

Plants and their Pathogens

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Comparative morphological and biochemical studies for two Romanian indigenous subspecies of *Achillea distans* Waldst. et Kit.

D. Benedec¹, M. Popovici¹, L. Vlase¹, C. Bele¹, B. Tiperciuc¹, M. Tamas¹, D. Hanganu¹

¹Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Pharmacognosy, Cluj-Napoca, Romania

dbenedec@umfcluj.ro

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Genus *Achillea* is represented by 33 species and subspecies in the flora of Romania. *A. millefolium* represents a cytogenetic and chemically polymorph aggregate of 12 species, characterized by a well defined morphological, anatomical and caryological features. In the present paper two samples of *Millefolii herba*, obtained from *Achillea distans* Waldst. et Kit. ex Willd. and *Achillea distans* Waldst. et Kit. subsp. *alpina* (Rochel) Soó were described morphologically by scanning electron microscopy (SEM) and it were also biochemically characterized. *Achillea distans* Waldst. et Kit. ex Willd. is an alpino-carpatho-balkan type species that vegetates on the upper limit of mountain forests and in subalpine shrubs. According to the length of ligulate florets and to their color, 2 subspecies are recognized: *A. distans* subsp. *distans* with white flowers and 2 mm length of ligulae and *A. distans* subsp. *alpina*, with pink flowers and 3 mm length of ligulae [1,2]. The secretory and protective tissues were detected in the flower head of *Achillea distans* subsp. *alpina* by SEM. The *glandular* trichomes are *biseriate*. The filamentous hairs were found only on the bracts, with a protective role. The bract has a serrated edge. The tubular corolla contains the echinate pollen grains, with form feature *Asteraceae* family, "Figure 1". *Achillea distans* subsp. *distans* contains the *biseriate glandular* trichomes, the filamentous tector hairs and the pollen grains. The pollen grains were found on the bracts. The edge of bract is serrated and it contains numerous filamentous tector hairs. The *glandular* trichomes were found inside the tubular flower, alongside many grains of pollen, "Figure 2". The identification and quantification of major phenolic compounds was performed by a HPLC-MS method. The total phenolic and flavonoidic content was determined spectrophotometrically. The antioxidant capacity (AC) was determined using the DPPH bleaching method [3]. The growth of silver nanoparticle (SNP)-based method for evaluating the antioxidant capacity of polyphenolic compounds from vegetal extracts was also used [4]. The reduction of Ag⁺ to spherical silver nanoparticles (SNPs) by polyphenols in the presence of trisodium citrate and silver seed was produced an intense absorption band of SNPs at 423 nm. The absorbance of SNPs allowed the quantitative spectrophotometric detection of polyphenols. The results of this method were correlated with those of other antioxidant methods (DPPH assay, Folin-Ciocalteu method). In *A. distans* subsp. *distans* flowers tincture, the phenolic profile showed the presence of the phenolic acids and the aglycones of flavonoids in large amounts, while in *A. distans* subsp. *alpina* flowers extract, the flavonoids predominated and they are represented especially by the glycosides and the aglycones. The total polyphenolic (TPC) and flavonoids content values were summarized for *Achillea* extracts in "Table 1". The highest amount of the total polyphenols was determined in the tincture of *A. distans* subsp. *alpina* (80.13±0.72) followed by *A. distans* subsp. *distans* tincture (60.64±0.31). Concerning the content of flavonoids, contrary, the extract of *A. distans* subsp. *distans* (29.32±0.24) was richer in flavonoids, than the extract of *A. distans* subsp. *alpina* (22.75±0.23). The antioxidant effect of *A. alpina* subsp. *alpina* was higher than the activity of *A. distans* subsp. *distans* "Table 1". The comparative study showed significant differences, both qualitative and especially quantitative, between the two taxa of *Achillea distans*, and it could be used as a potential taxonomic marker in order to distinguish the species.

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Samples	TPC (mg gallic acid/g plant material)	Flavonoid (mg rutin/g plant material)	DPPH (%)	SNP (μg gallic acid/g plant material)
<i>A. distans</i> subsp. <i>distans</i>	60.64 \pm 0.31	29.32 \pm 0.24	73.43 \pm 1.07	0.15 \pm 0.001
<i>A. distans</i> subsp. <i>alpina</i>	80.13 \pm 0.72	22.75 \pm 0.23	89.92 \pm 0.92	0.17 \pm 0.018
Trolox			90.60% \pm 0.65	

Table 1. Total phenolic (TPC) and flavonoids content in *A. distans* and antioxidant activity
Each value was obtained by calculating average of three experiments with a standard deviation.

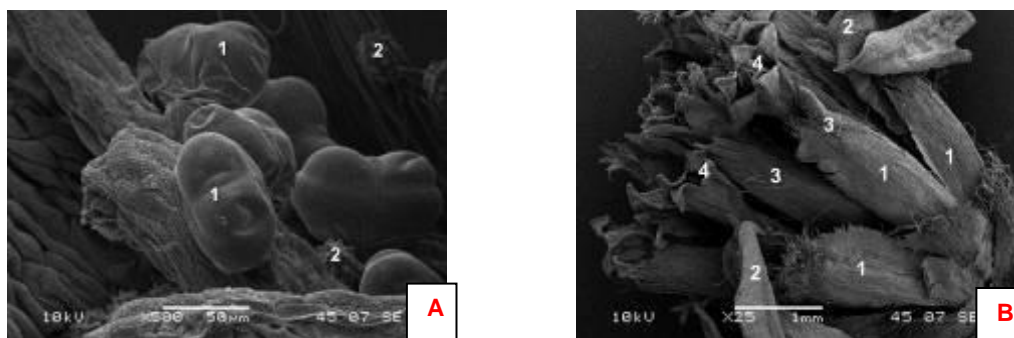


Figure 1. A. *Achillea distans* subsp. *alpina* (tubular flower): 1-biseriate glandular trichomes, 2-pollen grains. B. *Achillea distans* subsp. *alpina*: 1= bract, 2- ligulate flower, 3- filamentous tector hairs, 4- tubular flowers

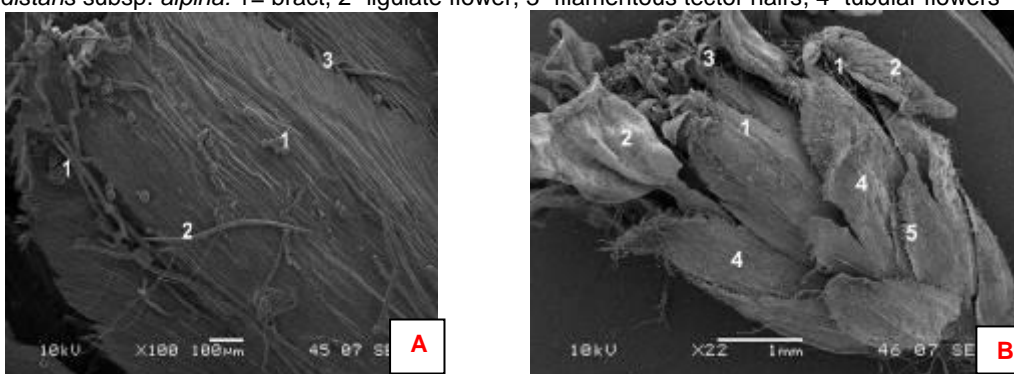


Figure 2. A. *Achillea distans* subsp. *distans* (bract): 1-pollen grains, 2-filamentous hairs, 3-serrated edge. B. *Achillea distans* subsp. *distans* (calatidiu): 1- tector hairs, 2- ligulate flower, 3-tubular flower, 4-bract, 5-serrated edge of bract