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Assessment of antioxidant potential, verbascoside, and sugar composition of the floral parts of *Verbascum sinuatum*

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Abstract

In this study, the antioxidant capacity of the hydromethanolic extract obtained from the floral parts of *Verbascum sinuatum* was evaluated, and its total phenolic, flavonoid, verbascoside, and sugar contents were determined. The antioxidant capacity of the extract was evaluated using DPPH, ABTS, and FRAP assays. Total phenolic and flavonoid contents were measured spectrophotometrically, while the verbascoside and sugar contents of the extract were quantified by the HPLC-UV and HPLC-RID methods, respectively. According to the results, the DPPH radical scavenging activity of the extract was found to be IC_{50}= 30.70 ± 1.76 µg/mL, and the ABTS value was IC_{50}= 17.31 ± 0.65 µg/mL. The FRAP activity was determined as 63.94 ± 0.98 mg TE/g extract. The total phenolic content was measured as 45.23 ± 0.77 mg GAE/g, and the total flavonoid content as 6.31 ± 0.50 mg QE/g. According to the HPLC-UV analysis, the verbascoside content of the extract was found to be 9.72 ± 0.85 mg/g. Additionally, the results demonstrated that fructose (190.39 ± 3.12 mg/g) was the most abundant sugar in the floral extract of *V. sinuatum*, followed by glucose (132.91 ± 2.47 mg/g) and a relatively small amount of sucrose (7.21 ± 0.36 mg/g). As a result, the hydromethanolic extract of *V. sinuatum* floral parts exhibited a considerable level of antioxidant activity, contained significant amounts of phenolic compounds, particularly verbascoside, and was rich in hexose sugars, especially fructose and glucose. These findings suggest that this plant may be considered a promising natural source of antioxidants and bioactive compounds.

Keywords: *Verbascum sinuatum*, verbascoside, antioxidant activity, sugar composition, HPLC analysis

1. Introduction

The genus *Verbascum* (Scrophulariaceae), commonly known as mullein (in Turkish: Sığır kuyruğu), includes over 360 species worldwide. It is especially widespread in the Mediterranean region and Western Asia. Türkiye is considered a major center of diversity for *Verbascum*, with a notably high number of species and an impressive rate of endemism, hosting around 255 species, of which approximately 200 are endemic [1,2]. This high level of endemism highlights both the evolutionary importance of the genus and the uniqueness of the flora of Türkiye.

The *Verbascum* species have played an important role in traditional medicine. Infusions and decoctions prepared from their leaves and flowers have been used to treat a wide-range of ailments. Most commonly, they have been used as expectorants and antitussives in respiratory conditions such as coughs, bronchitis, and asthma [3,4]. Additionally, they have been used topically for their anti-inflammatory, analgesic, and wound-healing effects, often to relieve skin irritations, burns, and minor injuries [5–8]. Some species have also been used as natural dyes and in traditional practices [9].

Recent studies have confirmed traditional uses by demonstrating various biological activities in *Verbascum* species. The antioxidant, anti-inflammatory [10,11], antimicrobial [12,13], antiviral [14], analgesic [15], and anticarcinogenic [16,17] properties largely attributed to the complex phytochemical composition of the genus have been previously reported. The key groups of these compounds include iridoid and phenylethanoid glycosides [18], flavonoids [19], saponins [20,21], and polysaccharides [22]. Iridoid glycosides, such as aucubin and catalpol, are often linked to anti-inflammatory and hepatoprotective activities [23]. Phenylethanoid glycosides like verbascoside, isoverbascoside, and poliumoside are known for their potent antioxidant [18] and anti-inflammatory effects [24]. Flavonoids also

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contribute to antioxidant capacity and inflammatory regulation, while saponins are thought to support expectorant activity [25].

Verbascum sinuatum (known in Turkish as bodanotu) is a biennial herbaceous species belonging to the family Scrophulariaceae. It is distributed across roadsides, fallow fields, steppe areas, and coastal sandy regions in Turkey, flowering between May and October, typically at elevations from sea level to 1100 m [26].

V. sinuatum has been reported to contain various iridoid glycosides, including aucubin, catalpol, and its glycosides, sinuatoside, aucuboside, pulverulentoside, harpagide, and harpagoside [27], as well as phenylethanoid glycosides such as verbascoside and isoverbascoside [28]. In addition, several flavonoids (apigenin, rutin, and quercetin) and organic acids (chlorogenic acid, *p*-coumaric acid, cinnamic acid, and gallic acid) have been quantified in the aerial parts of *V. sinuatum*, supporting its high phenolic content and associated antioxidant activity [19]. The therapeutic effects of *V. sinuatum* probably result from the combined action of its compounds, which encourages further research into its pharmaceutical and nutraceutical applications.

The present study focused on the floral parts of *V.* sinuatum, aiming to determine their antioxidant activity, verbascoside content, and sugar composition. Among the various glycosides reported in this species, verbascoside was selected for quantitative analysis, as it is the most abundant and pharmacologically relevant phenylethanoid glycoside and is widely regarded as a marker compound in phytochemical investigations. Floral tissues, known to accumulate phenolic compounds involved in defense, pigmentation, and pollinator attraction, also contribute significantly to nectar chemistry and floral secretions. To the best of our knowledge, this is the first study to simultaneously assess the antioxidant capacity, verbascoside content, and sugar profile (fructose, glucose, and sucrose by HPLC-RID) of *V. sinuatum* flowers. This targeted approach not only provides insight into the phytochemical and ecological roles of floral metabolites, particularly in relation to pollinator interactions, but also highlights the pharmacological and nutraceutical potential of *V. sinuatum* as a natural source of bioactive compounds.

2. Material and methods

2.1. Plant materials

The floral parts, including petals and stamens, were collected in July 2024 from Yalnızbağ, Erzincan, Türkiye (39°47'51.6"N 39°24'58.9" E). A voucher specimen was identified by Prof. Dr. Ali Kandemir and deposited in the herbarium of Erzincan Binali Yıldırım University (EBYU) under the accession number EBYU-0000036. The samples were air-dried for one week in the shade and then ground into a fine powder for further analysis.

2.2. Extraction

Dried and powdered floral parts (100 g) were extracted in 70% methanol (v/v) using ultrasound-assisted extraction (UAE) for 30 min, followed by overnight maceration at room temperature, repeated three times, following established protocols for *Verbascum* spp. with minor modifications to the previously reported UAE/hydroalcoholic procedure [29]. After filtration, the combined extracts were evaporated under reduced pressure, yielding 10.2 g of a dark brown semi-solid crude extract, with a yield of 10.2%. The extract was stored in a dark-colored bottle at +4 °C until further analyses, including antioxidant activity assays and HPLC analysis.

2.3. Determination of antioxidant activity

2.3.1. Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of the extract were determined using spectrophotometric methods. A stock solution of the extract was prepared at a concentration of 1 mg/mL in methanol. For the TPC assay, the Folin–Ciocalteu reagent was used following a modified colorimetric method. Briefly, 100 µL aliquot of the extract was mixed with 100 µL of Folin–Ciocalteu reagent and 300 µL of sodium carbonate solution (10%). After 30 minutes of incubation in the dark, absorbance was measured at 765 nm using a microplate reader. Gallic acid was used to obtain the calibration curve, and results were expressed as mg gallic acid equivalents (GAE) per g of extract [30,31]. For the TFC assay, the aluminum chloride colorimetric method was employed. A 100 µL aliquot of the extract solution was mixed with 100 µL of aluminum chloride (1%) and 100 µL of sodium acetate solution (1M). After 30 minutes of incubation in the dark, absorbance was recorded at 415 nm. Quercetin was used as the standard, and results were expressed as mg quercetin equivalents (QE) per g of extract. All measurements were performed in triplicate (n=3) [32,33].

2.3.2. Radical scavenging assays (DPPH· and ABTS·+)

The antioxidant capacity of the extract was evaluated using DPPH and ABTS radical scavenging assays. Both assays were conducted in 96-well microplates using the same sample volumes and extract concentrations. A stock solution of the extract was prepared at a concentration of 1 mg/mL in methanol and diluted as needed. Trolox, BHA, BHT, and ascorbic acid were used as positive controls in both methods. For the DPPH

assay, the procedure was based on the method of [34,35], with slight modifications. Briefly, 50 µL of a 0.26 mM DPPH solution in ethanol was added to 100 µL of extract at various concentrations. The mixtures were incubated for 30 minutes in the dark, and absorbance was measured at 517 nm using a microplate reader. The ABTS assay was conducted following the protocol described by [36,37] with minor adjustments. $ABTS^{\bullet+}$ radicals were generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12–16 hours. The working solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 50 µL of the $ABTS^{\bullet+}$ solution was combined with 100 µL of extract at varying concentrations and incubated for 20 minutes in the dark. Absorbance was recorded at 734 nm. For both assays, radical scavenging activity (RSA) was calculated using the following formula:

RSA (%) = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control reaction (without the test sample), and A_1 is the absorbance in the presence of the extract or standard. RSA values were plotted against concentrations (µg/mL), and IC_{50} values were determined. All measurements were performed in triplicate and are presented as mean ± standard deviation.

2.3.3. FRAP activity

Reducing power was determined using the method of [38,39], with minor modifications. Stock solutions of extracts (1 mg/mL) were prepared, and 100 µL was mixed with phosphate buffer (0.2 M, pH 6.6) to a total volume of 1.25 mL. Subsequently, 1.25 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added, and the mixture was incubated at 50 °C for 20 minutes. After incubation, 1.25 mL of 10% TCA solution and 0.25 mL of 0.1% $FeCl_3$ solution were added to the reaction mixture. The final absorbance was measured at 700 nm using UV–Vis spectrophotometer. Reducing power was calculated as µmol Trolox equivalents per gram of extract using a Trolox calibration curve. All analyses were conducted in triplicate, and the results are presented as mean ± standard deviation.

2.4. HPLC-UV quantification of verbascoside

The analysis was carried out using a Thermo Scientific Ultimate 3000 HPLC system equipped with a Chrometsil C18 analytical column (4.6 × 150 mm, 5 µm particle size), following a slightly modified version of the protocol previously described by [40]. The mobile phase consisted of solvent A (2.5% formic acid in deionized water) and solvent B (methanol). The separation was performed at a flow rate of 1.0 mL/min using the following gradient program: initial isocratic elution at 95:5 (A: B) for 2 minutes, a linear gradient to 5:95 (A: B) over 20 minutes, isocratic elution at 0:100 (A: B) for 3 minutes, and re-equilibration to initial conditions (95:5 A: B) for 3 minutes. The column temperature was maintained at 35 °C, and detection was carried out at 330 nm. The verbascoside standard used for quantification had previously been isolated and characterized via preparative HPLC, as reported in our earlier study [41]. Calibration was based on six concentrations of verbascoside (5, 10, 25, 50, 100, and 200 µg/mL), yielding a linear regression equation of $y = 0.2427x - 0.1757$ with an R^2 value of 0.9996. The linear range was determined as 5–200 µg/mL, with a limit of detection (LOD) of 3.36 µg/mL and a limit of quantification (LOQ) of 10.19 µg/mL. For sample preparation, the extracts were dissolved in deionized water at a concentration of 5 mg/mL, filtered through a 0.22 µm syringe filter, and transferred into HPLC vials for injection.

2.5. HPLC-RID quantification of sugars

The sugar composition of the floral extract was analyzed using a Shimadzu Prominence HPLC system equipped with an LC-20AR pump and an LC-20RID refractive index detector. Separation was carried out on an Agilent Zorbax NH_2 column (150× 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and deionized water (75:25, v/v), delivered isocratically at a flow rate of 1.0 mL/min. The column temperature was maintained at 30 °C using a column oven. Sample injection was performed manually using a 20 µL fixed-volume loop [42].

For quantification, standard calibration curves were constructed for fructose, glucose, and sucrose at concentrations of 10, 20, 30, 40, and 50 mg/mL. Floral extract samples were prepared at a concentration of 50 mg/mL in deionized water and filtered through a 0.22 µm PTFE syringe filter prior to injection. The retention times and peak areas of the sugars in the samples were compared with those of authentic standards for identification and quantification. The analysis was performed in triplicate, and results were expressed as mean ± standard deviation. Sugar contents were calculated and reported as mg of sugar per gram of extract (mg/g extract).

2.6. Statistical analysis

All antioxidant activity data were expressed as mean ± standard deviation (n = 3) and statistically analyzed using one-way ANOVA using SPSS version 19 software.

3. Results and discussions

3.1. Antioxidant activity of *V. sinuatum* floral extract

The antioxidant activity results of the floral extract are presented in Table 1. The hydromethanolic extract of *V.*

sinuatum floral parts exhibited notable antioxidant activity, although it was lower than the reference antioxidants. The IC_{50} values for DPPH and ABTS radical scavenging assays were found to be 30.70 ± 1.76 µg/mL and 17.31 ± 0.65 µg/mL, respectively. The results show that the extract is more sensitive to cationic radicals, as indicated by the lower IC_{50} value in the ABTS assay compared to DPPH. Compared to standards such as Trolox, BHA, BHT, and ascorbic acid, which showed much lower IC_{50} values (ranging from 6.33 to 10.70 µg/mL), the extract demonstrated moderate radical scavenging potential. *V. sinuatum* extract exhibited a ferric reducing capacity of 63.94 ± 0.98 mg TE/g extract, which is significantly lower than the values for the synthetic and natural antioxidants tested in the FRAP assay. For instance, ascorbic acid showed the highest FRAP activity (394.17 ± 0.98 mg TE/g), followed by BHA (338.57 ± 0.31 mg TE/g) and BHT (257.80 ± 1.24 mg TE/g). Despite its relatively lower antioxidant power compared to standards, the extract contained a considerable amount of total phenolics (45.23 ± 0.77 mg GAE/g) and flavonoids (6.31 ± 0.50 mg QE/g). These secondary metabolites are known contributors to antioxidant activity, suggesting that the moderate performance of the extract may be attributed to these compounds, particularly phenolics.

When compared with previous findings, our results show both consistencies and differences. [43] reported that *V. sinuatum* aerial parts contained the highest total phenolic content (118.2 ± 2.46 mg GAE/g DW) among three *Verbascum* species, while our analysis of floral tissues yielded a lower value (45.23 ± 0.77 mg GAE/g extract). This discrepancy can be explained by the difference in plant material: [43] evaluated the whole aerial parts (stems, leaves, and flowers), whereas the present study focused exclusively on the floral organs, which may accumulate phenolics at comparatively lower levels. Interestingly, in terms of flavonoids, our extract contained a slightly higher amount (6.31 ± 0.50 mg QE/g) compared to the aerial part value reported for *V. sinuatum* (4.87 ± 0.06 mg QE/g), suggesting that flavonoids are more concentrated in the flowers, where they play a role in pigmentation and pollinator attraction. With respect to antioxidant capacity, [43] showed that *V. sinuatum* aerial parts displayed strong DPPH radical scavenging activity (average inhibition 89.95%), comparable to other *Verbascum* species such as *V. nudicaule* and *V. speciosum*. In our study, the floral extract exhibited an IC_{50} value of 30.70 ± 1.76 µg/mL in the DPPH assay, which indicates moderate activity when compared to standards such as Trolox, BHA, and ascorbic acid, but is in line with the antioxidant profile previously reported for the genus. These comparisons suggest that although the absolute values differ depending on plant parts and extraction conditions, both our results and earlier studies consistently highlight *V. sinuatum* as a species rich in phenolics and capable of exerting notable antioxidant effects.

3.2. Verbascoside content of *V. siniatum* floral extract

The verbascoside content of the floral methanol extract was determined to be 9.72 ± 0.85 mg/g extract by HPLC-UV at 330 nm. As shown in the HPLC-UV chromatogram (Fig. 1), the floral hydromethanolic extract of *V. sinuatum* exhibited a major peak corresponding to verbascoside at 13.390 min, consistent with the retention time of the standard (13.303 min). This indicates that verbascoside is one of the dominant phenylethanoid glycosides in the extract. In addition to verbascoside, several minor peaks were also observed, showing the presence of other related phenylethanoid compounds previously reported in *V. sinuatum*, such as isoverbascoside and forsythoside [44]. The high verbascoside content (9.72 mg/g extract) accounts for a substantial portion of the total phenolic content (45.23 mg GAE/g, see Table 1), suggesting that phenylethanoid glycosides are major contributors to the phenolic content of the extract. The relatively low flavonoid content (6.31 mg QE/g, see Table 1) further supports the predominance of non-flavonoid phenolics in *V. sinuatum* floral tissues.

Recent studies have focused on optimizing extraction techniques, such as ultrasound-assisted extraction, to maximize phenolic yields from *V. sinuatum* [45]. Research has indicated that different extraction times and solvent ratios can significantly influence the mg/g yield of verbascoside, emphasizing the importance of extraction parameter selection to enhance the bioactivity of the extracts [46]. Reports have shown that the phenolic content in extracts derived from *V. sinuatum* can exceed 8.53 mg/g, which is relevant for therapeutic applications

Table 1. Antioxidant activities and total phenolic and flavonoid contents in the methanol: water (7:3) extract of *Verbascum sinuatum* floral parts.

Samples and standards	DPPH IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})	Total phenolics mg GAE g^{-1} Extract	Total flavonoids mg QE g^{-1} Extract	FRAP mg TE g^{-1} Extract
V. sinuatum	30.70 ± 1.76^{b*}	17.31 ± 0.65^{b}	45.23 ± 0.77	6.31 ± 0.50	63.94 ± 0.98^{c}
Trolox	8.50 ± 0.77^{a}	7.07 ± 0.15^{a}	-	-	-
BHA	8.04 ± 0.69^{a}	6.33 ± 0.19^{a}	-	-	338.57 ± 0.31^{a}
BHT	10.70 ± 0.73^{a}	9.42 ± 0.63^{a}	-	-	257.80 ± 1.24^{b}
Ascorbic acid	9.91 ± 0.87^{a}	8.25 ± 0.41^{a}	-	-	394.17 ± 0.98^{a}

*The different letters in the same column indicate statistically significant differences at the $p < 0.05$ level.

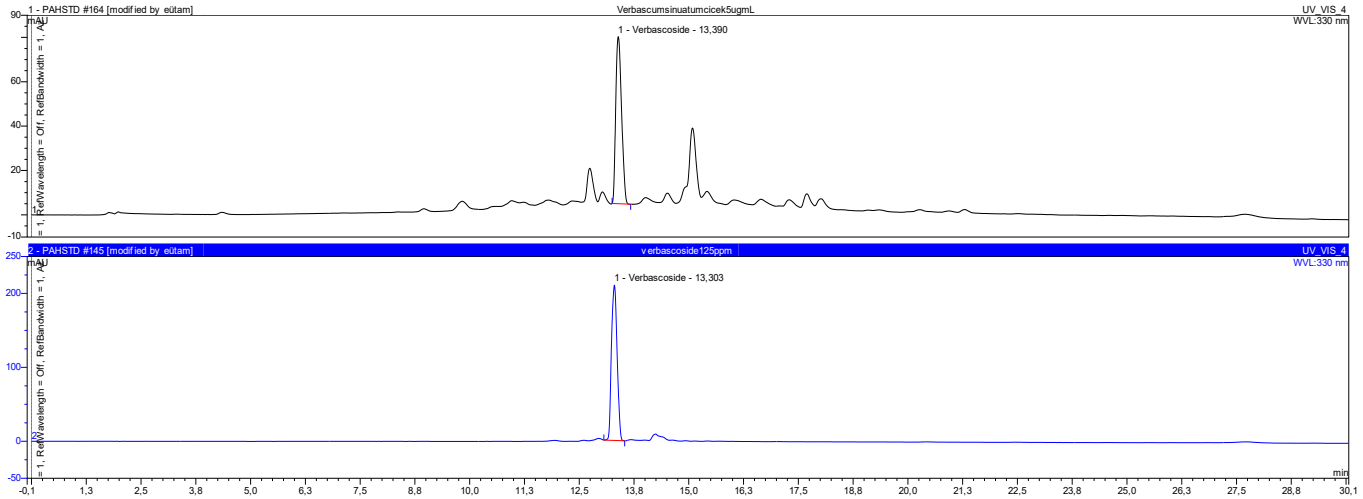

Figure 1. HPLC-UV chromatograms of floral part extract of *V. sinuatum* (top) and standard verbascoside (bottom) at 330 nm

where a high phenolic concentration is closely associated with increased antioxidant capacity [47]. In our study, the verbascoside concentration of 9.72 mg/g was found specifically from the floral part of the plant, indicating that targeted extraction from specific organs may yield phenolic contents comparable to or higher than those reported in the literature for whole-plant extracts.

3.3. Fructose, glucose, and sucrose levels in the floral extract

The HPLC-RID chromatograms of the floral extract and sugar standards (fructose, glucose, and sucrose) are shown in Fig. 2. The results demonstrated that fructose (190.39 ± 3.12 mg/g) was the most abundant sugar, followed by glucose (132.91 ± 2.47 mg/g) and a relatively small amount of sucrose (7.21 ± 0.36 mg/g) in the floral methanol extract of *V. sinuatum.* This sugar profile indicates a hexose-dominant composition, which is generally associated with pollinator preference and may also reflect ecological or metabolic adaptations of the species. To the best of our knowledge, this is the first report of sugar composition in *V. sinuatum*. As sugar composition has not yet been reported for this genus, literature on nectar chemistry in other plant families provides useful comparisons: *orchids* and members of

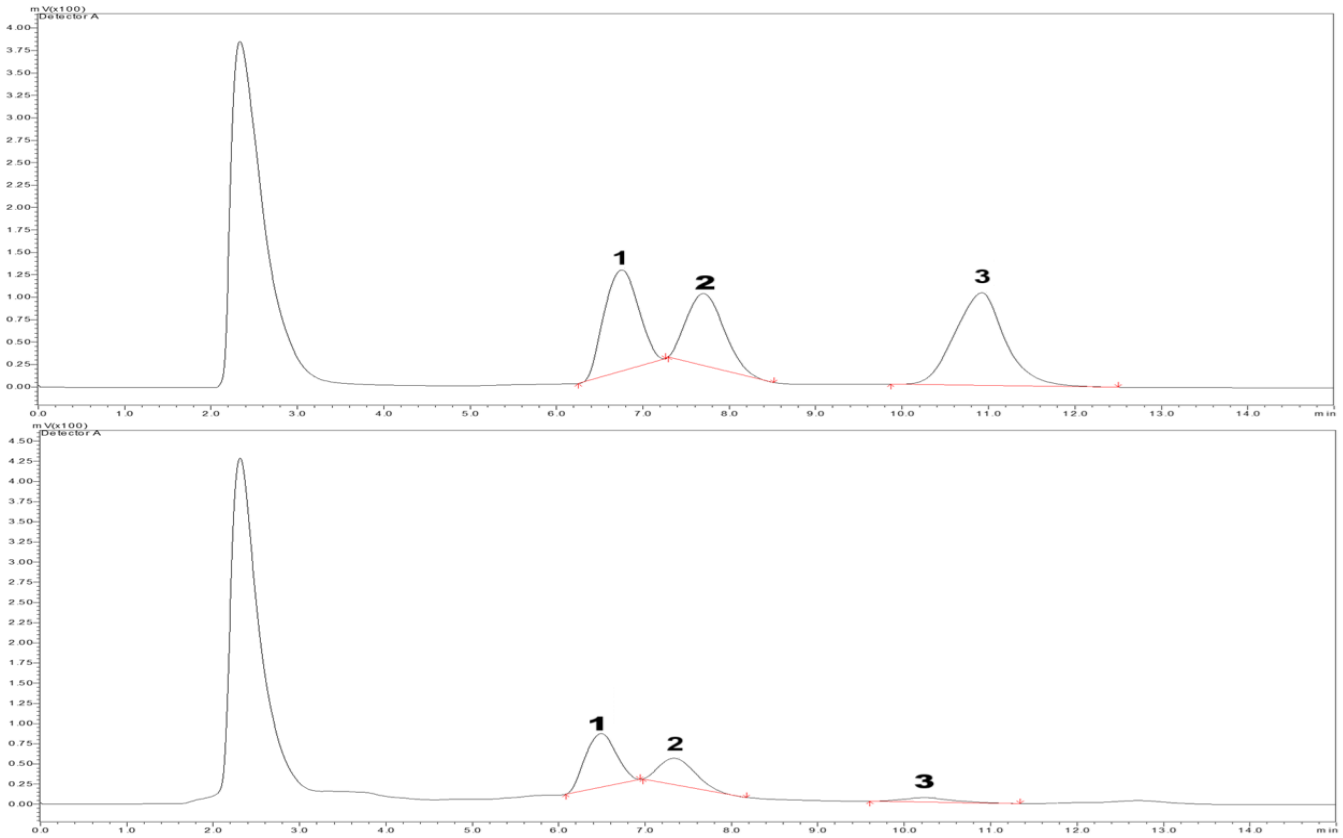

Figure 2. HPLC-RID chromatograms of the standards (top; 1: fructose, 2: glucose, 3: sucrose) and the floral part extract of *V. sinuatum* (bottom)

Lamiaceae also exhibit hexose-dominant profiles in which fructose and glucose are the predominant sugars [48]. The similarity of *V. sinuatum* to these families suggests that a hexose-based nectar composition may represent an ecological strategy that supports pollinator attraction and contributes to reproductive success.

4. Conclusion

The findings of this study demonstrate that the hydromethanolic extract obtained from the flowers of *V. sinuatum* possesses moderate antioxidant capacity determined by DPPH, ABTS, and FRAP assays. The extract contains considerable amounts of phenolic compounds and flavonoids, with verbascoside identified as a major bioactive constituent. In addition to its antioxidant properties, the floral extract was found to be rich in sugars, particularly fructose and glucose, indicating a hexose-dominant composition. Notably, this study provides the first report simultaneously addressing the antioxidant activity, verbascoside content, and sugar composition of the floral parts of *V. sinuatum*. This novelty adds to the phytochemical knowledge of the species and highlights its dual relevance: the pharmacological potential of bioactive phenylethanoid glycosides such as verbascoside and the ecological significance of a hexose-dominant sugar profile that is associated with pollinator attraction and plant–pollinator interactions.

Overall, these results indicate that *V. sinuatum* flowers may represent a promising natural source of antioxidants and bioactive compounds with potential applications in the pharmaceutical, nutraceutical, and food industries. Further research, including *in vivo* studies and bioactivity-guided isolation, is warranted to better elucidate the therapeutic and ecological potential of this species.

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