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CHEMICAL PROFILING AND BIOLOGICAL ACTIVITIES OF *ECHEVERIA RUNYONII* ROSE EX E WALTER

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Abstract

Medicinal plants are a rich source of secondary metabolites chemical compounds that can be used to develop new drugs and effectively treat serious health conditions. This investigation employed Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the ethanolic and ethyl acetate extracts of *Echeveria runyonii* and to assess their analgesic, anti-inflammatory, and antispasmodic properties. The bioactive components in the extracts were identified by matching their mass spectra to those in the National Institute of Standards and Technology (NIST) database. Analgesic effects were evaluated using the hot plate (HP) and acetic acid-induced writhing (AAI) methods. Anti-inflammatory activity was assessed using the carrageenan-induced paw edema model (PEM), while antispasmodic effects were examined through the gastrointestinal motility test (CMT). GC-MS analysis revealed several bioactive compounds, including pharmacologically active constituents such as glycerin, cetene, trehalose, 1,2,3-propanetriol 1-acetate, pyridine-2,4-diol, 3,5,6-trimethyl-, 4H-pyran-4-one, 2,3-dihydro-3,5-dimethyl-, (E)-5-eicosene, and bis (2-ethylhexyl) phthalate. The AAI method demonstrated significant peripheral analgesic activity (p<0.001) at a 300 mg/kg dose, while the HP test showed an increase in reaction time (p<0.001) after 90 minutes. Anti-inflammatory testing revealed a reduction in paw volume by 42.86% at the same dose. The CMT indicated significant inhibition of charcoal movement, with the ethanolic extract reducing movement by 59.88% at 300 mg/kg. The identified compounds, including hexadecane and 1-octadecene, may contribute to the plant's observed bioactivity. This study underscores the promising medicinal potential of *E. runyonii*, although further research is warranted to elucidate the mechanisms underlying these effects.

Key words: GC-MS analysis; Analgesic; Anti-inflammatory; Antispasmodic activities; *Echeveria runyonii*

Introduction

According to the World Health Organization (WHO), around 80% of people in underdeveloped nations receive their medical care through traditional medicine (Kasilo & Nikiema, 2014). Globally, interest in traditional and complementary medicine has steadily grown, as noted by Ayanaw *et al*., (2023). This rise in interest stems from increasing awareness of the potential side effects, adverse reactions, and costs associated with modern pharmaceuticals. Due to their therapeutic and nutritional qualities, plants are valued for their bioactive compounds, which offer a broad range of health benefits (Sasikumar *et al*., 2022; Adil *et al*., 2024).

Natural substances have played a crucial role in the development of many highly effective drugs used in modern medicine. Through evidence-based research, scientists have discovered and optimized naturally derived bioactive compounds, improving their physicochemical, pharmacological, and pharmacokinetic properties to more effectively treat various diseases (Ema *et al*., 2023). Preliminary analysis of medicinal plants is essential for identifying their chemical profiles and biological activities.

Gas Chromatography–Mass Spectrometry (GC-MS) has gained substantial attention for its ability to detect a broad spectrum of biologically active compounds, even from small quantities of plant extracts. These compounds include alkaloids, nitro-compounds, alcohols, long-chain hydrocarbons, esters, steroids, amino acids, organic acids, and other phytoconstituents (Nabi *et al*., 2022). GC-MS is widely used in pharmaceutical research to isolate and characterize bioactive molecules from diverse natural sources (Mariyammal *et al*., 2023). Although plants are renewable sources of pharmacologically important compounds, their vast chemical diversity often complicates the identification of specific bioactive constituents. To overcome this challenge, chromatographic techniques such as GC-MS are extensively employed to separate, identify, and quantify active compounds from various plant parts and solvent extracts (Safdar *et al*., 2021).

Chronic pain is a significant medical concern and remains a key focus of contemporary scientific research. Pain has a dual role, functioning as both a protective and adaptive mechanism for the body. It typically presents as a symptom, signaling the body to take necessary actions to avoid further harm. However, pain is a complex and multifaceted experience that can develop into chronic pathological pain syndrome. The pain frequently gets worse in this state, impairing everyday functions like eating and sleeping and exacerbating psychological problems like sadness and anxiety. Pain management in contemporary medicine entails the complex interplay of numerous physiological and psychosocial elements Adil *et al*., (2020).

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Inflammation is a vital immune response that is essential to the progression of chronic conditions. It is triggered when the body reacts to harmful stimuli, leading to symptoms like pain, swelling, redness, and warmth. During the early stages of inflammation, blood vessels dilate, neutrophils, and lymphocytes are recruited to the site of injury (Yeshi *et al*., 2022). Inflammation is a protective process that eliminates the cause of tissue damage. However, when it becomes chronic, it can lead to diseases such as rheumatoid arthritis, asthma, inflammatory bowel disease, cardiovascular disorders, and cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) like naproxen and aspirin, as well as glucocorticoids, are used to relieve inflammation, pain, and fever by inhibiting COX-1 and COX-2 enzymes involved in prostaglandin production. Yet, prolonged NSAID use can cause side effects, including gastrointestinal and cardiovascular complications (Alam *et al*., 2023; Mat *et al*., 2023). For plant-derived products to serve as effective anti-inflammatory agents, they must be able to inhibit the enzymes (Jahan *et al*., 2022; Noor *et al*.,2025).

Even with advancements in sanitation and public health awareness, diarrhea remains a significant worldwide health concern. Over 4.4 billion instances were reported globally in 2016, resulting in 1.6 million fatalities, making it the tenth most common cause of death. The scale of these figures has considerable economic and social consequences (Shah *et al*., 2023). The WHO defines diarrhea as having three or more loose or watery stools in a single day. In Pakistan, the toll is particularly devastating, with over 150,000 lives lost annually due to severe and recurrent bouts of diarrhea. These episodes not only claim lives but also leave a lasting impact on the youngest and most vulnerable, contributing to malnutrition in newborns and hindering their growth and development, casting a long shadow over their future potential (Kulić *et al*., 2023). Diarrhea, especially common in children, is defined as passing three or more loose stools per day. In adults, it is often accompanied by painful intestinal spasms. Conventional treatments, which primarily block muscarinic receptors, can cause various side effects. This underscores the need to explore traditional medicines as potential alternatives, warranting scientific research into their effectiveness for treating diarrhea and intestinal spasms (Wahid *et al*., 2022).

Commonly used to lessen excessive smooth muscle contractions that can cause conditions including genitourinary tract spasms, gastrointestinal cramps, and bronchial spasms, antispasmodic drugs are also known as spasmolytics. Many synthetic antispasmodics, such as opioid receptor modulators like trimebutine, calcium channel blockers like pinaverium, direct smooth muscle relaxants like papaverine, and anticholinergic drugs like butyl scopolamine, have been approved by regulatory bodies around the world Adil *et al*., (2024).

Echeveria runyonii Rose ex E. Walther belongs to family Crassulaceae commonly known as Silver Spoons *Echeveria*, Mexican Hens and Chicks. *E. runyonii* is a succulent that grows quickly and produces rosettes of pinkish-white to silvery-grey leaves. It is native to the Mexican state of Tamaulipas and is found in Baluchistan, Pakistan. Numerous cultivars have been identified and raised. Because some *Echeveria* species have a mucilaginous sap that may have soothing qualities, they have been applied topically to treat minor burns, skin irritations, and insect bites. Furthermore, some people believe succulents like *Echeveria* might have antibacterial or anti-inflammatory qualities in the future (Godoy Beltrán, 2021).

This study will assess the plant for drug standardization and evaluate its pharmacological effects, aiming to identify its potential for future drug development.

Methodology

Plant collection: *Echeveria runyonii* was collected from Balochistan, Pakistan, located at 30.183270° N and 66.996452° E and identified using the Flora of Pakistan with the assistance of plant taxonomist Ghulam Jelani. The specimen was preserved in the herbarium for future reference under Voucher Muhammad Naseer Bot.2051 (QUSIT).

Plant extraction: The selected plant was shade-dried at room temperature and ground into a fine powder. A 50 g portion of the powdered plant material was soaked separately in 250 mL of ethanol and ethyl acetate solvents. The mixtures were kept at room temperature for two weeks, with occasional shaking each day. The saturated plant material was initially filtered through plain filter paper and subsequently through Whatman No. 41 filter paper. The solvents were completely evaporated under reduced pressure using a rotary evaporator to obtain the crude extracts. The resulting extracts were stored at 4°C in a refrigerator (Ambrin *et al*., 2024).

Gas chromatography-mass spectrometry (GC/MS): The ethanol and ethyl acetate extracts of *Echeveria runyonii* were analyzed using GC-MS at the Central Research Laboratory (CRL) to identify active compounds. A 1 µL sample was injected into an RTx-5 column (30 m × 0.32 mm) using a GC-MS system (Perkin Elmer, Clarus 500, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The analysis was conducted using a temperature gradient method, starting at 75°C with a ramp rate of 50°C per minute, followed by a final hold at 175°C for seven minutes. The mass-to-charge ratios (m/z) of the bioactive compounds were compared with reference mass spectra from known organic compounds (Naseer *et al*., 2025).

Analgesic Activity

Acetic acid-induced writhing: The analgesic properties of the ethyl acetate and ethanolic extracts of *Echeveria runyonii* were evaluated using a modified method based on Bilal *et al*., (2023). Crude extracts (10 mg) were dissolved in 25 mL of ethanol or ethyl acetate. Five groups of mice, each consisting of five animals, were established. Group 1 (control) received an intraperitoneal injection of normal saline (10 mg per kg of body weight). Group 2 was administered diclofenac sodium (25 mg/kg) as the standard reference drug. Groups 3, 4, and 5 received 100, 200, and 300 mg per kg of body weight, respectively, of the ethyl acetate or ethanolic extracts. An intraperitoneal injection of 0.6% v/v acetic acid was administered to the mice one hour

prior to oral administration of the extracts. The number of writhing episodes was recorded for 20 minutes following the injection. The analgesic effect was calculated as a percentage using the standard formula:

$$\% \text{ Analgesic effect} = \frac{100 - \text{Number of writhing in treated animals}}{\text{Number of writhing in control animals}} \times 100$$

Eddy's hot-plate method: This experiment was conducted using a methodology similar to that described by Aziz *et al.* (2019). Five groups, each consisting of five randomly selected male albino mice, were established. Group 1 (control) received an intraperitoneal injection of normal saline (10 mL/kg), while Group 2 was administered the reference drug, diclofenac sodium (25 mg/kg). Groups 3, 4, and 5 received oral doses of ethanolic or ethyl acetate extracts at 100, 200, and 300 mg per kg of body weight, respectively. The mice were placed on a thermal plate maintained at 55 ± 0.5°C to record baseline reaction times for each group. The animals' responses to the heat were assessed based on behaviors such as jumping or paw licking. After treatment, reaction times were measured again at 30, 60, and 90 minutes.

$$\text{Elongation (\%)} = \frac{\text{(Latency (test) - Latency (control)}}{\text{Latency (test)}} \times 100$$

Anti-inflammatory activity: Following the method described by Shamala *et al*., (2022), the anti-inflammatory properties of *Echeveria runyonii* ethanol and ethyl acetate extracts were evaluated in a mouse model of carrageenan-induced paw edema. To prepare the extracts, 10 mg of crude extract was dissolved in 25 mL of either ethanol or ethyl acetate. Five groups of mice, each containing five animals, were established. Acute inflammation was induced by injecting 0.1 mL of a 1% carrageenan solution into the hind paw. Paw size was measured using a plethysmometer at regular intervals for up to 180 minutes. As a standard anti-inflammatory treatment, diclofenac sodium (10 mg per kg of body weight) was administered orally to all animals two hours prior to the carrageenan injection. The percentage suppression of inflammation for each extract was calculated using the following formula, and the average increase in paw volume was recorded.

Vc−Vt % inhibition of paw edema x 100 Vc,

where the test compound-treated mice's paw volume increased by Vt, whereas the mice in the control group noted a Vc increase in paw volume.

Antispasmodic activity: The ethanolic and ethyl acetate extracts of *Echeveria runyonii* were evaluated for their antispasmodic properties using the methodology described by Mamgain *et al*., (2023). Ten milligrams of each crude extract were dissolved in twenty-five milliliters of the respective solvent to prepare the extracts. Five groups of mice, each containing five animals, were established. Each mouse received 1 mL of castor oil orally to induce diarrhea. One hour later, the standard drug atropine sulfate (10 mg/kg body weight) was administered to Group II, while saline (10 mL/kg) was given to Group I as the control group. Groups III, IV, and V received intraperitoneal doses of the ethanolic or ethyl acetate extracts at 100, 200, and 300 mg per kg of body weight, respectively. After another hour, each mouse was administered 10 mL of a 5% gum acacia suspension containing charcoal meal (C.M.). The percentage of intestinal transit was calculated by measuring the distance traveled by the charcoal from the pylorus to the cecum, assessed when the mice were sacrificed one hour later.

(%) (D/L) ×100

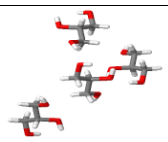
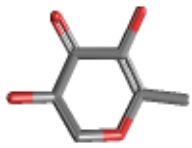
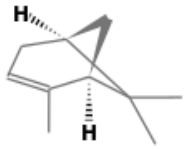
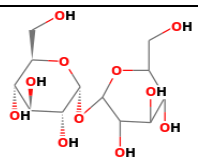
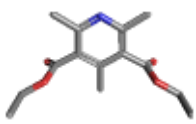
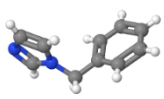
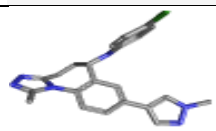
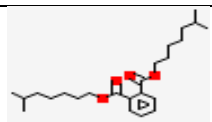
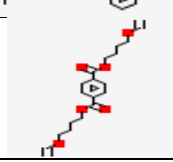
where L is the intestinal length in meters and D is the distance that charcoal covers in meters.

Data interpretation: The SPSS software version 22.0 was used to analyze the data using Dunnett's t-test. Statistical analyses were conducted using p-values of less than 0.001, 0.01, and 0.05.

Results

GC-MS analysis: Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to identify the phytochemical constituents present in the ethanolic and ethyl acetate extracts of *Echeveria runyonii*. The analysis revealed a diverse range of bioactive compounds in both extracts, with the ethanolic extract yielding fourteen distinct compounds and the ethyl acetate extract containing eight. The results are illustrated in Figures 1 and 2 and summarized in Tables 1 and 2. In the ethanolic extract, the most abundant compound was Diisooctyl phthalate, accounting for 23.96% of the total composition. Other major constituents included 9-Octadecenoic acid, (E)- (12.97%), 1,4-Benzenedicarboxylic acid (12.86%), 4-Benzylimidazole-5-(1-propenoic acid) (6.86%), Hexanedioic acid, bis(2-ethylhexyl) ester (6.86%), Pyridine-2,4-diol, 3,5,6-trimethyl- (6.20%), and Trehalose (6.18%) (Table 1, Fig. 1). These compounds are known for their various biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties. In the ethyl acetate extract, the predominant compound was Bis(2-ethylhexyl) phthalate, which constituted 44.72% of the total identified components. Other notable compounds included 1,3-Benzenedicarboxylic acid (39.65%), Hexanedioic acid (11.91%), 1, 2, 3-Propanetriol, 1-acetate (0.98%), 1-Octadecene (0.73%), 5-Eicosene, (E)- (0.73%), Hexadecane (0.68%), and Cetene (0.60%) (Table 2, Fig. 2). These phytochemicals are also associated with potential pharmacological effects such as antimicrobial and cytotoxic activities. The presence of such compounds indicates that *E. runyonii* possesses a rich phytochemical profile, suggesting its potential application in pharmaceutical and therapeutic formulations.

Table 1. (GC-MS) Analysis of Ethanolic extract of *Echeveria runyonii*.

S. No.	Compounds	Formula	RT/Min	(%) Area	Structure	Si	M/weight	Probability
1.	Glycerin	$C_3H_8O_3$	3.853	5.53		2420	92.09	64
2.	4H-Pyran-4-one, 2,3-dihydro-3,5-...	$C_6H_8O_4$	6.911	3.21		20640	144.12	90
3.	(1R)-2,6,6-Trimethylbicyclo [3.1....	$C_{10}H_{16}$	7.884	1.93		15852	136.2340	84
4.	Trehalose	$C_{12}H_{22}O_{11}$	13.608	6.18		177856	342.2965	52
5.	Pyridine-2,4-diol, 3,5,6-trimethyl-	$C_{14}H_{19}NO_4$	17.275	6.20		26471	265.30	30
6.	9,12-Octadecadienoic acid (Z, Z)-...	$C_{18}H_{32}O_2$	18.446	2.69		139724	280.4455	99
7.	9-Octadecenoic acid, methyl este...	$C_{19}H_{36}O_2$	18.529	3.43		141310	296.4879	99
8.	4-Benzylimidazole-5-(1-propenoic...	$C_{10}H_{10}N_2$	18.786	6.86		95610	158.20	47
9.	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	18.856	12.97		129353	282.4614	99
10.	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	19.095	5.02		129352	282.4614	94
11.	Hexanedioic acid, bis (2-ethylhex	$C_{22}H_{42}O_4$	21.016	6.16		196965	370.6	95
12.	Alpha- [4-Chlorophenyl]-1-methy...	$C_{22}H_{21}ClN_6$	21.802	2.99		93043	404.9	38
13.	Diisooctyl phthalate	$C_{24}H_{38}O_4$	22.087	23.96		207658	390.6	91
14.	1,4-Benzenedicarboxylic acid, bi...	$C_{20}H_{26}O_6$	23.725	12.86		207813	362.4	91

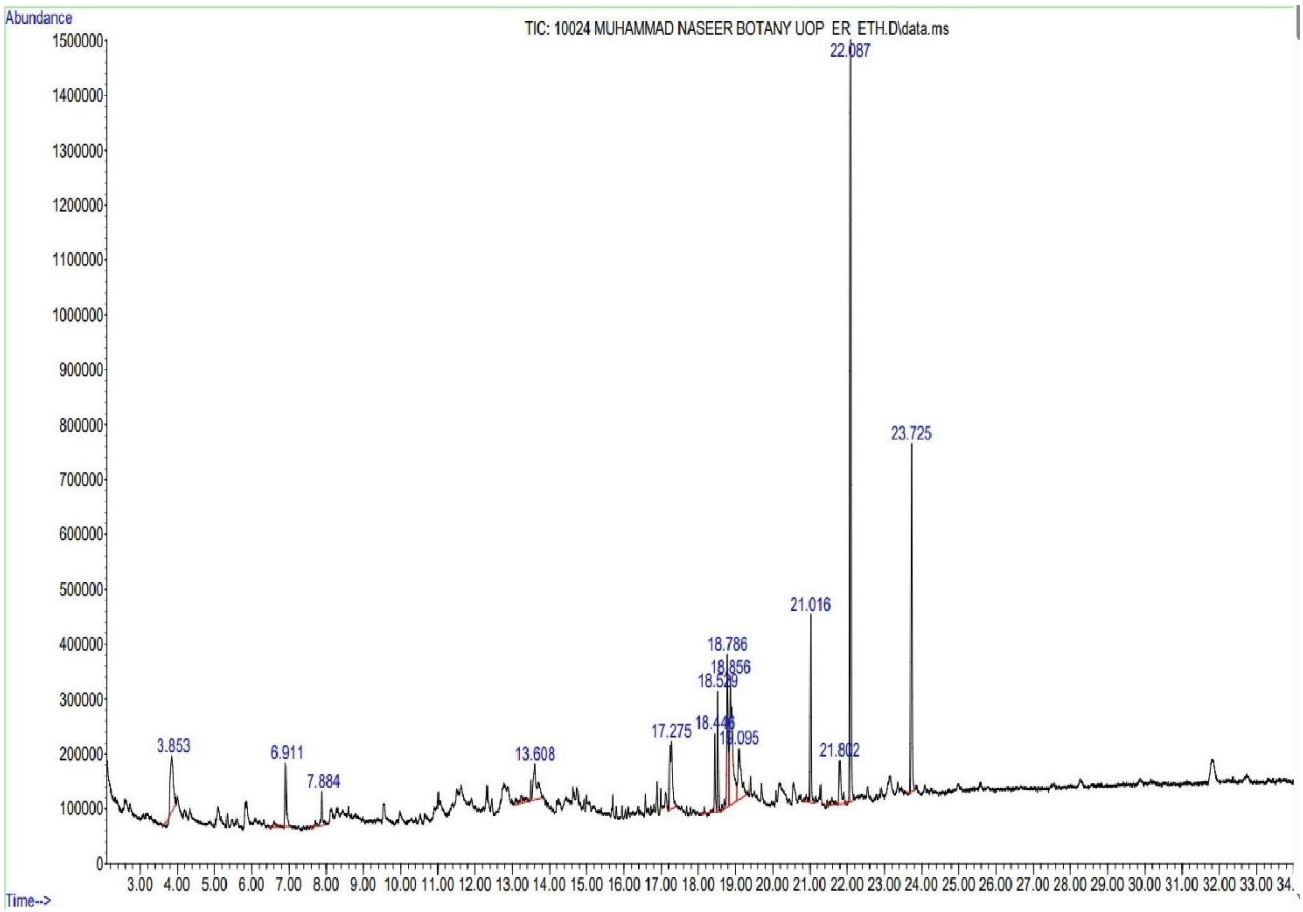


Fig. 1. GC-MS chromatogram of *Echeveria runyonii* ethanolic extract.

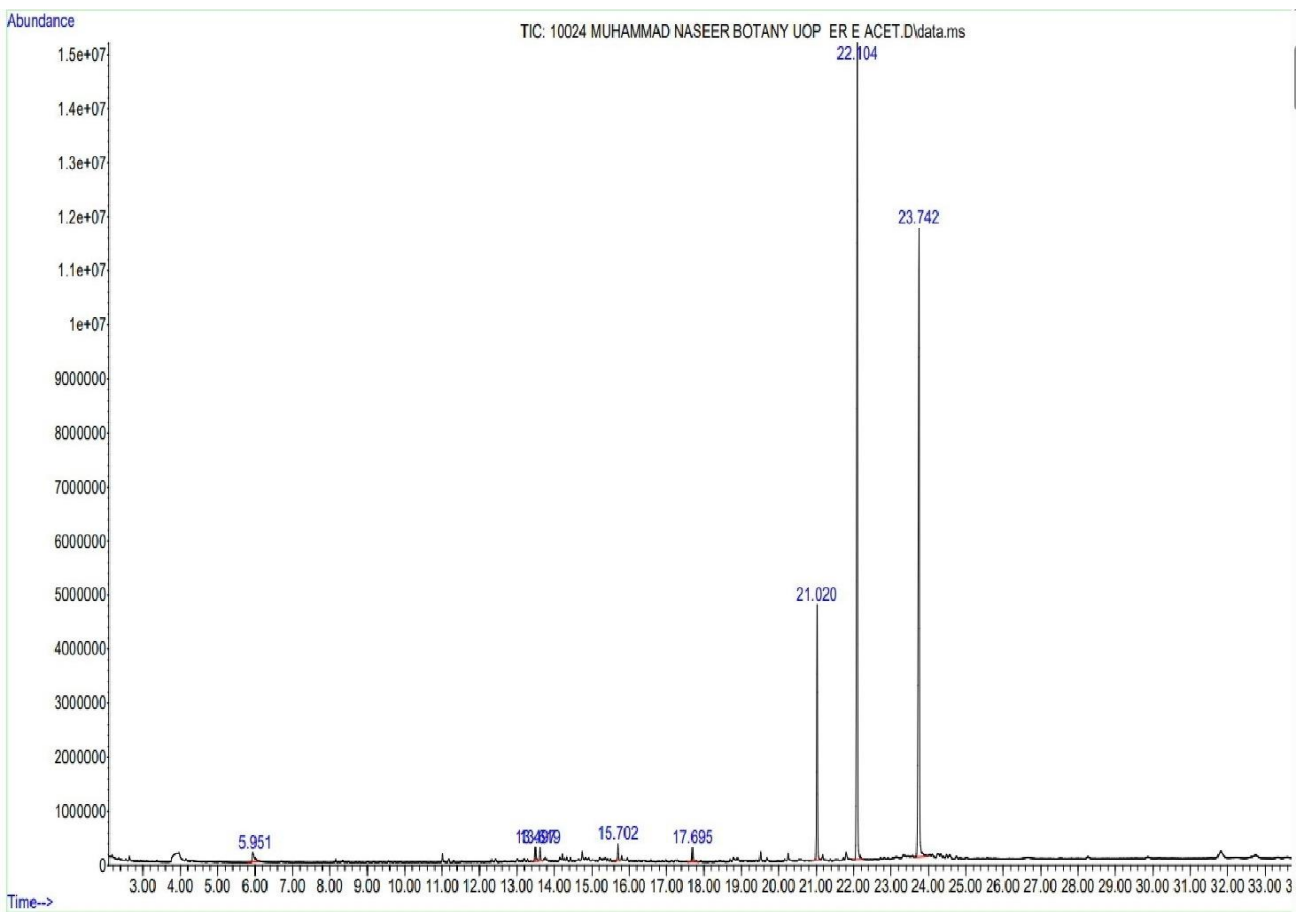
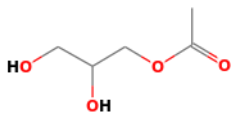
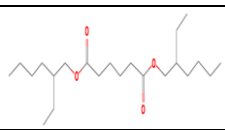
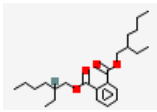
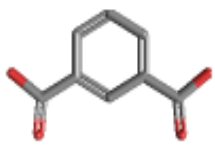


Fig. 2. GC-MS chromatogram of *Echeveria runyonii* of ethyl acetate extract.

Table 2. GC-MS analysis of ethyl acetate extract of *Echeveria runyonii.*

S. No	N/compounds	F/compound	RT/Min	Area (%)	Compound Structure	Si	M/weight	Probability
1.	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	5.951	0.98		15025	134.1305	53
2.	Cetene	$C_{16}H_{32}$	13.497	0.60		81242	224.4253	94
3.	Hexadecane	$C_{16}H_{34}$	13.619	0.68		83027	226.44	95
4.	1-Octadecene	$C_{18}H_{36}$	15.702	0.73		104182	252.5	99
5.	5-Eicosene, (E)-	$C_{20}H_{40}$	17.695	0.73		127769	280.5316	99
6.	Hexanedioic acid, bis (2-ethylhex...	$C_{22}H_{42}O_4$	21.020	11.91		196971	370.5665	95
7.	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	22.104	44.72		207664	390.6	91
8.	1,3-Benzenedicarboxylic acid, bi...	$C_8H_4Na_2O_4$	23.742	39.65		207811	210.09	94

Analgesic effect

Writhing method: At a dosage of 300 mg/kg, both the ethyl acetate and ethanolic extracts of *Echeveria runyonii* significantly and dose-dependently reduced the number of writhing responses in mice during the acetic acid-induced writhing test (p<0.001). The ethanolic extract exhibited inhibition rates of 22.44%, 39.28%, and 70.91% at doses of 100, 200, and 300 mg/kg body weight, respectively. Similarly, the ethyl acetate extract produced inhibition rates of 24.48%, 40.81%, and 77.04% at the same corresponding doses. The positive control, diclofenac sodium, achieved an inhibition rate of 84.18%, indicating potent analgesic activity. Notably, the ethyl acetate extract demonstrated a stronger analgesic effect compared to the ethanolic extract, highlighting its potential as an effective natural pain reliever (Table 3).

Hot-plate method: The ethanolic and ethyl acetate extracts of *Echeveria runyonii* significantly (p<0.001) reduced paw-licking behavior in mice 90 minutes after oral administration at doses of 100, 200, and 300 mg/kg body weight. In the ethanolic extract group, inhibition rates were 40.95%, 46.07%, and 50.59% at 100, 200, and 300 mg/kg, respectively. The ethyl acetate extract produced even greater inhibition rates of 48.78%, 52.85%, and 57.22% at the same dosages. For comparison, the positive control, diclofenac sodium, achieved a 66.41% reduction in licking behavior. Notably, the ethyl acetate extract demonstrated the strongest analgesic effect among the test samples, underscoring its promise as a potent natural analgesic agent (Table 4).

Anti-inflammatory: The ethanolic and ethyl acetate extracts of *Echeveria runyonii* produced a significant (p<0.001) and dose-dependent reduction in paw edema volume 90 minutes after administration at doses of 100, 200, and 300 mg/kg body weight. At these concentrations, the ethanolic extract showed inhibition rates of 26.28%, 34.22%, and 42.86%, while the ethyl acetate extract exhibited inhibition rates of 21.56%, 29.15%, and 39.35%, respectively. These effects were comparable to those of diclofenac sodium, the positive control, which achieved the highest inhibition rate of 72.41%. Although both extracts were less effective than diclofenac sodium (administered at 10 mg/kg body weight), the ethanolic extract consistently demonstrated greater anti-inflammatory activity than the ethyl acetate extract (Table 5).

Antispasmodic activity: At doses of 100, 200, and 300 mg/kg body weight, the ethanolic and ethyl acetate extracts of *Echeveria runyonii* significantly ($p\leq$0.001) reduced the intestinal transit of charcoal meals in mice. Their effects were comparable to those of atropine sulfate (10 mg/kg), which also significantly ($p\leq$0.001) inhibited charcoal meal movement. At these doses, the ethyl acetate extract exhibited inhibition rates of 27.37%, 37.59%, and 47.92%, while the ethanolic extract achieved inhibition rates of 20.47%, 36.28%, and 59.88%, respectively. Among all treatments, atropine sulfate produced the highest inhibition at 65.36%. Notably, the ethanolic extract at 300 mg/kg resulted in the most pronounced reduction in charcoal meal transit, with an inhibition rate of 59.88% (Table 6).

Table 3. Analgesic activity of ethanolic and ethyl acetate extract of *Echeveria runyonii* by writhing method.

Groups	Dose (mg/kg)	Median count of writings	Rate of inhibition (%)
N. Saline	10 ml/kg	39.20 ± 1.30384	---------
D. Sodium	25 mg/kg	6.20 ± 1.48324	84.18 (%)
Ethanolic extract	100 mg/kg	30.40 ± 1.140	22.44 (%)
	200 mg/kg	23.80 ± 1.30384	39.28 (%)
	300 mg/kg	11.40 ± 2.50998	70.91 (%)
E. Acetate extract	100 mg/kg	29.60 ± 1.51658	24.48 (%)
	200 mg/kg	23.20 ± 1.30384	40.81 (%)
	300 mg/kg	9.00 ± 0. 70711	77.04 (%)

Table 4. Analgesic properties of ethyl acetate and ethanolic extracts of *Echeveria runyonii* assessed using the hot-plate method.

Medication	Doses (mg/kg)	Licking Numbers (mean ± SEM)			Licking Decrease by %
		30 min	60 min	90 min	
N. Saline	10ml/kg	26.00 ± 1.58114	24.80 ± 1.788	15.60±8.809	……
D. Sodium	25 mg/kg	8.20 ± 1.64317	7.40 ± 1.34164	6.70 ± 1.337	66.41 (%)
Ethanolic extract	100 mg/kg	17.80 ± 1.64317	11.60 ± 4.59952	9.80 ± 2.82056	40.95 (%)
	200 mg/kg	16.40 ± 1.140	10.50 ± 3.50397	8.90 ± 1.85293	46.07 (%)
	300 mg/kg	14.60 ± 1.140	9.80 ± 2.859	8.40 ± 1.577	50.59 (%)
Ethyl acetate extract	100 mg/kg	12.40 ± 5.399	11.40 ± 4.376	10.20 ± 3.224	48.78 (%)
	200 mg/kg	11.60 ± 4.599	10.50 ± 3.597	9.20 ± 2.201	52.85 (%)
	300 mg/kg	10.70 ± 3.653	9.70 ± 2.626	8.00 ± 1.333	57.22 (%)

Table 5. Anti-inflammatory properties of E. acetate and ethanolic extracts from *Echeveria runyonii*.

Medication	Doses (mg per kg)	Volume of the paw after drug administration (Mean ± SEM).			
		Thirty minutes	Sixty minutes	Ninety minutes	percentage of inhibition (%)
N. Saline	10ml/kg	0.9680 ± 0.03834	0.9860 ± 0.00548	0.9700 ± 0.03937	……..
D. Sodium	10 mg/kg	0.3300 ± 0.07649	0.2920 ± 0.05357	0.1840 ± 0.08532	72.41 (%)
E. extract	100 (mg per kg)	0.8520 ± 0.03114	0.7500 ± 0.02915	0.5520 ± 0.05263	26.28 (%)
	200 (mg per kg)	0.7380 ± 0.04494	0.6480 ± 0.05933	0.5360 ± 0.02408	34.22 (%)
	300 (mg per kg)	0.6760 ± 0.05857	0.5980 ± 0.08672	0..4400 ± 0.03937	42.86 (%)
E. acetate extract	100 (mg per kg)	0.8480 ± 0.04764	0.7720 ± 0.05848	0.6720 ± 0.05848	21.56 (%)
	200 (mg per kg)	0.7700 ± 0.04472	0.6920 ± 0.05541	0.6080 ± 0.05070	29.15 (%)
	300 (mg per kg)	0.7060 ± 0.01140	0.6060 ± 0.01140	0.4600 ± 0.05477	39.35 (%)

Table 6. Antispasmodic activity of ethanolic and ethyle acetate extract of *Echeveria runyonii*.

Medication	Doses (mg/kg)	Average intestinal measurement	Average charcoal transit distance	Percentage of inhibition (%)
N. Saline	10ml/kg	54.36	48.60 ± 1.78045	10.59 (%)
Atropine sulphate	10 mg/kg	53.12	18.40 ± 1.53948	65.36 (%)
Ethanolic extract	100 mg/kg	51.48	40.940 ± 1.71114	20.47 (%)
	200 mg/kg	55.28	35.220 ± 2.96176	36.28 (%)
	300 mg/kg	57.04	22.880 ± 3.10355	59.88 (%)
E. Acetate extract	100 mg/kg	55.6	40.380 ± 3.27597	27.37 (%)
	200 mg/kg	56.34	33.10 ± 2.21246	37.59 (%)
	300 mg/kg	53.04	27.620 ± 1.42021	47.92 (%)

Discussion

Plant extract chemo-profiling mainly uses (GC-MS) analysis" especially in pharmaceutical biotechnology and pharmacognosy. Recent studies highlight the value of combining (GC-MS) for the investigation of complex mixtures, including hydrocarbons, secondary metabolites, and essential oils. With this method, several chemicals in these combinations may be separated, identified, and detected (Xie *et al*., 2023). The ethanolic and ethyl acetate extracts of *Echeveria runyonii* revealed a range of bioactive compounds with varying retention times (min). The ethanolic extract contained approximately 14 compounds, some of which have notable therapeutic potential. These included Diisooctyl phthalate, often found in medical tubing, and 9-Octadecenoic acid (E), It is known to reduce inflammatory reactions by modifying the pathways for NF-κB and MAPK signaling in RAW264.7 macrophages stimulated by LPS (Khan *et al.,* 2025). Additionally, 1,4-Benzenedicarboxylic acid, which is employed in the aliphatic diol-based polyester manufacturing process, was also discovered (Reddy *et al*., 2017), and 4-Benzylimidazole-5-(1-propenoic acid), which has applications in anticancer treatments, antihypertensives, antivirals, antifungals, anti-HIVs, anticonvulsants, and antidiabetics (Islam *et al*., 2023). Additionally, 3,5,6-trimethyl serves as a solvent for aromatic halogenation and nitration, essential for preparing pesticides and pharmaceuticals (Ohtake & Wang, 2011). While Trehalose is utilized as a sweetener, stabilizer and medicines industries (Thomas *et al*., 1984). The ethyl acetate fraction, analyzed by GC-MS, revealed 8 compounds, including Bis(2-ethylhexyl) phthalate, which acts as an apoptosis inhibitor, an androstane receptor agonist, and a plasticizer (Sakuta & Nakamura, 2019). Hexanedioic acid is used in producing nylon 6,6 polymer for fibers and plastics (Chambers *et al*., 2017). While 5-Eicosene (E) is recognized for its strong antimicrobial properties, and Cetene is employed in treating serious health conditions (Madi` eye *et al*., 2023). The analgesic effects of *E. runyonii* may be linked to various bioactive compounds, such as 9-Octadecenoic acid (E), Benzylimidazole-5-(1-propenoic acid), and 5-Eicosene (E), all identified in the plant extracts. Acetic acid-induced constriction of the abdomen test is utilized to evaluate peripherally acting analgesics, as pain is triggered by the release of endogenous substances and other pain mediators, including prostaglandins (Yassine *et al*., 2016). The test for writhing caused by acetic acid is linked to increased levels of lipoxygenase products and prostaglandins (PGF2α and PGE2) in peritoneal fluids (Ambrin *et al*., 2024). Both ethanolic and ethyl acetate extracts of *E. runyonii* significantly (p<0.001) decreased writhing responses in mice across all dosages in comparison group, according to the writhing caused by acetic acid test used in this investigation. Both the ethanolic and ethyl acetate extracts demonstrated significant percentage inhibition at 100, 200, and 300 Milligrams for each kilogram. These outcomes are in line with earlier research by Ambrin *et al*., (2024), which found that ethanolic and ethyl acetate extracts from *Oenothera biennis* and *Ziziphus mauritiana* had comparable inhibitory effects. Furthermore, in comparison to the C. group, both extracts demonstrated a substantial analgesic effect after 90 minutes in the (HP) test at a dosage of 300 Milligrams for each kilogram (Table 3). This aligns with findings from Alam *et al*., (2023), who reported marked analgesic effects for *Ageratina glabrata*. Inflammation is a natural physiological response that protects the body from tissue damage, with two primary types: acute and chronic (Chen *et al*., 2019). Anti-inflammatory medications are frequently evaluated using the carrageenan-induced inflammation paradigm, with diclofenac sodium serving as a popular reference. Diclofenac sodium is a widely utilized NSAID known for its effectiveness against pain and inflammation (Rahman *et al*., 2023). In the rat paw model, acute inflammation is triggered by a subcutaneous carrageenan injection, which releases mediators such as histamine, prostaglandins, and serotonin, causing pain and fever (Zhao *et al*., 2018). *E. runyonii* extracts in ethanol and ethyl acetate showed substantial anti-inflammatory effects at a 300 mg/kg dose after 90 minutes, comparable to those of diclofenac sodium, aligning with similar results reported for *Juniperus sabina* (Qadir *et al*., 2014). Drug effects on peristalsis can be evaluated with the charcoal meal test (Sumitha *et al*., 2022). The hydrolysis of castor oil generates ricinoleic acid can cause intestinal mucosal irritation and inflammation, which can result in diarrhea. This is because the production of water and electrolytes as well as gastrointestinal motility are driven by prostaglandin release (Ventura-Martinez *et al*., 2020). In comparison to atropine sulfate (10 Milligrams for each kilogram), which also significantly decreased the movement of the charcoal meal (p≤0.001), the ethyl acetate and ethanolic extracts of *E. runyonii* revealed notable antispasmodic effects, with doses of 100, 200, and 300 Milligrams for each kilogram notably reducing the distance traveled by the charcoal meal (p≤0.001). Udobang *et al*., (2022) demonstrated comparable outcomes using the ethanolic extract of *S. anomalum*, which is consistent with these findings.

Conclusion

This study provides compelling evidence for the medicinal potential of *Echeveria runyonii*, demonstrating that its ethanolic and ethyl acetate extracts contain a range of bioactive compounds with significant analgesic, anti-inflammatory, and antispasmodic properties. GC-MS analysis successfully identified several pharmacologically relevant metabolites, and the efficacy of the extracts was substantiated through robust experimental methodologies, including the hot plate and acetic acid-induced writhing tests, as well as carrageenan-induced paw edema and gastrointestinal motility assays. These findings suggest that *E. runyonii* may serve as a valuable source for the development of novel therapeutic agents. Future studies should focus on isolating and characterizing individual bioactive constituents to assess their safety and efficacy in clinical settings, elucidate their specific mechanisms of action, and explore the potential for developing synergistic formulations. Investigating the broader therapeutic applications of *E. runyonii* could enhance its role in modern medicine and contribute to the discovery of new treatments for pain, inflammation, and gastrointestinal disorders.

Author contributions

M. Naseer; Writing – Original Draft Preparation, Conceptualization, Resources, Validation and Data Curation, M. Adil; Supervision, Project Administration, Writing – Review & Editing and Visualization,

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