



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Article

GC-MS Analysis of Ethanolic Extracts *Gliricidia Sepium*

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ARTICLE INFO

Published: 13 Mar. 2025

Keywords:

Gliricidia sepium, Ethanolic extracts, GC-MS

DOI:

10.5281/zenodo.15018901

ABSTRACT

The GC-MS analysis of *Gliricidia sepium* revealed the presence of 48 compounds. This research examined the chemical composition of *Gliricidia sepium* leaves, flowers, and seeds using gas chromatography-mass spectrometry (GC-MS) on their ethanolic extracts. In this study, the ethanolic extract of *Gliricidia sepium* leaves, flowers, and seeds was tested to explore their chemo profiling through gas chromatography-mass spectrometry (GC-MS). The investigation employed gas chromatography-mass spectroscopy to analyze *Gliricidia sepium*, a plant known for its various medicinal properties. The primary objective was to identify the phytochemicals present in the plant through GC-MS analysis. The results confirmed the presence of multiple phytochemicals in the extracts. Considering the absorption and persistence of specific compounds found in these plant extracts, they could be suggested as a potential natural treatment for addressing conditions affecting the chorioallantoic membrane in *Gallus gallus domesticus*.

INTRODUCTION

In health sciences and therapeutic protocols worldwide, ancient and historical texts have documented extensive knowledge about diseases and treatments, drawing from information provided by Ayurveda and traditional folk theories (Vaidya A. D. et al., 2007). *Gliricidia sepium*, also called quick stick or undirmari in Marathi, is a medium-sized tree that is a legume and a member of the Fabaceae family. It can grow to a height of 10 to 12 meters, and its bark can range from whitish to deep red-brown (Hughes, 1987).

This species has been extensively introduced in tropical regions, primarily through planting efforts by forest departments, resulting in a significant increase in its global population (Kagya-Agyemang et al., 2007). Notably, *Gliricidia sepium* plays a vital role in environmental sustainability by fixing nitrogen, which contributes to the enrichment of the soil (Chadhokar, 1982). Expensive treatment of wound injuries creates a heavy burden to poor Filipinos. In line with this, the latter resorted to alternative herbal remedies that are not only tested to heal wounds but are also readily available. One of these

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



herbal remedies is the plant *Gliricidia sepium* Jacques Steudel (Fam Fabaceae) that can be found almost everywhere. The sap of the bark, leaves and roots has been cited by folkloric literatures as a wound healing promoter (Carandang *et al.*, 2015). The most widely used method for identifying and quantifying secondary metabolites is GC-MS. This study aimed to explore the biochemical components using GC-MS/MS analysis. The technique produces a representative spectral output for all compounds extracted from the sample.

MATERIAL AND METHODS:

Collection of the plant material:

Gliricidia sepium was collected from Panhala, Kolhapur (16°50'06.4"N 74°07'39.9"E) situated in the Western Ghats of India. The plant material was identified by Department of Botany, Sadguru Gadage Maharaj College, Karad.

Preparation of extract:

The aerial Parts like leaves, flowers and seeds of *Gliricidia sepium* were shade dried after washing and then grinded in grinder to a make fine powder. The fine powder from leaves, flowers and seeds will be exhaustively extracted by soxhlet extractor, using solvent. Prepared extract was further used for GC-MS/MS analysis of different compounds from *Gliricidia sepium* plant species.

GCMS Analysis:

The ethanol extracts obtained from leaves, flowers and seeds of *Gliricidia sepium* were exposed to GC-MS analysis. GCMS analysis of this extract was performed using a Shimadzu Japan TQ 8050 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a

RTX TQ 8050 column (60 m X 0.25 mm ID X 1 µm, composed of 100% Dimethyl polysiloxane) for GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Especially pure Helium gas (99.99 %) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 2 µl was employed. Injector temperature 250°C; Ion-source temperature 200° C. The oven temperature was programmed from 50°C (isothermal for 2 min.) with an increase of 5°C/min to 180° C, then 5°C/min to 250°C, ending with a 8 min. isothermal at 260° C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 500 m/z. Total GC running time was 48 minutes the relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was GC-MS Real Time Analysis (Phukan *et al* 2017).

RESULTS AND DISCUSSION

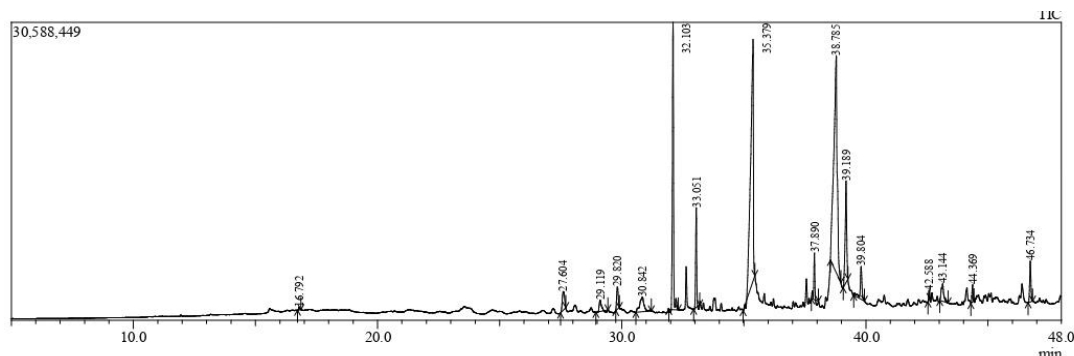
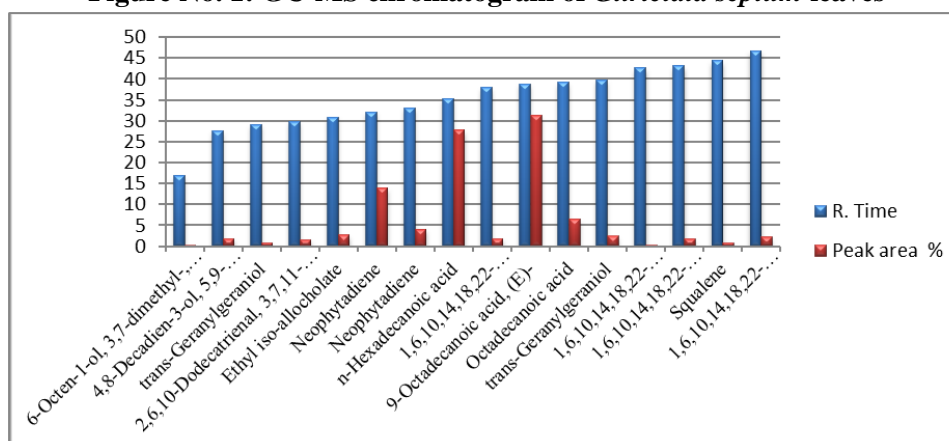
The ethanolic extract of the leaves of *Gliricidia sepium* was analyzed using gas chromatography and mass spectrometry (GC-MS) resulting in the identification of a total of 16 components (Table 1, Fig. 1, and Fig. 2). The table also shows the compounds' retention time, peak area, and percentage. The major phytochemical compounds were 9-Octadecanoic acid, (E)- (31.18%), n-hexadecanoic acid (27.79%), Neophytadiene (14.03%) shows more area % peak and Squalene, 1,6,10,14,18,22-Tetraacosahexaen-3-ol, 2,6,10, trans-Geranylgeraniol, 6-Octen-1-ol, 3,7-dimethyl-, formate shows less area % peak in analysis.

Table N0. 1 : Phytocomponents identified in the leaves extract of *Gliricidia sepium*.

| No. | Name of compound | R. Time | Peak area % |
|-----|---|---------|-------------|
| 1 | 6-Octen-1-ol, 3,7-dimethyl-, formate | 16.792 | 0.14 |
| 2 | 4,8-Decadien-3-ol, 5,9-dimethyl- | 27.604 | 1.83 |
| 3 | trans-Geranylgeraniol | 29.119 | 0.84 |
| 4 | 2,6,10-Dodecatrienal, 3,7,11-trimethyl-, (E,E)- | 29.820 | 1.54 |
| 5 | Ethyl iso-allocholate | 30.842 | 2.66 |
| 6 | Neophytadiene | 32.103 | 14.03 |
| 7 | Neophytadiene | 33.051 | 4.10 |
| 8 | n-Hexadecanoic acid | 35.379 | 27.79 |



| | | | |
|----|---|--------|-------|
| 9 | 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10 | 37.890 | 1.81 |
| 10 | 9-Octadecanoic acid, (E)- | 38.785 | 31.18 |
| 11 | Octadecanoic acid | 39.189 | 6.56 |
| 12 | trans-Geranylgeraniol | 39.804 | 2.40 |
| 13 | 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10 | 42.588 | 0.36 |
| 14 | 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10 | 43.144 | 1.73 |
| 15 | Squalene | 44.369 | 0.83 |
| 16 | 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10 | 46.734 | 2.20 |

Figure No. 1: GC-MS chromatogram of *Gliricidia sepium* leavesFigure No. 2: Graphical representation of percentage of area detected by GC-MS in leaves of *Gliricidia sepium*

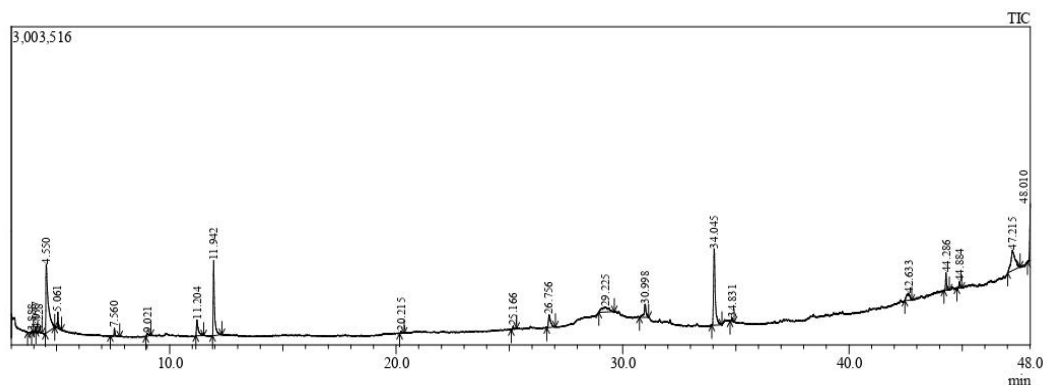
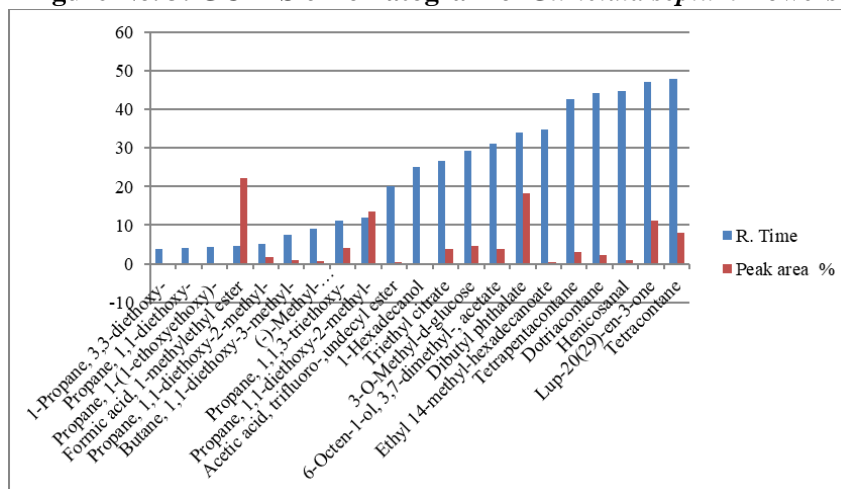
The ethanolic extract of the flower of *Gliricidia sepium* was analyzed using gas chromatography and mass spectrometry (GC-MS) resulting in the identification of a total of 21 components (Table 2 and Fig. 2). The table also shows the compounds' retention time, peak area, percentage. The major phytochemical compounds were Formic acid, 1-

methyl ethyl ester (22.14%), Dibutyl phthalate (18.22%), Propane, 1,1-diethoxy-2-methyl (13.43), Lup-20(29)-en-3- shows more area % peak in analysis.

Table N0. 2 : Phytocomponents identified in the flowers extract of *Gliricidia sepium*.

| No. | Name of compound | R. Time | Peak area % |
|-----|----------------------------------|---------|-------------|
| 1 | 1-Propane, 3,3-diethoxy- | 3.888 | 0.03 |
| 2 | Propane, 1,1-diethoxy- | 4.077 | 0.25 |
| 3 | Propane, 1-(1-ethoxyethoxy)- | 4.228 | -0.38 |
| 4 | Formic acid, 1-methylethyl ester | 4.550 | 22.14 |
| 5 | Propane, 1,1-diethoxy-2-methyl- | 5.061 | 1.83 |

| | | | |
|----|---|--------|-------|
| 6 | Butane, 1,1-diethoxy-3-methyl- | 7.560 | 1.05 |
| 7 | (-)-Methyl-3,3diethylcyclopropane-1,trans-2 | 9.021 | 0.74 |
| 8 | Propane, 1,1,3-triethoxy- | 11.204 | 3.96 |
| 9 | Propane, 1,1-diethoxy-2-methyl- | 11.942 | 13.43 |
| 10 | Acetic acid, trifluoro-,undecyl ester | 20.215 | 0.33 |
| 11 | 1-Hexadecanol | 25.166 | 0.22 |
| 12 | Triethyl citrate | 26.756 | 3.72 |
| 13 | 3-O-Methyl-d-glucose | 29.225 | 4.50 |
| 14 | 6-Octen-1-ol, 3,7-dimethyl-, acetate | 30.998 | 3.73 |
| 15 | Dibutyl phthalate | 34.045 | 18.22 |
| 16 | Ethyl 14-methyl-hexadecanoate | 34.831 | 0.51 |
| 17 | Tetrapentacontane | 42.633 | 3.09 |
| 18 | Dotriacontane | 44.286 | 2.26 |
| 19 | Henicosanal | 44.884 | 1.01 |
| 20 | Lup-20(29)-en-3-one | 47.215 | 11.23 |
| 21 | Tetracontane | 48.010 | 8.14 |

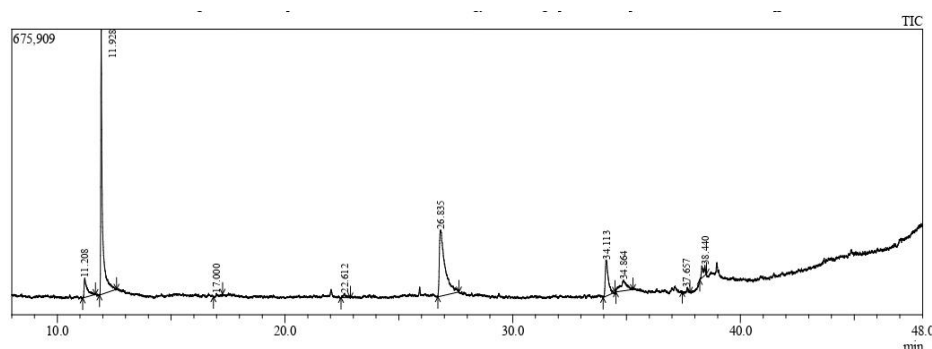
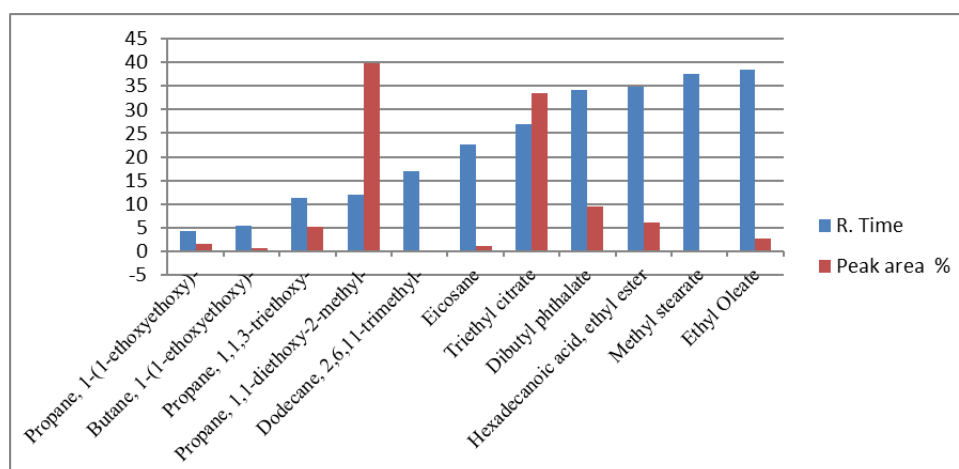
Figure No. 3: GC-MS chromatogram of *Gliricidia sepium* flowersFigure No. 4: Graphical representation of percentage of area detected by GC -MS in flowers of *Gliricidia sepium*

The ethanolic extract of the seeds of *Gliricidia sepium* was analyzed using gas chromatography and mass spectrometry (GC-MS) resulting in the identification of a total of 11 components (Table 3 and Fig. 3). The table also shows the compounds'

retention time, peak area, percentage. The major phytochemical compounds were Propane, 1,1-diethoxy-2-methyl- I(39.85%), Triethyl citrate (33.47%) shows more area % peak.

Table N0. 3 : Phytocomponents identified in the seeds extract of *Gliricidia sepium*.

| No. | Name of compound | R. Time | Peak area % |
|-----|---------------------------------|---------|-------------|
| 1 | Propane, 1-(1-ethoxyethoxy)- | 4.184 | 1.50 |
| 2 | Butane, 1-(1-ethoxyethoxy)- | 5.328 | 0.72 |
| 3 | Propane, 1,1,3-triethoxy- | 11.208 | 5.24 |
| 4 | Propane, 1,1-diethoxy-2-methyl- | 11.928 | 39.85 |
| 5 | Dodecane, 2,6,11-trimethyl- | 17.000 | -0.24 |
| 6 | Eicosane | 22.612 | 1.09 |
| 7 | Triethyl citrate | 26.835 | 33.47 |
| 8 | Dibutyl phthalate | 34.113 | 9.50 |
| 9 | Hexadecanoic acid, ethyl ester | 34.864 | 6.03 |
| 10 | Methyl stearate | 37.657 | 0.20 |
| 11 | Ethyl Oleate | 38.440 | 2.64 |

**Figure No. 5: GC-MS chromatogram of *Gliricidia sepium* seeds****Figure No. 6: Graphical representation of percentage of area detected by GC -MS in seeds of *Gliricidia sepium*****CONCLUSION:**

- The GC-MS analysis of *Gliricidia sepium* revealed the presence of various bioactive compounds with potential medicinal and industrial applications. Key phytochemicals detected which are known for their antimicrobial, antioxidant, anti-inflammatory,

and therapeutic properties. These findings validate the traditional use of *Gliricidia sepium* in herbal medicine and suggest its potential for pharmaceutical and agrochemical applications.

- Further research, including biological activity assays and toxicity studies, is recommended

to explore the full therapeutic potential of these compounds and develop value-added products from *Gliricidia sepium*.

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HOW TO CITE: Santosh Jagatap*, Dr. B. S. Wali, GC-MS Analysis of Ethanolic Extracts *Gliricidia Sepium*, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 3, 1186-1191. <https://doi.org/10.5281/zenodo.15018901>

