Analele Universităţii din Craiova, seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series) Vol. XLVI 2016

HISTO-ANATOMICAL AND PRELIMINARY TLC INVESTIGATIONS ON *TRIBULUS TERRESTRIS* L. (*ZYGOPHYLLACEAE*) SPECIES

CORNELIA BEJENARU[1], GEORGE DAN MOGOŞANU[2]*, ANDREI BIŢĂ[2], LUDOVIC EVERARD BEJENARU [2]

[1]Department of Vegetal & Animal Biology, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova

[2]Department of Pharmacognosy & Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, e-mail: george.mogosanu@umfcv.ro

Keywords: Tribulus terrestris L., histo-anatomy, polyphenols, thin layer chromatography

ABSTRACT

Using microphotography technique, the cross-sections of root, aboveground stem and leaf were obtained and investigated for Tribulus terrestris L. (Zygophyllaceae) plants in blossom, collected in June 2016, from the surroundings of Craiova City, Dolj County (southwestern Romania). The polyphenols content of the aerial parts (Tribuli herba) was analyzed by thin layer chromatography. One rutin derivative was identified starting from the 12 peculiar chromatographic bands.

INTRODUCTION

Tribulus terrestris L., Goat's head, Devil's thorn, *Zygophyllaceae* family, is an annual herbaceous species, widespread mainly in the steppe, pastures, meadows and sandy or sandy-rocky areas in the Central Europe, Mediterranean zone, South East Asia, America, India, Africa, Australia [4].

T. terrestris contains various active principles (steroidal saponins, sterols, flavonoids, polysaccharides, alkaloids, tannin) [10, 13] with various pharmacological actions, mainly aphrodisiac [5], anti-inflammatory [10], antioxidant [8], antimicrobial [11], immunomodulatory, hepatoprotective, hypolipidemic, hypoglicemiant [9, 10].

In the specialty papers, there are scarce and incomplete data concerning *T. terrestris* histo-anatomy [7, 14].

The aim of our paper was the histo-anatomical investigation of the root, aboveground stem and leaf of *T. terrestris* and the preliminary analysis of the polyphenols content from the aerial parts (*Tribuli herba*).

MATERIAL AND METHOD

Histo-anatomical investigation

The vegetal material was harvested from *T. terrestris* plants in blossom, in June 2016, from the surroundings of Craiova City, Dolj County (southwestern Romania).

Fixation and preservation of roots, aboveground stems and leaves were achieved in 70% ethanol. Cross-sections sections were obtained using botanical razor.

After washing with distilled water, the cross-sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture (Genevese reagent) was used for the staining of cross-sections. Depending on the chemical composition of cell membranes, the reactive induced various stains: pink to red for cellulose and mucilage, pale red for cytoplasm, yellow for suberin and brown for lignin [2].

Stained and mounted cross-sections were analyzed on a Krüss binocular photon microscope (objectives ×4, ×10, ×40) and then photographed using a Sony DSLR-A380 digital system adapted to the microscope.

The description of microscopic cross-sections was achieved according to classical authors [12].

Thin-layer chromatography (TLC) analysis

Preliminary analysis of polyphenols was performed on the aerial parts of *T. terrestris* species (*Tribuli herba*), using a CAMAG (Muttenz, Switzerland) system, in the following experimental conditions [1, 3, 6]: stationary phase TLC silica gel 60 F_{254} 20×10 precoated glass plates (Merck, Darmstadt, Germany) pre-washed with chloroform–methanol (1:1, *v/v*); mobile phase chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes) in a vapor-equilibrated chromatographic tank (20×10 cm twin trough chamber, CAMAG); sample – 20% methanolic extract of *Tribuli herba*; standards (Merck) – 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin; migration distance 80 mm; sample (1–10 µL) and standards (2 µL) application – CAMAG Linomat 5 semi-automatic system (spray gas nitrogen, dosage speed 150 nL/s and band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, resolution 100 µm/step; measurement mode – absorption; spectra acquisition, processing and quantification analysis – winCATS ver. 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical investigation

Root

In cross-section, the root has circular shape and secondary structure due to the two lateral meristems: phellogen and libero-ligneous cambium. In cross-section, from the outside towards the inside of the root, the following histological sequence was evidenced. Suber is made of 2–3 layers of flattened cells with suberin-impregnated walls. Sometimes suber is exfoliated. Phellogen has circular shape, consisting of two layers of flattened cells with thin and corrugated walls. Phelloderm is made of 2–3 layers of heterodiametric cells, uniformly placed, without intercellular spaces. Cortical parenchyma of the primary structure is well represented, consisting of large parenchyma cells, delimiting small intercellular meatus. At this level, several bundles of sclerenchyma fibers are distinguished. Conducting tissues are arranged in two concentric rings, separated by libero-ligneous cambium. Phloem tissue forms a thin, external ring, made of sieve tubes, annex cells and phloem parenchyma. Libero-ligneous cambium has circular shape, consisting of flattened cells with cellulosic thin walls. Xylem tissue forms the internal ring made of some metaxylem vessels of different sizes, disseminated into the libriform tissue. In longitudinal-radial sections, metaxylem exhibits reticulate, ringed and spiral thickenings. Protoxylem is pushed toward the center of the root adjacent to the medullary parenchyma. Medullary rays are multi-cellular, uniseriate, cellulosic (Figure 1).

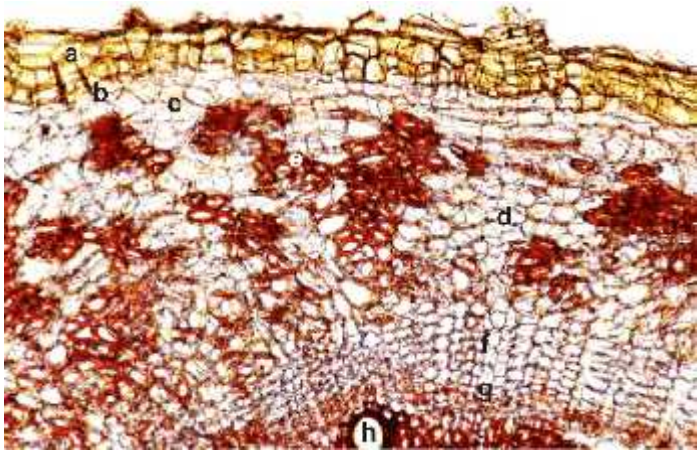

Figure 1. Cross-section through T. terrestris root: (a) suber; (b) phellogen; (c) phelloderm; (d) cortical parenchyma; (e) bundles of sclerenchyma fibers; (f) phloem tissue; (g) libero-ligneous cambium; (h) metaxylem (Congo red–chrysoidine staining, ×100).

Aboveground stem

In cross-section, into the upper third, the aboveground stem has slightly circular sinuous shape and secondary structure generated by the intra- and inter-fascicular libero-ligneous cambium. In cross-section, from the outside towards the inside of the aboveground stem, the following histological sequence was observed. Epidermis consists of a single layer of isodiametric cells with thin radial walls and slightly thickened tangential external and internal walls. The external walls are bulged and covered by a thick cuticle with papilliform relief. From point to point, we found unicellular, long and bowed tector hairs, some of them situated at the rib's level and implanted in a multi-cellular epidermal pedestal. Hypodermis is made of a single layer of small cells, uniform in size, alternately stacked with the epidermal cells. Cortical parenchyma is represented by 5–7 layers of large, heterodiametric cells, which generate small intercellular spaces. At this level are evidenced sclerenchyma caps that protect the conducting fascicles in periphloemic position. The conducting tissues are organized into multiple collateral-open libero-ligneous fascicles of various sizes. Phloem tissue is represented by sieve tubes, annex cells and phloem parenchyma; at the level of primary phloem, it is protected by some sclerenchyma caps. Xylem tissue consists of large caliber metaxylem vessels scattered in the libriform mass on the internal side of libero-ligneous cambium. At the fascicle base, small diameter protoxylem is accompanied by xylem parenchyma. Libero-ligneous cambium of circular sinuous shape is made of flattened cells. Medullary rays are wide and lignified, with the increasing move towards secondary structure. Medullary parenchyma is well developed, made of large cells with thin, cellulosic walls, of meatus type (Figures 2 and 3).

Figure 2. Cross-section through T. terrestris aboveground stem: (a) epidermis; (b) tector hair; (c) hypodermis; (d) cortical parenchyma; (e) sclerenchyma cap; (f) phloem tissue; (g) libero-ligneous cambium; (h) metaxylem; (i) medullary ray; (j) protoxylem; (k) medullary parenchyma (Congo red–chrysoidine staining, ×100).

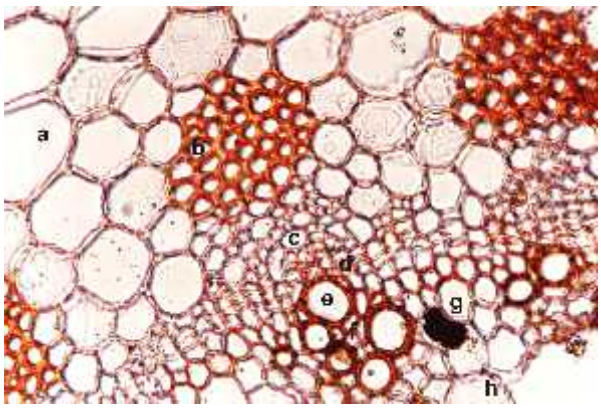

Figure 3. Cross-section through T. terrestris aboveground stem: (a) cortical parenchyma; (b) sclerenchyma cap; (c) phloem tissue; (d) libero-ligneous cambium; (e) metaxylem; (f) libriform tissue; (g) medullary ray; (h) medullary parenchyma (Congo red–chrysoidine staining, ×100).

Leaf's limb

In cross-section, from the outside towards the inside of leaf's limb, the following histological sequence is observed. Upper epidermis is made of a single layer of elongated heterodiametric cells with thickened tangential external and internal walls and thin radial walls. A thin cuticle covers the epidermis. Rare unicellular tector hairs, long and with sharp peak, are found at the main rib level. Mesophyll is uniform, consisting of a single layer of elongated cells, without intercellular spaces, ordered in palisade, rich in chloroplasts, under the upper epidermis and of two layers of smaller cells, slightly elongated, also without intercellular spaces and rich in chloroplasts, above the lower epidermis. The conducting tissue is represented by libero-ligneous fascicles evenly distributed into the mesophyll. On the outside, an assimilatory fascicular sheath protects the libero-ligneous fascicles. Lower epidermis is made of a single layer of oval cells, with thin radial walls and slightly thickened external and internal walls. At this level, stomata are observed from point to point. The leaf has hypostomatic bifacial equifacial structure (Figures 4 and 5).

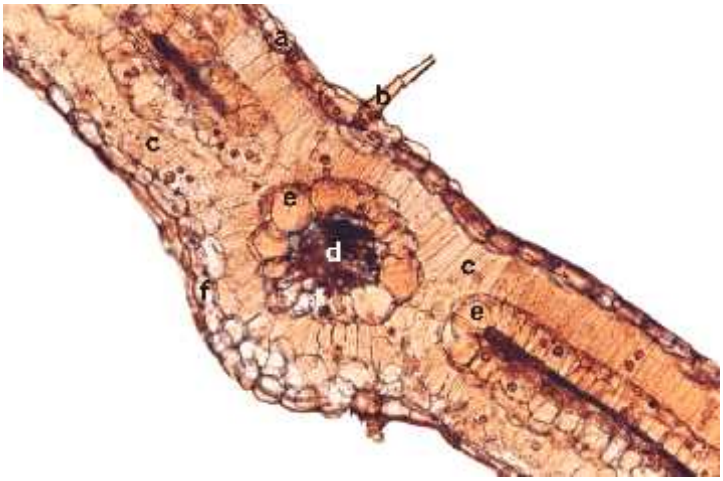

Figure 4. Cross-section through T. terrestris leaf's limb: (a) upper epidermis; (b) tector hair; (c) palisade parenchyma; (d) libero-ligneous conducting fascicle; (e) assimilatory fascicular sheath; (f) lower epidermis (Congo red–chrysoidine staining, ×100).

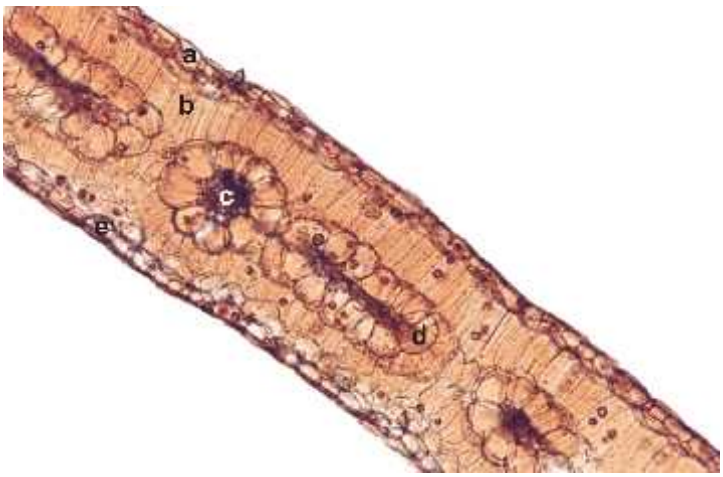

Figure 5. Cross-section through T. terrestris leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) libero-ligneous conducting fascicle; (d) assimilatory fascicular sheath; (e) lower epidermis (Congo red–chrysoidine staining, ×100).

TLC analysis

The experimental data on the preliminary TLC analysis of polyphenols from *Tribuli herba* are highlighted in Figures 6–8. One rutin derivative (R_f 0.05, 161.86 mg/100 g of dried vegetal product) was identified starting from the 12 peculiar chromatographic bands.

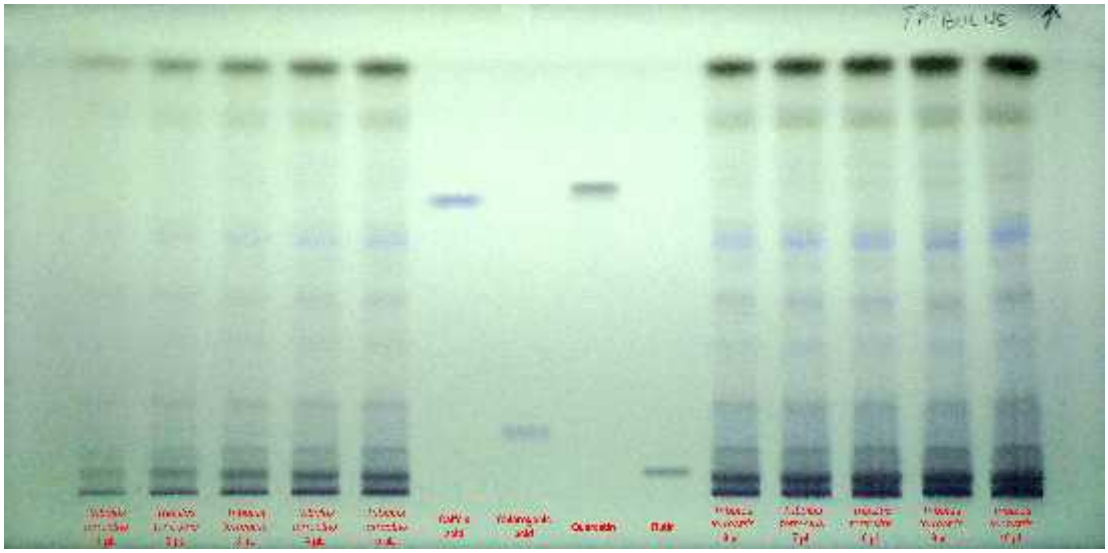

Figure 6. TLC chromatogram of polyphenols from Tribuli herba methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

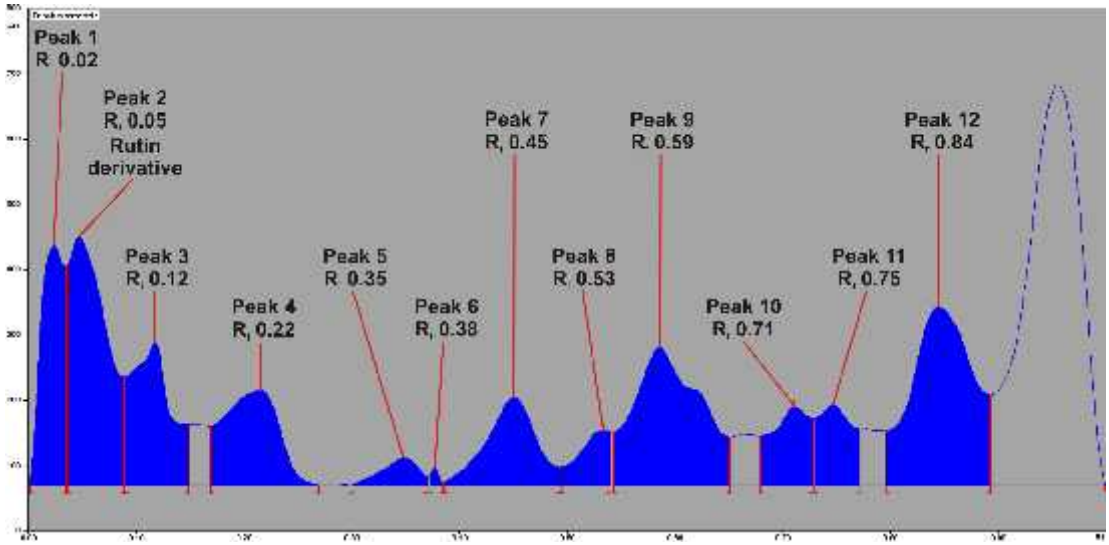

Figure 7. Densitogram of polyphenols (UV 254 nm) separated from Tribuli herba methanolic extract.

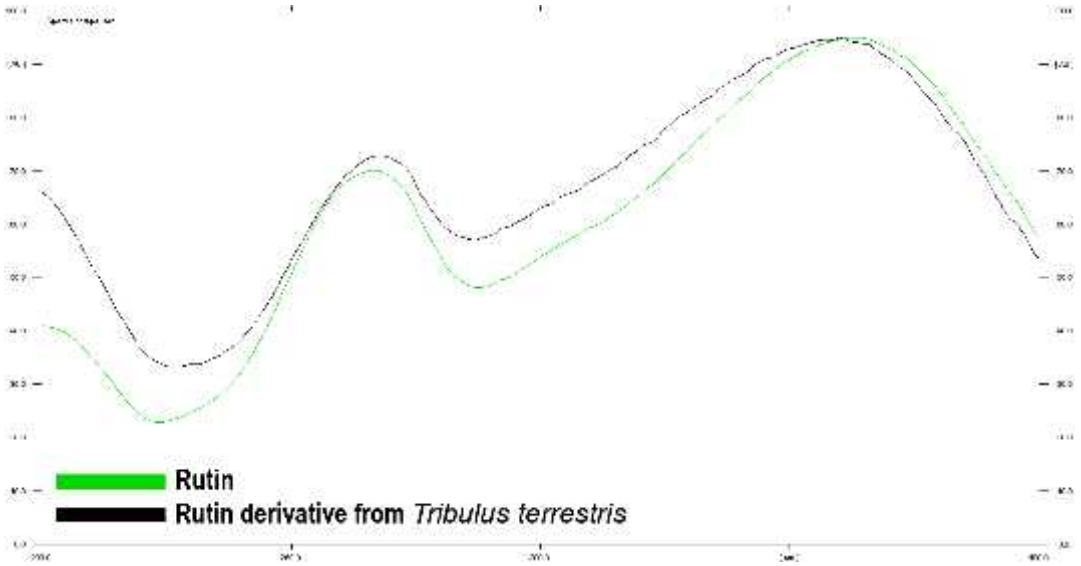

Figure 8. Rutin derivative in situ UV spectra of standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of the root, aboveground stem and leaf of *Tribulus terrestris* species and the preliminary TLC analysis of *Tribuli herba* polyphenols were accomplished. The root has circular shape and secondary structure. Into the upper third, the aboveground stem has slightly circular sinuous shape and secondary structure due to the intra- and inter-fascicular libero-ligneous cambium. The leaf's limb has hypostomatic bifacial equifacial structure. Starting from the 12 peculiar chromatographic bands, one rutin derivative was identified by TLC.

BIBLIOGRAPHY

1. **Altemimi, A., Watson, D.G., Kinsel, M., Lightfoot, D.A.**, 2015 – *Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a TLC-densitometric method*, Chem. Cent. J. 9:39.
2. **Andrei, M., Paraschivoiu, R.M.**, 2003 – *Microtehnică botanică*, Ed. Niculescu, Bucureşti, 2003, 222 pag.
3. **Bojić, M., Simon Haas, V., Sarić, D., Maleš, Z.**, 2013 – *Determination of flavonoids, phenolic acids, and xanthines in mate tea (Ilex paraguariensis St.-Hil.)*, J. Anal. Methods Chem. 2013:658596.
4. **Ciocârlan V.**, 2000 – *Flora ilustrată a României. Pteridophyta et Spermatophyta*, ediţia a 2-a revizuită şi adăugită, Ed. Ceres, Bucureşti, 1138 pag.
5. **de Souza, K.Z., Vale, F.B., Geber, S.**, 2016 – *Efficacy of Tribulus terrestris for the treatment of hypoactive sexual desire disorder in postmenopausal women: a randomized, double-blinded, placebo-controlled trial*, Menopause 23(11):1252–1256.
6. **Gîrd, C.E., Nencu, I., Costea, T., Duţu, L.E., Popescu, M.L., Ciupitu, N.**, 2014 – *Quantitative analysis of phenolic compounds from Salvia officinalis L. leaves*, Farmacia 62(4):649–657.
7. **Niculescu, M., Făgăraș M.**, 2015 - *The grassland natura 2000 habitats found in Lespezi quarry and the surroundings, Dambovita county,* 15th International Multidisciplinary Scientific GeoConference SGEM 2015, www.sgem.org, SGEM2015 Conference Proceedings, ISBN 978-619-7105-39-1 / ISSN 1314-2704, June 18-24, 2015, Book5 Vol. 1, 877-882 pp, DOI: 10.5593/SGEM2015/B51/S20.116, http://www.sgem.org/sgemlib/spip.php?article6369&lang=en
8. **Niculescu, M., Făgăraș, M., Popescu,C.,** 2016 - *Diversity, distribution and ecology of the invasive alien plants in the ,,Cotmeana platform,, protected area, Romania,* Conference proceedings of the 16th International Multidisciplinary Scientific Geoconference (SGEM2016)
9**. Niculescu, M., Grecu, F., Popescu, C.,** 2014 - *The corology, ecology and phytosociology of the sands plant communities and natural habitats in the sandy area from Dabuleni, Dolj county –Romania*, 14th SGEM GeoConference on Ecology, Economics, Education And Legislation, www.sgem.org, SGEM2014 Conference Proceedings, ISBN 978-619-7105-18-6/ISSN 1314-2704, June 19-25, 2014, Vol. 2, 697-702 pp http://www.sgem.org/sgemlib/spip.php?article4905&lang=en
DOI: 10.5593/SGEM2014/B52/S20.092
10. **Nikolova A., Vassilev A.**, 2011 – *A study on Tribulus terrestris L. anatomy and ecological adaptation*, Biotechnol. Biotechnol. Equipm. 25(2):2369–2372.
11. **Reshma, P.L., Lekshmi, V.S., Sankar, V., Raghu, K.G.**, 2015 – *Tribulus terrestris (Linn.) attenuates cellular alterations induced by ischemia in H9c2 cells via antioxidant potential*, Phytother. Res. 29(6):933–943.
12. **Samani, N.B., Jokar, A., Soveid, M., Heydari, M., Mosavat, S.H.**, 2016 – *Efficacy of Tribulus terrestris extract on the serum glucose and lipids of women with diabetes mellitus*, Iran. J. Med. Sci. 41(3):S5.

13. **Shahid, M., Riaz, M., Talpur, M.M., Pirzada, T.**, 2016 – *Phytopharmacology of Tribulus terrestris*, J. Biol. Regul. Homeost. Agents 30(3):785–788.
14. **Soleimanpour, S., Sedighinia, F.S., Safipour Afshar, A., Zarif, R., Ghazvini, K.,** 2015 – *Antibacterial activity of Tribulus terrestris and its synergistic effect with Capsella bursa-pastoris and Glycyrrhiza glabra against oral pathogens: an in-vitro study*, Avicenna J. Phytomed. 5(3):210–217.
15. **Toma, C., Rugină, R.**, 1998 – *Anatomia plantelor medicinale. Atlas*, Ed. Academiei Române, Bucureşti, 1998, 320 pag.
16. **Wang, Z.F., Wang, B.B., Zhao, Y., Wang, F.X., Sun, Y., Guo, R.J., Song, X.B., Xin, H.L., Sun, X.G.**, 2016 – *Furostanol and spirostanol saponins from Tribulus terrestris*, Molecules 21(4):429.
17. **Zhang, J., Wang, Z., Zhang, X., Jin, J., Yang, F.**, 2000 – *The descriptions, microscopic and TLC identification of Tribulus terrestris L.*, J. Chin. Med. Mater. 23(11):675–677.