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GC-MS Studies in Ethyl acetate extract of Rare, Endemic Medicinal plant *Crotalaria ramosissima* - A Comparative Study

Gokila T[1*], Arul Sheeba Rani. M[2], Sincy Joseph[3], Anitha T[4] and Kiruthika S[5]
1, 2 and 5 - Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu, India.
3 and 4 - Department of Botany, Auxilium College (Autonomous), Vellore, Tamil Nadu, India.
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Abstract

Gas chromatography mass spectrometry (GC-MS) analysis was carried out to investigate the phytochemical composition of the stem and root of *Crotalaria ramosissima*. The study revealed the presence of a diverse range of bioactive compounds, with 28 compounds identified in the stem ethyl acetate extract and 30 compounds detected in the root ethyl acetate extract. These compounds belong to various chemical classes such as alkaloids, flavonoids, terpenoids, fatty acids, and phenolic derivatives, which are known for their significant pharmacological activities. Many of the identified constituents exhibit potent antimicrobial, antioxidant and anti-inflammatory properties, suggesting their potential role in therapeutic applications. The variation in compound distribution between stem and root indicates differential biosynthesis and accumulation of metabolites in plant parts. The presence of these bioactive molecules supports the traditional medicinal use of *Crotalaria ramosissima* and highlights its importance as a promising source of natural drugs. This study provides a scientific basis for further investigation into the isolation, characterization, and biological evaluation of these compounds. Overall, the GC-MS profiling emphasizes the plant's potential in pharmaceutical and nutraceutical development.

INTRODUCTION

Medicinal plants have long served as a cornerstone in the development of traditional and modern healthcare systems. Across diverse cultures, plant-derived compounds have been utilized for their therapeutic properties, contributing significantly to the treatment and prevention of various diseases. The increasing interest in natural products has led to extensive research focused on identifying bioactive compounds from plants and understanding their pharmacological potential. Among the various analytical techniques available, gas chromatography–mass spectrometry (GC–MS) has emerged as a powerful and reliable tool for the qualitative and quantitative analysis of phytochemicals present in plant extracts, (Alan *et al*., 2022).

GC–MS combines the separation capabilities of gas chromatography with the detection and identification features of mass spectrometry, making it highly effective in profiling complex mixtures of volatile and semi-volatile compounds. This technique enables researchers to identify a wide range of chemical constituents, including alkaloids, flavonoids, terpenoids, steroids, fatty acids, and phenolic compounds, (Paulpriya *et al*., 2014). These phytochemicals are often responsible for the biological activities exhibited by medicinal plants, such as antimicrobial, antioxidant, anti-inflammatory, anticancer, and antidiabetic effects. Therefore, GC–MS analysis plays a crucial role in phytochemical research by providing detailed insights into the chemical composition of plant materials.

The genus *Crotalaria*, belonging to the family Fabaceae, comprises many species widely distributed in tropical and subtropical regions. Many species within this genus are known for their medicinal, ecological, and agricultural importance. Plants of the genus *Crotalaria* have been traditionally used for treating a variety of ailments, including infections, inflammation, skin diseases and digestive disorders. Additionally, some species are utilized as green manure, soil stabilizers, and in pest control due to their bioactive constituents, (Felix *et al*., 2023).

Crotalaria ramosissima is a lesser-explored species within this genus that has gained attention for its potential medicinal properties. Despite its traditional use in folk medicine, there is limited scientific information available regarding its phytochemical composition and pharmacological activities. This highlights the need for comprehensive studies to explore its chemical profile and validate its therapeutic potential. Investigating different parts of the plant, such as the stem and root, is particularly important because the distribution and concentration of bioactive compounds can vary significantly within the plant, (Lanchana *et al.,* 2025).

The application of GC–MS in phytochemical analysis provides a comprehensive understanding of the chemical constituents present in plant extracts. By comparing the chromatographic profiles of stem and root extracts, researchers can identify variations in compound composition and abundance. This information can be used to determine which part of the plant has greater medicinal potential and to guide further studies on bioactivity and compound isolation. Additionally, GC–MS data can serve as a reference for quality control and standardization of plant-based products. Previous studies on related species of *Crotalaria* have reported the presence of various bioactive compounds with significant pharmacological activities. However, detailed GC–MS analysis of *Crotalaria ramosissima*, particularly focusing on its stem and root, remains limited. This gap in knowledge underscores the importance of conducting systematic research to explore its phytochemical profile and evaluate its potential applications, (Lanchana *et al.,* 2024).

The present study aims to perform GC–MS analysis of the stem and root extracts of *Crotalaria ramosissima* to identify and characterize the bioactive compounds present. By analyzing the chemical composition of both plant parts, the study seeks to highlight the diversity of phytochemicals and their associated biological activities. The identification of compounds with antimicrobial, antioxidant, and anti-inflammatory properties will provide a scientific basis for the traditional use of this plant and support its potential use in pharmaceutical and nutraceutical applications.

MATERIALS AND METHODS

Extraction of Sample:

The leaves of selected plant material were shade dried under the room temperature and powdered using a mixer and stored in an air-tight container. The stem and root powder was extracted by using Ethyl acetate by using a hot extraction method. A total of 30 g of each powder was extracted with 300 ml solvents in a Soxhlet apparatus. The residue was removed by filtration through What Mann No. 1 filter paper. These extracts were subjected to GC MS analysis to find the compound present, (Prameela *et al*., 2015).

Instrument Method:

The PERKIN ELMER CLARUS SQ8C, were engaged for analysis. The instrument was set as follows, Injector port temperature set to 280 o C, Interface temperature set as 290 o C, source kept at 200 o C. The oven temperature is programmed as available, 70 ^{o}C for 2 mins, 130 ^{o} C at 4 ^{o} C/min, up to 300 ^{o} C at 5 ^{o} C/min. Split ratio is set as 1:50 and the injector used was spitless mode. The DB- 35 MS Nonpolar column was used whose dimensions were 0.25 mm OD x0.25 µm ID x 30 meters length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200 o C and 40 motor vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

Identification of compounds:

Interpretation of mass spectrum of GC -MS was done using the data base of National Institute Standard and Technology (NIST4) and WILEY9. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt Library.

RESULTS AND DISCUSSIONS

The present study employed gas chromatography–mass spectrometry (GC–MS) analysis to investigate the phytochemical composition of the stem and root ethyl acetate extracts of *Crotalaria ramosissima*. The analysis revealed a rich diversity of bioactive compounds distributed across both plant parts, highlighting the plant's potential as a valuable source of pharmacologically active molecules. A total of 28 compounds were identified in the stem ethyl acetate extract, while the root ethyl acetate extract exhibited a slightly higher diversity with 30 compounds. The variation in the number and nature of compounds between the stem and root indicates differential accumulation of secondary metabolites, which may be attributed to the distinct physiological roles and metabolic activities of these plant parts.

GC–MS Profiling of Stem Extract

The GC–MS chromatogram of the stem ethyl acetate extract demonstrated the presence of 28 distinct peaks corresponding to various phytochemical constituents. These compounds belong to multiple chemical classes, including fatty acids, esters, alcohols, hydrocarbons, phenolic compounds and terpenoids. The presence of such a wide range of chemical groups suggests that the stem is metabolically active and contributes significantly to the plant's defense and adaptation mechanisms.

Among the identified compounds, fatty acids and their derivatives constitute a major proportion. These compounds are widely known for their antimicrobial and anti-inflammatory properties. Fatty acids can disrupt microbial cell membranes, thereby inhibiting the growth of pathogenic organisms. In addition, certain long-chain fatty acids are known to modulate inflammatory responses by influencing the production of cytokines and other signaling molecules, (Malathi and Ravindran, 2015).

Phenolic compounds detected in the stem extract are of particular importance due to their strong antioxidant activity. These compounds act as free radical scavengers, thereby protecting cells from oxidative damage. Oxidative stress is associated with various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. The presence of phenolic constituents in the stem extract suggests its potential use in preventing or managing oxidative stress-related disorders, (Hala *et al.,* 2024). Terpenoids identified in the stem extract also contribute significantly to its biological activities. Terpenoids are known for their antimicrobial, antiviral, and anti-inflammatory effects. They play a crucial role in plant defense by deterring herbivores and inhibiting the growth of pathogens. In addition, some terpenoids have been reported to possess anticancer properties by inducing apoptosis and inhibiting tumor growth. Alcohol and esters present in the stem extract further enhance its pharmacological profile. These compounds are often associated with antimicrobial and antioxidant activities. Esters are known to exhibit anti-inflammatory effects and may contribute to the therapeutic potential of the plant, (Vennila *et al*., 2023).

The diversity of compounds detected in the stem extract indicates that this plant part is a rich reservoir of bioactive molecules with multiple therapeutic properties. The combination of antimicrobial, antioxidant and anti-inflammatory compounds suggests a synergistic effect, which may enhance the overall efficacy of the ethyl acetate extract, (Figure- 1 and Table-1).

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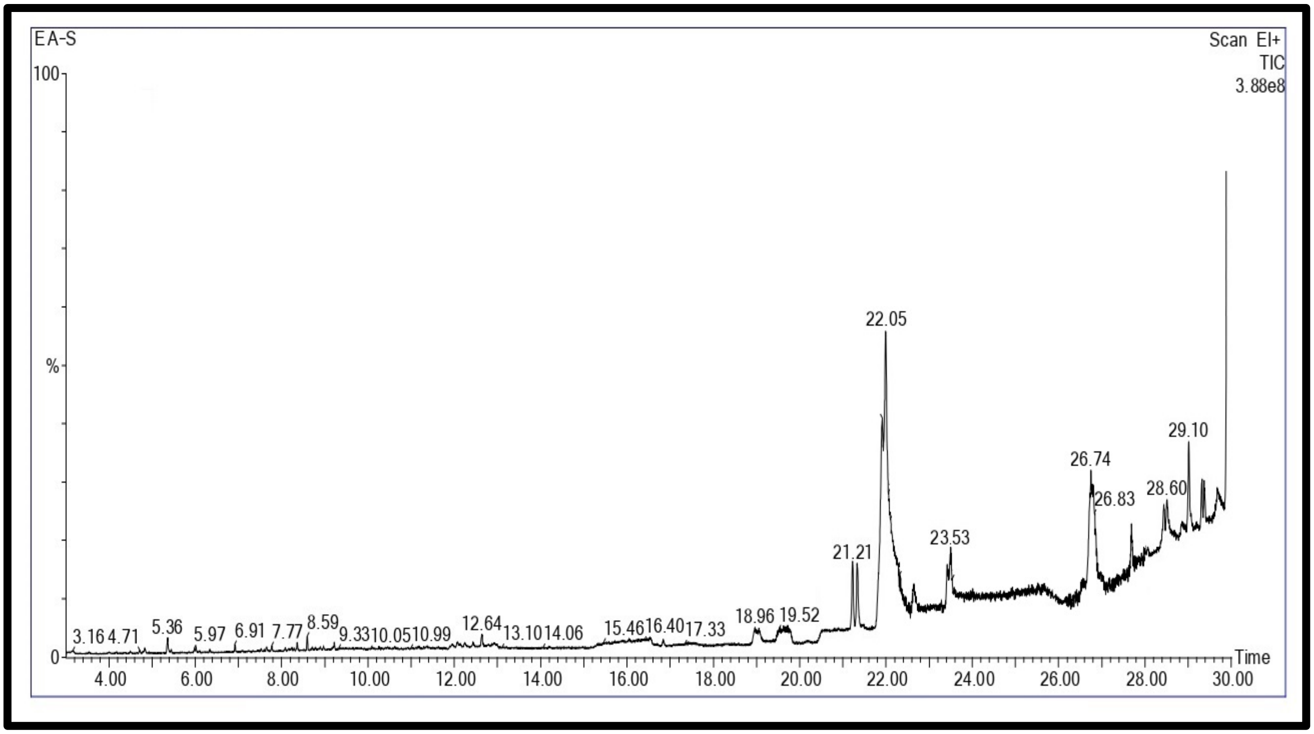
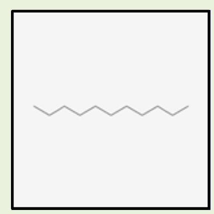
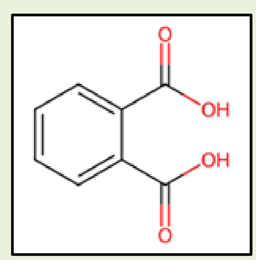
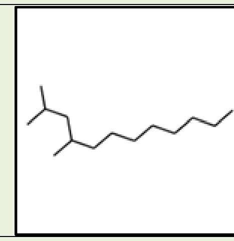
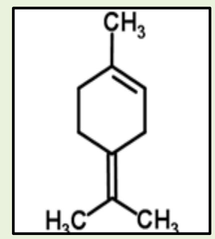
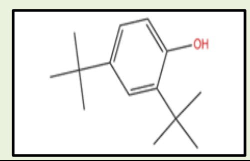
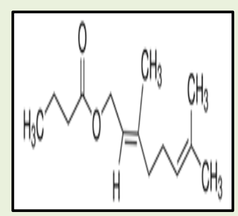
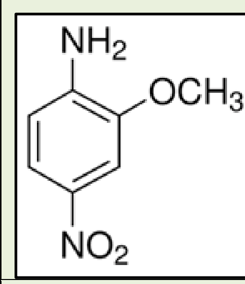
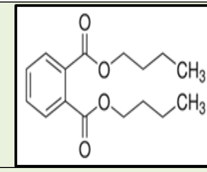
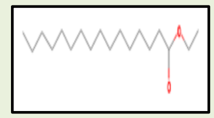
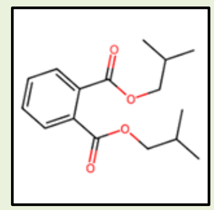
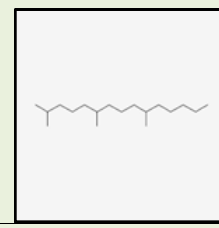
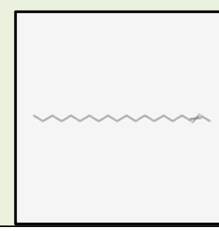


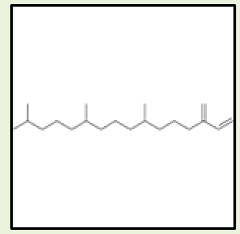
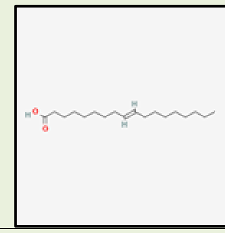
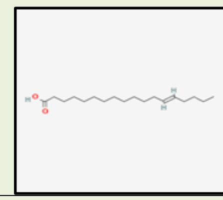
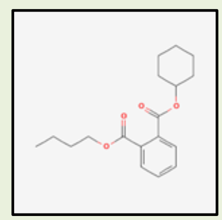
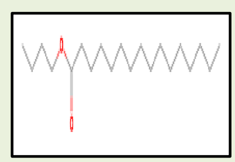
Fig.1 GC-MS TIC Chromatogram

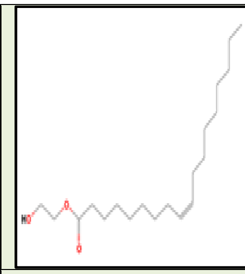
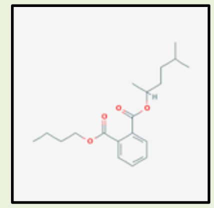
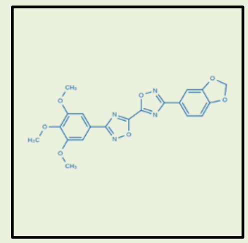
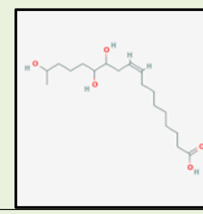
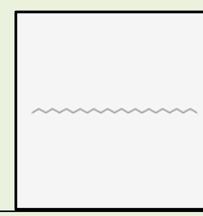
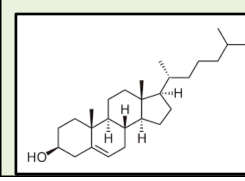
Table: 1 GC MS analysis of *Crotalaria ramosissima* - Stem ethyl acetate extract

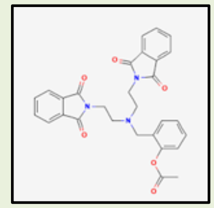
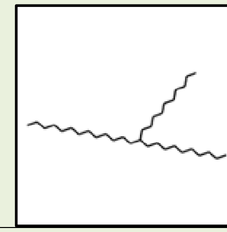
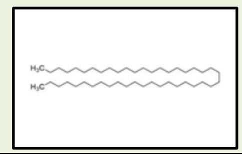
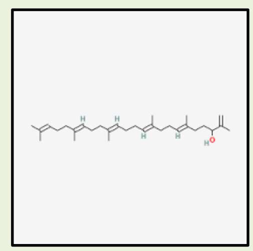
S. no	List of the Medicinal Compound	RT	MOLICULAR FORMULA	Group of Classified	Therapeutics value	STRUCTURE OF THE COMPOUND
1	Undecane	5.36	C11H24	Alkane hydrocarbon	Anti-allergic, Antifungal, Antibacterial and anti-inflammatory	
2	1,2-Benzenedicarboxylic acid	5.97	C8H6O	Aromatic dicarboxylic acid	Anticancer, antifungal, and antimicrobial activities in various mono- and diesters, including diisooctyl phthalate and diisobutyl phthalate, against plant pathogens	

					and cancer cell lines	
3	2,4-Dimethyldodecane	6.91	C14H38	Alkanes	Flavor or aroma compound	
4	Terpinolene	7.77	C10H16	Monoterpene	Antioxidant, anti-inflammatory, anticancer, antimicrobial (including antifungal and antiviral), and insecticidal	
5	Phenol, 2,4-bis(1,1-dimethylethyl)-	8.59	C14H22O	Phenol	Antioxidant, antibacterial and anti-inflammatory	
6	Geranyl butyrate	9.33	C14H24O2	Saturated fatty acid	Anticancer, used as a fragrance ingredient in perfumes, colognes, and body care products	
7	2-Methoxy-4-nitroacridone	10.05	C14H10N2O4	Ketone	Anticancer	
8	Dibutyl phthalate	12.64	C16H22O4	Phthalate ester	Antimicrobial and antimalarial	

						
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.06	C20H40O	Diterpenoid	Antimicrobial, anti-inflammatory, antioxidant, and antinociceptive	
10	Hexadecanoic acid, ethyl ester	15.46	C18H36O2	Long-chain fatty acid	Anti-inflammatory, anticancer, and antifungal	
11	1,2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester	16.40	C16H22O4	Carboxylic acid	Anticancer and Anti-proliferative	
12	2,6,10-Trimethyl,14-ethylene-14-pentadecne	17.33	C20H38	Isoprenoid	Antimicrobial, Antioxidant and Anticancer	
13	Eicosene	18.96	C20H40	Long-chain alkene	Antimicrobial, Anticancer, anti-inflammatory and antifungal	

14	Neophytadiene	19.02	C20H38	Diterpene	Anxiolytic, anticonvulsant, antidepressant, Anti-inflammatory, antioxidant, Neuroprotective, Antimicrobial, Antimalarial and sedative	
15	Elaidic acid	19.11	C18H34O2	Unsaturated fatty acid	Antitumor, anti-inflammation and antioxidant	
16	trans-13-Octadecenoic acid	19.52	C18H34O2	Long-chain fatty acid	Anti-inflammatory, antioxidant, and antibacterial	
17	1-Butyl 2-cyclohexyl phthalate	21.21	C18H24O4	Phthalate ester	Inhibit cell proliferation, cause DNA damage, and induce chromosomal abnormalities	
18	Hexadecenoic acid, butyl ester	21.25	C20H40O	Fatty acid ester	Antimicrobial and antioxidant, act as a neurotransmitter in the central nervous system.	
19	9-Octadecenoic acid (z)-, 2-hydroxyethyl ester	22.05	C20H38O3	Fatty Acid Methyl Ester	Anti-inflammatory, Antimicrobial,Antineoplastic, Antiurolithic, Antiobesity, Antiprotozoal,	

					Antifibrinolytic, antialcoholic, Antipsoriatic, Antioxidant.	
20	Phthalic acid, 5-methylhex-2-yl butyl ester	22.61	C19H28O4	Phthalate ester	Aminoglycoside antibiotic, Neuroprotective3	
21	1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl]-	23.53	C18H16N2O4	Heterocyclic aromatic azole	Anti-inflammatory antimicrobial, anti cancer, and antiviral activities, as well as potential for antihypertensive and analgesic effects	
22	5,8,12-trihydroxy-9-octadecenoic acid	24.56	C18H34O5	Long-chain fatty acid	Anticancer, Anti-inflammatory, Antiviral and antimicrobial	
23	Pentacosane	26.74	C25H52	Alkane-saturated hydrocarbons	anti-cancer	
24	Cholesterol	26.83	C27H46O	Sterol	Antimicrobial and vitamin D synthesis	

25	Phenmethylamine, 2-acetoxy-n,n-bis[2-(benzoimid- 2-yl)ethyl]-	28.60	C29H25N3O6	Benzimidazolyl	Anti-termitic	
26	Tetracosane, 11-decyl-	29.10	C34H70	Alkane	Antioxidant and anticancer	
27	n-Tetratetracontane	29.35	C44H90	Long-chain alkane	Antimicrobial, antioxidant, and larvicidal properties.	
28	22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl	29.39	C50H76N2O6	Triterpene	Anticancer, Antimicrobial, Antioxidant Chemopreventive, Pesticide Anti- tumor and Sunscreen	

GC–MS Profiling of Root Ethyl Acetate Extract

The GC–MS analysis of the ethyl acetate root extract revealed the presence of 30 compounds, indicating a slightly higher level of chemical diversity compared to the stem. Like the stem extract, the root extract contained compounds belonging to various chemical classes, including fatty acids, alkaloids, phenolics, terpenoids and hydrocarbons. The presence of alkaloids in the root extract is particularly noteworthy, as these compounds are known for their potent pharmacological activities. Alkaloids often exhibit antimicrobial, analgesic and anticancer properties. They can interfere with the metabolic processes of microorganisms, thereby inhibiting their growth and proliferation. The detection of alkaloids in the root extract suggests its potential use in the treatment of infectious diseases and other health conditions.

Fatty acids and their derivatives were also prominently present in the root extract. These compounds contribute to the antimicrobial and anti-inflammatory properties of the extract. In addition, certain fatty acids have been reported to possess antioxidant activity, further enhancing the therapeutic potential of the root. Phenolic compounds identified in the root extract play a crucial role in neutralizing free radicals and reducing oxidative stress. The antioxidant activity of these compounds is essential for protecting cells from damage and maintaining overall health. The presence of phenolics in both stem and root extracts indicates that the plant possesses strong antioxidant potential. Terpenoids detected in the root extract further contribute to its biological activities. These compounds are known for their ability to modulate inflammatory responses and inhibit the growth of microorganisms. Some terpenoids also exhibit anticancer properties, making them valuable candidates for drug development, (Renuka, 2013).

The root extract also contained various hydrocarbons and other minor compounds that may contribute to its overall bioactivity. Although these compounds may not have significant pharmacological effects individually, they may act synergistically with other bioactive molecules to enhance the therapeutic potential of the extract, (Figure- 2 and Table-2).

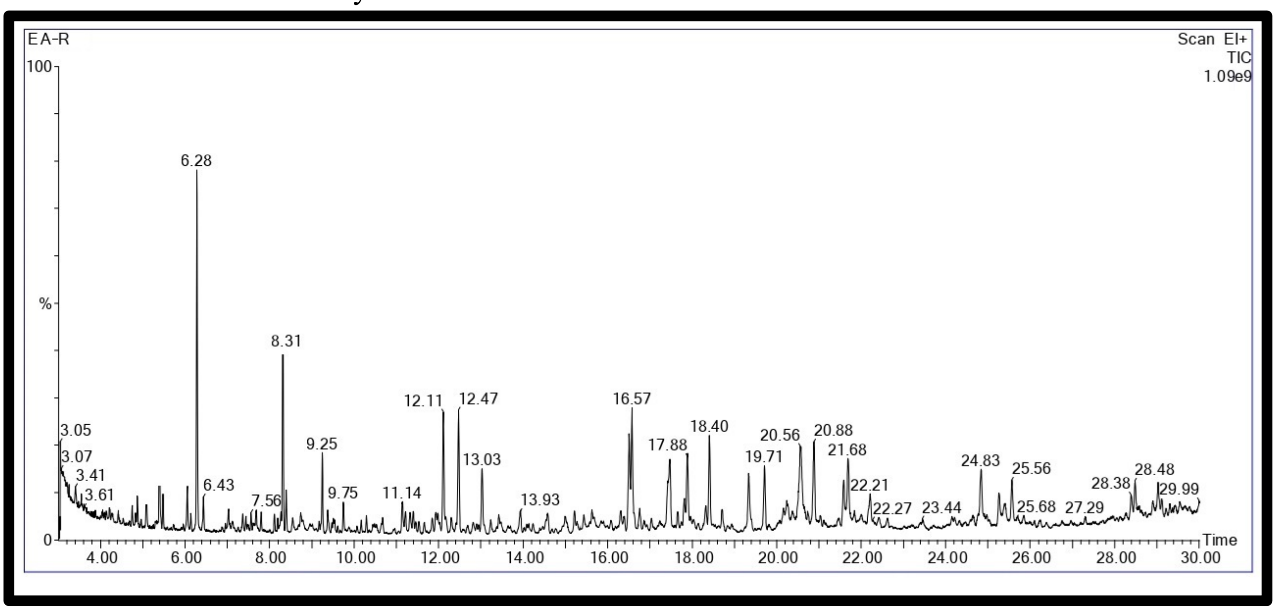
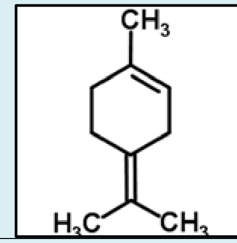
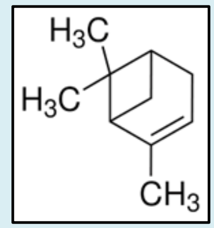
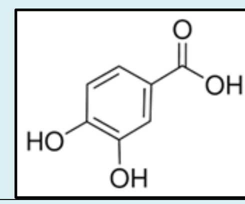
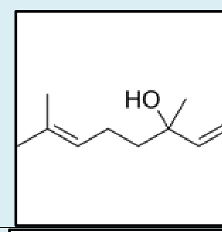
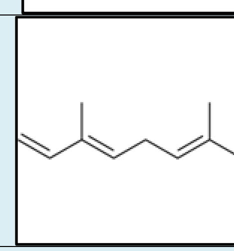
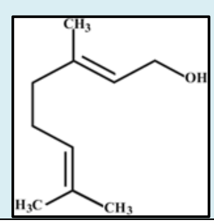
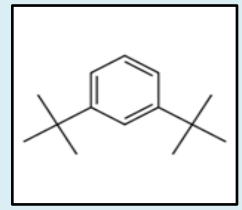
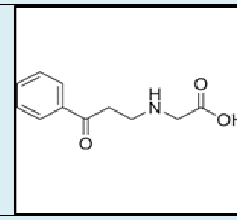
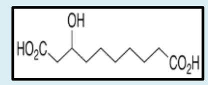
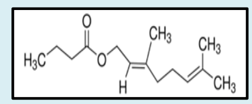
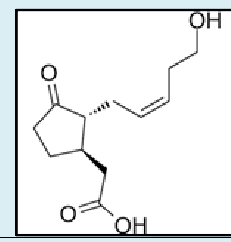
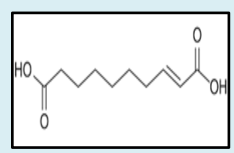
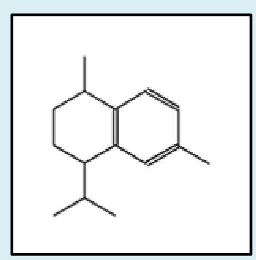


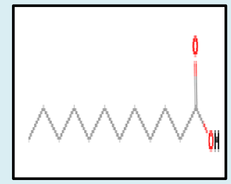
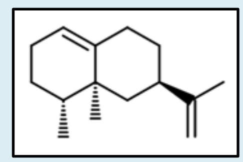
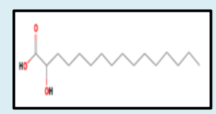
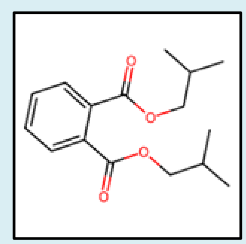
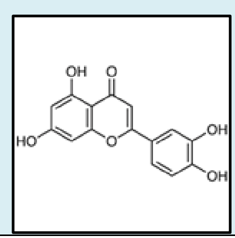
Fig.2 GC-MS TIC Chromatogram

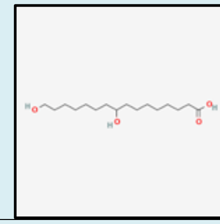
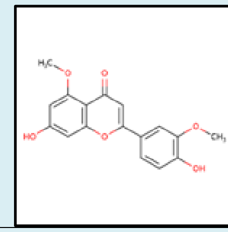
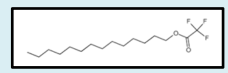
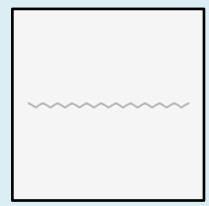
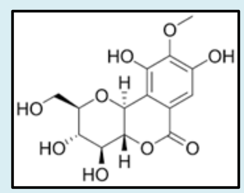
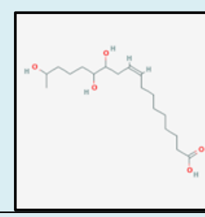
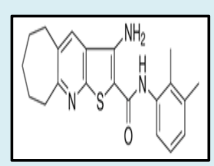
Table: 2 GC MS analysis of *Crotalaria ramosissima* - Root Ethyl Acetate extract

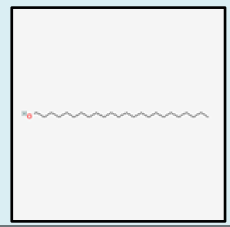
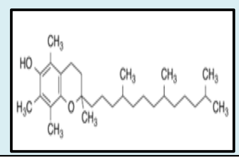
S. No	List of the Medicinal Compound	RT	Molicular Formula	Group of classified	Therapeutics value	Structure of the compound

1	Terpinolene	3.41	C10H16	Monoterpene	Antioxidant, anti-inflammatory, anticancer, antimicrobial (including antifungal and antiviral), and insecticidal	
2	α-Pinene	4.92	C10H16	Terpenoids	Treatment of bladder, kidney, and urinary stones, Antimicrobial, Anticoagulative/antiplatelet, anti-inflammatory, Anti-tumor, Antioxidant, Gastroprotective activity, and Neuroprotective activity	
3	3,4-Dihydroxybenzoic acid	5.41	C7H6O4	Phenolic acid	Antioxidant, anti-inflammatory, anticancer, and antimicrobial	
4	Linalool	5.45	C10H18O	Terpene alcohol	Anti-inflammatory, antioxidant, anticancer, antimicrobial, and neuroprotective	
5	β-Ocimene	6.05	C10H16	Monoterpene	Antifungal, antitumor, and anticonvulsant effects	
6	Geraniol	6.17	C10H18O	Terpenoid alcohol	Antimicrobial (antibacterial and antifungal), anti-inflammatory, antitumor and antioxidant	

7	Benzene, 1,3-bis(1,1-dimethylethyl)-	6.28	C14H22	Monocyclic benzene	Agonistic activity	
8	Phenylpropionylglycine	6..43	C11H13NO3	Acyl glycine	Anti-adipogenic anti- and inflammatory	
9	3-hydroxy-sebacic acid	7.02	C10H18O5	Dicarboxylic acid	Anti-inflammatory and anti-cancer	
10	Geranyl butyrate	7.56	C14H24O2	Saturated fatty acid	Anticancer, used as a fragrance ingredient in perfumes, colognes, and body care products	
11	12-hydroxyjasmonic acid	8.11	C12H18O4	Carboxylic acid	Antioxidant	
12	Decenedioic acid	8.31	C10H16O4	Medium-chain fatty acid	Antimicrobial, anti-anaphylactic, and metabolic effects	
13	Calamenene	8.42	C15H22	Sesquiterpenoid	Antimicrobial (against bacteria and fungi like MRSA and Candida albicans), antioxidant, and antiproliferative (against ovarian cancer cells), anti-inflammatory, analgesic, and insecticidal	

14	Dodecanoic acid	9.25	C12H24O2	Medium-chain fatty acid	Anti-apoptotic antimicrobial, antifungal, antiviral and antioxidant	
15	n-Hexadecanoic acid	9.75	C16H32O2	Unsaturated fatty acid	Antioxidant, nematicide, pesticide, antiandrogenic	
16	Valencene	10.68	C15H24	Sesquiterpene	Anti-inflammatory, antioxidant, antiallergic, antitumor (anticancer), and antimicrobial	
17	2-Hydroxyhexadecanoic acid	11.14	C16H32O3	Saturated long-chain hydroxyl fatty acid	Anti-inflammatory and anti-cancer	
18	1,2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester	11.95	C16H22O4	Carboxylic acid	Anticancer and Anti-proliferative	
19	Heneicosane	12.11	C21H44	Fatty acid	Antimicrobial and Antioxidant	
20	5,7,3’,4’-Tetrahydroxyflavone	12.25	C15H10O6	Flavonoid	Antioxidant, anti-inflammatory, and potential anti-cancer	

21	9,16-dihydroxy-palmitic acid	12.47	C16H32O4	Long-chain fatty acid	Anti-inflammatory and anticancer	
22	Luteolin 5,3'-dimethyl ether	12.63	C17H14O6	Flavonoid	Antioxidant and anti-inflammatory	
23	4-Trifluoroacetoxytetradecane	13.03	C16H29F3O2	Alkane hydrocarbon	Anti-diabetic, diuretic, anti-obesity, anti-cancer, antimicrobial, anti-inflammatory	
24	Tricosane	13.93	C23H48	Straight-chain alkane	Antimicrobial and anti-inflammatory	
25	Bergenin	16.57	C14H16O9	Polyphenol	Anti-infective, anti-cancer, anti-diabetic, neuroprotective, hepatoprotective, anti-urolithiatic, anti-hyperuricemic, and anti-bradykinin	
26	5,8,12-trihydroxy-9-octadecenoic acid	19.71	C18H34O5	Long-chain fatty acid	Anticancer, Anti-inflammatory, Antiviral and antimicrobial	
27	3-Amino-n-(2,3-dimethylphenyl)-6,7-dihydro-5h-cyclopenta[b]thieno[3,2-	22.27	C19H19N3OS	Thienopyridine	Antimicrobial and potential anticancer	

	e]pyridine-2-carboxamide					
28	Pentacosane	24.83	C25H52	Unbranched -chain alkane	Anti-cancer	
29	n-Tetracosanol -1	25.68	C24H50O	Long-chain primary fatty alcohol	Antioxidant, antibacterial, antifungal, anticancer, antimutagenic, nematocidal, and wound healing properties	
30	alpha.-tocopherol	28.38	C29H50O2	Nutrient compound/ Vitamin E	Antioxidant, anti-inflammatory and anticancer	

Comparative Analysis of Stem and Root Extracts

A comparative analysis of the GC–MS profiles of stem and root extracts reveals both similarities and differences in their phytochemical composition. While both plant parts contain a wide range of bioactive compounds, the root extract exhibited a slightly higher number of compounds (30) compared to the stem (28). This suggests that the root may have a greater capacity for the synthesis and accumulation of secondary metabolites. The presence of common compounds in both stem and root extracts indicates that certain metabolic pathways are active throughout the plant. These compounds likely play a fundamental role in the plant's defense mechanisms and overall survival. However, the variation in the types and concentrations of compounds between the two plant parts suggests that each part has specialized functions and contributes differently to the plant's physiology, (Tshepiso *et al*., 2017).

The higher diversity of compounds in the root extract may be attributed to its interaction with the soil environment. Roots are exposed to various microorganisms and environmental stresses, which may stimulate the production of secondary metabolites as a defense mechanism. These compounds may help protect the plant from soil-borne pathogens and enhance its adaptability to environmental conditions. In contrast, the stem is primarily involved in structural support and the transport of nutrients and water. Although it also produces bioactive compounds, the diversity may be slightly lower compared to the root. However, the compounds present in the stem still contribute significantly to the plant's overall pharmacological potential.

Biological Significance of Identified Compounds

The compounds identified in both stem and root extracts of *Crotalaria ramosissima* exhibit a wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. These activities are of great importance in the prevention and treatment of various diseases. The antimicrobial activity of the identified compounds suggests that the plant extracts may be effective against a variety of pathogenic microorganisms. This is particularly important in the context of increasing antibiotic resistance, which has become a major global health concern. Plant-derived antimicrobial agents offer a promising alternative to conventional antibiotics. The antioxidant activity of the compounds plays a crucial role in protecting cells from oxidative damage. Free radicals generated during normal metabolic processes can cause cellular damage and contribute to the development of chronic diseases. Antioxidants neutralize these free radicals and help maintain cellular integrity. The anti-inflammatory properties of the identified compounds further enhance the therapeutic potential of the plant. Chronic inflammation is associated with various health conditions, including arthritis, cardiovascular diseases, and diabetes. Compounds that can modulate inflammatory responses may help in the management of these conditions, (Nyoman *et al*., 2022).

CONCLUSION

The findings of this study highlight the potential of *Crotalaria ramosissima* as a source of bioactive compounds for pharmaceutical and nutraceutical applications. The identification of compounds with multiple biological activities suggests that the plant may be used in the development of new drugs and therapeutic agents. Further research is needed to isolate and characterize individual compounds from the extracts and evaluate their biological activities through in vitro and in vivo studies. Such studies will provide a deeper understanding of the mechanisms of action of these compounds and their potential applications in medicine. In addition, the synergistic effects of the compounds present in the extracts should be investigated. The combined action of multiple compounds may enhance the overall efficacy of the extract and provide a more effective treatment option. The comparative analysis of stem and root extracts indicates that both plant parts contribute to the overall therapeutic potential of the plant, with the root showing slightly higher diversity. These findings provide a scientific basis for further research and development of plant-based drugs derived from *Crotalaria ramosissima*.

In conclusion, the GC-MS analysis of the stem and root extracts of *Crotalaria* *ramosissima* revealed a diverse array of bioactive compounds with significant pharmacological potential. The presence of 28 compounds in the stem and 30 compounds in the root highlights the rich phytochemical diversity of the plant. The identified compounds exhibit antimicrobial, antioxidant, and anti-inflammatory properties, supporting the traditional use of the plant in medicine. The investigation of *Crotalaria ramosissima* through GC–MS analysis represents an important step toward understanding its phytochemical composition and therapeutic potential. The comparative analysis of stem and root extracts provides valuable insights into the distribution of bioactive compounds within the plant. With the growing interest in natural products and their applications in healthcare, studies like this play a vital role in identifying new sources of medicinal compounds and promoting the sustainable use of plant resources.

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