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ARTICLE

CHEMICAL AND ANTIOXIDANT CAPACITY EVALUATION OF *CENTAUREA JACEA* EXTRACTS FROM PLANTS HARVESTED IN ARAD COUNTY, ROMANIA

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Abstract: *The phytochemical composition and biological activity of the plant extracts obtained in different solvents (water, 70% ethanol, methanol, and diethyl ether) of the brown knapweed (Centaurea jacea L.) harvested in Romania were obtained. The chemical composition of the extracts was determined by gas chromatography–mass spectrometry analysis, by total phenolic and flavonoid content. The antioxidative potential was determined using ferric-reducing antioxidant power (FRAP) and 2,2-dyphenyl-1-picrylhydrazyl (DPPH) scavenging methods. The methanolic extracts revealed the highest antioxidant activity by FRAP and DPPH assays. This study revealed, once again, the importance of the geographic and climate region of harvesting the plants used for biomedical applications.*

Keywords: *Centaurea jacea*, extracts, GC-MS, antioxidant capacity, total phenolic content; FRAP assay, DPPH assay.

INTRODUCTION

Centaurea jacea L. (brown knapweed) is an Asteraceae family herbaceous plant. It is one of the most abundant plant families on the planet, with over 23,000 species (Wilson and Randall, 2005), but only herbaceous species are present in Romania. Numerous edible, ornamental, medicinal, industrial, and even invasive species belong to this family (Roche and Roche, 1991).

Centaurea jacea L. is a perennial plant native to Europe that reproduces solely via seeds. The leaves are lance-shaped and can reach a maximum length of 15 cm at the plant's base. The blossoms are typically pinkish-purple (Fig. 1) and occasionally white. The blooming period begins in July and continues through October. Each flower can produce up to twelve dark brown seeds that produce pollen and nectar, making this an attractive plant for beekeepers. The bracts encircling the flower range in color from light to dark brown and reflect a metallic golden hue when in bloom (Ayad and Akkal, 2019; Wilson and Randall, 2005). Several studies described the morphology, and phenotypes of Centaurea plants from diverse areas around the globe (Arnelas et al., 2018; DiTommaso et al., 2021; Dydak et al., 2009; Francini et al., 2008; Vanderhoeven et al., 2002; Villodre and Garcia-Jacas, 2000; von Cossel et al., 2021), including the cytogenetic analysis of several populations of *Centaurea jacea* (Dydak et al., 2009)

Traditional medicine employs the genus *Centaurea* L. extensively (Ayad and Akkal, 2019; Martkoplishvili and Kvavadze, 2015; Sharonova et al., 2021). In Russia, the blossoms of *Centaurea cyanus* L. (cornflower) are frequently employed as a diuretic. In Scottish medicine, *Centaurea cyanus* L. and *Centaurea scabiosa* L. (greater knapweed) are also employed as diuretics and tonics. Traditional Turkish medicine uses *Centaurea pulchella*, *Centaurea drabifolia*, and *Centaurea solstitialis* to treat abscesses, hemorrhoids, peptic ulcers, and colds. It is confirmed that *Centaurea* L.

species have biological potential with antimicrobial, antifungal, antiviral, antihelicobacterial, antioxidant, anti-inflammatory, and cytotoxic action (Albayrak et al., 2017; Ayad and Akkal, 2019; Bouafia et al., 2021; Ceyhan Güvensen et al., 2019; Martkoplishvili and Kvavadze, 2015; Milosevic et al., 2010; Özcan et al., 2019; Sharonova et al., 2021; Zater et al., 2016; Zavada et al., 2021). The research suggests that obtaining knapweed extracts for various applications, such as medical and food uses, mostly involves utilizing dried aboveground biomass, including leaves and flowers. At the same time, the biological activity of essential oils from various *Centaurea* L. species has been determined and includes antitumor, antidiabetic, anti-inflammatory, analgesic, antidepressant, antirheumatic, antioxidant, antimicrobial, and enzymatic properties.

The chemical composition of plants belonging to the genus *Centaurea* L. varies greatly based on species and geographic location. There is a scarcity of studies on plants belonging to the genus *Centaurea*, considered representatives of the flora in Central Russia (Sharonova et al., 2021). Several researchers investigated the indigenous Centaurea from Turkey, such as *Centaurea antiochia* var., *Centaurea hypoleuca (Özcan et al., 2019)*, *Centaurea amaena Boiss. & Balansa (Albayrak et al., 2017)*, *Centaurea aksoyi Hamzaoglu & Budak*, and *Centaurea babylonica* L. (Albayrak et al., 2017; Ceyhan Güvensen et al., 2019). Additionally, investigations have been conducted on *Centaurea pumilio* L. (Zater et al., 2016) and 26 other species from Africa (Ayad and Akkal, 2019). Flavonoids, lignans, alkaloids, phenolic compounds, steroids, and terpenes are the most distinct biologically active compounds. Despite this, pharmacological and chemical research on *Centaurea* L. plants is still limited. For many taxa, phytochemical composition data are insufficient or absent. A recent study, published in 2021 (Sharonova et al., 2021), sought to determine the phytochemical composition and biological activity of extracts derived from *Centaurea cyanus* L., *Centaurea jacea* L., and *Centaurea scabiosa* L. in order to assess their potential application in organic agriculture. The brown knapweed flowers extract included the fewest compounds, whereas the extract of bigger knapweed had the highest number of compounds. The content of extracts derived from the flowers of *Centaurea scabiosa* L. varied greatly depending on the solvents used. Extraction using non-polar solvents such as petroleum ether and MTBE revealed the highest number of components. During the initial evaluation phase, it was determined that the methanol extracts of recently collected knapweed biomass exhibited limited antimicrobial efficacy against phytopathogenic microorganisms. The minimum inhibitory concentrations ranged from 1250 to 10,000 µg/mL, while the minimum bactericidal and fungicidal concentrations were 2500 µg/mL or higher. The species *Centaurea scabiosa* L. exhibited the highest level of activity.

The antimicrobial activity of *Centaurea scabiosa* L. flower extracts, obtained using methyl tert-butyl ether (MTBE), exhibited the highest efficacy against the phytopathogens under investigation. The minimum inhibitory concentrations (MICs) ranged from 60 to 120 µg/mL, while the minimum fungicidal concentration (MFC) was determined to be 120 µg/mL. The minimum bactericidal concentration (MBC) was also found to be 250 µg/mL. The studied plants exhibited varying levels of antioxidant activity, with *Centaurea jacea* L. displaying the lowest activity. This finding was consistent with the levels of individual flavonoids and the overall flavonoid content present in the extracts. The application of knapweed extracts at a dosage of 0.1% did not exhibit any inhibitory effect on the germination of garden cress seeds. However, it did have an impact on the growth of the resulting seedlings. Notably, the most pronounced phytotoxic effect was observed when using extracts derived from larger knapweed.

The objective of this study was to add knowledge base on the current understanding of the phytochemical composition and biological activity of extracts derived from the bloomed plant of *Centaurea jacea* L. harvested in Romania. We aimed to accomplish the following objectives: (1) to examine the phytochemical composition of the plants extracts obtained in different solvents in order to

identify and detect biologic active compounds by chromatographic and spectrophotometric methods and (2) to evaluate the antioxidant capabilities of the extracts using ferric ion reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays (Fig. 1).

MATERIALS AND METHODS

2.1. Materials

All employed chemical reagents and solvents were of an appropriate analytical grade and were acquired from Sigma-Aldrich and Merck.

Plants of *Centaurea jacea* L. were collected from two different locations in Arad County, Romania, in 2022. The first batch of plants was collected from the resort of Moneasa, and the second batch was collected from Pecica. The plants were washed, dried at room temperature in the dark, and kept in a cool dry place.

Both flowers and leaves were detached from stems and grounded with an electric grinder. After grinding, each sample was divided into 4 parts. Plant extracts were made from dried biomass by a single maceration process. The maceration process used a ratio of plant material: solvent of 1: 10, and the following solvents: 1 – distilled H_2O; 2 - 70% ethanol; 3 – methanol; 4 - diethyl ether. The maceration was done for 3 hours at 19 ± 1 °C with continuous stirring, followed by filtration. PVDF-type filters were used for aqueous extracts and PTFE-type filters (45 µm) were used for the other three macerates. Thus 16 different types of extracts were obtained. These were stored at +4 °C until further analysis.

GC-MS analysis

The chemical composition of three extracts (aqueous extract; 70% ethanolic extract; methanolic extract, as they showed the highest antioxidant capacity) was determined using a gas chromatograph (GC, Shimadzu 2010, Kyoto, Japan) and a mass spectrometer (MS, TQ 8040, Shimadzu, Kyoto, Japan). All chemical constituents were identified using the spectra libraries NIST 14 and Wiley 09 as described in (Popa et al., 2021).

Determination of total polyphenolic content (Folin-Ciocalteu method)

To determine the total polyphenolic content of the extracts, 1 mL of 1:25 diluted sample was mixed with 0.5 mL Folin-Ciocalteu reagent, 2 mL $NaCO_3$ (20%), and 5 mL dist. H_2O. The reaction mixture was stirred and kept at room temperature in the dark for 90 min, and the absorbance was read at λ = 765 nm against the blank, which was prepared under the same conditions. Data was recorded using a UV-VIS double-beam spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany). The results were expressed as mg gallic acid equivalents (GAE)/g dry weight (DW).

Determination of flavonoid content

To determine the flavonoid content of the extracts, 0.250 mL of sample was mixed with 1.250 mL sodium acetate solution (100 g/L), 0.750 mL aluminium chloride solution (25 g/L), and 0.250 mL dist. H_2O. The reaction mixture was stirred and kept at room temperature in the dark for 15 minutes, and the absorbance was recorded at λ = 434 nm relative to the control sample, which was prepared under the same conditions. Data was recorded using a UV-VIS double-beam spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany). The calibration curve was performed using rutin as standard in the concentration range 0.02-0.4 mM, and the results were expressed in mg rutin equivalent/g dry weight.

Antioxidant capacity

FRAP assay - 200 µL sample and 1.5 mL FRAP reagent (freshly prepared by mixing 300 mM acetate buffer solution (pH 3.6), 20 mM $FeCl_3$, and 10 mM TPTZ (in 40 mM HCl)) were reacted in the dark for 20 minutes. Aqueous extracts were diluted 1:5. Absorbance was recorded at λ = 593 nm relative to H_2O with a spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany) and the calibration curve was performed with Trolox (concentration range 0-0.5 mM).

DPPH assay - The assessment of the radical scavenging activity of the extracts was conducted by employing the 1,1-diphenyl-2-picrylhidrazyl radical (DPPH) and afterwards measuring the absorbance at $\lambda = 517$ nm using a spectrophotometer. The reaction duration was set at 1 hour, and the experiment was carried out in a dark environment, following the methodology and calculation outlined in (Popa et al., 2021).

All analyses were performed in triplicates, and the results are reported as mean ± standard deviation.

RESULTS AND DISCUSSIONS

In this study we obtained four extracts in different solvents (water, 70% ethanol, methanol, and diethyl ether) of leaves and flowers from plants of *Centaurea jacea* L. harvested in western part of Romania, in Moneasa and in Pecica, in Arad county.

The extracts of leaves and flowers of *Centaurea jacea* L., respectively, obtained in three solvents (aqueous extract; 70% ethanolic extract; and methanolic extract), as determined by GC-MS analyses, have a different chemical profile, conclusion that is supported in previously published reports (Albayrak et al., 2017; Bouafia et al., 2021; Sharonova et al., 2021; Zater et al., 2016). In the leaves' extracts (Table 1) 27 compounds with more than 0.5% concentration were totally identified in the analyzed samples, in diverse concentrations.

The major compounds were: glycidyl palmitate, 9,12,15-octadecatrienoic acid, ethyl ester; 1,E-11,Z-13-octadecatriene; 2,4-di-tert-butylphenol; glycidyl oleate; isomenthol; phytol; isopropyl octacosyl ether; decane and its derivatives; heptacosane; gitogenin acetate; benzeneacetic acid, 2-tetradecyl ester. In the flowers extracts 24 compounds were totally identified in the analysed samples, in diverse concentrations. The major compounds were: methyl (Z)-5,11,14,17-eicosatetraenoate; heptadecane, 2,6,10,14-tetramethyl; 1,8,11-heptadecatriene; 9,12-octadecadienoic acid; 2,4-tert-butylphenol; heptacosane; and minor compounds: fumaric acid, 2-isopropylphenyl pentadecyl ester; undecane and its derivatives, etc. The phytochemical compositions of the *Centaurea jacea* L. flowers' ethanolic extract presented 13 compounds in the samples obtained from Verkheuslonsky Municipality, Republic of Tatarstan, Russian Federation, in 2020. The main compounds from these sample, separated in a TraceGold TG-5 MS fused silica GC column, were 3',5,6-trihydroxy-3,4',7-trimethoxyflavone (60.42%), 2-hydroxy-5-methylbenzaldehyde (8.02%)' palmitic acid (6.49%), vitexicarpin (5.83%), catechol (5.65%) (Sharonova et al., 2021).

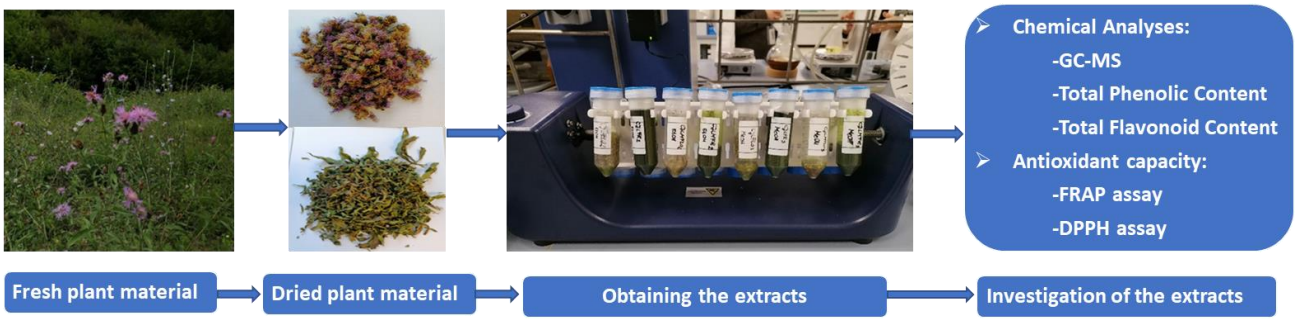

Figure 1. The presentation of the work plan followed in this study: material plant and the methods employed to obtain the extracts in four different solvents (water, 70% ethanol, methanol, and diethyl ether) and their analyses.

The chemical profile of the extracts obtained in this study reveals the presence of alkanes, esters, phenols, aldehydes, ketones, ethers and acids. The concentration of the compounds depends on the solvent used for extraction and on the geographical localisation of the grown plants.

Table 1. The chemical composition, as determined by GC-MS of the leaves' samples harvested in Moneasa and Pecica. The first letter is from the sample location: M is from Moneasa and P is from Pecica; L is for leaves, 1 - aqueous extract; 2 – 70% ethanolic extract; 3 – is a methanolic extract.

R.T. (min.)	**Name**	**PL1**	**PL2**	**PL3**	**ML1**	**ML2**	**ML3**
12.555	Decane, 2,9-dimethyl-	1.09	1.96	2.33	n.d.	n.d.	n.d.
12.905	Decane	0.57	1.73	n.d.	n.d.	n.d.	n.d.
15.118	Octane, 2-methyl-	0.95	1.31	n.d.	n.d.	n.d.	n.d.
19.611	Decane, 2,6,6-trimethyl-	0.97	2.18	3.95	3.95	n.d.	n.d.
20.113	Decane, 2,6,7-trimethyl-	1.35	4	n.d.	n.d.	n.d.	n.d.
20.396	Heptane, 2,3,5-trimethyl-	0.7	1.52	n.d.	n.d.	n.d.	n.d.
21.836	Heptadecane, 2,6,10,14-tetramethyl-	1.18	0.56	2.05	5.05	6.76	n.d.
22.24	Isopropyl octacosyl ether	2.41	1.5	2.71	n.d.	12.67	n.d.
22.56	Oxalic acid, cyclobutyl tetradecyl ester	1.06	4.09	n.d.	n.d.	n.d.	n.d.
23.759	Undecane, 2,8-dimethyl-	0.99	2.02	n.d.	3.94	4.95	n.d.
27.797	Hexadecane, 8-hexyl-8-pentyl-	1.25	9.91	n.d.	n.d.	n.d.	n.d.
28.362	Tetracontane, 3,5,24-trimethyl-	0.85	1.99	n.d.	3.55	n.d.	12.77
28.46	Hexadecane	2.99	2.74	5.36	10.68	11.56	n.d.
28.685	Gitogenin acetate	0.6	1.89		3.43	5.32	n.d.
29.055	2,4-Di-tert-butylphenol	6.23	1.4	6.86	20.99	22.48	34.29
29.637	Hexadecane	0.98	1.51	3.04	n.d.	n.d.	4.28
33.509	Eicosane	0.61	18.79	n.d.	3.15	3.14	3.96
33.598	Heptacosane	1.55	3.13	5.81	n.d.	7.89	4.86
34.24	Benzeneacetic acid, 2-tetradecyl ester	0.92	1.56	n.d.	4.69	n.d.	n.d.
34.607	Heptadecane, 2,6,10,15-tetramethyl-	1.02	2.33	n.d.	n.d.	n.d.	n.d.
36.813	Phytol	1.1	0.56	n.d.	6.9	6.92	n.d.
41.465	1,8,11-Heptadecatriene, (Z,Z)-	1.05	3.36	n.d.	n.d.	n.d.	n.d.
41.571	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	31.14	1.26	26.84	n.d.	n.d.	n.d.
41.882	Glycidyl oleate	3.91	1.9	8.63	n.d.	n.d.	3.62
42.74	Glycidyl palmitate	1.09	14.86	3.43	13	12.27	7.04
43.057	Isomenthol	2.07	1.69	4.13	9.3	6.04	6.67
43.371	1,E-11,Z-13-Octadecatriene	31.37	8.48	21.4	2.07	n.d.	3.83

Table 2. The chemical composition, as determined by GC-MS of the flowers' samples harvested in Moneasa and Pecica. The first letter is from the sample location: M is from Moneasa and P is from Pecica; F is for flowers, 1 - aqueous extract; 2 – 70% ethanolic extract; 3 – is a methanolic extract.

R.T. (min.)	Name	MF1	MF2	MF3	PF1	PF2	PF3
12.897	Undecane, 4,7-dimethyl-	3.14	2.78	n.d	1.46	2.29	1.35
19.607	Undecane, 3,7-dimethyl-	1.97	2.02	n.d	2.92	2.71	0.77
20.409	Decane, 2,9-dimethyl-	2.09	4.03	n.d	2.54	5.52	1.55
21.833	Isobutyl tetratriacontyl ether	3.33	1.24	1.72	3.44	1.92	0.35
22.244	Dodecane, 4,6-dimethyl-	7.48	1.5	3.35	4.64	5.91	0.63
23.757	Fumaric acid, 2-isopropylphenyl pentadecyl ester	2.34	5.06	1.27	1.5	4.43	0.29
27.777	Heptadecane, 2,6,10,14-tetramethyl-	3.4	0.87	0.9	1.84	2.18	1.2
28.466	Tridecane	7.04	6.33	3.88	2.29	3.8	2.42
29.061	2,4-Di-tert-butylphenol	2.02	1.24	5.18	8.56	3.49	0.76
29.531	Undecane, 5,5-dimethyl-	16.42	1.33	0.45	7.39	2.05	4.07
29.647	Heptacosane	1.4	0.9	1.18	1.09	2.02	5.96
32.747	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol	2.27	0.86	1.99	2.27	15.09	1.01
33.523	Hexatriacontane	1.38	1.66	1.31	0.75	3.31	1.79
33.596	Heptacosane	4.29	2.21	2.31	3.7	3.35	1.86
34.578	Tridecane, 6-methyl-	1.9	4.11	0.97	4.15	2.34	1.38
38.801	Methyl (Z)-5,11,14,17-eicosatetraenoate	0.66	22.1	0.4	n.d.	0.48	n.d.
38.938	Heptadecane, 2,6,10,15-tetramethyl-	5.17	1.08	1.45	n.d.	2.53	12.34
40.707	Pentadecanoic acid	2.75	1.46	4.79	23.3	1.59	4.63
41.568	Methyl (Z)-5,11,14,17-eicosatetraenoate	4.46	16.34	28.28	7.7	1.1	7.93
43.398	1,8,11-Heptadecatriene	11.27	3.96	26.65	3.82	1.02	9.65
43.453	9,12-Octadecadienoic acid	5.02	4.6	3.92	1.57	19.77	32.48
44.247	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.alpha.)-	6.05	1.4	1.36	3.83	7.26	1.6
45.794	Glycidyl palmitate	4.15	5.72	6.26	7.64	3.41	3.84
45.884	Octacosane	n.d.	2.73	2.38	3.6	2.43	2.14

As shown in Table 3, the highest amount of total polyphenols was recorded for methanolic extracts obtained from flowers (11.3243±0.1294 - 1.2089±0.0094 mg gallic acid equivalent/g of dry weight for flowers from Moneasa extracted in methanol and diethyl ether, respectively) and leaves (15.6468±0.3321 - 2.4314±0.2200 mg gallic acid equivalent/g of dry weight for leaves from Moneasa extracted in methanol and from Pecica extracted in diethyl ether, respectively), followed by aqueous extracts, ethanolic extracts, and diethyl ether extracts, respectively. A previously report determined for ethanolic flower extract 163.7 ± 2.25 mg GAE/L, while we determined a large concentration in the range 120.89±0.94 mg GAE/L (PF4, diethyl ether extracts from flowers harvested in Pecica) - 1132.43±12.94 mg GAE/L (MF3, methanolic extract from flowers harvested in Moneasa).

The chemical profile of the extracts is responsible for the biological activity of these extracts. There are many methods to determin the antioxidant properties of the biomolecules from plants.

The methanolic extracts revealed the highest antioxidant activity by DPPH and FRAP methods (maximum values of 1.6535±0.0040 and 1.4850±0.0122 mM TROLOX equivalent/g of dry weight for leaves extracted in methanol from Moneasa-ML3 and Pecica-PL3, respectively, and minimum value of 0.0320±0.0007 mM TROLOX equivalent/g of dry weight for flowers extracted in diethyl ether from Moneasa-MF4).

The most modest antioxidant activity was reported for the diethyl ether extracts for FRAP method and aqueous extracts for the DPPH method, respectively.

The chemiluminescent activity tests conducted on the ethanolic extracts derived from the flowers of the *Centaurea* plants in the study published by Sharonova et al. demonstrated limited antioxidant properties. The observed antioxidant effects were relatively weak, the effective concentration threshold for these effects was determined to be 0.01 mg/mL. Ethanol extracted *Centaurea jacea* flowers were most capable of binding free radicals in total reactive antioxidant potential (TRAP) method as compared with the extracts from *Centaurea cyanus* and *Centaurea scabiosa* (Sharonova et al., 2021). The authors concluded that this result was well corelated with the concentration of total flavonoid in the investigated samples. In the present study, could be also other biomolecules, beside polyphenols and flavonoids, that lead to the antioxidant capacity of the extracts.

CONCLUSIONS

The chemical profile of *Centaurea jacea* L. extracts produced using four different solvents exhibits variation, as supported by previously published literature. The chemical composition of the extracts indicates the existence of alkanes, esters, phenols, aldehydes, ketones, and acids. According to the data, the methanolic extracts from flowers and leaves exhibited the highest concentration of total polyphenols. In descending order, this was followed by aqueous extracts, ethanolic extracts, and diethyl ether extracts. The flavonoid content analysis revealed a significant concentration of flavonoids in the flower and leaf samples when extracted using methanol, with ethanol extraction yielding slightly lower levels. The biological activity of the extracts was closely linked to their chemical composition. Methanolic extracts demonstrated superior antioxidant activity according to DPPH and FRAP assays, with leaves from Moneasa (ML3) and Pecica (PL3) showing the highest values, and flowers from Moneasa (MF4) displaying the lowest. The least potent antioxidant activity was observed in diethyl ether extracts using the FRAP method and in aqueous extracts using the DPPH method. The methanolic extracts of both flowers and leaves from *Centaurea jacea* will be further considered to be used in future applications due to their antioxidant activity.

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Table 3. The total polyphenols, flavonoids, and antioxidant capacity (by DPPH and FRAP assays) of the samples harvested in Moneasa and Pecica. The first letter is from the sample location: M is from Moneasa and P is from Pecica; L is for leaves, F is from flowers; 1 - aqueous extract; 2 – 70% ethanolic extract; 3 – is a methanolic extract; 4 – diethyl ether extract.

Sample	**Total polyphenolic content**	**Total flavonoid content**	**Antioxidant capacity**	
	mg gallic acid equivalent / g of DW	mg rutin equivalent / g of DW	**FRAP assay**	**DPPH assay**
			mM TROLOX equivalent / g of DW	Inhibition (%)
ML1	12.9936±0.7069	2.208±0.004	0.4167±0.0046	4.55±0.15
MF1	11.1571±0.3416	1.211±0.007	0.3184±0.0087	10.17±0.21
PL1	12.0160±0.6510	2.959±0.365	0.5077±0.0047	3.51±0.08
PF1	8.5737±0.1406	0.730±0.083	0.3884±0.0056	4.86±0.02
ML2	8.3647±0.1489	4.250±0.081	0.1325±0.0199	36.87±0.91
MF2	6.2615±0.0783	2.083±0.004	0.6068±0.0296	32.47±0.79
PL2	8.3301±0.3883	5.613±0.011	1.1007±0.0225	36.47±0.38
PF2	8.6448±0.1812	3.711±0.007	0.6207±0.0350	38.55±0.93
ML3	15.6468±0.3321	8.663±0.025	1.6535±0.0040	87.82±0.12
MF3	11.3243±0.1294	3.887±0.027	1.2516±0.0086	92.02±0.1
PL3	12.0256±0.2738	5.743±0.010	1.4850±0.0122	87.51±0.16
PF3	10.2763±0.0508	3.814±0.004	0.8901±0.0088	87.34±0.96
ML4	2.5712±0.1072	0.531±0.117	0.1091±0.0101	18.74±0.58
MF4	1.2089±0.0094	0.178±0.067	0.0320±0.0007	3.70±0.06
PL4	2.4314±0.2200	0.681±0.009	0.1658±0.0043	23.97±0.83
PF4	2.6993±0.1700	1.088±0.0014	0.1176±0.0204	27.49±1.05

DW = dry weight. n. r. = not recorded. The values depicted are expressed in mean ±standard deviation. Triplicate measurements were performed.

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