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MORPHO-HISTOLOGICAL STUDIES OF THREE ROMANIAN *SOLIDAGO* SPECIES

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Abstract

Solidago species, originally from North America, are successfully used in medicine due to their pharmacological effects. The most common part of *Solidago* plants used for its pharmacological effects is the herba, and in spite of their multiple uses, there is insufficient information about their taxonomical description in scientific literature. Due to this reason, the stems, leaves and flowers of *Solidago virgaurea*, *Solidago gigantea* and *Solidago canadensis* were studied more deeply by using scanning electron microscopy and optical microscopy. The detected morpho-histological features of Romanian *Solidago* species add new scientific data to the available records of the species. Compared with the known data, our results confirmed significant and distinctive features of these taxa which can be used as taxonomical markers for the identification of these selected species. In addition, in order to demonstrate the diuretic action of the three *Solidago* species, a comparative analysis was performed concerning the diuretic and saluretic action of the extracts and substances isolated from these plants.

Key words: *Solidago* species, Leaves, Flowers, Pollen, Morpho-histology, Diuretic and saluretic action.

Introduction

Solidago species (Asteraceae), originally from North America, are also successful worldwide invaders (Weber, 1997). *S. gigantea* was first recorded in 1977, *S. canadenis* in 1998, both in Lithuania, while *S. virgaurea* in 1905 in Austria (Szymura & Szymura, 2013). In addition, there are about 120 *Solidago* taxa known which can be classified in natives and invaders. In central Europe, one native taxa (*S. virgaurea* L.) and three invaders were found so far (*S. gigantea* Aiton, *S. canadensis* L., *S. altissima* L). These three species are morphologically similar to each other. Native species like *S. virgaurea* are found in different locations from those of non-native ones (e.g. shadow forests and mountain meadows). Thus, the morphological characteristics also differ and this fact provides them a competitive advantage against alien *Solidago* species.

The taxonomy of many introduced species is imperfectly known and the misidentification of the target species can also cause incorrect phytochemical results or failed biological control attempts. Kapoor & Beaudry (1966) created a morphological description of Asteraceae and *Solidago* taxa. The distinctions of these were made based on only the external morphology of plants, information which were complemented by Sieren (1970) who supplied some data about venation, trichomes, and other epidermal features of Euthamia (Kapoor & Beaudry, 1966; Anderson & Creech, 1975). The leaf anatomy frequently reflects the environmental adaptation. Thick cuticles, considerable mechanical tissue, water storage parenchyma, and small surface-to-volume ratio, all indicate the xeromorphy. Plants with isolateral leaves usually receive intense solar radiation. Leaf thickness often is related to droughty or saline habitats (Anderson & Creech, 1975).

Since environmental conditions differ among European countries, the histological features of *Solidago* species can also be different. European *Solidago* species are well studied, as they are invaders, but it is important to emphasize that there are not a lot of studies about the Romanian *Solidgo* species in the scientific literature (Dobjanschi *et al.,* 2019). Since taxonomical relationships in *Solidago* species were based mostly on similarities and differences in floral morphology, the aim of our work was to complement this information with new morphological and histological features of flowers, leaves and stem of the three Romanian *Solidago* species by using scanning electron microscopy (SEM) and optical microscopy respectively.

The diuretic action of *Solidago* species has been known since the last century (Harnischfeger & Stolze, 1983; Thiem & Goslinska, 2002). *Virgaurea* herba, harvested during flowering is very appreciated in phytotherapy in several countries for its diuretic effect. In medicine it is used as an infusion, decoction or hydro-alcoholic extract for the treatment of nephritis, urinary retention, kidney stones, urinary tract infections and high blood pressure (Tămaş *et al.,* 1990; Chodera *et al.,* 1991; Halberstein, 2012). Due to the high demand for *Solidaginis virgaureae herba*, there is now a tendency to replace *S. virgaurea* with two *Solidago* species, cultivated or subspontaneous: *S. gigantea* and *S. canadensis*. Thus, the second perspective of this work was to analyze the diuretic and saluretic action of the Romanian *Solidago* species.

Materials and Methods

Plant material: The plant material from the three *Solidago* species (*Solidago* herba) was collected during their blooming period in Cluj, which is a province in Transylvania, presenting hills with a continental climate, and was identified by prof. dr. Tămaş from Faculty of Pharmacy, Cluj-Napoca, Romania.

Sample preparation for SEM analysis: For both SEM and histological studies, the plant materials were preserved in a 96% ethanol-acetic acid mixture (3:1) and kept at 4°C. The leaves and the flowers were subsequently dried and covered with metal under vacuum, then were studied by using a scanning electron microscope (JEOL-JSM 5510 LV).

Sample preparation for histological analysis: Due to anatomical developmental differences of stems and leaves, plant samples (stems and leaves) of *Solidago* species were collected in different seasons. Stem samples were collected in May, when there was a transition between the primary and secondary structure of stems, which allowed us to prepare vertical sections (12 µm) by a rotary microtome (Anglia Scientific 0325). The leaf samples were collected from the medical plant collection of the Faculty of Pharmacy, Cluj-Napoca, Romania, during the blooming period. Staining was performed in Toluidine blue 0.2%, green iodine-carmine alum and Genevez reagent. Slides were covered with Canada balsam and then studied by Olympus digital camera using 6x, 10x and 20x microscope objectives for the leaves and 3x, 10x and 40x for the stem. The used method for embedding and preparation of the studied plant parts were described previously in the scientific literature (Toma & Rugină, 1998; Palade *et al.*, 2000).

Sample preparation for the study of the diuretic and saluretic action: The plant material (herba of the three *Solidago* species) was dried and then ground to a fine powder (sieve VI) (Anon., 1976) followed by a final preparation step to obtain the final compound made of: a fluid extract (1:1 with 70^0 alcohol), a 1.4% infusion and saponins. The diuretic action was tested on female white rats Wistar Bratislava, weighing about 150 grams, by using the techniques used in the literature (Cummings *et al.*, 1969; Chodera *et al.*, 1991). The experimental animals were divided into eight lots (12 animals per lot) in Modi diuretic devices. Water and food intake was suppressed by 18 hours prior experience. 1 ml of an aqueous solution was administrated by gavage to each animal. The control group received distilled water, group II received furosemide as a reference, and groups III-VIII received aqueous solutions containing freshly prepared fluid extract, infusion or saponin. The animal urine was collected in graduated cylinders from each device after 24 hours since the substances administration and the diuretic index was calculated based on the determined urine volume. The quantitative analysis of sodium and potassium ions was performed in order to determine the saluretic action by using a flame photometer (PFP7/C Clinical Flame Photometer, Jenway), after that the amount excreted during 24 hours per kg animal body mass was calculated and the saluretic index was determined.

Results

Since the herba (collected during the blooming period) is the therapeutically active part of the *Solidago* species, the stems, leaves and flowers of *S. virgaurea*, *S. gigantea* and *S. canadensis* were studied more deeply as far as the morpho-anatomical and pharmacological aspects are concerned. The abaxial leaf surface, pappus, pollen grains and stems of these three Romanian *Solidago* species were compared and the results are illustrated in the Figs. 1-7. Also, a comparative analysis was performed concerning the diuretic and saluretic action of the extracts and substances isolated from these plants.

SEM analysis: According to the SEM analysis, there are some distinguishable differences between the three Romanian *Solidago* species. SEM images of the abaxial leaf surface of *S.virgaurea*, *S. gigantea* and *S. canadensis* revealed non-glandular trihomes, mesomorphic stomata and abaxial epidermal cells. The abaxial epidermis of the leaf of *S. virgaurea* and *S. gigantea* presented non-glandular and unicellular trihomes, while the leaves of *S. canadensis* had both non-glandular and glandular trichomes (Fig. 1).

The tubular flower of *S. virgaurea* presented two stigma without evident stigmatic papillae. In addition, an abundant, thin and branched cellulosic pappus was present on the lower part of flower (Figs. 2A, a).

The tubular flower of *S. gigantea* showed petals with round edges. The petal and stigma surfaces were covered by pollen grains with spherical shape. These tricolporate pollen grains (20/15 µm) were echinate which means that they were ornamented with spikes and striated (Fig. 3). The pappus and pollen grains of *S. gigantea* showed significant differences when compared with *S. Canadensis*. Its pappus was less but strongly branched, divergent and the cells from the filaments were more numerous (Figs. 2B, b).

The tubular flower of *S. canadensis* presented protector hairs and a lot of pollen grains (15/11.6 µm) on the surface of stigma (Figs. 2C, c). These pollen grains were also tricolporate, which is a characteristic of Asteraceae family, but these were smaller than those of the other studied species (Fig. 3).

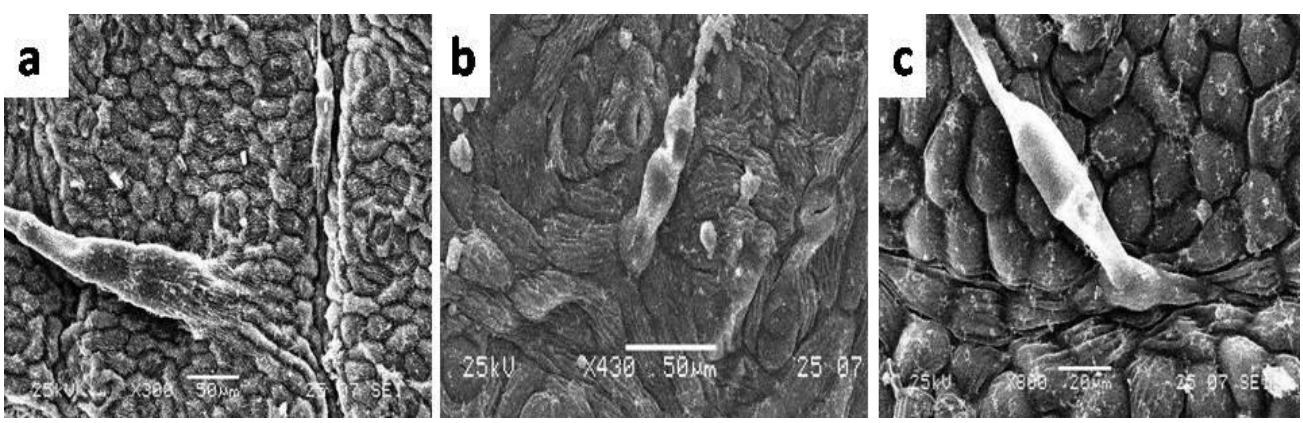

Fig. 1. SEM images of the abaxial leaf surface of *S. virgaurea* (a), *S. gigantea* (b) and *S. canadensis* (c).

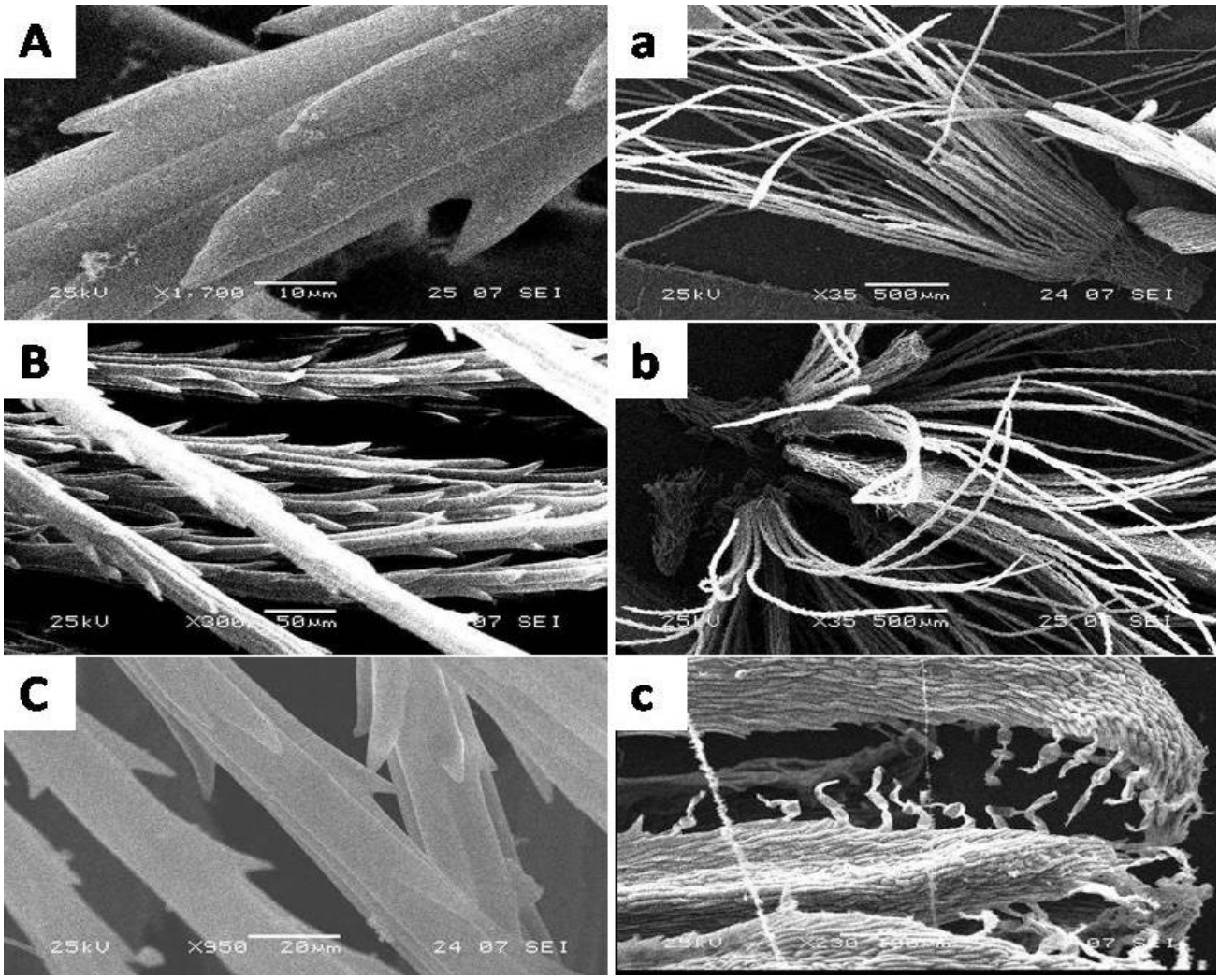

Fig. 2. SEM images of the pappus of *S. virgaurea* (A,a), *S. gigantea* (B,b) and *S. canadensis* (C,c)

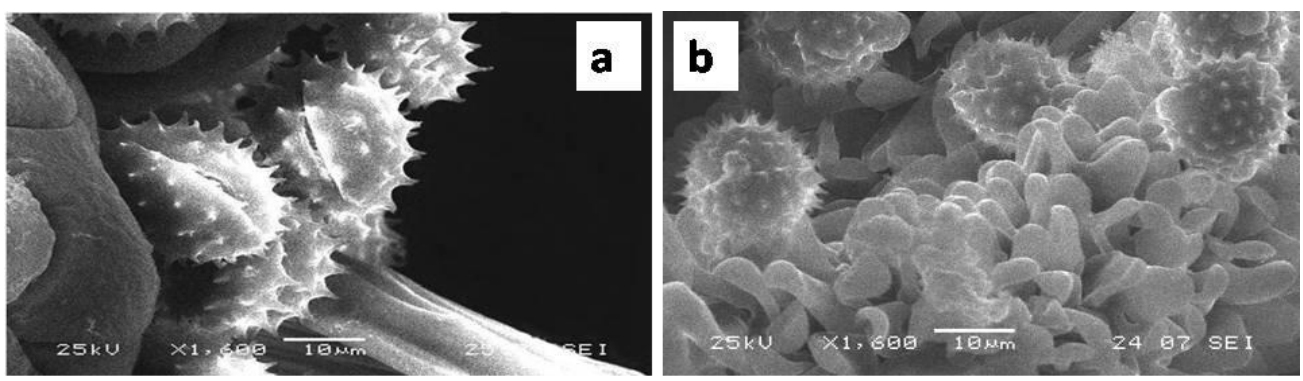

Fig. 3. SEM images of pollen grains of *S. gigantea* (a) and *S. canadensis* (b).

Histological analysis

Histological features of stems: The stem of each species presented a isodiametric parenchyma and collenchymatous cells surrounded the vascular bundles, which consisted of a phloem and xylem composed of an open collateral structure without sclerenchymatous cells but separated with cambium (Fig. 4). The structure of stem sections was the same in each case, but their shape was different. The stem of *S. virgaurea* was pentagonal, that of *S. gigantea* was circular, while *S. canadensis*'s stem presented an ovoid shape. In addition, secretory cells were observed in the internal zone of the cortex of *S. virgaurea.* In contrast to this, secretory cells were observed in both the internal zone of cortex and the medullary ray of *S. gigantea.* The cortex of *S. canadensis* did not present secretory cells. The piths and medullary rays of each plant were highly developed and the cortex of *S. gigantea* presented lacunes, while of *S. virgaurea* had some vascular bundles.

Even though the structure of the vascular bundles is the same in all three cases, *S. canadensis* showed glandular trichomes which were not observed at the other two *Solidago* taxa (Fig. 5).

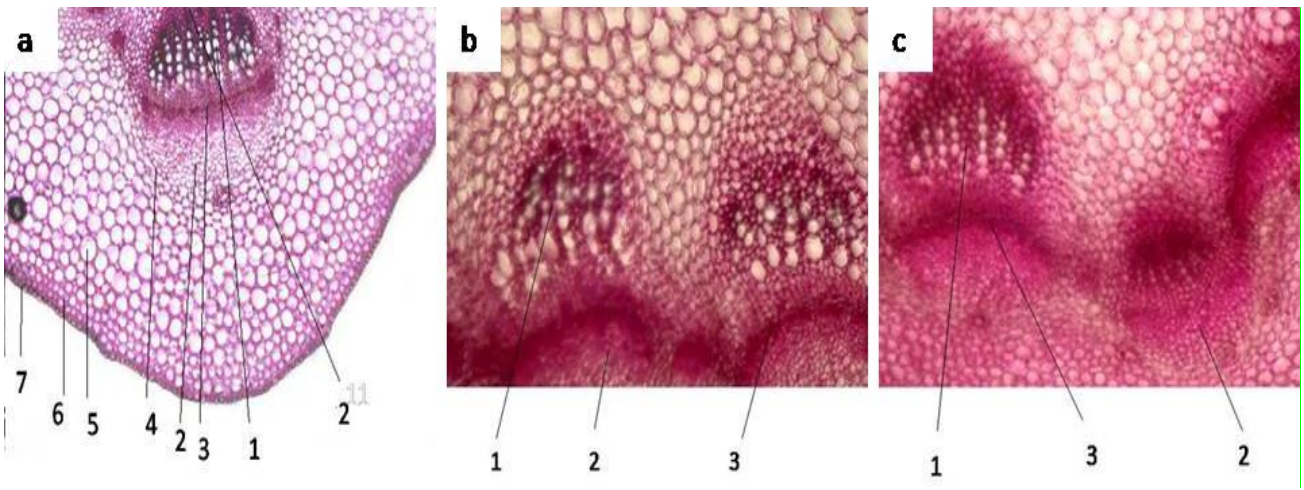

Fig. 4. Histological studies of stem of *S. virgaurea* (a), *S. gigantea* (b) and *S. Canadensis* (c) x40; 1-xylem, 2-phloem, 3-cambium, 4-endodermis, 5- parenchyma, 6- collenchyma, 7-epidermis.

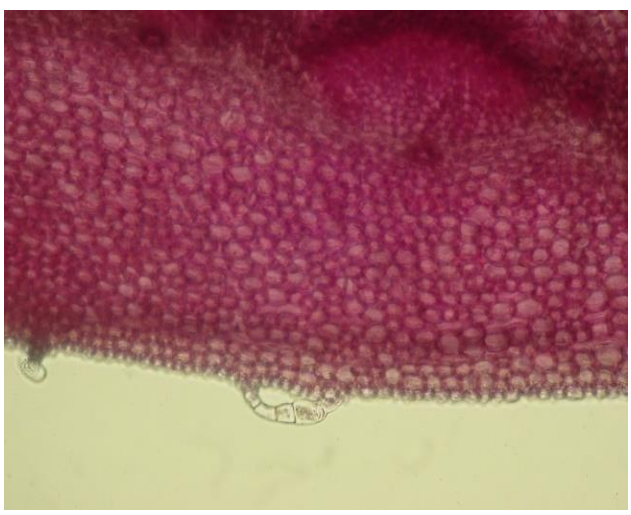

Fig. 5. Glandular trichomes of *S. Canadensis* x40.

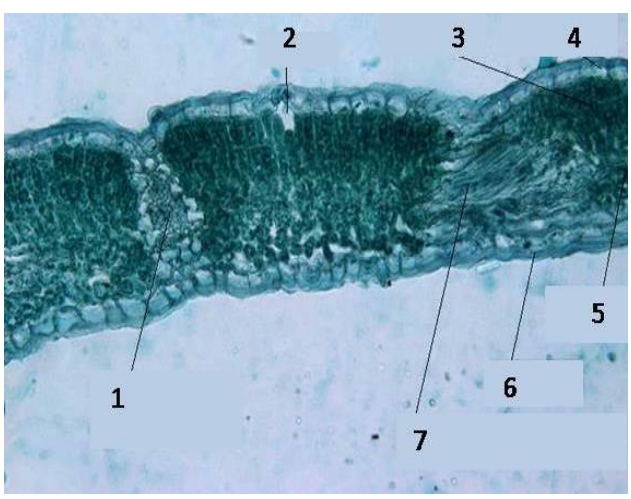

Fig. 6. Transversal leaf section of *S. virgaurea;*1-vascular bundle; 2 -stomata;3 -palisade cells; 4 -adaxial epidermis; 5 -spongy cells; 6 -abaxial epidermis;7- vascular bundle x40.

Histological features of leaves: The most important distinctive anatomical characters of the leaves structure were the heterogenous mesophyll and midrib, and the presence or absence of non-glandular and glandular trichomes. Even though the leaves of *S. canadensis* and *S. gigantea* described equifacial leaf structure, *S. virgaurea* showed dorsiventral and bifacial structure which was in accordance with data obtained by Anderson LC (Anderson & Creech, 1975). Leaf sections of *S. canadensis* showed non-glandular and glandular trichomes with multicellular bases, while *S. virgaurea* and *S. gigantea* described only non-glandular trichomes with unicellular bases on the abaxial leaf surface. However, the midrib of *S. virgaurea* and *S. gigantea* was surrounded by endodermis and collenchymatous tissues that were observed in each of the three leaf sections. This collenchima was also identified by Anderson LC (Anderson & Creech, 1975).

Each transversal leaf section of the *Solidago* species presented a one-cell-layer epidermis with stomata and they were covered by cuticles, similarly to earlier reported data (Anderson & Creech, 1975). In addition, the heterogeneous mesophyll of *S. virgaurea* presented elongate palisade and ovoidal spongy cells with less intercellular spaces. The ratio of the layers of palisade and spongy cells was 3:4 (Fig. 6).

Even though the midrib of *S. virgaurea*, *S. gigantea* and *S. canadensis* showed vascular bundles composed by a xylem and phloem leading to an open collateral structure with sclerenchymatous cells on the lower part of it, the secondary veins did not show a sclerenchyma. In addition, the abaxial part of vascular bundle was protected by collenchymatous cells as presented by Dobjanschi L (Dobjanschi & Tămaş, 2005).

The equifacial leaf structures of *S. gigantea* and *S. canadensis* were composed of palisade cells, which were rich in chloroplasts, and the ratio of their layers was 3:2 and 3:3 (Fig. 7). This information completes the previous results, indicating that the number of non-glandular trichomes was smaller on the abaxial leaf surface of *S. canadensis*, and the leaf blade section of this taxa seemed like the letter ”V”, while the leaf blades of other two taxa had horizontal arrangements (Dobjanschi & Tămaş, 2005).

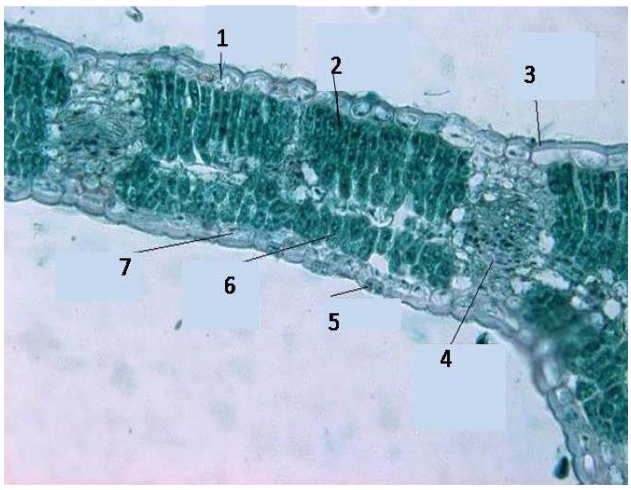

Fig. 7. Transversal leaf section of Solidago gigantea; 1-adaxial epidermis; 2-palisade cells; 3-cuticle; 4-vascular bundle; 5-stomata; 6-palisade cells; 7-abaxial epidermis x40.

Diuretic and saluretic action: The diuretic effect of the extracts, infusion and saponins isolated from *Solidago* species was compared to the control and standard groups, and the results are presented in Table 1.

It was observed that the control group animals show a lower diuresis than those from the standard group which were treated with furosemide (D.I. = 1.98). By analyzing the diuretic action of the vegetal extracts obtained from the three *Solidago* species, we noticed that the fluid extract of *S. virgaurea* showed the highest diuretic index (D.I. = 2.1), even higher than that of furosemide, meanwhile the fluid extracts of *S. gigantea* and *S. canadensis* presented a lower diuretic index. Regarding the diuretic index of the saponins, it appeared that the saponin isolated from *S. virgaurea* had a higher diuretic index (D.I. = 1.99) than the saponin isolated from *S. canadensis* (D.I. = 0.79), which is similar to that of furosemide.

The saluretic effect of the saponins and extracts obtained from *Solidago* species are presented below and compared with the control and standard groups (Table 2).

Analyzing the obtained data, it can be observed that the fluid extract obtained from *S. virgaurea* showed an increased urinary excretion of sodium (S.I. = 3.15) and potassium (S.I. = 2.56) ions, and the values were superior to the control and standard groups. The infusion of *S. virgaurea* presented a lower saluretic index compared to the fluid extract and to the saponin isolated from this plant.

Discussion

SEM analysis: Our results were in accordance with the previously published data by Szymura (Szymura & Szymura, 2013). In earlier reports, the leaves of *S. canadensis* and *S. gigantea* were also described. They had one pair of main lateral veins near the base, running parallel with the midrib, while leaves of *S. virgaurea* presented many pairs of short and often inconspicuous lateral veins (Birute & Jolita, 2016). According to Szymura, these species are shorter plants with smaller inflorescences than the other two, which is correlated with the amount of available soil nutrients and the environmental zone (Szymura & Szymura, 2013). According to Birute, the studied species also had high pollen viability (Birute & Jolita, 2016). *S. canadensis* also presented more stigmatic papillae than the tubular flowers of *S. virgaurea.* Our findings were in concordance with Weber who demonstrated that inflorescences of *S. gigantea* can easily be distinguished from that of the closely related *S. canadensis* by its longer rhizomes, brownish white pappus, glabrous stems and the denser inflorescence architecture (Weber & Jakobs, 2005).

Histological analysis: Since histological studies showed significant anatomical differences among the stems of *Solidago* taxa, this description can be considered as a distinguishing criterion for these three *Solidago* species. According to Birute 2016, the stem color of *Solidago* species varied from green to brown, with a median green in *S. canadensis* and intermediate in *S. virgaurea* (Birute & Jolita, 2016). In addition, the stem and of leaves' pubescence can also be used for the differentiation of these plants (Weber, 1997).

In previously reported data, *S. gigantea* and *S. canadensis* presented more abundant collenchymatous cells around the midvein and sheath extension than *S. virgaurea*, while bundle sheath fibers were described only on the adaxial and abaxial part of midvein of *S. canadensis* (Anderson & Creech, 1975). In contrast with our results, secretory cavities of leaves of *S. gigantea* and *S. canadensis* were identified by Loran and they were mostly schizogenous and positioned on the adaxial part of veins. These cavities were not identified in *S. virgaurea* and storage parenchyma was identified only in *S. gigantean* (Anderson & Creech, 1975).

Diuretic and saluretic action: We observed that there was a similarity between the diuretic index of the extracts and the diuretic index of saponins isolated from the *Solidago* species. The infusion of *S. virgaurea* had a lower diuretic index than the fluid extract and saponin isolated from the same species. This may be due to the fact that the extraction of the active principles is lower in the infusion than in the fluid extract. The saluretic indices for both sodium and potassium in the fluid extracts of *S. gigantea* and *S. canadensis* were lower than the fluid extract of *S. virgaurea.* The saponin from *S. canadensis* presented a very low saluresis, even lower than the control group, which may indicate that it inhibited the saluresis.

Other studies also highlighted the diuretic and saluretic effect of *Solidago* species. An ethnobotanical study about the use of wild and cultivated plants from Bosnia and Herzegovina also indicated the diuretic effect of *Solidago* species (Saric-Kundalic *et al.,* 2010). The flavonoid fractions from the flowers of three *Solidago* species from Poland showed diuretic and saluretic activity after being administrated per oral to rats (Chodera *et al.,* 1991).

In conclusion, the abaxial leaf surface, pappus, pollen grains, stems and leaves of three Romanian *Solidago* species were compared from the point of view of their morpho-histological features, by using microscopic

methods. The structure of the pappus, heterogenous mesophyll and midrib, the presence or absence of non-glandular and glandular trichomes on the abaxial leaf surface and the shape of stems were the most important distinctive anatomical characters. The diuretic action of the fluid extract of *S. virgaurea* was stronger than that of the extracts of *S. canadensis* and *S.gigantea* species, the diuretic index being even a little bit higher than that of furosemide. The saluretic effects presented by the fluid extract and by the infusion of *S. virgaurea* were stronger than the saluretic effect of the extracts of *S. gigantea* and *S. canadensis.* Based on this work, we can conclud that *Solidago* species present significant taxonomical differences and pharmacological effects which highlight the potential role of these species as a new source of natural medicines and treatments.

Table 1. The diuretic index of the fluid extracts, infusion and saponins isolated from *Solidago* species.

Group (administered substance)	Concentration (g %)	Diuresis (mg/kg body mass/24h)	Diuretic index (D.I.)
Control group (distilled water)	-	9.4	-
Standard group (furosemide)	0.0057	18.4	1.98
Research group III (S. virgaurea fluid extract)	1.4	20.0	2.1
Research group VI (S. virguarea infusion)	1.4	13.8	1.4
Research group V (S. virgaurea saponin)	1.4	18.5	1.99
Research group VI (S. gigantea fluid extract)	1.4	11.0	1.38
Research group VII (S. canadensis fluid extract)	1.4	7.9	0.9
Research group VIII (S. canadensis saponin)	1.4	6.3	0.79

Table 2. The saluretic index of the fluid extracts, infusion and saponins isolated from *Solidago* species.

Group	Concentration g %	Saluresis Na^+ mEg/kg/24h	Saluresis K^+ mEg/kg/24h	Saluretic index (S.I.) Na^+	Saluretic index (S.I.) K^+
Control group (distilled water)	-	1.66	0.28	-	-
Standard group (furosemide)	0.0057	4.54	0.38	2.74	1.32
Research group III (S. virgaurea fluid extract)	1.4	5.22	0.73	3.15	2.56
Research group VI (S. virguarea infusion)	1.4	3.11	0.43	1.87	1.57
Research group V (S. virgaurea saponin)	1,4	4.75	0.65	1.86	2.36
Research group VI (S. gigantea fluid extract)	1.4	1.93	0.28	1.55	1.31
Research group VII (S. canadensis fluid extract)	1.4	1.20	0.23	0.96	1.08
Research group VIII (S. canadensis saponin)	1.4	1.1	0.11	0.88	0.57

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