Original Article

Phytochemical Screening and Anti-inflammatory, Antioxidant, and Antimicrobial Investigations on Extracts of *Ixora javanica*

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Abstract

Background: *Ixora coccinea* Linn., belonging to *Rubiaceae* family, often known as red *Ixora* and jungle of *geranium*, is an evergreen shrub that may be found throughout India. The Indian Traditional Medical System, Ayurveda, as well as numerous folk medicines, use the flowers, leaves, roots, and stems to cure various ailments. **Aims and Objectives:** The present study sheds some light on the anti-inflammatory, antioxidant, and antimicrobial activities of different plant extracts of *Ixora javanica* (IJ) and on analyzing its major bioactive component by Gas chromatography–mass spectrometry (GC-MS). **Materials and Methods:** Cold maceration was performed for the extraction of dried flowers of IJ with the escalating polarity of the solvents (Petroleum ether, chloroform, ethyl acetate, and methanol), and different extracts were screened for antioxidant, anti-inflammatory, and antimicrobial activities. **Results:** The ethyl acetate and methanolic extracts showed significant antioxidant activity at a concentration of 400 µg/ml. The ethyl acetate and methanolic extracts of IJ showed similar anti-inflammatory activity at a dosage of 500 mg/kg body weight in rats compared to the standard drug indomethacin. The antimicrobial activity was also found against *Escherichia coli* at the concentration of 500 µg/ml. Further analysis of these two extracts by GC-MS, Methyl palmitate was identified as a major bioactive component responsible for anti-inflammatory activity. Moreover, the different extracts of IJ also showed antioxidant activity due to the presence of Vitamin E, and tocopherol. **Conclusion:** Overall, it was found that IJ extracts in methanol and ethyl acetate were effective at imparting the aforementioned pharmacological effects.

Keywords: Anti-inflammatory, antioxidant, gas chromatography–mass spectrometry, *Ixora javanica,* methyl palmitate, Vitamin-E

Introduction

Medicinal plants are the oldest source of essential medicines useful for human diseases because of their significant therapeutic value. The medicinal plants possess an exceptionally huge place as crude material for critical use of medications. They enclose phytoconstituents, which are utilized in the cure of numerous human diseases. Plants are considered an abundant source of environmentally created optional metabolites, which are powerful solutions for various diseases. A critical problem in pharmacotherapeutics is emerging in the form of multiple drug resistance. However, medicinal plants are considered rich sources for antimicrobial agents.[1] Hence, phytochemical evaluation of diverse plant species for medicinal leads to gain much consideration.

Ixora javanica (IJ) is a flowering plant that belongs to the family *Rubiaceae*. It can grow up to 11 m tall, and the trunk of the shrub can be 26 cm in diameter. The plant is sometimes grown as an ornamental. The edible flowers are gathered from the wild for local use. The plant is well known for its medicinal values, such as abrasives, astringents, and antiseptics. Conventionally, it was accepted as a beneficial integrant in tooth care commodity by the Arabian people.

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Antihyperlipidemic and antitumor potential of its stem was scientifically proven on rodents. The leaves and roots are utilized to cure diarrhea, hiccup, fever, bruises, constant ulcers, and skin infections.[2] Ayurvedic practitioners utilized the flower of IJ for curing peptic ulcers as well as syphilitic or auxiliary inflammatory ulcers.[3]

This shrub is accounted for the presence of a variety of chemical constituents like; anthocyanins in blooms; methyl ester of palmitic, stearic, oleic, and linoleic acids in root oil; Octadecadienoic corrosive, Saponins, and tannins from root bark, alkaloids, flavonoids, sterols, terpene, and phenols. Literature proclaimed that ether extract of *Ixora coccinea* exhibits good antimicrobial activity compared to methanol extract.[4] However, there was not much information available regarding the antimicrobial activity of IJ plant and its constituent. Hence, through this work, we focused on the antimicrobial activity, antioxidant, and anti-inflammatory activity along with the identification of chemical constituents by gas chromatography–mass spectrometry (GC-MS), which were not reported.

Materials and Methods

Chemicals and reagents

The entire chemicals and reagents utilized in the study were of analytical grade. Purified water obtained from the Milli Q water purification system (Millipore, Milford, MA, USA) was used in the study. The AR-Analytical Reagent Grade grade solvents such as methanol, petroleum ether, chloroform, ethyl acetate, and formic acid procured from Ranchem Private Limited were utilized in the study. Syringe filters of pore size of 0.22 µm used in the study were procured from Merck, Mumbai. The chemicals used in our experiment are Potassium dihydrogen orthophosphate, Potassium ferricyanide, Trichloro acetic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Disodium hydrogen orthophosphate, Sodium hydroxide, concentrated Hydrochloric acid, Ferric chloride ($FeCl_3$), Benzene, Hexene. Moreover, Methanol, Chloroform, Ethyl acetate, and Petroleum ether are used as a solvent in this work. All the chemicals are obtained from the Laboratories of Dr. B. C. Roy College of Pharmacy and Allied Health Sciences.

Preparation of plant extracts

Extraction of the components from the flowers of IJ was performed by cold maceration with the help of solvents of different polarities. Freshly collected flowers of IJ were dried in the shade and then coarsely powdered. The powdered flowers were defatted with petroleum ether (60–80) for 48 h. Successive extraction was performed with chloroform, ethyl acetate, and methanol and continued for 7–10 days. Extracts were then filtered through filter paper to remove the traces of particulate matters of flowers. All the extracts (Petroleum ether (IJPEE), Chloroform (IJCE), Ethyl acetate (IJEAE), and Methanol (IJME) extracts) were concentrated under vacuum by Rota evaporator and poured into the Petri-dishes to yield dry powder extracts. To identify the phytoconstituents included in the dried crude extracts, they underwent preliminary phytochemical screening. The crude extract was employed by GC-MS to determine the components responsible for the activity and to assess the antioxidant and antibacterial properties.

Phytochemical screening of different extracts

Preliminary phytochemical screening was performed to identify the phytoconstituents present in the extracts. Phytochemical tests, such as Killer–Killani test, Liebermann–Burchard test, Millon's test, and Dragendorff's test, were performed to determine the presence of alkaloids, glycosides, tannins, flavonoids, phenols, sterols, carbohydrates, and proteins.[5]

Antioxidant assessment of *Ixora javanica* extracts

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The DPPH free radical scavenging assay of different extracts (IJPEE, IJCE, IJEAE, and IJME) was performed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) procedure as described by Dey and Ghosh.[6] First, a solution of 0.1 mM DPPH in methanol was developed. Using the same solvent, samples of extracts were developed with various concentrations (50, 100, 200, 400, 600, 800, and 1000 µg/ml). For performing antioxidant assay, Ascorbic acid was used as a quality standard with various concentrations (50, 100, 200, 400, 600, 800, and 1000 µg/ml). Further, 1 ml of DPPH solution was added to the 3 ml of standard or different extracts (Petroleum ether, Chloroform, Ethyl acetate, and Methanol) in methanol in various concentrations as mentioned above. Similarly, the control was prepared devoid of the samples and kept as the positive control. The reaction mixture was strenuously stirred and conserved for half an hour in a darkened area at room temperature until the reaction gets completed. An ultraviolet (UV) spectrophotometer was utilized to measure absorbance at a wavelength of 517 nm.

$$\%\ \text{DPPH scavenging activity is} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

($A_{Control}$ = absorbance of control reaction, A_{sample} = absorbance in the presence of standard or sample).

Reducing power assay

According to the method explained by Dey and Ghosh,[6] the reducing power assay for the various extracts of IJ was performed with few modifications. Samples with different concentrations (50, 100, 200, 400, 600, 800, and 1000 µg/ml) were prepared in Mili-Q water. One milliliter of sample solution was mixed with 2.5 ml KH_2PO_4 buffer (0.2 M, pH 6.6) and K_3 (Fe [CN] $_6$) 2.5 ml. The above reaction blend was allowed to heat for 20 min at a temperature of 60°C, followed by cooling and the addition of 2.5 ml of 10% trichloroacetic acid. Then, the reaction blend was allowed to centrifuge at 2000 rpm for 10 min. A volume of 2.5 ml of the upper layer

were separated out and 2.5 ml of Mili-Q water and 0.5 ml of $FeCl_3$ solution (0.1%) freshly prepared were added to it. Using an UV spectrophotometer, absorbance was recorded at 700 nm. Similarly, control was processed beyond the samples. As mentioned before, ascorbic acid served as the reference point for the various concentrations. After testing, the data were shown as a percentage (%) of inhibition. An increase in the reaction blend's absorbance is shown by a rise in the reducing power of extracts at varying concentrations.

$$\% \text{ reducing power} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

($A_{control}$ = absorbance of control reaction, A_{sample} = absorbance in the presence of standard or sample).

$$\% \text{ DPPH scavenging activity is} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

% ($A_{control}$ = absorbance of control reaction, A_{sample} = absorbance in the presence of standard or sample.

Assessment of antimicrobial activity of different extracts of *Ixora javanica*

Antimicrobial activity

While performing the antimicrobial study, the test microbe utilized in the study were Gram-negative bacteria *Escherichia coli* (R122) and Gram-positive bacteria *Staphylococcus aureus* (ATCC6532), *Salmonella Typhi* (*S. typhi* 59) and *Streptococcus pneumonia* (NTCC839). Three milliliters of nutrient broth were taken, and 300 ml of each stock culture was added to it. Cultures were kept for incubation for overnight (24 h) at 37°C. After incubation, using a sterile physiological solution, bacterial suspension (inoculum) was diluted so that the final cell concentration reaches to 10^5 CFU/mL.[7]

Antimicrobial activity on *Ixora javanica* (extracts) by disc diffusion method

The antibacterial activity of various extracts of IJ was evaluated as per the method discussed by Dey and Ghosh[6] against *E. coli* (R122) and *S. aureus* (ATCC6532), *S. typhi* (*S. typhi* 59) and *S. pneumonia* (NTCC839). The disc diffusion technique was used, and amoxicillin (200 µg/ml) was used as standard.

Assessment of anti-inflammatory activity of different extracts of *Ixora javanica*

Animal details

Albino rodents (Wistar strain, mean weight 200–250 g) were utilized for this examination. They were sheltered in the animal house for a number of weeks and they were contented with standard natural conditions. The animals were given standard research center nourishment and water *Ad libitum* and kept up at a characteristic day and night cycle. The test convention was endorsed by the CPCSEA, New Delhi, after the endorsement of the Institutional Animal Ethics Committee.

Preparation of dose

The extract of IJ flowers at a dose of 2000 mg/kg body weight was thought to be safe because it showed no signs of toxicity.[8] A dose of 100 mg and 200 mg per kg body weight was taken, and the suspension was prepared using CMC. One percent w/v carrageenan was prepared in normal saline used for inflammation.

Animal grouping and dosing

For performing the analgesic and anti-inflammatory activity in all models, rats were arbitrarily segregated into groups (negative control, positive control, and two test groups) and each group contained six animals. The first group was allocated as control and administered distilled water at an amount of 10 ml/kg. Group second was allocated as positive control and received a standard drug (Indomethacin) at a dose of 20 mg/kg. The rest two groups received distinct doses (100 mg/kg, 200 mg/kg) of methanolic extract for the carrageenan-induced paw edema model. OECD-Organization for Economic Cooperation and Development 425 guidelines were followed for the determination of dose after the evaluation of the plant for acute toxicity. Drugs were administered orally.

Carrageenan-induced rat paw edema method

The anti-inflammatory activity was assessed utilizing carrageenan-initiated paw edema in rodents as depicted with slight adjustments; acute inflammation was initiated by infusing carrageenan (1% w/v carrageenan in typical saline, 50 µl) into the plantar surface of the right rear paw of the rat.[5,9] At first, the rats were separated into five groups. In the individual group, the rats were pretreated with standard medication, the vehicle and the extracts, 60 min before infusion of carrageenan. The acute phase of the inflammatory reaction was quantified in terms of displacement of water (ml) by edema using a digital plethysmometer (Orchid) at times 0, 1, 2, 3, 4, and 5 h, respectively, after carrageenan injection. The percent inhibition of edema was computed in contrast to the animals in the control group by utilizing the following formula.

The percentage of anti-inflammatory activity was computed using the formula inclined below:

$$100\times (1 - Vt/V_c)$$

V_c is the control group mean; V_t is the testing group mean.

Gas chromatography coupled with mass spectroscopy

The equipment used for GC-MS/MS was of Shimadzu GC-MS Model Number: QP2010S. The column used for the study was Rxi-5Sil MS, (30 m × 0.25 mm × 0.25 µm). The column oven temperature was 60°C, and injection temperature was 260 C, the sampling time was 2.00 min, the flow control mode was linear velocity, the pressure was 57.4 kPa, the column flow was 1.00 mL/min, GC Program (GC-MS-QP2010) and ion source temperature was 200°C, interface temperature was: 280°C and solvent cut time was 5.00 min, the run time was set for 49 min.

The mass fragmentation started at 50.00 and end 650.00 m/z: The ethyl acetate, petroleum ether, and methanol extracts were subjected to GC-MS. GC-MS data were used to compare with libraries used NIST 11 and WILEY 8.

Results

Phytochemical screening of different extracts

Every extract was screened to know the main phytoconstituents present in it. According to the early phytochemical screening results, the methanolic extract is more abundant in phenolic compounds, flavonoids, and other chemicals. In Table 1, the phytoconstituents of various extracts were enumerated. Other therapeutic effects might be brought on by certain phytochemical combinations (secondary metabolites) present in plant extracts.

Antioxidant assessment of *Ixora javanica* extracts

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

When the antioxidant is present with the DPPH in the DPPH scavenging assay, a color shift occurs, turning the purple DPPH into a yellow substance. The deeper the yellow color of DPPH, the more striking the antioxidant activity of the extract that has been investigated. The reaction among the DPPH and the antioxidant test molecule leads to the formation of a stable complex which gives the color. The standard ascorbic acid expressed 96.29% prohibition at a concentration of 600 ppm, while Ethyl acetate disclosed 80.77. The extracts with petroleum ether and chloroform expressed a relatively lower percentage of inhibition. The percentage of inhibition for the extracts and the standard data are compiled in Table 2 and Figure 1. IC_{50} values for all the extracts are given in Table 3.

Table 1: Phytoconstituents of different extracts of *Ixora javanica*

Chemical components	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Steroids	+	+		+
Triterpenes	+	+	+	+
Alkaloids			+	+
Tannins				+
Glycosides			+	+
Saponins		+		+
Flavonoids		+	+	+
Carbohydrates		+	+	+

+: Presence of compounds, - The absence of compounds in high quantity

Reducing power activity

When the antioxidant compounds are tested with the help of a reducing power assay, the iron (Fe^{3+}) in $FeCl_3$ converts to ferrous (Fe^{2+}), the formation of a metal ion combination with iron determines the iron's oxidation state. This is the key principle behind the reducing power assay done for any antioxidant compound. In this study, ascorbic acid was chosen as the standard or positive control. Ascorbic acid exhibited 71.48% prohibition at a concentration of 800 ppm, while the ethyl acetate and methanol exhibited 69.99% and 72.11%. Other extracts such as chloroform and petroleum ether, exhibited a reduced percentage of inhibition compared to the standard as well as ethyl acetate and methanol extracts. The percentage of inhibition of all the extracts and standard data are encapsulated in Table 4 and Figure 2. Ascorbic acid, the reference standard, matches the activity with that of ethyl acetate and methanol extracts. IC_{50} values for all the extracts are given in Table 3.

Antimicrobial activity

Assessment of antimicrobial activity

The antimicrobial activity of IJ extracts was carried out for four different extracts i.e., petroleum ether, ethyl acetate, chloroform, and methanol, at a concentration of (500 µg/ml). The antimicrobial activities of various extracts were studied with Amoxicillin and were taken as standard. The antimicrobial activity was examined using a zone of inhibition of bacterial growth for different extracts. The results [Table 5] and the Figures 3 and 4 are shown below. In the present study, Gram-negative bacteria have shown or not shown microbial activity against Gram-positive bacteria shown or not shown activity against different extracts. The antimicrobial activity was estimated by calculating the zone of inhibition with different extracts against Gram-positive and Gram-negative stains Figures 3-5. The Gram-positive bacteria *E. coli* used in the study had shown antimicrobial activity in methanolic extract as 10 mm.

Assessment of anti-inflammatory activity of different extracts of Ixora javanica

Anti-inflammatory activity of ethyl acetate and methanol extracts of IJ by carrageenan-induced paw edema at different time intervals is given in Table 6. Anti-inflammatory activity

Table 2: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay by different extracts of *Ixora javanica*

Concentration (µg/mL)	Ascorbic	Pet ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
50	48.006	7.26	10.45	37.63	7.38
100	63.89	24.23	10.47	44.99	27.85
200	93.74	67.81	24.42	49.39	38.33
400	96.74	69.36	50.26	69.99	70.47
600	96.29	75.29	69.42	80.77	72.21
800	96.42	75.79	92.53	89.25	96.28
1000	98.58	90.12	96.11	96.23	97.22

of ethyl acetate and methanol extracts of IJ in terms of % inhibition of paw volume is given in Table 7. Anti-inflammatory activity of ethyl acetate and methanol extracts of IJ by carrageenan-induced paw edema at different time intervals is given in Figure 6. Anti-inflammatory activity of ethyl acetate and methanol extracts of IJ in terms of % inhibition of paw volume is given in Figure 7.

Chromatographic isolation of ethyl acetate and methanol fraction using Gas chromatography–mass spectrometry/mass spectrometry

The results obtained from GC-MS analysis led to the identification of phytoconstituents present in ethyl acetate and methanol extract of IJ. The GC-MS spectra of ethyl acetate [Figure 8] and methanol [Figure 9] indicated the presence of Methyl palmitate, 11-Tricosene, Octadecyl Fluoride, Methyl Linolelaidate, Methyl 9-Octadecenoate, 9-Eicosene, (E), Methyl 12-Methyltetradecanoate, 1,1-Dichloro-2-Dodecanol, Dioctyl Phthalate, Unknown Terpene and Docosane in the ethyl acetate fraction of *I. javanica* given in Table 8. The methanol fraction of IJ contains 2-Ketoisohexanoic acid, Chinic acid, 7, 9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, Methyl palmitate, Dibutyl phthalate, Mome inositol, Glycerol. beta.-palmitate, 1,2-Benzenedicarboxylic acid, 5.Alpha.-Androstan-3.alpha.-ol-17-one glucuronide, (3. BETA.,23E)-9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate, 5,7-Ergostadienol, 23-(Phenylsulfonyl)lanosta-8,24-dien-3-ol, Octadecanoic acid, 2,3-dihydroxypropyl ester, Decan dioic acid, bis (2-ethylhexyl) ester, Heptadecanoic acid, heptadecylic ester, 3',6-dihydro-(3.beta.,5.beta.,6.alpha.,22. xi.,23.xi.)-Cyclopropa[5,6]-33-norgorgostan-3-ol, 2,4a, 8,8-Tetramethyldecahydrocyclopropa [d] naphthalene, 3. beta.,22z-Chola-5,22-dien-3-ol, Stigmast-4-en-3-one and Vitamin E in Table 9.

Table 3: IC_{50} values of all the extracts using 2,2-diphenyl-1-picrylhydrazyl and reducing power assay

Extracts	IC_{50} value for DPPH assay	IC_{50} value for reducing power assay
Ascorbic acid	56.4	187
Methanol	273.81	307
Chloroform	398.6	508
Ethyl acetate	296	412
Petroleum ether	530	556

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Discussion

Methanol and ethyl acetate extracts of IJ shown promising antioxidant activity compared to ascorbic acid as a standard. Antioxidant potential was evaluated by DPPH and the Reducing power method. Both this method shows quite similar IC_{50} value as compared to ascorbic acid. Thus, from the above study, we can conclude both of these extracts are having antioxidant potential. Antimicrobial activity of methanol extract of IJ was found to be active against *Staphylococcus pneumonia, S. aureus,* and *E. coli* with a zone of inhibition of 7.3, 8.5, and 10 mm at a concentration of 500 µg/ml. All the other extracts were also evaluated for antimicrobial assay, but no extracts showed any potential against gram-positive and gram-negative bacteria. Hence, there might be the presence of antimicrobial compounds in the methanolic extract, which is responsible for antimicrobial activity. Anti-inflammatory activity was evaluated for all the extracts by the paw edema method. The test drug was given in two different doses 250 mg/kg (T1) and 500 mg/kg (T2). Methanolic extract of IJ has promising anti-inflammatory activity. The test drug (T2) was found

Table 4: Reducing power assay by different extracts of *Ixora javanica*

Concentration (µg/mL)	Ascorbic	Pet ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
50	40.25	1.815	2.58	9.4	11.07
100	46.43	3.63	5.15	18.81	22.15
200	50.71	7.26	10.35	37.63	44.31
400	54.19	24.23	10.47	44.99	55.21
600	58.003	40.23	24.42	49.39	56.23
800	71.48	59.33	50.26	69.99	72.11
1000	74.18	60.01	69.42	70.21	73.09

Table 5: Antimicrobial activity of all extracts of *Ixora javanica*

Extracts	*Staphylococcuspneumonia*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhi*
Petroleum ether (PE)	–	–	–	–
Ethyl acetate (EA)	–	–	–	–
Chloroform	–	–	–	–
Methanol (mm)	7.3	8.5	10	–
Standard (amoxicillin)	–	74	27	–

EA: Ethyl acetate, PE: Petroleum ether

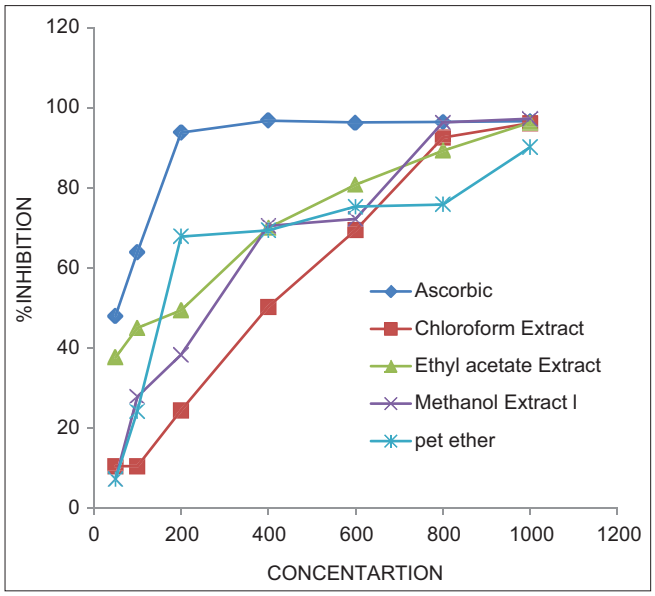

Figure 1: This graph represents the all extracts % of Inhibitions in DPPH assay. DPPH: 2,2-diphenyl-1-picrylhydrazyl

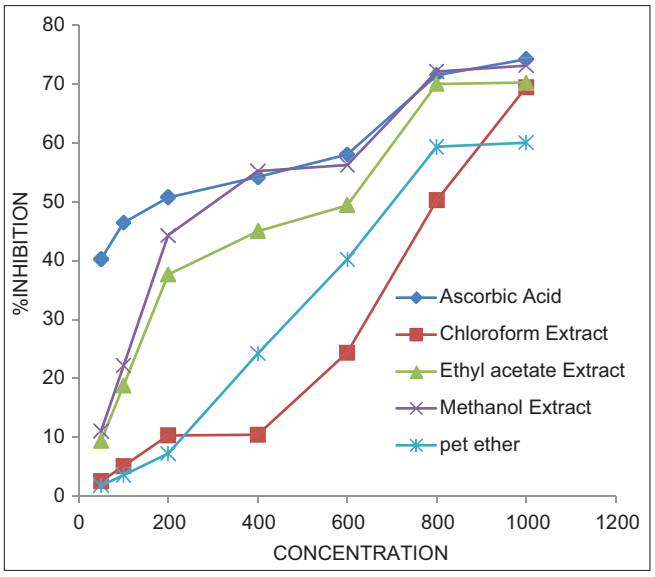

Figure 2: This graph represents the all extracts % of Inhibitions by reducing power assay

Figure 3: Zone of inhibition of different extracts (PE, EA, CH and ME) of *I. javanica* on *S. aureus*. Zone of inhibition of different extracts (PE, EA, CH and ME) of *I. javanica* on *E. coli*. CH: Chloroform, ME: Methanol, PE: Petroleum ether, EA: Ethyl Acetate, *S. aureus*: *Staphylococcus aureus*, *I. javanica: Ixora javanica, E. coli: Escherichia coli*

Figure 4: Zone of inhibition of different extracts of PE, EA, CH, ME of *I. javanica* on *S. typhi*. zone of inhibition of different extracts of PE, EA, CH, ME of *I. javanica* on *S. pneumoniae*. CH: Chloroform, ME: Methanol, PE: Petroleum ether, EA: Ethyl acetate, *I. javanica: Ixora javanica, S. pneumonia: Streptococcus pneumonia*

Table 6: Anti-inflammatory activity of and ethyl acetate and methanol extracts of *Ixora javanica* flowers in carrageenan-induced paw edema

Treatment group	Dose (mg/kg)	30 min	1 h	2 h	3 h	4 h
Control		0.043	0.047	0.050	0.052	0.051
Standard	20	0.023	0.022	0.021	0.017	0.015
Test 1 (methanol)	100	0.030	0.029	0.027	0.025	0.023
Test 2 (methanol)	200	0.026	0.025	0.022	0.020	0.018
Test 1 (EA)	100	0.032	0.031	0.029	0.026	0.025
Test 2 (EA)	200	0.028	0.026	0.025	0.022	0.021

EA: Ethyl acetate

to have very promising activity when compared to that of the standard drug indomethacin. Hence, we can conclude that the methanolic extract has anti-inflammatory activity, i.e., anti-inflammatory compounds may be present in the methanolic extract, which is culpable for anti-inflammatory activity. The GC-MS data of the methanol and ethyl acetate extracts shows nearly 32 compounds, among which 5,7-Ergostadienol suggest the presence of steroidal hormones present in the flower parts of *Ixora javanica*in methanol extract. Methanol extract of the flowers of IJ was found to have rich in Vitamin E. Methyl palmitate is a major chemical constituent present in both the extracts of methanol and ethyl acetate of IJ. Both the extracts of IJ

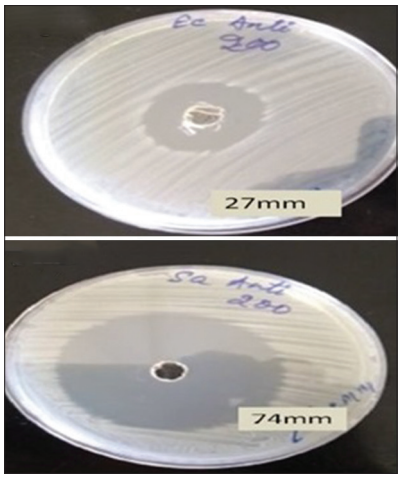

Figure 5: Zone of inhibition of amoxicillin (standard) on *E. coli.* Zone of inhibition of Amoxicillin (standard) on *S. aureus*. *S. aureus*: *Staphylococcus aureus*, *E. coli: Escherichia coli*

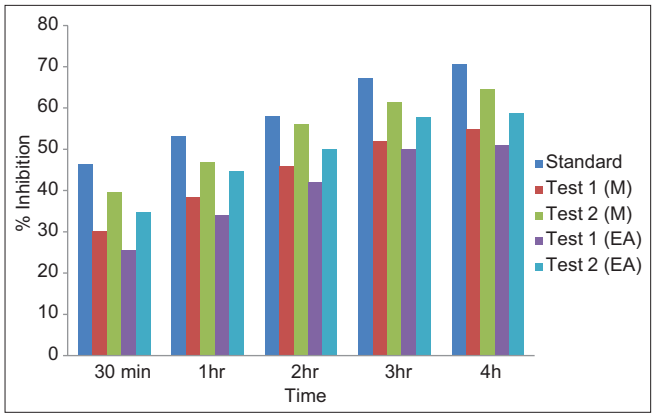

Figure 7: Anti-inflammatory activity of and EA and methanol extracts of *I. javanica* flowers in terms of % inhibition by carrageenan-induced paw edema. EA: Ethyl acetate, *I. javanica: Ixora javanica*

Table 7: Anti-inflammatory activity of and ethyl acetate and methanol extracts of *Ixora javanica* flowers in terms of % inhibition by carrageenan-induced paw edema

Percentage inhibition	Dose (mg/kg)	30 min	1 h	2 h	3 h	4 h
Standard	20	46.512	53.191	58.000	67.308	70.588
Test 1 (methanol)	100	30.233	38.298	46.000	51.923	54.902
Test 2 (methanol)	200	39.535	46.809	56.000	61.538	64.706
Test 1 (EA)	100	25.581	34.043	42.000	50.000	50.980
Test 2 (EA)	200	34.884	44.681	50.000	57.692	58.824

EA: Ethyl acetate

have potential anti-inflammatory activity this is due to the existence of Methyl palmitate as a primary phytoconstituent in IJ. The methyl palmitate serves as an anti-inflammatory agent for the extracts (methanol and ethyl acetate) of IJ. This research finding can help to develop a new potent anti-inflammatory agent due to the presence of IJ. Recent

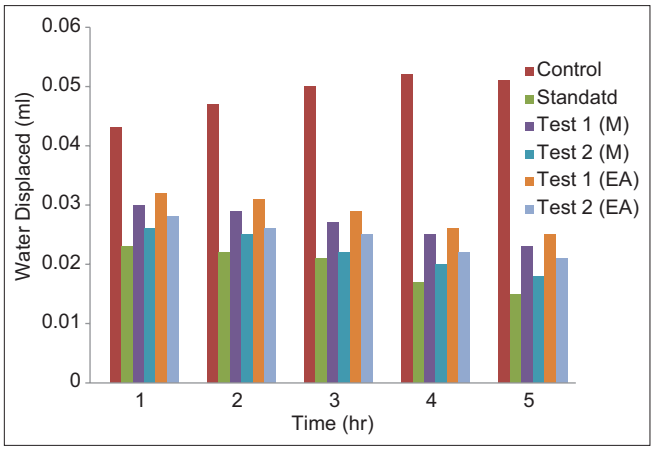

Figure 6: Anti-inflammatory activity of and EA and methanol extracts of *I. javanica* flowers in carrageenan-induced paw edema. EA: Ethyl acetate, *I. javanica: Ixora javanica*

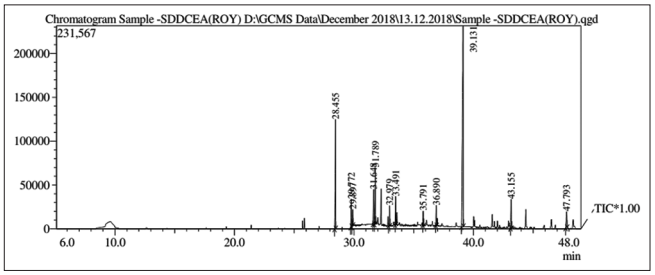

Figure 8: GC-MS chromatogram of EA extract of *I. javanica.* GC-MS: Gas chromatography–mass spectrometry, EA: Ethyl acetate, *I. javanica: Ixora javanica*

research findings suggested that methyl palmitate has its potency as a potent vasodilator released in the retina.[10] Vitamin E or tocopherol was found in methanol extract, which is a potent antioxidant.

Conclusion

The IJ plant is an immense treasure of a variety of ailments. The present study was focused on the evaluation of various extracts of IJ for antioxidant, antimicrobial, and anti-inflammatory activities. Overall, the methanol and ethyl acetate extracts of IJ were found to be active for imparting the above said pharmacological activities. The GC-MS analysis of methanol and ethyl acetate extracts of IJ revealed the presence of methyl palmitate as a major bioactive component responsible for anti-inflammatory activity. Moreover, the different extracts of IJ exhibited antioxidant activity due to the presence of vitamin E (tocopherol). Therefore, IJ can be further evaluated for molecular pharmacological studies.

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Table 8: Compounds present in the ethyl acetate extract of *Ixora javanica* using gas chromatography-mass spectrometry analysis

Peak	Name	Retention time	Area	Area (%)	Height	Height (%)	Base peak m/z
1	Methyl palmitate	28.455	299,711	14.67	124,815	19.05	74.10
2	11-tricosene	29.772	78,674	3.85	31,977	4.88	55.10
3	Octadecyl fluoride	29.897	45,506	2.23	18,955	2.89	57.10
4	Methyl linolelaidate	31.648	102,745	5.03	39,399	6.01	67.10
5	Methyl 9-octadecenoate	31.789	171,189	8.38	64,303	9.81	55.05
6	9-octadecenoic acid (Z)-	32.979	52,110	2.55	23,400	3.57	55.05
7	9-eicosene, (E)-	33.491	76,834	3.76	32,435	4.95	55.05
8	Methyl 12-methyltetradecanoate	35.791	37,871	1.85	16,364	2.50	74.05
9	2-dodecanol, 1,1-dichloro-	36.890	51,545	2.52	22,994	3.51	57.10
10	Dioctyl phthalate	39.131	979,757	47.95	228,964	34.94	149.05
11	Unknown terpene	43.155	81,350	3.98	33,221	5.07	69.10
12	Docosane	47.793	66,062	3.23	18,432	2.81	57.10

Table 9: Compounds present in the methanol extract of *Ixora javanica* using gas chromatography-mass spectrometry analysis

Peak	Name	Retention time	Area	Area (%)	Height	Height (%)	Base peak m/z
1	2-ketoisohexanoic acid	22.350	2,540,299	2.69	157,056	2.31	73.05
2	Chinic acid	25.636	60,427,609	63.88	860,226	12.64	60.05
3	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	27.972	589,354	0.62	183,153	2.69	57.10
4	Methyl palmitate	28.353	576,605	0.61	210,248	3.09	74.10
5	Dibutyl phthalate	28.955	702,418	0.74	221,946	3.26	149.10
6	Mome inositol	29.574	1,390,462	1.47	147,619	2.17	73.10
7	Glycerol .beta.-palmitate	38.769	1,362,367	1.44	206,684	3.04	57.05
8	1,2-benzenedicarboxylic acid	38.960	3,966,219	4.19	1,332,285	19.57	149.05
9	5.alpha.-androstan-3.alpha.-ol-17-one glucuronide	40.007	3,313,697	3.50	476,132	7.00	69.10
10	9,19-cyclolanost-23-ene-3,25-diol, 3-acetate, (3.beta.,23E)-	40.135	5,656,042	5.98	540,716	7.94	81.15
11	5,7-ergostadienol	40.250	2,610,043	2.76	478,123	7.02	55.10
12	23-(phenylsulfanyl) lanosta-8,24-dien-3-ol	40.631	559,061	0.59	113,063	1.66	109.15
13	Octadecanoic acid, 2,3-dihydroxypropyl ester	41.901	619,890	0.66	61,837	0.91	73.05
14	Decan dioic acid, bis (2-ethylhexyl) ester	42.874	964,704	1.02	345,617	5.08	57.10
15	Heptadecanoic acid, heptadecyl ester	43.225	572,753	0.61	73,252	1.08	55.10
16	Cyclopropa[5,6]-33-norgorgostan-3-ol, 3',6-dihydro-, (3.beta.,5.beta.,6.alpha.,22.xi.,23.xi.)-	44.372	881,599	0.93	135,001	1.98	55.05
17	2,4a, 8,8-tetramethyldecahydrocyclopropa[d] naphthalene	44.732	2,257,703	2.39	287,498	4.22	123.15
18	Stigmast-4-en-3-one	45.500	822,110	0.87	163,145	2.40	124.15
19	Chola-5,22-dien-3-ol, (3.beta.,22z)-	47.877	715,991	0.76	126,882	1.86	69.10
20	Vitamin E	48.312	2,101,458	2.22	355,660	5.23	165.15

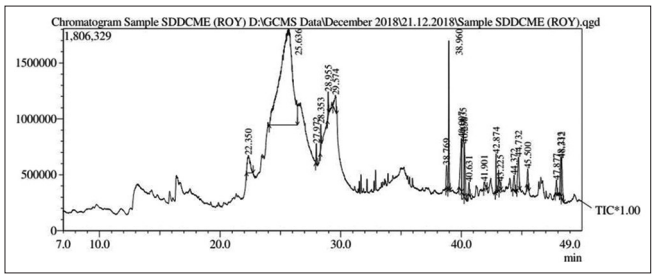

Figure 9: GC-MS chromatogram of methanol extract of *Ixora javanica*. GC-MS: Gas chromatography–mass spectrometry

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Nil.

Conflicts of interest

There are no conflicts of interest.

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