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ANALYSIS OF PHYTOCHEMICAL PARAMETERS WITH POTENTIAL PHARMACEUTICAL APPLICATIONS IN THREE *VIOLA* SPECIES, SUBGENUS *VIOLA*

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Abstract: *V. odorata* is widely recognized for its medicinal applications, with its compounds utilized in the treatment of various ailments as well as in cosmetic preparations. Both *V. suavis* and *V. alba* are found in habitats similar to those of *V. odorata* and possess comparable phytochemical profiles. This paper presents a comparative analysis of key phytochemical compounds of pharmacological interest among these species, focusing on total phenols, anthocyanins, and total flavonoids. To complement the research on polyphenols, the antioxidant capacity of plant extracts was analysed using DPPH.

Keywords: polyphenols, flavonoids, *V. alba*, *V. suavis*, *V. odorata*, DPPH.

INTRODUCTION

The genus *Viola* L. (*Violaceae* Batsch.) is divided into about 16 subgenera. The *Viola* subgenus (x=10, 11, 12, 13) contains taxa native to the temperate zone of the Northern Hemisphere, and has approximately 18-22 species (Marcussen and Borgen, 2000; Jonsell *et al.,* 2009).

Most of these species are perennial herbaceous plants, which generally have stolons (Ballard *et al.,* 1999). The flower has an almost cylindrical style, ending with a rostral, hook-shaped stigma. In this group, seed dispersal is carried out only by ants (Marcussen and Borgen, 2000; Sârbu *et al.,* 2001; Jonsell *et al.,* 2009).

The species of this subgenus are taxonomically complex. This complexity arises from their phenotypic plasticity, frequent interspecific hybridization, and regional differentiation caused by varying environmental conditions. As a result, it can be difficult to distinguish among taxa belonging to *V. odorata*, *V. alba,* and *V. suavis* (Marcussen and Borgen 2000; Mereďa *et al.,* 2008).

Depending on the presence or absence of stolons, the *Viola* group is divided into two categories: flagellatae (with stolons) and eflagellatae (without stolons) (Ballard *et al.,* 1999; Marcussen and Borgen, 2000). *V. odorata*, *V. suavis,* and *V. alba* have stolons and, for this reason, they are part of flagellatae.

V. odorata was designated as the "type species" of this subgenus by Britton and Brown in 1913 (Jonsell *et al.,* 2009; Espeut, 2020). It is cosmopolitan, growing in a wide range of areas, especially in thickets, bushes, meadows, forest edges, clearings, and along watercourses. It prefers hilly and plain regions (Beldie *et al.,* 1955). The species is characterized by a dense, articulated rhizome, long lateral stolons, and leaves arranged in a rosette with ovate-lanceolate stipules (Ballard *et al.,* 1999; Jonsell *et al.,* 2009; Chifu *et al.,* 2001, 2006). Due to diverse habitat conditions, *V. odorata* exhibits substantial morphological variability. Varieties may display flowers in different colours, including lilac, white, pale pink, sulphur, blue, reddish, and variegated (Jonsell *et al.,* 2009).

Like *V. odorata*, *V. suavis* is a perennial plant that blooms in March and April. Its range extends from plains (steppe zones) to mountain (beech) regions. This species has short, densely articulated rhizomes that are 4–5 mm thick. Its aboveground stolons can reach 15 cm in length, and sometimes it produces underground stolons. Other features include long fimbriated stipules and bracteoles located on the lower half of the flowering peduncle (Mereďa *et al.,* 2008; Jonsell *et al.,* 2009; Sârbu *et al.,* 2013).

V. alba occupies similar habitats and demonstrates high phenotypic plasticity, much like *V. odorata* and *V. suavis*. It blooms at the same time as the other two species (March and April). The species is distinguished by flowers with white petals, nearly ovate and acute leaves, linear-lanceolate and long-fimbriated stipules, light green coloration, and relatively long stolons (about 30 cm) that do not root at the nodes. (Jonsell *et al.,* 2009; Sârbu *et al.,* 2013).

V. odorata is well-known for its medicinal properties. Its compounds are used to treat a variety of ailments and in cosmetic applications. For example, extracts containing salicylic acid and rutin are applied in the treatment of skin conditions (Rimkiene *et al.,* 2003). The plant's flavonoids such as violantine, which has anti-inflammatory effects, are used to prepare creams for eczema, acne, or ulcers (Parekh and Chandra, 2007; Hammami *et al.,* 2011, 2012; Burzo, 2015). Polyphenolic compounds with antibacterial and anti-inflammatory properties are also present. These extracts serve as adjuvants in treating lung conditions, gastrointestinal disorders, heart conditions, urinary tract issues and can help reduce blood pressure (Appell, 2000; Rimkiene *et al.,* 2003; Pilberg *et al.,* 2016; Dastagir *et al.,* 2023).

Frequently, the habitats of these three species overlap. They are found in open deciduous forests, forest edges, scrublands, and river meadows. Because of this, taxa from *V. odorata* can be confused with *V. suavis* and/or *V. alba*, and vice versa (Jonsell *et al.,* 2009; Chifu *et al.,* 2001, 2006). In traditional medicine, these species

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are used to treat the same conditions and to produce similar extracts.

Phytochemical studies on *V. suavis* are few, and for *V. alba*, they are even scarcer. Through this study, we aim to address some of these research gaps by providing new information about the quantitative composition of total polyphenols, total anthocyanin pigments, and total flavonoids in these three species. Specifically, this study aims to answer the following questions: (1) How do the levels of total polyphenols, anthocyanin pigments, and flavonoids compare among *V. odorata, V. suavis*, and *V. alba*? (2) Are there significant differences in these compounds that could aid in distinguishing these species?

METHODS

The plant material was collected from spontaneous flora (Table 1) from three different populations for each species, during the flowering phenophase in the North-East of Romania. To ensure unbiased sampling, a randomized grid method was used: sampling sites were selected by overlaying a grid, then plants were collected from randomly assigned grid intersections. The plant material was taxonomically determined according to Sârbu et al., 2013.

Table 1.

The collecting locations for the plant material

Species	Plant Population (PP)	Collecting area	Habitat type
V. odorata L.	PP1	Cioatele, Vaslui County	Meadow
	PP2	Breazu Forest, Iași County	Deciduous forest
	PP3	Mălini, Suceava County	Deciduous forest edge
V. suavis Bieb.	PP1	Mălini, Suceava County	Meadow
	PP2	Cozla Mountain, Neamț County	Mixed forest (deciduous and coniferous)
	PP3	Bârnova Forest, Iași County	Deciduous forest edge
V. alba Bess.	PP1	Bârnova Forest, Iași County	Deciduous forest edge
	PP2	Cozla Mountain, Neamț County	Mixed forest (deciduous and coniferous)
	PP3	Breazu Forest, Iași County	Deciduous forest

Selection for plant health was based on explicit criteria: only individuals showing no visible signs of disease (e.g., no lesions, spots), uniform leaf coloration, absence of mechanical damage or senescence, and with intact, fully open flowers were included.

For anthocyanin extraction, fresh flowers (1 g) were mixed with 20 mL of 95% ethyl alcohol acidified with 0.1N HCl. The mixture was agitated on a shaker at 100 rpm for 24 hours at room temperature (20–22°C) in the dark. Anthocyanin content was quantified spectrophotometrically at 515 nm (Jia *et al.*, 1999; Wrolstad *et al.*, 2005).

Total phenols and flavonoids were extracted using both aqueous and 50% ethanolic solvents at concentrations of 0.5%, 1%, 2.5%, and 5% (w/v). For each extraction, 1 g of air-dried, mechanically ground aerial plant material (flowers removed) was combined with 20 mL of solvent, agitated as above for 24 hours in darkness, followed by filtration. Standardized conventional methods were used for the quantitative analysis of total phenolics and flavonoids (Jia *et al.*, 1999; Herald *et al.*, 2012; Lobiuc *et al.*, 2017).

Antioxidant activity was assessed using the 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method, chosen for its sensitivity and reliability in measuring radical neutralisation capacity (Blois, 1958; Lobiuc *et al.*, 2017).

Statistical analysis was performed using Microsoft Excel v2406 and OriginPro 9.4.

RESULTS AND DISCUSSIONS

Anthocyanin content

Interest in researching anthocyanin pigments stems from their well-documented antioxidant properties. These compounds are known to support ocular, neurological, and cardiovascular health. Additionally, anthocyanins provide protective actions against solar radiation (Vermerris and Nicholson, 2006). Anthocyanin pigments are linked to long-term human health benefits because of their potent antioxidant activity and their potential to lower the risk of chronic diseases. For example, scientific studies have demonstrated that diets rich in anthocyanins are associated with a reduced risk of cardiovascular disease. (Hollman *et al.,* 1995; Wallace and Giusti, 2015).

The anthocyanin pigments are responsible for the red and blue colours seen in flowers and fruits. Their presence is closely associated with the survival of the species, as these pigments play an important role in processes such as pollination and seed dispersal (Soare *et al.,* 2011; Iwashina, 2013; Wallace and Giusti, 2015).

As expected, high values were obtained from intensely purple (*V. odorata*) and blue-purple petals (*V. suavis*). For *V. odorata,* the quantitative values for the anthocyanin were, on average, between 0.79±0.3 mg/g and 1±0.3 mg/g. Most of the *V. odorata* plants collected had intensely purple petals. Differences were observed in PP3, plant material collected from the edge of a deciduous forest (ecotone habitat), from where plants with creamy-white flowers and variegated purple petals were harvested (Fig. 1).

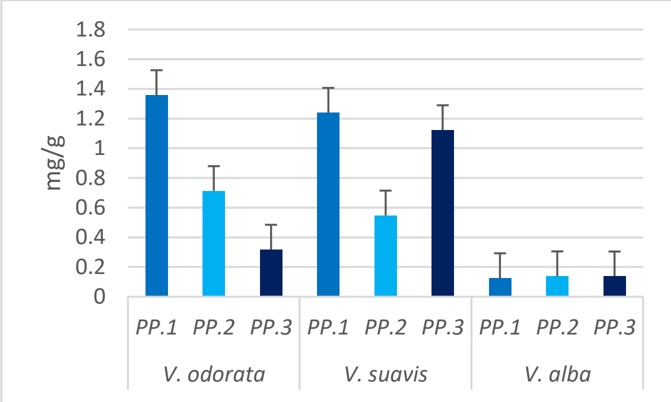

Fig. 1. The total anthocyanins expressed as mg/g fresh weight ± standard error determined for *V. odorata, V. suavis, and V. alba,* for three plant populations (PP1, PP2, PP3).

Because of this situation, the values for total anthocyanins for the PP3 population were the lowest, at approximately 0.3±0.01mg/g. In the case of the second population, the flowers of *V. odorata* were a mixture of indigo, blue-purple, pale purple, variegated, and creamy white. Therefore, the average value observed was 0.7±0.2 mg/g fresh weight.

Most of the flowers of *V. suavis* were blue-purple, pale purple, and variegated. The average values for total anthocyanin content were between 0.5±0.04 mg/g (PP2) and 1.2±0.09 mg/g (PP1).

The fluctuation of the quantitative values of anthocyanin compounds, both between species and between populations of the same species, may also be due to soil quality or water stress, since they are harvested from different habitats. The type of nutrients and soil pH have a direct influence on the colour of the petals (Glover, 2009; Dalrymple *et al.,* 2020).

Since *V. alba* has mainly white petals, it has the lowest average values for the anthocyanin compounds of approximately 0.13±0.004 mg/g, compared with *V. odorata* and *V. suavis*. In nature, "pure" white is not found, as pale shades of yellow are often present, resulting in white-cream hues. The "white" petals contain flavones and flavonols, types of plant pigments that influence petal coloration by absorbing specific wavelengths of light, thereby contributing to the characteristic off-white/ cream-white shades. These compounds, while largely colourless or only faintly yellow, accumulate in the petals and impart a subtle pale-yellow hue, preventing the appearance of a truly pure white. Examples include luteolin, quercetin, apigenin, and kaempferol (Iwashina *et al.,* 2010; Iwashina, 2013).

Total phenol content

Polyphenolic compounds are recognized in modern medicine for their anti-inflammatory and antibacterial effects, as well as for their strong antioxidant capacity (Parekh and Chandra, 2007; Hammami *et al.,* 2011, 2012; Burzo, 2015). Researchers have also demonstrated the potential of certain phenolic compounds to combat vascular and neuronal inflammatory processes (Parekh and Chandra, 2007; Burzo, 2015; Williamson, 2017; Dayani *et al.,* 2022). New studies highlight the applicability of polyphenols from medicinal plants in the treatment of neurodegenerative diseases, such as Alzheimer's or Parkinson's (Cory *et al.,* 2018; Aquilano *et al.,* 2008).

To determine an optimal concentration for analysis, various dilutions of the analysed biological material were performed, and it was identified that the highest values were recorded for the smaller concentrations of 0.5% and 1% (w/v) for both types of plant extracts (aqueous and ethanolic).

For the aqueous extracts (Fig. 2), the highest values at 0.5% (w/v) were for *V. suavis* (25±1 mg/g GAE), followed by *V. odorata* with 21±2 mg/g GAE, and *V. alba* (16±1 mg/g GAE).

For the 5% (w/v) aqueous extracts, the highest value was found to be for *V. suavis* (15±2 mg/g GAE), followed by *V. odorata* (14±1 mg/g GAE) and *V. alba* (11±1 mg/g GAE).

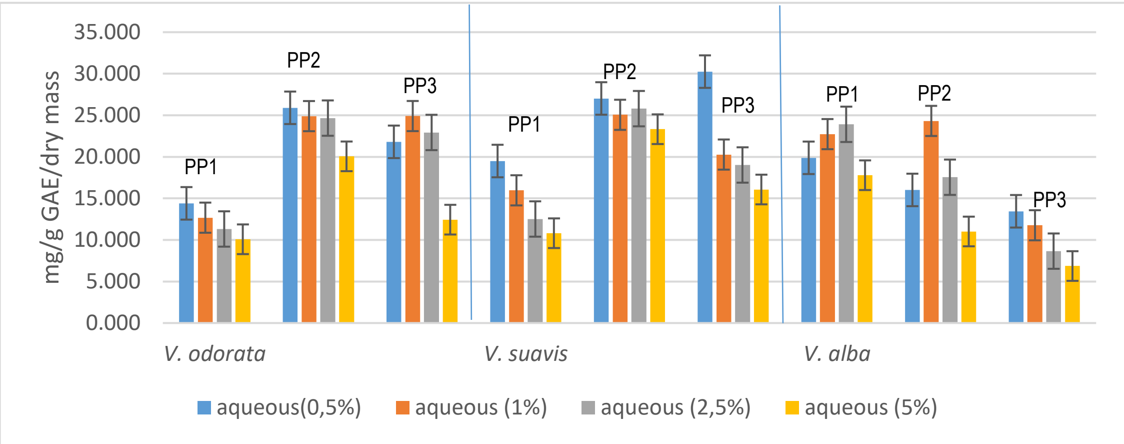

Fig. 2. The total amount of polyphenols expressed as mg/g GAE/dry mass ± standard error determined for *Viola* aqueous extracts for four concentrations (w/v), for three plant populations (PP1, PP2, PP3).

These data show that, for this type of aqueous plant extract, increasing the extract concentration does not lead to a proportional increase in the efficiency of phenolic compound extraction. For all four plant extract concentrations, there was no statistical difference regarding the total phenolic content between *V. odorata* and *V. suavis*. For the hydroalcoholic extracts (Fig. 3), we observed a similar pattern in extraction yield (expressed as mg of extract per g of plant material) as noted in previous studies. Specifically, the extraction yield for *V. odorata* decreased from 20–21 mg/g to 17–18 mg/g when the plant-to-solvent ratio was increased. This decrease may be due to the reduced solvent availability per unit of plant material, which limits the extraction of soluble compounds.

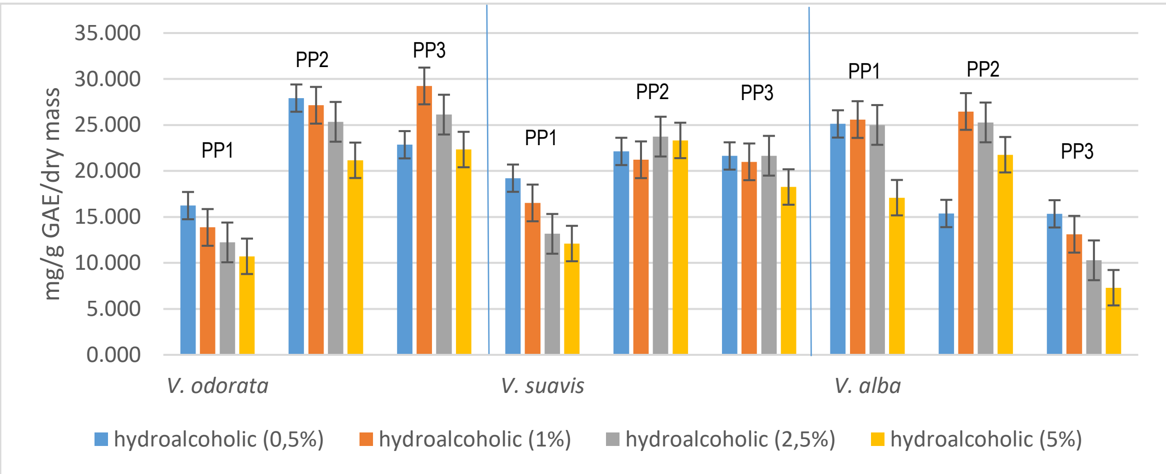

Fig. 3. The total amount of polyphenols expressed as mg/g GAE/dry mass ± standard error determined for *Viola* hydroalcoholic extracts for four concentrations (w/v), for three plant populations (PP1, PP2, PP3).

Similar to the aqueous extract, the highest extraction efficiency was for 0.5% and 1% plant-to-solvent ratios. For a 0.5% ratio, the highest value was observed for *V. odorata* (21±1 mg/g GAE) and the smallest for *V. alba* (18±1 mg/g GAE). The total amount of polyphenols recorded for the 5% hydroalcoholic extracts was between 18±1 mg/g GAE (*V. odorata*) and 15±2 mg/g GAE (*V. alba*).

Analysing the quantitative values of polyphenols extracted from three plant populations revealed that meadow habitats yielded the lowest amounts for both types of plant extract. In contrast, plants from forest and forest edge habitats showed higher polyphenol levels. The analysis of the aqueous extracts, plant material collected from the meadow (plant population 1 -PP1), for both *V. odorata* and *V. suavis*, showed that lower values were recorded for all plant-to-solvent ratios. For *V. odorata,* the values were comprised between 14±0.3 mg/g GAE (0.5%w/v), 12±0.2 mg/g GAE (1% w/v), 11±0.1 mg/g GAE (2.5% w/v), and 10±0.03 mg/g GAE (5% w/v). The plant material collected from deciduous forest (PP2, PP3) contained approximately 23±0.6 mg/g GAE (0.5% w/v), 24±0.3 mg/g GAE (1% w/v), 23±1 mg/g GAE (2.5% w/v), and up to 20±1 mg/g GAE (5% w/v) total polyphenols.

Regarding *V. suavis,* the values for total phenols content obtained by analysing the samples from PP1 (meadow type habitat) were between 19±0.7 mg/g GAE (0.5% w/v) and 10±0.1 mg/g GAE (5% w/v). Similarly to the values obtained for *V. odorata*, the values obtained for *V. suavis* collected from deciduous forest (PP2, PP3) were comprised between 28±1 mg/g GAE (0.5% w/v) and 19±1 mg/g GAE (5% w/v).

Unlike these two species, *V. alba* was not collected from a meadow-type habitat. The plant material was collected from mixed forests and deciduous forests. Regardless, the values for total polyphenols in the aqueous extracts were lower than those of the previous species. The registered values were between 13-19 mg/g GAE (0.5% w/v), 11-24 mg/g GAE (1% w/v), 9-23 mg/g GAE (2.5% w/v), and 7-17mg/g GAE (5% w/v).

Regarding the hydroalcoholic extracts, the same patterns are found for all three species. For PP1 - *V. odorata,* the values were comprised between 16±1 mg/g GAE (0.5%w/v), 13±1 mg/g GAE (1% w/v), 12±0.1 mg/g GAE (2.5% w/v), and 10±0.5 mg/g GAE (5% w/v). For the plant material collected from PP2 and PP3, the values for total phenol content were approximately 24±1 mg/g GAE (0.5% w/v), 28±1 mg/g GAE (1% w/v), 25±1 mg/g GAE (2.5% w/v), and 21±1 mg/g GAE (5% w/v).

For *V. suavis* collected from the meadow, the values obtained from hydroalcoholic extracts were of approximately 19±1 mg/g GAE (0.5%w/v), 16±1 mg/g GAE (1% w/v), 13±1 mg/g GAE (2.5% w/v), and 12±1 mg/g GAE (5% w/v). Similarly with *V. odorata*, the data regarding the total phenols for PP2 and PP3 from *V. suavis* showed intervals between 18±0.1 mg/g GAE (5% w/v) and 22±1 mg/g GAE (0.5%w/v).

The hydroalcoholic extracts for *V. alba* revealed close values to those obtained for *V. odorata* and *V. suavis.* The type of solvent used influenced the efficiency of the extraction. The number for total phenols content was between 15-25 mg/g GAE (0.5% w/v), 13-25 mg/g GAE (1% w/v), 10-25mg/g GAE (2.5% w/v), and 7-21 mg/g GAE (5% w/v).

It seems that for the plant material collected from inside a deciduous forest, for both types of plant extracts, *V. alba* registered the lowest values. This result may be due to the soil quality, since it is known that polyphenols are involved in plant adaptation to less favourable conditions, such as nutrient-poor soils (Di Ferdinando et al., 2014).

Total flavonoid content

Flavonoids are pigments that contribute to the formation of the colour of vegetative organs, with an important role in protecting the plant from various pests and environmental factors (Samanta *et al.,* 2011).

Due to their antioxidant properties and more, this category of compounds is of great interest to modern medicine (Burzo, 2015). For example, certain flavonoids have been shown to reduce oxidative stress and inhibit inflammatory pathways implicated in neurodegeneration (Cory *et al.,* 2018; Aquilano *et al.,* 2008).

The total flavonoids were analysed for two types of plant extract at four plant-to-solvent ratios (Fig. 4).

For the aqueous extract, the average values for *V. odorata* were about 9 mg/g QE for all four extract concentrations. The lowest listed values were for PP1, plant material gathered from meadow, between 4±0.9 mg/g QE (5% w/v) and 6±0.1 mg/g QE (0.5% w/v).

The highest values (up to 14±0.7 mg/g QE, 1% w/v) were registered for plant material collected from a deciduous forest.

More or less, the same data was obtained from the aqueous extract of *V. suavis.* Lower values registered for plant material of PP1, between 8±0.1 mg/g QE (5% w/v) and 9±0.3 mg/g QE (0.5% w/v). For the other populations, the values were around 10-13 mg/g QE.

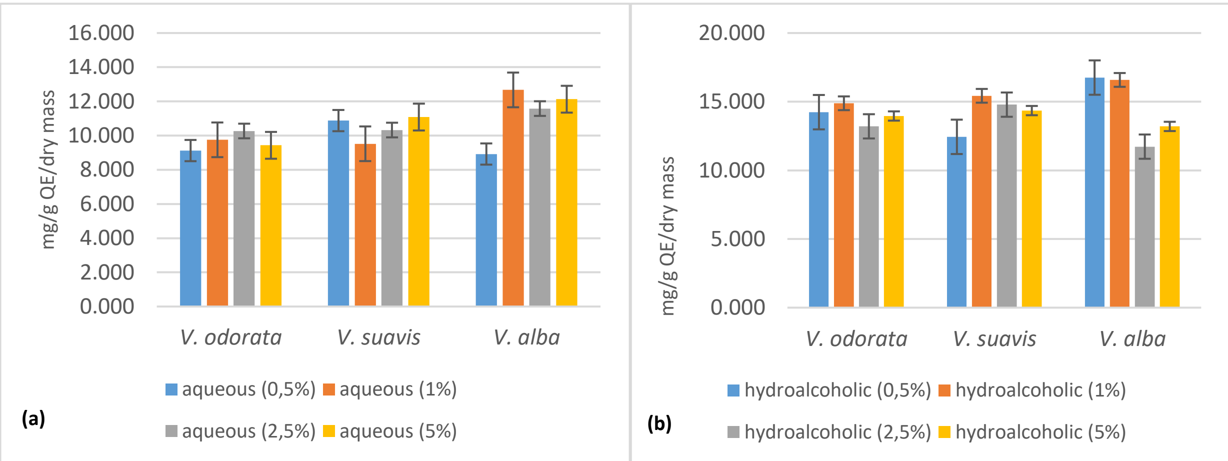

Fig. 4. The total flavonoids expressed in mg/g quercetin/dry mass ± standard error in aqueous (a) and hydroalcoholic (b) *Viola* extracts of four concentrations

For *V. alba,* the data regarding the total flavonoids from aqueous plant extract were lower for PP1 (deciduous forest edge-ecotone type habitat) – up to 10±0.2 mg/g QE (2.5 %-5% w/v) compared to the other two populations – up to 15±0.1 mg/g QE (1% w/v).

Regarding the hydroalcoholic *Viola* extracts, the data are similar to those obtained for aqueous plant extract. For *V. odorata* and *V. suavis*, lower flavonoid compound values were observed in plant material harvested from meadow-type habitats. Specifically, *V. odorata* showed up to 12±0.1 mg/g QE (5% w/v), while *V. suavis* showed up to 11±0.1 mg/g QE (5% w/v). For the other plant populations, the average values for *V. odorata* were about 15±0.1 mg/g QE (0.5% w/v), 17±0.4 mg/g QE (1% w/v), and 14±0.1 mg/g QE (2.5% and 5% w/v). Comparatively, the data for *V. suavis* were approximately 14±0.3 mg/g QE (0.5% w/v), 17±0.2 mg/g QE (1% and 2.5% w/v), and 15±1 mg/g QE (5% w/v).

The hydroalcoholic *V. alba* extracts obtained from the PP1 had lower values compared with PP2 and PP3, which were between 12±0.4 mg/g QE (5% w/v) and 16±0.8 mg/g QE (0.5% w/v). The highest average values were obtained for PP2, plant material collected from a mixed forest (deciduous and coniferous): 18±0.02 mg/g QE (0.5% w/v), 19±0.3 mg/g QE (1% w/v), and 11±0.1 mg/g QE (2.5% and 5% w/v). A reason for this difference in values may be that stress factors influence the expression of some genes that are involved in the synthesis of flavonoids. The soil of a mixed forest is influenced by the type of plants that live in it, and conifers directly influence the pH of the soil (Winkel-Shirley, 2002; Di Ferdinando *et al.,* 2014; Samanta *et al.,* 2011).

Therefore, the fluctuation of quantitative values recorded between species and between populations may be due to the type of flavonoids biosynthesized in response to external stimuli (Karim et al., 2018).

Another reason for these differences may be due to the type of solvent and the grinding method of the plant material, which influences the size of the particle that comes into contact with the solvent (John *et al.,* 2006; Panja, 2018).

The antioxidant capacity

The highest values of the antioxidant capacity (Fig. 5) were recorded for *V. alba* and *V. suavis*; meanwhile, *V. odorata* had the lowest values.

Analysing the data, the most efficient extraction was achieved at a 2.5% plant-to-solvent ratio, followed by 1% plant-to-solvent ratio. For three extract concentrations (1%, 2.5% and 5% w/v), a dilution was necessary for an efficient analysis (1/5, respectively, 1/10 dilution).

The highest values of the antioxidant capacity for *V. alba* aqueous extracts were 15±0.1 mg/g GAE – 35 % inhibition rate (1% w/v), and about 10±0.1 mg/g GAE – 28 % inhibition rate (2.5% w/v extract). For the hydroalcoholic extracts, the inhibition rate of 1% (w/v) extract was about 21% (11±0.6 mg/g GAE), and around 27 % (5±0.3 mg/g GAE).

At the opposite end, the lowest values were registered for *V. odorata*. The inhibition rate for aqueous extracts of 1% (w/v) was between 5-7 % (3±0.1 mg/g GAE), and the extract was diluted by 1/5 times. For the aqueous extracts of 2.5% (w/v), the antioxidant capacity was between 2-3±0.2 mg/g GAE with an inhibition rate of about 6-10%.

Analysing the data obtained at the population level for all three species, we note that there is no statistically significant difference between them.

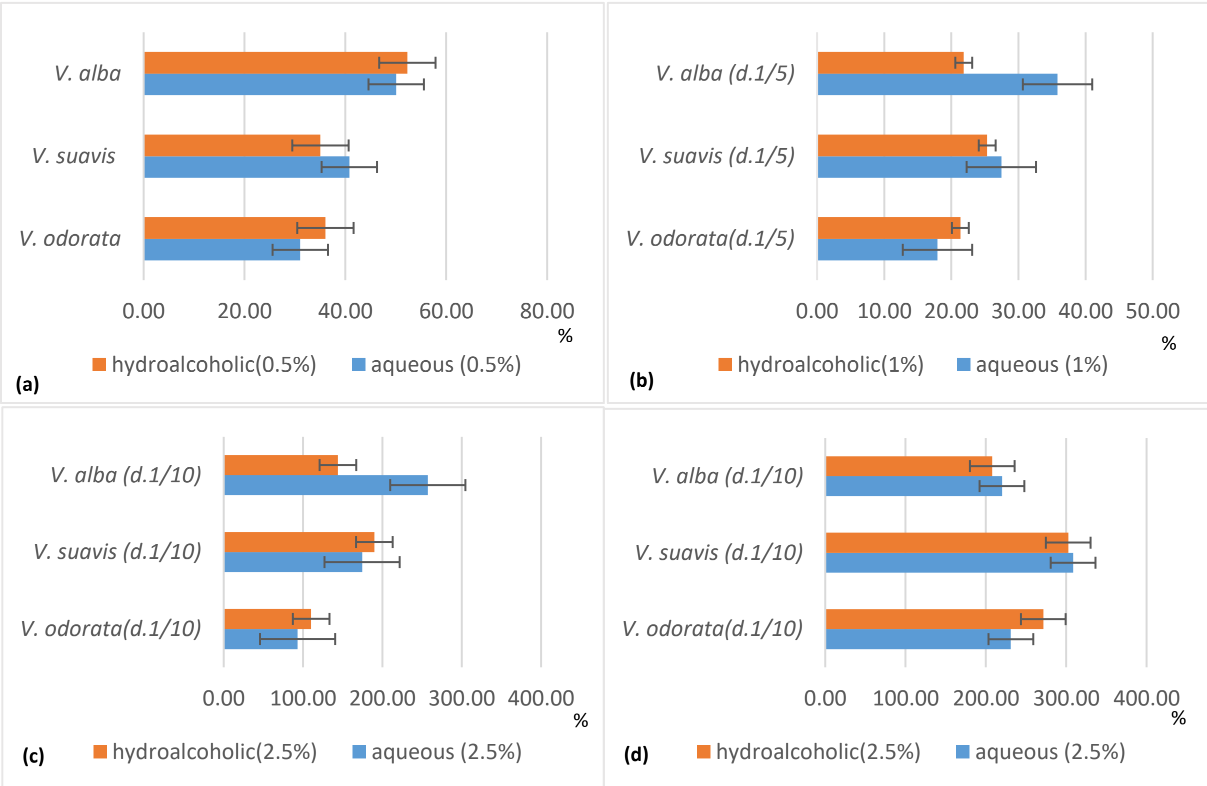

Fig. 5. Antioxidant capacity expressed through *Inhibition Rate* (%) in aqueous and hydroalcoholic *Viola* extracts of various concentrations: (a) 0.5%, (b) 1%, (c) 2.5% and (d) 5% w/v. (d = dilution of the plant extract)

The antioxidant capacity of the plant extracts is directly influenced by the presence of the polyphenolic compounds, which are recognized as having antimicrobial, anti-inflammatory, and antioxidant properties. Since free radicals are considered mediators in cardiovascular, neurological diseases, and various types of cancer, the antioxidant capacity of a compound is considered particularly important in counteracting free radicals (Parekh *et al.,* 2005; Parekh and Chandra, 2007; Hammami *et al.,* 2011, 2012; Burzo, 2015). The differences between the values of the results obtained for both between species and between populations of the same species can also occur due to the quality of the plant material as well as the stress factors acting on the plant at the time of harvesting (Słomka *et al.,* 2008; Hassanvand *et al.,* 2021).

The inhibition rate values varied depending on the type of solvent used (water or 50% ethanol) and the concentration of the plant extract. The method chosen for determining the antioxidant capacity greatly influenced the efficiency of the compound's extraction. In general, the values obtained closely matched the measured amounts of total phenols and flavonoids (Fig. 6). The inhibition rate values increased in parallel with the total amount of phenols and flavonoids measured, indicating a positive correlation between these variables.

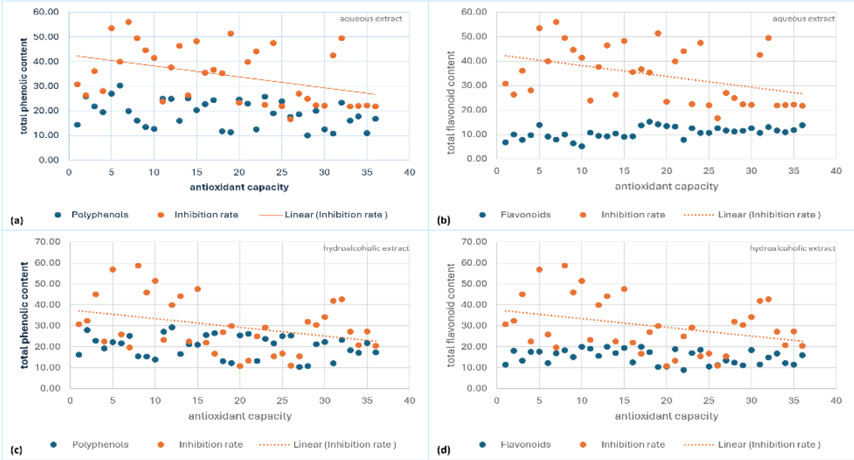

Fig. 6. Correlation between antioxidant capacity and total polyphenols content [left- (a) and (c)], respectively, between antioxidant capacity and total flavonoid contents [right – (b) and (d)], for both types of extract.

CONCLUSION

V. odorata is one of the most well-known species of the *Viola* genus, and numerous studies were conducted from a biochemical perspective. By comparison, studies on phytochemistry and pharmacology are fewer for *V. suavis*, and even less for *V. alba*. Therefore, through this study, we present new findings on the total amounts of phenols, flavonoids, and anthocyanins, as well as the antioxidant capacity of two types of plant extracts, analyzed from the perspective of four concentrations.

The quantitative analysis of anthocyanin pigments revealed their presence, but in low values, in *V. alba*, a species with cream-white flowers.

The quantitative analysis of phenols and flavonoids revealed lower values in the species collected from meadows and the deciduous forest ecotone. In comparison with *V. odorata*, both *V. alba* and *V. suavis* contain a significant amount of polyphenols. In general, for all biocompounds analysed, we can distinguish a significant difference between *V. alba* and the other two species.

Based on the quantitative analysis of polyphenols and on the types of habitats to which *V. suavis* and *V. odorata* can adapt, we can conclude that these two species have economic potential as raw materials for the extraction of biologically active compounds.

The analysed *Viola* species contains a significant amount of compounds from the class of polyphenols, biocompounds important for the pharmaceutical and cosmetic industries. Our quantitative results, based on simple extraction methods, demonstrate notable levels of polyphenols, flavonoids, and anthocyanin pigments. If advanced extraction techniques (ultrasound-assisted extraction, supercritical fluid extraction) are used, along with other solvent types, the efficiency of the biocompound extraction can be greatly increased.

AUTHORS CONTRIBUTIONS

Conceptualization, E.R., A.L., and M.M.Z.; methodology, E.R., A.L., and M.M.Z.; data collection, E.R.; data validation, E.R., A.L., and M.M.Z.; data processing, E.R. and A.L.; writing—review and editing, E.R., A.L., and M.M.Z.

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FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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