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GENERAL DATA REGARDING *TRAGOPOGON DUBIUS* SCOP. SPECIES - PHARMACOGNOSTIC ANALYSIS

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ABSTRACT: *Tragopogon dubius* scop., familiarly known as goat's beard, is a herbaceous dicotyledonous plant, which belongs to Asteraceae family. The species is studied because there are no sufficient data in the autochthonous literature and the information we have only refers to the macroscopic characteristics, ecology, area and variability and its use in the folk medicine, as well. For this purpose, the species, which was harvested within an area close to Siutghiol lake in Constanta district, was identified and dried according to the instructions in the literature in order to obtain *Tragopogoni dubiusi radix* and *Tragopogoni dubiusi cauli et folium* vegetal products. The macroscopic analysis confirmed the description of the species in the literature and the anatomo-histological study highlighted the presence of the laticifer vessels, which characterize the Asteraceae family. The global chemical analysis of the two vegetal products highlighted the presence of certain chemical compounds that justifies some of the use of the species in the traditional medicine. Also, the determinations showed the highest solubility of the active principles in ethanol 50%, which recommends the obtaining, analysis and test of these alcoholic extracts with possible therapeutic potential.

Keywords: *Tragopogon dubius*, Asteraceae family, macroscopic analysis, transverse section, pharmacognostic analysis.

INTRODUCTION:

The autochthonous literature does not provide sufficient data about this species and the information we have only refers to the macroscopic characteristics, ecology, area, variability (Săvulescu *et al*., 1965; Beldie, 1979; Pârvu, 2002; Ciocârlan, 2000) and its use in the folk medicine (Pârvu, 2002). Because the *Tragopogon dubius* Scop. (goat's beard) species is part of a complex study, we considered important to supplement the existing information with the pharmacognostic analysis of the two vegetal products, which consisted in macro- and microscopic analysis, global chemical analysis and preliminary determinations (loss on drying and substances soluble in different solvents). To find the therapeutic application of any vegetal species implies deep knowledge of that species; for this, the correct identification of the plant (macroscopic examination) and the anatomo-histological study (microscopic examination) are preliminary stages without which there is no substantiation for the successive phases.

According to the classification system taken from the "The Illustrated Flora of vascular plants from east of Romania, Determinator" (Sârbu *et al*., 2001), *Tragopogon dubius* Scop. has the following systematic classification: Vegetable Kingdom, Magnoliophyta Division, Magnoliopsida Class, Asteridae Sunclass, Asterales Order, Asteraceae Family, Tragopogon Genus, *Dubius* Scop. Species.

Tragopogon dubius Scop. is a pontico-mediteranean species, annual-biennial, terophite-hemiterophite, mesotherm to moderate termophile, heliophite, mesophite, which is ph amphitolerant. It has average to high needs for heat and vegetates in full sun. It does not stand shadow. The species prefers soils that are dry-moist to moist, eubasic, deficient in humus, even skeletal. It has a broad ecologic spectrum regarding the ionic reaction of the soil. Phytocenologically, it belongs to *Festuco-Brometea*, Car. *Sysimbrion*, *Mesobromion* (Pârvu, 2002; Beldie, 1979).

This species is frequently found in the plain, on hillocks, in the steppe-subarea of the chestnut oaks, on lawns, in the bushes, in the ploughed fields, in the locust tree plantations, among grassy rocks. In Romania, it can be found in Cluj, Braşov, Hunedoara, Banat, Argeş, Dobrogea, Galaţi, Bacău, Suceava (Săvulescu *et al*., 1965). In Dobrogea, it grows in the Măcin Mountains (Pricopan Peak) (Popescu *et al*., 2008). It is also found in the vascular flora list of Iaşi, Osoi, Popricani, Tomeşti, Vulturi (Huţanu, 2004). It spreads over the entire Basarabia (Negru and Ioniţa, 2009) and Bulgaria (Vladimirov and Tsoneva, 2006); it grows in Europe, Asia Minor, the Caucasus (Săvulescu *et al*., 1965).

According to the analysed data in the Romanian literature, *Tragopogon dubius* Scop. has not been studied from a chemical point of view. In the rural areas, the parts of the plant that grow in the air are used because of their sudorific, depurative, diuretic, and antidiarrheic properties. The active principles in these parts enhance transpiration and activate the toxine elimination through the sudoriparous glands and kidneys. These principles act upon renal epithelium, enhancing the quantity of urine eliminated in time; externally, they act upon inflammatory ailments such swellings, and cure certain cutaneous diseases. The Romanian literature describes the decoction made from the plant, which is prepared differently, depending on the ways of administration (external or internal). Literature data state that the girls in the country use the decoction to wash themselves in order to have smooth skin (Pârvu, 2002). *Tragopogon major* Jacq. has been used for a long time in galenics to treat

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dermatosis,including the parasitic one, in humans and animals (Râpeanu *et al*., 2001).

MATERIALS AND METHODS:

The material analysed was *Tragopogon dubius* Scop., harvested in the period of full flowering, in the sunlight, close to Siutghiol Lake, Constanta County, in May and July 2011. The plants were pull up by hand. To determinate the plant, we used "The Flora of RPR",volume X (Săvulescu *et al*., 1965), "The Illustrated Flora of Romania. Pteridophyta and Spermatophyta" (Ciocârlan, 2000) and "The Flora of Romania. Illustrated determinator of vascular plants", volume II (Beldie, 1979). The identity of the plant was confirmed by Mariana Arcuş, Doctor in Biology, titular of General, Vegetal and Animal Biology Discipline at the Faculty of Pharmacy, Ovidius University, Constanta. Most of the harvested material was dried at the room temperature in order to obtain the vegetal products *Tragopogoni dubiusi radix* and *Tragopogoni dubiusi cauli et folium*.

To establish the structure of the tissue and the anatomic elements of the root, stem and leaf, transverse sections were performed. The piece of the organ was introduced into two fragments of elder marrow, which were previously cut with the anatomic razor. The obtained sections were clarified with Javel water and fixed with acidulated water in acetic acid 1%. They were double stained with iodine green and potassium-alum carmine and then placed in gelatinous mass (Bucur *et al*., 2002); the sections were examined with optical microscope (Optika, SN 2272 52) first with 10X lens and then with 40X lens; afterwards, pictures were taken with a Panasonic photodigital camera.

The microscopic examination in powder, which aims to determine the organ that is the vegetal product and to identify the anatomic elements that characterize the studied vegetal product, was made by placing the vegetal product on the object slide over 5-6 drops of aqueous solution of chlorhydrate 70% and maintaining the slide upon a flame until white pungent chlorhydrate-like smelling smoke appeared (Bucur *et al*., 2002). The slide covered with a lamella is examined with optical microscope.

The global chemical analysis of the two vegetal products (*Tragopogoni dubiusi radix* and *Tragopogoni dubiusi cauli et folium*) involved successive extractions with solvents of different polarity and their separation by chemical methods, followed by the performing of specific reactions that help identify different groups of active principles or certain chemical constituents (Istudor *et al*., 1995).

The preliminary determinations involved the loss on drying and the quantification of the soluble substances, according to FR X. To determine the loss on drying, the vegetal product was kept in weighting ampules in the drying oven at 105°C for 3-4 hours and successively weighted until the constant mass. To determine the soluble substances the vegetal product was kept in contact with the proper solvent for 23 hours; afterwards, it was filtered and the filtrate was kept in the drying oven at 105°C until the constant mass (FR X, 2012).

RESULTS AND DISCUSSIONS:

The macroscopic examination confirmed that the species harvested was *Tragopogon dubius* Scop. (Fig.1) and allowed to highlight the following characteristics:

- the main root is a thick tap root with thin secundary roots that have remains of old leaves at the upper end;-
- the stem is erect, simple or branched, glabrate, green, cylindrical; when broken, it releases an abundant milk-like substance; it has numerous leaves and is finely striated;
- basal leaves during flowering, which usually miss or are dried; the leaves on the stem slightly decreased upwards, lineal or lineal-lanceolate, sessile, semiamplexicaul, sometimes almost vaginant then very slightly and long diminished into a fine sharp point;
- long floral peduncle, fistular, nude, gradually thickened up to anthodium; large anthodium, with long involucre; 8, rarely more uniseriate involucral folioles that grow together at the base, glabrate, smooth; involucral folioles longer than flowers, lanceolate, slightly narrowed from the base; lingulate flowers, hermaphrodite, bright-yellow;
- large akene fruit, without pappus, 3 cm long, with 5 marked ribs and other 5 attenuate ribs between, all thorny denticulated, with a smooth rostrum slightly thickened at its summit, equal to or longer than the lower part of the akene; feathery, interlaced creamy white pappus, 2 cm long.

Fig. 1 *Tragopogon dubius* Scop. – macroscopic examination (original) 1 – the whole plant; 2 – root; 3,4 – stem and leaves; 5 – inflorescence; 6,7 – fruit.

The microscopic analysis of the transverse sections highlighted the following:

- the root (Fig. 2):

- circular-costate contour of the transversal section, with attenuate ribs;
- rhizodermis largely exfoliated;
- under the rhizodermis there is the pluristratified suber, followed by the secondary cortex

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which is made of small cells, more or less rounded, with thickened walls, some of them with inulin spherocrystals;

- the primary cortex also pluristratified, made of large tangentially elongated cells with thin walls and intercellular spaces;
- unistratified visible endodermis with thickened cell walls ;
- central cylinder made of xylemic-phloemic fascicles with an early form of secundary structure;
- phloem which forms radially elongated fascicles, made of perforated tubes, annexed cells and many laticifer vessels, being separated by the parenchyma of the medullary rays dilatation;
- wood makes a central xylemic body which has groups of radial continous or discontinous strings of vessels separated by cellulosic parenchyma and libriform fibres; the vessels are encircled by xylemic parenchyma;
- strings of xylemic vessels separated by pluriseriated medullary rays of cellulosic parenchyma;
- in the center of the root there is the pith, made of polyhedral cells without intercellular spaces;
- a xylemic body made of meta- and protoxylem developed in its center.

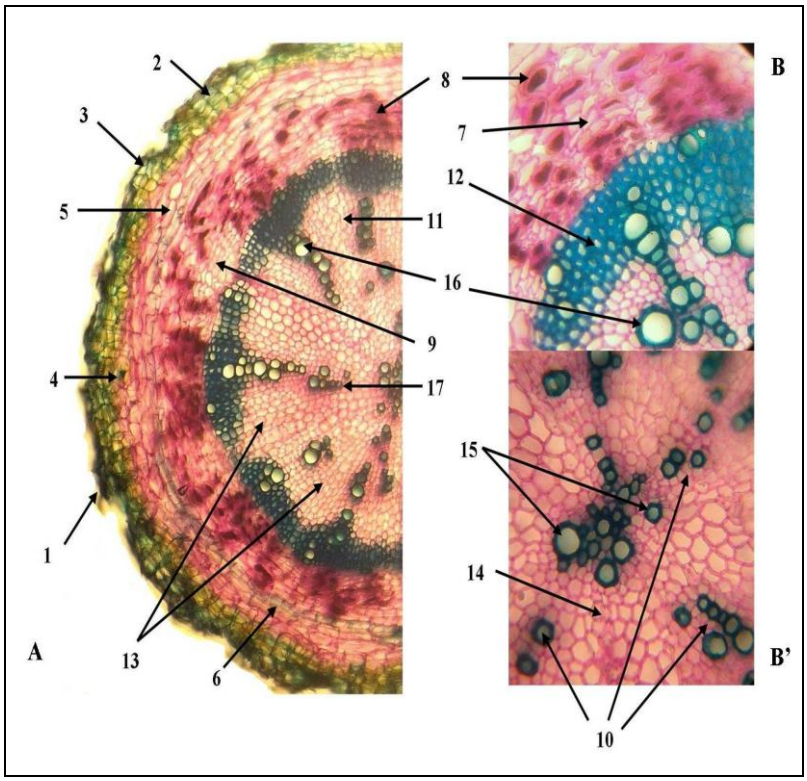

Fig. 2 *Tragopogon dubius* Scop. – A. root transverse section (100x) (original); B, B'. detail of root transverse section (400x) (original): 1 – rhizodermis, 2 – pluristratified suber, 3 – secondary cortex (felodermis), 4 – inulin spherocrystals, 5 – primary cortex, 6 – endodermis, 7 –phloem with laticifers, 8 – laticifer vessels, 9 – dilatation parenchyma, 10 – radial strings of xylemic vessels, 11 – cellulosic parenchyma, 12 – xylemic parenchyma, 13 – pluriseriated medullar rays of cellulosic parenchyma, 14 – pith, 15 – xylemic body, 16 – metaxylem vessels, 17 –rotoxylem vessels.

- **the stem (Fig. 3, Fig. 4):**
  - circular-costate contour of the transversal section, with attenuate ribs;
  - the unistratified epidermis has isodiametric cells with thickened internal and external walls; the external ones are covered by a visible cuticle;
  - in the ribs there is a cholenchyma type cortex and parenchyma-assimilating type in rest;
  - the conducting tissue forms xylemic fascicles of open collateral type, which are separated by xylemic and sclerotic medullar rays;
  - the phloem is thin, made of perforated tubes, annexed cells and few cells of xylemic parenchyma;
  - the conducting fascicles have a thick cord of sclerenchyma elements at both ends;
  - the parenchymatic-cellulosic medulla of meatus type is partially resorbed in the central position, resulting a large aeriferous cavity;
  - both in the cortex and in the perimedullar area there are laticifer canals of different diameters which are placed mainly in front of the conducting fascicles.

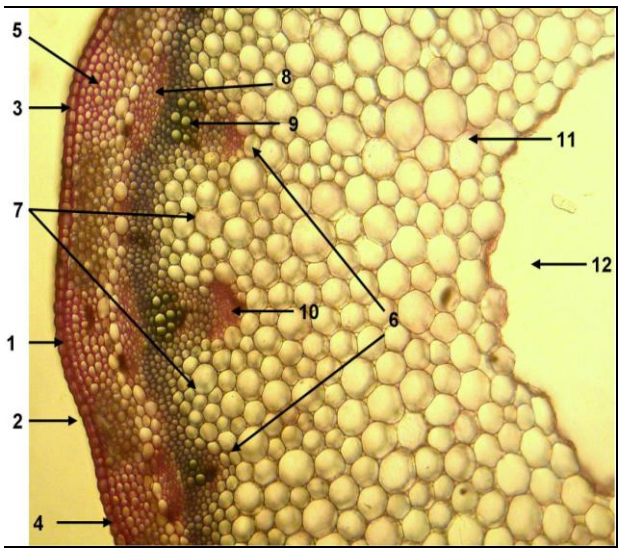

Fig. 3 *Tragopogon dubius* Scop. – fragment of stem transverse section (100x) (original) 1 – ribs, 2 – vallecula, 3 – epidermis with isodiametric cells, 4 – cuticle, 5 – cholenchyma type cortex, 6 – xylemic fascicles, 7 – sclerotic medullar rays, 8 – phloemic cord, 9 – xylemic cord, 10 – sclerenchymatous pod, 11 – medulla of meatus type, 12 – medullar lacunae

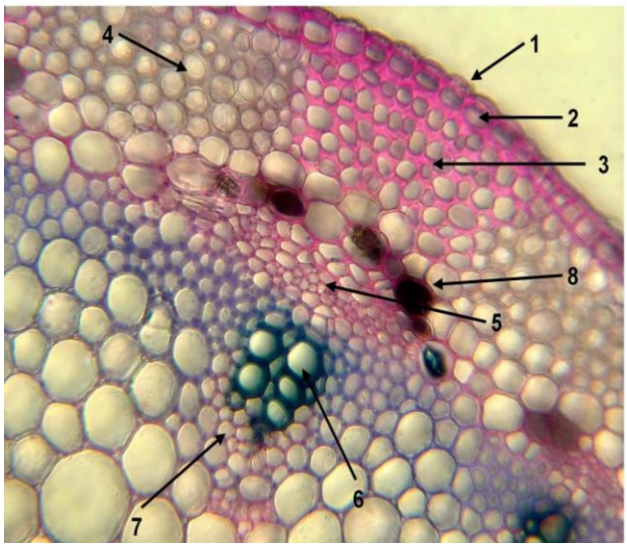

Fig. 4 *Tragopogon dubius* Scop. – detail of stem transverse section (400x) (original) 1 – cuticle, 2 – epidermis with isodiametric cells, 3 – collenchymatous cortex, 4 – assimilating cortex, 5 – phloemic cord, 6 – xylemic vessels, 7 – sclerenchyma pod, 8 – laticifer vessels

- leaf (Fig. 5):

- the transverse leaf section shows an aspect of slightly ondulated strip, with a slightly protuberant median vein on the lower face, covered by a single xylemic fascicle;
- both upper and lower epidermis are unistratified and made of isodiametric cells with thickened internal and external walls, the external ones being covered by a finely striated cuticle; from place to place, stomata appear on the lower epidermis;
- mesophyll is homogenous; towards the upper epidermis, it is made of large, rounded or polygonal cells with numerous aeriferous spaces between them;
- towards the lower epidermis and near the fascicles, it is made of smaller isodiametric cells;
- the conducting fascicle in the median vein is of close collateral type; it is made of three parallel strings of protoxylem vessels encircled by xylemic parenchyma and by a phloem cord made of perforated tubes, companion cells and phloem parenchyma; at both ends of the fascicle we can see a collenchymatous pod, which is thinner towards the upper epidermis and much more developed at the extremity of phloem; here we can also see a string of laticifer vessels;
- laterally to the median vein, there are the secondary veins; each of them are made of one much smaller single conducting bundle.

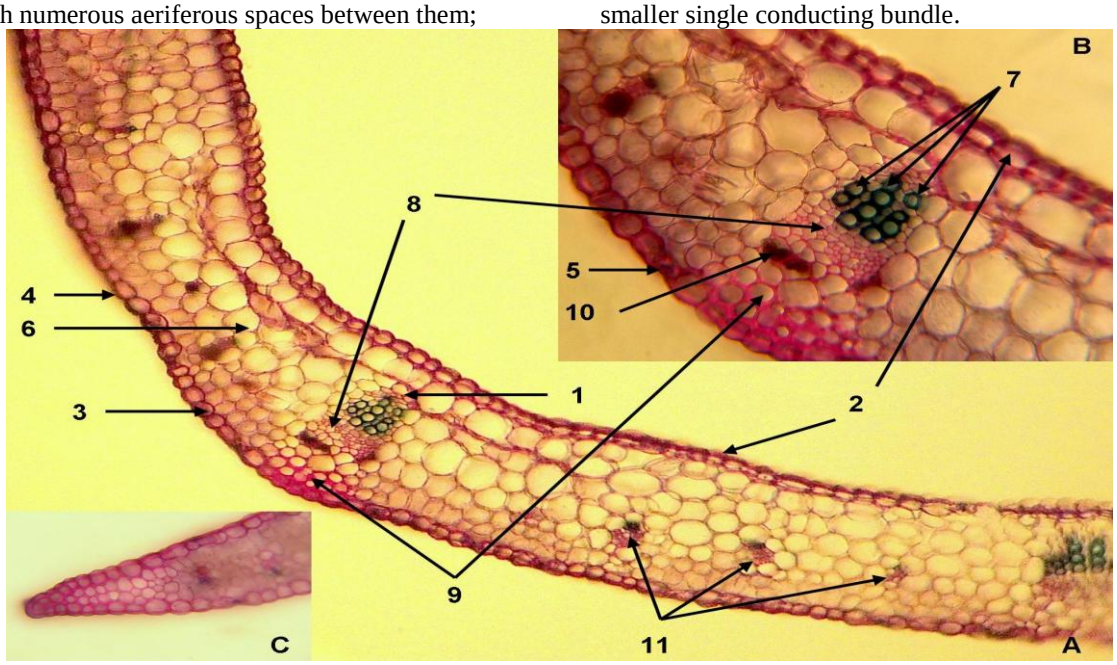

Fig. 5 *Tragopogon dubius* Scop.- A - leaf transverse section (100x) (original); B - detail of leaf transverse section (400x) (original); C - tip of the foliar limbus (100x) 1 – midvein, 2 – upper epidermis, 3 – lower epidermis, 4 – cuticle, 5 – stomata, 6 – homogenous mesophyll, 7 - strings of radial xylemic vessels, 8 – phloemic cord, 9 – collenchymatous pod, 10 – laticifer vessels, 11 – secundary veins

The microscopic examination in powder highlighted: for the root – xylemic vessels (Fig. 6), for the stem and the leaves – anomocytic stomata, xylemic fibres, fragments of parenchyma, large xylemic vessels (Fig. 7).

The histological analysis was made on the powder microscopic preparation clarified with chloralhydrate reagents.

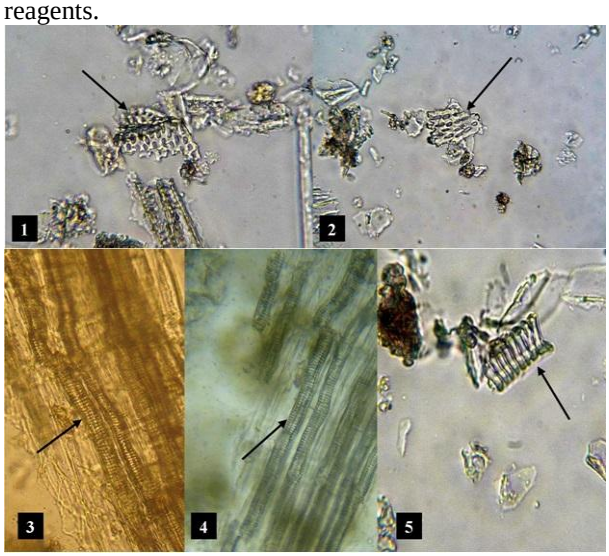

Fig. 6 *Tragopogoni dubiusi radix* – anatomic elements from powder microscopic preparation (original) 1, 2 – pitted xylemic vessels (100x), 3, 4 – spiral xylemic vessels (100x), 5 – scalariform xylemic vessels (100x)

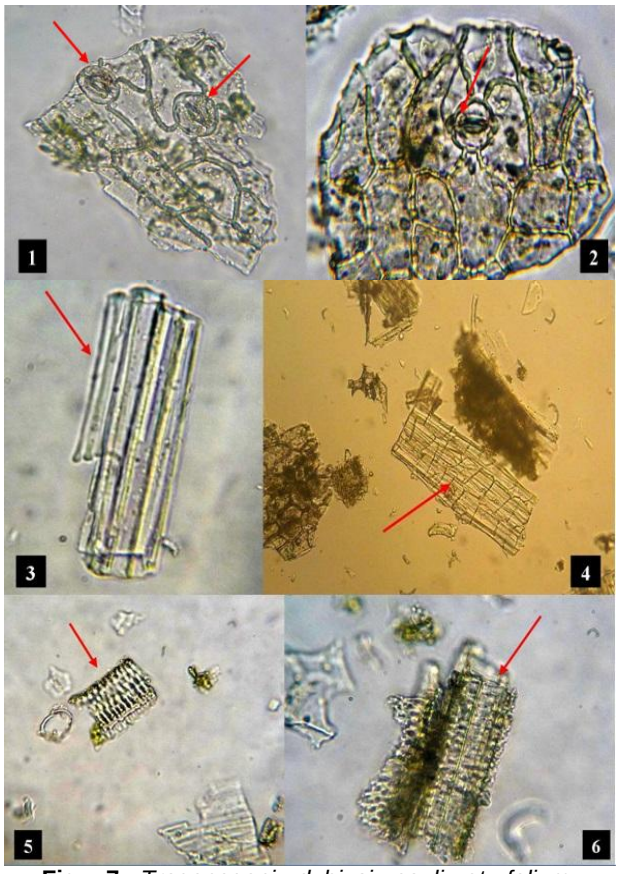

Fig. 7 *Tragopogoni dubiusi cauli et folium* - anatomic elements from powder microscopic preparation (original) 1, 2 - anomocytic stomata (400x), 3 - xylemic fibres (400x), 4 - fragments of parenchyma (100x), 5, 6 - large xylemic vessels (100x)

The global chemical analysis uses the successive extraction method, with solvents of different polarity, from the powder of dry plant material. The specific identification was made using the three solutions (etheric solution, alcoholic solution/alcoholic hydrolised solution and aqueous solution).

The results highlighted the following chemical compounds (Table I):

Table 1.

The results of the global chemical analysis

No.	Vegetal product	Analysed solution	Identified active principles
1.	*Tragopogoni dubiusi radix*	Etheric	● triterpenes, carotenoids, fatty acids
	Tragopogoni dubiusi cauli et folium		● volatile oil, triterpenes, carotenoids, fatty acids
2.	*Tragopogoni dubiusi radix*	Alcoholic	● tannins, reducing compounds
	Tragopogoni dubiusi cauli et folium		● tannins, reducing compounds
3.	*Tragopogoni dubiusi radix*	Alcoholic hydrolised	● triterpenic heterosides
	Tragopogoni dubiusi cauli et folium		● triterpenic heterosides
4.	*Tragopogoni dubiusi radix*	Aqueous	● polyuronides, reducing compounds, oses and polyoses, catehic tanines
	Tragopogoni dubiusi cauli et folium		● polyuronides, reducing compounds, oses and polyoses, catehic tanines

The plant material, signify by aerial plant and root, have the same chemical composition, volatile oil found only in aerial plant. The both part of the plant may be used in phytotherapy. The presence of tannins justifies the antidiarrheal use of the aerial plant in the traditional medicine; the presence of the polyuronides explains the empirical use of the decoction from *Tragopogoni dubiusi herba* for a smooth skin.

According to the preliminary determinations, the active principles in the two vegetal products are highly soluble in ethanol 50%; the loss on drying keeps within the limits provided by the literature: max. 10-15% for radix and max. 12-13% for herba (Istudor, 1998) (Table 2).

Table 2.

The results of the preliminary determinations

No.	Preliminary determinations	Quantity (g%)	
		Tragopogoni dubiusi radix	*Tragopogoni dubiusi cauli et folium*
1.	Loss on drying	10,0472	11,5545
2.	Substances soluble in water	34,5542	21,6931
3.	Substances soluble in ethanol 40%	37,4422	26,0518
4.	Substances soluble in ethanol 50%	**37,7675**	**26,4558**
5.	Substances soluble in ethanol 70%	35,8185	25,8939
6.	Substances soluble in absolute ethanol	29,0426	17,4742
7.	Substances soluble in ethyl ether	12,4571	20,1438

The laticifer vessels were also found in other species of the Asteraceae family: in the transverse section of the mature root of *Chicorium inthybus* L., the laticifers are in the secondary phloem (Zaman and Basar, 2013); the powder analysis of the root and leaf of *Taraxacum officinale* Weber (Popescu *et al*., 2010) and the stem, leaves of *Centaurea cyanus* L. (Chiru *et al*., 2013) the laticifer tubes are associated to annular and spiral vessels.

The secretory structures are an important element in the identification of genus in the Asteraceae family. They are mainly described in the roots of these species. In the stem transverse section of *Cichorium inthybus* L., the secretory structures are placed on the cortex (Akçin, 2007); in the root transverse section of *Hieracium lasiochaetum* (Bornm. & Zahn) Sell & West they are in the cells of the cortex; in *Mikania glomerata* Spreng the secretory structures are tubular in shape and in *Porophylum ruderale* (Cass) they appear as secretory cavities (Akçin and Akçin, 2009).

The root transverse section of *Tragopogon dubius* Scop. highlighted the presence of inulin spherocrystals in the secondary cortex; they are also present in the root of *Cichorium inthybus* L. (Zaman and Basar, 2013), *Taraxacum officinale* Weber (40% in autumn) (Istudor, 2001) and the above soil part of *Bellis perennis* L. (Pârvu, 2006).

The stem and leaves examination in powder showed anomocytic stomata, which are also present in *Taraxacum officinale* Weber (Popescu *et al*., 2010), *Cichorium inthybus* L. (Akçin, 2007), *Tussilago farfara* L. (Akçin, 2007), *Hieracium lasiochaetum* (Bornm. & Zahn) Sell & West. (Akçin and Akçin, 2009), *Centaurea cyanus* L. (Chiru *et al*., 2013), *Chrysanthemum carinatum* L., (Ali *et al*., 2010). For the Asteraceae family, the examinations found anomocytic and anisocytic stomata, as identified in the leaf of *Tragopogon latifolius* Boiss. (Akçin, 2007).

The results of the global chemical analysis showed similar active principles for the two vegetal products; the difference is the presence of the volatile oil in *Tragopogoni dubiusi cauli et folium*.

The reaction for the identification of the polyuronides was intensively positive. They were identified in the chemical structure of numerous asteraceae: in the roots of *Arctium lappa* L. (0.06-0.18% mucilages) (Stănescu *et al*., 2004), in floral somites of *Centaurea cyanus* L. (Pârvu, 2006), in the roots of *Taraxacum officinale* Weber (1,1%) (Istudor, 2001), in the leaves and flowers of *Tussilago farfara* L., (7-8% asoturic mucilage in leaves, 7% mucilage in flowers) (Istudor, 1998).

CONCLUSIONS:

Tragopogon dubius Scop corresponds to the description of this species in the literature.

The microscopic examination highlighted the tissue and elements that characterise this type of vegetal product and the other asteraceae with latex: lacitifer vessels, cells with inulin spherocrystals; the laticifers are also present in *Cichorium inthybus* L. in the root transverse section, *Taraxacum officinale* Weber in powder of leaf and stem, *Centaurea cyanus* L. in the stem transverse section, leaves and bracts.

The comparative phytochemical examination of *Tragopogoni dubiusi radix* and *Tragopogoni dubiusi cauli et folium* showed that the entire plant had the same active principles, excepting the volatile oil, which is only in the above soil part.

The presence of tanins justifies the antidiarrheal use of the aerian part in the traditional medicine; the presence of the polyuronides explain the empirical use of the decoction from *Tragopogoni dubiusi herba* for a smooth skin.

The active principles in the analysed vegetal products are most highly soluble in ethanol 50%, which recommends the obtaining, analysis and test of these alcoholic extracts with possible therapeutic potential.

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