ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXII, N 3, 7 SECTIO DDD 2009

Danylo Halytsky Lviv National Medical University, Department of Pharmacognosy and Botany

NATALIYA VOROBETS

Investigation on Tribulus terrestris L. for carotenoids and chlorophyll content

Badania zawartości karotenoidów i chlorofilu w *Tribulus terrestris* L.

Tribulus terrestris L. (in English, Pincture Vine), *Zigophyllaceae,* which is an annual creeping herb growing in sandy and rocky places occurs as a weed in the southern part of Ukraine and Bulgaria. It is spread in China, Japan, Korea, and India, the western part of Asia, South Europe, Africa and Australia. It has long been quite popular in the folk medicine of Oriental countries, India, China and Bulgaria as a medicinal plant in the treatment of sexual deficiency. Its extract demonstrates a wide range of biological activities. The active components of *T. terrestris* are steroid saponins of furostanol type, mainly protodioscin, protogracillin and their methoxy derivatives [6]. The most important and investigated action is stimulation of *libido sexualities* and spermatogenesis. There were also proved immunomodulating, antitumor, antiviral, hepatoprotective and antiurolithatic effects [2]. Another type of compounds from *T. terrestris* are flavonoids and tannins (rutin, astragalin, tribuloside, tritulosin and glycosides of kaempferol [Dinchev et al., 1982]; α-amino acids and its derivatives; some quantities of indol type alkaloids in the herb [5]. Rutin (10%) proved to be a good angiothensic and venotonic agent; alkaloids – sedative action. The extracts of *Tribulus* contain numerous not investigated compounds which can demonstrate the protective and antioxidative effect. For this reason our investigations on the phytochemical composition of *T. terrestris* are concentrated on characterization of carotenoids and chlorophylls.

MATERIAL AND METHODS

Tribulus terrestris L. was harvested in Dnipropetrovsk region of Ukraine and in Bulgaria (from the company Cd „Sharkovi I sie" – Sofia). Analytical methods were used for quantitative determination of pigments in the whole plant. *a* and *b* chlorophyll content and carotenoids were evaluated in the general extract without their previous division. Acetone was employed in 100 % pigment extracting. Optic density was defined in λ= 662; 644; 440.5 nm. The pigment concentration was estimated by Kholm-Vettstein formula [4]. The silufol-type plates, 15x15 cm served as the basis for the obtained pigment division. They were placed into a chromatographic camera, previously saturated with benzine, acetone, petroleum ether, hexane, in volumetrical correlations 10:10:3:10. The pigment zones were identified with Rf regard.

RESULTS

The results obtained with these methods indicate that plants of *Tribulus terrestris* species from both investigated regions contain chlorophylla *a* and *b* (Table 1). The concentration of total chlorophyll a and b was 1.88–2.20 ± 0.08 mg·g^{-1} dry weight.

Table 1. Chlorophyll concentration of *Tribulus terrestris* L. growing in Ukraine and Bulgaria (mg·g^{-1} dry weight)

Region of harvesting	Chlorophyll a	Chlorophyll b	Total chlorophyll (a+b)
Ukraine	0.59 ± 0.032	0.07 ± 0.005	0.65 ± 0.062
Bulgaria	0.78 ± 0.052	0.05 ± 0.002	0.83 ± 0.093

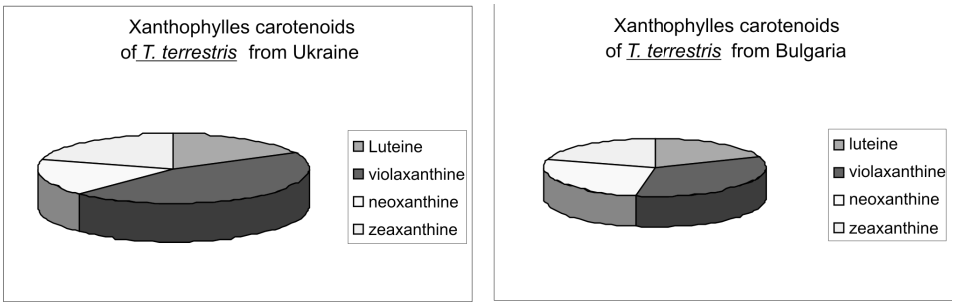

Fig. 1. Correlations between xanthophyll carotenoids of *Tribulus terrestris* L., %

Total carotenoids content was in the range of 0.21 ± 0.026 mg·g^{-1} dry weight in plants from Ukraine and in the range 021 ± 0.034 mg·g^{-1} dry weight of thhose growing in Bulgaria. Xanthophyll carotenoids, namely lutein, neoxanthin, zeaxanthine and violaxanthin were found in all investigated plants. A correlation between various groups is found in the figure. Of these xanthophylls, violaxanthine appears in the highest concentration, usually comprising between 33 and 45% of total xanthophylls.

In plants from Ukraine, there was more violaxanthine and a smaller amount of neoxanthine compared to plants from Bulgaria. Luteine and neoxanthine comprise a smaller proportion of the total xanthophylle pool.

The analyses showed the presence of β-carotene in all plants. Its amount was 10.01±1.22 μg·g^{-1} dry weigh and 47.2±1.98 μg·g^{-1} dry weigh in plants from Ukraine and Bulgaria, respectively.

DISCUSSION

The current state of knowledge of the functional role of chlorophylls and carotenoids in human organism suggests that they can protect from various diseases. Carotenoids and chlorophylls are in a group of antioxidants. They cannot synthesize in an animal's organism *de novo* and probably must receive from food or feed additives [8]. The functions of chlorophylls are mostly as healing wounds and ulcers, improvement of pancreatic and thyroid glands function, prevention of cell pathologies. Carotenoids are fat-soluble antioxidants that may protect polyunsaturated fatty acids, such as n-3 fatty acids from oxidation, and are potentially important in Alzheimer's disease prevention and treatment [9]. Persson et al. [7] found that those who have very low plasma levels of alpha-carotene and beta-carotene are at a higher risk of gastric cancer. Carotenoid zeaxanthin accumulates in the

human macula lutea and protects retinal cells from blue light damage [1]. Diets rich in lutein and zeaxanthin are moderately associated with a decreased prevalence of nuclear cataract in older womem [3]. Despite its low level in *T. terrestris*, an increased intake of this carotenoid can contribute to a decreased risk of age-related macular degeneration and nuclear cataract.

The value and level of the group of chemicals known as antioxidant that operate by reducing the concentration of harmful, stress-induced, reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical can be of use for the treatment of many various diseases. Recent investigations suggest that the sum of carotenoids and chlorophyll belongs to the factors that prevent chronic diseases. Further research is needed to confirm these possible associations.

REFERENCES

1. Bub A. et al.: Zeaxanthin is bioavailable from genetically modified zeaxanthin potatoes. Eur. J. Nutr., 47, 99, 2008.
2. Dinchev D. et al.: A short review of photochemical content and biological activity of *Tribulus terrestris* L.: Ninth International Symposium of Immunonology of reproduction. 2, 2000.
3. Moeller S. M. et al.: Associations between age-related nuclear cataract and lutein and zeaxanthin in the diet and serum in the carotenoids in the age-related eye disease study (CAREDS), an ancillary study of the women's health initiative. Arch. Ophthalmol., 126, 354, 2008.
4. Musiyenko M. M. et al.: Spectrophotometrychni metody v praktytsi phiziolohiyi, biokhimiyi ta ekollohiyi Roslyn. Fitosotsiotsentr, Kyiv 2001.
5. Obreshkova D. et al.: Alkaloids of *Tribulus terrestris* L. Pharmacia, XLVI, 19, 1999.
6. Obreshkova D. et al.: Comparative analytical investigation of *Tribulus terrestris* preparations. Pharmacia, XLV, 10, 1998.
7. Persson C. et al.: Plasma levels of carotenoids, retinol, and tocopherol and the risk of gastric cancer in Japan: a nested case-control study. Carcinogenesis, 2008.
8. Petit H. et al.: Ontogeny of carotenoid metabolism in the prawn *Penaeus japonicus* Bate (1888) (*Crustacea Penaeides*). A qualitative approach. Compar. Biochem. And Physiol. B, 99, 3, 667, 1991.
9. Wang W. et al.: Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity. J. Alzheimer Dis., 13, 31, 2008.

SUMMARY

A comparative investigation in respect to the qualitative and quantitative composition of carotenoids and chlorophylls from *Tribulus terrestris* L. wild growing in Ukraine and Bulgaria was conducted. There were no significant differences in the concentration of chlorophyll *a, b* and carotenoids such as luteine, zeaxanthine in *T. terrestris* from the investigated regions, though there were bigger amounts of violaxanthine and smaller amounts of neoxanthine in plants from Ukraine.

STRESZCZENIE

Przeprowadzono badania porównawcze składu jakościowego i ilościowego karotenoidów i chlorofilu w dziko rosnącym na terenie Ukrainy i Bułgarii *Tribulus terrestris L*. Nie stwierdzono istotnych różnic w stężeniu chlorofilu a, b i karotenoidów, takich jak luteina, zeaksantyna w *T. terrestris* z badanych regionów, jednakże rośliny pochodzące z Ukrainy zawierały większe ilości wiolaksantyny i mniejsze neoksantyny.