured when animals did not showed any limb or body movements, hung passively and completely motionless except for those movements caused by respiration[23, 24].

2.6.3. Open-field test (OFT)

The locomotor activity was performed by OFT according to the method described by Herrera-Ruiz et al in order to detect any link between locomotor activities and antidepressant activity

of the *I. coccinea* extracts. The OFT was performed on mice which received those treatments, used to determine immobility time in FST/TST 60 min before being observed in the open-field. Animals were placed individually in a box (30×30×15 cm), with the floor divided into 9 equal squares. After the 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[25, 26].

2.7. Phytochemical investigation of active extracts by Gas chromatography-mass spectrometry (GC–MS)

The GC–MS analysis of methanol extract of *I. coccinea* was performed at SAIF Panjab University Chandigarh, India. Chemical composition of the extracts was determined using an Thermo Scientific TSQ 8000 Gas Chromatograph-Mass Spectrometer with a direct capillary interface fused with silica capillary column TG 5MS (30 m X 0.25 mm, 0.25 µm). Methanol extract of *I. coccinea* were injected with helium was used as carrier gas at constant rate 1mL/min, 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 by NIST (National Institute of Standards and Technology) mass spectral databases.

2.8. Statistical analysis

All experimental results are given as the mean ± standard error of the mean (SEM).To compare 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.

3. Result

3.1. 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.

3.2. Acute toxicity studies

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

3.3. Antidepressant Activity

3.3.1. Effects of *I. coccinea* extracts on the immobility time in the force swim test

The methanol extract of *I. coccinea* showed 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 time of methanol extract of *I. coccinea* was found to be 138.00±6.763, 124.7±6.36 sec. and for chloroform extract of *I. coccinea* 172.70±6.259 and 160.00±7.849 sec. for the doses of 100 and 200mg/kg/ day on 7th day respectively. While, chloroform extract of *I. coccinea* was not reduced immobility time significantly. The group treated with fluoxetine showed good activity (111.83±4.826s). No significant difference was observed in immobility time of *I. coccinea* extracts on 4th day and 7th day in FST.

3.3.2. Effect of *I. coccinea* extracts on the immobility time in the tail suspension test

In the TST, the methanol extract of *I. coccinea* showed a significantly decreases immobility time, compared with the vehicle-treated control group (180.00±6.23 sec.) (Figure 2). 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. While chloroform extract of *I. coccinea* were not showed significant effect on immobility time (161.3± 8.044 and158.50 ±5.476sec). Imipramine, a non-selective reuptake inhibitor, used as positive control, significantly decreased the immobility time during test session. (106.50±5.156sec.). No significant difference observed in immobility time of *I. coccinea* extracts on 4th day and 7th day in TST.

3.3.3. Effects of *I. coccinea* extracts in the open field test

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

3.4. Gas chromatography-mass spectrometry analysis of pharmacological active extract of *I. coccinea*

The results obtained from GC-MS analysis lead to the identification phytoconstituents present in methanol extract of *I. coccinea*. The GC-MS spectra (Fig.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, Triacontane, Diisooctyl phthalate, Tetracosane, Stigmast-4-en-3-one, Squalene and β-Sitosterol (Table 1).

4. Discussion

Although *I. coccinea* has been used to treat nervous shock in traditional medicine, its specific neuropharmacological activities have not been demonstrated yet. The forced swim test and tail suspension test 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 behaviour reflects a state of despair or lowered mood, which can be reduced by agents which are therapeutically active in human depression. The TST also induces a state of immobility in animals similarly that in the FST. Fluoxetine is classical selective serotonin reuptake inhibitors SSRIs, it is bound at the primary site of pre-synaptic serotonin transporter (SERT) with a very high affinity, and it has higher serotonergic activity than the classical SSRIs[24]. Imipramine prevents reuptake of nor adrenaline and serotonin resulting in their increased availability in the synapse and therefore an increase in adrenergic and serotonergic neurotransmission[31].

The psychostimulants, convulsants and anticholinergics are able to increases locomotor activity in OFT and give a false positive result in TST and FST[32]. The agents which shows hyperkinesis effect also produces false positive effect in TST and FST by reducing the immobility time[33]. Therefore, OFT was used to exclude these false effects that could be associated with psychostimulants, convulsants and anticholinergics or hyperkinesis[2]. The main difference between antidepressants and psychostimulant is that antidepressants would not increased locomotor activity[34]. In addition, the finding suggested that reduction of immobility time elicited by methanol extract in FST as well as in TST was specifically arises via its antidepressant mechanism. In TST and FST methanol extract of *I. coccinea* decreased immobility time which not due to any psychostimulant, anticholinergic, convulsant effect or hyperkinesis activity.

The methanol extract of *I. coccinea* decreases immobility time while chloroform extract not showed any effect in TST as well as FST. The immobility in TST and FST, referred to be as behavioural despair in animals, is believed to reproduce a condition similar to human depression[35].

In the present study, methanol extract of *I. coccinea* was analyzed by GC-MS. Till date no reports exist on the GC-MS analysis of *I. coccinea* stems. From GC-MS analysis, bioactive extract that show significant antidepressant activity contains fatty acid or esters like 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 like Stigmast-4-en-3-one, Squalene and β-Sitosterol and phenolics like 2-Methoxy-4-vinylphenol, 3,4-Dimethoxy-6-methylpyrocatechol, 4-(3-hydroxy-1-propenyl)-2-methoxy- Phenol. The phenolic compounds shows good antidepressant activity [36, 37] and due to these phytoconstituents of methanol extract of *I. coccinea* showed prominent antidepressant activity.

5. Conclusion

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

Acknowledgement

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Authors' contributions

Surana AR were performed all experimental work and preparation of manuscript.

Wagh RD were design the study, interpret the data and edit the manuscript.

Conflict of Interest

No conflict of interest

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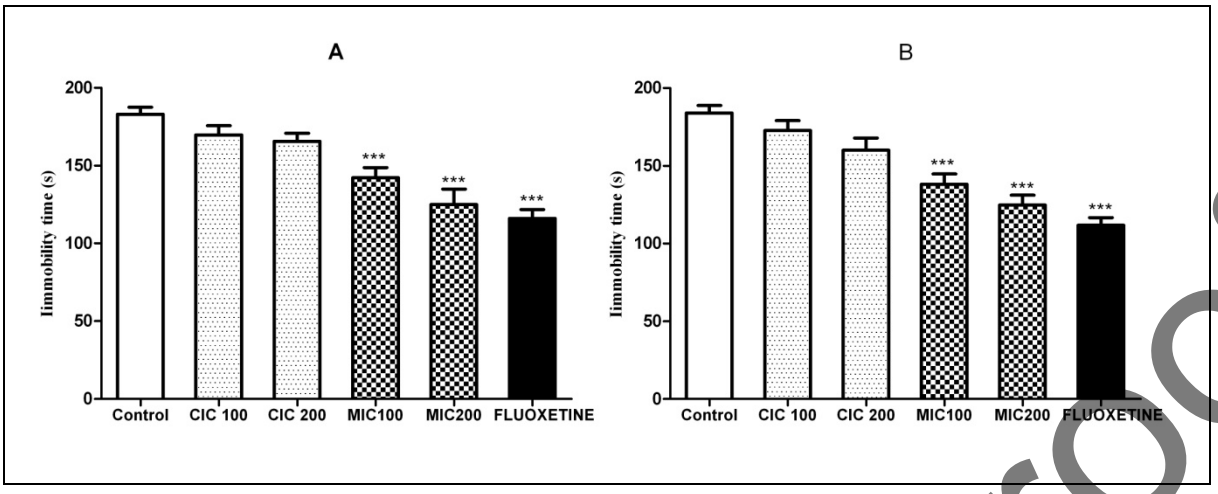

Fig. 1: Effects of *I. coccinea* extracts (100 and 200mg/kg) and fluoxetine (10mg/kg) on the immobility time in the forced swim test on 4th day (A) and 7th day (B). The results are expressed as the mean ± SEM, n= 6 in each group. ***p< 0.001 VS the vehicle-treated control group. (CIC- Chloroform extract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

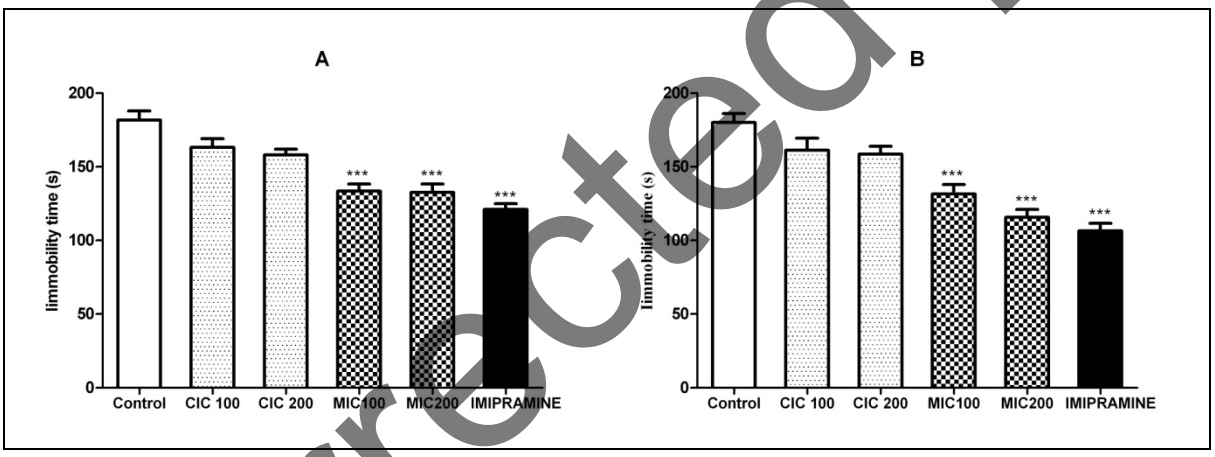

Fig. 2: Effects of *I. coccinea* extracts (100 and 200mg/kg) and imipramine (10mg/kg) on immobility time in the tail suspension test on 4th day (A) and 7th day (B) in mice. The results are expressed as the mean ± SEM, n= 6 in each group. ***p< 0.001 VS the vehicle-treated control group. (CIC- Chloroform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

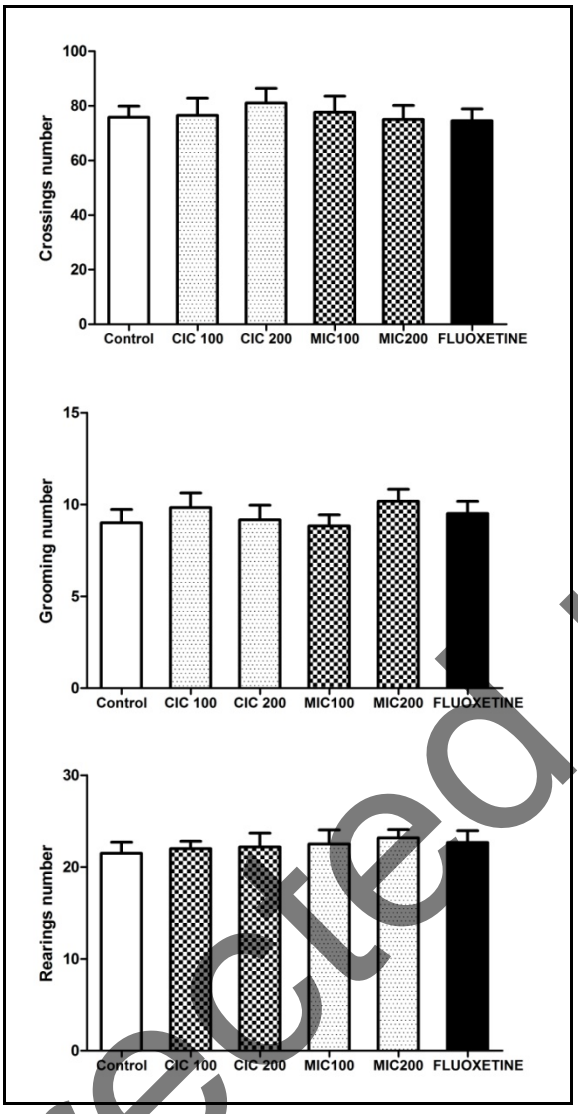

Fig. 3: Effects of *I. coccinea* extracts (100 and 200mg/kg) and fluoxetine (10mg/kg) after 7 days administration on the number of crossing, rearing and grooming in open field test in mice. The results are expressed as the mean ± SEM, n= 6 in each group. (CIC- Chloroform extract of *I. coccinea*, MIC- Methanol extract of *I. coccinea*)

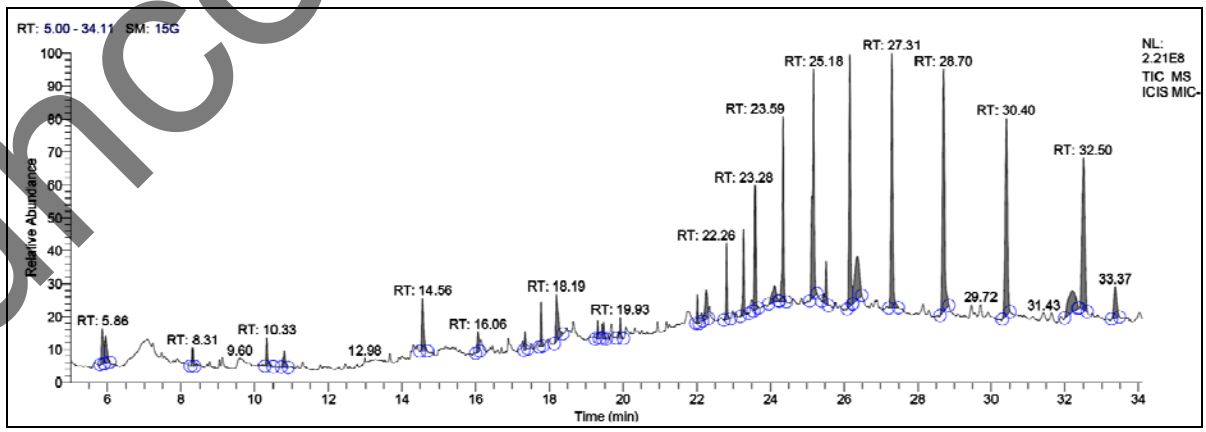

Fig 4: GC-MS chromatogram of methanol extract of *I. coccinea*

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	$C_9H_{10}O_2$	150.17
2.	14.56	3,4-Dimethoxy-6-methylpyrocatechol	$C_9H_{12}O_4$	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