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Research Article

FIELD-CULTIVATED MEDICINAL PLANTS OF *ACHILLEA MILLEFOLIUM* GROUP: A SOURCE OF BIOACTIVE COMPOUNDS

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Summary: Medicinal plants of *Achillea millefolium* group in Bulgarian wild flora are widely used in both traditional medicine and pharmaceutical, cosmetic and nutritive industries. They are intensively collected from the natural habitats, thus leading to a number of negative effects, such as ecological risk of extinction of native sources, and high heterogeneity of the collected market samples. We explored the possibilities for field cultivation of wild *A. millefolium* group species as a promising approach to produce homogenous samples of valuable plants without damage to the natural environment. Our preliminary studies have identified as promising two species of *A. millefolium* group in Bulgaria - *A. collina* and *A. asplenifolia.* The species *A. collina* and *A. asplenifolia*, each with two populations, were studied, while cv. "Proa", bred for field cultivation, was used as a standard. Seeds were collected from the natural habitats, and those of cv. Proa were purchased from the firm "Pharmasaat GmbH", Germany. Seedlings were produced in a greenhouse and then transferred to the experimental field of the Institute of Plant Physiology and Genetics near Sofia. Plants were harvested at full blossoming stage. Essential oil yield and chamazulene content in the oil, contents of total flavonoids and chlorogenic (3-O-caffeoyl quinic) acid as well as antiradical and antioxidant activities in flower heads were determined. Principal Component Analysis (PCA) of data was performed. The results showed that field-grown plants of the studied *A. millefolium* group species produced significant amounts of bioactive compounds, particularly flavonoids and chlorogenic acid which highly correlated with the antiradical activity. PCA pointed to the relatedness of populations within each species, and the distant positions of the species. The populations of *A. asplenifolia* were distinguished by higher contents of total flavonoids, essential oil, chamazulene and chlorogenic acid as well as by higher antiradical and antioxidant activities as compared to *A. collina*, while being closer by all parameters to cv. "Proa". The data point to *A. asplenifolia* as a promising species for field cultivation.

Keywords: *Achillea collina*; *A. asplenifolia*; bioactive compounds; field cultivation.

Abbreviations: AO – antioxidant; AR – antiradical; DPPH$^{\cdot}$ – 2,2′-diphenyl-1-picrylhydrazyl radical; FID – flame ionization detector; FRAP – ferric reducing antioxidant power; GC – gas chromatography; HPLC – high performance liquid chromatography; MS – mass spectrometry; PCA – principal component analysis.

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INTRODUCTION

Achillea genus (Asteraceae) (yarrow) has been named after the mythical invulnerable hero Achiles who was heeling his soldiers by applying infusions of these plants. The remarkable therapeutical properties of yarrow have been recognized since the antiquity. It has been largely used in folk medicine and presently in traditional practice as well as in pharmaceutical, cosmetic and nutritive industries. The plants exhibit a rich pattern of curative properties ranging from antiphlogistic to spasmolytic, choleretic and antimicrobial. They are relevant to the extremely versatile spectrum of secondary metabolites, such as terpenoids including sesquiterpene lactones, flavonoids, caffeoylquinic acids, coumarins, polyacetylenes, tocopherols, tannins, sterols, etc. (Benedek et al., 2006; Lakshmi et al., 2011; Saeidnia et al., 2011; Dias et al., 2013). Many of them are polar compounds, which determines the usage of yarrow in water and alcoholic solvents as teas, infusions, decoctions, and tinctures (Benedek et al., 2006).

Essential oil is considered as a particularly valuable constituent of yarrow determining many of its curative actions. It consists of a wide range of compounds predominantly with terpenoid character, the active principle being chamazulene, derived from proazulenes (sesquiterpene lactones) in the drug (Rauchensteiner et al., 2004). Flavonoids and caffeoylquinic acids are estimated as components responsible for the spasmolytic and choleretic effects, and as determinants of the significant antioxidant and antiradical capacity of the drug (Benedek et al., 2006, 2007; Vitalini et al., 2011; Nikolova et al. 2013).

The genus *Achillea* is widely distributed over the Northern hemisphere extending from centers of diversity in Southeast Europe to Southwest Asia. In Bulgaria the genus is presented by 19 species. Six of them belong to *Achillea millefolium* group (or aggregate), namely *A. asplenifolia* Vent., *A. setaceae* Waldst and Kit, *A. collina* J. Becker ex Heimerl, *A.millefolium* L., *A. distans* Waldst. and Kit ex Willd., and *A. pannonica* Scheele. Data point to *A. collina* being the most largely distributed species in the country, and question the notion of the predominance of *A. setaceae* (Saukel et al., 2003; Vitkova et al., 2005).

Presently the increasing demand of yarrow drug is predominantly met by collecting wild-growing plants from their natural habitats. However, this leads to a number of negative effects such as the important ecological risk of extinction of the natural sources as well the non-homogeneity of the quality of the collected samples which are a mixture of various wild species at different stages of development and maturity. There are reports about the extremely large variability of commercial samples of yarrow as evaluated by the wide span of chemical indices, about 50% of them below the limits indicated in European Pharmacopoeia (Benedek et al., 2008). Moreover, according to Rauchensteiner et al. (2004) only 90% of market samples meet the requirements about proazulene content of the drug.

For this purpose attempts have been made to cultivate plants of wild yarrow species in field conditions, i.e. at proper agrotechnical requirements,

thus ensuring favorable environment for growth, development and quality of plants, and for obtaining a homogenous drug (Spinarova and Petřikova, 2003; Karlova, 2006). Moreover, breeding experiments have been carried out resulting in the development of cultivars providing high-grade and homogenous drug for the market. Vitkova et al. (2005) established the possibility of successful cultivation of wild species of *A. millefolium* group in an experimental field located in Sofia region.

On this basis the aim of the present work was to investigate the content of important bioactive compounds in two field-grown azulenogenic wild species of *A. millefolium* group – *A. collina* and *A. asplenifolia*, to compare it with that of cv. Proa, bred for field cultivation, and to estimate the intra- and interspecific variability of these taxa. Essential oil yield and chamazulene content in the oil, contents of total flavonoids and chlorogenic acid as well as antioxidant and antiradical activities were determined.

MATERIALS AND METHODS

Plants

Seeds were collected from natural habitats of the two species in Sofia region – *A. collina* (2n=36) and *A. asplenifolia* (2n=18). Each species was presented by two populations – *A. collina* 102 (Vitosha Mt.) and *A. collina* 3802 (village Dolni Lozen), *A. asplenifolia* 9602 (village Kutina) and *A. asplenifolia* 10403 (village Bezden). Seeds from cv. Proa were purchased from the firm "Pharmasaat GmbH", Germany. Experiments were carried out in 2009. Seedlings were produced in a greenhouse and then transferred to the experimental field of the Institute of Plant Physiology and Genetics near Sofia at 570 m a.s.l. The type of soil in the experimental field is alluvial meadow with pH 6.5. Seedlings were grown in garden soil in pots 12 cm in diameter. Six-months seedlings were planted in the experimental field in 4 replicates, each of them containing 10 plants. The distance between plants and between rows was 40 cm. During the first year the plants formed floral stems. Plants were harvested at the stage of full blossoming. Flower heads were cut, air-dried, milled, and used for chemical analyses.

Essential oil

A sample of 100 g was hydrodistilled in a Clevenger-type apparatus for 2 h to give the corresponding dark blue colored oil. The oil was dried over anhydrous sodium sulfate and kept at ca. 4°C until analysis.

Chamazulene content in the essential oil

GC and GC-MS analyses were applied. GC analyses were carried out on a HP 5890 gas chromatograph (FID), carrier gas nitrogen, linear velocity 25 cm/s, split ratio 1:100, fused silica capillary column HP-5MS (poly-5%-diphenyl-95%-dimethylsiloxane), 30 m x 0.25 mm, 0.25 μm film thickness. The injector and detector temperature was 260°C, column temperature was programmed from 50°C to 230°C at a rate of 4°C/min, and 10 min at 230°C. Quantitative data were obtained from the electronic integration of the FID peak areas.

GC-MS analyses were performed on

a HP 6890 instrument. The GC conditions and the capillary column used were as described above but the carrier gas was helium. The components of the oil were identified by comparison of their mass spectra and retention indices with those published in the literature (Adams, 1995; Joulian and Konig, 1998) and presented in a library developed by us.

Chlorogenic acid

Chlorogenic acid content was determined by applying HPLC (High Performance Liquid Chromatography) as described by Dagnon and Edreva (2003). A sample of 0.2 g was mixed with 10 ml 70% (v/v) aqueous methanol, sonicated for 10 min, filtrated under vacuum, passed through a membrane filter 0.45 μm and adjusted to 10 ml. The instrumentation used for HPLC analysis consisted of quaternary mixer Smartline Manager 5000, pump Smartline 1000 and photodiode array detector 2800 (Knauer, Germany). The separation was achieved on a Kromasil C18, 15 cm×4.6 mm i.d., 5 μm particle size (Supelco, USA). The chromatographic separation was carried out using 0.1% trifluoroacetic (TFA) acid solution in acetonitrile as solvent B. As solvent A was used mixture from 90 parts water and 10 parts 0.1% TFA acid solution in acetonitrile with the following gradient elution program: 0 – 10 min, 100% – 90% A (0 – 10% B), 10 – 18 min, 89% A (11% B), 18 – 25 min, 85% A (15% B), 25 – 40 min, 45% A (55% B). The polyphenols were monitored at 340 nm and 352 nm. The flow rate was set by 1.0 ml/min; the sample injection volume was 10 μl. The chlorogenic acid was identified by using the retention time of pure standard compound (Sigma).

Total flavonoids

The method of Zhishen et al. (1999) was used. One gram of material was grinded in 10 ml 80% (v/v) aqueous ethanol. An aliquot of 1 ml was mixed with 4 ml water, 0.3 ml 5% $NaNO_2$, 0.3 ml 10% $AlCl_3$, 2 ml 1M NaOH, and adjusted to 10 ml with H_2O. The resulting red coloration was measured at 510 nm. Rutin (Sigma) was used as a standard.

Antiradical, or free radical scavenging activity

It was determined in 80% (v/v) aqueous ethanolic extract (the same as for total flavonoids) by the 2,2′-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) test (Brand-Williams et al., 1995). A volume of 0.01 ml extract and 1.99 ml $DPPH^{\cdot}$ solution in methanol (6×10^{-5}M) were mixed, and the decrease of absorbance was followed up at 515 nm. Trolox (Sigma) was used as a standard.

Antioxidant activity

The method of Benzie and Szeto (1999) (the FRAP test) was applied using the same extract as for total flavonoid determination. A volume of 0.05 ml of extract was mixed with 1.50 ml FRAP reagent and 0.15 ml water. A blue coloration developed, the absorbance being read after 15 min at 593 nm. The FRAP reagent was a mixture of 0.3 M acetate buffer pH 3.6, 0.02M $FeCl_3.6H_2O$, and 0.01M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04M HCl in a ratio of 25:2.5:2.5. $FeSO_4$ was used as a standard.

Principal Component Analysis (PCA)

It was based on six variables, namely essential oil yield, chamazulene content in the oil, contents of total flavonoids and

chlorogenic acid, antioxidant activity, antiradical activity, and was performed according to the program Statistica 8.0.

Correlation analysis

The antioxidant and antiradical activities were subjected to correlation analysis with the content of total flavonoids and chlorogenic acid by using ANOVA multifactor analysis.

Statistical Analysis

Data are expressed as means ± standard error (±SE), $n = 4$. Different letters indicate significant differences assessed by the Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis. The statistical software package StatGraphics Plus version 5.1 for Windows was used.

RESULTS AND DISCUSSION

Essential oil

As mentioned above, essential oil is a major valuable component of yarrow, responsible for a large array of therapeutic properties (Saeidnia et al., 2011). Important diversity of essential oil components was observed, this pointing to their applications as reliable chemotaxonomic markers (Rauchensteiner et al., 2004). In flower heads of field-grown *A. asplenifolia* Konakchiev et al. (2005) identified chamazulene, β-pinene, sabinene and β-caryophyllene as main components of the essential oil, while in field-grown *A. collina* chamazulene, borneol, lavandulyl acetate, Z-thujone, 1,8-cineole were determined. Antibacterial and antifungal activities were shown in the oil (Konakchiev et al., 2005, 2006). In the present study, the yield of essential oil in flower heads varied from 0.25% in *A. collina* 102 to 0.45% in *A. asplenifolia* 9602. This yield was higher than the limit indicated in European Pharmacopoeia (0.2%) (Benedek et al., 2008). Both populations of the species possessed similar oil yields (0.25% – 0.26% for the populations of *A. collina* and 0.44% – 0.45% for those of *A. asplenifolia*). The average values for the species were 0.26% and 0.45% for *A. collina* and *A. asplenifolia*, respectively. Thus low intraspecific variability and more significant interspecific difference were demonstrated. Cv. Proa approached to *A. asplenifolia* 9602 by 0.41% yield of essential oil (Fig. 1A). In field-cultivated wild species of *A. millefolium* group Spinarova and Petřikova (2003) registered variability of essential oil between 0.09% to 0.80% for *A. collina* and 0.13% to 0.55% for *A. asplenifolia*. In commercial samples of yarrow herba originating from cultivated plants Benedek et al. (2008) reported an essential oil yield of 2% to 5.88%. The variability of essential oil yield in flos from commercial samples was between 1% to 4% (Benedek et al., 2008). Hence, field cultivation may result in increasing the essential oil yield to values higher than the required limit of 0.2%.

Chamazulene content in the oil

Chamazulene, an active principle of the essential oil, responsible for its antiphlogistic action, originates from a class of sesquiterpene lactones (proazulenes) in the drug, and is produced upon drug steam distillation. Chamazulene determines the dark-blue color of the essential oil, and is considered as a marker of its high-grade quality (Rauchensteiner et al., 2004). Todorova et

al. (2006) and Trendafilova et al. (2006) studied the azulenogenic potential of *A. asplenifolia* and *A. collina*, and identified new members of sesquiterpene lactone family. The chamazulene content in the oil of the investigated by us plants varied from 2.8% in *A. collina* 102 to 17.50% in *A. asplenifolia* 1043. The populations of the species were not very different (2.80% and 4.45% for the populations of *A. collina*; 11.58% and 17.50% for those of *A. asplenifolia*). The average values of chamazulene for the species were 3.62% for *A. collina* and 14.54% for *A. asplenifolia*, respectively. Thus, lower intraspecific than interspecific variability of chamazulene was established. Cv. Proa was closer to *A. asplenifolia* 10403 showing 15.23% chamazulene content (Fig. 1B). Konakchiev et al. (2005, 2006) reported values of 25.60% for chamazulene content in the oil of field-grown *A. asplenifolia* and 20.80% chamazulene in the oil of field-grown *A. collina*. This discrepancy with our data points to the suggestion that in the last case better field and climatic conditions occurred incresing the chamazulene content.

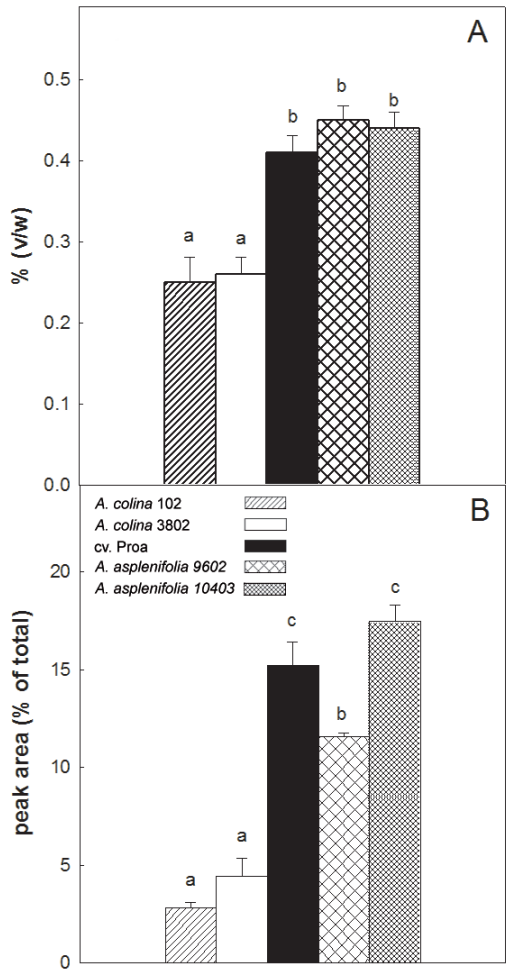

Figure 1. Essential oil yield (%, v/w) (A) and chamazulene content in the oil (peak area, % of total) (B) in flower heads of field-grown wild *Achillea* species and cv. Proa.

Total flavonoids and chlorogenic acid

Phenylpropanoids belonging to flavonoid (C_6–C_3–C_6) and cinnamic acid derivative (C_6–C_3) classes, such as caffeoylquinic and dicaffeoylquinic acids significantly contribute to the pharmacological importance of yarrow, being determinants of its spasmolytic, choleretic and antiplasmodial effects. High diversity of these constituents was reported which is a rationale of their relevance as chemotaxonomic markers (Benedek et al., 2007; Vitalini et al., 2011). In aerial parts of wild-growing plants from *A. millefolium* aggregate Benedek et al. (2007) identified ten major flavonoids. In *A. asplenifolia* they established rutin, apigenin, apigenin 7-O-glucoside, luteolin 7-O- glucoside, and luteolin 4-O-glucoside.. In *A. collina* these authors showed the occurrence of apigenin 7-O-glucoside, luteolin 7-O-glucoside and luteolin 7-O-glucuronide. In flos and herba of field-grown *A. collina* Karlova (2006) reported the presence of 7-O-glucosides of apigenin and luteolin as

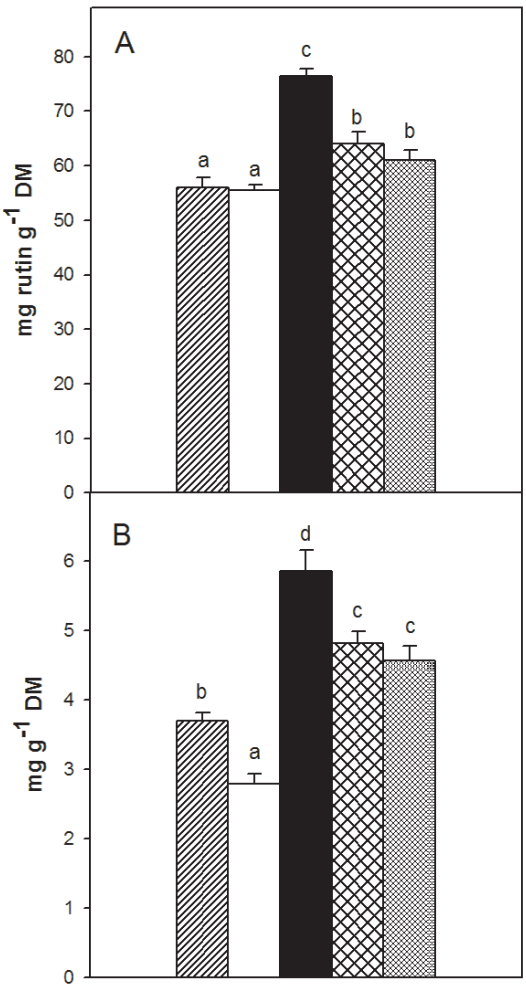

Figure 2. Flavonoid content (mg rutin g^{-1} DM) (A) and chlorogenic acid content (mg g^{-1} DM) (B) in flower heads of field-grown wild *Achillea* species and cv. Proa. For legend see Fig. 1.

well as the occurrence of both aglycones. In flower heads of wild *A. collina* and *A. asplenifolia* species Trendafilova et al. (2007) observed flavonoids such as quercetagetine derivatives. The analysis of HPLC pattern of our plant material is in progress. The occurrence of the flavonoids rutin and apigenin was already proven (data not shown).

In flower heads of the yarrow plants studied by us the total flavonoid content varied from 55.50 mg/g in *A. collina* 3802 to 76.45 mg/g in cv. Proa. This amount was higher than the reference limit in European Pharmacopoeia (0.3%, or 3 mg/g) (Benedek et al., 2008). Both populations of the species showed similar values (55.50 mg/g – 55.99 mg/g) for the populations of *A. collina*, and 61.02 – 64.00 mg/g for the populations of *A. asplenifolia*. The average values for the species were 55.70 and 62.50 mg/g for *A. collina* and *A. asplenifolia*, respectively. Therefore, low intraspecific variability and larger interspecific difference are obvious. Cv. Proa approached to *A. asplenifolia* 9602 showing 76.45 mg/g total flavonoid content (Fig. 2A).

In flower heads of field-cultivated species of *A. millefolium* group Spinarova and Petřikova (2003) reported values for total flavonoid content of 16.0 mg/g to 31.1 mg/g in *A. asplenifolia*, and 21.0 mg/g to 32.6 mg/g in *A. collina*. In commercial flos samples the total flavonoid content varies from 0.3 mg/g to 11.1 mg/g (Benedek et al., 2008) i.e. it is lower than the values obtained by us and by Spinarova and Petřikova (2003). Field cultivation hence looks as a promising tool to manipulate the accumulation of flavonoids in yarrow.

The chlorogenic acid determination revealed trends similar to total flavonoids (Fig. 2 – A, B). The variation in its content was between 2.79 mg/g in *A. collina* 3802 to 5.85 mg/g in cv. Proa. Both populations of the species exhibited similar values (2.79 mg/g in *A. collina* 3802 to 3.70 mg/g in *A. collina* 102, and 4.56 mg/g in *A. asplenifolia* 10403 to 4.82 in *A. asplenifolia* 9602). The average values for the species were 3.25 mg/g and 4.69 mg/g in *A. collina* and *A. asplenifolia*, respectively. Hence, a lower intraspecific variability and higher

interspecific difference are evident. Cv. Proa was close to *A. asplenifolia* 9602 showinga chlorogenic acid content of 5.85 mg/g (Fig. 2B). For wild-growing nine species of *A. millefolium* group plants Benedek et al. (2007) reported the presence of chlorogenic acid in all species of the group, including *A. collina* and *A. asplenifolia*. The variability of this polyphenol in the group is in the range of 3.4 mg/g to 27.5 mg/g. Variability in wild plants is evidently more important than in field-grown ones; hence, field cultivation may produce a more homogenous drug regarding chlorogenic acid. In the above experiment Benedek et al. (2007) established the occurrence of four isomers of dicaffeoylquinic acid, namely 3,4-, 3,5-, 1,5- and 4,5- dicaffeoylquinic acids. In *A.collina* all four isomers were shown while *A. asplenifolia* lacked the 1,5-isomer. The dicaffeoylquinic acids exert an important choleretic effect, therefore, they highly contribute to the significant pharmaceutical value of yarrow (Benedek et al., 2007; Dias et al., 2013). In our plant material big amounts of 3,5- and 4,5-dicaffeoylquinic acids were demonstrated, their sum ranging between 5 and 10 mg/g (Dagnon, unpublished results).

Antioxidant and antiradical activities

Damage to biomolecules caused by oxidative stress is considered as a key event triggering pathological states of the organisms. This explains the increasing demand of products, capable to counteract oxidative damage and diseases, referred to as antioxidants and free radical scavengers (Halliwell and Gutteridge, 1995). Medicinal plants including yarrow are rich sources of substances endowed with antioxidant and antiradical properties. Flavonoids and caffeoylquinic acids, along with essential oil, are regarded as important determinants of the antioxidant /antiradical protective potential of yarrow (Sökmen et al., 2004; Konyaliogly and Karamenderes, 2005; Tuberoso et al., 2009; Vitalini et al., 2011).

In our experiments the antioxidant (AO) activity varied from 247. 88 μmoles FRAP in *A. collina* 102 to 457.53 μmoles FRAP in *A. asplenifolia* 9602, whereas the variability of the antiradical (AR) activity was between 229.72 μmoles trolox in *A. collina* 3802 to 391.73 μmoles trolox in cv. Proa. Both populations of the species showed similar AR activity – from 229.72 to 232.86 μmoles trolox for the populations of *A. collina*, and from 324.23 to 337.54 μmoles trolox for the populations of *A. asplenifolia*. The AO activity of the populations was more divergent – from 247.89 to 298.88 μmoles FRAP in the populations of *A. collina*, and from 346.34 to 457.53 μmoles FRAP in the populations of *A. asplenifolia*. The average values for the AO activity of the species were 273.39 and 401.93 μmoles FRAP in *A. collina* and *A. asplenifolia*, respectively, whereas the average values for AR activity were 231.29 and 330.89 μmoles trolox for *A. collina* and *A. asplenifolia*, respectively. Hence, lower intraspecific than interspecific variability was observed, this trait being better expressed in AR than in AO activity. Cv. Proa was closer to *A. asplenifolia* 9602 showing AO activity of 338.55 μmoles FRAP, and closer to *A. asplenifolia* 10403 with AR activity of 391.73 μmoles trolox (Fig. 3 - A, B).

DPPH$^{\cdot}$ test was predominantly applied to evaluate the AR activity in

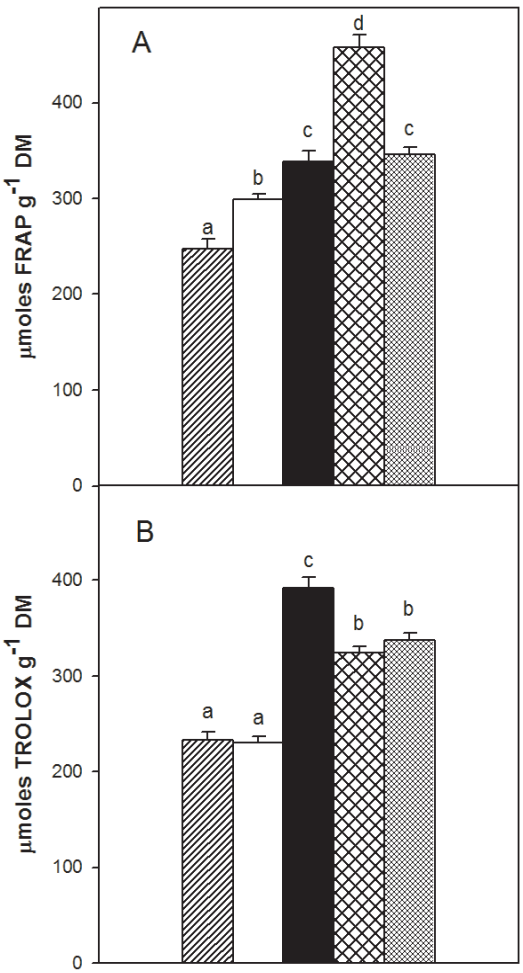

Figure 3. Antioxidant activity (µmoles FRAP g^{-1} DM) (A) and antiradical activity (µmoles TROLOX g^{-1} DM) (B) in flower heads of field-grown wild *Achillea* species and cv. Proa. For legend see Fig. 1.

different species of *Achillea*, such as *A. biebersteinii*, *A. alexandri-regis*, *A.ligustica*, *A. millefolium*, *and A. distans*. Positive relationships were established between the free radical scavenging activity and the capacity of *Achillea* extracts to exert antimicrobial effects, and to protect cells from and prevent oxidative damage (Sökmen et al., 2004; Kundakovič et al., 2006; Tuberoso et al., 2009; Vitalini et al., 2011; Benedec et al., 2013), this justifying the large usage of yarrow in folk and modern medicine.

Correlation analysis

Our data show that strong correlations exists between the AR activity and the contents of total flavonoids and chlorogenic acid. They are described by the equations y = 6.8006x – 135.66 with a correlation coefficient R^2 = 0.9709 for total flavonoids, and y = 57.761x + 52.302 with a correlation coefficient R^2 = 0.9048 for chlorogenic acid. Benedec et al. (2013) reported about a positive relation between data of DPPH$^{\cdot}$ test and the total polyphenol content of two Romanian subspecies of *A. distans*. Ardestani and Yazdanparast (2007) established a strong correlation between the contents of total flavonoids and polyphenols, and the reducing power of ethanolic extracts of *A. santolina.* In our experiments no correlations were found between the AO activity and the contents of total flavonoids and chlorogenic acid.

Principal component analysis (PCA)

The PCA score plot of field-grown *A. asplenifolia*, *A. collina* and cv. Proa (Fig. 4) clearly indicates the relatedness between the populations of *A. asplenifolia* as well as between the populations of *A. collina* which is in accordance with our claiming of low intraspecific variability of the taxa studied. On the contrary, both species are clearly remote which allows claiming the occurrence of interspecific variability. Our data corroborate the analysis of Benedek et al. (2007) based on the distribution of phenolic compounds in *A. millefolium* aggregate, pointing to the distant position of the diploid *A. asplenifolia* forming a separate cluster from the tetraploid *A. collina*. In addition,

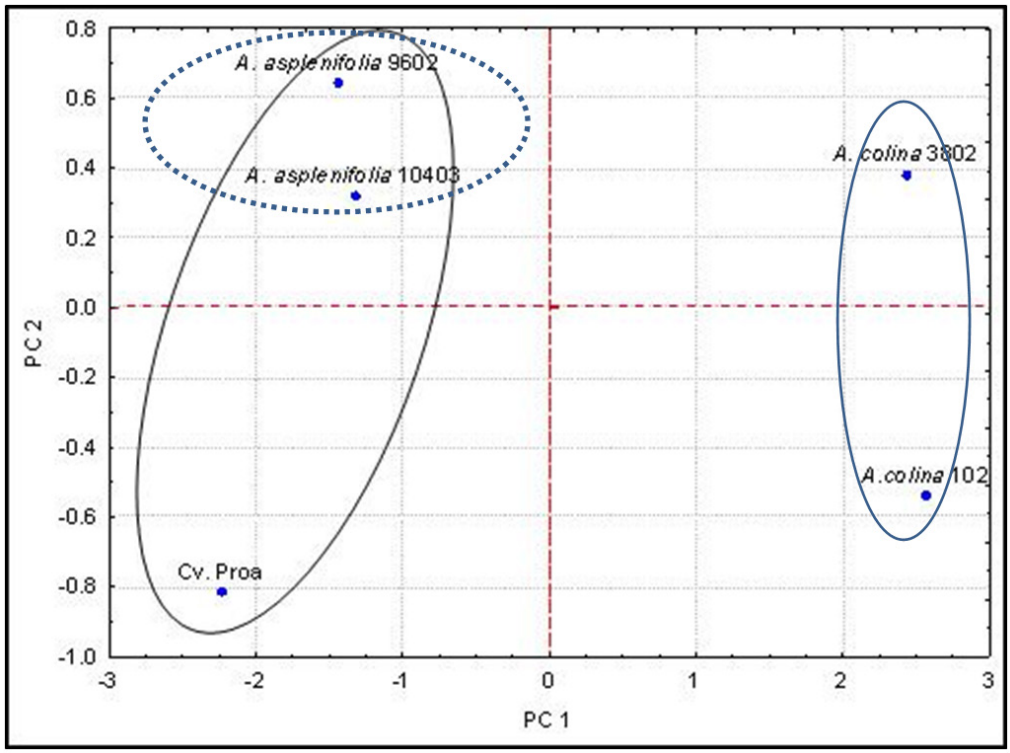

Figure 4. Principal Component Analysis (PCA) score plot of field-grown wild *Achillea* species and cv. Proa. Percentage variance described: PC1 (88.9%), PC2 (6.7%). The classification is based on six variables, namely essential oil yield, chamazulene content in the oil, flavonoid and chlorogenic acid contents, antioxidant and antiradical activities.

our results prove the closer position of the commercially supplied cv. Proa to *A. asplenifolia* as compared to the species *A. collina* (Fig. 4).

CONCLUSION

Flower heads of field-cultivated species *A. asplenifolia* and *A. collina* as well as cv. Proa are a good source of bioactive compounds, particularly flavonoids and chlorogenic acid. The flavonoid and chlorogenic acid contents of ethanolic extracts highly correlate with the AR activity, i.e. with the capacity to scavenge damaging free radicals. Low intraspecific variability and larger interspecific differences with regard to bioactive components point to the species being isolated and distant enough to serve as a homogenous source of raw plant material for the market. *A. asplenifolia* is richer in valuable components than *A. collina*. This species is closer to the commercially supplied cv. Proa, and hence is a promising plant for field cultivation.

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