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OPTIMISED EXTRACTION AND ANTIOXIDANT PROFILING OF *Ixora javanica* VIA SPECTROSCOPY AND GC–MS

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ABSTRACT

Oxidative stress occurs when the human body produces free radicals, which can lead to disease. Consequently, there is interest in finding natural antioxidants from medicinal plants. *Ixora javanica* (commonly known as *Bunga Jenjarum* in Southeast Asia) has a traditional history of use for treating fever, infections, and inflammations; however, its antioxidant properties remain underexplored. This study aimed to investigate the phytochemical constituents and antioxidant activity of extracts from *Ixora javanica* flowers prepared with methanol, a methanol–acetone mixture, hexane, and a hexane–ethyl acetate mixture. The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) using a 96-well plate. The methanol–acetone mixture contributed the best solvent system activity, as evidenced by the lowest IC_{50} value of 48.73 µg/mL, and methanol was the second-best solvent system with an IC_{50} value of 50.21 µg/mL, while non-polar solvent systems exhibited weak effects. The antioxidant assay indicates that the methanol–acetone mixture as a solvent revealed the presence of flavonoid and phenolic compounds in *Ixora javanica*. Fourier-Transform Infrared (FTIR) spectroscopy analysis determined the functional groups, such as hydroxyl, O–H (~3282 cm^{-1}) and carbonyl, C–O (~1070 cm^{-1}), indicating that *Ixora javanica* possesses bioactive compounds. Gas Chromatography–Mass Spectrometry (GC–MS) analysis identified 19 phytochemical constituents, including 2-butoxyethanol and 2,4-di-tert-butylphenol, which may contribute to the overall antioxidant activity of *Ixora javanica*. The observations suggest that the bioactive compounds and antioxidants of *Ixora javanica* have significant phytochemicals for the functional foods, nutraceuticals, and pharmaceutical industries, and that these need to be consistently validated in traditional uses.

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Introduction

Excessive production of free radicals gives rise to oxidative stress, which leads to cellular damage and increases the progression of many chronic pathologies such as cancer, cardiovascular diseases, and neurodegenerative disorders (Chandimali *et al*., 2025). Therefore, there is considerable interest in natural plant-derived antioxidants as safe and sustainable alternatives to synthetic antioxidants. Researchers value the flowers of *Ixora javanica* because they have been proven to be medicinal and have effects such as the treatment of cancer, reducing inflammation, and providing antioxidant benefits, as well as flavonoids and anthocyanins, which have a wide range of health benefits (Dontha *et al*., 2016; Nadeem *et al*., 2024). Flavonoids, phenolic acids, carotenoids, and tocopherols fall into the category of antioxidants that mostly neutralise the free radicals that harm the human body (Qaisi *et al*., 2024). Flavonoids, phenolic acids,

carotenoids, and tocopherols are considered to be among the antioxidants that neutralise the free radicals that cause damage to the human body (Qaisi *et al*., 2024)

Phytochemicals are extracted with loss of vitamins and antioxidants (if the process is not performed correctly and under favourable conditions) during stress and after stress, due to the pressure and negative influences of environmental factors, which, among others, show the recovery and conversion of reactive oxygen species. Thus, an efficient extraction method is necessary to maximise the recovery of bioactive compounds and to reliably estimate antioxidant capacity (Shebis *et al*., 2013). Among these methods, the DPPH radical scavenging assay, TPC, and TFC are the most widely used quantitative techniques for assessing antioxidant activity and anti-free-radical efficiency (Seruji *et al*., 2023). These are supported by analytical methods such as FTIR spectroscopy, Ultraviolet-Visible (UV-Vis) spectroscopy, and GC–MS, which permit the identification of bioactive compounds and functional groups associated with antioxidant potential (Dey *et al*., 2023; Indira *et al*., 2024). While there is previous research on various aspects of *Ixora javanica*, no data are available on the extraction of flavonoids and phenolic compounds (Oktaviyanti *et al*., 2020).

Additionally, FTIR spectroscopy has not been used to investigate the bioactive constituents of *Ixora javanica* extracts in all the studies. The objective is to identify the antioxidant activity of *Ixora javanica* extracts using DPPH, TFC, and TPC. UV-Vis spectroscopy, FTIR spectroscopy, and GC–MS were used to analyse phytochemical compounds to better understand phytochemicals. *Ixora javanica* has been used traditionally, and preliminary phytochemical reports indicate its medicinal benefits; however, no detailed studies have yet reported solvent extraction optimisation, antioxidant assays, or spectroscopic characterisation. This study, therefore, investigates the antioxidant activities and phytochemical composition of *Ixora javanica* flowers using solvents of varying polarities. The antioxidant capacities were evaluated using techniques such as DPPH, TPC, and TFC, followed by FTIR, UV-Vis, and GC–MS analysis. It revealed the presence of functional groups and major bioactive components in each plant extract.

Materials and Methods

Preparation of Flower Extract

Fresh flower samples of *Ixora javanica* (Figure 1) were collected from a residential area at Bandar Puteri Jaya, Sungai Petani, Kedah, and a residential area in front of SJK(C) Hua Hsia, Bagan Datuk, Perak. The flowers (4 kg) were washed, dried in an oven at 50°C, and ground into a fine powder. Powdered samples of 500 g were soaked twice in different solvents: Hexane, mixtures of hexane-ethyl acetate (1:1 v/v), methanol, and mixtures of methanol–acetone (1:1 v/v) for three days. The extracts were filtered and evaporated under reduced pressure using a rotary evaporator at 40°C to remove solvents without degrading the compounds. Crude extracts were weighed to determine the extraction yield and stored at 4°C until analysis.

Figure 1: The picture of red *Ixora javanica*

Phytochemical Screening

Additionally, Rajayan *et al*. (2024) investigated the secondary metabolites, including phenolic compounds, flavonoids, tannins, and alkaloids, in *Ixora javanica* using a phytochemical screening method. Flavonoids, phenolics, alkaloids, terpenoids, and steroids of the plant were confirmed using the Libermann-Burchard, Dragendroff, Shinoda, Wagner, and Mayer tests (Dey *et al*., 2023).

Antioxidant Activity

DPPH Radical Scavenging Assay

The free radical scavenging activity of red *Ixora javanica* extracts was tested using the DPPH assay (Azizan *et al*., 2020). The crude extract (5 mg) was dissolved in methanol. The samples were then sonicated and vortex-mixed so that uniform aliquots could be easily prepared. 100 µL of methanol and 100 µL of the sample solution were added to the 96-well plate. In triplicate, 100 µL of the DPPH solution was added to each well. Quercetin was added as a positive control. A microplate reader was used to measure the absorbance at 517 nm after incubation in the dark at room temperature for 30 minutes (Rikhabchand & Dayaram, 2017). The results are expressed as the mean ± standard deviation of the percentage inhibition of DPPH at different concentrations, with gallic acid as a positive control (Jaafar *et al*., 2022). The percent inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = (AB\text{-}AS) / AB \times 100, \quad (1)$$

where AB and AS are the absorbance of the reagent blank and the tested samples, respectively.

Total Flavonoid Content

An aluminium chloride colorimetric test was used to determine the flavonoid content. A solution was prepared by combining 120 µL of distilled water, 10 µL of 5% sodium nitrate, and 20 µL of the sample extract, which was 5 mg/mL in methanol. After a 6-minute incubation, 10 µL of a 10% aluminium chloride solution was added. Following an additional 6 minutes, 40 µL of sodium hydroxide (1 mol/L) was introduced to the mixture in the well. The plate was subsequently stored at room temperature in the dark for 15 minutes. Finally, the absorbance was measured at 510 nm using a microplate reader (Sari *et al*., 2023; Umar *et al*., 2023).

Total Phenolic Content

The method allows accurate determination of phenolic content by minimising potential interferences and optimising reaction conditions for reliable results. The revised method is also applicable to various plant extracts, as described in related studies (Luaces *et al*., 2021). The Folin-Ciocalteu reagent method was used to determine the TPC. The samples were dissolved in methanol to a final concentration of 5 mg/mL. A 20 µL aliquot of the extract was combined with 100 µL of Folin-Ciocalteu reagent (incubated for 5 minutes) and 80 µL of 7.5 % (w/v) Na_2CO_3 solution. After 2 hours of incubation at room temperature, the absorbance was determined at 760 nm by a spectrophotometer. Standard curves were constructed from eight different concentrations of gallic acid (Sari *et al*., 2023).

FTIR Spectroscopy

20 mg of crude extracts, re-dissolved in 1 mL of analytical acetone, were analysed using a Shimadzu Corp IR Tracer-100 FTIR spectrometer equipped with 40 interferograms and a scan range of 600 to 4,000 cm^{-1} at a resolution of 4 cm^{-1}. The Attenuated Total Reflectance (ATR) crystal centre was loaded with about 0.1 mL of the sample (1 mg/mL). At room temperature, samples were dried for approximately 40 seconds. A spectrum of the ATR crystal was recorded before each sample scan using the same instrumental conditions as the background. After each sample scan, the ATR plate was cleaned with acetone and a dust-free tissue. Six biological replicates and three technical replicates were used for the FTIR analysis of the samples. All FTIR values were transformed to American Standard Code for Information Interchange (ASCII) files, and multivariate data analysis was performed (Jaafar *et al*., 2022)

Ultraviolet-Visible Spectroscopy

Scanning of the flower extracts with various solvents was performed using UV-Vis spectroscopy. The extraction solvent was mixed with the sample, which was diluted twice at room temperature. Absorbance spectra were collected in the range of 200 to 600 nm using a UV-Visible spectrophotometer. Subsequently, 2 mg of the extract was precisely measured and re-dissolved in a suitable solvent. Methanol served as the blank for calibrating both methanol and the mixture of methanol with acetone for the crude extracts. At the same time, hexane was utilised as the blank for calibrating hexane and the hexane-ethyl acetate mixture for the crude extract prior to conducting the solvent baseline analysis.

Gas Chromatography–Mass Spectrometry

The GC–MS analysis was carried out using an Agilent Technologies GC–MS (GC-7890A, MS 5975C) with a fused silica capillary column measuring 15 m x 0.2 mm ID x 1 µm. The instrument was set to an initial temperature of 110°C and maintained at this temperature for 2 minutes. At the end of this period, the oven temperature rose to 280°C at 5°C per minute and was maintained for 9 minutes. The injection port temperature was set to 250°C, and the helium flow rate was 1 mL/min. The ionisation voltage was 70 eV. The samples were injected in split mode at a ratio of 10:1. The mass spectral scan range was set at 30 to 450 m/z. Using computer searches on a NIST version 2.1 MS data library and comparing the spectrum obtained through GC–MS, the compounds present in the plant's sample were identified. Interpretation of the GC–MS mass spectrum was conducted using the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 patterns. The spectra of the unknown components were compared with those of known components stored in the NIST library. The names, molecular weights, and structures of the components of the test materials were determined (Tyagi & Agarwal, 2017).

Results and Discussions

Extraction of Ixora javanica Flowers

The extraction of *Ixora javanica* flowers was influenced by the polarity of the solvent used (Table 1). The highest amount of crude extract (6.3 g) was obtained with a mixture of methanol and acetone, followed by methanol (5.7 g), hexane (3.2 g), and hexane–ethyl acetate (2.3 g). The polar media, especially the methanol and methanol–acetone mixture, were more efficient at extracting a greater number of crude extracts, as they can dissolve phenolics and flavonoids, which are abundant in *Ixora javanica* (Mahasuari *et al*., 2020). The large amount of recovered polar and semipolar compounds from the choice of methanol–acetone indicates that the application of binary solvents is advantageous.

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Table 1: Weight of different types of solvent extracts

Solvent	Weight of Crude Extract (g)
Hexane	3.2
Hexane–ethyl acetate	2.3
Methanol	5.7
Methanol–acetone	6.3

Phytochemical Analysis of Ixora javanica Flower Extracts

Phytochemical analysis of *Ixora javanica* flower extracts using different solvents is shown in Table 2. Flavonoids, phenolics, terpenoids, steroids, and alkaloids were quantitatively extracted from the methanol extract, demonstrating that methanol was effective for extracting these compounds. On the other hand, the hexane extract contained only flavonoids and terpenoids; the hexane–ethyl acetate and methanol–acetone extracts presented a broader spectrum of secondary metabolites.

These results emphasise the effect of solvent polarity on phytochemical recovery. Owing to its highly polar nature, methanol proved to be an efficient solvent for extracting phenolics and flavonoids (antioxidants) (Dontha *et al*., 2016; Dey *et al*., 2023). The existence of alkaloids and steroids in methanol extracts is consistent with previous reports on *Ixora coccinea* and *Ixora chinensis*, which found that polar solvents (methanol) were more effective than non-polar ones for the extraction of the bioactive compounds present in these extracts (Kharat *et al.,* 2023; Rajayan *et al*., 2024). In binary solvent systems, e.g., methanol–acetone at a volume ratio of 1:1 v/v, increased diversity in phytochemicals was observed, suggesting that, due to solvent combination (mixing), the solubilisation of both polar and semipolar constituents may be facilitated. This section highlights the need to select the best solvent to achieve the highest phytochemical content.

Table 2: Phytochemical composition of different extracts of *Ixora javanica*

Chemical Components	Hexane	Hexane–Ethyl Acetate	Methanol	Methanol–Acetone
Flavonoid	+	-	+	+
Phenolic	-	-	+	+
Terpenoid	+	+	+	+
Steroid	+	+	+	+
Alkaloid	+		+	+

Note: + Presence of compounds; - absence of compounds.

Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

The methanol–acetone extract exhibited the highest TPC value of 216.54 mg/g GAE [Figure 2 (a)] and TFC value of 153.82 mg/g QE [Figure 2 (b)], respectively, followed by the methanol extract with lower values of these parameters. On the other hand, phenolic compounds and flavonoids were less abundant in the hexane and hexane–ethyl acetate extracts. These results indicate that phenolic compounds and flavonoids are the primary antioxidants in *Ixora javanica*. Therefore, the findings of the present study corroborate the traditional use of *Ixora javanica* as a free radical scavenger (Dontha *et al.*, 2015).

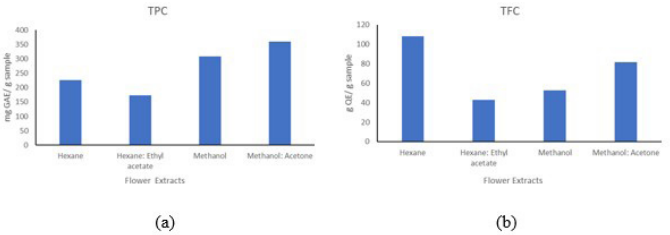


Figure 2: Bioassay of (a) TPC and (b) TFC from different types of solvent *Ixora javanica* extracts

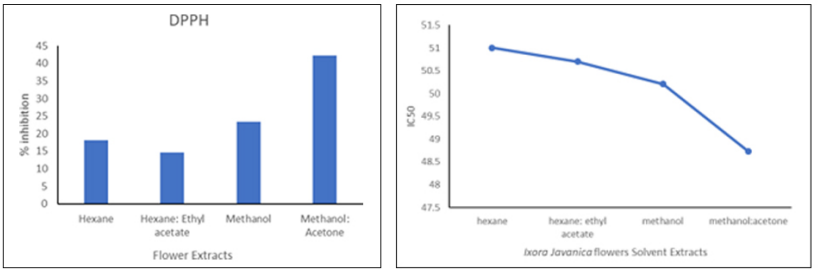
Table 3: TPC and TFC data analysis for different solvent extracts

Crude Extract	TPC ± SD mg (GAE/g)	TFC ± SD mg (QE/g)
Hexane	204.09 ± 40.28	96.53 ± 22.27
Hexane–ethyl acetate	167.82 ± 16.00	41.15 ± 3.21
Methanol	266.71 ± 108.36	47.21 ± 12.97
Methanol–acetone	332.27 ± 27.69	77.55 ± 7.02

DPPH Radical Scavenging Activity

IC_{50} values at various concentrations (25 to 350 mg/g) for all extracts, as estimated by the DPPH test [Figure 3(a)], tended to be dose-dependent. The methanol and acetone eluted extracts showed the lowest IC_{50} value (48.73 µg/mL), indicating the highest antioxidant activity compared to the other extracts tested, followed by the methanol extract (IC_{50} = 50.21 µg/mL) [Figure 3 (b)]. However, the hexane and hexane–ethyl acetate extracts showed lower activity, with higher IC50 values than those of the other extracts.

All these observations indicate that the antioxidant activity of *Ixora javanica* could be due to the presence of phenolic compounds and flavonoids, as revealed by the phytochemical screening. Higher extraction yields with methanol–acetone as the extracting solvent indicate that mixed-polarity solvents are effective for extracting several antioxidants (Lee *et al*., 2024). In addition, low IC50 values below 100 µg/mL support the notion that Ixora javanica possesses a high self-antioxidant potential, as reported in previous studies of other medicinal herbs (Kharel & Sharma, 2020). The superior performance of the methanol–acetone mixture suggests that mixed-polarity solvents enhance the extraction of both polar and semipolar antioxidant compounds. Phytochemical screening corroborated these findings, revealing a higher abundance of phenolic and flavonoid compounds in the more polar extracts. Such constituents are well known for their radical-scavenging capacity via hydrogen-atom or electron donation.



(a) (b)

Figure 3: Bioassay of (a) DPPH radical scavenging activity and (b) IC_{50} of the *Ixora javanica* solvent extracts

Correlation between DPPH–TPC

Methanol–acetone extract had the most potent antioxidant activity (DPPH % inhibition = 42.21%; IC_{50} = 48.73 µg/mL) and the highest TPC (332.27 ± 27.69 mg GAE/g). Surprisingly, TPC also showed the highest statistical correlation (R^2 = 0.9317) with DPPH (Figure 3), indicating that phenolic compounds are significant contributors to antioxidant activity. Torey *et al*. (2010) also reported the hydrogen-donating ability of the phenolic hydroxyl group as a mechanism for neutralising free radicals.

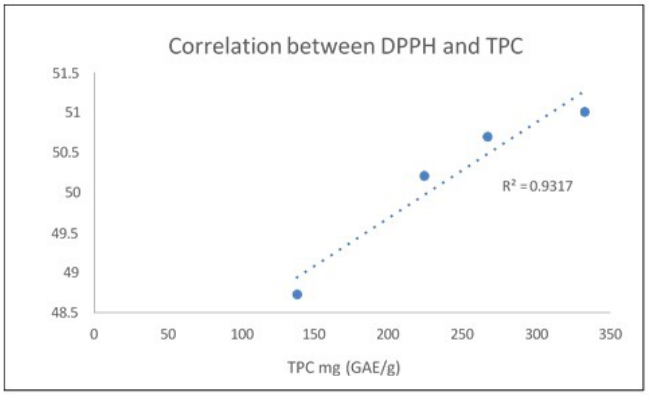


Figure 4: Correlation analysis of DPPH assay and TFC

Spectroscopy Analysis

Fourier Transform Infrared Spectroscopy

The FT-IR analysis provided significant information about the functional groups in *Ixora javanica* extracts. All solvent extracts exhibited broad absorption peaks in the range of 3,282 to 3,372 cm^{-1}, attributable to the stretching vibrations of the O–H group, which belong to hydroxyl stretches from phenolics and flavonoids. The peaks ranging from 2,851 to 2,919 cm^{-1} were assigned to aliphatic C–H stretching. In comparison, a strong band at ~1,725 cm^{-1} could be attributed to the C = O stretching modes characteristic of ester, ketone, aldehyde, and carboxylic acid functional groups. The strong absorption peak at about 1,162 cm^{-1} was attributed to C–O stretching due to the presence of alcohols and ethers.

Methanol showed the strongest O–H stretching in the extracts, indicating that its polarity facilitated the extraction of hydroxyl groups and other phenolic moieties. This is in line with the higher values of total phenolic and flavonoid contents determined in methanol extracts. These functional groups are known features of bioactive phytochemicals and reconcile the phytochemical and antioxidant assays. Similar modes of absorption were reported for other phenolic-rich medicinal plants (Johnson *et al*., 2020), suggesting that *Ixora javanica* is a potential source of natural antioxidants.

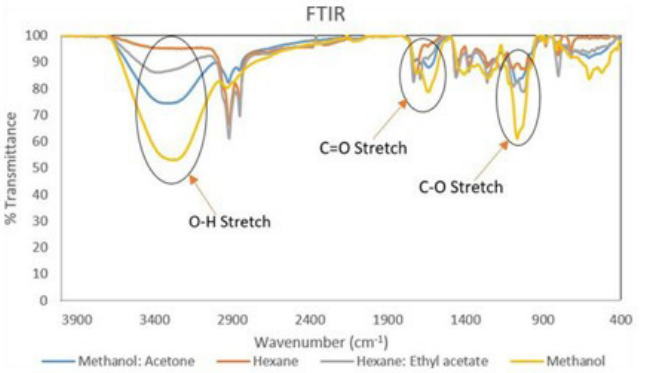


Figure 5: FTIR spectra of *Ixora javanica*'s flower crude solvent extracts

Ultraviolet-Visible Spectroscopy

Phenolic and flavonoid substances in methanol and Methanol–acetone extracts of *Ixora javanica* have been identified using UV-Vis spectroscopy. The absorption bands between 286 and 333 nm are due to n → π* (and π → π*) transitions of the phenolic absorption bands and provide evidence of a high degree of π-conjugation effects (Shebis *et al*., 2013). This high absorbance is consistent with phenolic-rich extracts, as confirmed by phytochemical analyses and antioxidant assays. On the other hand, hexane or hexane–ethyl acetate extracts showed lower absorbance in the phenolic region, but they exhibited specific bands between 446 and 669 nm (indicating the presence of *β*-carotene and other lipophilic compounds). This implies that although non-polar solvents extracted carotenoids preferentially, their contribution to antioxidant activity (DPPH scavenging) was lower than that of hydrophilic radical scavengers. Overall, UV-Vis spectra provide spectrophotometric confirmation of phytochemical results. The antioxidant activities of *Ixora javanica* are predominantly due to phenolics and flavonoids.

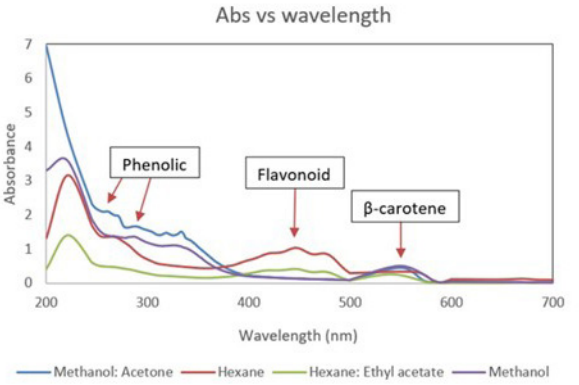


Figure 6: UV-Vis spectral overlay of *Ixora javanica* extracts

Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS analysis of methanol (Figure 7) and methanol–acetone extracts (Figure 8) of *Ixora javanica* identified 19 compounds, many of which are known for their antioxidant and pharmacological activities (Table 4). The major components identified were 2-butoxyethanol (17.5 % peak area) and 2,4-di-tert-butylphenol. On the other hand, 2,4-di-tert-butylphenol, reported as a phenolic compound in our extracts, has been known for its potent radical scavenging (Zhao *et al*., 2017) and anti-lipid peroxidative activities, which enhance the antioxidant activities observed in the DPPH, TPC, and TFC assays. Comparison of the chromatographic profiles of the methanol and methanol–acetone extracts (Figure 9) showed that the solvent used influenced the nature of the compounds that were extracted. In the methanol extraction, relatively polar aromatic antioxidants, such as 2,4-di-tert-butylphenol, were selectively extracted, whereas the methanol–acetone mixture extracted more semipolar compounds, such as trimethylbenzene derivatives and trimethylsilyl ethaneperoxoate. This indicates that the solvent's polarity not only affects the yield of extracts but also influences the qualitative chemical composition of *Ixora javanica*. The GC–MS profiling supported the implications from phytochemical and antioxidant analyses that phenolic and aromatic antioxidants were the major constituents. The occurrence of these known anti-inflammatory and lipid peroxidation inhibitors demonstrates the pharmacological potential of *Ixora javanica,* warranting its use in future nutraceutical and therapeutic formulations.

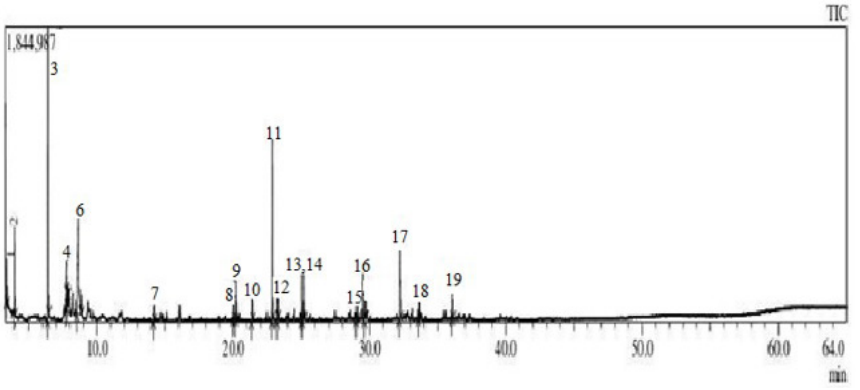


Figure 7: GC–MS Chromatogram of methanol crude extract

Table 4: GC–MS analysis of bioactive compound in the methanol crude extract

Peak	Compound Name	Retention Time	Area (%)	Molecular Weight (g/mol)	Base Peak (m/z)
1	Dimethylsilanediol	3.364	1.78	92	77.05
2	Ethyl mandelate	3.935	4.61	180	106.95
3	2-Butoxyethanol	6.413	17.48	118	57.15
4	1-Ethyl-3-methylbenzene	7.766	5.61	120	105.05
5	1-Ethyl-2-methylbenzene	7.859	2.08	120	105.05
6	1,2,4-Trimethylbenzene	8.632	20.35	120	105.05
7	Methyl phenylphosphonofluoridate	14.180	1.20	174	91.00
8	1-Tetradecene	20.014	1.40	196	55.25
9	Tetradecane	20.228	3.22	198	57.15
10	Dimethyl phthalate	21.384	1.86	194	163.05
11	2,4-Di-tert-butylphenol	22.865	15.42	206	191.20
12	Bibenzyl	23.255	1.83	182	91.05
13	(Z)-5-Nonadecene	25.017	3.83	266	83.15
14	Hexadecane	25.199	3.60	226	57.15
15	2-Octyl-1-decanol	29.046	1.15	270	57.10
16	Eicosane	29.676	1.71	282	57.20
17	Methyl hexadecanoate 1-	32.227	9.03	270	74.10
18	Nonadecene	33.622	1.90	266	57.10
19	Methyl octadecanoate	36.091	1.95	298	74.05

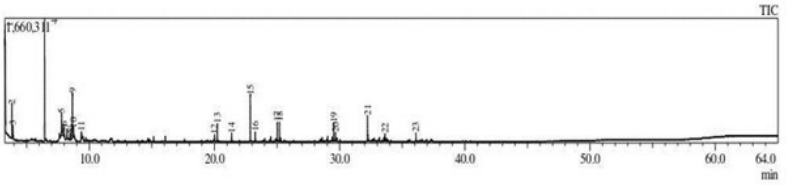


Figure 8: GC–MS chromatogram of methanol–acetone crude extract

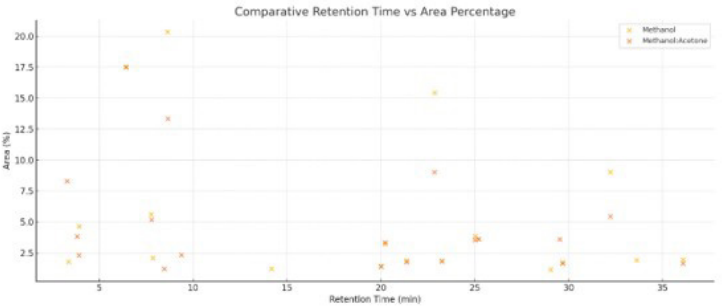


Figure 9: The comparative time versus area (%) of the methanol and methanol–acetone extracts of *Ixora javanica*

Conclusions

In conclusion, *Ixora javanica* flowers are a potential source of natural antioxidants, and the phenolic content and antioxidant activity of the methanol–acetone extract were higher than those of the other two extracts. On the other hand, spectroscopic and chromatographic studies confirmed the presence of bioactive components of diverse chemical types, which may be responsible for the plant's ethnomedicinal use and may also reflect its pharmacological activity. Further studies should concentrate on the purification of individual components, in vivo verification, and toxicological evaluation to promote the development of this species as a functional food or drug.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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