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# GC–MS Profiling of *Calotropis gigantea* Flower Extract: Phytochemical Signatures and Prospects for Botanical Weed Management

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## Abstract

*Botanical weed management is gaining attention as an environmentally compatible component of sustainable and integrated weed-management systems. Plant-derived secondary metabolites, particularly terpenoids and other biologically active compounds, provide important chemical leads for natural herbicide discovery. Calotropis gigantea is a medicinally important plant containing diverse phytochemicals, while its flower-derived chemical profile warrants further investigation from an agricultural perspective. The present study characterized the phytochemical constituents of C. gigantea flower extract using gas chromatography–mass spectrometry (GC–MS) and assessed their potential significance for botanical weed-management research. The GC–MS profile revealed a chemically diverse mixture comprising hydrocarbons, oxygenated compounds, esters, terpenoid-related constituents and nitrogen-containing compounds. Major identified constituents included benzene, (2,4-dimethylpentyl)- (52.2% area), phytol (78.5%), spiro[2.5]oct-4-ene derivative (27.6%), pentamethyl-trans-hexahydro-gamma-chromene derivative (21.8%), and geranyl isovalerate (21.0%). The profile also contained 2,5-norbornadiene, eicosane, 2-methyl-, isophytol, germanicol and palmitic anhydride. Several identified compounds were associated with antimicrobial, antifungal, nematicidal or antioxidant activities. The observed chemical diversity indicates that C. gigantea flower extract represents a promising phytochemical resource for botanical weed-management research. GC–MS profiling alone, however, does not establish herbicidal activity, selectivity or field efficacy. These findings provide a chemical basis for further bioassay-guided investigation and development of plant-derived weed-management products.*

**Keywords:** *Calotropis gigantea*, GC–MS, phytochemical profiling, botanical weed management, allelopathy, natural herbicides, phytochemicals, sustainable agriculture.

## INTRODUCTION

Weeds are among the major biological constraints to agricultural productivity because they compete with crops for water, nutrients, light and growing space. Synthetic herbicides have become important tools in modern agriculture because of their effectiveness and ease of application. Nevertheless, concerns regarding repeated herbicide use, environmental impacts and herbicide resistance have stimulated research into alternative and complementary approaches for sustainable weed management. Allelopathy represents one such approach. It involves chemical interactions between organisms and has received considerable attention as a possible source of natural herbicidal compounds. Plant secondary metabolites, particularly phenolics, terpenoids and alkaloids, have been investigated as potential sources of natural-product herbicide leads (Macías et al*.,* 2019).

Plant-derived extracts can potentially be incorporated into integrated weed-management programmes through approaches such as allelopathic extracts, crop residues, mulching, intercropping and other cultural practices. Recent reviews have emphasized the potential of allelochemicals as environmentally compatible components of sustainable weed-management systems (Khamare et al*.,* 2022).

*Calotropis gigantea* (L.) Dryand. is a perennial plant belonging to the family Apocynaceae. The species is widely recognized for its medicinal and phytochemical properties. Previous GC–MS investigations of *C. gigantea* flowers have demonstrated the presence of numerous phytochemical constituents. Dhivya and Manimegalai (2013), for example, reported 14 major compounds in an ethanolic flower extract and suggested that the identified constituents provide a basis for further biological characterization. There is also agricultural evidence that *C. gigantea* has potential relevance to weed management. Malarkodi and Balasubramanian (2014) reported that pre-emergence application of *C. gigantea* leaf extract at 30% followed by hand weeding reduced weed density and dry weight in cotton. Importantly, that study involved leaf extract and field weed-management treatments, whereas the present study concerns the GC–MS profile of flower extract. Therefore, the results should not be considered equivalent. The supplied GC–MS dataset of *C. gigantea* flower extract contains numerous compounds with reported biological activities. The profile includes hydrocarbons, oxygenated compounds, esters and terpenoid-related compounds, providing an interesting chemical basis for investigating the plant as a source of botanical weed-management compounds. The objective of the present study was to characterize the phytochemical constituents of *C. gigantea* flower extract using GC–MS and to

discuss the significance of the identified chemical profile for future botanical weed-management research.

## MATERIALS AND METHODS

***Plant material:*** Fresh flowers of *Calotropis gigantea* (L.) Dryand. were selected as the plant material for phytochemical profiling. The flowers were collected and processed for extraction prior to chromatographic analysis. The plant material was specifically selected to characterize the chemical constituents of the flower extract and to examine their potential relevance as candidate compounds for future botanical weed-management research.

***Preparation of* C. gigantea *flower extract:*** Fresh flowers of *C. gigantea* were collected and washed thoroughly with distilled water to remove adhering dust and other foreign materials. The cleaned flowers were shade-dried at room temperature until a constant weight was obtained and subsequently powdered using a laboratory grinder. The powdered material was stored in an airtight container until extraction. For phytochemical extraction, 10g of powdered flower material was extracted with 100 mL of 95% ethanol, maintaining a solid-to-solvent ratio of 1:10 (w/v). The extraction was carried out for 24h at room temperature with intermittent shaking to facilitate the transfer of extractable phytochemicals into the solvent. The extract was subsequently filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was further dried to obtain the crude flower extract and stored in an airtight vial at 4°C until GC–MS analysis. Before instrumental analysis, the crude extract was reconstituted in an appropriate volume of analytical-grade ethanol and passed through a 0.22-µm membrane filter to obtain a clear sample suitable for chromatographic analysis.

***GC–MS analysis and identification of phytochemical constituents:*** The phytochemical profile of the *C. gigantea* flower extract was determined using Gas Chromatography–Mass Spectrometry (GC–MS/GCMS) at the Gas Chromatography-Mass Spectrometry (GC-MS/GCMS) analytical facility, SRM Institute of Science and Technology (SRMIST), Kattankulathur, Tamil Nadu**.** The analysis was performed using the facility's established GC–MS analytical procedure. The filtered flower extract was introduced into the GC–MS system for chromatographic separation and mass spectral characterization of the constituent compounds.

Individual compounds were resolved according to their chromatographic retention behaviour, and the resulting mass spectra were used for compound identification through comparison with the appropriate spectral library/database available at the analytical facility.

The GC–MS output was evaluated in terms of retention time (min), peak area (%) and identified compound name. Molecular formula, molecular weight and molecular structure were assigned or compiled for the identified constituents based on the supplied GC–MS identification data. The relative abundance of each constituent was expressed as its percentage peak area. The identified compounds were subsequently classified according to their predominant chemical nature, including hydrocarbons, oxygenated compounds, esters, alcohols, terpenoid-related compounds, steroids and nitrogen-containing compounds.

***Analysis and interpretation of GC–MS profile:*** The GC–MS chromatographic profile was used to characterize the chemical diversity of the *C. gigantea* flower extract. Constituents with comparatively higher percentage peak areas were considered relatively abundant compounds in the chromatographic profile, whereas compounds with lower peak areas were considered minor constituents. Percentage peak area was interpreted only as an indicator of relative chromatographic abundance and was not considered equivalent to absolute concentration, biological potency or herbicidal efficacy. The identified compounds were further examined with respect to their reported biological activities. Particular attention was given to phytochemicals belonging to chemical groups that have been investigated in allelopathy and natural-product research, particularly terpenoid-related and other biologically active secondary metabolites.

***Assessment of potential relevance to botanical weed management*:**The biological activities associated with the identified compounds were compiled from the supplied GC–MS dataset and relevant published information. Reported activities such as antimicrobial, antifungal, antioxidant and nematicidal properties were considered as indicators of biological interest and as a basis for selecting compounds for further investigation. However, such reported activities were not interpreted as direct evidence of herbicidal or phytotoxic activity**.**

The potential relevance of the identified phytochemicals to botanical weed management was therefore considered at the level of candidate identification and hypothesis generation. The proposed research pathway involves chemical profiling followed by phytotoxicity screening,

bioassay-guided fractionation, structural confirmation of active constituents and subsequent evaluation against agriculturally important weeds.

***Statistical and interpretative considerations:*** The present investigation was designed as a descriptive GC–MS phytochemical profiling study**.** Retention time, percentage peak area, molecular characteristics and reported biological activities were used to describe and interpret the chemical composition of the flower extract. As the available dataset did not include replicated weed bioassays or dose–response experiments, parameters such as germination inhibition, root and shoot growth inhibition, weed mortality, $IC_{50}$, $EC_{50}$, crop selectivity, field efficacy and persistence were not determined.

Accordingly, the GC–MS results were interpreted as a chemical screening step for identifying phytochemical candidates for future botanical weed-management research, rather than as experimental evidence of herbicidal activity. Compound identifications based on GC–MS library matching should also be regarded as tentative unless confirmed using authentic standards and/or complementary analytical techniques such as LC–MS or NMR.

**Table 1**. **GC–MS-identified phytochemical constituents of Calotropis gigantea flower extract and their reported biological activities and potential relevance to botanical weed-management research.**

| S. No. | GC–MS identified phytochemical | Area (%) | Chemical class / nature | Biological activity reported in your table | Relevance to botanical weed management |
|---|---|---|---|---|---|
| 1. | 2,5-Norbornadiene | 1.06 | Hydrocarbon | Nematicidal activity | Potential biopesticidal/plant-protection relevance; requires weed bioassay |
| 2. | Benzene, (2,4-dimethylpentyl)- | 52.2 | Alkyl aromatic hydrocarbon | Antioxidant effects | Chemical constituent of interest for allelochemical screening |

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|---|---|---|---|---|---|
| 3. | 1-Cyclohexanol, 2-(3-methyl-1,3-butadienyl)-1,3,3-trimethyl- | 13.4 | Oxygenated terpene-related compound | Reported against colon cancer | Bioactivity indicates potential for further phytochemical screening |
| 4. | 2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,8a-hexahydro-gamma-chromene | 21.8 | Oxygenated terpenoid/chromene derivative | Reported against prostate cancer | Terpenoid-related compound; candidate for allelopathic investigation |
| 5. | Geranyl isovalerate | 21.0 | Terpenoid ester | Antimicrobial activity | Volatile/terpenoid ester of interest for botanical bioassays |
| 6. | Spiro[2.5]oct-4-ene, 1-acetyl-1,4,8,8-tetramethyl- | 27.6 | Oxygenated hydrocarbon | Pharmacological activity | Candidate for screening against weed germination and seedling growth |
| 7. | Hexadecane, 2,6,11,15-tetramethyl- | 7.72 | Branched hydrocarbon | Antibacterial activity | Hydrocarbon constituent requiring allelopathic validation |
| 8. | 6-(3,3-Dimethyl-oxiran-2-ylidene)-5,5-dimethyl-hex-3-en-2-one | 19.2 | Oxygenated compound | Anti-cancer/ADME properties | Bioactive compound; potential candidate for phytotoxicity screening |
| 9. | 1,4-Methanoazulene-9-methanol derivative | 13.2 | Sesquiterpenoid-type compound | Reported against liver cancer | Terpenoid-related compound of interest for allelochemical studies |
| 10. | Eicosane, 2-methyl- | 8.36 | Long-chain hydrocarbon | Antifungal activity | Hydrocarbon associated with biological activity; weed bioassay required |

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| 11. | Aspidospermidin-17-ol derivative | 11.9 | Nitrogen-containing alkaloid | Anti-tumour and antimalarial | Alkaloid-type phytochemical; candidate for phytotoxicity screening |
| 12. | Heptadecane, 2,6,10,15-tetramethyl- | 6.22 | Branched hydrocarbon | Anti-inflammatory | May contribute to biological activity; direct weed effect unconfirmed |
| 13. | 1-Decanol, 2-hexyl- | 4.33 | Long-chain alcohol | Antibacterial activity | Lipophilic compound potentially relevant to botanical screening |
| 14. | Cyclohexanone derivative | 11.3 | Oxygenated cyclic compound | Reported against colon cancer | Candidate for seed-germination/seedling assays |
| 15. | Isophytol | 1.72 | Diterpene alcohol | Antimicrobial activity | Terpenoid compound with potential allelochemical interest |
| 16. | 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone | 6.91 | Nitrogen-containing polycyclic compound | Apoptosis/cancer-related activity | Bioactive compound; requires phytotoxicity validation |
| 17. | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 91.5 | Oxygenated spiro compound | Anti-cancer properties | Major detected constituent; important candidate for bioassay-guided weed research |
| 18. | Germanicol | 0.19 | Triterpenoid | Reported against colon cancer | Triterpenoid candidate for allelopathic investigation |

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|---|---|---|---|---|---|
| 19. | Heneicosane, 11-(1-ethylpropyl)- | 2.75 | Long-chain hydrocarbon | Antimicrobial activity | Candidate for screening against weed germination |
| 20. | 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester | 2.98 | Phthalate ester | Cytotoxicity | Requires careful confirmation and phytotoxicity testing |
| 21. | Estra-1,3,5(10)-trien-17β-ol | 34.0 | Steroidal compound | Reported against liver cancer | Steroid-type phytochemical; potential biological significance |
| 22. | Palmitic anhydride | 8.70 | Fatty-acid derivative | Antimicrobial and antioxidant | Lipid-derived bioactive compound; candidate for allelopathic studies |
| 23. | Phytol | 78.5 | Diterpene alcohol | Cytotoxic activity | Important terpenoid constituent for further botanical screening |
| 24. | Toluene | 17.6 | Aromatic hydrocarbon | Antibacterial activity | Biological activity reported; direct herbicidal effect not established |

## RESULTS AND DISCUSSION

### *GC–MS profile of C. gigantea flower extract*

The GC–MS analysis demonstrated a chemically diverse profile in the flower extract. The identified constituents included hydrocarbons, oxygenated compounds, esters, terpenoid-related compounds and nitrogen-containing compounds. (Table 2)

**Table 2. Selected phytochemicals identified in *Calotropis gigantea* flower extract by GC–MS**

| S. No. | Retention time (min) | Area (%) | Compound | Molecular formula | Molecular weight |
|---|---|---|---|---|---|
| 1. | 3.69 | 1.06 | 2,5-Norbornadiene | $C_7H_8$ | 92.06 |
| 2. | 3.845 | 52.20 | Benzene, (2,4-dimethylpentyl)- | $C_{13}H_{20}$ | 176.15 |

| | | | | | |
|---|---|---|---|---|---|
| 3. | 42.22 | 13.40 | 1-Cyclohexanol, 2-(3-methyl-1,3-butadienyl)-1,3,3-trimethyl- | $C_{14}H_{24}O$ | 208.18 |
| 4. | 42.29 | 21.80 | Pentamethyl-trans-hexahydro-gamma-chromene derivative | $C_{14}H_{24}O$ | 208.18 |
| 5. | 42.66 | 21.00 | Geranyl isovalerate | $C_{15}H_{26}O_2$ | 238.19 |
| 6. | 42.71 | 27.60 | Spiro[2.5]oct-4-ene derivative | $C_{14}H_{22}O$ | 206.16 |
| 7. | 43.13 | 7.72 | Hexadecane, 2,6,11,15-tetramethyl- | $C_{20}H_{42}$ | 282.32 |
| 8. | 44.02 | 19.20 | Oxirane-containing ketone derivative | $C_{12}H_{18}O_2$ | 194.13 |
| 9. | 44.10 | 13.20 | Methanoazulene methanol derivative | $C_{15}H_{26}O$ | 222.19 |
| 10. | 44.56 | 8.36 | Eicosane, 2-methyl- | $C_{21}H_{44}$ | 296.34 |
| 11. | 46.38 | 1.72 | Isophytol | $C_{20}H_{40}O$ | 296.30 |
| 12. | 47.21 | 0.19 | Germanicol | $C_{30}H_{50}O$ | 426.38 |
| 13. | 48.24 | 8.70 | Palmitic anhydride | $C_{32}H_{62}O_3$ | 494.46 |
| 14. | 50.79 | 78.50 | Phytol | $C_{20}H_{40}O$ | 296.30 |

***Note.*** Values are derived from the supplied GC–MS dataset. The complete source table contains additional compounds.

***Relative abundance of major constituents***

The chromatographic profile showed considerable variation in the relative abundance of individual constituents. Among the compounds reported in the supplied dataset, phytol represented a prominent peak with an area of 78.5%, while benzene, (2,4-dimethylpentyl)-

represented 52.2%. Other relatively prominent constituents included the spiro[2.5]oct-4-ene derivative (27.6%), the pentamethyl-trans-hexahydro-gamma-chromene derivative (21.8%) and geranyl isovalerate (21.0%).

Relative peak area provides an indication of the chromatographic abundance of detected constituents, but it should not be interpreted directly as a measure of biological potency. A compound present at a lower percentage may nevertheless possess substantial biological activity, while a highly abundant compound may not necessarily be responsible for a particular biological effect.

***Diversity of identified phytochemicals***

The GC–MS profile indicates substantial chemical diversity in the flower extract. The compounds include aliphatic hydrocarbons, aromatic compounds, oxygenated metabolites, esters and terpenoid-related compounds. The early chromatographic region includes 2,5-norbornadiene and a benzene derivative, while later retention times contain several oxygenated and terpenoid-related constituents.

The presence of multiple chemical classes is relevant to natural-product research because botanical extracts can contain complex mixtures in which individual compounds or combinations of compounds may contribute to biological activity.

***Compounds with reported biological activities***

The supplied dataset associates several compounds with biological activities. 2,5-Norbornadiene is reported with nematicidal activity, while geranyl isovalerate is reported with antimicrobial activity. The hexadecane derivative is associated with antibacterial activity. Eicosane, 2-methyl- is reported with antifungal activity, whereas isophytol is reported with antimicrobial activity. Palmitic anhydride is associated with antimicrobial and antioxidant properties, while phytol is reported with cytotoxic activity in the supplied dataset.

These biological annotations demonstrate that the extract contains compounds with recognized biological relevance. However, they should not be interpreted as evidence that the flower extract itself possesses herbicidal activity (Sparks et al*.,* 2017).

***Relevance to botanical weed management***

The development of botanical weed-management products requires identification of plant metabolites capable of affecting weed establishment, growth or development. Allelochemical research has particularly emphasized phenolics, terpenoids and alkaloids as important chemical

groups in natural-product herbicide discovery (Macías et al., 2019). The present GC–MS profile includes several terpenoid-related compounds, including phytol, isophytol and germanicol. Consequently, the chemical profile provides a rational starting point for bioassay-guided botanical weed-management research.

The proposed research pathway is:

*C. gigantea* flowers → extraction → GC–MS profiling → identification of candidate metabolites → phytotoxicity screening → isolation/confirmation of active compounds → formulation → botanical weed-management evaluation. This approach is consistent with the broader natural-product herbicide research strategy described by Macías et al. (2019), where chemical characterization and isolation of allelochemicals are important components of natural herbicide discovery.

***Evidence from previous Calotropis weed-management research***

The relevance of *C. gigantea* to weed management is further supported by previous agricultural research, although that evidence concerns a different plant part. Malarkodi and Balasubramanian (2014) reported that *C. gigantea* leaf extract was evaluated as a pre-emergence weed-management treatment in cotton. Among the leaf-extract treatments, 30% extract followed by hand weeding at 40 days after sowing resulted in lower weed density and dry weight compared with lower extract concentrations and an unweeded control. This previous work supports the agricultural relevance of the species, while the present study contributes a GC–MS-based chemical perspective specifically for the flower extract (Rajamohan et al., 2014). The distinction is important: the previous study demonstrated field-level effects of a leaf extract, whereas the present study identifies phytochemicals in a flower extract. Therefore, the two findings should be considered complementary rather than equivalent.

***Botanical weed-management prospects***

The present GC–MS profile suggests that *C. gigantea* flower extract deserves consideration as a candidate source for botanical weed-management chemistry.

Three aspects are particularly relevant.

***Chemical diversity:*** The presence of several chemical classes increases the possibility of identifying biologically active constituents through subsequent fractionation and screening.

***Terpenoid-rich profile:*** Terpenoids have attracted considerable attention in natural herbicide and allelochemical research. The detection of phytol, isophytol and germanicol therefore provides a useful basis for further investigation.

***Potential for integrated weed management:*** Allelopathic plant materials can potentially complement cultural, mechanical and chemical weed-management practices rather than necessarily functioning as stand-alone herbicides. Reviews have identified allelopathy as a potential component of sustainable and integrated weed-management systems. (Khamare et al., 2022).

***Limitations of the present study***

The present study is limited to GC–MS profiling. Therefore, the following conclusions cannot be made from the current dataset: direct weed germination inhibition, weed mortality, root or shoot growth inhibition, herbicidal dose-response, $IC_{50}$ or $EC_{50}$ values, crop selectivity, field efficacy, persistence in soil, formulation stability. GC–MS identification should therefore be considered a chemical screening step, rather than proof of botanical herbicidal activity. In addition, some compound identifications obtained through GC–MS library matching require confirmation using authentic standards and/or complementary analytical techniques such as LC–MS, NMR or targeted quantitative analysis before attributing biological activity to a specific compound.

**CONCLUSION**

The GC–MS profiling of *Calotropis gigantea* flower extract revealed a chemically diverse mixture containing hydrocarbons, oxygenated compounds, esters and terpenoid-related constituents. The profile included phytol, benzene, (2,4-dimethylpentyl)-, geranyl isovalerate, isophytol, germanicol, palmitic anhydride, eicosane, 2-methyl-, and several other compounds.

Several compounds identified in the extract have reported biological activities, including antimicrobial, antifungal, nematicidal and antioxidant properties. These findings provide a useful phytochemical basis for exploring *C. gigantea* as a botanical resource for weed-management research.

The study should, however, be interpreted strictly as a GC–MS profiling investigation. The presence of biologically active phytochemicals does not establish herbicidal efficacy. Future research should therefore employ bioassay-guided fractionation to determine whether the identified constituents or their combinations possess phytotoxic activity against important

agricultural weeds. Overall, the chemical diversity observed in *C. gigantea* flower extract supports its further investigation as a potential source of natural-product leads for sustainable and integrated botanical weed management.

**Declarations and statements**

**Conflict of Interest**

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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**Data Availability**

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions**

MA: Conceptualization, study design, interpretation of GC–MS data, literature review, preparation of the manuscript, critical revision, and finalization of the manuscript;

ED: Contribution to phytochemical data compilation, literature review, interpretation of the identified compounds, manuscript preparation, and critical revision.

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