Phytochemical Characterization and Radical Scavenging Activity of *Roylea cinerea* Leaves

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Abstract

Roylea cinerea, a medicinal plant belonging to the Lamiaceae family, is widely recognized for its pharmacological properties including anti-inflammatory, antimicrobial and antioxidant activities. This study aimed to comprehensively analyse the phytochemical composition and antioxidant potential of Roylea cinerea leaves using qualitative and quantitative assessments. Various solvents including chloroform, acetone, methanol and ethanol were employed to extract key bioactive compounds such as alkaloids, flavonoids and phenolics. The phytochemical screening confirmed the presence of these compounds across all solvents, with acetone demonstrating the highest efficiency in extracting alkaloids and flavonoids, while also exhibiting the highest phenolic content. The antioxidant activity of the extracts was evaluated using the DPPH assay which indicated that methanol and ethanol extracts exhibited the most potent free radical scavenging activity, followed by chloroform. HPLC analysis further validated the separation and identification of major bioactive compounds, although incomplete separation of late-eluting compounds was observed in acetone and methanol extracts.

This research validates the traditional medicinal uses of Roylea cinerea and underscores its potential for pharmaceutical and nutraceutical applications. The strong antioxidant properties of the plant suggest its relevance in combating oxidative stress-related disorders such as cancer, neurodegenerative diseases and cardiovascular conditions. The study contributes to the growing interest in natural product research by providing insights into the bioactive potential of Roylea cinerea, paving the way for future drug discovery and the development of plant-based therapeutics. Further investigations into the isolation and characterization of individual compounds will enhance the understanding of the plant's therapeutic efficacy.

Keywords: Phytochemicals, antioxidation, DPPH, HPLC, *Roylea cinerea*.

Introduction

Roylea cinerea, a member of the Lamiaceae family, is a medicinally significant plant known for its diverse pharmacological properties[14]. Traditionally, it has been used in herbal medicine for treating various ailments due to its anti-inflammatory, antimicrobial and antioxidant activities which are attributed to its rich phytochemical profile including alkaloids, flavonoids and phenolic compounds. Its anti-inflammatory properties make it a potential candidate for managing conditions like arthritis and other inflammatory diseases[7] while its antimicrobial activity has been highlighted in studies demonstrating its effectiveness against drug-resistant bacterial and fungal infections[14]. Additionally, the plant exhibits strong antioxidant potential, contributing to its protective effects against oxidative stress-related diseases such as cancer, neurodegenerative disorders and cardiovascular conditions.

Ethnobotanically, *Roylea cinerea* has been widely used in traditional medicine for wound healing, skin infections, respiratory ailments, gastrointestinal disorders and fever, showcasing its broad therapeutic relevance[22]. The plant's pharmacological benefits have led to increasing interest in its pharmaceutical and nutraceutical applications, particularly in the formulation of herbal medicines[16], dietary supplements and functional foods. As research on natural products expands, *Roylea cinerea* holds significant promise for the discovery of novel bioactive compounds that could contribute to drug development for chronic diseases[23]. With continued advancements in phytochemical analysis and biotechnology, this plant is poised to play a crucial role in modern medicine, offering sustainable and innovative healthcare solutions.

The present study aims to comprehensively analyse the phytochemical composition and antioxidant potential of *Roylea cinerea* through a systematic approach. By employing qualitative and quantitative analyses, this research seeks to identify the key bioactive constituents responsible for its therapeutic effects. Advanced techniques such as High-Performance Liquid Chromatography (HPLC) further enhance the accuracy and reliability of the phytochemical profiling. The significance of this research lies in its potential to validate the traditional uses of *Roylea cinerea* and to explore its applications in pharmaceutical and nutraceutical industries. Understanding the chemical composition and biological activities of this plant could pave the way for the development of novel natural remedies and contribute to the growing field of plant-based therapeutics.

Material and Methods

Sample Collection and Identification: The fresh leaves of *Roylea cinerea* were collected from the adjoining area of

Panchkula-Badiyal Road, Haryana, ensuring that they were harvested in their optimal physiological state. The plant species was authenticated by a qualified botanist, who verified its taxonomic identity based on morphological characteristics. A voucher specimen was prepared and deposited in a recognized herbarium for future reference.

Sample Extraction: Plant leaves were oven-dried at 40°C for 24 hours. The dried plant sample was finely pulverized using a mortar and pestle. Subsequently, 30 g of pulverized sample was subjected to separate extractions using 150 mL of solvents of increasing polarity (chloroform, acetone, methanol, ethanol and water)[6]. The extraction process was carried out for 20 cycles using a continuous extraction system[23] (Pyrex® Soxhlet apparatus). All filtrates were pre-concentrated under reduced pressure using the rotary evaporator at 60˚C to yield the crude extracts.

Qualitative Screening of Phytochemicals: Conventional phytochemical tests were employed due to their cost-effectiveness, simplicity and minimal resource requirements, making them a suitable choice for preliminary phytochemical screening. The obtained plant extracts were further analysed qualitatively for the presence of selected phytochemicals using various chemical reagents.

Quantification of Alkaloid content: 1 mL of the plant extract was accurately weighed into a 250 mL beaker, followed by the addition of 40 mL of 10% acetic acid. The mixture was covered and allowed to stand for 4 hours to facilitate extraction. The solution was then filtered and the filtrate was concentrated using a water bath to reduce the volume to one-quarter of its original size.

Concentrated ammonium hydroxide was added dropwise to the concentrated extract until precipitation occurred[2]. The solution was then allowed to settle and the precipitate was collected by filtration, followed by washing with dilute ammonium hydroxide to remove impurities. The reaction mixture was incubated in the dark for 30 minutes and the absorbance was measured at 512 nm using a spectrophotometer. The alkaloid content was calculated as caffeine equivalents CAF/ g of dry plant material based on a standard curve of Caffeine (mg/ ml)[8].

Quantification of Flavonoid content: The total flavonoid content was determined using the aluminium chloride colorimetric method. 1 mL aliquot of each plant extract was diluted to 10 mL with the corresponding solution. Subsequently, 1 mL of the diluted extract was combined with 100 µL of 10% aluminium chloride solution and 100 µL of 1 M potassium acetate. The reaction mixture was incubated at room temperature for 30 minutes, after which the absorbance was measured at 415 nm using a UV-visible spectrophotometer. The flavonoid concentration was calculated as quercetin equivalents QUE/g of dry plant material, based on a standard calibration curve of quercetin (mg/mL)[12].

Quantification of Phenolic content: The total phenolic content was determined using the Folin–Ciocalteu reagent. 1 mL aliquot of each plant extract was diluted to 10 mL with the corresponding solution. Subsequently, 1 mL of the diluted extract was transferred to a test tube, followed by the addition of 0.5 mL of Folin–Ciocalteu reagent (diluted to 50% with distilled water) and 4 mL of 1 M sodium carbonate solution. The reaction mixture was incubated at room temperature for 40 minutes and the absorbance was measured at 765 nm using a UV-visible spectrophotometer. The total phenolic content was expressed as gallic acid equivalents GAE/g of dry plant material, based on a standard calibration curve of gallic acid (mg/mL)[21].

Determination of Free Radical Scavenging Activity: The free radical scavenging activity of *Roylea cinerea* plant extracts was evaluated using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay, as measured by UV–Vis spectrophotometry[10]. In this assay, 800 µL of 0.1 M Tris-HCl buffer (pH 7.4) was added to a test tube followed by the addition of 200 µL of the plant extract and 1 mL of 0.1 mM DPPH solution. The components were thoroughly mixed to ensure uniformity. A control solution was prepared by combining 800 µL of 0.1 M Tris-HCl buffer (pH 7.4) with 1.2 mL of methanol which served as a blank[13].

All reaction mixtures were incubated at room temperature in the dark for 30 minutes to allow for the completion of the reaction. Following incubation, the absorbance of the test samples was measured at 517 nm against the blank. The absorbance recorded in the presence of the plant extract was designated as ‘As’, while the absorbance of the control solution (where methanol replaced the extract) was denoted as ‘Ac’. The percentage inhibition of free radical activity by the plant extracts was calculated using the following equation:

Free radical scavenging activity (%) = [Ac- As] / Ac x 100

where Ac=Absorbance of the control and As=Absorbance of the sample, at 517nm[4]. This assay provided a quantitative measure of the antioxidant potential of *Roylea cinerea* extracts based on their ability to scavenge DPPH free radicals.

High-performance liquid chromatography: High-performance liquid chromatography (HPLC) was employed to analyse the phytochemical composition of the plant extracts under optimized instrumentation and chromatographic conditions[6]. A reverse-phase C18 column (250 mm × 4.6 mm, 5 µm particle size) was utilized for separation. The mobile phase consisted of a gradient or isocratic system comprising solvent A (water with 0.1% formic acid) and solvent B (either acetonitrile or methanol), ensuring effective compound resolution. The flow rate was maintained at 1.0 mL/min, with an injection volume of 10 µL. The column temperature was set at 25°C to maintain stability and reproducibility during the analysis. Detection

wavelengths were selected based on the maximum absorbance (λmax) of each phytochemical, typically ranging between 200 – 400 nm. The total run time was adjusted between 30–60 minutes, depending on the number of compounds present and the efficiency of separation[20].

Results and Discussion

Qualitative analysis of Phytochemicals: The extraction analysis revealed the presence of alkaloids, flavonoids and phenolic compounds in all tested solvents (Table 1). Chloroform successfully extracted all three classes of compounds, indicating its efficiency as an extraction medium. Similarly, acetone demonstrated the ability to extract alkaloids, flavonoids and phenolic compounds, suggesting its suitability for phytochemical extraction.

Methanol also facilitated the extraction of these bioactive compounds, further confirming its effectiveness as a solvent. Ethanol exhibited similar results, effectively extracting alkaloids, flavonoids and phenolic compounds. Water, despite its lower efficiency in antioxidant extraction, was also found to extract all three classes of compounds. These findings suggested that each solvent possessed the capability to extract alkaloids, flavonoids and phenolic compounds with potential variations in efficiency.

Quantitative analysis: Different solvents were utilized to extract essential bioactive compounds and their presence was confirmed through phytochemical screening. The phytochemicals content was assessed based on the standard calibration graphs of caffeine for alkaloids, quercetin for flavonoids and gallic acid for phenolic compounds (Figure 1).

The total alkaloid content varied significantly among the different solvents, with acetone exhibiting the highest extraction efficiency, averaging 145 mg/g (Figure 2). This finding suggested that acetone was particularly effective in alkaloid extraction, likely due to its strong solubility characteristics. Water also demonstrated a high extraction capacity, with an average alkaloid content of 129 mg/g, possibly attributed to its polar nature. Ethanol (88 mg/g) and chloroform (79 mg/g) showed moderate efficiency, whereas methanol yielded the lowest alkaloid content at 54 mg/g, likely reflecting its lower affinity for alkaloid compounds. These results identified acetone and water as the most effective solvents for alkaloid extraction.

The total flavonoid content analysis across the solvents demonstrated significant differences in extraction efficiency. Acetone exhibited the highest average flavonoid content at 27 mg/g, identifying it as the most effective solvent for flavonoid extraction, likely due to its high polarity and strong solvating ability for flavonoid compounds. Water followed with a moderate average of 19 mg/g, indicating a substantial but lower efficiency compared to acetone.

Methanol yielded an average flavonoid content of 14 mg/g, suggesting a lower extraction efficiency than water and acetone, yet still achieving reasonable results. Chloroform, with an average of 11.5 mg/g, displayed moderate extraction capabilities but proved less effective than polar solvents. Ethanol recorded the lowest average flavonoid content at 8.53 mg/g, indicating it was the least effective solvent for flavonoid extraction in this study.

These findings suggested that solvent polarity played a crucial role in flavonoid extraction, with highly polar solvents such as acetone demonstrating superior performance in recovering these compounds. The total phenolic content varied significantly among the different solvents. Acetone exhibited the highest phenolic content, with an average of 114 mg/g, indicating its superior efficiency in extracting phenolic compounds. Ethanol, water and chloroform followed, with average phenolic contents of 72 mg/g, 69 mg/g and 56 mg/g respectively, suggesting moderate extraction capabilities. Methanol demonstrated the lowest phenolic content, averaging 45 mg/g. These findings highlighted acetone as the most effective solvent for phenolic extraction, likely due to its polarity and solvent-solute interaction properties.

DPPH Analysis: The results of the DPPH assay provided a comprehensive evaluation of the antioxidant extraction efficiency of different solvents across various concentrations, ranging from 20 µL to 140 µL. The standard calibration curve of ascorbic acid was plotted to determine the DPPH activity of various solvent extractions of the sample (Figure 3). Each solvent exhibited distinct trends in its ability to extract antioxidants as reflected in their absorbance readings (Figure 4).

Table 1
Qualitative analysis of Phytochemicals in different extracts of *Roylea cinerea.* All the solvents used for sample extraction showed the presence for the tested phytochemicals.

Sample Extraction	Alkaloids	Flavonoids	Phenolic compounds
Chloroform	+	+	+
Acetone	+	+	+
Methanol	+	+	+
Ethanol	+	+	+
Water	+	+	+

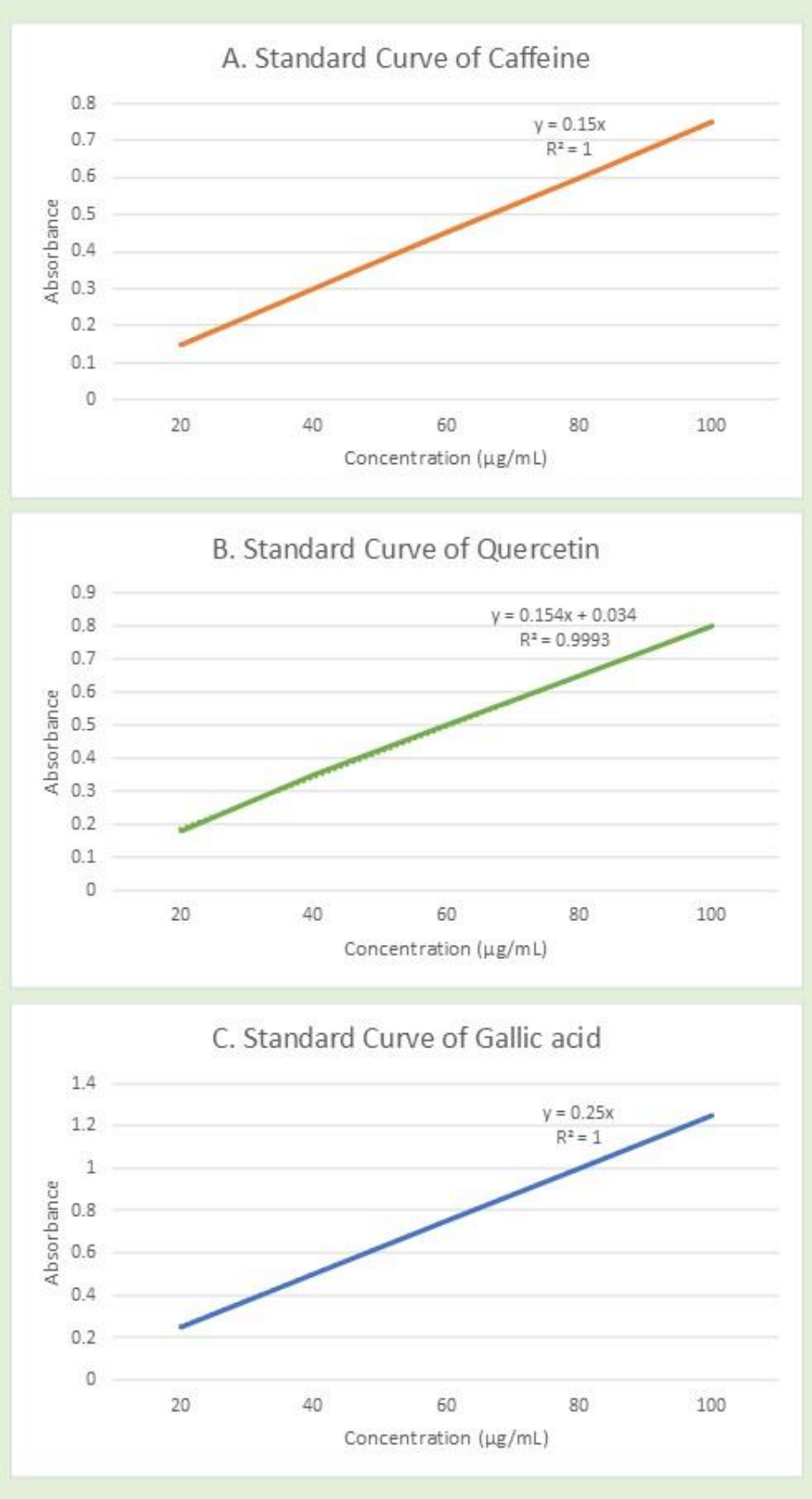

Figure 1: Standard curves of Caffeine, Quercetin and Gallic acid. (A) The alkaloid content was calculated as Caffeine equivalents CAF/ g of dry plant material based on a standard curve of Caffeine (mg/ ml). (B) The flavonoid content was calculated as Quercetin equivalents QUE/ g of dry plant material based on a standard curve of Quercetin (mg/ ml) (C) The phenolic content content was calculated as Gallic acid GAE/ g of dry plant material based on a standard curve of Gallic acid (mg/ ml)

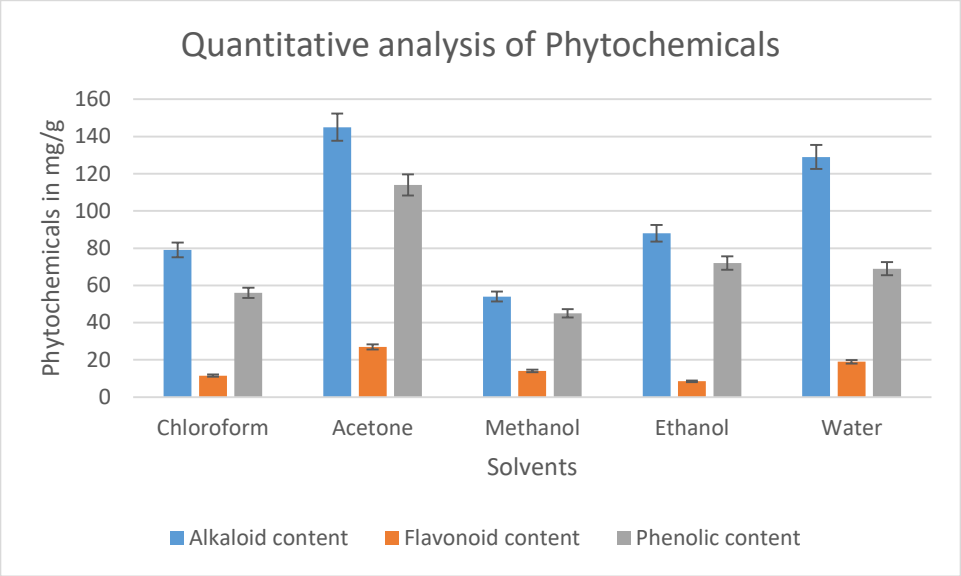

Figure 2: Quantitative analysis of Phytochemicals. Acetone exhibited the highest average alkaloid, flavonoid and phenolic content identifying it as the most effective solvent for phytochemical extraction, likely due to its high polarity and strong solvating ability for phytochemicals.

Chloroform demonstrated a steady increase in absorbance, beginning at 12.02 inhibition% at 20 µL and rising sharply to 51.13 at 140 µL. This consistent upward trend indicated that chloroform effectively extracted antioxidants, particularly as the concentration increased. The average % inhibition across all concentrations was 30.42, suggesting that chloroform maintained relatively high antioxidant activity. These findings implied that chloroform served as a strong medium for antioxidant extraction, especially at higher volumes.

Acetone, in contrast, exhibited lower absorbance values than chloroform, ranging from 6.43 inhibition% at 20 µL to 21.83 at 140 µL. The trend for acetone appeared more irregular, with fluctuations such as 4.62 at 60 µL and 10.45 at 80 µL. These variations suggested that acetone's efficiency in extracting antioxidants was inconsistent. The average %inhibition for acetone was calculated as 11.51, indicating moderate extraction capability but lower effectiveness compared to chloroform. Thus, while acetone facilitated antioxidant extraction, it was not the most efficient solvent in the DPPH assay.

Methanol exhibited a strong antioxidant extraction capacity, with % inhibition values increasing significantly from 23.19 at 20 µL to 57.65 at 100 µL. After reaching its peak at 100 µL, the absorbance values stabilized around 35–36 at 140 µL. This sharp increase followed by a slight decrease suggested that methanol was highly effective in the initial stages but potentially reached a saturation point beyond 100 µL, where additional volume did not enhance antioxidant extraction. Methanol's average % inhibition of 37.82 positioned it among the most efficient solvents in this assay.

Ethanol showed a consistent increase in absorbance, starting at 46.47 inhibition% at 20 µL and peaking at 63.42 at 100 µL. However, at higher concentrations, ethanol exhibited a slight decline in absorbance, decreasing to 44.06 at 140 µL. These findings suggested that ethanol performed optimally at moderate concentrations (around 100 µL), but its extraction efficiency diminished at higher volumes.

Despite this decline, ethanol maintained an average % inhibition of 55.63, making it one of the most effective solvents for antioxidant extraction. Its high antioxidant activity at lower volumes further indicated that ethanol efficiently extracted antioxidants without requiring large volumes.

In contrast to the other solvents, water exhibited minimal absorbance throughout the assay. Beginning at 1.08 inhibition% at 20 µL, its absorbance remained close to zero even at higher concentrations, peaking at only 0.73 at 100 µL and further decreasing to 0.63 at 140 µL. The average absorbance for water was 0.82, significantly lower than that of the other solvents. These findings indicated that water was the least effective solvent for antioxidant extraction in the DPPH assay. The poor extraction efficiency was likely attributed to water's polar nature, which limited its interaction with antioxidant compounds.

Overall, the DPPH assay demonstrated the varying effectiveness of solvents in antioxidant extraction. Methanol, ethanol and chloroform emerged as the most efficient solvents, with ethanol displaying the most consistent performance across different volumes.

Although methanol exhibited the highest peak absorbance, it showed a slight decline beyond 100 µL, suggesting a potential saturation threshold. Chloroform maintained a steady increase in antioxidant activity, reinforcing its effectiveness.

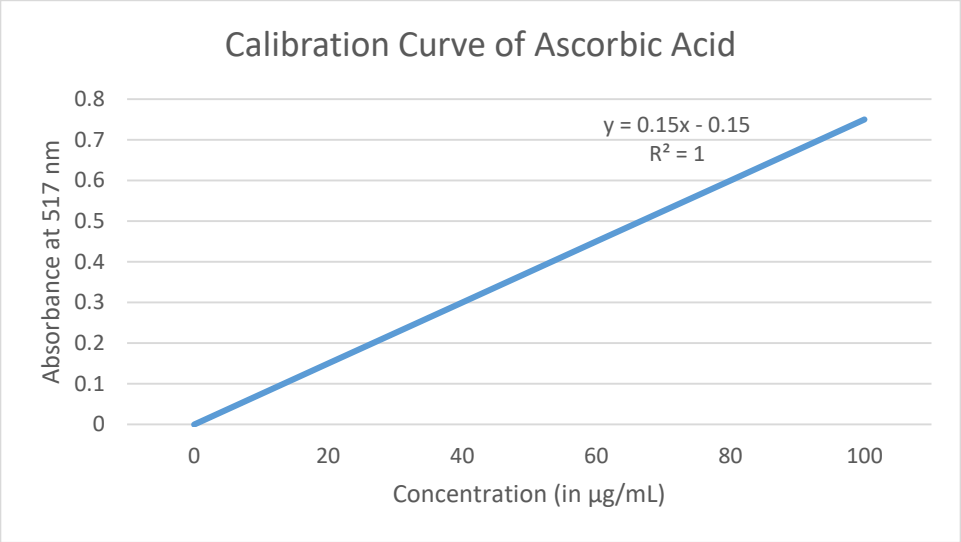

Figure 3: Standard curve of Ascorbic acid

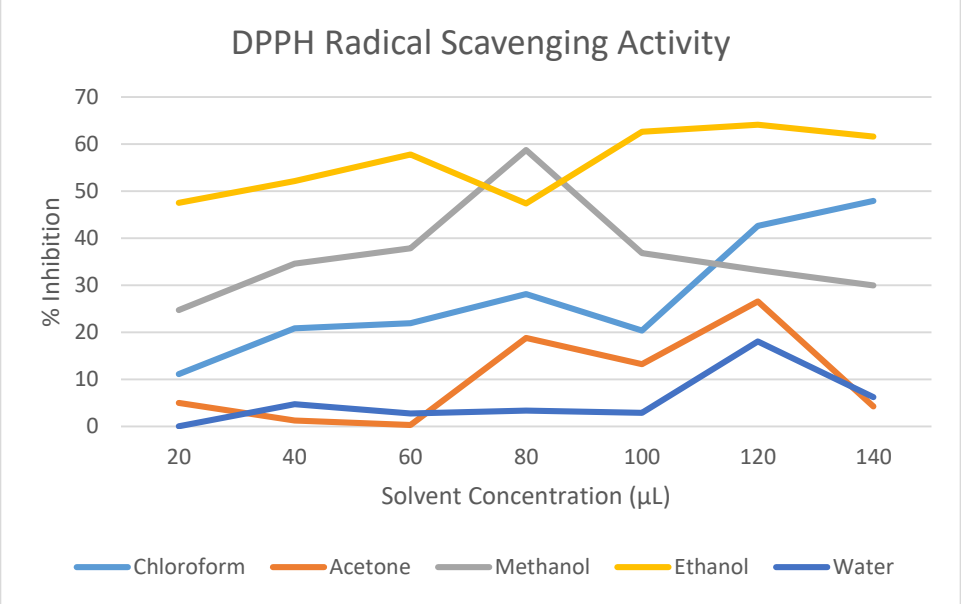

Figure 4: DPPH radical Scavenging Activity. It represents comprehensive evaluation of the antioxidant extraction efficiency of different solvents across various concentrations, ranging from 20 µL to 140 µL.

Acetone, while capable of antioxidant extraction, exhibited an irregular pattern and lower absorbance values compared to the top-performing solvents. Finally, water was confirmed as the least effective solvent, with negligible antioxidant extraction capabilities. These results provided valuable insights into the solvent-dependent variations in antioxidant extraction efficiency, highlighting ethanol and methanol as the most effective solvents.

HPLC Analysis

Chloroform extracts of sample: The HPLC data for all extractions is give in appendix 1. The chloroform extraction of the sample revealed a range of retention times (tR) from 0.53 to 8.68 minutes, indicating that the compounds eluted at varying rates, likely due to differences in their polarity and affinity for the stationary phase (Figure 5). The retention factors (k) exhibited an increasing trend, with lower k values corresponding to early retention times. This trend suggested that highly polar compounds eluted first whereas nonpolar compounds such as Beta-Sitosterol (k = 3.62) and linoleic acid (k = 5.49) eluted at later times.

The standard deviation (σ) values ranged from 0.27 to 4.07 seconds, with higher values correlating to broader peaks, thereby affecting resolution. The efficiency (N) remained relatively high across all measurements, with values ranging from 13,769 to 16,392, indicating a high number of theoretical plates and efficient chromatographic separation. Resolution (Rs) values exceeded 1.5 for most peaks, signifying effective separation between compounds. Notably, unknown compounds with higher Rs values such as peak 11 with Rs = 17.47, demonstrated baseline separation.

Compounds including caffeic acid, beta-sitosterol, apigenin glycosides and linoleic acid were successfully identified, with their retention times aligning with expected values. Overall, the data confirmed successful separation, efficient column performance and good peak resolution for most compounds in the sample.

Acetone extracts of sample: The HPLC analysis of acetone extraction of the sample provided a comprehensive evaluation of phytochemicals, with tR ranging from 0.53 to 14.08 minutes (Figure 6). This variation in retention times indicated the presence of compounds with differing polarities. The retention factor (k) values followed a similar pattern, with higher k values observed for later-eluting compounds. For instance, geraniol (k = 9.18) exhibited stronger interactions with the nonpolar C18 stationary phase, resulting in delayed elution.

The standard deviation (σ) of the peaks varied, with higher values recorded for late-eluting compounds, suggesting broader peaks and potentially more complex interactions within the column. Notably, unknown compounds corresponding to peaks 15, 16 and 17 displayed σ values exceeding 6 seconds, indicating significantly broader peaks. This affected the Rs between these peaks, leading to poor separation, as seen in Rs = 0.17 between Peaks 16 and 17.

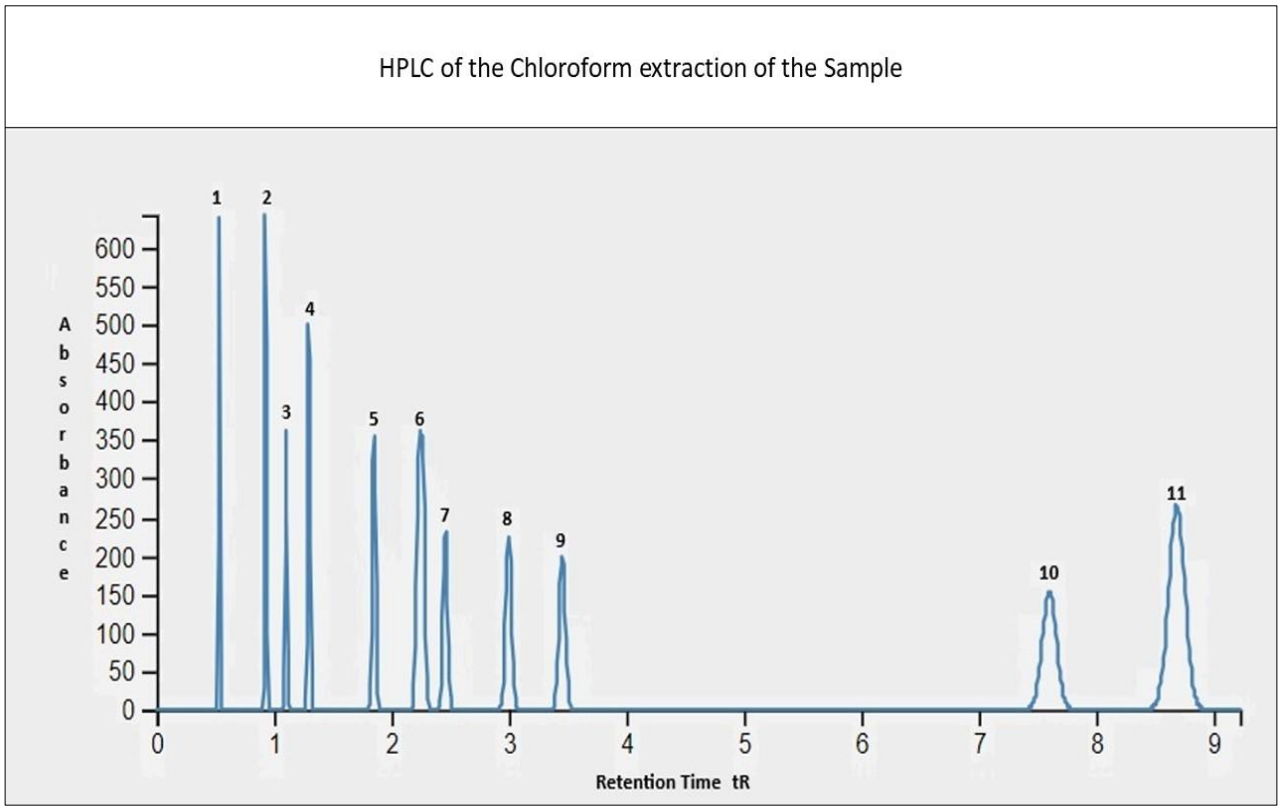

Figure 5: Chromatogram of Chloroform extracts of sample

APPENDIX 1
HPLC data for Chloroform extract of the sample

Peak	Compounds	k	tR (min)	σ (s)	kw	S	N	Rs
1	Unknown	0.4321	0.5318	0.2719	1	0	13769	
2	Unknown	0.7434	0.9272	0.4478	4.1676	4.3097	15432	4.5712
3	Caffeic Acid	1.0702	1.1009	0.5271	5.3127	4.0057	15703	4.9452
4	Unknown	1.4343	1.2946	0.6162	8.7216	4.5128	15891	4.7141
5	Unknown	2.4756	1.8483	0.8727	25.728	5.8528	16148	9.518
6	Unknown	3.1903	2.2285	1.0496	27.213	5.359	16227	5.432
7	Unknown	3.2584	2.2646	1.0665	40.761	6.3163	16232	0.5089
8	Beta-Sitosterol	3.6169	2.4553	1.1554	45.819	6.3477	16258	2.4756
9	Apigenin Glycosides	4.6317	2.995	1.4073	49.74	5.9347	16305	5.7524
10	Linoleic Acid	5.4868	3.4498	1.6197	64.132	6.1465	16329	4.2115
11	Unknown	13.2823	7.5954	3.5598	217.06	6.9843	16388	17.4686
12	Unknown	15.3184	8.6783	4.0669	441.64	8.4036	16392	3.9938

HPLC data for Acetone extract of the sample

Peak	Compounds	k	tR (min)	σ (s)	kw	S	N	Rs
1	Unknown	0.2246	0.5318	0.2719	0.3291	9.4965	13769	
2	Quercetin	0.7434	0.9272	0.4478	4.1676	4.3097	15432	4.5712
3	Unknown	0.8584	0.9883	0.4756	71.1943	11.0452	15543	1.9292
4	Unknown	1.4343	1.2946	0.6162	8.7216	4.5128	15891	7.4555
5	β-Sitosterol	2.3968	1.8064	0.8532	68.8073	8.3929	16136	8.9986
6	Campesterol	2.4756	1.8483	0.8727	25.7281	5.8528	16148	0.7202
7	Unknown	2.6638	1.9485	0.9193	82.7407	8.5899	16173	1.6336
8	Luteolin	3.1903	2.2285	1.0496	27.2133	5.359	16227	4.0013
9	Unknown	3.2584	2.2646	1.0665	40.761	6.3163	16232	0.5089
10	Unknown	3.6169	2.4553	1.1554	45.8185	6.3477	16258	2.4756
11	Carnosic Acid	4.6317	2.995	1.4073	49.7395	5.9347	16305	5.7524
12	Naringenin Glycoside	7.0812	4.2976	2.0162	73.603	5.8531	16356	9.6913
13	Geraniol	9.1775	5.4125	2.5379	639.0611	10.6081	16373	6.5893
14	Unknown	11.3473	6.5664	3.078	338.1357	8.4862	16383	5.6232
15	Unknown	24.3132	13.4618	6.3073	636.15	8.161	16399	16.3987
16	Unknown	25.3285	14.0017	6.5602	637.5795	8.0644	16399	1.2346
17	Unknown	25.4728	14.0785	6.5961	2040.5	10.9583	16399	0.1745

HPLC data for Methanol extract of the sample

Peaks	Compounds	k	tR (min)	σ (s)	kw	S	N	Rs
1	Unknown	0.0484	0.5576	0.283	356.5413	22.2593	13972	
2	Unknown	2.4756	1.8483	0.8727	25.7281	5.8528	16148	22.1856
3	Unknown	2.6638	1.9485	0.9193	82.7407	8.5899	16173	1.6336
4	Unknown	3.1903	2.2285	1.0496	27.2133	5.359	16227	4.0013
5	Unknown	4.8993	3.1373	1.4737	116.1459	7.9144	16314	9.2503
6	Unknown	6.2968	3.8805	1.8211	71.5975	6.0775	16345	6.1218
7	Geraniol	6.8466	4.1729	1.9579	104.0148	6.802	16353	2.2398
8	Unknown	7.0812	4.2976	2.0162	73.603	5.8531	16356	0.9282
9	Unknown	9.1775	5.4125	2.5379	639.0611	10.6081	16373	6.5893
10	Unknown	13.5143	7.7188	3.6176	761.0127	10.0772	16389	9.5629
11	Unknown	15.3184	8.6783	4.0669	441.6422	8.4036	16392	3.5387
12	Unknown	18.8707	10.5674	4.9516	362.2167	7.3866	16396	5.7228
13	Isorhamnetin	25.3285	14.0017	6.5602	637.5795	8.0644	16399	7.8527
14	Unknown	26.0315	14.3756	6.7353	711.3233	8.2695	16399	0.8326

HPLC data for Ethanol extract of the sample

Peaks	Compounds	k	tR (min)	σ (s)	kw	S	N	Rs
1	Protocatechuic Aldehyde	0.4142	0.7521	0.3688	8.7081	7.6139	14972	
2	Unknown	0.8584	0.9883	0.4756	71.1943	11.0452	15543	7.4495
3	Unknown	1.0702	1.1009	0.5271	5.3127	4.0057	15703	3.2044
4	Rosmarinic Acid	1.1379	1.1369	0.5436	13.8645	6.2504	15745	0.9935
5	Apigenin	3.2584	2.2646	1.0665	40.761	6.3163	16232	15.861
6	Unknown	4.6317	2.995	1.4073	49.7395	5.9347	16305	7.7849
7	Unknown	5.4868	3.4498	1.6197	64.1317	6.1465	16329	4.2115
8	Unknown	8.859	5.2431	2.4586	193.3171	7.7072	16372	10.9412
9	Unknown	9.1775	5.4125	2.5379	639.0611	10.6081	16373	1.0013
10	Unknown	9.2852	5.4697	2.5647	251.0742	8.2433	16374	0.3347
11	Unknown	13.5143	7.7188	3.6176	761.0127	10.0772	16389	9.3257
12	Pterostilbene	15.3184	8.6783	4.0669	441.6422	8.4036	16392	3.5387
13	Xanthones	18.8707	10.5674	4.9516	362.2167	7.3866	16396	5.7228
14	Unknown	25.3285	14.0017	6.5602	637.5795	8.0644	16399	7.8527
15	Unknown	35.6976	19.5161	9.1432	984.1218	8.2917	16401	9.0467
16	Unknown	89.5938	48.1786	22.57	5483.264	10.2854	16403	19.049

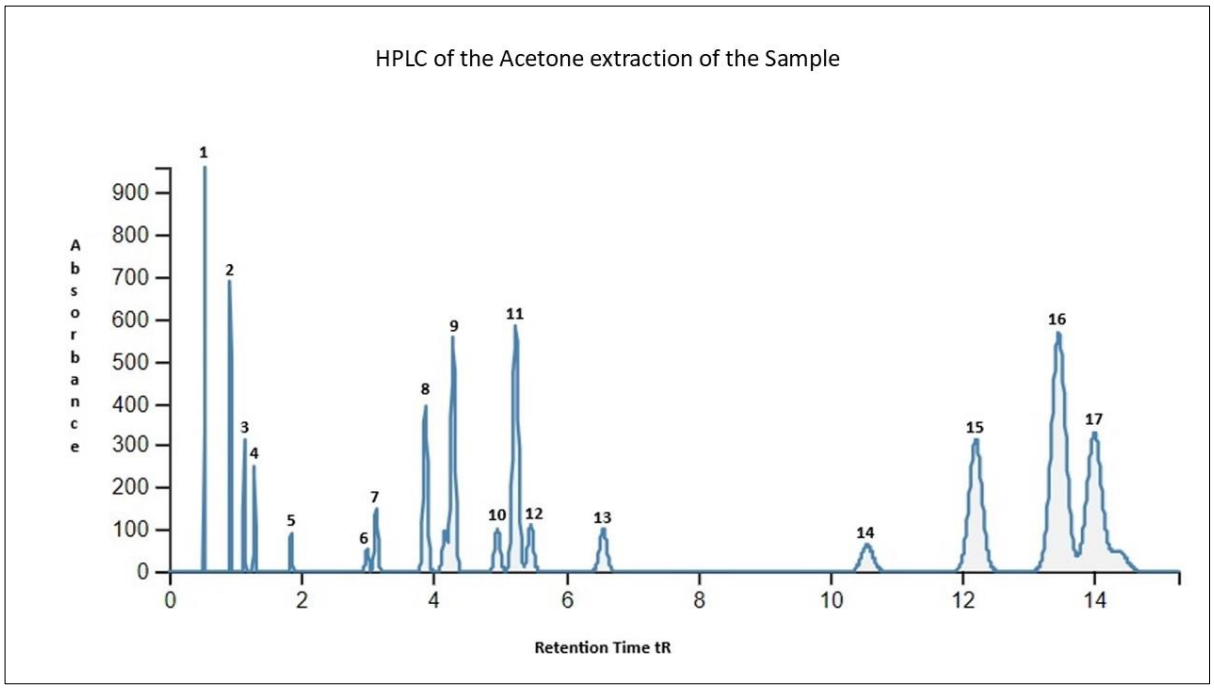

Figure 6: Chromatogram of Acetone extracts of sample

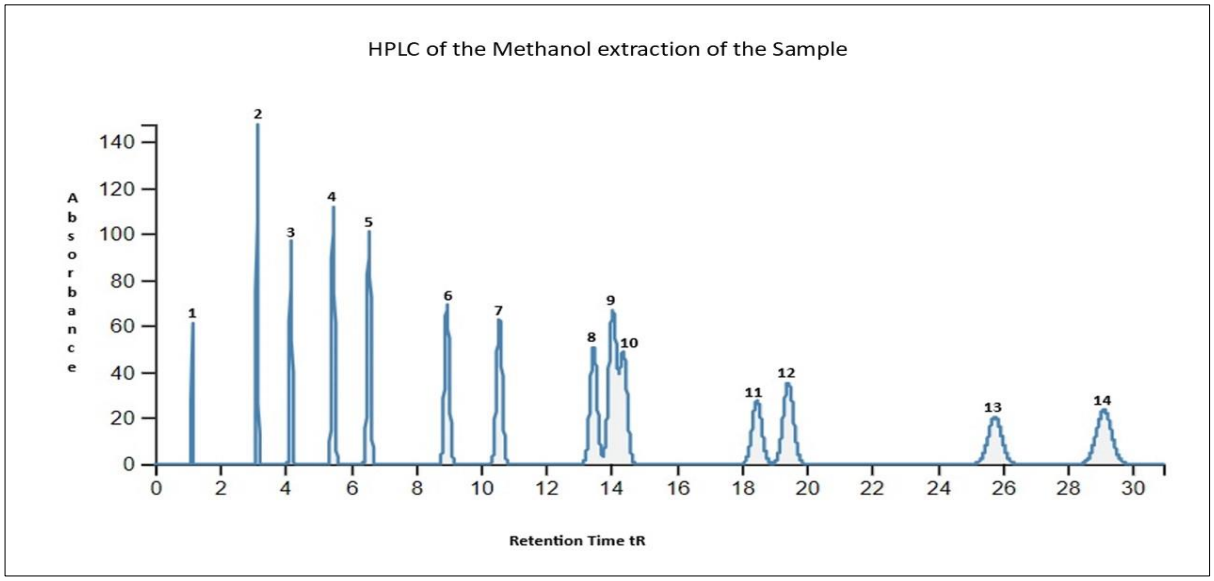

Figure 7: Chromatogram of Methanol extracts of sample

The selectivity (S) values demonstrated variability in separation between adjacent peaks, with higher values (e.g. 10.61 for geraniol) indicating enhanced separation efficiency. Column efficiency (N) remained consistently high, ranging from 13,769 to 16,399, suggesting effective chromatographic performance and successful separation for most peaks. However, selectivity and resolution values for closely eluting compounds, particularly unknowns in peaks 15, 16 and 17, revealed limitations in resolving these late-eluting compounds.

Identified compounds, including quercetin, β-Sitosterol, campesterol, carnosic acid, naringenin glycoside and geraniol exhibited retention times and retention factors consistent with their chemical properties. Overall, the data confirmed successful separation for the majority of compounds, although further optimization may be required to enhance the resolution of late-eluting compounds.

Methanol extracts of sample: The HPLC analysis of methanol extracts of the sample revealed a diverse range of tR from 0.56 to 14.38 minutes. The lowest k value (0.05) corresponded to the first peak and the highest (26.03) was observed for the last unknown compound (Figure 7). This trend reflected a strong interaction between later-eluting compounds and the stationary phase.

The σ values, ranging from 0.28 to 6.74 seconds, indicated variations in peak width. Peaks with higher σ values, such as those for isorhamnetin (σ = 6.56) and the last unknown

compound (σ = 6.74), exhibited broader peak shapes, potentially due to complex interactions or lower analyte concentrations. N remained consistently high, between 13,972 and 16,399, demonstrating strong separation performance and a high number of theoretical plates.

The S values varied significantly, with the highest selectivity (22.26) observed in the first peak. Rs values were generally satisfactory, particularly for peaks 2 and 5, which exhibited Rs values of 22.19 and 9.25 respectively, indicating excellent separation.

However, the later-eluting peaks (13 and 14) displayed lower Rs values, such as Rs = 0.83, suggesting incomplete separation and potential peak overlap. Identified compounds including geraniol and isorhamnetin, exhibited retention times and k values consistent with their expected chromatographic behavior. Overall, the dataset confirmed effective separation for most compounds, though some late-eluting peaks may require further method optimization to enhance separation and resolution.

Ethanol extracts of sample: The HPLC analysis of ethanol extracts of sample revealed a wide range of tR from 0.75 to 48.18 minutes. The k values varied significantly, ranging from 0.41 for protocatechuic aldehyde, suggesting high polarity and rapid elution, to 89.59 for the last unknown compound, indicating strong retention and nonpolar characteristics (Figure 8). The standard deviation (σ) values spanned from 0.37 to 22.57 seconds. N remained consistently high, ranging from 14,972 to 16,403, confirming strong chromatographic performance and effective analyte separation.

S values exhibited substantial variability, with the highest values observed for early-eluting peaks such as the first peak (22.26) while mid-range peaks displayed lower selectivity. Rs values were generally satisfactory, with particularly high resolution observed for the last unknown compound (Rs = 19.05), ensuring effective separation from adjacent peaks. However, compounds with low Rs values, such as rosmarinic acid (Rs = 0.99) and a mid-range unknown compound (Rs = 0.33), indicated incomplete separation, suggesting the need for method optimization.

Identified compounds including protocatechuic aldehyde, rosmarinic acid, apigenin, pterostilbene and xanthones, exhibited characteristic retention times and effective separation. Overall, the dataset confirmed efficient compound separation, though further adjustments may be necessary to enhance the resolution of closely eluting peaks.

Conclusion

The comprehensive phytochemical analysis revealed that different solvents exhibited varying efficiencies in extracting alkaloids, flavonoids and phenolic compounds. Qualitative assessment confirmed the presence of these bioactive compounds across all solvents, with chloroform, acetone, methanol and ethanol demonstrating strong extraction capabilities. Quantitative analysis further highlighted acetone as the most effective solvent for alkaloid and flavonoid extraction, while it also exhibited the highest phenolic content. The DPPH assay indicated that ethanol and methanol were the most efficient solvents for antioxidant extraction, with chloroform also demonstrating strong activity.

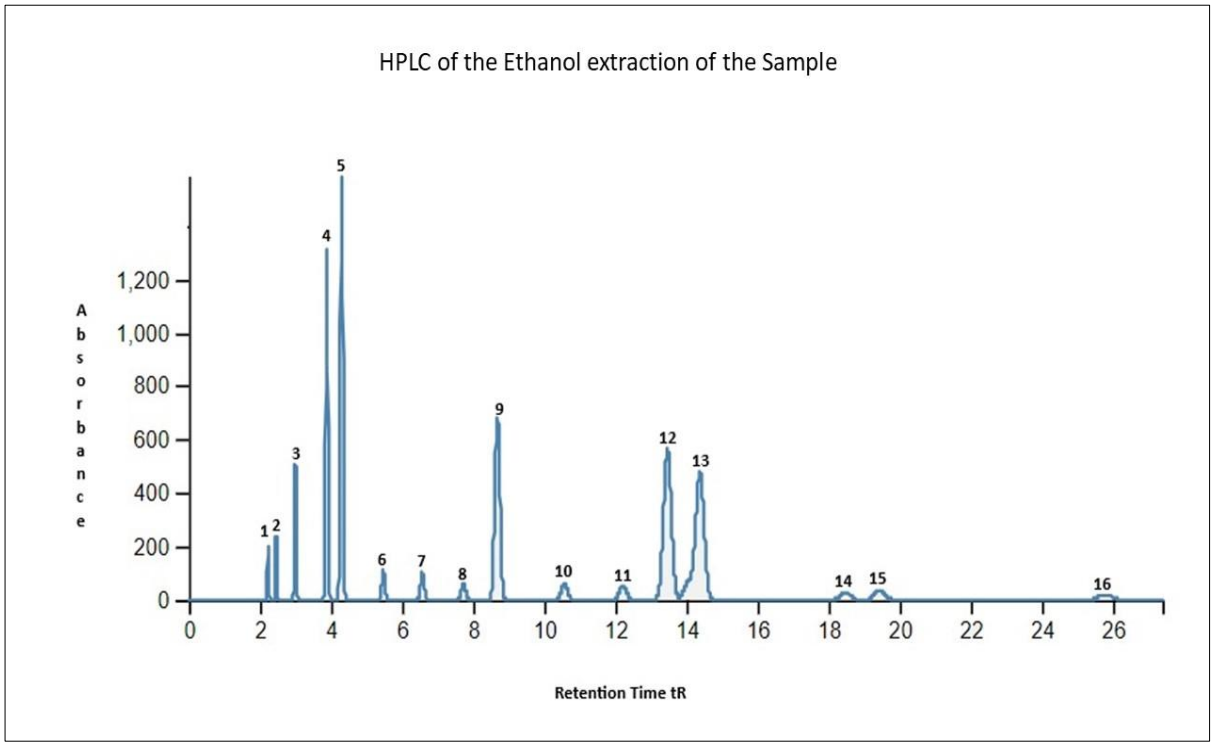

Figure 8: Chromatogram of Ethanol extracts of sample

HPLC analysis confirmed the successful separation of key phytochemicals, with high resolution and efficiency in most cases, although late-eluting compounds in acetone and methanol extracts exhibited incomplete separation. These findings collectively emphasize the solvent-dependent variations in phytochemical extraction, antioxidant activity and chromatographic resolution. The study highlights the importance of selecting an appropriate solvent based on the specific target compounds, with acetone proving highly efficient for alkaloid, flavonoid and phenolic extraction, while ethanol and methanol demonstrated superior antioxidant recovery.

The results underscore the need for optimized solvent selection to enhance the yield, bioactivity and separation efficiency of bioactive compounds, which are crucial for applications in pharmaceuticals, nutraceuticals and functional food development.

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References

1. Abubakar A.R. and Haque M., Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes, *Journal of Pharmacy and Bioallied Sciences*., **12(1)**, 1 **(2020)**

2. Ajanal M., Gundkalle M.B. and Nayak S.U., Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer, *Ancient Science of Life*, **31(4)**, 198 **(2012)**

3. Alamgir A.N.M. and Alamgir A.N.M., Methods of Qualitative and Quantitative Analysis of Plant Constituents, Therapeutic Use of Medicinal Plants and Their Extracts: Volume 2: Phytochemistry and Bioactive Compounds, 721 **(2018)**

4. Ak T. and Gülçin I., Antioxidant and radical scavenging properties of curcumin, *Chemico-Biological Interactions*, **174(1)**, 27 **(2008)**

5. Chanu W.K., Chatterjee A., Singh N., Nagaraj V.A. and Singh C.B., Phytochemical screening, antioxidant analyses and *in vitro* and *in vivo* antimalarial activities of herbal medicinal plant-*Rotheca serrata* (L.) Steane & Mabb, *Journal of Ethnopharmacology*, **321**, 117466 **(2024)**

6. Dhiman S., Dhiman B. and Pathania M.S., Preliminary quantification of phytochemicals in methanolic extract of *Roylea cinerea* (D. Don) Baillon by using HPTLC technique, *Journal of Pharmacognosy and Phytochemistry*, **10(1)**, 1569 **(2021)**

7. Frezza C., Venditti A., Serafini M. and Bianco A., Phytochemistry, chemotaxonomy, ethnopharmacology and nutraceutics of *Lamiaceae*, *Studies in Natural Products Chemistry*, **62**, 125 **(2019)**

8. Gonfa T., Teketle S. and Kiros T., Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and *in vitro* antioxidant activity evaluation of *Cadaba rotundifolia* Forssk leaf extracts, *Cogent Food & Agriculture*, **6(1)**, 1853867 **(2020)**

9. Gupta M., Thakur S., Sharma A. and Gupta S., Qualitative and quantitative analysis of phytochemicals and pharmacological value of some dye-yielding medicinal plants, *Oriental Journal of Chemistry*, **29(2)**, 475 **(2013)**

10. Jha D.K., Panda L., Ramaiah S. and Anbarasu A., Evaluation and comparison of radical scavenging properties of solvent extracts from *Justicia adhatoda* leaf using DPPH assay, *Applied Biochemistry and Biotechnology*, **174**, 2413 **(2014)**

11. Li M., Bai L., Peng S., Sun F., Wang L., Liu H. and Yan H., Simple quantitative analytical methods for the determination of alkaloids from medicinal and edible plant foods using a homemade chromatographic monolithic column, *Journal of Chromatography B*., **1128**, 121784 **(2019)**

12. Maleš Ž., Plazibat M., Bilušić Vundać V. and Žuntar I., Qualitative and quantitative analysis of flavonoids of the strawberry tree-*Arbutus unedo* L. (Ericaceae), *Acta Pharmaceutica*, **56(2)**, 245 **(2006)**

13. Mradu G., Saumyakanti S., Sohini M. and Arup M., HPLC profiles of standard phenolic compounds present in medicinal plants, *International Journal of Pharmacognosy and Phytochemical Research*, **4(3)**, 162 **(2012)**

14. Pundir S. and Mahindroo N., *Roylea cinerea* (D. Don) Baillon: Ethnomedicinal uses, phytochemistry and pharmacology: A review, *Journal of Ethnopharmacology*, **232**, 193 **(2019)**

15. Prasad R. and Sharma O.P., Qualitative test on ethanolic extract of *Coccinia indica* for profiling secondary metabolites, *International Journal of Scientific Research and Review*, **07(1)**, 147 **(2019)**

16. Rawat R. and Vashistha D.P., *Roylea cinerea* (D. Don) Baillon: A traditional curative of diabetes, its cultivation prospects in Srinagar Valley of Uttarakhand, *International Journal of Advanced Pharmaceutics, Biological and Chemical Sciences*, **2(2)**, 372 **(2013)**

17. Roghini R. and Vijayalakshmi K., Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*, *International Journal of Pharmaceutical Sciences and Research*, **9(11)**, 4859 **(2018)**

18. Sankhalkar S. and Vernekar V., Quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L., *Pharmacognosy Research*, **8(1)**, 16 **(2016)**

19. Sembiring E.N., Elya B. and Sauriasari R., Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb., *Pharmacognosy Journal*, **10(1)**, 123-127 **(2018)**

20. Soumya V. and Kiranmayi P., Potential of *Catharanthus roseus* applied to remediation of disparate industrial soils owing to accumulation and translocation of metals into plant parts, *International Journal of Phytoremediation*, **25(6)**, 746 **(2023)**

21. Soumya V., Sowjanya A. and Kiranmayi P., Evaluating the status of phytochemicals within *Catharanthus roseus* due to higher metal stress, *International Journal of Phytoremediation*, **23(13)**, 1391 **(2021)**

22. Upadhyay G., Tiwari N., Maurya H., Upadhyay J., Joshi R. and Ansari M.N., *In vivo* wound-healing and antioxidant activity of aqueous extract of *Roylea elegans* leaves against a physically induced burn model in Wistar albino rats, *3 Biotech*, **11(10)**, 442 **(2021)**

23. Yadav R.N.S. and Agarwala M., Phytochemical analysis of some medicinal plants, *Journal of Phytology*, **3(12)**, 10-14 **(2011)**.

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