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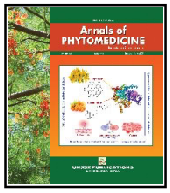

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Phytochemical characterization of *Ixora coccinea* L. flower extract using FTIR spectroscopy and GC-MS analysis for identification of bioactive compounds

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Abstract

This study investigated the chemical composition and bioactive properties of Ixora coccineaL. flower extracts using gas chromatographymass spectrometry (GC-MS) and fourier transform infrared (FTIR) spectroscopy. The goal was to identify the major compounds in both ethanolic and aqueous extracts and assess their potential pharmacological activities. GC-MS analysis identified 38 chemical compounds in the ethanolic extract and 45 in the aqueous extract, belonging to various classes such as fatty acids, alcohols, sterols, phenolic compounds, and nitrogen- and sulfur-containing heterocyclic compounds. Notably, the most abundant compound in the ethanolic extract was n-butanoic acid, 2-ethylhexyl ester (33.55%), followed by D-nannitol (25.01%) and dl-a-tocopherol (18.02%). These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties, suggesting that the ethanolic extract has potential therapeutic applications in managing oxidative stress, inflammation, and microbial infections. The aqueous extract contained 45 compounds, with 3,3-dimethyl-2-butanol, trimethylsilyl ether (11.77%) being the most abundant, followed by 3-ethylbenzonitrile (10.64%) and squalene (5.35%). The presence of bioactive compounds like squalene and dl-a-tocopherol further indicates the plant's potential for pharmaceutical and cosmetic applications due to their moisturizing and antioxidant properties. FTIR spectroscopy revealed key functional groups in both extracts, including hydroxyl (O-H), carbonyl (C=O) and alkane (C-H) groups, which are characteristic of phenolic compounds, alcohols, fatty acids and lipids. These functional groups are known for their antioxidant and anti-inflammatory activities. The broad O-H stretch at 3348 cm^{-1} and C=O stretch at 1635 cm^{-1} confirm the presence of bioactive compounds that contribute to the plant's medicinal properties.The findings of this study suggest that *I. coccinea* flowers contain a diverse range of bioactive compounds with potential therapeutic properties. The antioxidant and anti-inflammatory effects of compounds like D-mannitol and dl-a-tocopherol indicate the plant's usefulness in treating oxidative stress-related diseases and inflammatory conditions. The antimicrobial potential of the plant also makes it an attractive candidate for further pharmacological exploration.

1. Introduction

Plants have long served as a cornerstone of human healthcare systems, offering natural remedies for a wide range of diseases and health conditions. The traditional knowledge surrounding medicinal plants forms the foundation of many modern pharmaceuticals and continues to inspire the discovery of novel therapeutic agents. Among the numerous plants used in traditional medicine, *Ixora coccinea* L., a species belonging to the Rubiaceae family, holds significant ethnomedicinal importance. Commonly referred to as Jungle Flame, Flame of the Woods, or Jungle Geranium, *I. coccinea* is an evergreen shrub native to South and Southeast Asia (Rasajna *et al*., 2025). It is widely recognized not only for its ornamental beauty but also for its extensive use in traditional medicine systems such as Ayurveda, Siddha, and folk practices. Various parts of the plant, including the flowers, leaves, stems, and roots, have been utilized for treating a broad spectrum of ailments. The flowers are known for their astringent and febrifuge properties and are traditionally used to manage diarrhea, dysentery, and gastrointestinal disturbances. The root extracts have been employed for their anti-inflammatory and antimicrobial activities, particularly in treating skin infections, ulcers, and chronic wounds. In traditional Southeast Asian medicine, infusions made from the flowers are also recommended for treating headaches, fevers, and hypertension. The leaves are applied externally to promote wound healing, reduce inflammation, and alleviate pain. Beyond therapeutic applications, *I. coccinea* flowers hold ceremonial value in various cultures, being used in religious rituals to symbolize purity and vitality. Despite its widespread traditional use, scientific

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studies focusing on the phytochemical constituents of *I. coccinea* flowers remain limited, highlighting the need for systematic research to validate and explore its medicinal potential (Nair *et al*., 2018).

The increasing global interest in natural products and herbal medicines has amplified the importance of identifying and characterizing bioactive compounds from medicinal plants. Bioactive phytochemicals, including alkaloids, flavonoids, phenolics, terpenoids, tannins, and saponins, are responsible for a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic effects. As the burden of chronic diseases continues to rise and issues such as antimicrobial resistance become more pressing, the search for new, effective, and safe therapeutic agents has become a priority for researchers worldwide (Ghoshal *et al*., 2022). Medicinal plants like *I. coccinea* offer promising sources of such agents, provided their chemical profiles are properly understood. Traditional knowledge often provides valuable clues about the therapeutic potential of a plant, but without scientific validation through phytochemical studies, these claims remain largely anecdotal. Identifying the bioactive compounds present in medicinal plants is crucial not only for understanding their mechanisms of action but also for developing standardized herbal formulations and isolating novel lead compounds for drug development. Phytochemical profiling ensures the quality, efficacy, and safety of herbal products, which are increasingly demanded by regulatory authorities and consumers alike. Moreover, detailed phytochemical investigations can uncover synergistic interactions between different compounds, providing insights into the holistic efficacy often observed in traditional medicine. Thus, comprehensive phytochemical analysis of *I. coccinea* flower extracts is a necessary step toward bridging traditional medicinal knowledge with modern pharmacological science (Shreelakshmi *et al*., 2022).

To achieve a thorough understanding of the phytochemical constituents of medicinal plant extracts, advanced analytical techniques are essential. Among these, fouriertransform infrared (FTIR) spectroscopy and gas chromatography-mass Sspectrometry (GC-MS) are two powerful and complementary tools widely used in natural product research. FTIR spectroscopy is a rapid, non-destructive analytical technique that provides valuable information about the functional groups and molecular structures present in a sample (Axiotis *et al*., 2021). When infrared radiation passes through a sample, specific frequencies areabsorbed depending on the vibrational energies of the chemical bonds, producing a unique spectrum that serves as a molecular fingerprint. FTIR is particularly useful for preliminary phytochemical screening, as it allows the identification of characteristic functional groups such as hydroxyl, carbonyl, amine, and aromatic groups. This can give insights into the presence of classes of compounds like alcohols, phenols, carboxylic acids, esters, and ketones. In the context of *I. coccinea* flower extract, FTIR analysis offers a rapid means of detecting the functional groups associated with potentially bioactive phytochemicals, laying the groundwork for more detailed investigations (Ugbabe *et al*., 2018). Gas chromatography-mass spectrometry (GC-MS), on the other hand, is a highly sensitive and specific technique ideal for the separation, identification, and quantification of volatile and semi-volatile organic compounds. In GC-MS analysis, the sample is vaporized and carried by an inert gas through a chromatographic column where components are separated based on their volatility and interaction with the column material. The separated compounds then enter the mass spectrometer, where they are ionized and fragmented, generating mass spectra that can be compared to reference libraries such as the National Institute of Standards and Technology (NIST) database for compound identification. GC-MS enables the detailed profiling of complex mixtures and is particularly effective in identifying compounds such as terpenoids, fatty acids, esters, hydrocarbons, and phenolic derivatives. In the analysis of *I. coccinea* flower extract, GC-MS can provide a detailed inventory of its chemical constituents, revealing specific compounds that may be responsible for its therapeutic effects (Nwachukwu *et al*., 2019).

Together, FTIR and GC-MS offer a comprehensive phytochemical profiling approach, with FTIR identifying broad functional groups and GC-MS pinpointing specific molecular structures. Given the ethnomedicinal significance of *I. coccinea* and the lack of comprehensive phytochemical studies focusing specifically on its flowers, the present study was designed to systematically investigate the chemical composition of *I. coccinea* flower extract using FTIR spectroscopy and GC-MS analysis. The main objectives of the study were to utilize FTIR spectroscopy to identify the major functional groups present in the methanolic flower extract and to employ GC-MS analysis to detect and characterize individual volatile and semi-volatile phytoconstituents (Punetha and Vuppu, 2023). By integrating these two analytical techniques, the study aims to provide a detailed chemical profile of *I. coccinea* flowers, thereby contributing to the scientific validation of their traditional uses and highlighting bioactive compounds of potential pharmaceutical importance. Additionally, the study seeks to establish a phytochemical basis for the observed medicinal properties of *I. coccinea* flowers, offering insights that could inform further research into their pharmacological activities. Identification of key bioactive compounds not only strengthens the scientific foundation of traditional medicine but also opens avenues for the development of new drugs and health supplements derived from natural sources. Through a detailed investigation of the chemical constituents of *I. coccinea* flower extract, this research aspires to bridge the gap between traditional ethnomedicinal knowledge and modern scientific understanding, reaffirming the relevance of medicinal plants in contemporary healthcare and drug discovery (Guddi and Sarkar, 2024).

2. Materials and Methods

2.1 Authentication of plant material

Dr. R. Ramasubbu, Associate Professor, Department of Biology, Gandhigram Rural Institute, Gandhigram, Dindigul, conducted the entire botanical authentication and identification of the plant specimen. The Voucher Specimen is Catalogued and stored at the GUD Herbarium.

2.2 Collection and preparation of plant material

The flowers of *I. coccinea* were collected during the peak flowering season from healthy plants and only mature, disease-free flowers were selected for the study to ensure the consistency and quality of the extract. After collection, the flowers were thoroughly washed under running tap water followed by distilled water to remove any surface contaminants such as dust, soil particles, and microbial load. The cleaned flowers were then shade-dried at ambient room temperature (25-28°C) for approximately 10-14 days to preserve

the integrity of the phytoconstituents. Shade drying was preferred over direct sunlight to prevent the degradation of thermolabile bioactive compounds. Once completely dried, the flowers were ground into a fine powder using a mechanical grinder. The powdered material was sieved through a mesh to ensure uniform particle size and subsequently stored in airtight, amber-colored glass containers at room temperature until further use.For the extraction process, about 100 g of the powdered *I. coccinea* flower material were subjected to maceration using methanol (analytical grade, Merck) as the solvent. Methanol was chosen due to its high polarity and excellent ability to extract a wide range of phytochemicals, including flavonoids, phenolics, and terpenoids. The powdered plant material was soaked in 500 ml of methanol in a clean, dry conical flask and kept on a rotary shaker at 150 rpm for 72 h at room temperature to facilitate efficient extraction. The mixture was filtered through Whatman No. 1 filter paper to remove plant debris. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to yield a semi-solid methanolic extract. The crude extract was weighed, the yield percentage was calculated, and the extract was stored at 4°C in an airtight container for subsequent phytochemical analyses (Guddi*et al*., 2024).

2.3 Extraction methods

2.3.1 FTIR spectroscopy analysis

The FTIR spectroscopy analysis was performed using a Thermo Fisher Scientific Nicolet iS50 FTIR Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), operating in the mid-infrared range of 4000-400 cm^{-1}. The instrument was equipped with a deuterated triglycine sulfate (DTGS) detector and a standard sample compartment. All measurements were carried out at room temperature. The instrument was calibrated with a background scan prior to each analysis, and the spectral resolution was set at 4 cm^{-1} to ensure high-quality data acquisition. The software used for data acquisition and processing was OmniSpecta (Thermo Fisher Scientific). The FTIR system was routinely checked for alignment and calibration to maintain optimal performance. For FTIR analysis, a small quantity of the methanolic extract was dried to remove residual solvent, ensuring a more accurate reading. The dried sample was finely ground with spectroscopic grade potassium bromide (KBr) in a ratio of 1:100 (sample:KBr) and compressed into a thin, transparent pellet using a hydraulic press under high pressure. The KBr pellet method was selected to minimize scattering and obtain clear absorption peaks.The FTIR spectra were recorded at a resolution of 4 cm^{-1} with 32 scans per sample to ensure high signal-to-noise ratio and accuracy. Background spectra were collected under identical conditions and automatically subtracted from sample spectra by the software to correct for atmospheric interference such as water vapor and carbon dioxide. The acquired spectra were analyzed by identifying characteristic absorption bands corresponding to different functional groups. Peaks were assigned based on standard reference tables and literature data, providing insights into the major classes of phytochemicals present in the extract (Filimonov *et al*., 2014; Kamaljeet *et al*., 2024).

2.4 GC-MS analysis

GC-MS analysis was carried out using a Agilent 7890A Gas Chromatograph coupled with an Agilent 5975C Mass Selective Detector (MSD) (Agilent Technologies, Santa Clara, CA, USA). The GC was equipped with a capillary column HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness), which is widely used for the separation of volatile organic compounds. The mass spectrometer operated in electron impact ionization mode at 70 eV, with a mass range of 50-600 m/z for full scan analysis. The carrier gas was high-purity helium (99.999%), which was supplied at a constant flow rate of 1.0 ml/min. The system was controlled using Agilent ChemStation software for data acquisition, processing, and compound identification.The oven temperature program was optimized to achieve efficient separation: it was initially held at 50°C for 2 min, then ramped at a rate of 10°C/min to 280°C, where it was maintained for 10 min. The injector temperature was set at 250°C, and 1 µl of the methanolic extract, diluted appropriately with methanol, was injected in split mode (split ratio 10:1). The mass spectrometer was operated in full scan mode, scanning a mass range of 50-600 m/z. The ion source and quadrupole temperatures were maintained at 230°C and 150°C, respectively.The mass spectra generated by GC-MS analysis were compared against those in the national institute of standards and technology (NIST) mass spectral library for tentative identification. Compounds were identified based on their molecular weights, fragmentation patterns, and matching scores with library spectra. Only compounds with a high degree of match probability (>90%) were considered reliable identifications. In cases where multiple matches were possible, compounds were assigned based on retention time, peak intensity, and relevance to known phytochemicals reported in previous studies of related plant species. The relative abundance of each identified compound was determined based on the area under the corresponding chromatographic peaks (Dutta *et al*., 2020).

3. Results

3.1 Fourier transform infrared (FTIR) spectroscopic analysis of bioactive components in *I. coccinea*flower extract

FTIR spectroscopy was employed to investigate the presence of various functional groups in the extracts of *I. coccinea* flowers. This technique is vital for understanding the molecular structure and the interactions between the bioactive compounds within the extract. The FTIR spectra were recorded across the range of 4000 cm^{-1} to 400 cm^{-1}, which allowed for the identification of distinct absorption bands linked to different functional groups. The spectral data obtained from the flower extracts of *I. coccinea* highlighted the presence of various functional groups, aiding in the identification of bioactive compounds (Table 1). In the FTIR spectrum of the aqueous extract, several significant peaks were observed, reflecting the presence of various functional groups. A broad absorption band at 3348 cm^{-1} corresponds to the O-H stretching vibrations of hydroxyl groups, commonly associated with phenolic compounds and flavonoids. The peak at 1635 cm^{-1} corresponds to the stretching vibration of the C=O group, indicating the presence of carbonyl compounds. Additionally, peaks at 686 cm^{-1}, 601 cm^{-1}, 547 cm^{-1}, 432 cm^{-1}, and 408 cm^{-1} represent various bending and stretching vibrations of organic and inorganic components in the extract (Figure 1).

Similarly, the FTIR spectrum of the solvent extract revealed a range of characteristic peaks, confirming the presence of several functional groups. Absorption peaks at 3934 cm^{-1} and 3340 cm^{-1} are attributed to O-H stretching vibrations, which are indicative of alcohols and phenols. The bands at 2970 cm^{-1} and 2885 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of C-H bonds, suggesting the presence of alkanes. The peak at 1651 cm^{-1}corresponds

to C=O stretching, suggesting the presence of ketones, aldehydes, and esters. Additionally, peaks at 1450 cm^{-1}, 1419 cm^{-1}, 1381 cm^{-1}, and 1327 cm^{-1} correspond to the bending vibrations of C-H bonds in alkanes. Peaks at 1273 cm^{-1}, 1087 cm^{-1}, and 1041 cm^{-1} are indicative of alcohols, ethers, and esters. The fingerprint region of the spectrum shows peaks at 879 cm^{-1}, 686 cm^{-1}, 594 cm^{-1}, 501 cm^{-1}, and 478 cm^{-1}, which are associated with various functional groups (Figure 2).

3.1.1 Comparative analysis of aqueous and solvent extracts

A comparative analysis of the FTIR spectra from the aqueous and solvent extracts of *I. coccinea* flowers reveals distinct differences in their chemical compositions. The aqueous extract primarily exhibits strong absorption bands related to hydroxyl (O-H) and carbonyl (C=O) groups, which are characteristic of phenolic compounds and flavonoids. These compounds are likely responsible for the antioxidant properties of the plant. In contrast, the solvent extract shows prominent peaks related to O-H groups from alcohols and phenols, along with C-H stretching vibrations from alkanes and C=O stretching from ketones, aldehydes, and esters. This suggests that the solvent extract contains a wider variety of chemical constituents, including aliphatic and aromatic compounds. These differences indicate that the choice of extraction solvent has a significant impact on the types and concentrations of bioactive compounds extracted, which may influence their potential applications in pharmaceuticals, cosmetics and nutraceuticals.

Table 1: FTIR Spectroscopy of *I. coccinea* natural dye using different solvents

S. No.	Peak value (cm^{-1})	Bond	Functional group	Appearance of peak
			Aqueous extract	
1	3348	O-H	Hydroxyl groups (Phenols, Flavonoids)	S
2	1635	C=O	Ketones, aldehydes, amides	S
3	686	C-H	Alkenes, aromatic compounds	S
			Solvent extract	
1	3934	O-H	Alcohols, phenols	W
2	3340	O-H	Alcohols, phenols, carboxylic acids	S
3	2970	C-H	Alkanes (asymmetric stretching)	W
4	2885	C-H	Alkanes (symmetric stretching)	W
4	1651	C=O	Ketones, aldehydes, esters	S
5	1450	C-H	Alkanes (bending)	W
6	1419	C-H	Alkanes (bending)	W
7	1381	C-H	Alkanes (methyl groups)	W
8	1327	C-H	Alkanes, alcohols	W
9	1273	C-O	Alcohols, ethers, esters	W
10	1087	C-O	Alcohols, ethers, esters	S
11	1041	C-O	Alcohols, ethers, esters	W
12	879	C-H	Aromatic compounds	W
13	686	C-H	Alkenes, aromatic compounds	W

Note: S-Strong, W-Weak

3.2 GC-MS analysis of bioactive compounds in *I. coccinea* flower extract

The ethanolic extract of *I. coccinea* flowers was analyzed using gas chromatography-mass spectrometry (GC-MS), which led to the identification of 38 distinct chemical compounds. These compounds were characterized based on their molecular formula, molecular weight, retention time, and relative peak area percentages. The results from the GC-MS analysis, including retention times (RT) and the percentage relative composition of each compound, are summarized in Table 2.The compound identified in the highest abundance was n-Butanoic acid, 2-ethylhexyl ester, which accounted for 33.55% of the total extract and had a retention time of 13.020 min. Following this, D-mannitol was the second most prevalent compound, contributing 25.01% at a retention time of 14.274 min. Another notable compound was dl-α-tocopherol (vitamin E), which represented 18.02% of the extract and had a retention time of 15.863 min. Additional compounds identified in significant amounts included β-sitosterol (6.38% at 20.407 min) and 4,5-dimethylthiazole S-oxide (2.20% at 10.286 min). The analysis also revealed several oxygenated compounds, including benzoic acid (1.18% at 10.808 min) and hexadecanoic acid (3.36% at 13.028 min), which are often associated with antioxidant and anti-inflammatory properties. Moreover, sulfur- and nitrogen-containing heterocyclic compounds were detected, such as methylguanidine (0.04% at 11.797 min) and 5H-1-pyrimidine-3-carboxylic acid (0.11% at 10.153 min). The identification of these bioactive compounds, particularly dl-α-tocopherol and β-sitosterol, suggests

that the extract may have significant antioxidant and potential pharmacological effects (Figure 3).

Similarly, the aqueous extract of *I. coccinea* flowers was also analyzed using GC-MS, which identified 45 distinct chemical compounds. These compounds were characterized based on their molecular weight, molecular formula, retention time, and peak area percentage, as shown in Table 3. The most abundant compound in the aqueous extract was 3,3-dimethyl-2-butanol, trimethylsilyl ether, which accounted for 11.77% of the total extract and had a retention time of 10.553 min. Following this compound, 3-ethylbenzonitrile (10.64% at 9.887 min) and 1-phenylcyclopentanecarbonitrile (7.11% at 10.775 min) were also identified in significant amounts. Other important compounds detected included diethyl phthalate (6.96% at 10.387 min) and phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester (5.35% at 16.441 min). Additionally, oxygenated compounds such as benzoic acid, 2-amino-4-methyl- (0.65% at 8.842 min) and hexadecanoic acid (0.62% at 13.303 min) were identified. Furthermore, sulfur- and nitrogen-containing heterocyclic compounds, such as dihydro capsaicin (0.45% at 16.764 min) and 1-dimethyl (phenyl) silyloxypropane (0.52% at 16.652 min), were detected. The identification of bioactive compounds like squalene, dl-α-tocopherol, and terephthalic acid di(2-ethylbutyl) ester in the aqueous extract further suggests the potential antioxidant and pharmacological activities of the *I. coccinea* flowers (Figure 4).

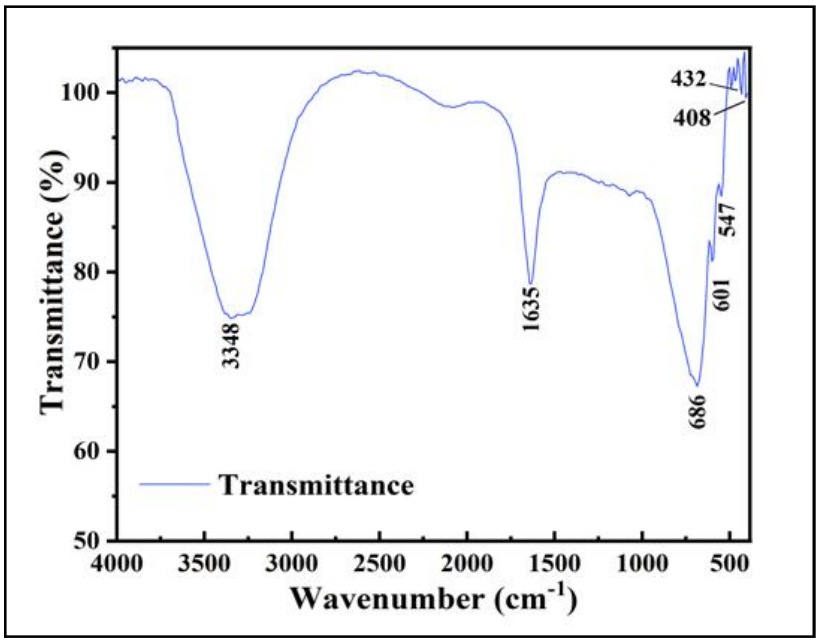

Figure 1: FTIR analysis of aqueous extract of *I. coccinea* natural dye.

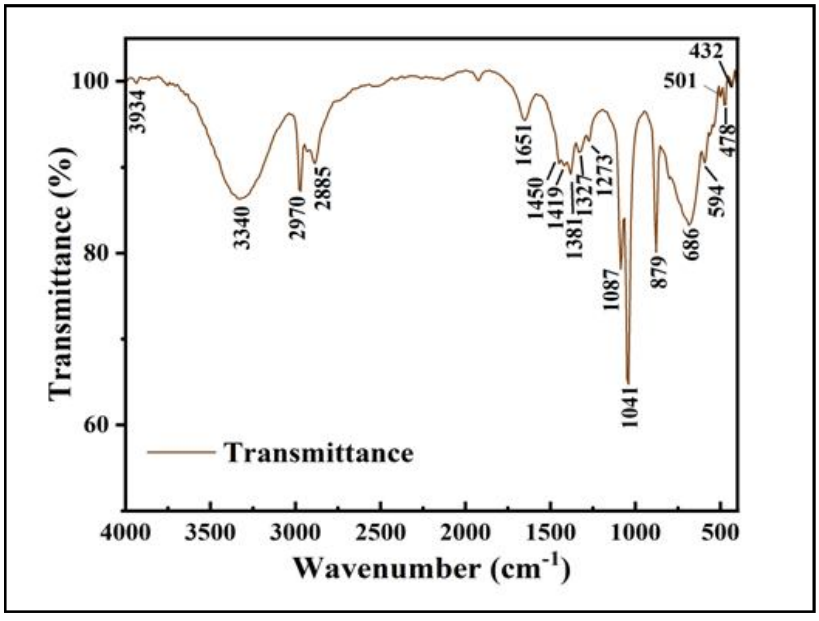

Figure 2: FTIR analysis of ethanolic extract of *I. coccinea* natural dye.

In both the ethanolic and aqueous extracts of *I. coccinea* flowers contain a variety of bioactive compounds, many of which have potential antioxidant, anti-inflammatory, and other pharmacological properties. The GC-MS analysis has provided valuable information regarding the chemical composition of these extracts, suggesting their possible applications in pharmaceuticals, nutraceuticals, and other therapeutic areas.

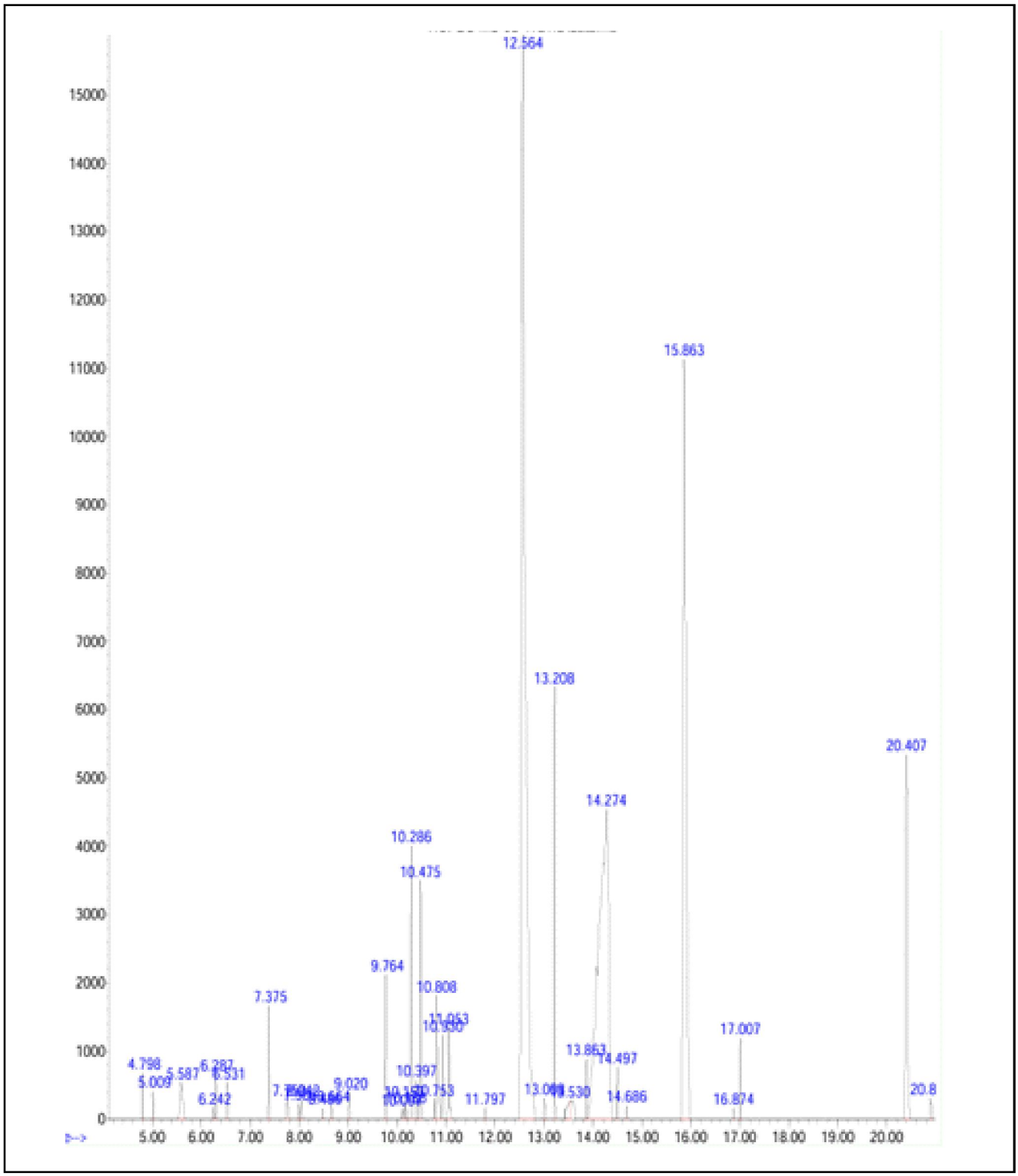

Figure 3: GC-MS Chromatogram of the ethanolic extract of natural dye from *I. coccinea*.

Table 2: GC-MS analysis of the ethanolic extract of natural dye from *I. coccinea*

S.No.	Retention time	Compound name	Molecular formula	Molecular weight (g/mol)	Area (%)
1	4.798	Ethanethiol	C_2H_6S	62.134	0.18
2	5.009	2-Cyclopenten-1-one	C_5H_6O	82.1005	0.16
3	5.587	Methanamine	CH_3NH_2	31.06	0.84
4	6.242	2-Propen-1-amine	C_3H_7N	57.0944	0.04
5	6.287	Ethene	C, H,,	28.05	0.31
6	6.531	2,5-Furandione	$C_4H_2O_3$	98.06	0.26
7	7.375	4H-Pyran-4-one	$C_5H_4O_2$	96.08	0.65
8	7.753	3-ethyl-Pyrimidine	C_7H_9N	107.15	0.28
9	7.986	2-Hydroxyethyl propyl sulfide	$C_5H_{12}OS$	120.213	0.11
10	8.042	2,4-Thiazolidinedione	$C_3H_3NO_2S$	117.13	0.13
11	8.486	Isothiazole	C_3H_3NS	85.12	0.04
12	8.664	2-Butynamide	C_4H_5NO	83.090	0.09
13	9.020	1,3-Cyclohexadiene-1-carboxaldehyde	C_7H_8O	108.1378	0.20
14	9.764	Benzenamine	$C_6H_5NH_2$	93.13	0.87
15	10.097	2-ethoxy-2-Propenoic acid	$C_5H_8O_3$	116.11	0.04
16	10.153	5H-1-Pyrindine-3-carboxylic acid	$C_{11}H_{13}NO_2$	191.23	0.11
17	10.186	2,4-Thiazolidinedione	$C_3H_3NO_2S$	117.13	0.05
18	10.286	4,5-Dimethylthiazole S-oxide	C_5H_7NOS	129.18	2.20
19	10.397	2-Amino-4-methyl-4-pentenoic acid	$C_6H_{11}NO_2$	129.16	0.15
20	10.475	3,4-Difluoroaniline	$C_6H_5F_2N$	129.11	1.76
21	10.753	3-Methyl-1,2,4-thiadiazole	C_2 H_2 N, S	100.15	0.21
22	10.808	Benzoic acid	$C_7H_6O_2$	122.123	1.18
23	10.930	9,12-Octadecadiynoic acid	$C_{18}H_{28}O_2$	276.41	0.54
24	11.053	3-Methyl-thiazole	C_4H_7NS	101.17	0.82
25	11.797	Methylguanidine	$C_2H_7N_3$	73.0971	0.04
26	12.564	n-Butyric acid 2-ethylhexyl ester	$C_{12}H_{24}O_2$	200.3178	33.55
27	13.008	N-Ethylformamide	C_3H_7NO	73.0938	0.21
28	13.208	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	3.36
29	13.530	Guanidine	CH_5N_3	59.07	0.59
30	13.863	Ethyl 4-methylbenzoate	$C_{10}H_{12}O_2$	164.2011	0.50
31	14.274	D-Mannitol	$C_6H_{14}O_6$	182.17	25.01
32	14.686	Propanamide	C_3H_7NO	73.095	0.29
33	15.863	2,4-Pentadienenitrile	C_5H_5N	79.10	0.10
34	15.863	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.71	18.02
35	16.874	isocyanato- methane	C_2H_3NO	57.0513	0.04
36	17.007	3-(Methylthio)propanoic acid methyl ester	$C_5H_{10}O_2S$	134.197	0.45
37	20.407	β-Sitosterol	$C_{29}H_{50}O$	414.71	6.38
38	20.896	6(2h)-Benzofuranone	$C_8H_6O_2$	134.13	0.24

Table 3: GC-MS analysis of the aqueous extract of natural dye from *I. coccinea* flower

S.No.	Retention time	Compound name	Molecular formula	Molecular weight (g/mol)	Area (%)
1	4.498	Butane, 2,2'-thiobis-	$C_8H_{18}S$	146.29	0.93
2	4.620	Ethoxyacetaldehydediethylacetal	$C_8H_{18}O_3$	162.22	0.53
3	4.743	(Dimethylamino)acetaldehyde dimethyl acetal	$C_6H_{15}NO_2$	133.19	0.34
4	5.354	Benzeneacetaldehyde	C_8H_8O	120.14	0.48
5	6.831	1,2-Benzenediol	$C_6H_6O_2$	110.11	1.65
6	6.876	Dodecane	$C_{12}H_{26}$	170.33	4.21
7	7.254	Silane, trimethyl[(1-methylhexyl)oxy]-	$C_{10}H_{24}OSi$	188.38	0.71
8	7.454	Phosphonic acid, methyl-, bis(trimethylsilyl) ester	$C_7H_{21}O_3PSi_2$	240.38	4.36
9	8.753	Tetradecane	$C_{14}H_{30}$	198.38	1.76
10	8.842	Benzoic acid, 2-amino-4-methyl-	$C_8H_9NO_2$	151.16	0.65
11	9.109	Benzene, 1,1'-(2-butene-1,4-diyl)bis-	$C_{16}H_{16}$	208.29	0.99
12	9.198	1,4-Di-O-acetyl-2,3,5-tri-O-methylribitol	$C_{12}H_{22}O_7$	278.30	0.55
13	9.309	3-Buten-2-ol, tert-butyldimethylsilyl ether	$C_{10}H_{22}OSi$	186.36	3.56
14	9.364	Silane, trimethyl(2-pentenyloxy)-, (Z)-	$C_8H_{18}OSi$	158.31	0.99
16	9.687	Benzene, (3-chloro-1-propenyl)-	C_9H_9Cl	152.62	2.91
17	9.764	1,3-Disilacyclobutane, 1,1,3,3-tetramethyl-	$C_6H_{16}Si_2$	144.36	2.58
18	9.887	3-Ethylbenzonitrile	C_9H_9N	131.17	10.64
19	10.009	3-Buten-1-ol, tert-butyldimethylsilyl ether	$C_{10}H_{22}OSi$	186.36	2.25
20	10.387	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.23	6.96
21	10.431	Hexadecane	$C_{16}H_{34}$	226.44	1.55
22	10.553	3,3-Dimethyl-2-butanol, trimethylsilyl ether	$C_9H_{22}OSi$	174.35	11.77
23	10.775	1-Phenylcyclopentanenitrile	$C_{12}H_{13}N$	171.23	7.71
24	10.820	3-Butenoic acid, 4-phenyl-	$C_{10}H_{10}O_2$	162.18	3.25
25	10.920	1H-Indene, 1-hexadecyl-2,3-dihydro-	$C_{25}H_{42}$	342.60	1.79
26	11.853	Benzyl Benzoate	$C_{14}H_{12}O_2$	212.24	2.44
27	11.942	Octadecane	$C_{18}H_{38}$	254.49	1.02
28	12.720	1-Undecyne	$C_{11}H_{20}$	152.27	0.72
29	12.986	Diphenyl sulfone	$C_{12}H_{10}O_2S$	218.27	0.34
30	13.053	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	0.62
31	13.308	Eicosane	$C_{20}H_{42}$	282.54	0.46
32	14.164	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	2.60
33	14.331	2-Chloroethyl linoleate	$C_{20}H_{35}ClO_2$	342.9	0.80
34	14.386	3-Octyne, 5-methyl-	C_9H_{16}	124.22	0.53
35	15.019	5-Acetoxymethyl-2,6,10-trimethyl-2,9-undecadien-6-ol	$C_{17}H_{30}O_3$	282.4	1.10
36	15.419	α-Amyrin	$C_{30}H_{50}O$	426.71	3.02
37	16.341	Palmitic acid â-monoglyceride	$C_{19}H_{38}O_4$	330.50	1.01
38	16.441	Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester	$C_{26}H_{42}O_4$	418.6 418.6	5.35

39	16.652	1-Dimethyl(phenyl)silyloxypentane	$C_{13}H_{22}OSi$	222.40	0.52
40	16.764	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307.42	0.45
41	17.275	Tetracosane	$C_{24}H_{50}$	338.65	0.96
42	17.419	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	$C_{11}H_{13}NO_3$	207.23	0.35
43	17.530	Terephthalic acid, di(2-ethylbutyl) ester	$C_{20}H_{30}O_4$	334.44	1.13
44	17.930	Squalene	$C_{30}H_{50}$	410.71	0.33
45	18.430	Tetratriacontane	$C_{34}H_{70}$	478.91	0.37

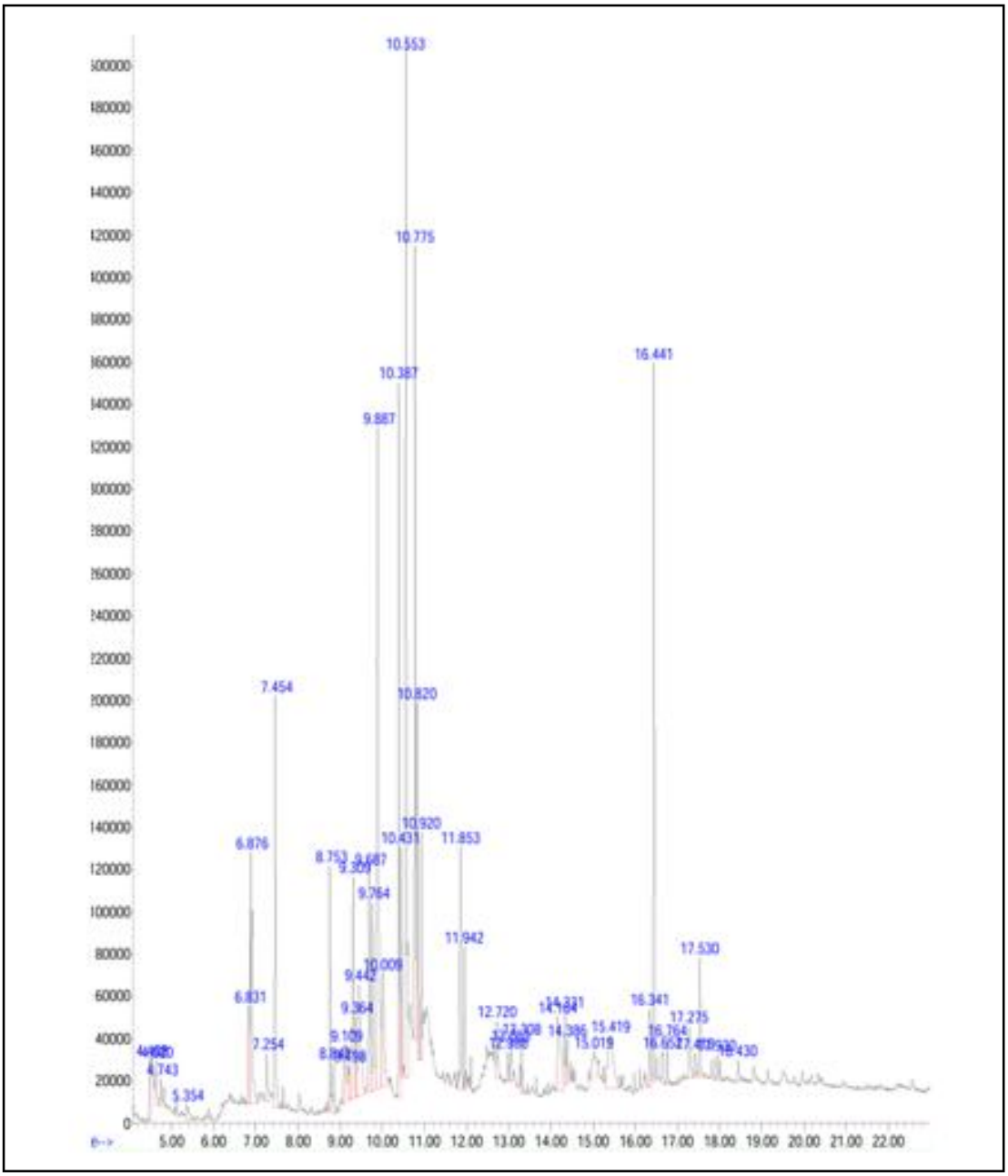

Figure 4: GC-MS Chromatogram of the aqueous extract of natural dye from *I. coccinea* flower.

4. Discussion

The study employed two widely used analytical techniques, gas chromatography-mass spectrometry (GC-MS) and fourier transform infrared (FTIR) spectroscopy, to examine the chemical composition of *I. coccinea* flower extracts. These techniques offer detailed insights into the phytochemical profile of the plant, aiding in the identification of bioactive compounds that could potentially have therapeutic applications. The combination of GC-MS and FTIR analysis enabled us to explore the diversity of chemical compounds within *I. coccinea* flowers and their possible pharmacological properties, such as antioxidant, anti-inflammatory, and antimicrobial activities (Pradhan and Dubey, 2021).

4.1 GC-MS analysis

In this study, GC-MS analysis was conducted on the ethanolic extract of *I. coccinea* flowers, leading to the identification of 38 distinct chemical compounds. These compounds fall into multiple categories, such as fatty acids, alcohols, phenolic compounds, sterols, and heterocyclic compounds containing sulfur and nitrogen. The most prevalent compound detected in the ethanolic extract was n-butanoic acid, 2-ethylhexyl ester, which accounted for 33.55% of the total peak area. This ester is known to play a key role in the production of fatty acids, which are essential for their anti-inflammatory and antioxidant properties (Kalusalingamand Balakrishnan, 2022; Abdulbaseer *et al*., 2024). The significant presence of n-butanoic acid suggests that the ethanolic extract of *I. coccinea* may have notable anti-inflammatory properties, which could be beneficial in the management of inflammatory conditions, such as arthritis, asthma, or other inflammatory diseases (Alburae *et al*., 2024). Another significant compound identified was D-Mannitol, a sugar alcohol found in various plants. It represented 25.01% of the total extract. Mannitol is recognized for its antioxidant properties, which help in reducing oxidative stress in biological systems. As oxidative stress is often associated with degenerative diseases, such as Alzheimer's disease and Parkinson's disease, the presence of mannitol in *I. coccinea* further supports the plant's potential for the treatment or prevention of neurodegenerative conditions. Additionally, dl-α-tocopherol, a form of vitamin E, was detected in the ethanolic extract, making up 18.02% of the composition. Vitamin E is a potent antioxidant that has long been studied for its ability to protect cells from oxidative damage. This compound's presence reinforces the potential of *I. coccinea* flowers as a source of antioxidants, offering protective effects against chronic diseases such as cancer and cardiovascular disorders (Singh *et al*., 2021).

Several other bioactive compounds were also identified, including β-sitosterol, which comprises 6.38% of the extract. β-sitosterol is a plant sterol with well-documented cholesterol-lowering effects, and it has been studied for its potential benefits in reducing cardiovascular disease risk. Another compound, 4,5-dimethylthiazole S-oxide (2.20%), is a sulfur-containing heterocyclic compound that may contribute to the antimicrobial properties of the extract (Segaran *et al*., 2021). The presence of benzoic acid (1.18%) and hexadecanoic acid (3.36%) further indicates the extract's potential for antioxidant and anti-inflammatory effects, as both of these compounds have been studied for their bioactive properties. Moreover, methylguanidine (0.04%) and 5H-1-pyrimidine-3-carboxylic acid (0.11%) were detected, suggesting that the ethanolic extract of *I. coccinea* could have antimicrobial properties, particularly supported by the presence of sulfur- and nitrogen-containing heterocyclic compounds.These compounds are often associated with various biological activities, including antimicrobial and antifungal effects, which could add to the plant's pharmacological versatility (Arokiarajan *et al*., 2024).

The aqueous extract of *I. coccinea* flowers also underwent GC-MS analysis, revealing a different set of 45 compounds. The most abundant compound in the aqueous extract was 3,3-dimethyl-2-butanol, trimethylsilyl ether, which accounted for 11.77% of the total composition. This compound is typically used as an industrial solvent and has applications in the synthesis of various chemical derivatives. The aqueous extract also contained notable compounds such as 3-ethylbenzonitrile (10.64%) and 1-phenylcyclo pentane carbonitrile (7.11%), adding to the diversity of the chemical profile. These compounds could contribute to the plant's biological activities, such as acting as precursors in the synthesis of bioactive molecules with potential pharmaceutical applications (Mohamed, 2021). Moreover, the presence of diethyl Phthalate (6.96%) and phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester (5.35%) in the aqueous extract suggests that the plant may have potential applications in industrial and pharmacological fields, as phthalates are commonly used as plasticizers and stabilizers in the manufacturing of polymers. Another interesting finding in the aqueous extract was the identification of squalene, a naturally occurring triterpene found in the essential oils of many plants. Squalene is widely used in the cosmetic and pharmaceutical industries for its moisturizing and skin-healing properties. Its presence in the aqueous extract of *I. coccinea* suggests that the plant could be a valuable source of compounds with cosmetic and dermatological applications. Additionally, dl-α-tocopherol was again identified in the aqueous extract, reinforcing the antioxidant potential of the plant (Gangal *et al*., 2023).

The identification of benzoic acid, 2-amino-4-methyl- and hexadecanoic acid in the aqueous extract further supports the antioxidant and anti-inflammatory properties of *I. coccinea* flowers. These compounds are known for their ability to reduce oxidative stress and inflammation, both of which are implicated in various chronic diseases, including cancer, diabetes, and cardiovascular disorders (Sánchez-Hernández *et al*., 2023). This highlights the therapeutic potential of the aqueous extract for use in managing diseases associated with oxidative stress and inflammation.Together, the findings from the GC-MS analysis of both ethanolic and aqueous extracts of *I. coccinea* provide valuable insights into the plant's chemical composition. The ethanolic extract, rich in compounds like n-butanoic acid, D-mannitol, and dl-α-tocopherol, appears to be particularly promising for its antioxidant and anti-inflammatory properties. Meanwhile, the aqueous extract, which contains a diverse range of compounds including squalene and phthalates, may offer benefits in both pharmaceutical and cosmetic applications (Raharjo *et al*., 2023).

4.2 FTIR analysis

Fourier transform infrared (FTIR) spectroscopy serves as a powerful analytical tool to identify the functional groups present in plant extracts. By measuring the absorption of infrared radiation at specific wavelengths, FTIR provides detailed information about the molecular structure of the compounds in the extract. In the case of *I. coccinea* flowers, FTIR spectra obtained from both ethanolic and aqueous extracts revealed significant absorption bands, each corresponding

to distinct functional groups. These functional groups can provide insight into the plant's potential therapeutic properties, including antioxidant, anti-inflammatory and antimicrobial activities.Both the ethanolic and aqueous extracts of *I. coccinea* displayed a prominent broad peak around 3348 cm^{-1}, which corresponds to the O-H stretching vibration of hydroxyl groups. This is a characteristic feature of phenolic compounds, alcohols, and other plant metabolites. The presence of hydroxyl groups in both extracts is of particular interest due to their association with the antioxidant activity of phenolic compounds (Thapa *et al*., 2022). Phenols are known to have the ability to scavenge free radicals, which play a key role in oxidative stress and the development of various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. The hydroxyl group's role in free radical scavenging suggests that *I. coccinea* could be a valuable source of natural antioxidants. Another significant feature observed in the FTIR spectra is the peak at 1635 cm^{-1}, corresponding to the C=O stretching vibration of carbonyl groups. Carbonyl groups are commonly found in compounds such as aldehydes, ketones, esters, and carboxylic acids, many of which possess biological activities. The presence of carbonyl groups in both the ethanolic and aqueous extracts suggests that *I. coccinea* may contain compounds with anti-inflammatory, antimicrobial, and antioxidant properties. For example, aldehydes and ketones are known for their ability to modulate inflammatory pathways, while carboxylic acids and esters are involved in various metabolic processes that could have therapeutic effects. These findings reinforce the plant's potential as a source of bioactive compounds with diverse pharmacological properties (Umaru *et al*., 2019).

Furthermore, FTIR analysis revealed absorption bands at 2970 cm^{-1} and 2885 cm^{-1}, corresponding to the C-H stretching vibration of alkane groups. Alkanes, which are commonly found in fatty acids and lipids, are known for their moisturizing and emollient properties. These functional groups suggest that *I. coccinea* may contain lipophilic compounds that could be useful in cosmetic formulations, especially for skin care products. Fatty acids, in particular, are recognized for their ability to improve skin hydration, promote healing, and reduce inflammation, making the presence of these compounds significant for both cosmetic and therapeutic applications. Additional peaks observed in the FTIR spectra, such as the C-H bending vibrations at 1450 cm^{-1} and 1381 cm^{-1}, further indicate the presence of alkane groups. These bands are often associated with the bending vibrations of C-H bonds in alkane structures. Moreover, an absorption band at 1087 cm^{-1}, which is attributed to C-O stretching, is commonly observed in compounds such as alcohols, ethers, and esters. These groups are frequently found in natural plant metabolites with diverse bioactive functions, including antimicrobial, antioxidant, and anti-inflammatory properties (Khaing *et al*., 2019). The presence of these functional groups highlights the complexity of *I. coccinea* flower extracts and suggests that they may possess a broad range of potential therapeutic applications. The FTIR analysis of *I. coccinea* flower extracts revealed a diverse range of functional groups, each indicative of bioactive compounds with potential medicinal properties. The presence of hydroxyl groups points to antioxidant activity, while carbonyl groups suggest anti-inflammatory and antimicrobial potential. Additionally, the identification of alkane and ester functional groups suggests that the plant could be utilized for its moisturizing and emollient properties in cosmetic applications. Collectively, these findings support the hypothesis that *I. coccinea* flowers may contain a wealth of bioactive compounds that could be explored for a variety of therapeutic purposes, including antioxidant, anti-inflammatory and antimicrobial treatments (Sharma *et al*., 2021).

4.2.1 Comparison of ethanolic and aqueous extracts

The GC-MS and FTIR analyses of the ethanolic and aqueous extracts of *I. coccinea* flowers provided complementary information about the chemical composition of the plant. The ethanolic extract was found to contain higher concentrations of compounds like n-butanoic acid, 2-ethylhexyl ester, D-mannitol and dl-α-tocopherol, which are known for their antioxidant and anti-inflammatory properties. This suggests that the ethanolic extract may be particularly effective for therapeutic applications related to oxidative stress and inflammation. In contrast, the aqueous extract contained a more diverse range of compounds, including squalene, squalene, and various phthalates, which may contribute to its moisturizing and potential pharmacological activities (Sivasakthi *et al*., 2021). The aqueous extract also showed a broader spectrum of heterocyclic compounds, which could indicate antimicrobial properties. Both extracts contain functional groups related to phenolic compounds, fatty acids, and alcohols, suggesting that *I. coccinea* flowers have potential applications in the development of antioxidant, anti-inflammatory and antimicrobial agents (Ghazali *et al*., 2023). The differences observed between the two extracts highlight the influence of the extraction method on the composition of the plant's bioactive compounds. Ethanol extraction, in particular, seems to be more effective in extracting antioxidant and anti-inflammatory compounds, while aqueous extraction appears to extract a wider variety of compounds with potential moisturizing and antimicrobial effects.

5. Conclusion

The study employing both GC-MS and FTIR spectroscopy has successfully identified a range of bioactive compounds in *I. coccinea* flower extracts, highlighting its significant pharmacological potential. The GC-MS analysis revealed the presence of key compounds such as n-butanoic acid, D-mannitol, dl-α-tocopherol and β-sitosterol, all of which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. FTIR spectra further supported these findings by indicating the presence of functional groups like hydroxyl, carbonyl and alkane groups, suggesting that the plant may possess diverse biological activities. These findings provide a solid foundation for future research into *I. coccinea* as a source of natural compounds with therapeutic applications. The antioxidant properties of compounds like dl-α-tocopherol and D-mannitol suggest potential benefits in treating oxidative stress-related conditions, while other compounds point to the plant's possible use in inflammatory and antimicrobial therapies. Looking ahead, further research should aim to isolate and characterize individual bioactive compounds from *I. coccinea* to better understand their specific therapeutic effects. In vitro and in vivo studies are needed to confirm the plant's efficacy in treating conditions such as neurodegenerative diseases, infections, and inflammatory disorders. It would also be valuable to investigate the impact of different extraction solvents on the yield and bioactivity of compounds, allowing for the optimization of extraction methods for use in pharmaceuticals and nutraceuticals. Additionally, exploring novel delivery systems, such as nanoparticles, could enhance the

bioavailability and effectiveness of the bioactive compounds. The sustainable cultivation and harvesting of *I. coccinea* are also important for ensuring a steady supply of plant material for research and development. Finally, clinical trials will be essential to validate the safety and efficacy of *I. coccinea* extracts in human populations, with the aim of obtaining regulatory approvals and creating standardized formulations. With its rich chemical composition and promising biological activities, *I. coccinea* has the potential to become a valuable source of natural medicine for the future.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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