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Research Article

Analysis of Phytochemical Constituents, Antibacterial, Antioxidant and GC-MS Profiling of *Crotalaria ramosissima* Leaf Extracts

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ABSTRACT

Crotalaria ramosissima Roxb. (Fabales**:** Fabaceae) is a common weed that grows prolifically in a few areas of Karnataka. The plant is used as an insect repellent in a grain storage room and *C. ramosissima* leaves are used to treat skin diseases. The purpose of the study was to investigate phytochemical constituents and evaluate their antibacterial and antioxidant properties along with bioactive compound profiling. Phytochemical screening of ethyl acetate, ethanol and methanol extracts revealed the presence of necessary phytochemical components, antimicrobial activity against plant pathogens showed best results from ethyl acetate extract with MIC 15.60 µg mL^{-1} against *Pseudomonas syringae* and *Xanthomonas oryzae* with MIC 31.25 µg mL^{-1}, confirmed with TLC bio-autography, DPPH antioxidant assay, showed the highest activity of IC_{50} 2.71 µg mL^{-1} from methanol extract with standard reference, Gas chromatography/Mass spectroscopy (GC-MS) used for profiling to detect chemical compounds from plant solvent extracts which showed the presence of 21 compounds, ethyl acetate extract identified with 1,2,4-Oxidiazole, 3-(1,3-bezodioxol-5-y-5-[2-(4-methoxyphenyl)-ethyl] which is heterocyclic aromatic compound of azole family-alkaloid, which is reported for the first time in *C. ramosissima*. The results revealed significant properties and the obtained 1,2,4-oxidiazole derivative can be a novel bio-control agent against microorganisms and for crop protection. It also retained current researcher's attention from its biological properties in pharmaceutical drug industry.

INTRODUCTION

Plants are the natural sources that are engraved with diverse structural compounds that researchers investigate to reveal potential bioactive properties leading to new drug discovery. These phytochemical studies of plants from the last few decades declared plant chemistry or phytochemistry, have become one of the disciplines and are advancing through new techniques that are leading the world through many of their applications.[1] Phytochemicals can be classified in a number of ways based on their biosynthetic origin, solubility properties, and the presence of functional groups.[2] Secondary metabolites of plants have indispensable biological functions in which they are not only used for themselves but have great importance in human health treatments. Among alkaloids vinblastine and vincristine are well-known examples with anticancerous properties,[3] artemisinin, etoposide and taxol are also sources of novel drug entities from plants.[4] The remedial properties of plants are from secondary metabolites, some of which are good bioactive compounds that are capable of against several ailments and diseases.[3] Currently, many drugs that have been discovered for several diseases are from plant origin, but there is still a lot of scope for bio-prospecting novel plant-origin drugs for several other diseases and ailments in pharmaceutical industries, agricultural and food industries, cosmetics, natural products, etc., depending on plants for getting raw materials. Drug discovery included two main strategies: Random screening and ethno-medicinal knowledge.

Crotalaria is used as traditional folk medicine, among 702 species all over the world, only 93 species of *Crotalaria*

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genus are distributed in India.[5] Primarily plants are used for hemp, fiber, green manure, animal feed (forage), and as ethno medicine for skin diseases.[6,7] The genus *Crotalaria* has great diversity in habit, ecological preferences, a combination of distinctive morphological characteristics and also possesses pyrrolizidine alkaloids.[8-10] Pyrrolizidine alkaloids (PAs) are necine base naturally occurring alkaloid group found in *Crotalaria* genus plant root, leaves, flowers, fruits and seeds where it has been identified as a contaminant in food and beverages, honey, herbal medicine, and meat. In addition, it has been used as forage for cattle, birds, and fish, resulting in significant hepatotoxicity in animals and also central nervous system problems in humans.[11] *Crotalaria ramosissima* Roxb. [Fig. 1] is found only in the Indian Peninsula and open, dry deciduous forests.[12-14] The plant flower is used to treat eczema,[14] the entire plant for foraging cattle and as pest control in grain storage rooms.[15,16]

The flavanone named crotaramosmin was isolated and characterized by Khalilullah *et al.* (1992) and trimethoxychalcone was isolated by Rao and Narkulla (2007)[15,16] for the first time from the aerial part of *C. ramosissima*. However, prenylated chalcones, flavanones, and dihydrochalcones were already reported. Essential oil was extracted from *C. ramosissima* from flowers. It contained 17 different components, three of which were unknown chief constituent's geraniol, β-ocimmene, calamenene, sesquiterpenes and calamenene. These compounds are naturally occurring potent bio-molecules against human pathogen microbes to treat several bacterial diseases.[15] Narendra *et al.* (2005) reported *in-vitro* anti-malarial activities and isolated crotaorixin and prenlyted chalcones from *C. medicagenia* and *C. ramosissima*.[17] The genus *Crotalaria* reported several bioactive compounds, which include terpenoids, flavonoids, anthraquinone, tannins and steroidal alkaloids. Pyrrolizidine alkaloids reported in genus *Crotalaria* which shown heptotoxic activities in animals and other useful pharmaceutical uses yet to be explored[18] and chalcones are the natural flavonoid reported in *C. ramosissima* in which recent years it retained attentions from researchers due to its pharmacological and anti-inflammatory activities.[19] *C. ramosissima* plant has potent bioactive compounds yet to explore for pharmaceutical uses current study focused on evaluating activities from it.

Materials And Methods

Collection of Plant and Extraction

Healthy plant material was collected in Pavagada, Tumkuru district (14°10'13.8"N 77°06'18.2"E) and authenticated by a botanist at the University of Mysore with the help of Plant Herbarium at the Botany Department, Manasagangothri. Dried plant leaves of *C. ramosissima* Roxb. [Fig. 1] were ground into powder, 25 g of sample powder was successively extracted with 250 mL of ethyl acetate, ethanol and methanol using soxhlet apparatus boiling point fixed between (40–50°C) for 8 hours. The collected extract was concentrated in vacuum evaporator pressure fixed at 22 to 26 mmHg with 40°C and stored at 4°C.

Phytochemical Analysis

Obtained extracts were analyzed for the presence and absence of phytoconstituents. Alkaloids-dragendorff's reagent test, pyrrolizidine alkaloid- Ehrlich reagent test, flavonoids-alkaline test, terpenoids-Libermann-Buchurd test, diterpenes-copper acetate test, saponins-Froth test, cardiac glycosides-Keller-Kilian test, phenolics-ellagic acid test, tanins-gelatin test, carbohydrates-Benedicts test and protein-ninhydrins all test reagents were prepared from standard protocols.[1, 20, 21]

Antibacterial Activity

Test organisms

Two plant pathogenic strains *Xanthomonas oryzae* (MTCC-11107) and *Pseudomonas syringae* (MTCC-2703) gram-negative strains, were collected from microbial type culture collection and Gene bank (MTCC), Chandigarh and used throughout the study. The concentration of test organisms inocula was adjusted to 0.5 Mc Farland standards (1.5×10^8 CFU/mL).[22]

Evaluation of antibacterial activity was carried out by disc diffusion method with slight modification.[23] Sterilized

Fig. 1: *Crotalaria ramosissima* Roxb

nutrient agar media plates seeded with 24 hours old bacteria cultures (1.5×10^8 CFU/mL), 6 mm sterile discs equidistantly placed in a media, each disc loaded with 40 µL (10 mg $disc^{-1}$) of different plant solvent extracts, antibiotic tetracycline (2 mg $disc^{-1}$) positive control and 50% DMSO (40 µL $disc^{-1}$) negative contro. Plates were incubated for 24 hours at 37 ± 2°C and inhibition zone was measured in mm and compared with positive control.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was carried out by micro dilution method with modification.[23] About 100 µL of plant solvent extracts and 100 µL of nutrient broth were dispensed to 96 well plates. A two-fold serial dilution with a concentration range (10–0.01 mg mL^{-1}) was already done and also 10 µL of bacterial inoculum suspension added to each well and incubated for 24 hours at 37 ± 2°C. MIC was confirmed by adding of 10 µL of 2,3,5-triphenyl tetrazolium chloride (TTC) at 2 $mgmL^{-1}$ to incubated cultures. The absorbance of the ELISA plate reader was taken at 620 nm after 30 minutes of incubation, where there was no color of pink taken as MIC.

Thin layer chromatography- bioautography

Thin layer chromatography (TLC) bioautography agar overlay bioassay method was used to assess a compound's biological activity.[24] About 50 µL (10 mg) of extracts spotted on TLC plates (Dimensions 12×1.5 cm) they dipped in a solvent system acetic anhydride: Benzene: Petroleum ether (1:4:5).[25] The chromatogram was air dried, and then clear bands were observed; its retention factor (Rf) was calculated. Nutrient agar media combined with 2 $mgmL^{-1}$ 2,3,5-triphenyl tetrazolium chloride (TTC) overlaid on TLC plates and bacteria suspension cultures ($1.5x10^8$ CFU/mL) spread on media after incubation at 37 ± 2°C for 24 hours. The inhibition zone observed with the pink background experiment was repeated thrice.

Antioxidant assay

- *2, 2-Diphenyl-1-picrylhydrazl (DPPH) assay*

C. ramosissima leaf extract antioxidant activity was evaluated by the DPPH assay method. The solution was prepared by dissolving 3.9 mg DPPH (0.01M) in 100 mL of methanol. The reaction mixture containing 1-mL plant extracts of different concentrations (20 µg–100 $µgmL^{-1}$) and 3 mL of DPPH solution, it was incubated for 30 minutes at room temperature in dark conditions. Ascorbic acid was used as the standard absorbance of solutions recorded at wavelength 517nm, percentage of inhibition was calculated from the formula.[26] The inhibition curves and IC_{50} were calculated by linear regression equation and R^2

$$\text{Inhibition Percentage (\%)} = \frac{AC - AS}{AC} X100$$

Where, AC -Absorbance of Control; AS-Absorbance of plant sample extracts.

- *GC-MS analysis*

Gas chromatography/mass spectroscopy (GC-MS) profiling of *C. ramosissima* plant solvent extracts was carried out on Shimadzu GC-MS chromatogram of Model-QP2010S of a Capillary column of ELITE-5MS of 30 metre length, 0.25 mm inner diameter and 0.25 µm thickness. GC-MS Solutions software was used to identify compounds and compared them with mass spectral data libraries from NIST 11 (National Institute of Standards and Technology, Washington, DC, USA) and Wiley8.

Statistical Analysis

Three replicates results of each experiment were analyzed by ANOVA using SPSS Inc.16.0. Significant effects were determined by F values ($p \leq 0.05$) Tukey's HSD test means.

Results And Discussion

Phytochemical analysis

Phytochemical analysis results revealed the presence of alkaloids, pyrrolizidine alkaloids, flavonoids, steroids, diterpenes, and cardiac glycosides in ethyl acetate, methanol and ethanol, phenolics was present in methanol, ethanol extract and the absence in ethyl acetate and saponins, carbohydrates, and proteins were absent in all extract (Table 1). The major phytoconstituents were confirmed by qualitative analysis.

Table 1: Preliminary phytochemical analysis of *C. ramosissima* ethyl acetate methanol and ethanol leaves extracts:

Phytochemical compounds and test	*Ethyl acetate extract*	*Methanol extract*	*Ethanol extract*
Alkaloids			
Dragendorff's test	+	+	+
Mayer's test	+	+	+
Wagner's test	+	+	+
Hager's test	+	+	+
Pyrrolizidine alkaloid Ehrlich reagent	+	+	+
Flavonoids			
Alkaline reagent test	+	+	+
Ammonia test	+	+	+
Steroids &Terpenoids			
Libermann-Burchurd test	+	+	+
Salkowski test	+	+	+
	+	+	+
Diterpenes			
Copper acetate test	+	+	+
Saponins			
Froth test	–	–	–
Cardiac glycoside			
Keller-Kiliani's test	+	+	+
Phenols			
Ellagic acid test	+	+	+
Tannins			
Gelatin test	+	+	+

+ Present, - Absent

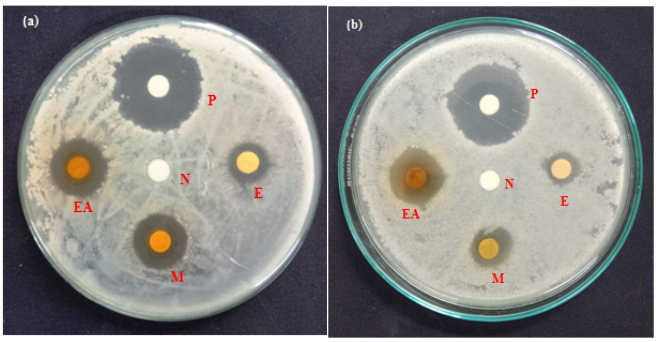
Antibacterial Activity, MIC and TLC Bioautography

Antibacterial activity was evaluated against phytopathogens *Pseudomonas syringae*, and *Xanthomonas oryzae. O*ut of three extracts, ethyl acetate and methanol extract reported antibacterial activity. Ethyl acetate extract showed an inhibition zone of 23 ± 0.5 mm and MIC of 15.60 µg mL^{-1} for *P. syringae*, 18 ± 0.5 mm zone of inhibition and MIC 31.25 µg mL^{-1} for methanol extract. Ethyl acetate extract showed an inhibition zone of 20 ± 0.5 mm and MIC 31.25 µg mL^{-1} for *X. oryzae*, 11 ± 05 mm zone of inhibition and MIC 62.25 µg mL^{-1} for methanol extract, ethanol extract less sensitive no inhibition zone formed results represented in Fig. 2(a), (b) and Table 2.

C. ramosissima extracts evaluated for antibacterial activity by TLC bioautography method ethyl acetate extracts showed totally 13 bands and Rf values of 0.45, 0.54, 0.62, 0.75, 0.78, 0.8, 0.88 showed inhibition zone for *P. syringae*, and Rf value of 0.78 band showed inhibition zone for *X. oryzae,* Fig. 3(a), (b) no inhibition zone observed in methanol, ethanol extracts. GC-MS profiling of ethyl acetate extract reported with the bioactive compounds majorly pthalic acids-1,2-benzene dicarboxylic acid renowned for antibacterial, antifungal and antiviral activities,[27] geranyl linalool also reported which is commercially important as an essential oil, as an antimicrobial agent[28] and 1,2,4-oxadiazole, 3-(1,3-bezodioxol-5-y)-5-[2-(4-methoxyphenyl)ethyl] which are potent antibacterial compounds reported in *C. ramosissima* for the first time where 1,2,4-oxidiazole derivatives exhibited antibacterial activity against *X. oryzae.* In previous report these compounds are known to have other properties like antiviral, antifugal[29,30] also which has unexplored activities in these compound. In previous studies other species, *C. sessiflora* reported for remarkable antibacterial activity against *Pseudomonas* species.[31] Ethyl acetate extracts reported 1,2,4-oxadiazole compounds, which are having broad spectrum biological applications.[32]

Antioxidant activity

C. ramosissima extracts were evaluated by DPPH assay with concentrations of (20–100 µgmL^{-1}) compared to standard ascorbic acid. Methanol extract offered highest scavenging activity with inhibition percentage 80.36 ± 1.58% and IC_{50} of 2.71 µg mL^{-1}, ethyl acetate extract 73.70 ± 1.68% and IC_{50} of 2.91 µgmL^{-1}, ethanol extract 74.22 ± 1.82% with IC_{50} of 2.91 µgmL^{-1}, standard ascorbic acid showed 85.76 ± 1.02% with IC_{50} value 2.41 µgmL^{-1}. The methanol extracts of *C. ramosissima* has showed good antioxidant activity by inhibiting free radicals, which is on par with ascorbic acid antioxidant activity. The results are significant ($p \leq 0.05$) Fig. 4. as all three extracts are good at scavenging free radicals. GC-MS analysis of methanol extract reported oxiranetanoic acid, methyl ester, hexadeconic acid, butyl ester, 10-octadecenoic acid and methyl ester fatty acids reported as antioxidants in previous studies.[33] However, in other studies *C. sessiliflora* showed antioxidant properties by the presence of hydroxyeucomic acid as strongest free radical-scavenging compound.[34]



NOTE: P-Positive control tetracycline antibiotic, N-Negative control, EA-Ethyl acetate *C. ramosissima* leaf extract, M-Methanol *C. ramosissima* leaf extract, E-Ethanol *C. ramosissima* leaf extract

Fig. 2: (a) Antibacterial activity of *P. syringae* (b) *X. oryzae*

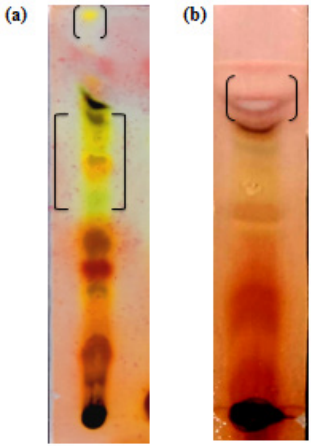


Fig. 3: (a) TLC-bioautography of *P. syringae* (b) *X. oryzae*

Table 2: Antibacterial activity of C. *ramosissima*

S No.	*Test organisms*	*Zone of inhibition (mm/60 µL)*				*MIC (µg/mL)*			
		Ethyl acetate extract	*Methanol extract*	*Ethanol extract*	*Standard*	*Ethyl acetate extract*	*Methanol extract*	*Ethanol extract*	*Standard*
1.	*X. oryzae*	16.33 ± 0.33^{e}	11.33 ± 0.33^{c}	1.66 ± 0.33^{b}	26.00 ± 0.0^{a}	31.25	62.50	NA	3.12
2.	*P. syringae*	17.66 ± 0.33^{b}	17.66 ± 0.33^{b}	5.00 ± 0.33^{c}	26.00 ± 0.0^{a}	15.60	15.60	NA	3.12

Each value is the mean for triplicates (n = 3) and ± indicate standard errors. Means followed by the same letter(s) within the same column are not significantly different ($p < 0.05$) according to Tukey's b HSD. Note: ND- No Activity determined

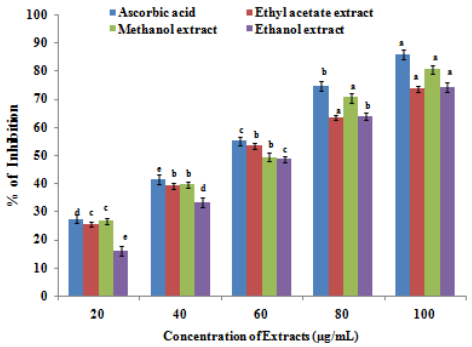


Fig. 4: Grapical representation of antioxidant activity DPPH assay ethyl acetate, ethanol, methanol of *C. ramosissima* leaf *followed by* bars are sharing same letters *are* not significantly different (p< 0.05) according to Tukeys HSD vertical bars are standard errors

C. retusa methanol extract showed significant antioxidant activity.[35] Ethyl acetate extract GC-MS analysis revealed squelenes-tetratetracontane, pentacosane, and tocopherol (vitamin E), which are potent in protecting cells and food from free radicals. Methanol extract showed the highest antioxidants compared to ethyl acetate extract and ethanol with the lowest antioxidants. Phthalic acids are not good antioxidants compared to other plant metabolites.

GC-MS Analysis

C. ramosissima leaf crude extracts GC-MS analysis revealed bioactive compounds chromatogram of ethyl acetate, ethanol and methanol extracts as represented in Figs 5(a), (b), (c) and Tables 3-6 described uses. Three solvent extracts revealed the presence of 1,2-benzenedicarboxylic acid (phthalic acids) highest percentage, followed by

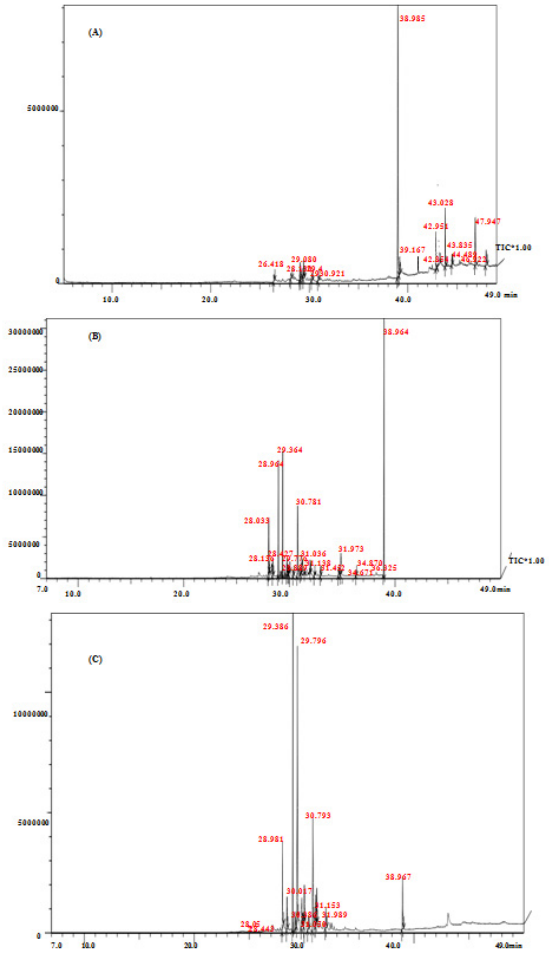


Fig. 5:GC-MS Chromatogram of (a) ethyl acetate, (c) ethanol, (b) methanol extract of *C. ramosissima* leaf

Table 3: GC-MS profile of C. *ramosissima* leaf ethyl acetate extract

Peak No	*Rentation time*	*Area of %*	*Compound name*	*Molecular formula*
1	26.418	1.12	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{18}H_{38}$
2	28.130	1.25	1,2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
3	29.080	3.48	Dibutyl phthalate	$C_6H_{28}(COOC_4H_9)_2$
4	29.167	2.20	Phthalic acid, 5-methylhex-2-yl butyl ester	$C_{19}H_8O_4$
5	29.429	3.18	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$
6	30.261	1.12	Geranyl linalool isomer	$C_{20}H_{34}O$
7	30.921	1.00	1-Butyl 2-cyclohexyl phthalate	$C_{18}H_{24}O_4$
8	38.985	33.73	1,2-Benzenedicarboxylic acid	C_8H_{60}
9	39.167	4.88	2-Methoxy-4-nitroacridone	$C_{14}H_9C_1N_2O_3$
10	42.854	6.52	3-Amino-n-(2,3-dimethylphenyl)-6,7-dihydro-5h-cyclopenta[b] thieno[3,2-e]pyridine-2-carboxamide	$C_{30}H_{27}N_3OS$
11	42.951	1.11	22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	$C_{30}H_{50}$
12	43.028	22.76	1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl]-	$C_{16}H_{12}N_2O_4$
13	43.835	6.15	Tetratetracontane	$C_{44}H_{90}$
14	44.489	1.23	22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-e)-	$C_{30}H_{50}O$
15	46.922	7.25	Pentacosane	$C_{25}H_{52}$
16	49.947	3.02	Dl-.alpha.-tocopherol	$C_{29}H_{50}O_2$
		100.00		

Table 5: GC-MS profile of C*. ramosissima* leaf methanol extract

Peak No	*Retention time*	*Area of %*	*Compound name*	*Molecular formula*
1	28.033	9.49	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$
2	28.136	1.23	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$
3	28.427	3.14	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$
4	28.964	9.70	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$
5	29.364	10.88	Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$
6	29.776	3.68	Diamyl phthalate	$C_{18}H_{26}O_4$
7	29.889	1.22	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
8	30.002	3.65	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$
9	30.369	1.10	Di-isopentylphthalate	$C_{18}H_{26}O_4$
10	30.781	12.03	1,2-benzenedicarboxylic acid, bis(2-methoxyethyl) ester	$C_{14}H_{18}O_6$
11	31.036	2.38	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
12	31.138	2.77	Diamyl phthalate	$C_{18}H_{26}O_4$
13	31.452	1.84	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
14	31.973	1.68	1,2-benzenedicarboxylic acid, bis(2-methoxyethyl) ester	$C_{14}H_{18}O$
15	32.360	0.51	2-(Heptyloxycarbonyl)benzoic acid	$C_9H_8O_5$
16	32.950	1.44	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O$
17	34.671	1.44	Monocrotaline	$C_{16}H_{23}NO_6$
18	34.870	3.83	Oxiraneoctanoic acid, 3-octyl-, methyl ester, trans-	$C_{19}H_{36}O_3$
19	36.325	0.82	Octadecanoic acid, butyl ester	$C_{22}H_{44}O$
20	38.964	27.16	1,2-benzenedicarboxylic acid	C_8H_{60}
		100.00		

Table 4: GC-MS profile of C*. ramosissima* leaf ethanol extract

Peak No	*Retention time*	*Area of %*	*Compound name*	*Molecular formula*
1	28.052	10.32	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$
2	28.443	3.87	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$
3	30.017	4.06	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O$
4	30.386	1.53	Phthalic acid, di(2-methylbutyl) ester	$C_{19}H_8O_4$
5	30.793	13.25	1,2-benzenedicarboxylic acid, bis(2-methoxyethyl) ester	$C_{14}H_{18}O$
6	31.050	3.07	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
7	31.153	3.69	Diamyl phthalate	
8	31.989	3.07	1,2-benzenedicarboxylic acid, bis(2-methoxyethyl) ester	$C_{14}H_{18}O$
9	38.967	5.98	1,2-benzenedicarboxylic acid	C_8H_6
		100		

Table 6: Biological applications of GC-MS profiled bioactive compounds of ethyl acetate, ethanol and methanol leaf extracts of *C. ramosissima*

S. No	*Extract*	*Compound name*	*Molecular Formula*	*Biological application*
1	Ethyl acetate extract, Ethanol extract, Methanol extract	1,2 Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	Pthalicacids- Antibacterial, antifungal, antiproliferative, Insecticidal-Diamond back moth,Mosquito repellent by acetylcholinesterase inhibition, and Phytotoxic[27].
2		Dibutyl phthalate	$C_6H_{28}(COOC_4H_9)_2$	
3		Phthalic acid, 5-methylhex-2-yl butyl ester	$C_{19}H_8O_4$	

4		1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	
5		1-Butyl 2-cyclohexyl phthalate	$C_{18}H_{24}O_4$	
6		1,2-Benzenedicarboxylic acid	C_8H_{60}	
7	Ethyl acetate extract	Geranyl linalool isomer	$C_{20}H_{34}O$	Metabolite, fragrance scents preparation, antimicrobial agent, and *insecticidal*[28]
8	Ethyl acetate extract	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{18}H_{38}$	Antileishmanial, antiproliferative, and Immnostimulant[40]
9	Ethyl acetate extract	3-Amino-n-(2,3-dimethylphenyl)-6,7-dihydro-5h-cyclopenta[b]thieno[3,2-e] pyridine-2-carboxamide	$C_{30}H_{27}N_{30}S$	Skin corrosion and organ toxicity Respiratory tract irritation[45]
10	Ethyl acetate extract	22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (alle)-	$C_{30}H_{50}$	Antibacterial, antiarthritic, anti-inflammatory, cytotoxic, insecticidal and chemopreventive[41]
11	Ethyl acetate extract	1,2,4-Oxadiazole, 3-(1,3-benzodioxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl]-	$C_{16}H_{12}N_2O_4$	Cancerous properties, haemolytic larvicidal activity of army worm, nematicidal, anti-Allodynic, anti-Insomnia, anticonvulsant, anti-Inflammatory, and antimicrobial[37,29]
12	Ethyl acetate extract	Tetratetracontane	$C_{44}H_{90}$	Semiochemical and a plant metabolite[41]
14	Ethyl acetate extract	Pentacosane	$C_{25}H_{52}$	unexplored
15	Ethyl acetate extract	Dl-.alpha.-tocopherol	$C_{29}H_{50}O_2$	Antioxidant[42]
16	Methanol extract	Monocrotaline	$C_{16}H_{23}NO_6$	Toxic,heptotoxic and cancerous [43]
17	Ethyl acetate extract	2-Methoxy-4-nitroacridone		*Acridine dye and* Skin corrosion[44]
18	Methanol extract	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$	Antioxidant, antimicrobial, and anti-inflammatory activities[41]
19	Methanol extract	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	Fattyacids-Antioxidant, antimicrobial, lubricants and decrease blood cholesterol[38, 39]
20	Methanol extract	Octadecanoic acid, butyl ester	$C_{22}H_{44}O$	
21	Methanol extract	Oxiraneoctanoic acid, 3-octyl-, methyl ester, trans-	$C_{19}H_{36}O_3$	unexplored

fatty acids, terpenoids, pyrrolizidine alkaloid. Ethyl acetate extract revealed 16 compounds 33.73% of 1,2-benzenedicarboxylic acid which belongs to phthalates is purified and the compound showed inhibitory effect on *Pseudomonas* genus[36] which is having properties of antibacterial, antifungal and phytotoxic activities,[27] 22.76% of 1,2,4-oxadiazole-3(1,3-benzodiaxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl] heterocyclic aromatic chemical compound of the azole family-alkaloid which is reported first in *C. ramosissima* plant, is having broad spectrum of applications in agricultural activities and also reported for its excellent antibacterial activity against *Xanthomonas oryzae* causative organism of bacterial blight[27,29,37] followed by 7.25% of pentacosane activities not explored and 1.12% of geranyl linalool is di-terpene with high fragrance repellent agent for insects, antimicrobial agent and used as essential oils.[28,32] Ethanol extract revealed only 12 compounds which are all pthalic acids 29.9% of 1,2-benzenedicarboxylic acid and 4.61% of diamyl phthalate and methanol extract reported 27.16% of 1,2-benzenedicarboxylic acid, 1.84% of 10-octadecenoic acid, methyl ester[38,39] 1.44% of hexadecanoic acid, butyl ester and 1.44% of monocrotaline pyrrolizidine alkaloid are reported in GC-MS analysis of *C. ramosissima* leaf extracts.

Conclusion

Crotalaria is folk medicine used to treat fever, cold, lung diseases, epidermal infections and skin diseases, but few bioactive compounds are considered toxic and can cause damage to livestock and human health problems. Among the high levels of hepatotoxic alkaloids that cause risk to livestock health are *C. novae-hollandiae*, *C. ramosissima*, *C. retusa* var, and *C. crispate*. A study of *C. ramosissima* illuminated an unexplored bioactive compound and its activity. Ethyl acetate extract exhibited antibacterial activity against phytopathogens *X. oryzae* and *P. syringae*, which can be used as biocontrol for plant diseases, along with antioxidants dyes are reported, which are helpful. Carcinogenic toxic compounds are also detected, which

are harmful to human beings. As per the GC-MS analysis report, pthalic acid contents are high in *C. ramosissima,* which are synthesized chemically for its plasticizer nature and application are antimicrobial, antioxidant, insecticidal and used as a mosquito repellent. *C. ramosissima* reported 1,2,4-oxadiazole-3(1,3-benzodiaxol-5-yl)-5-[2-(4-methoxyphenyl)ethyl] for first-time in ethyl acetate extract and as per reports methanol reported monocrotaline which is a problematic pyrrolizidine alkaloid. All three solvent extracts of the plant reported potent in biological activities yet to be explored for work in future studies.

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References

1. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science and business media, 1998.
2. Yamamoto K, Takahashi K, Mizuno H, Anegawa A, Ishizaki K, Fukaki H, Ohnishi M, Yamazaki M, Masujima T, Mimura T. Cell-specific localization of alkaloids in *Catharanthus roseus* stem tissue measured with Imaging MS and Single-cell MS. Proceedings of the National Academy of Sciences. 2016;113 (14):3891-3896. Available from: https://doi.org/10.1073/pnas.1521959113.
3. Rout GR, Das P. Effect of metal toxicity on plant growth and metabolism: I. Zinc. Sustainable agriculture Springer. 2009;873-884. Available from: https://doi.org/10.1007/978-90-481-2666-8_53
4. Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry. 2001;56 (3):237-43. Available from: https://doi.org/10.1016/S0031-9422 (00)00456-8.
5. Subramaniam S, Pandey AK. Reinstatement of *Crotalaria pellita* (Leguminosae, Papilionoideae) and a new combination for its variety. Phytotaxa. 2014;183(1):051-5. Available from: http://dx.doi.org/10.11646/phytotaxa.183.1.6.
6. Wortmann CS, Isabirye M, Musa S. *Crotalaria ochroleuca* as a green manure crop in Uganda. African Crop Science Journal. 1994;2(1). Available from: http://dx.doi.org/10.4314/acsj.v2i1.54683
7. Nwude N, Ibrahim MA. Plants used in traditional veterinary medical practice in Nigeria. Journal of Veterinary Pharmacology and therapeutics. 1980;3(4):261-73. Available from: https://doi.org/10.1111/j.1365-2885.1980.tb00491.x
8. Polhill RM. *Crotalaria* in Africa and Madagascar. CRC Press; 1982.
9. Stepanova AV, Oskolski AA, Tilney PM, Van Wyk BE. Wood anatomy of the tribe Podalyrieae (Fabaceae, Papilionoideae): Diversity and evolutionary trends. South African Journal of Botany. 2013;89:244-256. Available from: https://doi.org/10.1016/j.sajb.2013.07.023.
10. Verdoorn GH. Alkaloids as taxonomic characters in the tribe Crotalarieae (Fabaceae). Biochemical systematics and ecology. 1990;18(7-8):503-515. Available from: https://doi.org/10.1016/0305-1978(90)90122-V.
11. Pearson W. Pyrrolizidine Alkaloids in higher plants: hepatic veno-occlusive disease associated with chronic consumption. Journal of nutraceuticals, functional and medical foods. 2001;3(1):87-96. Available from: https://doi.org/10.1300/J133v03n01_06.
12. Subramaniam S, Pandey AK, Rather SA. A revised circumscription of the species in Bracteatae complex (section Calycinae) in the genus *Crotalaria* L.: evidence from nuclear and chloroplast markers. Plant Systematics and Evolution. 2015;2261-2290. Available from: https://doi.org/10.1007/s00606-015-1228-8
13. Thomas B, Kumar KM, George S, Rajendran A, Balachandran I. A new variety of *Crotalaria ramosissima* (Fabaceae) from Tamil Nadu, India. Asian Pacific Journal of Tropical Biomedicine. 2012;(3):S1412-S1414. Available from: https://doi.org/10.1016/S2221-1691(12)60427-4
14. Tirkey A. Some ethnomedicinal plants of family-Fabaceae of Chhattisgarh state. Indain Journal of Traditional Knowledge 2006;5(4):551-553. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/20063220770
15. Khalilullah MD, Sharma VM, Rao PS, Raju KR. Crotaramosmin, a new prenylated flavanone from *Crotalaria ramosissima*. Journal of Natural Products. 1992;55(2):229-31. Available from: https://doi.org/10.1021/np50080a013
16. Rao MS, Narukulla R. A new trimethoxychalcone from *Crotalaria ramosissima*. Fitoterapia. 2007;78(6):446-7. Available from: http://dx.doi.org/10.1016/j.fitote.2007.03.011
17. Narender T, Tanvir K, Rao MS, Srivastava K, Puri SK. Prenylated chalcones isolated from *Crotalaria* genus inhibits in vitro growth of the human malaria parasite *Plasmodium falciparum.* Bioorganic and medicinal chemistry letters. 2005;15(10):2453-5. Available from: https://doi.org/10.1016/j.bmcl.2005.03.081
18. Schrenk D, Gao L, Lin G, Mahony C, Mulder PP, Peijnenburg A, Pfuhler S, Rietjens IM, Rutz L, Steinhoff B, These A. Pyrrolizidine alkaloids in food and phytomedicine: Occurrence, exposure, toxicity, mechanisms, and risk assessment-A review. Food and chemical toxicology. 2020;136:107-111. Available from: https://doi.org/10.1016/j.fct.2019.111107
19. Xing N, Meng X, Wang S. Isobavachalcone: A comprehensive review of its plant sources, pharmacokinetics, toxicity, pharmacological activities and related molecular mechanisms. Phytotherapy Research. 2022;36(8):3120-42. Available from: https://doi.org/10.1002/ptr.7520.
20. Alabri TH, Al Musalami AH, Hossain MA, Weli AM, Al-Riyami Q. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. Journal of King Saud University-Science. 2014;(3):237-43. Available from: https://doi.org/10.1016/j.jksus.2013.07.002
21. Morsy N. Phytochemical analysis of biologically active constituents of medicinal plants. Main Group Chemistry. 2014;13(1):7-21. Available from: DOI:10.3233/MGC-130117.
22. Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial susceptibility testing protocols. Crc Press; 2007. Available from: https://doi.org/10.1201/9781420014495.
23. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016;71-9. Available from: https://doi.org/10.1016/j.jpha.2015.11.005
24. Jesionek W, Majer-Dziedzic B, Horváth G, Móricz ÁM, Choma IM. Screening of antibacterial compounds in *Thymus vulgaris* L. tincture using thin-layer chromatography—direct bioautography and liquid chromatography—tandem mass spectrometry techniques. JPC–Journal of Planar Chromatography–Modern TLC. 2017;131-5. Available from: https://doi.org/10.1556/1006.2017.30.2.7.
25. Dann AT. Detection of N-oxides of the pyrrolizidine alkaloids. Nature. 1960;186(4730):1051.Available from: https://doi.org/10.1038/1861051a0.
26. Islam MZ, Hossain MT, Hossen F, Mukharjee SK, Sultana N, Paul SC. Evaluation of antioxidant and antibacterial activities of *Crotalaria pallida* stem extract. Clinical Phytoscience. 2018;4:1-7. Available from: https:https://doi.org/10.1186/s40816-018-0066-y.
27. Huang L, Zhu X, Zhou S, Cheng Z, Shi K, Zhang C, Shao H. Phthalic acid esters: Natural sources and biological activities. Toxins. 2021;13(7):495. Available from: https://doi.org/10.3390/toxins13070495.
28. Chen W, Viljoen AM. Geraniol—A review of a commercially important fragrance material. South African Journal of Botany. 2010;76(4):643-51. Available from: https://doi.org/10.1016/j.sajb.2010.05.008.
29. Zhu L, Zeng H, Liu D, Fu Y, Wu Q, Song B, Gan X. Design, synthesis, and biological activity of novel 1, 2, 4-oxadiazole derivatives. BMC chemistry. 2020;14:1-2. Available from: https://doi.org/10.1186/s13065-020-00722-1.
30. Krolenko KY, Vlasov SV, Zhuravel IA. Synthesis and antimicrobial

activity of 5-(1 H-1, 2, 3-triazol-4-yl)-1, 2, 4-oxadiazole derivatives. Chemistry of Heterocyclic Compounds. 2016;52:823-30. Available from: https://doi.org/10.1007/s10593-016-1972-x.
31. Kumari R, Kumar S. Pharmacological, phytochemical and their application of *Crotalaria* L. Genus. 2022;15. Available from: https://dx.doi.org/10.2139/ssrn.4097263
32. Luo L, Liu D, Lan S, Gan X. Design, Synthesis, and Biological Activity of Novel Chalcone Derivatives Containing an 1, 2, 4-Oxadiazole Moiety. Frontiers in Chemistry. 2022;10:943062. Available from: https://doi.org/10.3389/fchem.2022.943062.
33. Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. Pharmacological research. 2008;57(6):451-5. Available from: https://doi.org/10.1016/j.phrs.2008.05.002.
34. Munim A, Negishi O, Ozawa T. Antioxidative compounds from *Crotalaria sessiliflora.* Bioscience, biotechnology, and biochemistry. 2003;67(2):410-4. Available from: https://doi.org/10.1271/bbb.67.410.
35. Anim MT, Larbie C, Appiah-Opong R, Tuffour I, Owusu KB, Aning A. Phytochemical, antioxidant and cytotoxicity of hydroethanolic extracts of *Crotalaria retusa* L. World Journal . of Pharmacy Research. 2016; 5990:162-179.
36. Rajamanikyam M, Vadlapudi V, Parvathaneni SP, Koude D, Sripadi P, Misra S, Amanchy R, Upadhyayula SM. Isolation and characterization of phthalates from *Brevibacterium mcbrellneri* that cause cytotoxicity and cell cycle arrest. Journal. 2017;16:375. Available from: https://doi.org/10.17179%2Fexcli2017-145.
37. Tayade AB, Dhar P, Kumar J, Sharma M, Chauhan RS, Chaurasia OP, Srivastava RB. Chemometric profile of root extracts of *Rhodiola imbricata* Edgew. with hyphenated gas chromatography mass spectrometric technique. PLoS One. 2013;8(1):e52797. Available from: https://doi.org/10.1371/journal.pone.0052797
38. Abubacker MN, Deepalakshmi T. In vitro antifungal potential of bioactive compound methyl ester of hexadecanoic acid isolated from *Annona muricata* linn (annonaceae) leaves. Bioscience Biotechnology Research Asia. 2013;10(2):879-84. Available from: http://dx.doi.org/10.13005/bbra/1211
39. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from *Thesium humile* Vahl. Journal of Materials and Environmental Science. 2015;6(10):2778-83.
40. Fletcher MT, McKenzie RA, Blaney BJ, Reichmann KG. Pyrrolizidine alkaloids in *Crotalaria* taxa from northern Australia: risk to grazing livestock. Journal of agricultural and food chemistry. 2009;57(1):311-9. Available from: https://doi.org/10.1021/jf8026099
41. de Felício R, de Albuquerque S, Young MC, Yokoya NS, Debonsi HM. Trypanocidal, leishmanicidal and antifungal potential from marine red alga Bostrychia tenella J. Agardh (Rhodomelaceae, Ceramiales). Journal of pharmaceutical and biomedical analysis. 2010;52(5):763-9. Available from: https://doi.org/10.1016/j.jpba.2010.02.018
42. Brigelius-Flohé R, Traber MG. Vitamin E: function and metabolism. The FASEB journal. 1999;13(10):1145-55. Available from: https://doi.org/10.1096/fasebj.13.10.1145.
43. Schrenk D, Gao L, Lin G, Mahony C, Mulder PP, Peijnenburg A, Pfuhler S, Rietjens IM, Rutz L, Steinhoff B, These A. Pyrrolizidine alkaloids in food and phytomedicine: Occurrence, exposure, toxicity, mechanisms, and risk assessment-A review. Food and chemical toxicology. 2020;136:107-111. Available from: https://doi.org/10.1016/j.fct.2019.111107.
44. Goni LK, Jafar Mazumder MA, Tripathy DB, Quraishi MA. Acridine and its derivatives: synthesis, biological, and anticorrosion properties. Materials. 2022;15(21):7560. Available from: https://doi.org/10.3390/ma15217560.
45. Shanab SM, Shalaby EA. Biological activities and anticorrosion efficiency of water hyacinth (*Eichhornia crassipes*). Journal of Medicinal Plants Research. 2012;6(23):3950-62. Available from: DOI: 10.5897/JMPR12.191

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