HPTLC Fingerprinting Profile and GC-MS Analysis of Methanolic Fruit Extract of Akre's Rhus coriaria (Sumac)

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Abstract— **The study was aimed to determine the chemical components, present in the methanolic fruit extract of Rhus coriaria that was sourced from Akre, Kurdistan, Iraq, using high-performance thin-layer chromatography (HPTLC) and gas chromatography-mass spectrometry (GC-MS). A mobile phase system composed of toluene, ethyl acetate, and formic acid in the ratio of 7:3:0.5 (v/v/v) was used to perform the HPTLC fingerprint analysis on the methanolic extract. The results showed that at 254 nm, 366 nm, and 520 nm, the component at peak 4, 1, and 13 have the largest peak area percentages at 39.62%, 13.3%, and 22.51%, respectively. The reports of GC-MS detected the presence of twenty-seven phytochemicals. The most prevalent components in the extract, includes 3-(Hexadecyloxy)propan-1-ol (7.56%), Phytyl decanoate (6.78 %), followed by (R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman (5.66%).**

Index Terms— **Rhus coriaria, Sumac, HPTLC, GC-MS, Akre.**

I. Introduction

Plants have traditionally been utilized as medicine and phytochemicals. Natural sources, especially edible therapeutic herbs, are ideal because they are cheap, safe, and accessible. Herbal remedies have been utilized for generations, and the global health business has become vital. Herbal remedies, bioactive pharmaceutical molecules, pharmacological tools, and patentable entities with higher activity and/or reduced toxicity remain as the goals of plant medicine. The safety, efficacy, and low side effects of herbal primary care medications make them popular.. Pharmacological biology acknowledges natural compounds, and plants as that have been the principal medicine source since ancient times. Ancient herbal remedies were potent medicinal agents due to their biological benefits and cultural beliefs. [1-13]

In polyphenol-rich plants like flavonoids, coumarins, and anthocyanins, pharmaceutical companies seek novel lead structures and standardized phototherapeutic agents. Herbal drug standardization and quality control are difficult due to phytoconstituent variability. Plant phytochemicals possess anti-bacterial, anti-inflammatory, analgesic, diuretic, antioxidant, and anti-fungal activity. Medicinal plant products have novel pharmacological potential due to their chemical variety and affordability. [1, 5, 8, 12-14]. The World Health Organization (WHO) reports that over 80% of the global population uses traditional medicine for basic healthcare. The WHO assists health ministries in implementing plant therapies, evaluating efficacy and safety, and monitoring raw material quality. The "Traditional Medicine Strategy" sets guidelines, conducts research, and integrates Traditional Medicine products into national healthcare systems. [2, 4, 7, 10, 11, 15].

Chromatographic fingerprints are crucial for ensuring the chemical integrity and authentication of herbal medicines due to their diversity in chemical components. Modern chromatographic methods like GC-MS and HPTLC help to identify, estimate, and standardize the drugs particularly the herbals. These methods provide effective verification and identification, even when chemically distinctive elements are not identical. Hybrid technologies like GC-MS provide structural verification data on new medicines and physiologically active secondary metabolites. Chromatographic fingerprinting is essential for quality control and assurance in herbal medicine. [3, 5, 6, 9, 16-22].

A small Anacardiaceous tree with approximately 250 species, Rhus coriaria L. grows in North Africa, the Mediterranean, the Caucasus, Afghanistan, Iran, and Central Asia. It thrives near northern Iraqi villages and in Kurdistan's lower woodlands. The plant is 0.5-3 meters tall and anti-carcinogenic, anti-inflammatory, antioxidant, antifungal, antibacterial, hypoglycemic, digestive, antidiabetic, and anticholinergic. Rhus coriaria is used traditionally to treat diarrhea, dysentery, ulcers, bleeding, and liver disease [23-33]. The present study was aimed to analyze and record the phytochemical contents in the methanol extract of Rhus coriaria fruit from Akre, known for its high-quality Sumac. The analysis was conducted using HPTLC and GC-MS techniques.

II. Materials and Methods

A. Collection and authentication of plant materials

Rhus coriaria L. fruits were harvested from Mountain Akre (36° 48′ 41.94″ N, 43° 48′ 0.9″ E), Duhok, Kurdistan, Iraq in July 2023. The fruits were recognized and certified at the Department of Pharmacognosy, Siddha Central Research Institute (CCRS), which is under the Ministry of Ayush, Government of India (PCOG002-ACF). The Rhus coriaria L. fruits were meticulously cleaned, finely sliced, and then dried in the shade to remove any excess moisture. The dried material was then crushed into a fine powder and stored in an airtight container to avoid any possible contamination from moisture.

DOI: http://doi.org/10.24086/biohs2024/paper.1425

B. Preparation of Extract

A total of 600 grams of air-dried and coarsely powdered fruit from the Rhus coriaria L. plant were extracted utilizing a sequential maceration procedure, with methanol and water separately. The extraction process lasted around 72 hours, after which it was concentrated using a vacuum evaporator, and then transported for further investigation. Phytochemical screening was conducted to identify the primary and secondary metabolites present in the crude plant medicine powder and extracts [34-36].

C. HPTLC Fingerprinting

The sample was subjected to sonication with 10 ml of ethanol for a duration of 10 minutes. The extracts that underwent filtration was transferred into a sample vial. An amount of 10, 15, and 20µl of the extract was applied to a silica-coated TLC plate (60 F254) using Camag's ATS4 applicator. The plate was developed by placing it in a 10×10 cm CAMAG twin trough chamber that had been saturated with the mobile phase comprised of toluene, ethyl acetate, and formic acid at a ratio of 7:3:0.5 v/v/v. After the development, the plate was imaged using Camag's TLC Visualizer, under 254 nm and 366 nm. The plates were scanned and the fingerprint profiles were generated, using 5% vanillin sulfuric acid as the detecting agent.

D. GC-MS Analysis

A 10 g sample of plant material was subjected to extraction using 40 mL of 70% (v/v) aqueous methanol in a shaker bath maintained at a temperature of 40 oC for a duration of 48 hours. The resulting mixture was then filtered and purified using ethanol. The GC-MS analysis of the methanolic Rhus coriaria fruit extract was carried out using a Shimadzu gas chromatography-mass spectrometer (GC-MS). The GC-MS consisted of a helium carrier gas and a 30-meter column with dimensions of 0.25 mm internal diameter and 0.25 µm df. We used a splitless injection mode and an injection temperature of 320.00 °C, with a column oven temperature of 50.0 °C. The flow control system is configured with the following parameters: linear velocity (31.6 cm/sec), pressure (33.5 kPa), total flow (50.0 mL/min), and column flow (0.76 mL/min). The ion source and interface temperatures are 230.00 and 320.00 °C, respectively, and 2.5 minutes was the solvent cut time with operating mode of 1.50 kV.

III. Result and Discussion

A. HPTLC Fingerprinting

By utilizing HPTLC, the constituents of the methanol extract of Rhus coriaria were distinctly separated. Using this technique, the plant of Akre's Rhus coriaria was differentiated from those of other species. The utilization of HPTLC profiles allows for the identification and differentiation of a specific plant from closely related species [19]. Utilizing the presence or absence of a chemical constituent to classify plants into taxonomic groups has been discovered to be beneficial. A range of solvent compositions was investigated for the mobile phase of HPTLC analysis with the aim of attaining peaks with consistent resolution and reproducibility. Chromatography was performed on a methanolic extract of Rhus coriaria in a chamber saturated with a mixture of toluene, ethyl acetate, and formic acid (7:3:0.5 v/v) serving as the solvent system or mobile phase. Methanolic Rhus coriaria extracts were fingerprinted using chromatographic techniques.

The HPTLC photo documentation of Rhus coriaria (Methanolic extract) at a wavelength of 254 nm revealed the presence of 9 spots. These spots were observed at specific Rf values of 0.06, 0.12, 0.17, 0.30, 0.32, 0.43, 0.69, 0.79, and 0.93, as depicted in Figure 1 and Table 1.At a wavelength of 366 nm, the extract showed 9 spots in the colors of blue, dark blue, and fluorescent blue. These spots appeared at the following Rf values: 0.04, 0.11, 0.17, 0.32, 0.37, 0.40, 0.49, 0.80, and 0.89. At a wavelength of 520 nm, specifically using derivatized vanillin-sulphuric acid, the extract displayed 12 spots in shades of purple and yellowish purple. These spots were observed at the Rf values 0.05, 0.20, 0.27, 0.34, 0.40, 0.42, 0.46, 0.57, 0.62, 0.69, and 0.77.

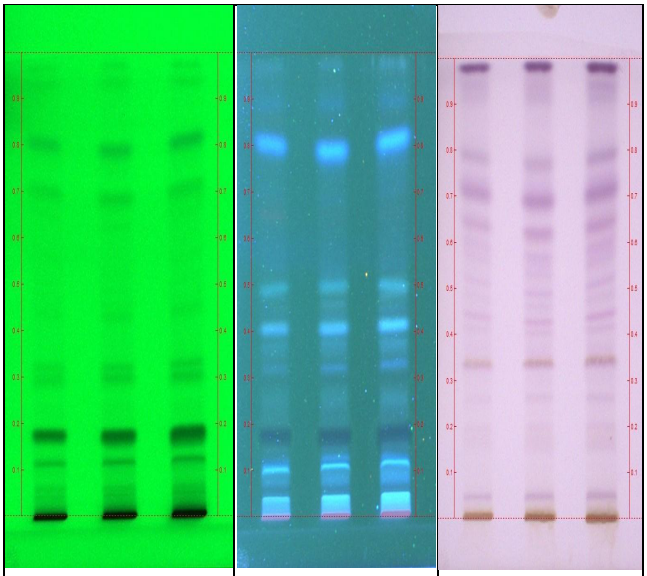

Fig. 1. HPTLC photo documentation of Rhus coriaria (Methanolic extract).

Table 1: Rf and color values of Rhus coriaria fruit extract.

S.NO.	R_f value(s)	Color	R_f value(s)	Color	R_f value(s)	Color
1	0.06	Dark	0.04	Blue	0.05	Purple
2	0.12	Dark	0.11	Blue	0.20	Purple
3	0.17	Dark	0.17	Dark blue	0.27	Purple
4	0.30	Dark	0.32	Blue	0.34	Yellowish Purple
5	0.32	Dark	0.37	Blue	0.40	Purple
6	0.43	Dark	0.40	Blue	0.42	Purple
7	0.69	Dark	0.49	Blue	0.46	Purple
8	0.79	Dark	0.80	Fluorescent Blue	0.49	Purple
9	0.93	Dark	0.89	Blue	0.57	Purple
10	-	-	-	-	0.62	Purple
11	-	-	-	-	0.69	Purple
12	-	-	-	-	0.77	Purple

The HPTLC fingerprint profile of the methanolic extract of Rhus coriaria, when scanned at a wavelength of 254 nm,

DOI: http://doi.org/10.24086/biohs2024/paper.1425

exhibited 14 distinct peak areas. These peaks ranged in Rf values from 0.03 to 0.96. Among these peaks, peak 4 displayed the highest maximum value at 559.8 AU. This value corresponds to a maximum percentage of 34.99% and an area of 15330.4 AU, which accounts for 39.62% of the total area (Figure 2 & Table 2).

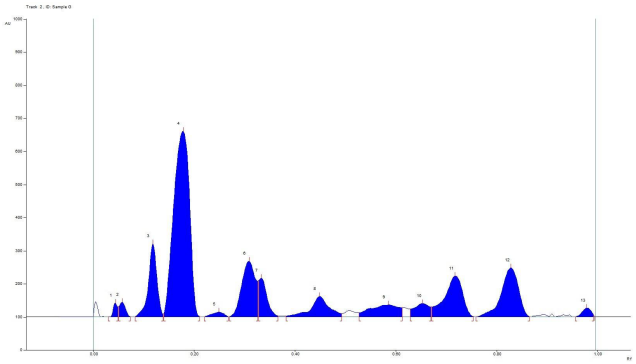

Fig. 2. HPTLC finger print profile of Rhus coriaria methanolic extract scanning at 254 nm

Table 2: Components detected in HPTLC of Rhus coriaria Methanolic extract at 254 nm

Peak	Start Position (R_f)	Start Height (AU)	Max Position (R_f)	Max Height (AU)	Max %	End Position (R_f)	End Height (AU)	Area (AU)	Area %
1	0.03	0.0	0.04	41.1	2.57	0.05	29.8	304.4	0.79
2	0.05	31.5	0.06	43.6	2.72	0.07	0.3	464.8	1.20
3	0.08	0.4	0.12	220.5	13.78	0.14	7.7	3218.5	8.32
4	0.14	9.8	0.18	559.8	34.99	0.21	0.6	15330.4	39.62
5	0.22	0.0	0.25	14.5	0.91	0.27	0.3	286.9	0.74
6	0.27	0.2	0.31	167.9	10.50	0.33	8.1	3952.3	10.21
7	0.33	109.1	0.33	116.5	7.28	0.37	2.9	1688.4	4.36
8	0.38	2.5	0.45	61.4	3.84	0.49	10.3	1949.2	5.04
9	0.53	12.3	0.59	35.9	2.25	0.61	27.5	1809.2	4.68
10	0.63	23.9	0.65	40.5	2.53	0.67	30.0	1042.4	2.69
11	0.67	30.1	0.72	123.4	7.72	0.75	1.1	3843.9	9.93
12	0.76	0.1	0.83	147.8	9.24	0.87	0.4	4399.5	11.37
13	0.96	0.0	0.98	26.7	1.67	0.99	6.8	405.5	1.05

The HPTLC fingerprint profile of the methanolic extract of Rhus coriaria, when scanned at a wavelength of 366 nm, exhibited 14 distinct peak areas. These peaks ranged in Rf values from 0.01 to 0.88. Peak 1 exhibits the greatest maximum value (418.6 AU), maximum percentage (16.64%), area (9504.9 AU), and area percentage (13.3%) as seen in (Figure 3 & Table 3).

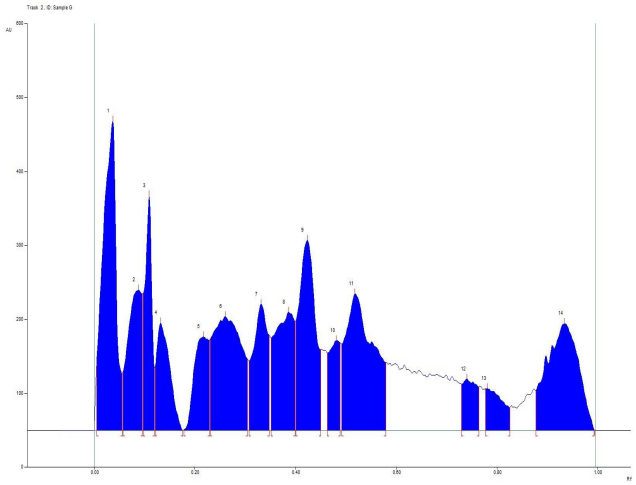

Fig. 3. HPTLC finger print profile of Rhus coriaria methanolic extract scanning at 366 nm.

Table 3: Components detected in HPTLC of Rhus coriaria methanolic extract at 366 nm.

Peak	Start Position (R_f)	Start Height (AU)	Max Position (R_f)	Max Height (AU)	Max %	End Position (R_f)	End Height (AU)	Area (AU)	Area %
1	0.01	95.5	0.04	418.6	16.64	0.06	76.7	9504.9	13.3
2	0.06	79.2	0.09	189.6	7.54	0.10	84.4	4635.8	6.49
3	0.10	185.1	0.11	317.4	12.62	0.12	84.5	3830.7	5.36
4	0.12	85.9	0.13	145.7	5.79	0.18	0.2	3325.2	4.65
5	0.18	0.8	0.22	126.9	5.04	0.23	21.9	3200.7	4.48
6	0.23	122.9	0.26	153.8	6.11	0.31	96.3	7627.8	10.68
7	0.31	93.8	0.33	170.3	6.77	0.35	28.6	4138.0	5.86
8	0.35	124.8	0.39	160.2	6.37	0.40	47.1	5355.0	7.5
9	0.40	148.6	0.42	257.4	10.23	0.45	9.1	7209.0	10.09
10	0.46	104.9	0.48	121.4	4.83	0.49	18.5	2292.0	3.21
11	0.49	116.7	0.52	184.5	7.33	0.58	92.2	8913.9	12.48
12	0.73	62.9	0.74	69.5	2.76	0.77	58.5	1735.3	2.43
13	0.78	56.3	0.78	56.4	2.24	0.83	31.1	1681.6	2.35
14	0.88	54.3	0.93	144.4	5.74	0.99	1.8	7944.0	11.12

The HPTLC fingerprint profile of the methanolic extract of Rhus coriaria, when scanned at 520 nm, exhibited 16 peaks with different areas. The peaks ranged from an Rf value of 0.03 to 0.96. Among these peaks, peak 13 displayed the highest maximum value of 277.2 AU, representing 14.35% of the total area. The area under peak 13 was measured to be 10857 AU, accounting for 22.51% of the total area. These findings are illustrated in (Figure 4 and summarized in Table 4).

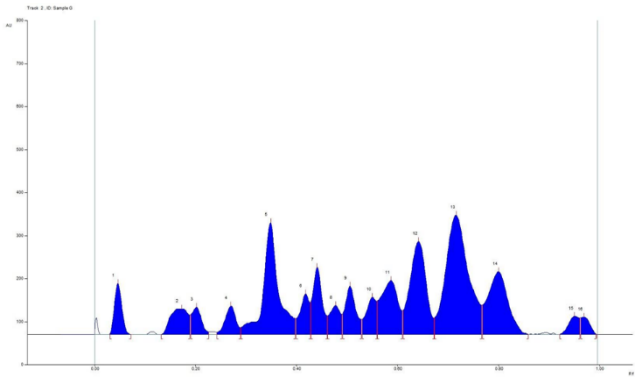

Fig. 4. HPTLC finger print profile of Rhus coriaria methanolic extract scanning at 520 nm.

Table 4: Components detected in HPTLC of Rhus coriaria methanolic extract at 520 nm.

Peak	Start Position (R_f)	Start Height (AU)	Max Position (R_f)	Max Height AU)	Max %	End Position (R_f)	End Height AU)	Area (AU)	Area %
1	0.03	1.8	0.05	118.9	6.16	0.07	0.1	1521.9	3.16
2	0.13	0.3	0.17	59.4	3.08	0.19	45.1	1837.8	3.81
3	0.19	45.6	0.20	63.2	3.27	0.23	5.8	1059.9	2.20
4	0.24	5.1	0.27	65.8	3.41	0.29	15.9	1212.3	2.51
5	0.29	16.0	0.35	260.3	13.48	0.40	37.1	6814.8	14.13
6	0.40	37.4	0.42	94.3	4.88	0.43	73.9	1521.8	3.16
7	0.43	76.0	0.44	155.4	8.05	0.46	43.4	2491.8	5.17
8	0.46	43.7	0.48	67.1	3.47	0.49	46.7	1231.4	2.55
9	0.49	47.5	0.51	113.0	5.85	0.53	34.9	2048.0	4.25
10	0.53	35.4	0.55	86.4	4.47	0.56	77.5	1520.4	3.15
11	0.56	77.9	0.59	125.4	6.5	0.61	55.1	3638.6	7.54
12	0.61	55.9	0.64	215.9	11.18	0.67	38.3	6036.1	12.52
13	0.67	39.1	0.72	277.2	14.35	0.77	68.2	10857	22.51
14	0.77	68.7	0.80	146.1	7.57	0.86	0.1	5029.4	10.43
15	0.92	0.3	0.95	42.4	2.19	0.96	38.6	779.6	1.62
16	0.96	38.7	0.97	40.2	2.08	0.99	0.8	628.3	1.30

DOI: http://doi.org/10.24086/biohs2024/paper.1425

B. GC-MS Analysis

Volatile compounds are essential for the functioning of medicinal plant healthcare systems. GC-MS has been utilized in various regions worldwide to document numerous volatile bioactives present in herbal extracts. The gas chromatogram displays the proportional concentrations of different chemicals being separated based on their retention time. The magnitude of the peak signifies the comparative levels of the constituents found in the plant. The mass spectrometer examines the compounds that are separated at various intervals to determine the characteristics and arrangement of the compounds. The mass spectra serve as distinctive patterns of the molecule, allowing for its identification using the data library [21].

In the current study, by employing gas chromatography-mass spectrometry (GC-MS), the components of Rhus coriaria fruits were identified (Figure 5). The active principles included in the Rhus coriaria fruit methanolic extract are listed in Table 5, along with their corresponding retention times (RT), molecular weights (MW), retention time and percentage area. In the active portion, 27 different components were found. The principal components consisted of 3-(Hexadecyloxy)propan-1-ol, Phytyl decanoate (6.78%), Eupomatenoid 5, and (R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman (5.66%).

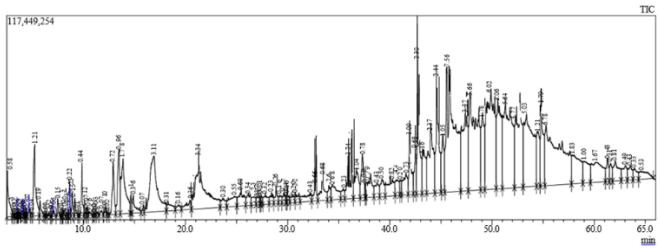

Fig. 5. GC-MS chromatogram of the methanolic fruit extract of Rhus coriaria.

Table 5: Major phytocompounds identified from the methanolic fruit extract of Rhus coriaria by GC-MS analysis

S.NO.	Peak	Name of the Component	Retention Time (Rt)	Molecule Formula	Molecular Weight	Area %
1	19	2(3H)-Furanone, 5-methyl-	5.245	$C_5H_6O_2$	98	1.21
2	48	Hexanoic acid, 3-butenyl ester	13.897	$C_{10}H_{18}O_2$	170	1.78
3	52	Butanoic acid, propyl ester	16.914	$C_7H_{14}O_2$	130	3.11
4	56	Kessanyl acetate	21.326	$C_{17}H_{28}O_3$	280	3.34
5	80	10,13-Octadecadienoic acid, methyl ester	35.889	$C_{19}H_{34}O_2$	294	1.24
6	81	9-Octadecenal, (Z)-	36.757	$C_{18}H_{34}O$	266	1.04
7	91	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	41.973	C25H42	342	1.09
8	93	Phenol, 3-(8-pentadecenyl)-, acetate, (Z)-	42.688	$C_{23}H_{36}O_2$	344	2.3
9	94	3,4,5,6,7,8-Hexahydrospiro[1-benzopyran-2,1'-cyclohexane]-6'-one	43.236	$C_{14}H_{20}O_2$	220	1.16
10	95	6-Monoacetylmorphine	44.029	$C_{19}H_{21}NO_4$	327	2.37
11	96	5-Butyl-5-ethylpentadecane	44.603	C21H44	296	2.44
12	97	2,2'-Thiodiacetamide, N,N'-dicyclohexyl-	45.191	C16H28N 2O2S	312	1.05
13	98	3-(Hexadecyloxy)propan-1-ol, TMS	45.582	$C_{22}H_{48}O_2Si$	372	7.56
14	99	Glycidol stearate	47.37	$C_{21}H_{40}O_3$	340	3.37
15	100	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman	47.885	$C_{28}H_{48}O_2$	416	5.66
16	101	Eicosane	49.069	$C_{20}H_{42}$	282	1.78
17	102	Eupomatenoid 5, TMS	49.907	$C_{23}H_{38}O_3Si$	366	6.02
18	103	.gamma.-Sitosterol	50.586	$C_{29}H_{50}O$	414	3.06
19	104	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,1	51.32	$C_{30}H_{52}O$	428	5.64
20	105	D:B-Friedo-18,19-secolup-19-ene, 3,10-epoxy-, (3.beta.,10.beta.)-	52.326	$C_{30}H_{50}O$	426	3.77
21	106	2,5-di-tert-Butyl-1,4-benzoquinone	53.38	$C_{14}H_{20}O_2$	220	5.03
22	107	Obtusifoliol	54.446	$C_{30}H_{50}O$	426	1.21
23	108	gamma.-Sitosterol	54.83	$C_{29}H_{50}O$	414	1.79
24	109	Phytyl decanoate	55.298	$C_{30}H_{58}O_2$	450	6.78
25	110	7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	57.881	$C_{14}H_{14}O_3$	230	1.83
26	111	4-Bromo-2-trimethylsilylthiazole	58.98	$C_6H_{10}BrNSSi$	235	1
27	112	Acetamide, N-cyclohexyl-2-[[1-(2-propenyl)-1H-1,2,3,4-tetrazol-5-yl]thio]-	60.154	$C_{12}H_{19}N_5OS$	281	1.67

IV. Conclusion

Rhus coriaria, or "sumac," has traditionally been used to treat many medical ailments. The study examined and documented the volatile bioactive components in Akre-region R. coriaria methanolic extract. The fruit of R. coriaria was dried in the shade, crushed into a powder, and macerated with methanol. HPTLC and GC-MS were performed on the methanolic extract. The HPTLC and GC-MS profiles of the methanolic extract showed a variety of secondary metabolites. Performing HPTLC fingerprint analysis on the methanolic extract, Peak 4, 1, and 13 had the highest peak area percentages at 39.62%, 13.3%, and 22.51% at wavelengths of 254 nm, 366 nm, and 520 nm, respectively. The GC-MS results identified twenty-seven phytochemicals. The study aims to investigate and document the phytochemical components in the methanol extract of Rhus coriaria fruit from Akre, renowned for its premium Sumac. The analysis was performed using (HPTLC) and (GC-MS) techniques.

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