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DETERMINATION OF PHYTOCONSTITUENTS IN THE LEAF EXTRACT OF *VERBESINA ENCELIOIDES* USING VARIOUS PHYTOCHEMICAL METHODS

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ABSTRACT
This study was carried out to identify the phytochemicals present in the leaves of *Verbesina encelioides*. Fresh leaves were collected from Jaipur, Rajasthan. The leaves were dried, powdered, and extracted with distilled water using sonication. The extract was tested for different phytochemicals. Carbohydrates, saponins, flavonoids, glycosides, alkaloids, terpenoids, phenols, and proteins were detected in the extract. FTIR analysis also showed distinct functional groups, including hydroxyl, C–H, carbonyl, C–N, and C–O. GC–MS analysis identified 39 compounds in the leaf extract. Linoleic acid was the major compound, with 38.56% of the total peak area. Hexadecanoic acid and octadecanoic acid were also present at 17.01% and 11.85%, respectively. Other compounds, such as phytol, methyl linoleate, and various fatty acid derivatives, were also detected. The results show that *V. encelioides* leaves contain a wide range of phytochemicals and other chemical compounds. These findings provide useful information about the plant's chemical composition and support further studies on its biological activities and potential use in the green synthesis of nanoparticles.

KEYWORDS: *Verbesina encelioides*, leaf extract, phytochemical, GCMS, FTIR.

1. INTRODUCTION

Plants serve as valuable sources of diverse bioactive compounds, including phenols, flavonoids, terpenoids, alkaloids, and saponins, which play important roles in determining the biological and functional properties of plant-derived materials (Saraf et al., 2026). These compounds may contribute to antioxidant, antimicrobial, anti-inflammatory, and other biological properties of plants. For this reason, phytochemical studies are useful for understanding the chemical composition and possible uses of medicinal plants.

Verbesina encelioides belongs to the family Asteraceae and is commonly known as golden crownbeard or wild sunflower. It is an annual herb widely distributed worldwide. In India, the plant has become an invasive weed, especially in dry and semi-dry regions. It can grow in wastelands, agricultural areas, and roadside habitats (Jain et al., 2008; Kataria et al., 2025).

Although *V. encelioides* is considered an invasive plant, it has attracted attention because of its phytochemical and medicinal properties. Previous studies have reported the presence of flavonoids, phenolic compounds, alkaloids, terpenoids, saponins, glycosides, and other compounds in different parts of the plant. These constituents may be responsible for some of its reported biological activities (Gorja and Bandla, 2024; Kataria et al., 2025).

Earlier studies have also reported antioxidant and antimicrobial activities of *V. encelioides* extracts. Jain et al. (2008) found that root extracts of the plant showed antimicrobial activity against several bacterial and fungal species. Antioxidant activity was also observed in the tested extracts. More recent work has further confirmed the presence of several phytochemicals and functional groups in *V. encelioides* extracts (Kataria et al., 2025).

Modern analytical techniques can provide more detailed information about plant extracts. FTIR analysis helps identify important functional groups present in an extract. GC–MS is useful for detecting and tentatively identifying different volatile and semi-volatile compounds. Recent studies on *V. encelioides* have reported the presence of phenolic compounds, flavonoids, phytosterols, triterpenes, and other chemical constituents in its leaves (Kaur et al., 2022).

Despite the available information, the chemical composition of *V. encelioides* can vary with the plant part, extraction method, and geographical location. More studies are needed to characterize its leaf extract using different analytical methods. The present study was therefore designed to investigate the phytochemical composition of the aqueous leaf extract of *V. encelioides*. Qualitative phytochemical tests, FTIR, and GC–MS were used to identify major classes of phytochemicals and chemical compounds present in the extract. The study provides further information on the chemical profile of *V. encelioides* leaves and their possible importance for future biological and green synthesis studies.

2. MATERIALS AND METHODS

2.1 Plant material collection

Fresh, healthy, and disease-free leaves of *Verbesina encelioides* (Cav.) were collected from the Delhi Road area of Amber, Jaipur, Rajasthan, India. The collected plant material was taxonomically authenticated by the Department of Botany, University of Rajasthan, Jaipur, India, and a voucher specimen was deposited in the departmental herbarium for future reference.

After collection, the leaves were washed thoroughly under running tap water to eliminate adhering dust and other surface impurities. The leaves were then immersed in sterile 0.9% (w/v) saline solution and repeatedly rinsed with deionized distilled water. Following washing, the excess surface moisture was removed by gently blotting the leaves dry (Anuradha et al., 2015). The cleaned leaves were subsequently shade-dried at room temperature for 8–10 days under well-ventilated conditions (Kamaraj et al., 2020).

For the determination of dry weight, 5 g of freshly collected leaves were placed in a laboratory cabinet dryer maintained at 40–50 °C and weighed periodically until a constant weight was obtained (Anuradha et al., 2015). The thoroughly dried leaves were pulverized into a fine powder using a laboratory grinder (Gupta et al., 2024). The powdered material was further passed through a 100-mesh sieve to obtain a uniform particle size and ensure sample homogeneity (Suresh et al., 2015). The final powdered leaf material was stored in airtight containers under suitable storage conditions

2.2 Preparation of aqueous leaf extract

An aqueous extract of *Verbesina encelioides* leaves was prepared using the previously dried and powdered plant material. Briefly, 10 g of the leaf powder was accurately weighed and suspended in 100 mL of distilled water. The resulting suspension was sonicated at 40 °C for 10 min to enhance the extraction and release of bioactive constituents into the aqueous phase (Shahzad et al., 2022). After sonication, the extract was allowed to cool to room temperature and subsequently filtered through Whatman No. 42 filter paper to remove residual plant debris and insoluble matter (Ganaie et al., 2014). The clear filtrate obtained was collected and stored at 4 °C until further use. The freshly prepared aqueous leaf extract was subsequently employed as a biological reducing and stabilizing agent during the green synthesis of nanoparticles.

2.3 Phytochemical analysis of the leaf extract

Preliminary phytochemical screening of the selected plant extract was conducted to assess the presence or absence of phytochemicals, including proteins, carbohydrates, phenols, flavonoids, alkaloids, glycosides, saponins, and terpenoids, using standard methods with minor modifications. For further analysis, the sample was sent to Accuphychem Analytics in Jaipur, Rajasthan, India, for GC-MS and FTIR analyses.

The extracts and the standards were analyzed by GC-MS using a Hewlett-Packard 6890/5973 GC-MS equipped with an Agilent 7890A/5975C GC-MS system and an HP-5 column, operated at 1000 eV ionization energy. Capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d.) Helium (He) gas was utilized as a carrier with a split ratio of 1:5. The temperature of the oven was set at 100 °C (for 3 minutes) to 280 °C at 1-40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 ml/min). Retention indices were determined using retention times of samples injected under the same chromatographic conditions. The substances in the standard and plant samples were identified by comparing their mass spectra and retention times with those reported in the literature and with mass spectra in the Wiley library (National Institute of Standards and Technology data bank).

3. RESULTS AND DISCUSSION

3.1 Phytochemical determination of the leaf extract

The preliminary phytochemical screening of the *Verbesina encelioides* leaf extract revealed the presence of several classes of bioactive constituents (Table 1, Figure 1). Fehling's test produced a red precipitate, indicating the presence of carbohydrates, while the formation of foam in the foam test confirmed the presence of saponins. The lead acetate test for flavonoids produced a green precipitate, indicating a positive reaction. The NaOH test for glycosides produced a red color, confirming their presence. Dragendorff's test yielded a brown precipitate, indicating the presence of alkaloids. The terpenoid test showed a dark brown color, suggesting the presence of terpenoids. The ferric chloride ($FeCl_3$) test produced a green color, confirming the presence of phenolic compounds. Furthermore, the ninhydrin test exhibited a dark purple color, indicating a positive result for proteins. Overall, the phytochemical screening demonstrated the presence of a diverse range of bioactive constituents, including carbohydrates, saponins, flavonoids, glycosides, alkaloids, terpenoids, phenolic compounds, and proteins in the *V. encelioides* leaf extract. The presence of these phytochemical constituents suggests that the extract contains a diverse range of potentially bioactive compounds, which may contribute to the functional properties of both the plant extract and the synthesized MgO nanoparticles (Tekman et al., 2025).

Table 1. Qualitative phytochemical profile of *Verbesina encelioides* leaf extract.

S.No.	Test Name	Observation	Result
1	Carbohydrate Test (Fehling's Test)	Red Precipitate	Positive
2	Saponin Test (Foam Test)	Foam formed	Positive

3	Flavonoid Test (Lead Acetate Test)	Green Precipitate	Positive
4	Glycoside Test (NaOH Test)	Red Colour	Positive
5	Alkaloid Test (Dragendorff's Test)	Brown Precipitate	Positive
6	Terpenoid Test	Golden Yellow Color	Positive
7	Phenol Test (Ferric Chloride Test)	Green Colour	Positive
8	Protein Test (Ninhydrin Test)	Dark Purple Color	Positive

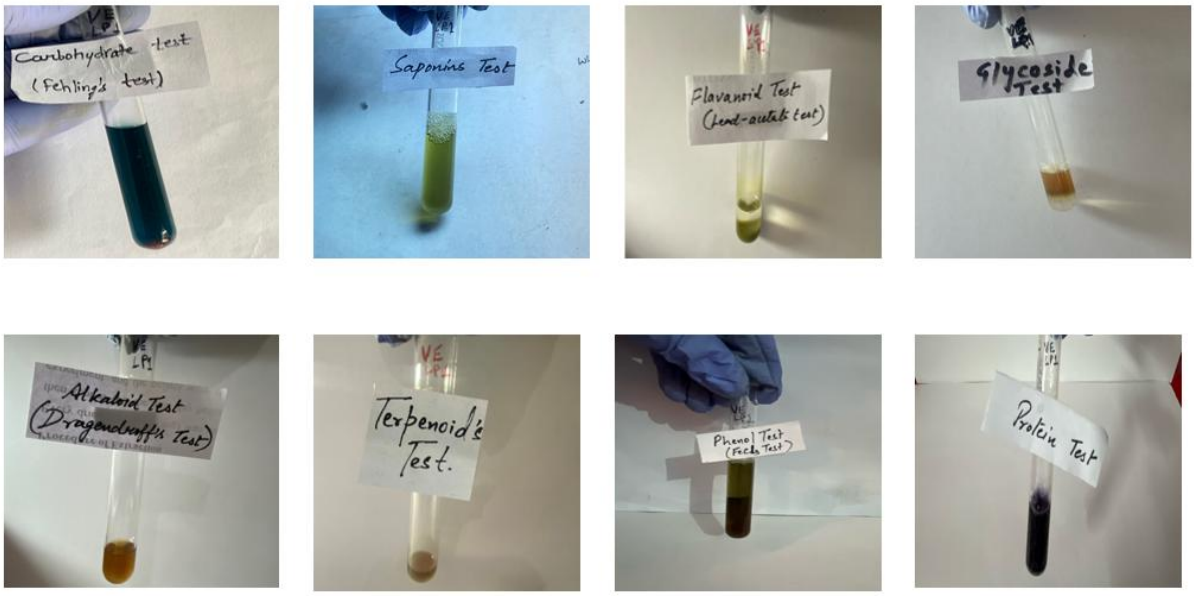


Figure 1. Qualitative phytochemical screening of *Verbesina encelioides* leaf extract.

3.2 FTIR analysis of the leaf extract

The FTIR spectrum of *Verbesina encelioides* leaf extract showed several characteristic absorption bands in the range of about 3851 to 550 cm^{-1} (Figure 2), indicating the presence of different functional groups corresponding to the phytochemical constituents of the extract (Table 2). Prominent bands appeared at 3851, 3742, 3618, and 3279 cm^{-1}. The absorption band observed at around 3279 cm^{-1} was mainly assigned to the stretching vibrations of O-H/N-H groups, indicating the existence of hydroxyl-containing compounds such as phenols and alcohols, as well as potential proteinaceous components. The peaks at 2989 cm^{-1} were mainly attributed to C–H stretching vibrations of aliphatic groups. A spectral feature at approximately 2317 cm^{-1} was detected in the region typically attributed to atmospheric CO_2 and was classified as a probable background contribution. The absorption bands at 1693 and 1648 cm^{-1} indicated the presence of carbonyl and/or conjugated functional groups. The band at 1577 cm^{-1} can be assigned to aromatic C=C stretches and/or N-H in-plane vibrations. The absorption at 1456 cm^{-1} was mainly attributed to the C-H bending vibrations. Additional bands at 1322 and 1157 cm^{-1} were assigned to C–N and C–O/C–O–C stretching vibrations, possibly due to phenolic compounds, alkaloids, ethers, alcohols, and carbohydrate-related entities. The C–O stretching vibrations exhibited a strong absorption at about 1050 cm^{-1}. Supplementary bands in the fingerprint region at 660, 606, and 550 cm^{-1} were detected, which can be attributed to skeletal and out-of-plane vibrational modes of several organic compounds. The full FTIR analysis revealed the presence of several functional groups in the leaf extract of *V. encelioides*, including hydroxyl, C–H, carbonyl, aromatic, C–N, and C–O groups. These functional groupings were consistent with the qualitative phytochemical analysis, which revealed the presence of phenols, flavonoids, alkaloids, glycosides, saponins, terpenoids, carbohydrates, and proteins. The presence of hydroxyl, carbonyl, and C–O groups suggests that the phytoconstituents in the leaf extract may be responsible for the extract's biological properties and for the reduction and stabilization of MgO nanoparticles during green synthesis (Muhaymin et al., 2024).

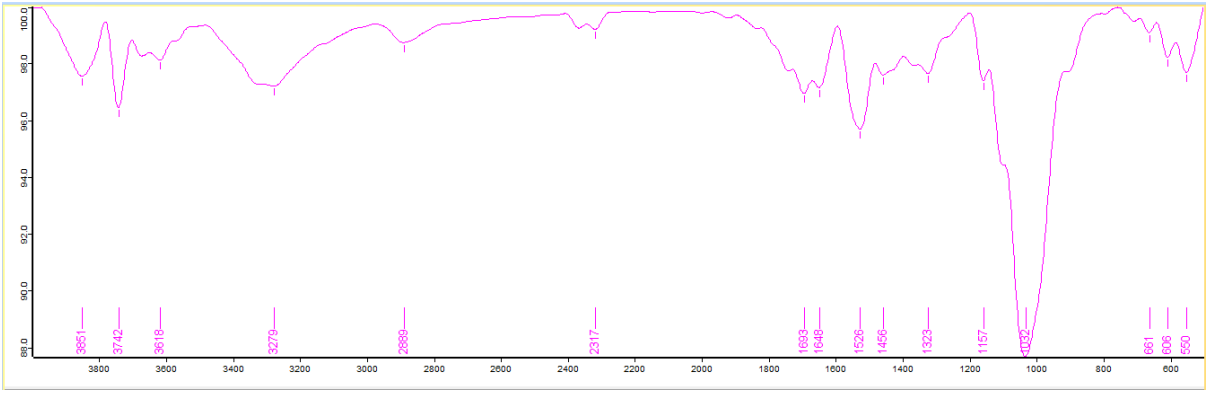


Figure 2. FTIR spectrum of the methanolic leaf extract of *Verbesina encelioides*.

Table 2. Functional groups identified in the *Verbesina encelioides* leaf extract by FTIR spectroscopy.

S. No.	Peak position (cm^{-1})	Tentative functional group assignment	Possible phytoconstituents
1	3851	O–H stretching	Free hydroxyl groups
2	3742	O–H stretching	Alcohols/phenolic compounds
3	3618	O–H stretching	Alcohols and phenols
4	3279	Broad O–H/N–H stretching	Phenols, alcohols, proteins
5	2989	C–H stretching	Aliphatic hydrocarbons
6	2317	O=C=O/atmospheric CO_2 region	Atmospheric CO_2 contribution
7	1693	C=O stretching	Carbonyl compounds, aldehydes/ketones
8	1648	C=C/C=O or amide I stretching	Aromatic compounds/proteins

3.3 GC-MS analysis of the leaf extract

Gas chromatography–mass spectrometry (GC–MS) analysis of the methanolic leaf extract of *Verbesina encelioides* revealed a complex chemical composition, with 39 identified chromatographic peaks (Table 3) corresponding to fatty acids, fatty acid esters, terpenoids, phenolic compounds, hydrocarbons, oxygenated compounds, and other organic compounds. The identified substances exhibited considerable variability in relative abundance, with some compounds being major constituents and many others present in relatively small amounts. GC–MS analysis showed that 9,12-octadecadienoic acid (Z, Z), or linoleic acid, was the major compound, accounting for 38.56% of the total peak area, with a retention time of 19.312 minutes. Hexadecanoic acid (palmitic acid) was the second most abundant compound at 17.516 minutes with 17.01% of the overall area. Another important component was octadecanoic acid (stearic acid), detected at 19.532 min and accounting for 11.85%. Other major library peaks were methenolone and trifluoroacetate (9.27%) at 18.177 min and 1-methylene-5α-androstan-3α-ol-17 (6.78%) at 17.939 min. The benzylammonium salt of 3-phenylheptanoic acid reached a peak concentration of 3.31%. The methyl ester of 9,12-octadecadienoic acid (Z, Z)- and phytol were identified at levels of 2.26% and 1.91%, respectively.

Other fatty acid derivatives identified were methyl stearate (1.09 %), 8,11,14-eicosatrienoic acid methyl ester (1.06 %), hexadecanoic acid methyl ester (0.73 %), 22-tricosenoic acid (0.39 %), and dodecanoic acid (0.10 %). The extract also contained terpenoids and phytol-related compounds, including neophytadiene (0.17%) and phytol (1.91%). Also, phenolic compounds such as 2-methoxy-4-vinylphenol (0.06%) and 2,6-dimethoxyphenol (0.20%) were identified. Detection of β-D-glucopyranose, 1,6-anhydro- (0.05%) also indicated the presence of carbohydrate-derived compounds. Relatively small individual amounts of long-chain hydrocarbons such as undecane, dodecane, tetradecane, hexadecane, eicosane, and heneicosane were observed.

The presence of unsaturated and saturated fatty acids, especially linoleic acid, palmitic acid, and stearic acid, indicates that the lipid-derived compounds are major components of the GC–MS detectable components in the leaf extract. Furthermore, phytol, neophytadiene, and phenolic compounds confirm the presence of bioactive terpenoid and phenolic constituents. These substances may jointly affect the extract's biological properties and may be relevant to its experimentally reported antioxidant, antibacterial, and other functional activities.

GC–MS analysis showed that the leaf extract of *V. encelioides* is a rich source of fatty acids and their derivatives, along with terpenoids, phenolic compounds, carbohydrates, and several other organic substances. The GC–MS results are consistent with the qualitative phytochemical evaluation and FTIR results, which confirm the presence of phenolic, flavonoid, alkaloid, terpenoid, glycosidic, saponin, carbohydrate, and protein-related components. The diversity of phytochemical composition provides a chemical basis for the extract's biological efficacy and

supports its potential as a natural reducing and stabilizing agent for the green synthesis of MgO nanoparticles (Ahmed et al., 2019; Sharmila et al., 2019).

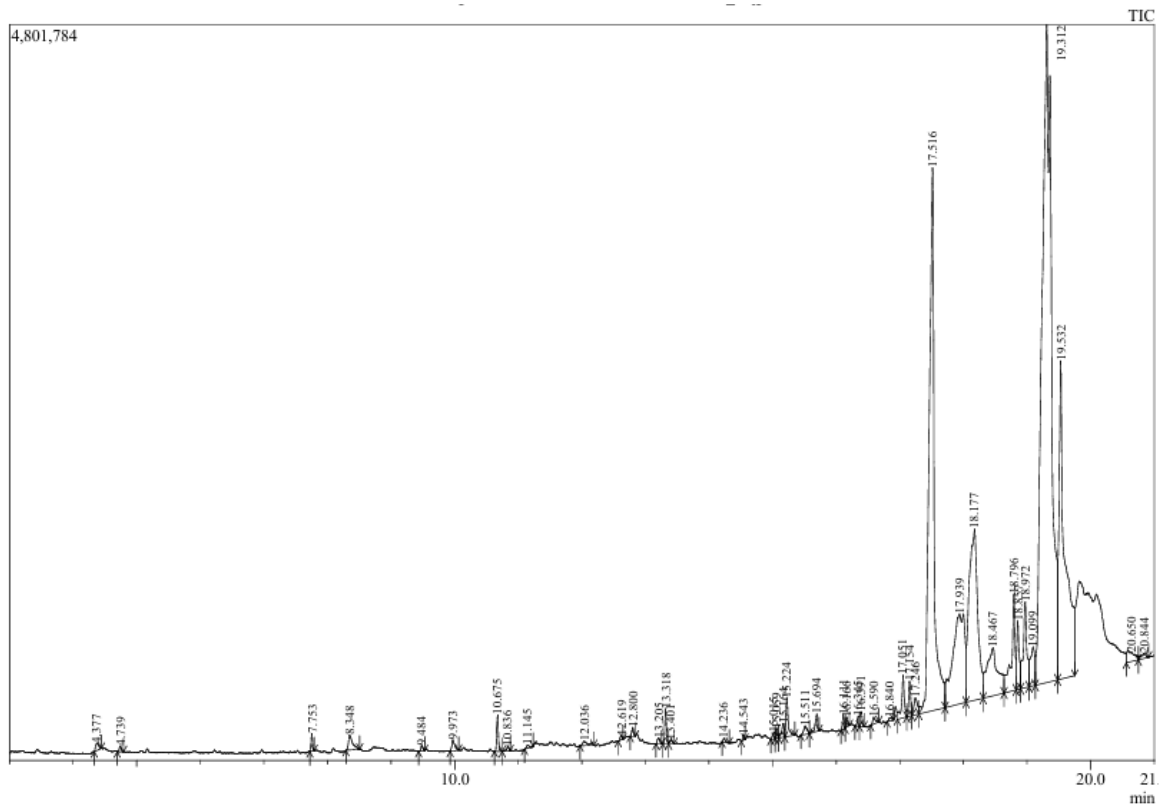


Figure 3. GC–MS chromatograph of the methanolic leaf extract of *Verbesina encelioides*.

Table 3. GC–MS profile of phytochemical constituents identified in the methanolic leaf extract of *Verbesina encelioides*.

S. No.	Retention time (min)	Tentatively identified compound	Area (%)
1	4.377	Glycerin	0.14
2	4.739	Undecane	0.07
3	7.753	Dodecane	0.17
4	8.348	2-Pentanol, TMS derivative	0.39
5	9.484	2-Methoxy-4-vinylphenol	0.06
6	9.973	Phenol, 2,6-dimethoxy-	0.20
7	10.675	Tetradecane	0.37
8	10.836	Propylphosphonic acid, fluoroanhydride, 4-methyl derivative	0.06
9	11.145	1-Hydroxymethyl-1,2,3,4-tetrahydronaphthalene	0.05
10	12.036	β-D-Glucopyranose, 1,6-anhydro-	0.05
11	12.619	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4-	0.05
12	12.800	Dodecanoic acid	0.10
13	13.205	Diethyl phthalate	0.09
14	13.318	Hexadecane	0.36
15	13.401	5-Isopropenyloxymethylene-3,3-dimethyl-cycl-	0.06
16	14.236	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy)-	0.05
17	14.543	Eicosane	0.05
18	15.015	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy)-	0.10
19	15.059	Hexadecane, 1-iodo-	0.12
20	15.164	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydro-	0.23
21	15.224	Tetradecanoic acid	0.56
22	15.511	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydro-	0.18
23	15.694	Heneicosane	0.26
24	16.111	Neophytadiene	0.17
25	16.166	2-Pentadecanone, 6,10,14-trimethyl-	0.12
26	16.345	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.14
27	16.391	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	0.20

28	16.590	Oxalic acid, 6-ethyloct-3-yl propyl ester	0.11
29	16.840	Oxalic acid, 6-ethyloct-3-yl propyl ester	0.07
30	17.051	Hexadecanoic acid, methyl ester	0.73
31	17.154	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-	0.45
32	17.246	22-Tricosenoic acid	0.39
33	17.516	Hexadecanoic acid	**17.01**
34	17.939	1-Methylene-5α-androstan-3α-ol-17	6.78
35	18.177	Methenolone, trifluoroacetate	9.27
36	18.467	3-Phenylheptanoic acid, benzylammonium salt	3.31
37	18.796	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2.26
38	18.857	8,11,14-Eicosatrienoic acid, methyl ester	1.06
39	18.972	Phytol	1.91

4. CONCLUSION

The present study showed that the leaves of *Verbesina encelioides* contain a wide range of phytochemicals. Qualitative screening confirmed the presence of carbohydrates, saponins, flavonoids, glycosides, alkaloids, terpenoids, phenols, and proteins. FTIR analysis further confirmed the presence of various functional groups, including hydroxyl, C–H, carbonyl, C–N, and C–O. GC–MS analysis identified 39 compounds in the leaf extract. Linoleic acid was the major compound, followed by hexadecanoic acid and octadecanoic acid. Other compounds, such as phytol, methyl linoleate, and various fatty acid derivatives, were also detected. These findings show that *V. encelioides* leaves have a diverse chemical composition. The results provide useful information for further studies on the plant's biological activities and its potential use in the green synthesis of metal nanoparticles.

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Author contributions

Syed Burhan Geelani: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, and Writing – original draft.
Arindam Saraf: Investigation, Data curation, Formal analysis, and Resources.
Anuradha Jayaraman: Supervision, Validation, and Writing – review and editing.
Indu Sharma: Supervision, Validation, and Writing – review and editing.
Sanjeevi Ramakrishnan: Supervision, Validation, and Writing – review and editing.

Conflict of interest

The authors have no conflict of interest to declare concerning the research, authorship, and publication of this article.

Data Availability Statement

All data generated and analyzed during this study are included in this published article and its supplementary information.

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