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Comparative analysis and antioxidant potential of hydroalcoholic extracts and infusions of cultivated ironwort

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ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ

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INTRODUCTION & AIM

Sideritis raeseri Boiss. & Heldr., native to the Balkan Peninsula, is renowned for its health-promoting properties and its composition since it is rich in bioactive diterpenoids and flavonoids. Its cultivation has rapidly expanded to meet the increasing demand for its aerial parts, particularly in beverage production and the pharmaceutical and cosmetic industries.

This study investigated the differences between ironwort extracts and infusions regarding their chemical composition and antioxidant capacity.

METHOD

Plant Material *S. raeseri* aerial parts, harvested from the Prefecture of Achaia (Peloponnese, Greece) and provided by the company "ΑΔΟLO", were naturally air-dried.

Extraction Ultrasound-assisted extraction was performed, first with petroleum ether to remove volatile and lipid contents and then with 50% aqueous methanol. Each step included three cycles of 20 min each, maintaining the temperature under 40°C.

Tea Infusion Preparation Infusions were prepared by steeping the plant material in water initially heated to 95°C for 10 minutes.

For both preparations, solvent removal was made in vacuo, and samples were stored at –20°C, under N_2.

Antioxidant Activity **Total Phenolic Content (TPC) Assay** The Folin-Ciocalteu method **[1]** was applied, and gallic acid was used as standard. Absorbance was measured at 750 nm. Results were expressed as mg of gallic acid equivalents per g of dry weight.

FRAP (Ferric Reducing Antioxidant Power) Assay: 60 μL of sample was mixed with 55 μL acetate buffer and 80 μL of FRAP reagent. The FRAP solution was prepared by mixing 70 mL of acetate buffer, 15 mL of TPTZ solution, and 15 mL of $FeCl_3$ solution **[2]**. Absorbance was measured at 595 nm after 5 min incubation. The results were expressed as μmol of Fe(II) per g of dry weight.

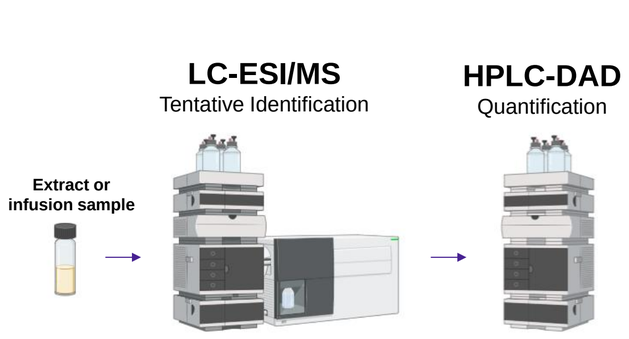
Table 1. **Calibration curve standards and equations for TPC and FRAP assays.**

Assay	Standard	Equation (1. for extracts, 2. for infusions)	R-Squared
TPC	Gallic acid	1. y = 1.2287x + 0.0321	0.996
		2. y = 1.2287x + 0.0321	0.996
FRAP	$FeSO_4•7H_2O$	1. y = 50.301x + 0.0206	0.995
		2. y = 69.202x + 0.0408	0.998

DPPH• Assay: 5 μL of sample was mixed with 195 μL of 0.1 mM DPPH reagent in methanol in a 96-well plate **[3]**. After a 30-minute incubation in the absence of light, absorbance was recorded at 540 nm. Ascorbic acid was used as a standard antioxidant compound. The results were expressed as % DPPH radical scavenging (%SCAV) from the equation: $\%SCAV = [(A_{control} - A_{sample})/A_{control}]*100$, where A_{sample} is the net sample absorbance and $A_{control}$ the absorbance of positive control. IC_{50} values represent the sample concentration required to scavenge 50% of the free DPPH radical.

LC-MS & HPLC-DAD analysis

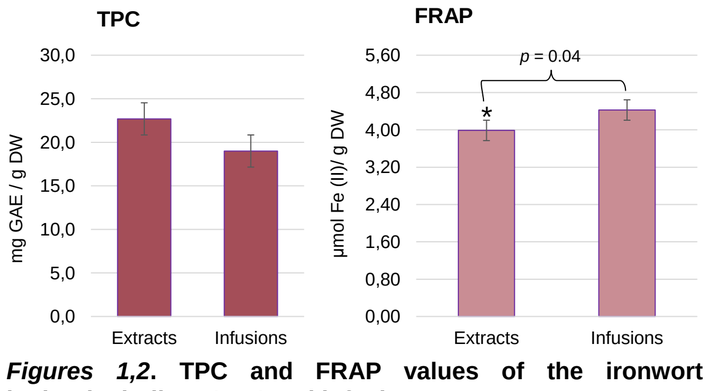
Column: C18 Polar (100 × 3.0 mm, 2.6 μm),
Temperature: 40°C
Mobile phase: A: 0.1% (v/v) formic acid in H_2O and B: 0.1% (v/v) formic acid in MeOH
Flow rate: 0.3 mL/min
Method: 0-3 min, 7% B; 3-5 min, 7-15% min; 5-9 min, 15-25% B; 9-31 min, 25-35% B; 31-37 min, 35-65% B; 37-47 min, 65% B; 48-53 min, 100, followed by a 7 min equilibration step at the original elution conditions.
External Standards: Melittoside (98%, purified in the laboratory, y = 20.896x + 248.48, R^2 = 0.999), Chlorogenic acid (≥99%, Extrasynthese, y = 70.224x - 27.218, R^2 = 0.999), Verbascoside (≥99%, PhytoLab, y = 37.671x + 10.079, R^2 = 0.999), Scutellarein (≥99%, Carbosynth, y = 102.47x - 364.11, R^2 = 0.997).



RESULTS & DISCUSSION

Extraction The mean extraction yield after UAE extraction with 50% aq. methanol was 18.17 ± 0.90%, while the mean yield after the infusion preparation was 0.17 ± 0.02%.

Antioxidant activity Both the extract and the infusion demonstrated significant antioxidant activity, as shown in Figure 1. They exhibited a similar TPC (extract: 22.69 ± 2.5 mg GAE/g DW, infusion: 19.00 ± 1.11 mg GAE/g DW) and close IC_{50} values in the DPPH• assay (extract: 2.17 ± 0.11 mg/mL, infusion: 2.58 ± 0.15 mg/mL), while in the FRAP assay, the infusion presented a significantly higher value (p<0.05) of 4.425 ± 0.381 μmol Fe(II)/g DW compared to that of the extract: 3.988 ± 0.332 μmol Fe(II)/g DW.



Figures 1,2. **TPC and FRAP values of the ironwort hydroalcoholic extract and infusion.**

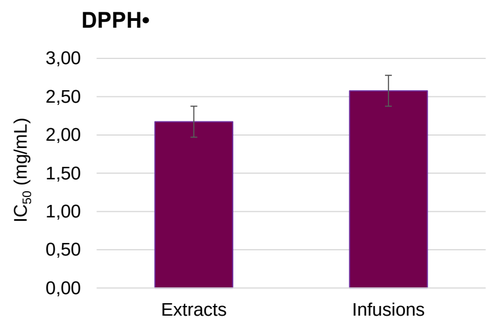


Figure 3. **DPPH• scavenging activity values of the ironwort hydroalcoholic extract and infusion.**

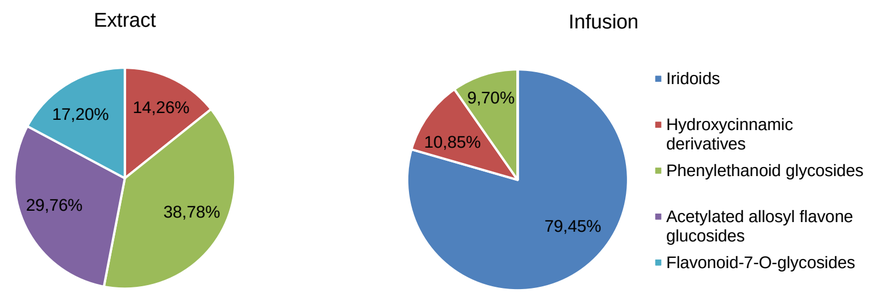
LC-MS & HPLC-DAD analysis

Among the identified compounds (Table 2) in the hydroalcoholic extract, phenyethanoid glycosides were the dominant group, with verbascoside being the leading component (22.21 ± 5.16 mg/g DW), followed by flavonoids, apigenin-7-*O*-(6''-*O*-4-coumaroyl)-β-glucopyranoside (echinacin) (16.21 ± 0.59 mg/g DW) and AcO-All-Glc-ISC-Me (15.93 ± 2.09 mg/g DW).
Although most iridoid compounds were not quantifiable with the UV-vis detector, melittoside was the most abundant metabolite in the infusion (31.39 ± 2.54 mg/g DW), followed by the hydrocinnamic derivative, chlorogenic acid 4.29 ± 0.88 mg/g DW.

Table 2. **List of polar metabolites in *S. raeseri*.**

Nº	Rt / min	MW	λmax / nm	Component	Extract	Infusion
1	4.66	524	210	Melittoside	•	•
2	7.73	354	215/240/290/325	Unknown		•
3	10.14	376	220/280	Unknown		•
4	12.27	354	215/245/290/330	Chlorogenic acid	•	•
5	12.80	402	210/230/275/315	Unknown		•
6	14.19	670	230/310	Unknown	•	
7	15.84	390	n.dtm.	Ajugoside	•	•
8	16.86	640	210/270/340	β-Hydroxyverbascoside isomer	•	•
9	17.61	712	210/280/325	Unknown	•	•
10	22.32	786	220/245/285/330	Echinacoside	•	•
11	22.89	756	220/245/285/330	Forsythoside B or Lavandulifolioside	•	•
12	23.63	624	220/245/285/330	Verbascoside	•	•
13	27.35	624	220/245/285/330	Verbascoside isomer	•	•
14	29.78	770	n.dtm.	Alyssonoside	•	•
15	32.65	668	210/255/275/300/340	AcO-All-Glc-HYP	•	
16	34.23	652	225/275/300/310/335	AcO-All-Glc-ISC/LUT	•	
17	37.77	682	210/255/275/300/340	AcO-All-Glc-HYP-Me	•	
18	38.56	624	225/275/305/325/370	All-Glc-ISC-Me	•	
19	38.78	666	225/275/305/325/371	AcO-All-Glc-ISC-Me	•	
20	40.34	578	210/230/270/320	Echinacin isomer	•	•
21	40.82	578	210/230/270/321	Echinacin isomer	•	•
22	40.92	694	220/275/340	$(AcO)_2$-All-Glc-ISC	•	
23	40.34	724	225/275/305/325/370	$(AcO)_2$-All-Glc-HYP-Me	•	
24	42.95	708	230/265/320	$(AcO)_2$-All-Glc-ISC-Me	•	

Abbreviations: All: allosyl, Glc: glucosyl, AcO: acetoxy, Me: methyl, HYP: hypolaetin, ISC: isoscutellarein, LUT: luteolin, n.dtm.: not determined.



Figures 4,5. **Main secondary metabolite categories in ironwort extracts and infusions.**

CONCLUSION

These findings indicate that mountain tea infusions confer equipotent antioxidant protection to the hydromethanolic extract despite compositional variations, which can, however, affect other biological properties, warranting further investigation. Overall, this study highlights the potential of *S. raeseri* as a valuable natural source of antioxidants for industrial applications.

REFERENCES & ACKNOWLEDGEMENTS

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