

STUDY OF THE ANTIBACTERIAL AND ANTIFUNGAL EFFECT OF THE TRAGOPOGON DUBIUS SCOP. (ASTERACEAE) AQUEOUS EXTRACTS

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ABSTRACT: The aim of this study was to determine the antibacterial and antifungal effect of two aqueous extracts obtained from different vegetal products of *Tragopogon dubius* Scop. species: *Tragopogoni dubiusi radix* and *Tragopogoni dubiusi cauli et folium*. Two aqueous extracts of different concentrations were obtained and tested on three bacterial strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Streptococcus mutans* - identified in saliva) and one fungal strain (*Candida albicans* ATCC 10231). In the case of *Candida albicans*, none of the two tested aqueous extracts of different concentrations had any inhibitory effect, which means they are not antifungal; on the other hand, strains of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans* are sensible to the tested extracts. The results indicate that the two tested extracts have a moderate bacteriostatic effect.

Keywords: Tragopogon dubius, antimicrobial activity, aqueous extracts.

INTRODUCTION:

The medicinal plants are a genuine treasure nature gives us; men have been used them since ancient times to prevent or heal various ailments, instinctively at first, scientifically later.

Men have always been concerned about the idea of a longer life; because they did not find the secret of eternal life, the civilisations that have lived on Earth along history found the secret of a long and healthy life. Redescovering this secret by himself or by learning from his predecessors, man was driven to the very heart of Nature. Here, there are the substances that can support life or distroy it (Bojor and Perianu, 2005).

The medicinal plants are the origine of a special area of modern therapeutics, known as phytotherapy. As a new branch of biological and medical science, it has become a very important prophylactic and curative means in modern therapeutics (Mohan, 2007).

At present, treatment of the infectious diseases with antimicrobial agents is a problem, as the medical studies show a significant increase of the incidence of secondary effects and of the resistance developed by the pathogenic microorganisms against many antibiotics. Thus, we focused upon the active biological compounds obtained from plants that can be used in natural medicine: alkaloids, flavones, isoflavones, tanins, glicosides and phenolic compounds (Hasson, 2011).

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. The Romanian literature does not provide sufficient data about this species and the information we have only refers to the plant characteristics, ecology, area and variability (Săvulescu *et al.*, 1965; Beldie, 1979; Pârvu, 2002; Ciocârlan, 2000) and its use in the folk medicine (Pârvu, 2002).

According to the analysed data in the 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). One of the benefic properties of this plant is smoothing the skin (Pârvu, 2002).

MATERIALS AND METHODS:

Tragopogon dubius Scop. (goat's beard) was harvested in the sunny weather of May, near Siutghiol Lake, Constanța county. The determinations were made in the laboratory of General, Vegetal and Animal Biology in the Faculty of Pharmacy, Ovidius University, Constanta (Săvulescu et al., 1965; Ciocârlan, 2000; Beldie, 1979). The harvested material was dried at the room temperature according to the regulations in the literature (Pârvu, 2002) and grinded asked by each organ (FR Χ. 2012: as http://medplanet.dbioro.eu) in order to obtain the vegetal products Tragopogoni dubiusi radix and dubiusi Tragopogoni cauli et folium.

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The aqueous extracts were made with the refluxing method (Ciulei *et al.*, 1995); for both vegetal products stock solutions 50% were obtained. From the stock solutions were made dilutions: 2%; 10%; 12,5%; 25%.

The microbiological study were performed on three bacterial strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Streptococcus mutans* identified in saliva) and one fungal strain (*Candida albicans* ATCC 10231) that were chosen because they are representative and can be found in different human ecological niche; *Streptococcus mutans* is frequently found in patients with dental cavities. The sensitivity of the mentioned species was tested as follows: the antimicrobial activity was quantified by using the tested substances in the quantity of 30 mg powder applied directly on Muller-Hinton agar medium and blood agar medium in Petri dishes where selected bacteria were inoculated. Next, the Petri plates were incubated at 37° C for 24 hours. The results were interpreted on the same principle as for qualitative antibiogram data (antibiotic sensitivity report). The data is based on the existence of a direct relationship between the level of sensitivity to the tested substances and to the diameter of the inhibition area developed around the substances tested.

RESULTS AND DISCUSSION:

The results we obtain indicate that the two aqueous extracts of different concentrations have antibacterial activity; the inhibition areas, measured in mm, being observed for three strains: *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans*, according to the Tables 1, 2 and 3:

Table 1.

Results of the testing of different dilutions of aqueous extract of Tragopogoni dubiusi radix

No.	Concentrations of aqueous extract <i>Tragopogoni dubiusi radix l</i> Quantity used	Microorganisms/inhibition area (mm)			
		Staphylococcus aureus	Escherichia coli	Candida albicans	
1.	2% / 10µl	R	R	R	
2.	10% / 10µl	R	R	R	
3.	12,5% / 10µl	R	R	R	
4.	25% / 10µl	R	S (2 mm)	R	

*R = resistant, S = sensitive

Table 2.

Results of the testing of different dilutions of aqueous extract of Tragopogoni dubiusi cauli et folium

	Concentrations of aqueous extract	Microorganisms/inhibition area (mm)		
No.	<i>Tragopogoni dubiusi cauli et folium l</i> Quantity used	Staphylococcus aureus	Escherichia coli	Candida albicans
1.	2% / 10µl	R	S (4 mm)	R
2.	10% / 10µl	R	S (4 mm)	R
3.	12,5% / 10µl	R	S (4 mm)	R
4.	25% / 10µl	R	S (4 mm)	R

*R = resistant, S = sensitive

Table 3.

Results of the testing of the stock solutions 50% of the two vegetal products

		Microorganisms/inhibition area (mm)		
No.	Aqueous extract / Quantity used	Staphylococcus	Escherichi	Candida
		aureus	a coli	albicans
1.	Sol. 50% <i>Tragopogoni dubiusi radix</i> / 20 µl	S (11 mm)	S (12 mm)	S (15 mm)
2.	Sol. 50% Tragopogoni dubiusi cauli et folium / 20 μl	S (10 mm)	S (17 mm)	S (13 mm)

*S = sensitive

According to the data given in Tables 1 and 2, in both *Candida albicans* and *Staphylococcus aureus* there is no inhibitor effect for the two extracts tested for concentrations of 2%, 10%, 12,5%, 25% in 10 μ l, which is demonstrated by the lack of the inhibition area, as shown below (Fig. 1, Fig. 2):



Fig. 1 Activity of *Tragopogon dubius* Scop. aqueous extracts as against *Candida albicans* sp. (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*, T.D.R. = *Tragopogoni dubiusi radix*)

50



Fig. 2 Activity of *Tragopogon dubius* Scop. aqueous extracts as against *Staphylococcus aureus* (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*, T.D.R. = *Tragopogoni dubiusi radix*)

Escherichia coli was tested similarly; the results show that it is sensitive only to the concentration of 25% of the *Tragopogon dubius* Scop. root extract, with 2 mm



Fig. 3 Activity of aqueous extracts of different concentrations of *Tragopogon dubius* Scop. root as against *Escherichia coli* (T.D.R. = *Tragopogoni dubiusi radix*)

For the strain of *Escherichia coli*, the four tested solutions obtained from the stem and leaves of *Tragopogon dubius* Scop. have an inhibitory effect; the

inhibition area, as shown in Fig. 3 and Fig. 4 (detailed image).



Fig. 4 Antimicrobial activity of aqueous extract 25% of *Tragopogon dubius* Scop. root as against *Escherichia coli* (detailed image) (T.D.R. = *Tragopogoni dubiusi radix*)

inhibition areas with 4 mm diameter are equal for the four concentrations we studied (Fig. 5, Fig. 6).



Fig. 5 Antimicrobial activity of aqueous extracts (12,5%; 25%) of *Tragopogon dubius* Scop. stem and leaves as against *Escherichia coli* (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*)



Fig. 6 Antimicrobial activity of aqueous extracts (2%; 10%) of *Tragopogon dubius* Scop. stem and leaves as against *Escherichia coli* (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*)

For the 20μ l of stock solutions 50%, the levels of sensitivity to the strains of *Staphylococus aureus*, *Escherichia coli* and *Streptococus mutans* are significant. The results (Table 3) show that the extracts act upon the gram-positive and gram-negative bacteria, and inhibit their development, with a moderate bacteriostatic effect (Fig. 7, Fig. 8, Fig. 9).

However, in the inhibition areas with different diameters there can be seen the development of some rare bacterial colonies; that might be explain by the mutants resistent to the tested solutions, which are selected in 24 h at 37° C in the population of sensitive bacteria.



Fig. 7 Antimicrobial activity of aqueous extracts (50%) of *Tragopogon dubius* Scop. as against *Staphylococcus aureus* (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*, T.D.R. = *Tragopogoni dubiusi radix*)



Fig. 8 Antimicrobial activity of aqueous extracts (50%) of *Tragopogon dubius* Scop. as against *Escherichia coli* (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*, T.D.R. = *Tragopogoni dubiusi radix*)



Fig. 9 Antimicrobial activity of aqueous extracts (50%) of *Tragopogon dubius* Scop. as against *Streptococcus mutans* (T.D.C.F. = *Tragopogoni dubiusi cauli et folium*, T.D.R. = *Tragopogoni dubiusi radix*)

Literature shows that the antimicrobial activity was also tested for other species of Asteraceae family: Achillea millefolium L., (Hasson, 2011), Calendula officinalis L. (Roopashree et al., 2008; Goyal et al., 2011), Taraxacum officinale (Jassim et al., 2012), Cichorium inthybus L. (Verma et al., 2013; Ghaderi et al., 2012). For Achillea millefolium L., the antimicrobial activity in vitro of the aqueous extract from flowers was tested as against other species of gram-negative bacteria (Pseudomonas aeruginosa, Salmonella enterica, Shigella flexneri) and grampositive bacteria (Micrococcus luteus, Enterococcus faecalis). The results show that the extract tested inhibits Pseudomonas aeruginosa, Micrococcus luteus (Hasson, 2011). The results of these studies are similar to those of our study on gram-negative bacteria, although the species we tested are different.

Also, literature presents the results of the research made on extracts of Calendula officinalis L. which demonstrate that this species has a significant antimicrobial activity as against the most frequent microorganisms in humans, the lack of toxicity being remarkable; the microbiological studies on the aqueous extract from flowers of Calendula officinalis L. proves the existence of the antimicrobial activity (Staphylococcus aureus ATCC 2267, Bacillus subtilis ATCC 6633, Pseudomonas aeruginosa ATCC 25619 and Escherichia coli ATCC 10536) (Roopashree et al., 2008).

Our study led to similar results: the stock solutions (50%) from both the root and the stem and leaves of *Tragopogon dubius* Scop. are the most sensitive, the results are comparative to the study made on the flowers of *Calendula officinalis* L.

Similar results regarding the antibacterial and antifungal activity of the root, stem, leaves and flowers of *Calendula officinalis* L. were highlighted in other studies, as well (Goyal *et al.*, 2011).

The biological activity of other species of the Asteraceae family was tested on other strains: for the extract of *Taraxacum officinale* leaves there were used *Proteus mirabilis, Escherichia coli, Staphylococcus aureus* (Jassim *et al.*, 2012); for the leaves of *Cichorium inthybus* L., - *Streptococcus pyogen, Staphylococcus aureus, Enterococcus* (Ghaderi *et al.*, 2012), *Escherichia coli* and *Pseudomonas aeruginosa* (Verma *et al.*, 2013).

The *Streptococcus mutans* strain sensitivity to aqueous extracts from the root of *Tragopogon dubius* Scop. leads to further studies on the posibility to include this solution into the mouthwash which is largely used to clean the oro-dental cavity at present.

CONCLUSIONS:

> The aqueous extracts of *Tragopogon dubius* Scop., tested in different concentrations, do not inhibit the strain of *Candida albicans*, thus, they do not have antifungal activity.

> The strain of *Staphylococcus aureus* is resistant to both dilutions of the aqueous extract from the root and that from stem and leaves; still, the tested strain is sensitive to the stock solutions (50%).

> *Escherichia coli* is sensitive to the extract from the root with high concentration (25%, 50%); and it is sensitive to the extract from stem and leaves with all concentrations.

The tested aqueous extracts (50%) inhibit the strain of *Streptococcus mutans* the effect being more obvious in the root of *Tragopogon dubius* Scop.

> The results show that the aqueous extracts obtained from the two vegetal products Tragopogoni dubiusi radix and Tragopogoni dubiusi cauli et folium can be used as antimicrobials pharmaceutical products for external use.

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