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ANXIOLYTIC ACTIVITY OF METHANOLIC LEAF EXTRACTS OF AMARANTHUS CRUENTUS LINN. AND RUMEX ACETOSA LINN. IN MICE

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Rumex acetosa Linn, Amaranthus cruentus Linn, Anxiolytic activity, EPMT, Caryophyllene, CB2.

ABSTRACT
Background: The current study aims to define the anti-anxiety properties of the leaves of *Amaranthus cruentus Linn.* and *Rumex acetosa Linn*. **Methodology:** Soxhlet extraction was used to extract the leaves of *Rumex acetosa Linn* and *Amaranthus cruentus Linn*. FTIR and GC-MS were used to investigate the fraction. The elevated plus maze test (EMPT) was used to assess the antianxiety property of methanolic extracts at 200 mg/kg and 400 mg/kg in Swiss albino mice. **Results and discussion:** Alkaloids, steroids, glycosides, amino acid flavonoids, carbohydrates, and phenolic tannins are found in both extracts. The percentage yields of petroleum ether, chloroform, and methanol extracts were 2.4%, 3.6%, and 10% for *Amaranthus cruentus*, and 2.1%, 3.2%, and 8% for *Rumex acetosa*, respectively. *Amaranthus cruentus* extracts were associated with significant increases ($P < 0.05$) in the amount of time and the number of entries in open arms. Open arm entries ($p < 0.01$) and time spent in the open arm ($p < 0.01$) were both increased by 200 mg/kg of *Rumex acetosa Linn* extract, while 400 mg/kg increased both of these variables ($p < 0.001$). Nevertheless, activity rises in tandem with the dosage of *Rumex acetosa Linn* extract. In EMPT, diazepam (2 mg/kg) significantly increased open arm entries (22.67) and time spent in open arms (3.273 min), while decreasing closed arm entries (6.33) and time spent in closed arms (1.582 min) compared to the control group, confirming its potent anxiolytic activity. **Conclusion:** The methanolic extracts of both plants exhibit significant anxiolytic properties.

INTRODUCTION

With a current global incidence of 7.3%, anxiety disorders are the most common mental illnesses (4.8–10.9%). The most common phobia is specific (10.3%), followed by generalized anxiety disorder (2.2%), social phobia (2.7%), and panic disorder (with or without agoraphobia) (6.0%). It is unclear whether these illnesses have become more widespread in recent decades. In general, women are more prone to develop emotional problems that begin in adolescence; they are 1.5 to 2 times more likely than men to have an anxiety disorder [1]. It has become a prominent area of study focus in psychopharmacology over the past decade [2]. Various medications, including alprax, clonazepam, and benzodiazepines, are available to address mental health

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conditions such as stress, anxiety, sleeplessness, and depression. However, despite their therapeutic efficacy, these currently used anxiolytics possess significant drawbacks such as withdrawal symptoms, sedation, cognitive impairment, and high abuse liability, creating an urgent need for safer alternatives. However, these medicines can cause amnesia, dependence, tolerance, and disruption of the respiratory, immune, and digestive systems, among other side effects [3].

Therefore, the exploration of herbal medicines with anxiolytic benefits is gaining global significance due to their better safety profile and holistic approach to restoring physiological balance. The limitations of traditional pharmacological treatment for anxiety disorders have motivated the quest for alternatives. Herbal medicines are one type of possible treatment. Phytochemical and behavioral studies have revealed that continuous therapy with many herbal remedies [4]. Chronic treatment with several herbal medications has been demonstrated to normalize stress hormone levels. Medicinal plants rich in phytochemicals such as flavonoids, phenolics, terpenoids, and alkaloids show promising anxiolytic and neuroprotective effects by interacting with γ-aminobutyric acid (GABA), serotonergic, and antioxidant pathways. Hence, identifying edible plants with nutraceutical and CNS-modulating properties may contribute to safer therapeutic strategies for anxiety management [5].

Recently, as a result of a rising global population and in addition to its benefits for the country, creating meals with improved health has received significant attention due to the rise in degenerative disorders [6]. *Amaranthus* is a C4 photosynthetic plant that can thrive in saline, alkaline, acidic, or poor soil and is drought-resistant. Apart from its high nutritional value, its leaves are from different *Amaranthus* species. The amaranth leaves, *Amaranthus cruentus,* from the family *Amaranthaceae* [7]. It is characterized by potent antifungal and antimicrobial properties, which likely help protect against fungi and other microorganisms. A perception of necessary vitamin and mineral deficiencies poses a serious barrier to economic expansion & human health [8]. Phytoconstituents like betacyanins, flavonoids, phenolic acids & peptides present in *A. cruentus* have been associated with antioxidant, neuroprotective & CNS-modulating activities, indicating their potential role in anxiety reduction. They are unstable and highly reactive due to the presence of an unpaired electron. Reactive oxygen species (ROS) are crucial to the development of many pathological diseases [9]. Amaranth, also known as amaranthus, is one of the oldest plant crops and has a great tolerance for drought, salinity, alkalinity, and acidic soil conditions. It belongs to the family Amaranthaceae, which comprises 850 species and 65 genera. *Amaranthus* is a genus of 50–60 species that are grown for their leaves. *Amaranthus cruentus*, also known as *A. caudatus*, *A. hybridusi*, *A. hypochondriacus*, *A. bilitum*, *A. tricolor*, *A. gangeticus*, *A. tristis*, *A. melonch*, *A. managostanus*, and *A. polygamous*, is one of the main Amaranthus species [10]. Amaranthus is a rapidly growing crop primarily grown in Latin America, Africa, and Asia. Amaranthine, a component of the broad class of substances known as betacyanins, is found in amaranth [11].

Despite its high concentrations of micronutrients and bioactive phytochemicals and its ability to flourish in challenging environmental conditions, amaranth remains an underutilized crop. Amaranthus leaves have good nutritional value, few antinutrient factors, and a large amount of bioactive compounds. These compounds may have antioxidant, antimicrobial, antifungal, antihyperglycemic, and antihypercholesterolemic properties, among other health advantages. Numerous studies of grain consumption have been associated with a lower risk of oxidative stress-related degenerative illnesses, namely atherosclerosis, cancer, diabetes, alzheimer's, constipation, gastrointestinal disease, heart attack, and chronic cardiovascular disease. Perceived deficiencies of essential vitamins (vitamins B1, B2, B3, B6, and B9) and minerals severely limit both human health and economic development [12]. Because of its high protein content and amino acid composition, amaranth has medical benefits such as cholesterol-reducing, antioxidant, anticancer, antiallergic, and antihypertensive activities [13].

Amaranth proteins possess antiamnestic, antithrombotic, opioid, regulating, antioxidant, ligand, immunostimulating, embryotoxic, protease inhibitory, and antihypertensive activities as a result of their active peptides [14]. This demonstrates that the amaranth lunasin peptide is more effective for cancer prevention. Contrary to earlier reports, the amaranth lunasin peptide is not present in soybeans with a Bowman-Birk protease inhibitor, but rather in lipid-transfer proteins. Lunasin-containing plants, including amaranth, support recent studies on amaranth as a dietary alternative, as they contain peptides that are beneficial to health [15]. From Kashmir to Kumaon, the

perennial herb *Rumex acetosa Linn*. (Polygonaceae) thrives throughout Asia and the Western Himalayas. It is also known as garden sorrel (English), chuukaa (Ayurvedic), and churkuy [16]. The phytochemical tests of the aerial sections revealed ascorbic acid, oxalic acid, and flavones such as rutin, vitexin, kaempferol, oxymethylanthraquinone, flavone C-glycosides, and flavone O-glycosides [17]. Hyperin, chrysophanein, proanthocyanidin, and phloroglucinol derivatives were produced by extracting several anthraquinones from the roots, including chrysophanol, physcion, and emodin[18]. There have been reports of the antibacterial, anticancer, diuretic, diaphoretic, insecticidal, antiseptic, and antipyretic properties of R. acetosa [18-21]. The plant's decoction is used to treat constipation, bronchitis, scurvy, arthritis, gastric ulcers, cramps, diarrhea, sore throats, and water retention, and the seeds are used as an astringent for hemorrhages [22].

Although these plants are widely consumed as leafy vegetables and are rich in bioactive phytoconstituents, their potential anxiolytic effects have not been scientifically validated. Evaluating the anxiolytic potential of methanol leaf extracts of Amaranthus cruentus and *Rumex acetosa Linn* will provide experimental evidence supporting their traditional use and nutraceutical value. The present investigation aims to identify safe, effective, and plant-based therapeutic alternatives to conventional anxiolytics, addressing the unmet medical need for treatments with fewer adverse effects and improved patient safety. The present study aims to evaluate the anxiolytic potential of methanol leaf extracts of *Amaranthus cruentus* Linn. and *Rumex acetosa* Linn. in mice, thereby exploring their potential as natural alternatives to conventional anxiolytic drugs.

MATERIALS AND METHODS

Authentication

Fresh specimens of *Amaranthus cruentus* L. were collected from the Miraj local region of Maharashtra. Dr. S. M. Shendage authenticated them at the Department of Botany, Balwant College, Vita, District Sangli (Outward No. 005/2022-2023). Similarly, fresh *Rumex acetosa Linn*. plants were obtained from the Miraj local area and identified by Dr. M. V. Kale at the Department of Botany, Jaysingpur College, Jaysingpur, District Sangli, under authentication number ASE/JCJ.

Preparation of Plant Material

Fresh leaves of *Amaranthus cruentus (Amaranthaceae)* and *Rumex acetosa (Polygonaceae)* were cleaned, air-dried in a shady area, and ground into a coarse powder with a pestle and mortar. Soxhlet extraction was performed on 100 g of crude powder in 250 ml of methanol. Solvent volume was selected based on the Soxhlet apparatus's capacity & efficient recycling during continuous extraction, ensuring adequate extraction despite the relatively low solvent-to-drug ratio. The solvent was evaporated to produce a dry extract. The resulting extract was kept in a tightly labeled container for future experiments [23].

Phytochemical Analysis: Phytochemical analysis was performed to detect flavonoids, glycosides, carbohydrates, terpenoids, alkaloids & amino acids as per reported literature [24].

Isolation and Purification of Extract

The separation and purification of plant components were largely accomplished through one or more fractionation operations using various chromatography techniques, such as thin-layer chromatography (TLC) and column chromatography (CT).

Column chromatography of the methanol extract of the plant

Silica gel (60-120 mesh size) was heated for an hour in a hot oven at 110 ± 1°C. 100 g of activated silica gel slurry was prepared in 300 mL of pet ether and transferred into the glass column (equipped with a glass wool plug). The limited-amount-of-solvent method could remain on top of the column (about 2cm). The air bubbles in the column have been extracted with gentle tapping to create a uniform adsorbent bed. The column chromatography details are presented in Table 1.

Table 1: Column chromatography details

Adsorbent	**Silica gel for column chromatography is activated 110±2**
Length of the column	45 cms
Length of the adsorbent	35
Diameter of the column	Outer 3 cms, inner 2.8 cms.
Rate of elution	1 ml /min.
Volume of the elute collected	20 ml each
Gradient elution	n-Hexane: Ethyl acetate: Formic acid

A quantity (5g) of extract was used for part isolation of *A. cruentus* (Amaranthaceae) and *R. actosa* (Polygonaceae). The extract was dissolved in a minimal amount of ether, then poured over the chromatographic bed coated with cotton wool. For

isolation, a solvent mixture of increasing polarity, beginning with petroleum ether, was eluted through the column, and then n-hexane: Ethyl acetate: Formic acid (5:4.5:0.5) at set volume fractions (e.g., 20ml); cumulative fractions from the column were collected. Further study of its identification was carried out using analytical methods such as FTIR and GC-MS [25].

Acute toxicity studies: A methanolic extract of *A. cruentus* and *R. acetosa* was tested for acute oral toxicity in accordance with the amended OECD Guideline 423. Three mice were administered the extract orally at doses of up to 2,000 mg/kg, and no toxicity was seen. As a result, 200 & 400mg/kg of extract were selected for the trial [26].

Anxiolytic activity: Elevated plus Maze method

All animal experiments were conducted and reported in accordance with the ARRIVE guidelines. All experimental procedures were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee (IAEC/AMCP/04/387/2023-2024). For the evaluation of anxiolytic activity, mature male Swiss albino mice (7–10 weeks old, weighing 35–45 g) were used. The experimental animals were obtained from the Laboratory Animal Research Centre and housed under standard laboratory conditions at 20–24°C with a 12-hour light/12-hour dark cycle, with free access to food and water throughout the study. The animals were randomly divided into three groups (n = 6 per group): normal saline (control), diazepam (2 mg/kg, reference standard), and treatment groups. The treatment groups received oral administration of *R. acetosa* (200 and 400 mg/kg) and *A. cruentus* (200 and 400 mg/kg) [27].

Statistical Analysis: The mean is used to represent the gathered data, and tables are used to present the results. One-way ANOVA was used to analyze the data. A p-value of less than 0.05 was considered significant in GraphPad Prism software.

RESULT AND DISCUSSION

Results

Phytochemical Screening

The phytochemical screening of *A. cruentus* Linn and *R. acetosa* Linn revealed the presence of alkaloids, steroids, glycosides, carbohydrates, amino acids, flavonoids & phenolic compounds in both plants, while fats & oils were absent, as shown in Table 2. Moreover, the percentage yields of petroleum ether, chloroform, and methanol extracts were 2.4%, 3.6%, and 10% for *A. cruentus* & 2.1%, 3.2% & 8% for *R. acetosa*, respectively.

Table 2: Phytochemical Analysis of *Amaranth cruntus Linn and Rumex acetosa Linn*

Phytochemical Constituents	*Amaranth cruntus Linn*	*Rumex acetosa Linn*
Alkaloids	+	+
Steroid	+	+
Glycosides	+	+
Carbohydrates	+	+
Amino acids	+	+
Flavonoids	+	+
Fat and oil	-	-
Phenolic	+	+

Spectral Analysis (FTIR)

The FTIR interpretation of *Amaranthus cruentus* Linn Fraction A (methanol) revealed characteristic peaks corresponding to different functional groups, as given in Figure 1. An absorption band at 2922.49 cm^{-1} indicated NH stretching, suggesting the presence of an amine salt. A strong peak at 1735.94 cm^{-1} corresponded to C=O stretching of an aldehyde group. The band at 1456.05 cm^{-1} was attributed to C–H stretching of alkanes, while a peak at 1375.08 cm^{-1} indicated S=O stretching of a sulfonamide group. The absorption at 1162.89 cm^{-1} was due to C–O stretching of an ester, and another band at 1091.79 cm^{-1} corresponded to C–O stretching of a secondary alcohol. Finally, the peak at 724.74 cm^{-1} indicated C–H bending, confirming the presence of a benzene derivative.

GC-MS/MS Analysis: Figure 2 shows the GC-MS/MS analysis of Fraction A (A. Cruntus Linn.), while Table 3 provides a summary of the retention times, peak areas (%) & mol. weights of all identified compounds in Fraction *A. Cruntus Linn.*

Spectral Analysis (FTIR): The FTIR interpretation of *Rumex acetosa* Linn. Fraction R (methanol) revealed characteristic peaks corresponding to different functional groups, as shown in the figure. 3. A broad absorption band at 3342.21 cm^{-1} indicated O–H stretching, suggesting the presence of phenolic compounds. The peak at 2924.18 cm^{-1} was attributed to C–H stretching of alkanes. A strong absorption band at 1718.36 cm^{-1} corresponded to C=O stretching of carboxylic acid groups. The peak observed at 1604.27 cm^{-1} indicated C=C stretching of an aromatic ring. The band at 1384.65 cm^{-1} was assigned to C–H bending vibrations. Furthermore, the absorption peak at 1242.53 cm^{-1} corresponded to C–O stretching, while the peak at 1033.76 cm^{-1} indicated C–O stretching of alcohols, confirming the presence of various oxygenated functional groups in the extract.

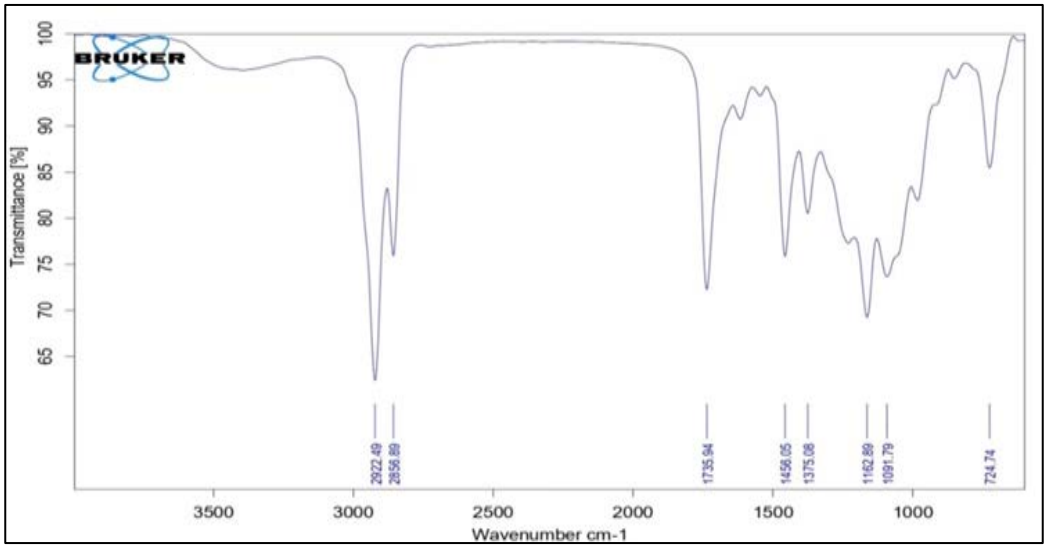
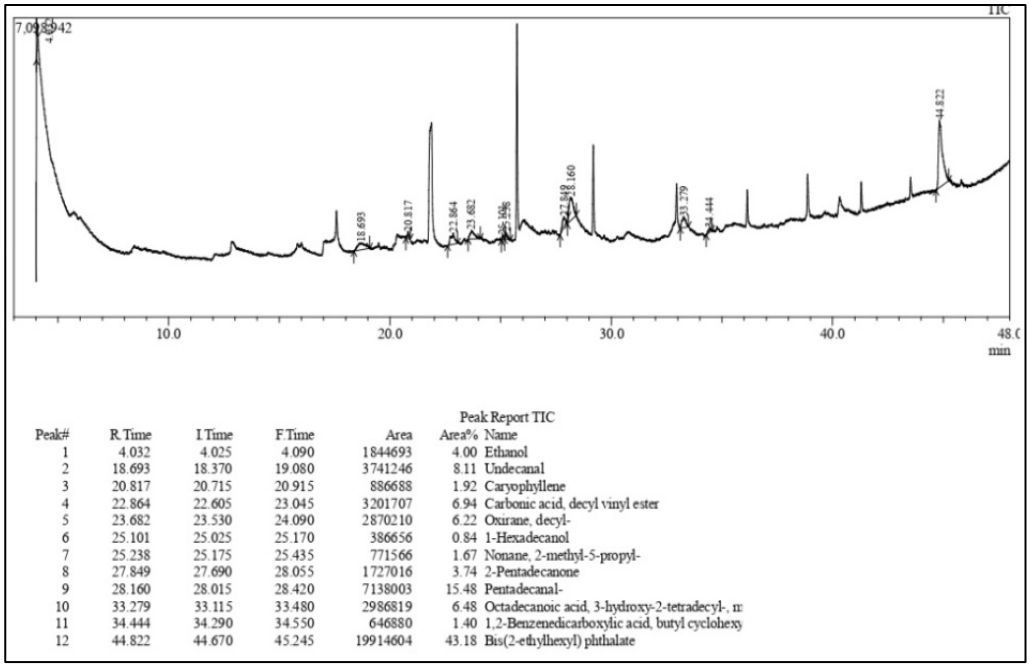


Figure 1:**FTIR Spectra of Fraction A** ***Amaranth Cruntus Linn***



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	4.032	4.025	4.090	1844693	4.00	Ethanol
2	18.693	18.370	19.080	3741246	8.11	Undecanal
3	20.817	20.715	20.915	886688	1.92	Caryophyllene
4	22.864	22.605	23.045	3201707	6.94	Carbonic acid, decyl vinyl ester
5	23.682	23.530	24.090	2870210	6.22	Oxirane, decyl-
6	25.101	25.025	25.170	386656	0.84	1-Hexadecanol
7	25.238	25.175	25.435	771566	1.67	Nonane, 2-methyl-5-propyl-
8	27.849	27.690	28.055	1727016	3.74	2-Pentadecanone
9	28.160	28.015	28.420	7138003	15.48	Pentadecanal-
10	33.279	33.115	33.480	2986819	6.48	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, m
11	34.444	34.290	34.550	646880	1.40	1,2-Benzenedicarboxylic acid, butyl cyclohexy
12	44.822	44.670	45.245	19914604	43.18	Bis(2-ethylhexyl) phthalate

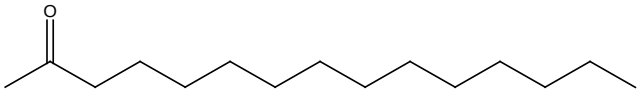
Figure 2: GCMS analysis of Fraction A ***Amaranth Cruntus Linn***

Table 3: summary of the retention times, peak areas (%), and molecular weights of all identified compounds in Fraction A ***Amaranth Cruntus Linn***

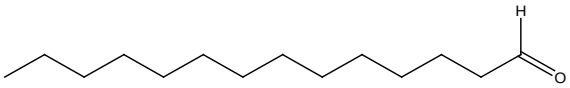
Name of molecule	Retention time	Peak area	Molecular weight
Ethanol	4.032	4.00	46
Undecanal	18.693	8.11	170
Caryophyllene	20.817	1.92	204
Carbonic acid, decyl vinyl ester	22.864	6.94	228
Oxirane, decyl-	23.682	6.22	184
1-Hexadecanol	25.101	0.84	242
Nonane, 2-methyl-5-propyl-	25.238	1.67	184
Pentadecanone	27.849	3.74	226
Pentadecanal-	28.160	15.48	226
Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester, (2R,3R)-	33.279	6.48	510
1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	34.444	1.40	304
Bis(2-ethylhexyl) phthalate	44.822	43.18	390

GC-MS – MS Spectra of fraction A reveal the presence of the following compounds

c

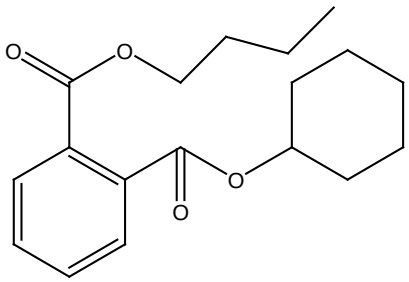


2-Pentadecanone



Pentadecanal

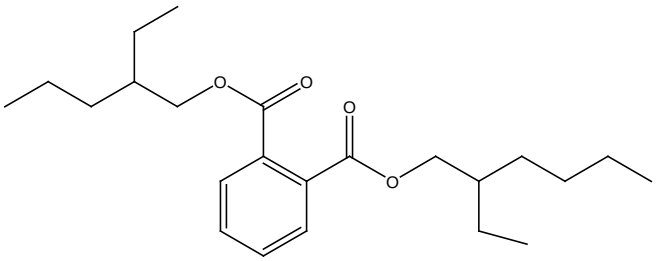
C



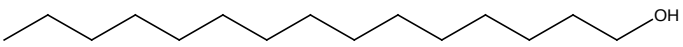
1,2 Bnzenedicarboxylic acid, butylcyclohxyl ester

C

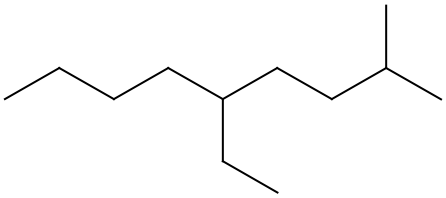
C



Bis (2-ethylhexyl) phalate



1-hexadencanol



2-methyl-5-propyl-Nonane

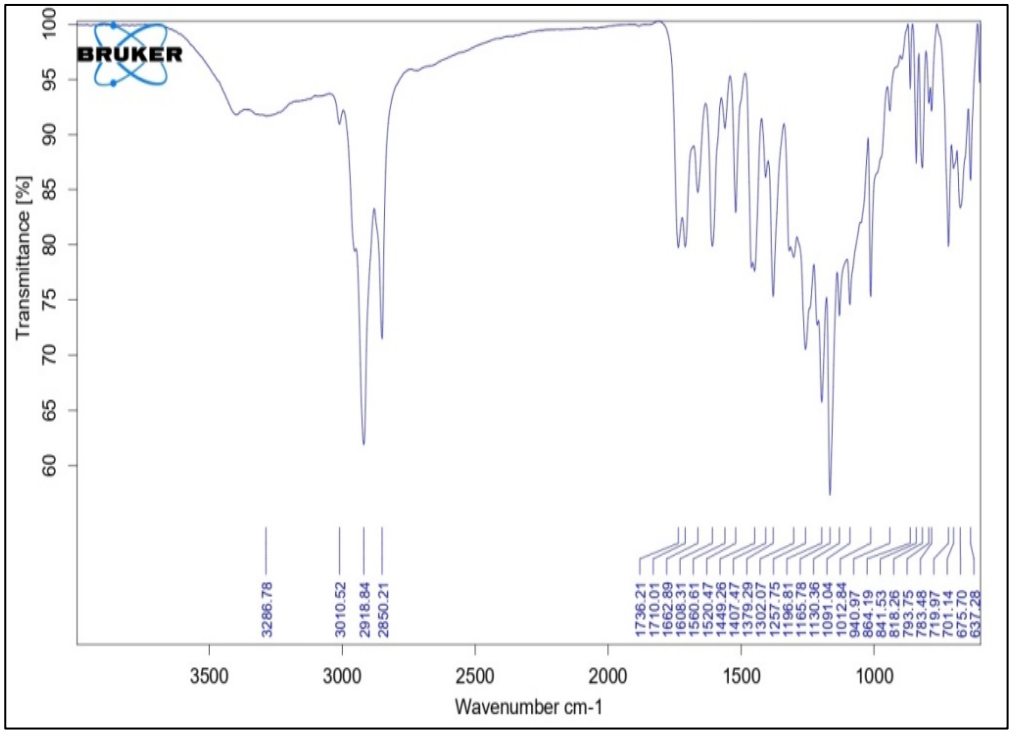
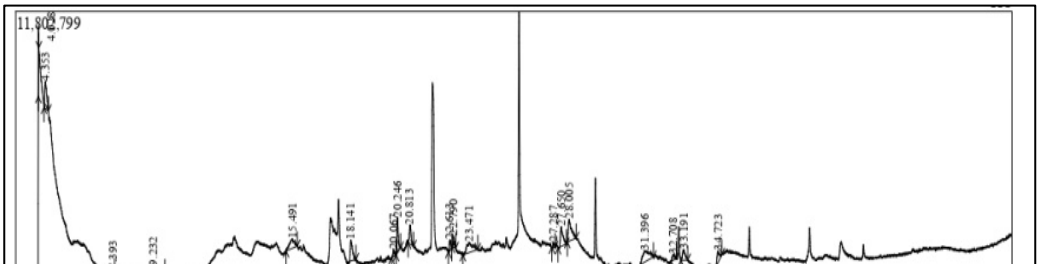


Figure 3: FTIR Spectra of *Rumex Acetosa Linn Fraction R*

GC-MS-MS Analysis

Figure 4 shows the GC-MS-MS Spectra of Rumex Acetosa Linn Fraction R, while Table 4: summary of the retention times, peak areas (%), and molecular weights of all identified compounds in Rumex Acetosa Linn Fraction R



Peak Report TIC

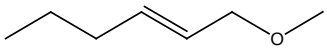
Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	4.028	4.020	4.045	1651522	2.39	3-Hexene, 1-methoxy-, (Z)-
2	4.353	4.280	4.480	5480737	7.92	Methanamine, N-methoxy-N-nitroso-
3	7.393	7.295	7.470	79532	0.11	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-
4	9.232	8.835	9.740	5928314	8.57	Octanal
5	15.491	15.210	15.725	4637686	6.70	1H-Pyrrole, 1-(2-furanylmethyl)-
6	18.141	18.000	18.375	4659916	6.73	Undecanal
7	20.067	19.980	20.175	846735	1.22	1-Tridecene
8	20.246	20.185	20.390	5402713	7.81	Tetradecane
9	20.813	20.730	20.970	3699448	5.35	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-
10	22.613	22.545	22.715	1622105	2.34	n-Pentadecanol
11	22.790	22.700	22.880	1377743	1.99	Heptadecane
12	23.471	23.200	23.880	6313879	9.12	Pentadecanal-
13	27.287	27.220	27.365	509927	0.74	1-Nonadecene
14	27.650	27.505	27.875	5210558	7.53	2-Pentadecanone
15	28.005	27.920	28.315	7385764	10.67	Pentadecanal-
16	31.396	31.210	31.810	7604820	10.99	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydr
17	32.708	32.610	32.845	1220826	1.76	2-Nonadecanone
18	33.191	33.050	33.365	2931513	4.24	Hexadecanoic acid, methyl ester

Figure 4: GC-MS-MS Spectra of *Rumex Acetosa Linn Fraction R*

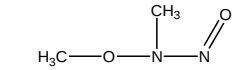
Table 4: summary of the retention times, peak areas (%), and molecular weights of all identified compounds in *Rumex Acetosa Linn Fraction R*

Name of compound	Peak R.Time	Peak Area%	Molecular weight
Hexene, 1-methoxy-, (Z)-	4.028	2.39	114
Methanamine, N-methoxy-N-nitroso-	4.353	7.92	90
Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-	7.393	0.11	222
Octanal	9.232	8.57	128
1H-Pyrrole, 1-(2-furanylmethyl)-	15.491	6.70	147
Undecanal	18.141	6.73	170
1-Tridecene	20.067	1.22	182
Tetradecane	20.246	7.81	198
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	20.813	5.35	204
n-Pentadecanol	22.613	2.34	228
Heptadecane	22.790	1.99	240
Pentadecanal-	23.471	9.12	226
1-Nonadecene	27.287	0.74	266
2-Pentadecanone	27.650	7.53	226
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	31.396	10.99	196
2-Nonadecanone	32.708	1.76	254
Hexadecanoic acid, methyl ester	33.191	4.24	270
Hexadecanoic acid, ethyl ester	34.723	3.81	284

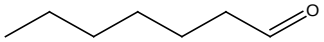
GC-MS – MS Spectra of fraction R reveal the presence of the following compounds



3-Hexene-1-methoxy



N-methoxy-N-nitroso methanamine



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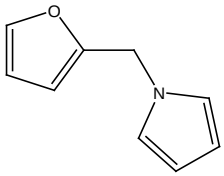
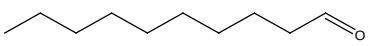
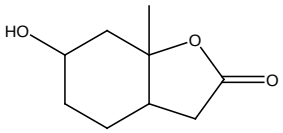


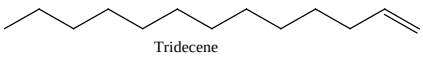
1-H pyrrole,1(2-furanyl methyl)



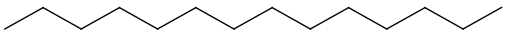
Undcanal



6-hydroxy-4,4,7a trimthyl5,6,7,7a tetrahydrobenzofuran-(4H) one



Tridecene



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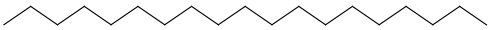
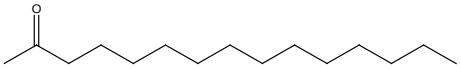
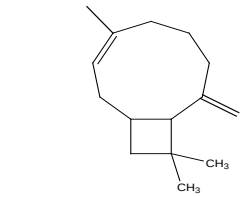


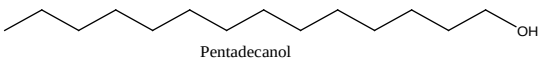
Nonadcane



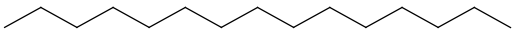
2-Pentadecanone



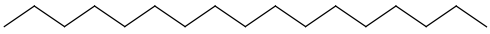
Bicyclo[7,2,0] undec-4-ene,4,11,11-trimthyl-8-methylene



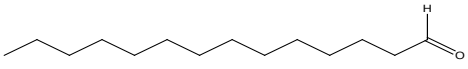
Pentadecanol



Pentacane



Heptadecane



Pentadecanal

Acute Toxicity Studies

When the limit test was conducted with a dose of 2000 mg/kg b.w. of Amaranthus cruentus Linn leaves and Reumx actosa leaves extract, no mortality was seen in mice. The doses selected for Anxiolytic activity are 200 mg and 400 mg/kg body weight.

Diazepam-treated mice made considerably more open arm entries ($P < 0.0001$) and spent more time in them. Mice administered extracts of Amaranthus cruentus at 200 mg/kg and 400 mg/kg showed substantial increases ($P < 0.05$) in time spent in the open arm and in the number of open-arm entries. Reumx actosa extract at 200 mg/kg increased open arm entries ($p < 0.01$) and time spent ($p < 0.01$), whereas 400 mg/kg increased open arm entries ($p < 0.001$) and time spent ($p < 0.001$). However, increasing the amount of Extract of Reumx actosa increases the number of open-arm entries, demonstrating that activity increases with dose, as shown in Table 5 and Fig. 5, compared to the vehicle-treated group [28].

Table 5: Effect of *Amaranthus cruentus Linn* and *Rumex acetosa Linn* methanol extract on the behavior of mice in the elevated plus maze test

Sr. No.	Group	n	Open Arm Entries	Closed Arm Entries	Open Arm Duration (min)	Closed Arm Duration (min)
1	Vehicle Control (Normal Saline)	6	7.50 ± SEM	20.67 ± SEM	1.232 ± SEM	3.488 ± SEM
2	Diazepam (2 mg/kg)	6	22.67 ± SEM ****	6.33 ± SEM ****	3.273 ± SEM ****	1.582 ± SEM ****
3	*A. cruentus* (200 mg/kg)	6	13.50 ± SEM *	14.50 ± SEM **	2.048 ± SEM *	2.897 ± SEM *
4	*A. cruentus* (400 mg/kg)	6	15.50 ± SEM *	14.00 ± SEM **	2.132 ± SEM *	2.660 ± SEM **
5	*R. acetosa* (200 mg/kg)	6	14.67 ± SEM **	14.00 ± SEM ***	2.075 ± SEM *	2.720 ± SEM **
6	*R. acetosa* (400 mg/kg)	6	17.17 ± SEM ***	12.17 ± SEM ***	2.632 ± SEM ***	2.397 ± SEM ***
7	F value		10.09	13.57	10.96	16.23

Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, with all groups compared against the vehicle control group. Level of Significance: P < 0.05 (*); P < 0.01 (**); P < 0.001 (***); P < 0.0001 (****)

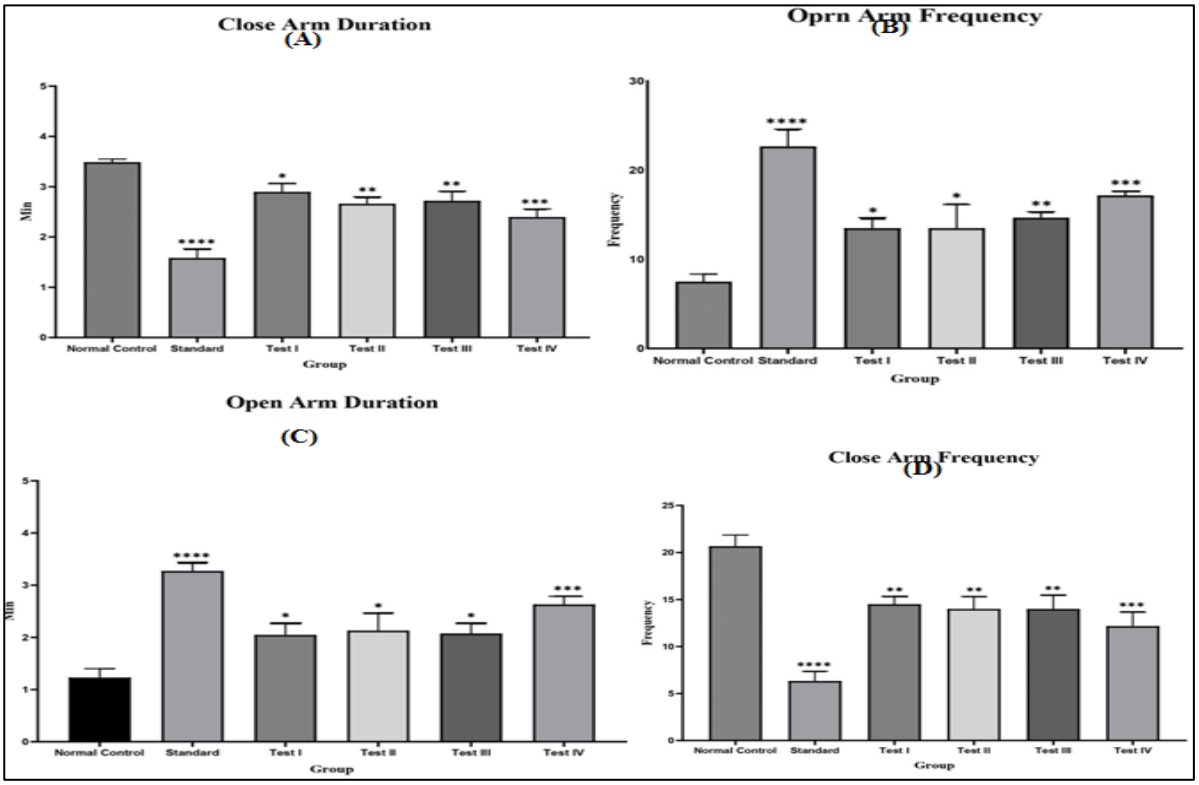


Figure 5: Impact of Rumex acetosa and Amaranthus cruentus Linn (200 and 400 mg/kg p.o.) on (a) open arm time (s), (b) closed arm time (s), (c) open arm entries, and (d) closed arm entries in the elevated plus maze test (n=6).

Discussion

A preliminary phytochemical analysis of Amaranthus cruentus methanol leaf extract revealed the presence of phenolic, flavonoid, glycoside, carbohydrate, terpenoid, alkaloid, and amino acids. Previous studies on Amaranthus cruentus Linn revealed the presence of polyphenols, tannins, flavonoids, steroids, terpenoids, saponins, and betalains. These phytoconstituents, particularly flavonoids and terpenoids, are widely reported to exert CNS-modulating effects by interacting with neurotransmitter systems, including GABAergic, serotonergic, and cannabinoid pathways, which are crucial for anxiety regulation [29]. Photochemical analysis of Rumex acetosa Linn. Methanol leaf extract revealed the presence of phenols, flavonoids, glycosides, carbohydrates, terpenoids, alkaloids, and amino acids. Previous studies on Rumex species have reported high levels of anthraquinones, naphthalenes, flavonoids, stilbenoids, triterpenes, carotenoids, and phenolic acids. The presence of strong antioxidant phytochemicals may contribute to anxiolysis by reducing oxidative stress in neuronal tissues, as elevated ROS has been associated with anxiety-induced behavioral alterations [30].

The chemical components of *A. cruentus* were identified using GC-MS analysis, including undecane, caryophyllene, carbonic acid decyl vinyl ester, Oxiran decyl, 1-Hexadecanol, Nonane, 2-methyl, and 5-propyl. 2-Pentadecanone, Pentadecanal, and Octadecanoic acid 3-hydroxy-2-tetradecyl, 1,2-Benzenediar-boxylic acid, butyl cyclohexyl, and bis(2-ethylhexyl) phthalate. In the GCMS-MS spectra of Rumex acetosa, different chemical constituents showed 3-Hexene, 1-methoxy, Methamine, N-methoxy, N-nitroso, Disioxane1,3-diethoxy, 1,1,3,3-tetrame-thyl, Octanol, 1H pyrrole 1 (furanylmethyl), Undecanal, 1-Tetradecane, Biscyclo(7,2) undeo-4ene, 4,11,11-trimethyl, n-Pentadcanol, 1-Nonadecane, 2-Pent 6-Hydroxy-4,47a-trimethyl -5,6,7,7-tetrahydroxy, Hexadecanoic acid methyl ester. *A. cruentus* GC-MS spectra indicate a peak for caryophyllene. Furthermore, the GC-MS spectra of *R. Acetosa* show the presence of Biscyclo(7,2)undec-4-ene, 4,11,11-trimethyl (caryophyllene). Caryophyllene has been shown to enhance GABA-mediated inhibitory neurotransmission and act as a full agonist at cannabinoid type 2 (CB2) receptors [31].

This suggests a dual mechanism through which extracts may exert anxiolytic activity: (1) potentiation of GABAergic signaling, leading to neuronal calmness; and (2) activation of the CB2 receptor system, which regulates emotional responses without psychoactive CB1-related effects.

The endocannabinoid system (ECS) could play a role in mood regulation [32] and anxiety disorders [33,34].

According to an increasing amount of data from genetic and pharmacological research. Endocannabinoids activate the CB1 and CB2 cannabinoid receptors, which are either presynaptic or postsynaptic in the brain and couple to the Gαi/o class of G-proteins [35]. Ever since the ECS was discovered, numerous researchers have looked at how CB1 receptors mediate all central nervous system effects, including anxiety and depression. The CB2 receptor was previously thought to occur only in immune cells and peripheral tissues, but it has now been identified in the brain [36]. It has been linked to illnesses associated with anxiety and depression [37]. From our findings, the presence of caryophyllene strongly correlates with CB2 receptor-mediated anxiolysis, suggesting that both plant extracts may regulate emotional behavior via ECS modulation. Caryophyllene, identified in both extracts, is a selective CB2 receptor agonist that may contribute to anxiolytic effects by modulating neuroinflammation and emotional responses, without CB1-mediated psychotropic effects. Additionally, interaction between the endocannabinoid system and GABAergic pathways may enhance inhibitory neurotransmission. Further, the presence of flavonoids and phenolic compounds may directly modulate GABAA receptors, similar to benzodiazepine action, thereby reducing neuronal excitability. The antioxidant properties of these phytoconstituents may also help reduce oxidative stress associated with anxiety. Thus, the anxiolytic activity may involve a combined mechanism of CB2 receptor activation, GABAergic modulation, and antioxidant effects.

Drugs that target Cannabinoid Receptor receptors may be employed as innovative pharmacological treatments to treat anxiety and depression, according to recently developed research. Additionally, because they don't have any negative CB1-mediated psychotropic effects, drugs that target CB2 receptors have drawn attention. Furthermore, in contrast to the current selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines (BZDs), which are the foundations of treatment for anxiety and depression, CB2 receptor modulators have several advantageous pharmacological effects despite severe

adverse effects, such as dependency, sedation, ataxia, and forgetfulness [38]. GABAergic neurotransmission in the brain is known to increase as a result of the pharmacological action of anxiolytics [39]. Therefore, flavonoids and terpenoids, such as caryophyllene, present in the extracts likely act synergistically to enhance GABA activity, thereby reducing anxiety-like behavior in mice. The elevated plus maze test is frequently used to evaluate a substance's ability to reduce anxiety in rodents [40].

Benzodiazepines' anxiolytic effects include GABA-A receptors [41]. It has also been demonstrated that flavonoids modulate GABA-A receptors, thereby mediating their calming effects [42]. The EPMT evaluates rodents' psychomotor performance and affective features. Since increases in open arm entry parameters are the most accurate markers of anxiolytic activity, results from the elevated plus maze following treatment with Amaranthus cruentus Linn. and Rumex acetosa Linn. (200 and 400 mg/kg) showed anxiolytic action. The observed increase in open-arm entries and time spent in open arms indicates reduced fear-driven behavior, confirming significant anxiolytic efficacy comparable to that of standard drugs. The anxiolytic effects of *Amaranthus cruentus* and *Rumex acetosa* methanolic extracts, although statistically significant, were lower than those of the standard drug, diazepam (2 mg/kg).

Diazepam exhibited a marked increase in open-arm entries and time spent in open arms, reflecting its well-established, potent anxiolytic action via GABAA receptor modulation. In contrast, the plant extracts demonstrated moderate anxiolytic activity, suggesting a comparatively lower efficacy. However, the observed effects of the extracts may be attributed to the presence of phytoconstituents such as flavonoids and terpenoids (e.g., caryophyllene), which are known to modulate GABAergic and endocannabinoid pathways. Importantly, while diazepam produces rapid and pronounced anxiolytic effects, it is also associated with adverse effects such as sedation, tolerance, and dependence. In comparison, plant-based extracts may offer milder but safer anxiolytic activity, potentially making them suitable for long-term use or as adjunct therapies. Thus, although the extracts are less potent than diazepam, their favorable safety profile and natural origin highlight their potential as alternative or complementary therapeutic agents. While total arm entries are an affected measure that reflects variations in anxiety or overall activity, time spent on the center platform appears to be linked to risk assessment and/or decision-making [43]. Thus, behavioral improvements observed in both extracts suggest their potential to modulate CNS pathways involved in emotional stability, stress coping, and decision-making. The presence of bioactive compounds with ECS and GABAergic modulatory actions, supported by behavioral evidence, indicates that methanol leaf extracts of Amaranthus cruentus and Rumex acetosa possess promising anxiolytic potential and may serve as safer herbal alternatives to conventional anxiolytics.

CONCLUSION

In conclusion, the findings of this study highlight the potential of *Amaranthus cruentus* Linn. and *Rumex acetosa* Linn. as promising sources of natural anxiolytic agents. Although the extracts demonstrated moderate activity relative to the standard drug, their favorable phytochemical profile and potential to reduce adverse effects make them attractive candidates for further development. Future studies should focus on the isolation and characterization of specific bioactive compounds, such as caryophyllene and flavonoids, as well as detailed pharmacological and clinical investigations to validate their efficacy and safety. These plants may serve as leads for developing safer, plant-based therapeutics for anxiety disorders.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Chitra Govind Rajput performed the experimental work and generated the data. Sachinkumar Vasantarao Patil monitored the project and provided the guidance. Pallavi Abhijeet Patil edited the manuscript and analyzed the data.

DECLARATION OF USE OF AI TOOLS

The authors used no AI tool. All content, analysis, and conclusions were reviewed and verified by the authors. The authors take full responsibility for the originality, accuracy, and integrity of the work

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