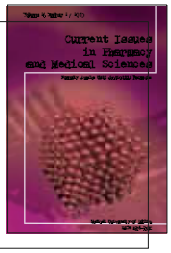
2013 © Curr. Issues Pharm. Med. Sci. Vol. 26, No. 2, Pages 215–220

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy



Sterols and triterpenoids in selected plant species of the family Cyperaceae

MARCIN CZOP[1], MAGDALENA JANYSZEK[2], ANNA OCH[3], MAREK OCH[3], ANNA BOGUCKA-KOCKA[3*]

[1]Clinical Genetics Department, Medical University of Lublin, Poland
[2]Department of Botany, University of Life Sciences, Poznań, Poland
[3]Chair and Department of Pharmaceutical Botany, Medical University of Lublin, Poland.

ABSTRACT

Triterpenes and sterol fractions were isolated from the herb *Scirpus maritimus* L. and *Carex Paradoxa* Willd. by typical extraction with petroleum ether. The fraction of sterols was investigated using Gas Chromatography/Mass Spectrometry (GC/MS) techniques. The occurrence of cholesterol, campesterol, stigmasterol, and β-sitosterol 5α-stigmast-7EN-3α-ol has been observed in the *Carex paradoxa* Willd. Their percentage in the herb has been determined by quantitative analysis. The presence of campesterol, stigmasterol and a mixture of sitosterols in the herb *Scirpus maritimus* L. was indicated and its percentage in the herb was determined. Thin Layer Chromatography (TLC) was carried out to identify triterpenoids contained in investigated plants. By comparison with the standards of lupeol and β-amyrine the presence of lupeol and an unknown compound in *Scirpus maritimus* Willd. has been indicated. In the separation of triterpenoids Column Chromatography (CC) was used. Melting point of an unknown compound was marked, but it was unidentified because of lack of appropriate standards.

Keywords: *Scirpus maritimus* L., *Carex Paradoxa* Willd., Sterols, Triterpenoids, GC-MS, TLC, CC

INTRODUCTION

Representatives of the order *Cyperales* are common plants and they are among quite invasive weeds, but they play a special role in the vegetation of temperate countries, and in cool mountain floras [5, 21, 18]. Mostly they grow in damp and open places such as the steppes and deserts [6]. In Poland, the family *Cyperaceae* includes more than 120 species, with the most numerous type of *Carex* covering almost 75% of all representatives of the order. Here it is represented by about 100 species of *Carex* [24]. Type *Carex* plants is concentrated in low and transitional mires and wet meadows and pastures. Sedges grow in forests or at the edges of water and alpine communities [23].

Carex paradoxa Willd. occurs mainly in fens and wet meadows, reeds; quite rarely in the lowlands, and it does not occur in the mountains [6].

Corresponding author
* Chair and Department of Pharmaceutical Botany,
1 Chodźki Str., 20-093 Lublin
e-mail: anna.kocka@umlub.pl

DOI: 10.12923/j.2084-980X/26.2/a.21

Plants of the genus *Carex* L. contain: oligostilbenes, alkaloids, flavonoids, phenolic acids, essential oils, fatty acids, saponins, tannins, waxes, resins, bitters, mineral salts, and starch [7-9, 14, 15, 18].

These plants are used in treating coughs, bacterial infections, metabolic disorders and inflammation. They show increasing perspiration and diuretic. Alcoholic extracts are used in bronchial catarrh, respiratory diseases, kidney and bladder diseases. They can be used externally in arthritic medical problems, eczema, alternatively in syphilis [17]. Due to the high content of silica, they are used as an aid for wounds and skin defects, in the treatment of lesions, mucous membranes and walls of blood vessels and prevent the formation of kidney stones [22].

Scirpus maritimus L. (*Cyperaceae*) occurs in temperate and cold climates. It grows in wet ditches and on the banks of the waters. In Poland it is spread mainly in the lowlands, it often occurs in the western part of the country and on the coast. It has low soil requirements and grows on sandy soils, loam and clay. It tolerates acidic soil, neutral and alkaline soils and salinity. It avoids shady places [1,3].

Scirpus maritimus L. root is used for its astringent and diuretic activity [2]. In Chinese traditional medicine it is used in the lack of menstruation, the treatment of postpartum pain, bloating and indigestion [27]. *Scirpus maritimus* L. has long been known as an edible plant because of high content of starch. It is eaten raw or cooked [4, 10, 11]. Seeds of this plant can also be eaten after cooking. They can be used as gruel [19]. Leaves of the plant are also used in weaving and basketry [19]. Seeds of *S. maritimus* L. show biological activity because of the presence of compounds from the group of stilbene: resveratrol, 3-hydroxyresveratrol, scirpusin A, scirpusin B and viniferin. Among them scirpusin B has the highest antileukemic activity. There are tannins in rhizome of the plant [12, 13]. Polysaccharides are also present in the plant [20].

Other species of the genus *Scirpus* contain carotenoids such as beta-carotene and lutein as well as sterol compounds: β-sitosterol and stigmasterol (*Scirpus silvaticus*), polyphenols, phenolic acids (*S. tuberosus, S. nodosus)*, essential oils (*S. americanus*), sugars, lipids, fiber (*S. grossus*). *Scirpus lacustris* provides a list of bitter extracts, including leucocyanidin and leucodelfinidin and catechin tannins. In the root, leucoantocyanides was detected [12, 13].

The aim of this study was isolation and analysis of chromatographic fractions of sterols and triterpenoids present in the herb *S. maritimus* L. and *Carex paradoxa Willd.*

MATERIALS AND METHODS

The raw material used for this study were herbs *S. maritimus* L. (295 g) and *C. paradoxa* Willd. (215 g) collected in the area of Poznań. Dried and crushed in accordance with the requirements of Polish Pharmacopoeia V (FP V).

Extraction and separation of sterols fraction

The material was extracted with petroleum ether in a Soxhlet apparatus for 60 hours. The solvent was evaporated under vacuum (40°C), in vacuum evaporators (Unipan-type-350) and water bath (Unipan-pro-365P). The concentrated extract was isolated and separated in a typical way of sterols and triterpenoids [16].

To isolate the fraction of sterols and triterpenoids, extracts from both plants were saponified with 10% ethanolic KOH solution. To each flask 100 mL of anhydrous ethanol and 20 g KOH (20% KOH) were added. In order to increase the boiling point 5 mL of toluene was added. Hydrolysis was carried out in a boiling water bath under reflux, keeping the mixture at boiling point for 9 h.

After hydrolysis, the ethanol was evaporated in a vacuum evaporator. Then about 200 mL water was added, the mixture was transferred to a separatory funnel and 10 fold was extracted with diethyl ether (50 mL). The unsaponifiable substances passed to the ether phase. The aqueous phase was discarded. The etheral phase was washed with water until neutral, in the presence of litmus paper (2-3 mL of water 6 fold). Ether extract was dried with sodium sulfate and concentrated in an evaporator to recive the fraction of inert compounds.

To the residue, after concentration of *Scirpus maritimus* L. herb extract and *Carex paradoxa* Willd. herb extract, 90° ethanol was added (70 mL and 150 mL respectively). In order to precipitate digitonides of sterols, to the flask with the extract of the herb *Scirpus maritimus* L. about 100 mL of 1% solution of digitonine in the 70° ethanol was added , and the flask containing the extract of – *Carex paradoxa* Willd. 200 mL of this solution were added. The prepared mixtures were heated for 1 hour in a water bath under reflux.

Sediments of digitonides precipitated were filtered and washed successively with 40 mL of anhydrous ethanol, 40 mL of diethyl ether (filtrates were left for further research). Then the sediment was washed with 50 mL of acetone, 50 mL of petroleum ether and 50 mL of chloroform (filtrate was discarded).

The received sediments of digitonides were dried at room temperature and then in a vacuum oven at 36°C to a constant weight.

The total sterol content in the studied raw materials was determined by the gravimetric method.

Digitonides of sterols. Sterols digitonides were phased in the process of acetylation for further analysis. Recently distilled acetic anhydride was added to sediments (15,7 mL *Scirpus maritimus* L. and 8 mL *Carex paradoxa* Willd.) and kept under reflux for 15 minutes at acetic anhydride boiling temperature (139°C). After cooling on ice, 50% ethanol was added (60.6 mL *Scirpus maritimus* L. and 28 mL *Carex paradoxa* Willd.), filtered and dried at room temperature. In the sediment, acetates of sterols remained, which were weighed and analyzed using Gas Chromatography/Mass Spectrometer (GC-MS).

Free sterols. In order to obtain free sterols hydrolysis of acetates was carried out. Sediments were added to 50% ethanolic KOH and heated in a boiling water bath for 2 hours. 100 mg *Scirpus maritimus* L. were added to 6 mL KOH and 50 mg of *Carex paradoxa* Willd. were added to 3 mL KOH. After cooling, water was added dropwise to the first turbidity. Free sterols were isolated from sediments, filtered and dried at room temperature. Sediments were weighed and then subjected to a crystallization of 96% ethanol. The resulting free sterols sediments were filtered, dried at room temperature, then weighed and analyzed using GC/MS.

Gas chromatography (*GC*). Gas chromatography was carried out using Carlo-Erba Instruments Chromatograph (Italy) type HRGC5300 Series Mega, equipped with the flame ionization detector FID and injector SSL.

The sterols were separated on the capillary column of 0.32 mm bore and 30 m length, packed with RTX-1 (0.25 μm size). The following optimum conditions of the analysis were stated: programming temperature of 200°C – 320°C (20 min isothermic), temperature of injector /detector 330°C, growth of temperature 6°C/min, flow-rate of carrier gas (N2) 1.5 mL/min. The sterols were identified on the basis of comparison of the retention times of the analyzed compounds with authentic standards.

Gas Chromatography/Mass Spectrometry (*GC/MS*). GC-MS analysis of sterol mixture was carried out using GC8000 FISONS INSTRUMENTS (Italy) equipped with MD800 mass detector, on-column injector and 30 m fused capillary column (0.32 mm) packed with RTX-1 (0.25μm size). The following optimum conditions of analysis were stated: programming temperature of 180°C – 320°C, growth of temperature 6 °C/min, flow-rate of carrier gas (He) 1.5 mL/min., EI+ , the source voltage 70 eV, ion source temperature 200°C. The sterols were identified on the basis of comparison of retention times and mass spectra of the analyzed compounds with authentic standards and literature data.

Analysis of triterpenoids

The filtrate obtained after the separation of sterols digitonids containing triterpene alcohols was concentrated in a vacuum evaporator. This was followed by crystallization from methanol to afford sediments: sediment of triterpenoids from *Scirpus maritimus* L. and sediment from *Carex paradoxa* Willd., which were filtered and dried to constant weight. The precipitate was dissolved in chloroform and the triterpenoids mixture was investigated by TLC and CC.

Thin layer chromatography (*TLC*). TLC was carried out on plates coated with silica gel (*DC-60 10x20 Fertigplatten Kiselgel – Merck, Germany*), impregnated with 10% silver nitrate and activated in an oven at 110°C for 1 h. The plates were developed in horizontal chambers of type DS in systems: toluene: ethanol: ammonium (95: 5: 5); chloroform: methanol (3: 7); chloroform: petroleum ether: acetic acid (75: 25: 0.5); benzene: ethyl acetate (85:15); benzene: petroleum ether (2: 3); chloroform: diethyl ether (19:1). The resulting chromatograms were analyzed after spraying reagent aniseed and 7.5% sulfuric acid in ethanol.

The spots were heated at 110°C for 10 minutes to make them visible. Triterpenoids were identified on the basis of comparison of the retention factors of the analyzed compounds with authentic standards (lupeol and β-amyrine).

Column chromatography (*CC*). Column chromatography was carried out using column 60 cm long and 2.5 cm in diameter, which was filled by sedimentation with silica gel- kiesegel MN-60, 0.05-0.2 mm/230-400 mesh ASTM (Merck, Germany). A fraction of triterpenoids was dispersed in chloroform. Chloroform: diethyl ether (19:1) was a developing phase of the system.

There were collected 40 fractions of 10 mL volume. Chromatographic fractions were monitored by TLC on silica gel in the same developing system. The plates were developed using the reagent anise and then warmed in the 110°C by 10 min. The presence of triterpene stains was found in the first 10 fractions. The fractions with the same chromatography image: from 3-6 a mixture of compounds, 7-10 single compound was combined and concentrated in a vacuum evaporator. Compounds were crystallized from ethanol and the uncorrected melting point was measured in the Boethius apparatus.

RESULTS AND DISCUSSION

As a results of the research there were 0.8980 g sterols digitonides (Tab. 1) obtained from 295 g of the herb *Scirpus maritimus* L., dried to constant weight. By multiplying this value by factor 0.2533 (average molecular weight ratio of β-sitosterol, stigmasterol and ergosterol to the average weight of digitonides of these sterols), it was calculated that 0.2275 g of sterols was in 295 g of raw material. Mass of sterols digitonides isolated from the herb *Carex paradoxa* Willd. was 0.4100 g (Tab. 1). Proceeding analogously, we calculated that the 215 g of herb *Carex paradoxa* Willd. contained 0.1038 g sterols. Converting this to a percentage of the total amount of free sterols in the raw materials we obtained the following values: sterol content in the herb *Scirpus maritimus* L. is 0.0771%; sterol content in the herb *Carex paradoxa* Willd. is 0.0481%.

Table 1. Weights of sediments free sterols before and after crystallization obtained from the herbs *Scirpus maritimus* L. and *Carex Paradoxa* Willd

	Scirpus maritimus L.	*Carex paradoxa* Willd.
Weight of sterol digitonids	0.898 g	0.410 g
Weight of sterol acetates	0.2362 g	0.0914 g
Weight of free sterols before crystallization	0.0741 g	0.0441 g
Weight of free sterols after crystallization	0.0253 g	0.0254 g

Sterol digitonides were acetylated. Mass of sterol acetates isolated from the herb *Scirpus maritimus* L. was 0.2362 g and from the herb *Carex paradoxa* Willd. 0.0914 g (Tab.1).

Weights of sediments free sterols before and after crystallization are given in Table 1. In the sterol fraction obtained from the herb *Scirpus maritimus* L. three sterol compounds were identified: campesterol (Fig. 3), stigmasterol (Fig. 4), sitosterol (most probably a mixture of β-sitosterol (Fig.5) and γ–sitosterol (Fig. 6)) (Tab. 2). However, in the fraction obtained from the herb *Carex paradoxa* Willd. five sterol compounds were identified: campesterol (Fig. 3), stigmasterol (Fig. 4), β-sitosterol

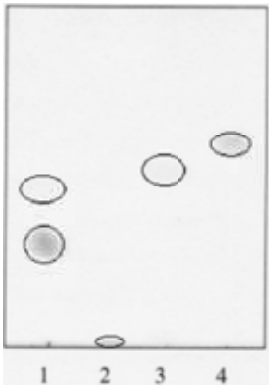


Fig. 1. TLC chromatogram of fraction of triterpenoids from *Scirpus maritimus* L. and *Carex paradoxa* Willd. in comparison with standards of lupeol and ß-amyrine in a system : chloroform : diethyl ether(19:1) (1 – Fraction of triterpenoids from *Scirpus maritimus* L.; 2 – Fraction of triterpenoids from *Carex paradoxa* Willd.; 3 – lupeol; 4 – ß-amyrine)

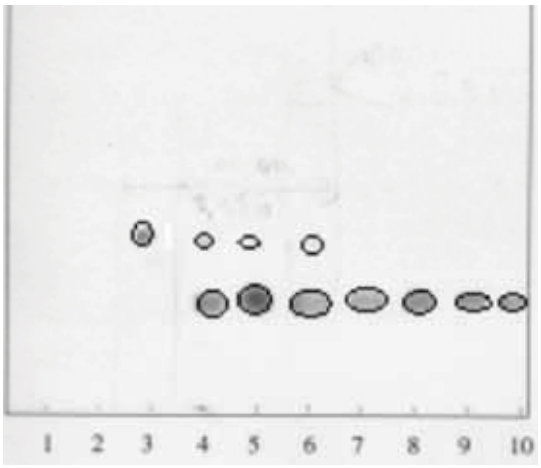


Fig. 2. TLC chromatogram of fraction 1-10 received from column chromatography of triterpenoids from *Scirpus maritimus* L. in a system : chloroform : diethyl ether(19:1)

THE STRUCTURES OF IDENTIFIED COMPOUNDS

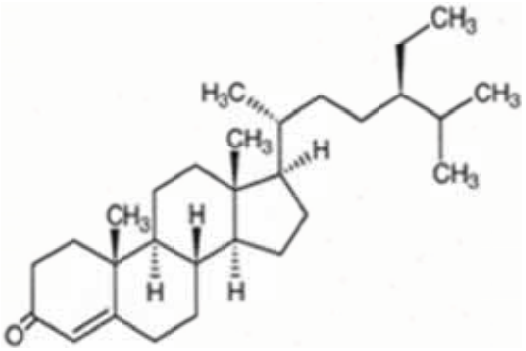


Fig. 3. Campesterol

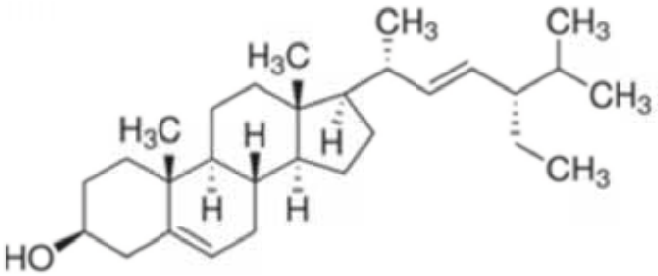


Fig. 4. Stigmasterol

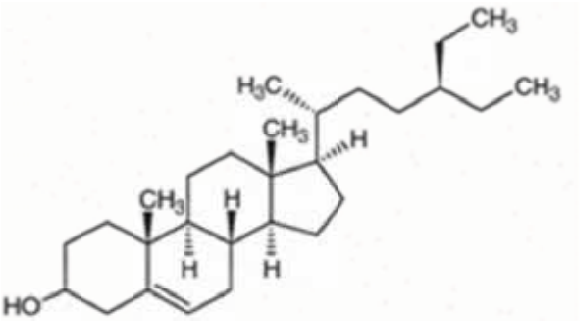


Fig. 5. ß-Sitosterol

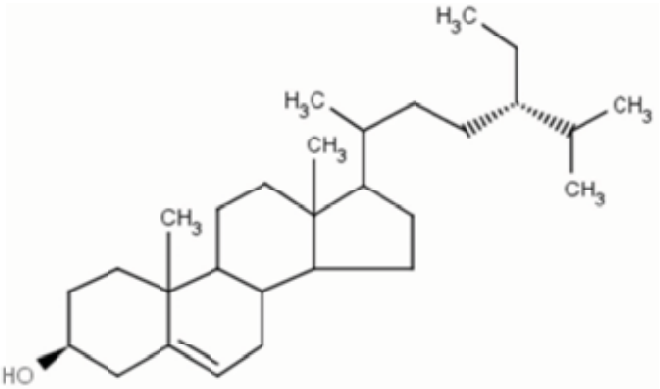


Fig. 6. γ–Sitosterol

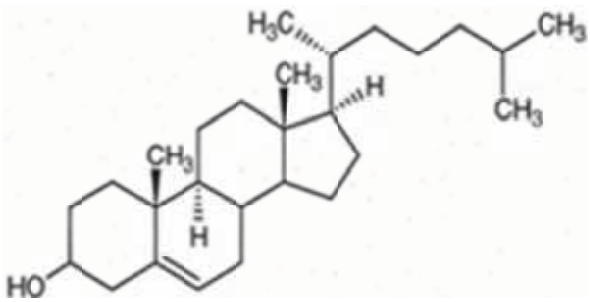


Fig. 7. Cholesterol

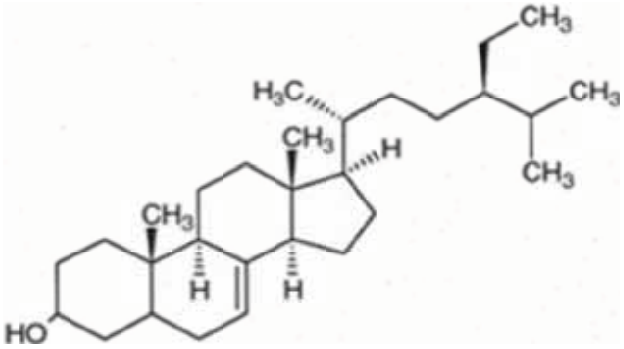


Fig. 8. Stigmast -7-en-3-ol

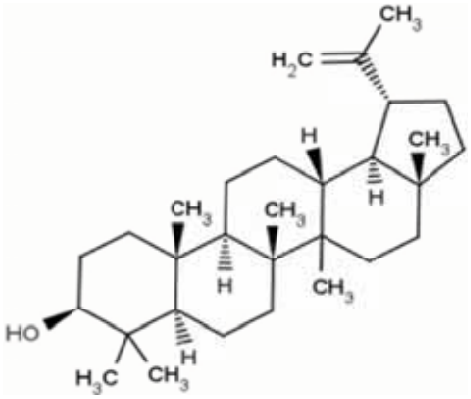


Fig. 9. Lupeol

(Fig. 5), cholesterol (Fig. 7) and 5α-stigmast-7EN-3α-ol (Fig. 8) (Tab. 2). β-sitosterol has been also found in *Cyperus rotundus* Linn., plant from *Cyperaceae* family [25].

In Tables 3 and 4 retention times, molecular ion mass and the percentage of the fraction are given. Sterol fractions were analyzed from both species containing a fraction of the aliphatic, saturated and unsaturated sterols, particularly rich and diverse in the herb *Scirpus maritimus* L. In the examined sterol fractions obtained from the herb *Scirpus maritimus* L. cholesterol and 5α-stigmast-7EN-3α-ol were not found, but an interesting and rare in the vegetable mixture of β – and γ – sitosterol were identified. The dominant compound in *Carex paradoxa* L. is β-sitosterol and in *Scirpus maritimus* Willd. mixture of β – and γ – sitosterol.

Table 2. The results of GC fractions of sterol acetates herbs *Scirpus maritimus* L. and *Carex paradoxa* Willd. (+ present; – missing)

P.N.	Chemical name of the compound	*Scirpus maritimus* L.	*Carex paradoxa* Willd.
1	Campesterol acetate	+	+
2	ß-sitosterol acetate	+	+
3	γ-sitosterol acetate	+	–
4	Stigmasterol acetate	+	+
5	Cholesterol acetate	–	+
6	5α-stigmast-7en-3α-yl acetate	–	+

Table 3. The results of GC fraction of free sterols *Carex paradoxa* Willd. herb (RT – retention time; M+ – molecular ion mass)

P.N.	Chemical name of the sterol	RT (min)	M+(m/e)	% of sterol fraction
1	cholesterol	20.65	386	3.5910
2	campesterol	22.60	400	11.5483
3	stigmasterol	23.16	412	14.5328
4	ß-sitosterol	24.25	414	58.0535
5	5α-stigmast-7en-3α-ol	24.83	414	0.5115

Table 4. The results of GC fractions of free sterols *Scirpus maritimus* L. herb (RT – retention time; M+ – molecular ion mass)

P.N.	Chemical name of the sterol	RT (min)	M+(m/e)	% of sterol fraction
1	Campesterol	22.51	400	11.3256
2	Stigmasterol	23.01	412	4.2601
3	Sitosterol (mixture ß- and γ)	24.27	414	70.3436

The fraction of triterpenoids was isolated from filtrate obtained after the separation of sterols digitonids: *Scirpus maritimus* L. (0.3278 g) and *Carex paradoxa* Willd. (0.0560 g). It was separated by CC and analyzed by TLC (Fig. 1, 2). The occurrence of two triterpenoids has been observed in this plant: lupeol and unidentified compound A. Compound A was crystallized from ethanol and the uncorrected melting point (MP) was measured in the Boethius apparatus (Compound A MP = 267-269°C). The presence of three triterpenoids has also been reported in *Cyperus scariosus* R. Br.: stigmasta-5,24(28)-diene-3-β-O-α-L-rhamnopyranosyl-O-ß-D-arabino-pyranoside, glycoside leptosidin 6-O-β-D-glucopyranosyl-O-α-2-rhamnopyranoside and 3-diacetoxy-19-hydroxy-urs-12-ene-24-O-β-D-xylopyranoside [26].

The presence of lupeol (Compound B) (Fig. 9) in *Scirpus maritimus* Willd. was identified by comparison with authentic lupeol standard and literature data. The presence of lupeol was evidenced by TLC analysis on comparison of the colors and positions of spots of this compound with those of authentic lupeol. Compound A has not been identified due to lack of appropriate standard.

REFERENCES:

1. Chittendon F.: *RHS Dictionary of Plants Plus Suplement.* Oxford University Press, 1951.
2. Chopra R.N., Nayar. S.L., Chopra. I.C.: *Glossary of Indian Medicinal Plants (Including the Suplement)*. Council of Scientific and Industrial Research, New Delhi, 1986.
3. Tootin C., Warburg.: *Flora of the British Isles.* Cambridge University Press, 1962.
4. Cribb A.B., Cribb I.W.: *Wild Food in Australia.* Fontana, 1976.
5. Escudero, M., Hipp, A., Waterway, M., Valente, L.: Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (Carex, Cyperaceae). *Molecular Phylogenetics and Evolution,* 63, 650–655, 2012.
6. Fiorentino A., D'Abrosca B., Pacifico S., Izzo A., Letizia M., Esposito A., Monaco P.: Potential allelopatic effects of stilbenoids and flavonoids from leaves of Carex distachya Desf. *Biochemical Systematics and Ecology,* 36, 691–698, 2008.
7. Fiorentino, D'Abrosca B., Pacifico S., Cefarelli G., Uzzo P., Monaco P.: Natural dibenzoxazepinones from leaves of Carex distachya: Structural elucidation and radical scavenging activity. *Bioorganic & Medicinal Chemistry Letters,* 17, 636–639, 2007.
8. Fiorentino A., D'Abrosca B., PacificoS., Natale A., Monaco P.: Structures of bioactive carexanes from the roots of Carex distachya Desf. *Phytochemistry,* 67, 971–977, 2006.
9. Fiorentino A., D'Abrosca B., Pacifico S., Iacovino R., Mastellone C., Di Blasiob B., Distachyasin M.P.: A new antioxidant metabolite from the leaves of *Carex distachya*. *Bioorganic & Medicinal Chemistry Letters,* 16, 6096–6101, 2006.
10. Harris B.C.: Eat the Weeds. *Pivot Health,* 1973.
11. Hederick U.P.: *Sturtevant's Edible Plants of the World.* Dover Publications, 1972.
12. Hegnauer R.: *Chemotaxonomie der Planzen.* Bd II. Basel und Stuttgart. 1963.
13. Hegnauer R.: *Chemotaxonomie der Planzen.* Bd IX. Basel Boston Berlin, 1990.
14. Ito T., Endo H., Shinohara H., Oyama M., Akao Z., Iinuma M.: Occurrence of stilbene oligomers in Cyperus rhizomes. *Phytotherapy*, 83, 1420–1429, 2012.

15. Ito T., Endo H., Oyama M., Iinuma M.: Novel isolation of stilbenoids with enantiomeric and meso forms from a cyperus rhizome. *Phytochemistry Letters,* 5, 267–270, 2012.
16. Jerzmanowska Z.: *Substancje roślinne. Metody wyodrębniania*. PWN Warszawa, 1967.
17. Jędrzejko K., Klama H., Żarnowiec I.: *Zarys wiedzy o roślinach leczniczych.* Śląska Akademia Medyczna, *Katowice,* 1997.
18. Li L., Henry G.E., Seeram N.P.: Identification and Bioactivities of Resveratrol Oligomers and Flavonoids from Carex folliculata Seeds. *J. Agric. Food Chem.,* 57, 7282–7287, 2009.
19. Moerman D.: *Native Americna Ethnobotany*. Timber Press. Oregon., 1998.
20. Moon Ch.K., Park K.S., Lee S.H., Yoon Y.P.: Antitumor activities of several phytopolysaccharides. *Arch. Pharmacal Res.*, 8(1), 42-4, 1985.
21. Reznicek A.A.:. Evolution in sedges (Carex, Cyperaceae). *Can. J. Bot.,* 68, 1409–1432, 1990.
22. Samochowiec L.: *Kompendium ziołolecznictwa*. Wydawnictwo medyczne Urban&Partner. Wrocław, 2002.
23. Stachak A.: *Botanika dla zootechników*. PWN. Warszawa, 1984.
24. Szweykowska A., Szweykowski J.: *Botanika*. Tom 2. Systematyka. PWN Warszawa, 1995.
25. Sener B., Bingol F.: Screening of natural sources for antiinflammatory activity. *Int. J. Crude Drug Res.* 26, 4, 197-207, 1988.
26. Sahu S., Singh J., Kumar S.: New Xylopyranoside From The Rhizomes Of Cyperus Scariosus. *International Journal of Chemical Engineering and Applications,* 1, 2, 2010.
27. Yeung, Him-Che.: *Handbook of Chinese Herbs and Formulas.* Institute of Chinese Medicine, Los Angeles, 1985.