Research Article

Phytochemical Investigation of Ethanolic Extract of Calotropis Gigantea Leaf Using GC–MS With Relevance To Wound Healing: An Experimental Study

Dr. Harishankar P V[1] , Dr. Laxmikant S D[2*], Dr. Ajit Chandrashekhar Lingayat[3], Dr. Athira Mohan K[4]

[1]Assistant Professor, Department of Shalya Tantra, KLE Ayurveda Medical College and Hospital, Chikodi, Karnataka, India. Email; 628harishankarpv@gmail.com
[2]Professor, Department of Shalya Tantra, KLE's Shri BMK Ayurveda Mahavidyalaya Belagavi, Karnataka, Email; India. shalyalsd@gmail.com
ORCID: https://orcid.org/0009-0006-0516-7960
[3]Associate Professor, Department of Dravyaguna, Kaher Shri B M K Ayurved Mahavidyalaya Belagavi, Karnataka, India. Email; ajitlingayat26@gmail.com
[4]Assistant Professor, Department of Rasa Sastra Evum Bhaishajya Kalpana, KLE Ayurveda Medical College and Hospital, Chikodi, Karnataka, India. Email; drkadathanadan5588@gmail.com
Corresponding Author:
Dr. Laxmikant S D Professor, Department of Shalya Tantra, KLE's Shri BMK Ayurveda Mahavidyalaya, Belagavi
ORCID: https://orcid.org/0009-0006-0516-7960 Email: shalyalsd@gmail.com

Abstract

Introduction: Chronic and delayed wounds continue to pose a significant challenge in healthcare, and medicinal plants provide multi-faceted alternatives to single-pathway synthetic drugs. In Ayurveda, Calotropis gigantea is recognised for its wound-healing properties, and contemporary studies confirm its antimicrobial, anti-inflammatory, and antioxidant capabilities; however, its ethanol-soluble phytochemical composition has not been analysed by GC-MS, and the link between this profile and its wound-healing pharmacology remains unexplored.

Aim of the study: To identify the phytochemical profile of Calotropis gigantea ethanolic leaf extract using GC-MS, and correlate these identified compounds with their reported significance in wound healing.

Materials and methods: This Experimental study was conducted at KAHERs Shri BM Kankanawadi Ayurved Mahavidyalaya, Belagavi, in collaboration with Amrith Labs (AYUSH-approved), Shimoga, Karnataka, from April 2025 to June 2025. The Ethanolic extract was macerated, filtered, and analysed by GC–MS (Shimadzu GCMS-QP2010SE; electron ionization; oven program 80–330 °C). Compounds were identified against the NIST 2020 library, and 80% similarity index (SI) was used as a cut-off threshold to indicate confident (>80%) and tentative identification (<80%).

Results: A total of 26 chromatographic peaks (retention time range: 4.986–37.600 min) were resolved by GC–MS, representing 100% of the total peak area, and 22 were confidently identified (SI percentages ranging from 80 to 97%), while the remaining were tentatively identified (SI [%] between 54 and 79%). Other constituents were lipid-based: Dodecanoic acid (2.14%, SI 90), oleic acid (1.50%, SI 86), α-linolenic acid(0.50%, SI 90), methyl linolenate (1.01%,SI94), methyl palmitate(0.44%,SI95), long-chain alkynes, glycerol diesters, esters of octanoic acid, and vinyl laurate. Two peaks were excluded as contaminants; the refined biological assay is literature-based.

Conclusion: A lipid-rich profile of fatty acids and esters with antimicrobial, anti-inflammatory, antioxidant, and tissue-regenerative qualities characterizes the ethanolic extract of C. gigantea leaves, offering scientific evidence for its traditional Vrana Ropana(wound healing) attribution, which is being evaluated in a comparative study. This phytochemical fingerprint provides a multi-targeted foundation for investigating C. gigantea as a plant-based wound-healing agent, supporting confirmation by confirmatory bioassay.

Keywords: Calotropis gigantea, Ayurveda, Fatty acids, Wound healing.

INTRODUCTION

Wound healing comprises Hemostasis, inflammation, proliferation, and remodeling, which are natural biological processes in the human body. Numerous variables may disrupt one or more stages of this process, leading to incorrect or compromised wound healing.[1].

The antibacterial, antioxidant, and anti-inflammatory qualities of medicinal plants high in flavonoids, terpenoids, polyphenols, alkaloids, and steroids help in the treatment of wounds. The complex stages of wound healing, including hemostasis, inflammation, proliferation, and remodeling, are thoroughly discussed in the literature in relation to the phytochemicals, emphasizing key cellular and molecular processes that are necessary for successful tissue repair. [2].

Calotropis gigantea, commonly known as gigantic milkweed, is a member of the Asclepiadaceae family. It is native to Bangladesh, India, China, Indonesia, the Philippines, Malaysia, Myanmar, Pakistan, Thailand, and Sri Lanka, and is found across continental and Southeast Asia. The main characteristics of C. gigantea

are its drought resilience, salt tolerance, and ease of cultivation.[3] In Ayurveda, it is said to possess Vrana Ropana (wound-healing) qualities, and contemporary research has reported its antibacterial, anti-inflammatory, and antioxidant characteristics, indicating its potential for therapeutic use. [4-5].

The phytochemical makeup of therapeutic plants significantly determines how well they heal wounds. Among these, fatty acids and their derivatives are crucial because they support tissue regeneration, control inflammation, and preserve the integrity of the skin barrier [6].

While unsaturated fatty acids promote cellular repair and regulate inflammatory responses, medium-chain fatty acids have antibacterial properties. By lowering dehydration and microbial invasion, lipid derivatives like glycerol and wax esters may provide additional wound protection.[7-9].

GC-MS phytochemical profiling helps link identified compounds to their biological functions and provides useful information about the chemical composition of plant extracts. Fatty acids, esters, and hydrocarbons are examples of volatile and semi-volatile phytoconstituents that can be reliably identified using gas chromatography–mass spectrometry (GC–MS). [10].

The present study aimed to characterise the phytochemical constituents of the Ethanolic leaf extract of Calotropis gigantea using GC–MS and to evaluate their relevance to wound healing. By correlating the identified compounds with established pharmacological activities, this study provides a scientific basis for the traditional Ayurvedic concept of Vrana Ropana, encompassing antimicrobial protection, inflammation control, and tissue regeneration. The findings may contribute to the development of plant-based therapeutics and provide a foundation for future experimental and clinical investigations.

MATERIALS AND METHODS:

This Experimental study was conducted at KAHERs Shri BM Kankanawadi Ayurved Mahavidyalaya, Belagavi, in collaboration with Amrith Labs (AYUSH-approved), Shimoga, Karnataka, from April 2025 to June 2025.

The plant material (Arka, Calotropis gigantea (L.) W.T. Aiton, Family: Apocynaceae) was authenticated at the Central Research Facility, Shri B.M.K. Ayurveda Mahavidyalaya, KLE Academy of Higher Education & Research, Shahapur, Belgaum. A voucher specimen has been deposited under CRF Code: CRF/Auth/142/2025-2026 in the institutional herbarium Fresh leaves of Calotropis gigantea were collected, identified, and authenticated by the Authentication Expert in CRF, KAHERs, Shri BM Kankanwadi Ayurved Mahavidyalaya, Belagavi. The leaves were thoroughly washed with distilled water to remove adhering impurities, shade-dried at room temperature until a constant weight was achieved, and pulverised into a fine powder using a mechanical grinder. The powdered material was stored in an airtight container at room temperature until extraction.

Based on the laboratory protocol, 100 g of the crushed leaf material was used with 500 mL of ethanol, and the extract was obtained using a Soxhlet Apparatus. The mixture was kept for drying in a water bath (80° Celsius). The Extract was used directly for GC–MS analysis.

Phytochemical analysis was performed using a Shimadzu GC- MS-QP2010SE Gas Chromatography–Mass Spectrometry system equipped with helium as the carrier gas. The oven temperature was programmed from 80°C to 330°C under optimised analytical conditions. The mass spectrometer was operated in electron ionisation mode, and mass spectra were recorded over an m/z range of 35–500.

Identification of Phytoconstituents and Data Analysis

Chromatographic peaks were identified by comparing their mass spectra with those available in the NIST 2020 Mass Spectral Library[11]. Compounds with a similarity index (SI) of ≥80% were considered positively identified. The relative abundance of individual phytoconstituents was determined from the percentage peak area in the total ion chromatogram (TIC). The identified compounds were subsequently interpreted based on published literature regarding their reported antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities.[12]

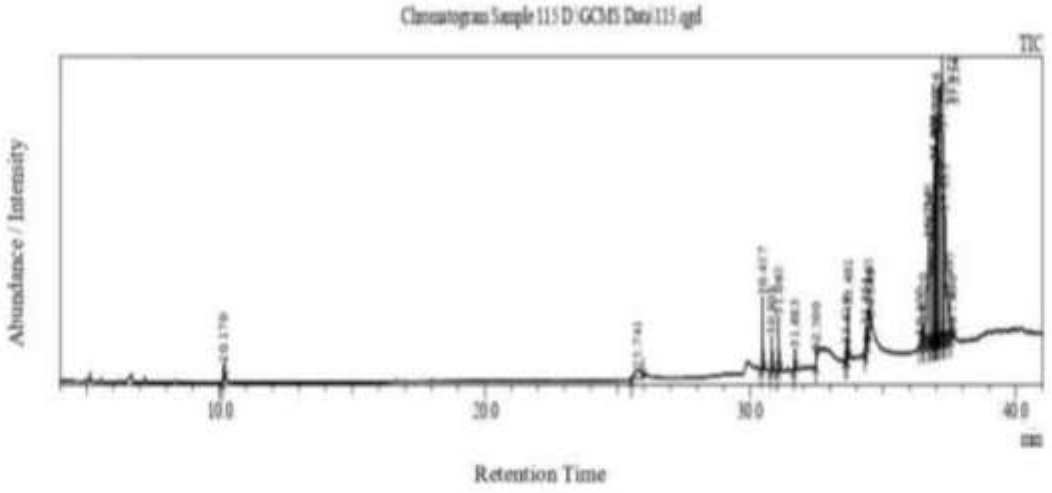
RESULTS

GC–MS analysis of the ethanolic extract of Calotropis gigantea leaves resolved 26 chromatographic peaks over a retention time range of 4.986–37.600 min (Table/Figure 1), collectively accounting for 100% of the total peak area (Table 1). Of the 26 peaks, 22 were confidently identified (SI 80–97%). The dominant confidently identified constituents were lipid-based compounds, particularly fatty acids and fatty acid

esters. Two long-chain alkynes, 1-octadecyne (2.70% cumulative, two peaks, SI 90–91) and 1-hexadecyne (0.78%, SI 90), were also confidently identified.

Two peaks were identified as exogenous, non-phytochemical constituents: trimethyl(2-propoxyethoxy) silane (peak 4, 0.67%, SI 79), consistent with instrumental column bleed or silylation background, and bis(2-ethylhexyl) phthalate (DEHP; peak 33, 0.41%, SI 80), a widely recognized plasticizer contaminant commonly introduced from laboratory plasticware during sample extraction or storage. Both are excluded from subsequent pharmacological interpretation.[Table/figure 2]

Table/Figure 1 - Total Ion Chromatogram (TIC) of ethanolic leaf extract of C. gigantea (Sample 115) showing 26 peaks (4.9-37.6 min). (Software used – Shimadzu GCMS Software)



Table/figure 2: Integration table of GC–MS Chromatogram of Sample of Calotropis gigantea Leaf Ethanolic Extract

Peak#	RTime	Area	Area%	Height	Height% CAS#	Name
1	25.741	4030080	1.89	239750	0.36 112-37-8	Undecamic acid
2	30.457	3486662	1.64	1905315	2.83 629-89-0	1-Octadecyne
3	30.808	1714591	0.80	908231	1.35 629-89-0	1-Octadecyne
4	31.065	2422255	1.14	1457700	2.17 629-89-0	1-Octadecyne
5	31.683	964871	0.45	566965	0.84 112-39-0	Hexadecanoic acid, methylester
6	32.500	356537	0.17	211814	0.31 628-97-7	Hexadecanoic acid, ethyl ester
7	33.610	571721	0.27	374387	0.56 2463-02-7	11,14-Eicosadienoicacid methyl ester
8	33.681	2200904	1.03	1356142	2.02 301-00-8	9,12,15-Octadecatrienoic acid, methyl ester,
9	34.354	1103466	0.52	583058	0.87 46340-1	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-
10	34.465	3276995	1.54	703807	112-80-1	OleicAcid

11	34.540	1482190	0.70	182178	0.27 593-39-5	6-Octadecenoicacid, (Z)-
12	36.405	2013853	0.94	204624	0.30 177717-46-3	1-Hydrox-y-3-(octanoyloxy)propan-2-yl decan
13	36.520	1874161	0.88	544565	0.81 0-00-0	Octanoic acid, 4-tridecyl ester
14	36.716	12250960	5.75	2719334	4.04 0-00-0	7,7-Dibutoxyheptanoic acid, butyl ester
15	36.745	10572791	4.96	2894663	4.30 0-00-0	Octanoic acid, 4-tridecylester
16	36.860	10006573	4.69	2494915	3.71 0-00-0	Octanoic acid, 4-tridecylester
17	36.931	13879475	6.51	4740481	7.04 177717-46-3	1-Hydroxy-3-(octanoyloxy)propan-2-yl decan
18	36.959	7994401	3.75	4687942	6.97 3623-05-0	2H-Azepin-2-one, hexahydro 4-methyl-
19	37.002	13564846	6.36	5400611	8.02 177717-46-3	l-Hydrox-y-3-(octanoyloxy)propan-2-yldecan
20	37.026	8200329	3.85	5777295	8.58 177717-46-3	1-Hydroxy-3-(octanoylox-y)propan-2-yldecan
21	37.072	27779769	13.03	6688742	9.94 0-00-0	Octanoic acid, 4-pentadecylester
22	37.135	5234712	2.45	4572218	6.79 73105-67-0	1-Iodo-2-methylundecane
23	37.234	42044948	19.72	7461123	11.09 177717-46-3	1-Hydroxy-3-(octanoyloxy)propan-2-yl decan
24	37.270	16507922	7.74	5502623	8.18 177717-46-3	l-Hydrox-y-3-(octanoyloxy)propan-2-yl decan
25	37.335	13969326	6.55	3308432	4.92 2146-71-0	Dodecanoic acid, ethenyl ester
26	37.495	5732764	2.69	999947	1.49 2146-71-0	Dodecanoic acid,ethenyl ester

DISCUSSION:

The GC-MS analysis of the ethanolic leaf extract from Calotropis gigantea pointed mainly to lipid-based phytoconstituents, with fatty acids, their esters, and glycerol derivatives. These compounds play a major part in helping wounds heal. Wound healing itself is a biological process that involves controlling inflammation, stopping microbes, helping tissue grow, and then remodeling the area, and the compounds found here seem to contribute to each of those stages. [13,14] What stood out was the high amount of glycerol fatty acid diesters, especially 1-hydroxy-3-(octanoyloxy) propan-2-yl decanoate. That particular molecule suggests there are bioactive lipids at work that might affect membrane stability, cellular signaling, and how tissue regenerates. These lipid structures resemble the components our body uses to repair cell membranes, and they could support re-epithelialization and boost fibroblast activity, both of which are essential for getting a wound to close properly.

The extract contains fatty acids like lauric acid, oleic acid, and alpha-linolenic acid, and these are known for fighting microbes and reducing inflammation. Lauric acid is a medium-chain fatty acid that kills Gram-positive bacteria effectively. This helps lower the number of microbes in wounds, which keeps infections from slowing down the healing process. [7,15]. In the same way oleic acid has demonstrated its ability to regulate inflammatory responses by affecting cytokine production and minimizing excessive inflammation, which is crucial for transitioning from the inflammatory phase to the proliferative phase of wound healing. Alpha-linolenic acid, an omega-3 fatty acid, helps in tissue regeneration. The presence of these fatty acids suggests that the extract has natural characteristics that can control important stages of wound healing.[16] Identification of vinyl esters like vinyl laurate and the long chain wax esters further add to the healing properties of the extract. These compounds have shown antifungal as well as antibacterial activity and also have the ability to build a barrier over the wound surface. This barrier not only protects the wound surface from invasion but also maintains the moist wound environment required for faster epithelialization. [7,17]. Another important aspect of wound healing is the mitigation of oxidative stress, which can impair cellular repair mechanisms. The polyunsaturated fatty acids identified in the extract, particularly linolenic acid and its derivatives, possess antioxidant properties that help neutralize reactive oxygen species. By reducing oxidative damage, these compounds facilitate fibroblast proliferation, collagen synthesis, and angiogenesis, all of which are essential for effective wound healing. The combined antioxidant and anti-inflammatory actions of these phytoconstituents create a favorable environment for tissue repair and regeneration.[18]

When compared with previously published GC–MS surveys of Calotropis gigantea leaves, the present phytochemical profile shows both similarities and differences. Long-chain fatty acids and their esters, particularly palmitate, oleate, and linolenate derivatives have been reported fairly consistently across solvent systems and are corroborated by the present findings [19].

Some of the main compounds found out in other GC MS studies of C. gigantea were hydrocarbons, terpenoids, phytosterols along with presence of alkaloids, terpenoids, saponins, tannins, and cardiac glycosides [20]. Unfortunately, the above-mentioned compounds were not found in the present study with acetone. This may be due to the solvent polarity.Methanol and ethanol having more polarity solubilizes glycosides, phenolics, saponins and alkaloidal salts. Moreover, the plant part, its geographical origin, season of harvest and extraction techniques also contribute to the compound's presence. In the present study, it mainly focuses on the lipid soluble fraction of C. gigantea leaves and so this should not be taken as a complete phytochemical profiling of the plant.

Plant extracts show their effect through a combination of multiple bioactive compounds rather than acting by single compound therapy [21]. This multitarget approach is beneficial in all ways considering a wound healing. It addresses many areas at once like infection control, inflammation modulation, oxidative stress reduction and tissue regeneration in a proper coordinated manner. Thus, the rich lipid profile of Calotropis gigantea leaf extract provides a proper therapeutic effect which contributes to the wound repair [22].

Even though the study was intended to focus on phytochemical identification, the given results strongly suggest wound healing potential of C. gigantea leaves. This was clearly backed up by the prior proved results of pharmacological activities of the plant in wound healing.

The study has four main limits. Firstly, GC MS was selected as the analytical platform due to its high resolution, cost effectiveness and robust capability to identify volatile and semi volatile constituents. The contrast is that GCMS is restricted to compounds that can be vaporized within the temperature program of the instrument and so cannot detect non-volatile, high molecular weight and thermolabile compounds. These include glycosides, saponins, tannins, and larger phenolic or polymeric compounds. Considering the importance, these compounds are much important with reference to C. gigantea. Other methods such as LC-MS or HPLC would be required for the same.

Secondly, compound identification relied on NIST20 library matching by similarity index rather than real reference standards.

Third, the reported percentages show relative peak area and not absolute amounts.

Fourth, the analysis was performed on a single ethanol extract from one geographic source and collection period. No replicates were used for comparison.

Finally, the wound healing effect were inferred from the past available information and was not tested in lab for any wound healing assay. Future studies should use reference standards. LC-MS or HPLC calibration

can be considered. C.gigantea collected from different geographical has to be analyzed. In vivo would healing action should be performed.

CONCLUSION

The GC–MS analysis of the ethanolic leaf extract of Calotropis gigantea revealed a diverse phytochemical profile predominantly comprising fatty acids, fatty acid esters, and other lipid-derived bioactive constituents. Several of the identified compounds have been reported in previous scientific literature to possess antimicrobial, anti-inflammatory, antioxidant, and tissue-repair-associated properties, suggesting their potential relevance to wound management. These bioactive constituents may contribute to different stages of wound healing by reducing microbial contamination, modulating inflammatory responses, minimizing oxidative stress, and supporting cellular processes involved in tissue repair and regeneration. The presence of multiple phytochemical constituents further suggests the possibility of complementary or synergistic biological effects, although such interactions have not yet been experimentally established. The findings provide a preliminary scientific basis for exploring Calotropis gigantea leaf extract as a potential source of bioactive compounds for the development of plant-based wound-care formulations. However, GC–MS profiling alone does not confirm wound-healing efficacy, and the therapeutic contribution of individual constituents remains to be established. Further investigations involving phytochemical quantification, antimicrobial and antioxidant assays, cytotoxicity evaluation, fibroblast proliferation and migration studies, in vitro wound-scratch assays, and suitable in vivo wound-healing models are necessary to establish its biological activity, safety, effective concentration, and therapeutic potential. Overall, the present study provides a foundation for future pharmacological investigations and formulation development involving Calotropis gigantea leaf extract in wound-healing applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

ETHICAL APPROVAL

This study did not involve any human participants or animal experimentation. Hence, ethical approval was not required.

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