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Characterization of Phytochemical compounds in *Crotalaria juncea* Leaf Extract Using GC-MS Analysis

Ambhore AT* and Pawade PN

Department of Botany, Smt. Narsamma Art, Commerce & Science College, Amravati
*Corresponding Author: Email: zoosaurabhm@gmail.com

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Abstract

This study aimed to explain the phytochemical profile of *Crotalaria juncea* leaf ethanol extract using Gas Chromatography Mass Spectrometry (GC-MS) analysis. *Crotalaria juncea*, It is commonly known as sun hemp and is widely cultivated in tropical and subtropical regions, especially in a for its medicinal properties, including anti-inflammatory and anti-diabetic effects. Leaves were collected from Borgaon, Amravati, India, and subjected to ethanol extraction. GC-MS analysis identified a complex mixture of sixteen compounds, with hexanoic acid, ethyl ester (36.22%) being the most abundant, followed by 2,2,4 trimethyl-1,3-pentanediol diisobutyrate (15.33%) and 2-myristynoyl pantetheine (9.70%). Other notable compounds included pregna-6,16 diene-11,20-diol, octanoic acid, ethyl ester, and β-N-acetylneuraminic acid derivatives. The identified compounds suggest a range of pharmacological activities, such as antimicrobial, anti-inflammatory, antiviral, and immune-modulating effects. This comprehensive phytochemical characterization underscores the therapeutic potential of *Crotalaria juncea* leaf extract, warranting further research to isolate these bioactive compounds and evaluate their individual and synergistic effects in various biological models. The findings contribute to a deeper understanding of the medicinal properties of *Crotalaria juncea* and support its potential use in pharmaceutical and nutraceutical applications.

Keywords: *Crotalaria juncea*, GC-MS analysis, phytochemical profiling, ethanolic extract, bioactive compounds.

1. Introduction

Crotalaria juncea is an important forage plant commonly consumed by grazing animals and belongs to the family Fabaceae (Leguminosae) [1]. It is widely distributed in tropical and subtropical regions, where it commonly grows in agricultural lands, roadsides, grasslands, and other disturbed habitats [2]. Besides its value as a forage species, *Crotalaria juncea* has long been recognized in traditional medicine for its diverse therapeutic properties, including anti-inflammatory, antipyretic, digestive, and respiratory health benefits.

Phytochemical analysis plays a crucial role in identifying the bioactive constituents present in medicinal plants and in understanding their potential therapeutic significance. Among the analytical techniques available, Gas Chromatography–Mass Spectrometry (GC–MS) is considered a powerful and reliable method for the identification and quantification of various phytochemical compounds in plant extracts. The medicinal importance of *Crotalaria juncea* is mainly attributed to the presence of several bioactive compounds such as alkaloids, flavonoids, phenolic compounds, terpenoids, glycosides, and saponins [3]. These phytochemicals are known to exhibit a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [4,5].

Although several studies have investigated the phytochemical composition and pharmacological properties of *Crotalaria juncea* using different extraction methods and analytical approaches, detailed GC–MS profiling of the leaf ethanol extract remains limited [6,7]. Therefore, the present study was undertaken to characterize the phytochemical constituents of the ethanol leaf extract of *Crotalaria juncea* through GC–MS analysis. The study aims to identify and quantify the major bioactive compounds responsible for the medicinal properties of the plant. The findings of this investigation may contribute to a better understanding of the therapeutic potential of *Crotalaria juncea* and support its future application in pharmaceutical, nutraceutical, and herbal formulations.

2. Materials and Methods

Collection of Plant *Crotalaria juncea* Material leaves were collected from Bankhead road, District Amravati, Maharashtra, India. Healthy and mature leaves were carefully handpicked to minimize contamination and damage.

Preparation of Leaf Extract

The collected *Crotalaria juncea* leaves were thoroughly washed with distilled water to remove any adhering dirt or debris. After drying at room temperature, the leaves were finely ground into powder using a mechanical grinder. The powdered plant material was then subjected to ethanol extraction using a Soxhlet extractor. Ethanol was chosen as the solvent due to its ability to extract a wide range of phytochemicals. The extraction process was carried out to ensure efficient extraction of bioactive compounds. The resulting ethanol extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to obtain a concentrated extract.

GC-MS Analysis

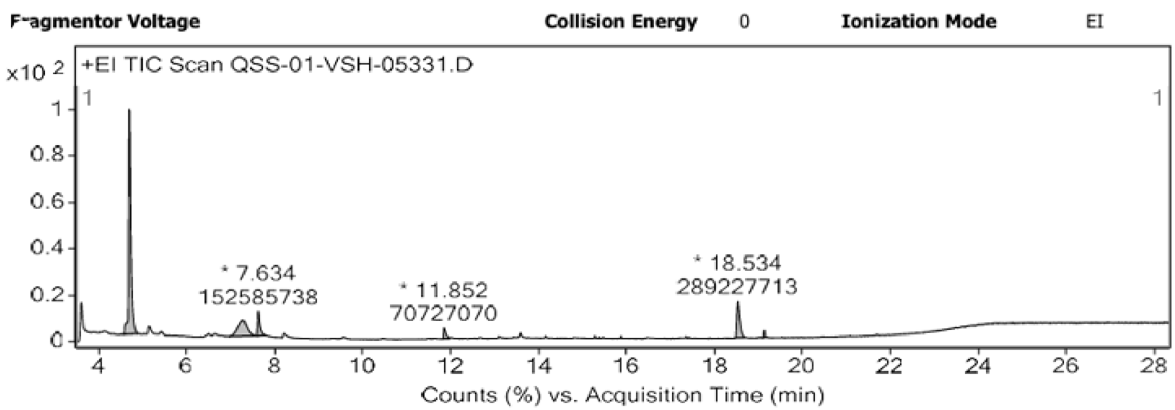
In the present investigation, Gas Chromatography–Mass Spectrometry (GC–MS) analysis was carried out to identify and characterize the phytochemical constituents present in the ethanol leaf extract of *Crotalaria juncea*. Prior to analysis, the crude extract was dissolved in a suitable solvent, commonly methanol, to obtain an appropriate concentration for efficient chromatographic separation. The GC–MS system, equipped with a suitable capillary column and optimized operating conditions such as oven temperature programming, carrier gas flow rate, and detector parameters, was employed to achieve accurate separation and detection of the compounds present in the extract.

The chromatographic conditions were carefully standardized to ensure proper resolution and reproducibility of peaks. Mass spectral analysis was performed using Electron Ionization (EI) mode, which generated characteristic fragmentation patterns for the detected compounds. Identification of the phytochemical constituents was accomplished by comparing the obtained retention times and mass spectra with reference data available in the NIST Mass Spectral Library and published literature. This analytical approach enabled the reliable identification of various bioactive compounds present in the ethanol leaf extract of *Crotalaria juncea* and provided valuable insight into its phytochemical composition and potential therapeutic significance.

3. Results and Discussion

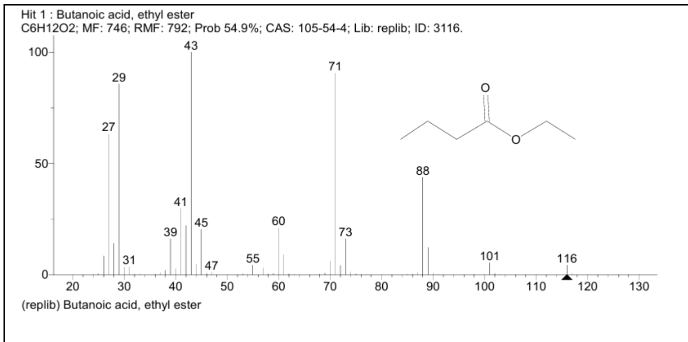
The GC-MS analysis of the ethanolic extract of *Crotalaria juncea* leaf revealed a complex mixture of compounds. A total of nine distinct compounds were identified, each characterized by its retention time (RT), molecular weight, molecular formula, and peak area percentage. The identified compounds and their corresponding data are presented in Table 1 and Fig.1. The most abundant compound in the extract was butonic acid. Ethyl ester, which accounted for 27.42625889%of the total peak area. This was Followed by Hexonic acid, ethyl ester, with a peak area percentage of 26.07170304% and, Dibenz[c,e] oxepin-5,7-dione, 3-ethenyl-1,2,3,8,9,10,11, 11b octahydro-11b-hydroxy-3,8,8 trimethyl which represented 24.14294588% of the extract. Other notable compounds included Ethyl 3-ethoxyacrylate (6.4356%), and Several compounds were present in smaller quantities, such as (-)-Methyl-3,3-dimethylcyclopro, 2,2,4-Trimethyl-1,3-pentanediol (1.1471%).

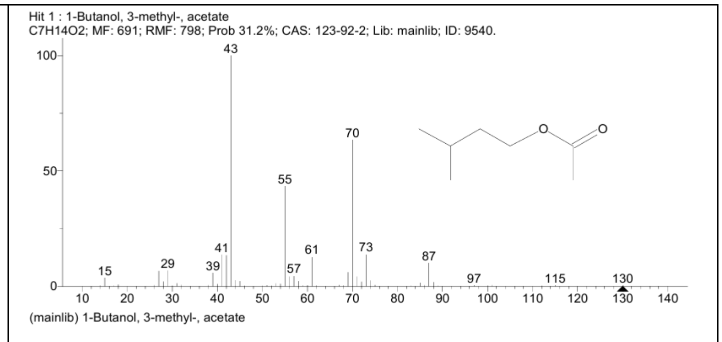
The compounds identified through GC-MS analysis exhibit several potential pharmaceutical and biological applications due to their diverse chemical properties. Silane, diethyl methyl derivatives are commonly associated with antimicrobial activity and are also used in pharmaceutical synthesis and drug delivery systems. Furan, 2,3-dihydro compounds possess antioxidant, antimicrobial, and anti-inflammatory properties, making them useful in medicinal chemistry. Ethyl 3-ethoxyacrylate is an important intermediate in the synthesis of pharmaceutical drugs and biologically active molecules. (-)-Methyl-3,3-dimethylcyclopropane derivatives are known for their role in the preparation of therapeutic agents and bioactive compounds with potential antimicrobial effects. Dibenz[c,e]oxepin-5,7-dione compounds exhibit notable anticancer, anti-inflammatory, and antimicrobial activities and are widely studied for medicinal applications.

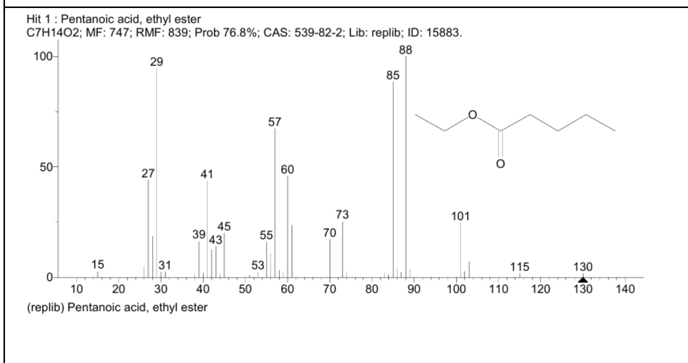


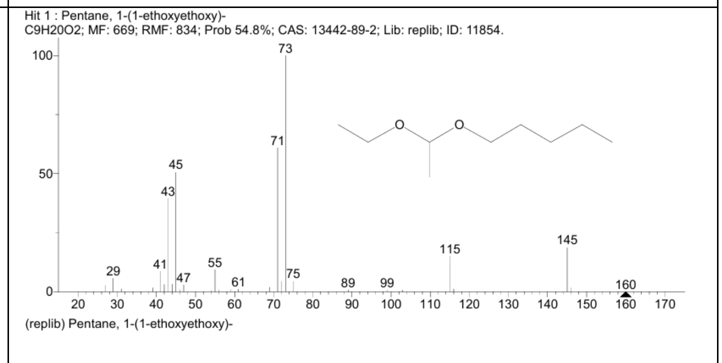
Integration Peak List

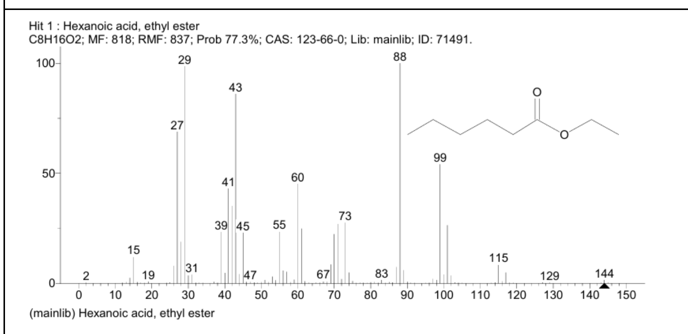
Peak	RT	End	Area	Area %	Name of Compound	MW
1	4.002	4.238	423,300,686	27.4263	Butanoic acid, ethyl ester	116
2	6.001	6.111	36,736,733	2.3802	1-Butanol, 3-methyl-, acetate	130
3	6.861	7.050	78,076,333	5.0587	Pentanoic acid, ethyl ester	130
4	10.680	10.898	120,532,281	7.8095	Pentane, 1-(1-ethoxyethoxy)-	160
5	11.736	11.990	402,394,283	26.0717	Hexanoic acid, ethyl ester	144
6	14.020	14.080	9,110,692	0.5903	Heptanoic acid, ethyl ester	158
7	15.283	15.329	5,661,817	0.3668	2-Hexadecanol	242
8	15.398	15.541	47,591,893	3.0835	Octanoic acid, ethyl ester	172
9	18.526	18.736	388,059,716	25.1429	Dibenz[c,e]oxepin-5,7-dione, 3-ethenyl-1,2,3,8,9,10,11,11b-octahydro-11b-hydroxy-3,8,8-trimethyl-, methyl ether	330
10	19.136	19.254	31,949,444	2.0701	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	286

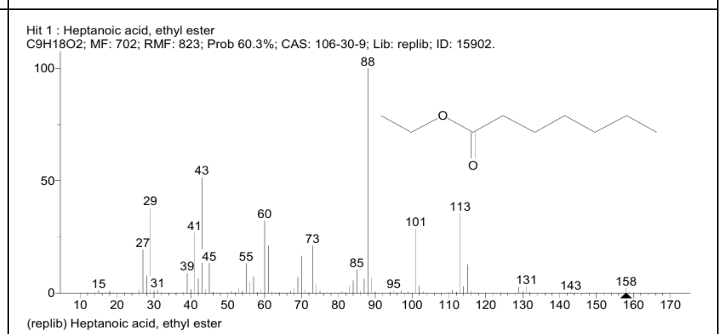

Hit 1 : Butanoic acid, ethyl ester
C6H12O2; MF: 746; RMF: 792; Prob 54.9%; CAS: 105-54-4; Lib: replib; ID: 3116.
(replib) Butanoic acid, ethyl ester

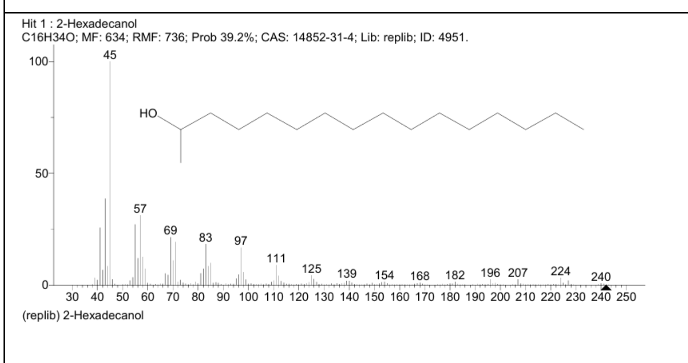

Hit 1 : 1-Butanol, 3-methyl-, acetate
C7H14O2; MF: 691; RMF: 798; Prob 31.2%; CAS: 123-92-2; Lib: mainlib; ID: 9540.
(mainlib) 1-Butanol, 3-methyl-, acetate

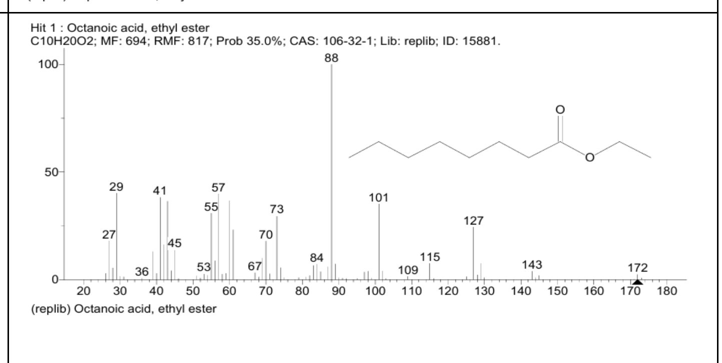

Hit 1 : Pentanoic acid, ethyl ester
C7H14O2; MF: 747; RMF: 839; Prob 76.8%; CAS: 539-82-2; Lib: replib; ID: 15883.
(replib) Pentanoic acid, ethyl ester

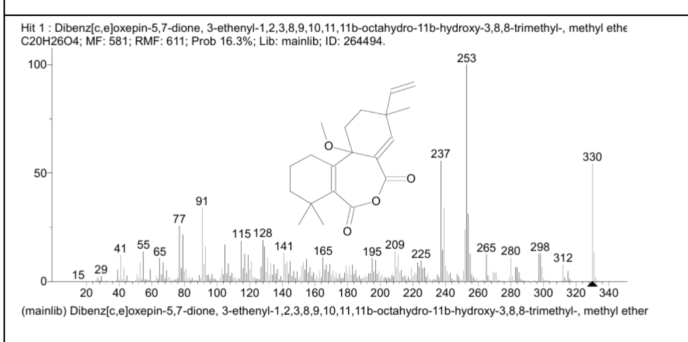

Hit 1 : Pentane, 1-(1-ethoxyethoxy)-
C9H20O2; MF: 669; RMF: 834; Prob 54.8%; CAS: 13442-89-2; Lib: replib; ID: 11854.
(replib) Pentane, 1-(1-ethoxyethoxy)-

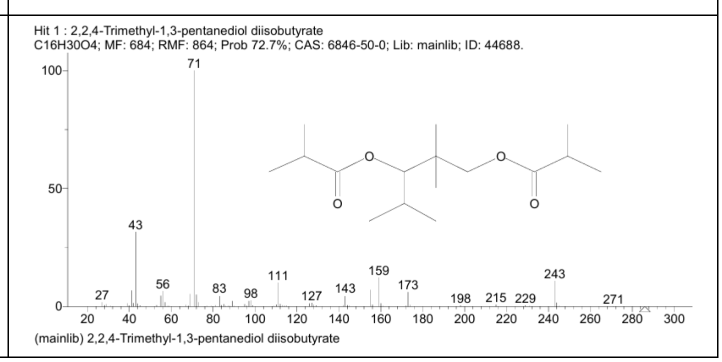

Hit 1 : Hexanoic acid, ethyl ester
C8H16O2; MF: 818; RMF: 837; Prob 77.3%; CAS: 123-66-0; Lib: mainlib; ID: 71491.
(mainlib) Hexanoic acid, ethyl ester


Hit 1 : Heptanoic acid, ethyl ester
C9H18O2; MF: 702; RMF: 823; Prob 60.3%; CAS: 106-30-9; Lib: replib; ID: 15902.
(replib) Heptanoic acid, ethyl ester


Hit 1 : 2-Hexadecanol
C16H34O; MF: 634; RMF: 736; Prob 39.2%; CAS: 14852-31-4; Lib: replib; ID: 4951.
(replib) 2-Hexadecanol


Hit 1 : Octanoic acid, ethyl ester
C10H20O2; MF: 694; RMF: 817; Prob 35.0%; CAS: 106-32-1; Lib: replib; ID: 15881.
(replib) Octanoic acid, ethyl ester


Hit 1 : Dibenz[c,e]oxepin-5,7-dione, 3-ethenyl-1,2,3,8,9,10,11,11b-octahydro-11b-hydroxy-3,8,8-trimethyl-, methyl ethe
C20H26O4; MF: 581; RMF: 611; Prob 16.3%; Lib: mainlib; ID: 264494.
(mainlib) Dibenz[c,e]oxepin-5,7-dione, 3-ethenyl-1,2,3,8,9,10,11,11b-octahydro-11b-hydroxy-3,8,8-trimethyl-, methyl ether


Hit 1 : 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
C16H30O4; MF: 684; RMF: 864; Prob 72.7%; CAS: 6846-50-0; Lib: mainlib; ID: 44688.
(mainlib) 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate

The compound 3-ethenyl-1,2,3,8,9,10,11,11b-octahydro-11b-hydroxy-3,8,8-trimethyl is structurally related to terpenoid compounds, which are recognized for antioxidant, anti-inflammatory, and anticancer properties. Similarly, 2,2,4-Trimethyl-1,3-pentanediol derivatives are utilized in pharmaceutical formulations, drug stabilization, and antimicrobial preparations. Overall, these phytochemical compounds indicate significant pharmaceutical potential and may contribute to the development of novel therapeutic agents.

Conclusion

The GC-MS analysis identified several phytochemicals with promising pharmaceutical potential. These compounds exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. They are also used in drug synthesis, drug delivery, pharmaceutical formulations, and stabilization. Overall, the identified compounds may serve as valuable candidates for the development of novel therapeutic agents.

Conflicts of interest: The authors stated that no conflicts of interest.

Correspondence and requests for materials should be addressed to **Ambhore AT**

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