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Determination of Phenolic Content and Antioxidant Activity of *Inula viscosa* L. Aiton

Research Article

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Abstract

The phenolic profile of three distinct extracts (petroleum ether (IVPE), acetone (IVA) and methanol (IVM)) derived from the aerial parts of the medicinal species *Inula viscosa* L. Aiton was determined using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). In this study, the analysis of phenolic compounds in petroleum ether and acetone extracts and the total phenolic content (TPC) for *I. viscosa* species were reported for the first time. Based on the results, IVA extract was found to be the richest in phenolic content (3189.14 mg/kg) followed by IVM (2509.39 mg/kg) and IVPE (184.37 mg/kg). Rhamnocitrin, dihydrokaempferol, isosakuranetin, nepetin, nepetin-7-glucoside, acacetin, quercetin, verbascoside, and taxifolin were the most prevalent phenolic compounds; the IVA extract contained the highest concentrations of these. In summary, *I. viscosa* extracts demonstrate significant phenolic content and considerable antioxidant activity. Furthermore, the TPC of these extracts was quantified using the Folin-Ciocalteu reagent, with IVA showing the highest phenolic content value (440.4 ± 0.001 mg GAE/g), followed by IVM (273.7 ± 0.001 mg GAE/g), and the lowest value was determined in IVPE (51.8 ± 0.001 mg GAE/g). The antioxidant capacities of the extracts were assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the cupric ion (Cu^{2+}) reducing capacity (CUPRAC) method. According to the DPPH results, IVA exhibited moderate-to-high radical scavenging activity at all concentrations and had the highest CUPRAC value (0.62 ± 0.04), consistent with its elevated phenolic

content. The results showed that *I. viscosa* extracts are rich in phenolic compounds and exhibit strong antioxidant properties.

Keywords: *Inula viscosa*, LC-MS/MS, Antioxidant activity, Phenolic compounds.

1. INTRODUCTION

Inula viscosa (L.) Aiton [*Dittrichia viscosa* (L.)] is widely recognized by its Turkish common names, including “yalancı sarıbaş,” “yapışkan inula,” “aromatik inula,” and “yapışkan andız otu” [1]. Belonging to the Asteraceae family, this species is widely distributed, particularly across the Mediterranean basin. Throughout history, it has been regarded as one of the most valuable medicinal plants due to its diverse pharmacological properties [2]. Within traditional healing practices, it has played a significant role in the treatment of cancer, diabetes, hypertension, respiratory diseases, skin disorders, wounds, and digestive system ailments [1].

According to the literature, the genus *Inula* comprises approximately 100 species worldwide. In Turkey, 27 species of this genus have been identified, seven of which are endemic. Recent studies have drawn considerable attention to *Inula* species due to their biological activities, and chemical analyses have demonstrated that these plants contain a wide variety of bioactive compounds, including terpenoids, flavonoids, and anthranilic acid derivatives [3–7]. Various studies have reported that these compounds exhibit antiproliferative, antioxidant, antitumoral, antimicrobial, and cytotoxic activities [4,8–16]. Furthermore, *I. viscosa* has been suggested as a valuable botanical source for the development of homemade fungicides [17–19].

Phenolic compounds are of significant interest for human health, primarily owing to their potent antioxidant and anti-inflammatory properties. These molecules play a crucial role in mitigating oxidative stress by modulating the activity of enzymes and other inflammatory mediators, thereby providing protection against a range of pathological conditions [11,20–21]. Moreover, evidence indicates that diets rich in phenolic compounds are associated with a lower incidence of cardiovascular, metabolic, and neurodegenerative disorders [20]. Consequently, studies focusing on the identification of phenolic compounds in plant extracts have increased, revealing a strong correlation between antioxidant activity and phenolic content. In recent years, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become a widely used method for the identification and quantification of phenolic acids and simple phenolic compounds [22–23].

The presence of compounds responsible for antioxidant, anti-inflammatory, antiproliferative, and antitumoral effects in these species further highlights the importance of this study. Many *Inula* species have not yet been investigated, or only limited studies have been conducted. Research on *Inula* species growing in Turkey is particularly scarce [24]. Consequently, future studies should prioritize the fractionation and isolation of compounds present in the aerial parts of *I. viscosa* extracts to fully identify their active constituents [1]. The isolation and characterization of bioactive compounds from *I. viscosa* are essential for identifying potential lead molecules. In this way, the plant emerges as a promising candidate for the development of new pharmaceutical compounds [25–26].

In this study, phenolic compounds in extracts obtained from *I. viscosa* were identified and quantified using LC–MS/MS, and their antioxidant activities were evaluated. For this purpose, extracts were prepared using three solvents with different polarities (petroleum ether, acetone, and methanol). Chromatographic analysis via LC–MS/MS was employed to identify and quantify phenolic acids and simple phenolics. The

antioxidant activities of the extracts were determined using DPPH and CUPRAC assays, thereby providing a solid basis for revealing the biological potential of *I. viscosa*.

2. MATERIALS AND METHODS

2.1 Plant material

I. viscosa is a species that can be easily recognized due to its characteristic camphor-like odor. The stems and leaves are covered with a sticky layer, and the plant bears small yellow flowers. The flowering period varies between June and November, depending on the geographical location where the plant grows [17].

In this study, approximately 10 kg of *I. viscosa* (L.) Aiton was collected before the flowering stage, including stems and leaves, from the Hasköy region of Akhisar/Manisa, 38°55'33.0"N, 27°55'35.3"E in the Aegean Region of Türkiye. The plant material was identified by Prof. Dr. Tuncay Dirmenci, Balıkesir University, Türkiye.

The stems and leaves were dried under shade conditions for 15–20 days and subsequently ground into powder using a grinder. The collected fresh plant material (approximately 10 kg) yielded about 3.75 kg after drying.

2.2 Extraction procedure

For the analysis of the components, 200 g portions of the dried plant material were separately macerated in closed glass containers with acetone, methanol, and petroleum ether for 15 days. The filtrates were concentrated by solvent evaporation, and the resulting extracts were coded as IVA, IVM, and IVPE, yielding 13.62 g, 23.61 g, and 6.99 g of dried extract, respectively. The samples were stored at 4°C pending subsequent analyses.

2.3 LC-MS/MS conditions

The phenolic composition of the plant extracts was analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Approximately 100 mg of each plant extract was dissolved in 5 mL of methanol, vortexed for 1 minute, and filtered through a 0.22 µm membrane. Thirty µL of internal standard solution (theophylline, 50 ppm in MeOH) was added to achieve a final concentration of 1.5 ppm.

Chromatographic separation was performed on a Troyasil C18 column (3.5 µm, 150 × 2.1 mm) at 25 °C using a gradient elution. The mobile phases were: A, 1% formic acid in water; B, 10% methanol – 10% water – 80% acetonitrile and the flow rate was 0.25 mL/min.

The Thermo TSQ Quantis, equipped with a heated electrospray ionization (H-ESI) source, was utilized for mass spectrometric analysis in both positive and negative ion modes. All analyses were conducted under controlled laboratory conditions at 23 ± 3 °C and 50 ± 15% relative humidity.

2.4 Determination of Total Phenolic Contents (TPC)

TPC was assayed using the Folin-Ciocalteu reagent. A saturated Na₂CO₃ solution was prepared by dissolving 35 g of anhydrous Na₂CO₃ in 100 mL of distilled water, allowing the mixture to stand overnight, and subsequently adding several Na₂CO₃·10H₂O crystals. Upon completion of crystallization, the resulting solution was filtered utilizing glass wool. The Folin–Ciocalteu reagent was obtained as a ready-to-use solution. Prior to analysis, the samples underwent centrifugation. The Folin reagent was introduced, the mixture was shaken and then allowed to incubate for 3 minutes. Following this, 1 mL of the saturated Na₂CO₃ solution was added, and the volumetric flask was brought to a final volume of 10 mL with distilled water. The flask was agitated once more and subsequently stored in the dark for 90 minutes. After 90

minutes, absorbance was measured at 720 nm using a spectrophotometer. Each sample was tested three times, and the TPC was calculated in terms of 'mg Gallic Acid Equivalent (GAE) per g of sample' [27].

2.5 Antioxidant activities

2.5.1 DPPH free radical scavenging method

The DPPH radical scavenging assay was employed to spectrophotometrically evaluate the free radical scavenging activity of the extracts [28-31]. For the DPPH method, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and α -tocopherol (α -toc) were used as standard compounds. The activity assessments were conducted at four distinct concentrations: 2 μ g/mL, 5 μ g/mL, 10 μ g/mL, and 20 μ g/mL. In brief, a 0.1 mM DPPH solution was prepared in methanol. Subsequently, 160 μ L of this DPPH solution was combined with 40 μ L of the sample solutions, which were also prepared in methanol at varying concentrations (2, 5, 10 and 20 μ g/mL). The reaction mixtures were subsequently allowed to stand in the dark for 30 min. Following this incubation period, the absorbance was measured spectrophotometrically at 517 nm. The radical scavenging potential of the samples against DPPH was then determined by comparison with the established standards.

2.5.2 The CUPRAC method

The reducing capacity of the extracts was evaluated using the CUPRAC method [30,32]. Briefly, a working solution was prepared by mixing 1 mM DMF, 10 mM CuCl₂, 7.5 mM neocuproine, 1 M NH₄CH₃COO buffer solution (pH 7.0), and distilled water at a volume ratio of 1:1:1:0.6. Subsequently, 180 μ L of this mixture was transferred into the wells, followed by the addition of 25 μ L of the test samples diluted 1:20 in ethanol (EtOH). All samples were incubated for 30 min at 25 °C. Finally, absorbance was measured at 450 nm against a reagent blank. Ethanol was used as the negative control, while curcumin served as the positive control.

3. RESULTS AND DISCUSSION

3.1 Phenolic contents

The phenolic compounds and their concentrations identified in *I. viscosa* extracts obtained with solvents of different polarities (IVA, IVM, and IVPE) are presented in Table 1. The five compounds: rhamnocitrin, dihydrokaempferol, isosakuranetin, nepetin-7-glucoside, and rosmarinic acid were determined as the most abundant compounds. Furthermore, the highest amounts of these five compounds were detected across all extracts. The chemical structures of these compounds are shown in Figure 1.

The richest extract in terms of phenolic content was determined as IVA (3189.14 mg/kg), followed by IVM (2509.39 mg/kg) and IVPE (184.37 mg/kg), respectively.

The solvent extraction method used in this study largely coincides with the method applied in similar studies in literature. The results clearly showed that solvent polarity is a determining factor in the extraction efficiency of phenolic compounds [33-35]. These results indicate that IVA and IVM are considerably richer in phenolic compounds compared to IVPE.

When evaluated on a compound basis, rhamnocitrin emerged as the most dominant phenolic compound in all samples, being detected at concentrations of 1942.06 mg/kg in IVA, 1640.75 mg/kg in IVM, and 156.18 mg/kg in IVPE. In addition, dihydrokaempferol, nepetin, isosakuranetin, acacetin, and quercetin were found at relatively high concentrations particularly in IVA and IVM, contributing to the diversity of their phenolic profiles. However, it has been observed that the IVPE sample generally has a low phenolic content. This is

because phenolic compounds are polar in nature due to the hydroxyl (–OH) groups in their structure and therefore dissolve better in polar solvents. In contrast, petroleum ether has low polarity, and therefore this extract contained the lowest amount of phenolic compounds.

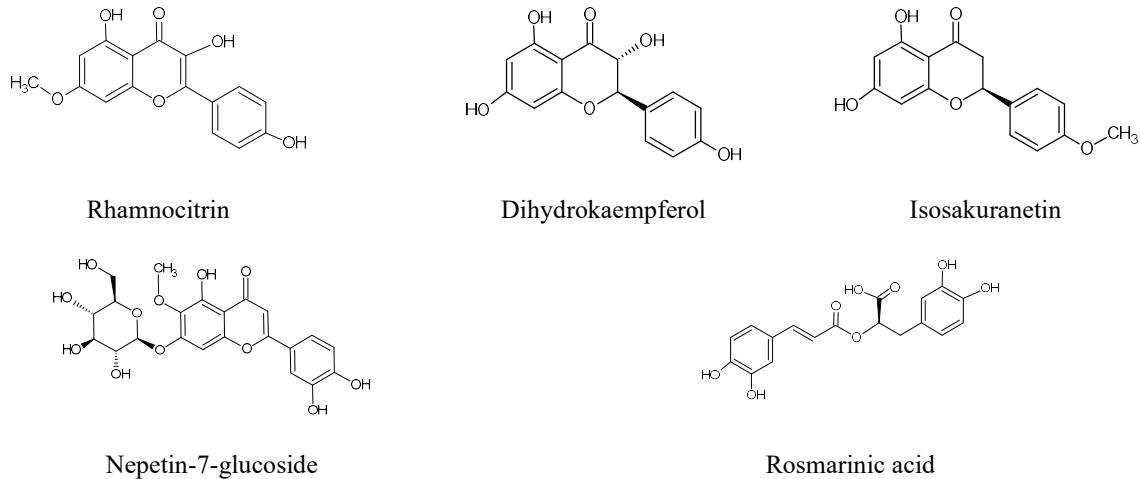


Figure 1. Chemical structures of the five most abundant phenolic compounds identified in *I. viscosa* extracts.

When evaluated in terms of flavonoids, quercetin, luteolin, naringenin, and eupatilin were present in varying proportions, indicating significant phenolic diversity that could influence the antioxidant capacity and biological activities of the samples. Furthermore, phenolic acids such as caffeic acid, salicylic acid, and 4-hydroxybenzoic acid were detected at particularly high levels in the IVM.

Taken together, these findings reveal marked differences in the phenolic compound profiles of *I. viscosa* extracts, with IVA and IVM showing higher concentrations and diversity of phenolics, thereby suggesting stronger potential for biological activity.

Table 1. Identified compounds and their concentrations (mg/kg) in plant extracts (IVA, IVM, IVPE)

Compound	IVA	IVM	IVPE
Flavonoids and Derivatives			
(+)-Catechin	<LOD*	0.35	0.34
(+)-Epicatechin	0.84	0.83	1.08
Acacetin	164.20	118.14	5.44
Apigenin-7-glucoside	0.53	0.31	<LOD*
Dihydrokaempferol	637.71	125.14	1.78
Eupatilin	43.36	30.68	1.74
Hyperoside	69.27	80.79	0.19
Isosakuranetin	515.47	437.75	64.36
Luteolin	8.32	11.46	<LOD*
Luteolin-7-rutinoside	48.30	19.24	0.13
Myricetin	1.68	0.60	0.25
Naringenin	30.44	13.76	0.04
Naringin	0.62	0.62	<LOD*
Nepetin	284.36	166.44	1.77
Nepetine-7-glucoside	170.23	268.22	0.28

Table 1. (Continued)

Phenolic Acids and Derivatives			
Orientin	0.11	0.82	<LOD*
Penduletin	45.97	32.43	1.76
Quercetin	162.73	50.97	1.25
Rhamnocitrin	1942.06	1640.75	156.18
Rutin	7.13	15.88	<LOD*
Scutellarein	3.92	0.1	0.57
Taxifolin	146.11	26.90	0.75
4-Hydroxybenzoic acid	6.06	78.75	<LOD*
Caffeic acid	19.74	20.52	0.31
Chicoric acid	0.40	1.42	<LOD*
Ellagic acid	0.28	<LOD*	<LOD*
p-Coumaric acid	0.84	0.84	0.28
Rosmarinic acid	7.44	8.36	10.25
Salicylic acid	9.16	57.72	1.52
Polyphenols and Glycosides			
Polydatin	0.54	0.54	0.53
Verbascoside	155.37	58.89	<LOD*
Anthraquinones			
Emodin	0.13	0.13	0.13
Amino Acids			
L-Theanine	< LOD*	0.04	< LOD*
Toplam (mg/kg)	3189.14	2509.39	184.37

*LOD:Limit of detection

Figures 2, 3, and 4 display the chromatograms for the compounds identified in each extract via LC-MS/MS.

3.2 Total Phenolic Content (TPC)

Standard gallic acid solutions prepared at increasing concentrations (40, 80, 120, 160, and 200 ppm) were analyzed under the same conditions. The absorbance values obtained were plotted against the concentrations, yielding the standard curve equation: $y=0.4925x-0.0812$ ($R^2=0.9775$). The TPC for each sample was derived from this equation, with results expressed in 'mg Gallic Acid Equivalent (GAE)/g sample'. The TPC contents were determined as 440.4 ± 0.001 mg GAE/g for IVA, 273.7 ± 0.001 mg GAE/g for IVM, and 51.8 ± 0.001 mg GAE/g for IVPE.

According to the results obtained, the TPC followed the order: IVA > IVM > IVPE. When the results are compared with literature, no data was found for acetone and petroleum extracts, while similar results were obtained for methanol extract. In addition, the TPC value determined for IVA was found to be higher than those reported for the methanol extract in many studies, whereas IVPE showed the lowest TPC value.

Eruygur et al. (2024) [25] reported the TPC value of 236.78 ± 7.63 mg GAE/g for methanol extract, while it was found to be similar for IVM (273.7 ± 0.001 mg GAE/g) in the present study, whereas the TPC value determined for IVA (440.4 ± 0.001 mg GAE/g) was considerably higher. Similarly, Qneibi et al. (2021) [36] reported the TPC value of the methanol extract prepared from the leaves of the plant as 193.07 ± 9.1 mg GAE/g. In a separate study, Trimech et al. (2014) [37] obtained diethyl ether, ethyl acetate, and methanol extracts from the plant's flowers, leaves, stems, and roots. Among these, the ethyl acetate extract exhibited the highest TPC values, which were quantified for the flowers, leaves, stems, and roots as 16.36 ± 3.08 , 8.48 ± 1.04 , 24.18 ± 2.07 , and 0.42 ± 0.03 mg GAE/g, respectively. Gökbüyük et al. (2013) [38] found the TPC

values of methanol extracts originating from the roots and above-ground parts of various *Inula* species as 176.9 ± 7.8 and 177.1 ± 3.6 mg GAE/g, respectively.

In another study (Ouahchia et al., 2020) [39], the TPC value of the methanol extract prepared from the flowers (251.33 ± 0.67 mg GAE/g) was consistent with our results, while the TPC value of the leaf part (387.33 ± 0.88 mg GAE/g) was higher.

When compared to studies in the literature, the TPC value of IVA was found to be higher than the TPC value reported for methanol extracts, and this can be attributed to the fact that the acetone extract was composed of large amounts of phenolic compounds.

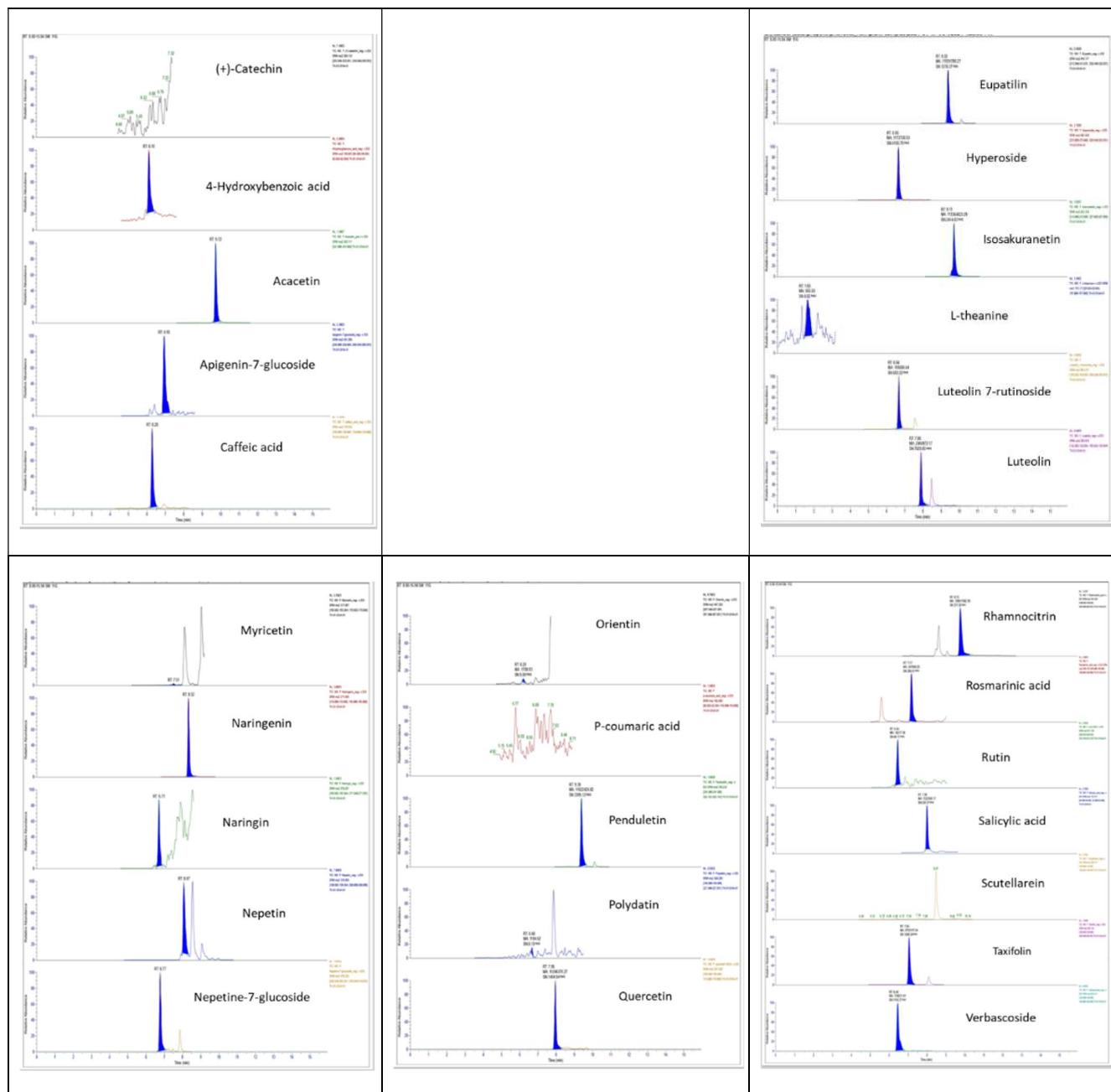


Figure 2. Chromatogram of *I. viscosa* acetone extract (IVA).

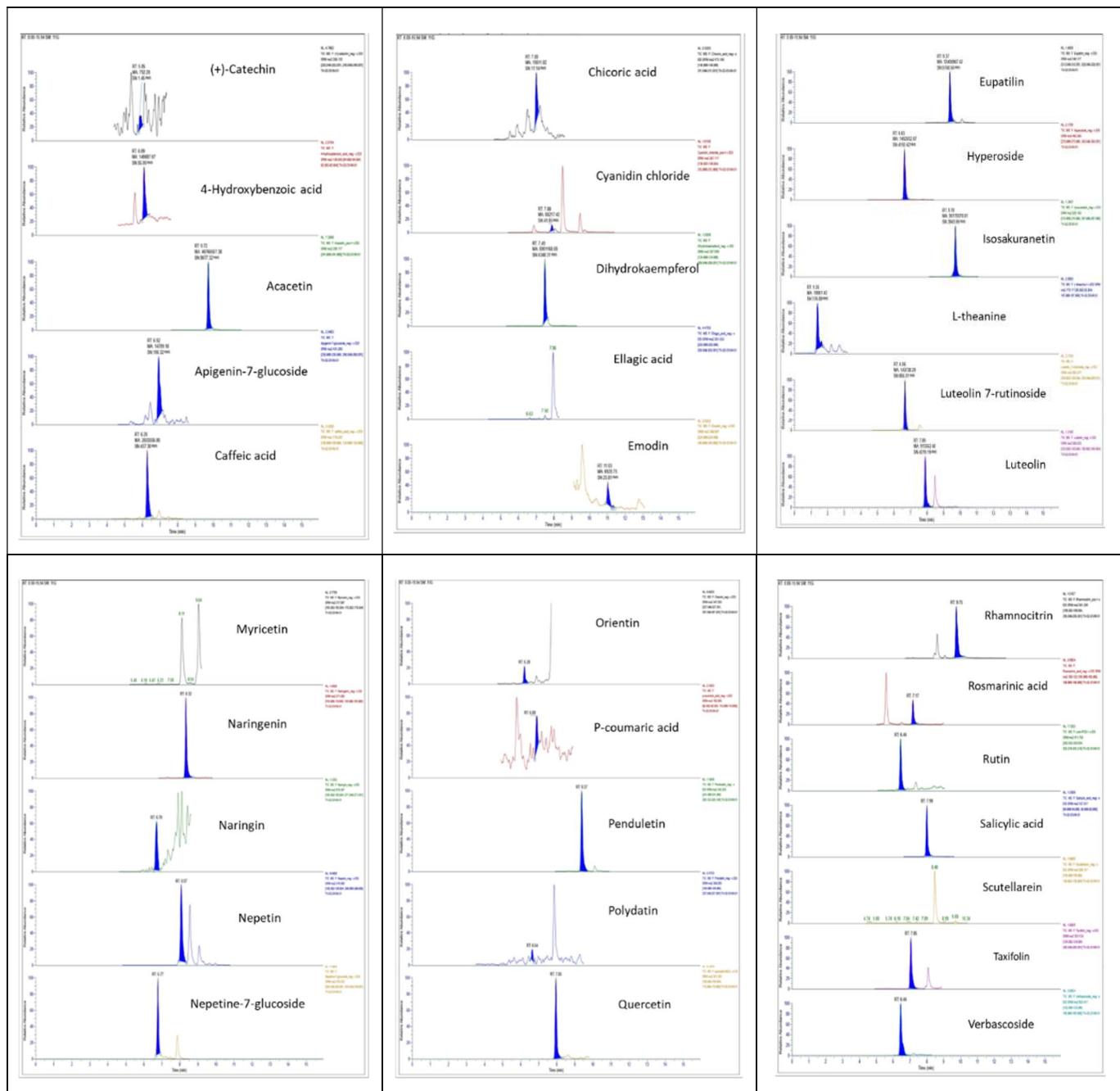


Figure 3. Chromatogram of *I. viscosa* methanol extract (IVM).

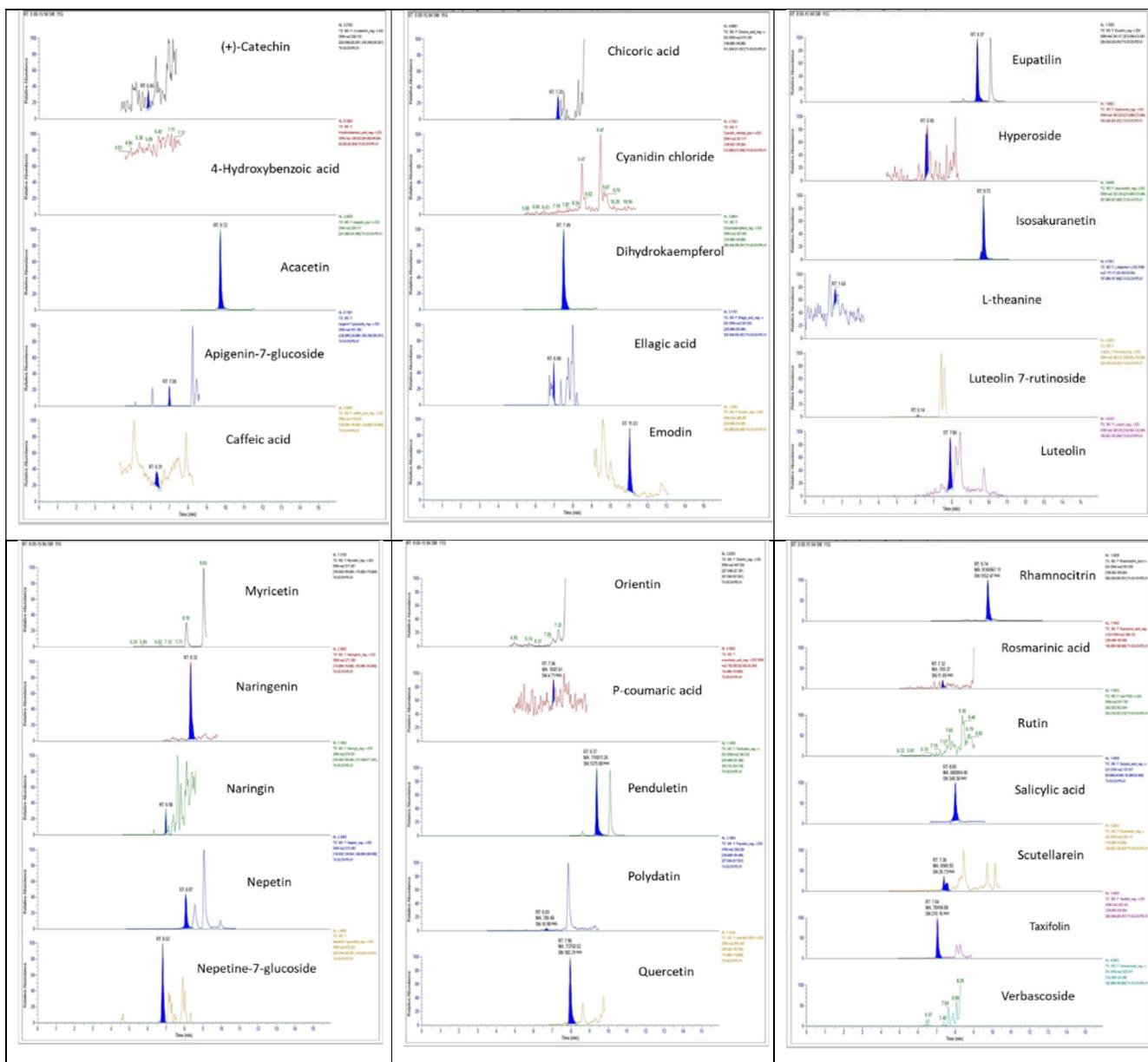


Figure 4. Chromatogram of *I. viscosa* petroleum ether extract (IVPE).

3.3 Antioxidant activity

The antioxidant potential of all extracts was measured using the DPPH radical scavenging and CUPRAC assays. The corresponding results are depicted in Figures 5. The DPPH radical scavenging activity was assessed at 10, 25, 50, and 100 ppm concentrations. Standard compounds BHT, BHA, and α -tocopherol were used in each method. Compared to standard compounds, BHT and BHA showed higher radical scavenging activity than the extracts while curcumin demonstrated strong radical activity, particularly at lower concentrations, and α -tocopherol exhibited lower or comparable activity relative to the extracts.

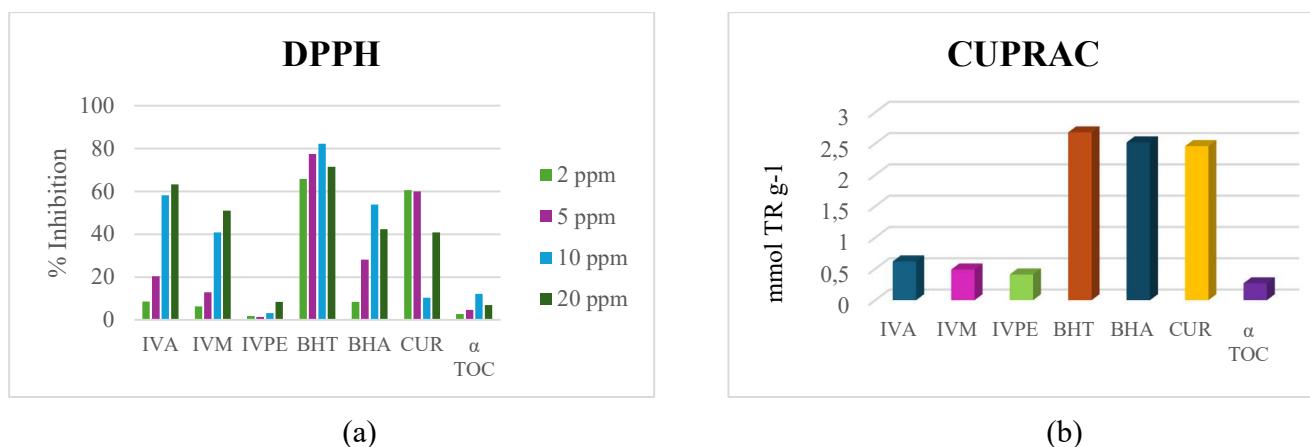
The DPPH results indicate that the antioxidant capacities of IVA, IVM and IVPE extracts are concentration dependent. IVA demonstrated the highest radical scavenging activity across all concentrations, reflecting moderate-to-high antioxidant potential, while IVM showed a similar trend with slightly lower activity.

Similarly, the literature also reports that methanol and acetone extracts possess high antioxidant capacity [25,40-41]. IVPE exhibited markedly lower radical scavenging capacity due to its low polyphenol content and nonpolar nature; this finding is supported by previous studies [42] and aligns with reports of limited antioxidant capacity in terpenoid-rich fractions [43]. These comparisons support the notion that extraction solvent and the chemical composition of the extracts play a decisive role in antioxidant activity.

According to CUPRAC method results, IVA (0.62 ± 0.04) showed the highest CUPRAC value, followed by IVM (0.49 ± 0.06) and IVPE (0.41 ± 0.03). All three extracts demonstrated higher antioxidant capacity than α -tocopherol but remained below that of standard compounds and curcumin. These findings indicate that the extracts possess significant antioxidant potential, consistent with previously reported activities of various extract and solvent types [43-45].

Findings from both assays indicate that *I. viscosa* extracts possess substantial antioxidant capacity. In the CUPRAC method, IVA exhibited the highest activity (0.62 ± 0.04), followed by IVM (0.49 ± 0.06) and IVPE (0.41 ± 0.03). Similarly, DPPH results confirmed that IVA had the strongest radical scavenging effect, with IVM and IVPE displaying lower activity levels.

Evaluation of both assays together highlights that IVA consistently exhibited higher activity, aligning with its rich phenolic content. Nonetheless, the antioxidant effects of *I. viscosa* extracts were lower than those of standard compounds (BHT, BHA) but higher than the natural antioxidant α -tocopherol. These data suggest that *I. viscosa* has potential as a natural source of antioxidants, with its biological activities strongly correlated to its phenolic profile.



IVA: *Inula viscosa* acetone, IVM: *Inula viscosa* methanol, IVPE: *Inula viscosa* petroleum ether

Figure 5. Antioxidant activity results of *I. viscosa* extracts (a) DPPH assay; (b) CUPRAC assay

4. CONCLUSION

The analysis of phenolic compounds revealed differences among the extracts. The IVA and IVM extracts exhibited high phenolic content, whereas IVPE showed comparatively low levels. Rhamnocitrin was identified as the dominant compound, and flavonoids such as dihydrokaempferol, nepetin, isosakuranetin, and quercetin were present in particularly high amounts in IVA and IVM. These findings indicate that the phenolic profiles of *I. viscosa* extracts are diverse, with IVA and IVM potentially exhibiting higher biological activity. In terms of total phenolic content (TPC), IVA and IVM were found to be rich in phenolic compounds, while IVPE yielded lower values. Overall, these results demonstrate that the aerial parts of the plant contain substantial levels of phenolics, suggesting their relevance for potential biological applications.

According to the literature review, this study represents the first report in which petroleum ether and acetone extracts of *I. viscosa* have been analyzed using LC-MS/MS and the Folin-Ciocalteu method, thereby contributing novel data to the existing literature.

When the results of the DPPH and CUPRAC assays are considered together, IVA displayed the highest antioxidant capacity in both tests, followed by IVM and IVPE. The antioxidant activity of *I. viscosa* extracts was lower than that of synthetic antioxidants but higher than or comparable to that of the natural antioxidant α -tocopherol. These results, which are consistent with the richness of phenolic compounds, underscore the potential of *I. viscosa* as a natural source of antioxidants.

Overall, the results demonstrate that *I. viscosa* extracts possess notable antioxidant potential, with activity varying among samples. In particular, the higher CUPRAC value of IVA compared to the other extracts reflects its greater phenolic richness.

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