Pharmacognosy Communications
Volume 2 | Issue 3 | Jul-Sep 2012
www.phcogcommn.org

Research Article

Study of the composition of the goutweed flowers essential oil, its renal effects and influence on uric acid exchange

O.O. Koyro,[1]* O.V. Tovchiga,[1] S. I. Stepanova,[2] S. Yu. Shtrygol'[1]

[1]Department of Pharmacology, National University of Pharmacy, 61002, Pushkinskaya Street 53, Kharkiv, Ukraine. [2]Department of Chemistry of Natural Compounds, National University of Pharmacy, 61002, Pushkinskaya Street 53, Kharkiv, Ukraine.

ABSTRACT: The composition of the goutweed flower essential oil from Kharkiv region (Ukraine) was studied using gas chromatography-mass spectrometry. Sesquiterpenes have been found to be the main group of the essential oil. The principal compounds contained in the oil were β-farnesene (43.94%), α-bergamoten (15.32%), (E, E)-α-farnesene (8.84%) and 1,5,9,9-tetramethyl-1,4,7-cycloundecatrien (5.51%). Diuretic and uricosuric activity of the goutweed flower essential oil in a dose of 1 ml/kg has been determined for the first time. It has been shown that the single dose increases creatinine, urea and uric excretion with the urine volume being unchanged. In the course of administration, a diuretic effect appears, creatinine excretion returns to the initial state while urea and uric acid excretion decreases. No significant differences between the effects of the aforesaid essential oil and the reference drug olimetin are observed. Thus, the essential oil may be involved in the diuretic and uricosuric activity of goutweed.

KEY WORDS: *Aegopodium podagraria* L., goutweed, diuretic agents, essential oil, uricosuric agents, uric acid

INTRODUCTION

Goutweed (Aegopodium podagraria L.) is a perennial herb of the carrot family (Apiaceae). It is indigenous to Europe, Western and Eastern Siberia, the Caucasus, the Sayans, Kazakhstan and Central Asia mountainous regions and has been naturalized in North America.

The interest in this plant has increased lately. The goutweed chemical composition and pharmacological activity have been studied. Hydroxycinnamic acids, flavonoids, coumarins, poliatsetilenes and micro- and macroelements have been determined in the goutweed.[1-4] The rich goutweed chemical composition explains a wide range of its pharmacological activity. It has been established that aqueous extract and the tincture (prepared with 70% ethanol) of the goutweed aerial part improve kidney excretory function, and have a positive influence on uric acid exchange. A nephroprotective action of the extract has been verified. It prevents lethality and histostructure kidney changes, normalizes the kidney concentration function, and reduces proteinuria and hyperazotemia.[5] The goutweed extract has hepatoprotective action in the carbon tetrachloride induced hepatitis.[6] The extract prevents lethality, reduces serum aminotransferases levels and normalizes protein level.[6]

In order to study the role of some groups of bioactive substances in the goutweed complex preparations pharmacological activity, we have investigated the goutweed essential oil chemical composition, renal effects and its influence on uric acid exchange. The goutweed essential oil composition varies depending on the growth stage and the harvesting and plant parts location.[7-9] All these factors encourage a deep insight into plant population habitats.

MATERIALS AND METHODS

Goutweed flowers were collected from natural population in Kharkiv region (Ukraine) in June 2007. Voucher specimens of the species were confirmed at the Departments of Botany

*Correspondence:
O.O. Koyro
Department of Chemistry of Natural Compounds,
National University of Pharmacy,
61002, Pushkinskaya Street 53,
Kharkiv, Ukraine.
Email: olgaokoyro@mail.ru
DOI: 10.5530/pc.2012.3.10

and Chemistry of Natural Compounds (National University of Pharmacy).

The air-dry plant material (100 g) was hydrodistilled by using 1500 ml of water for 3 hours in a Clevenger-type apparatus. The essential oil composition was established by gas chromatography-mass spectrometry (GC/MS) analyses. The analyses were carried out with the help of an Agilent-6980-5973N system. A split ratio 25:1 and fused-silica HP-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm) were used. The temperature program was from 60 to 240 °C at a rate of 3 °C/min, Helium as carrier gas (constant flow) was used at a pressure 65 kPa. Mass spectra were recorded at 70 eV. Mass range was from 40 to 400 m/z.

The identification of the essential oil components were carried out by comparison of their mass spectra with those of 'Flavor 2' and 'NIST 05A' libraries. The quantitative content of each essential oil component was calculated by the ratio of chromatographic peak areas which corresponded to the substance and standard.[10]

The study of the pharmacological activity of the goutweed flowers essential oil was conducted on healthy male albino mice. Adult noninbred mice (body weight 20-25 grams) were obtained from the Central Research Laboratorium of the National University of Pharmacy, Ukraine. All the experimental protocols were approved and in accordance with "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes." Mice were housed in a well-ventilated animal room under a controlled temperature and relative humidity, on a 12 h light/dark cycle. Food and water were supplied *ad libitum*. The mice were divided into two groups: group I received the goutweed flower essential oil at a dose of 1 mg/kg body weight and group II received olimetin ('Nizpharm,' Russian Federation) at a dose of 1 mg/kg and served as control. The preparations were given orally once a day for three days.

The animals were preliminarily adapted to the conditions of the experiment. Drinking water was given orally (5% of body weight) and, after forced diuresis, urine was collected for 2 h.[11] The creatinine urine concentration was measured using Jaffe reaction,[12] the urea concentration-diacetyl monooxime method,[13] the uric acid concentration-phosphotungstic reagent,[14] the excretion in all three cases being calculated. Intergroup comparisons were performed by using Student's t-test and intragroup comparisons were performed by using Wilcoxon's signed-rank test.

RESULTS AND DISCUSSION

Investigation of the goutweed flower essential oil chemical composition

The output of the goutweed flower essential oil (EF) equalled 0.22% of the dried flowers weight. The essential oil was oily to the touch with a light yellow color, characteristic pleasant odor and burning taste and a density less than that of water. The quantity of the compounds identified in the EF was 36. The essential oil composition is summarised in Table 1.

The main compounds of EF were *β*-farnesene (43.94%), α-bergamoten (15.32%), *(E, E)*-α-farnesene (8.84%) and 1,5,9,9-tetramethyl-1,4,7-cycloundecatrien (5.51%). Other

Table 1: Composition of goutweed flower essential oil

Compound	Retention time, min	Concentration, %	Compound	Retention time, min	Concentration, %
n-Hexanal	2.862	0.04	*cis*-Jasmone	23.188	0.16
Heptanal	4.429	0.24	*β*-Caryophyllene	24.049	1.45
α-Phellandrene	5.024	0.07	*β*-Cubebene	26.693	3.05
α-Pinene	5.201	0.1	*(+)*-Valencene	24.873	0.13
β-Pinene	6.316	0.2	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	25.506	5.51
6-Methyl-5-hepten-2-one	6.548	0.2	*(E)-β*-Farnesene	25.776	43.94
n-Octanal	7.021	1.52	*γ*-Muurolene	26.515	1.35
p-Cymene	7.739	0.59	*α*-Selinene	26.895	0.63
Limonene	7.874	0.8	*α*-Bergamotene	27.347	15.32
(Z)-β-Ocimene	8.165	0.18	*α*- Muurolene	27.503	0.36
γ-Terpinene	8.909	0.29	*(E,E)-α*-Farnesene	27.883	8.98
n-Nonanon	10.124	0.48	*α*-Amorphene	28.061	0.58
Linalool	10.424	0.07	*δ*-Cadinene	28.466	1.4
n-Nonanal	10.597	0.42	*cis-α*-bisabolene	29.251	0.12
p-Acetanisole	17.860	0.12	Spathulenol	30.679	3.26
α-Cubebene	21.060	0.08	Caryophyllene oxide	30.873	1.51
α-Copaene	22.187	0.27	Humulene epoxide II	31.886	2.01
β-Elemene	22.909	0.44	*γ*-Himachalene	33.651	0.35

Table 2: The influence of goutweed flower essential oil and olimetin on excretory kidney function

Parameter	Goutweed flowers essential oil, 1 mg/kg			Olimetin, 1 mg/kg		
	Initial state	Single dosing	After 3 days	Initial state	Single dosing	After 3 days
Diuresis, ml/10 g for 2 h	0.45 ± 0.04	0.43 ± 0.06 (–4%)	0.60 ± 0.03* (+33%)	0.53 ± 0.05	0.53 ± 0.06 (± 0%)	0.64 ± 0.05* (+20%)
Creatinine excretion, μmol/10g for 2 h	0.45 ± 0.07	0.60 ± 0.12* (+33%)	0.39 ± 0.04 (–13%)	0.44 ± 0.05	0.68 ± 0.21* (+54%)	0.50 ± 0.07* (+14%)
Uric acid excretion, μmol/10 g for 2 h	0.30 ± 0.05	0.38 ± 0.08* (+27%)	0.22 ± 0.04* (–27%)	0.23 ± 0.03	0.31 ± 0.08* (+35%)	0.26 ± 0.05* (+13%)
Urea excretion, mmol/10 g for 2 h	62.2 ± 9.6	73.8 ± 16.7 (+19%)	41.1 ± 7.6* (–34%)	49.2 ± 6.5	71.3 ± 16.7* (+44%)	46.3 ± 7.7* (–6%)

*significant differences according to the initial state; in parentheses are changes relative to the initial state. n = 7-9.

compounds were found to be present in smaller quantities (<4%). The content of the identified compounds corresponded to 96.22% of the total oil weight. Sesquiterpenes were found to be the main class of constituents of the essential oil. Their content in the EF amounted to 90.7%. The monoterpenes of the goutweed flowers were present in relatively low levels only (6.8%).

Study of the goutweed flower essential oil pharmacological activity

The express-method revealed that goutweed EF influenced renal function in test mice at relatively low doses. This supports to the known data on the "dose–effect" dependence for essential oils: the diuretic action of these substances is often not increased with the dose augmentation.[11,15,16] The dose of 1 mg/kg of goutweed EF had been chosen for the further investigation on the basis of previous studies. The capability to demonstrate diuretical properties in such doses can be regarded as an advantage of goutweed EF (for example, the Satureja montana subsp. montana essential oil has increased diuresis in much higher dosage 5 ml/100 g of 0.1% solution that corresponds to 50 mg/kg).[16]

As shown in Table 2, a single goutweed EF dose did not substantially change the diuresis in the test group compared to the control group. The significant increase in creatinine excretion (33%) following a single EF dose is the indicator of the glomerular filtration rate augmentation, with the urine volume being unchanged.[17] This can be obviously associated with a compensatory tubular reabsorption increase. However, after a single injection of the goutweed EF, the uric acid excretion was significantly higher (by 27%). In addition, there was a non-significant tendency for the urea excretion to augment (19%) following treatment with a single EF dose.

In the course of administration, a mild diuretic effect had appeared which was clearly associated with the inhibition of tubular reabsorption (as the creatinine excretion had not risen). Furthermore, the urea excretion had decreased and the uricosuric effect had disappeared following 3 days of EF treatment. The observed decrease in creatinine, uric acid and urea following 3 days of treatment may have been caused by their previous excretion enhancement after the first dose. Future studies should refer to the influence of goutweed EF on blood concentration of creatinine, uric acid and urea. As goutweed aqueous extract and a tincture exert hypoazotemic effect,[5] such studies are of great significance to understanding the plant metabolic effects.

No significant differences between the effects of the goutweed EF and the reference drug were observed both in a single goutweed EF dose and in course administration. Thus, the EF may be involved in the goutweed pharmacological effects, particularly in diuretic and uricosuric activity.

CONCLUSIONS

Using the gas chromatography-mass spectrometry method, it has been determined that the goutweed flowers essential oil wildly growing in the Kharkiv region (Ukraine) is made up of 36 compounds. Sesquiterpenes have been found to be the main group of the essential oil.

Diuretic and uricosuric activity of the goutweed flower essential oil has been determined for the first time. The goutweed flower essential oil may participate in the implementation of the pharmacological effects of goutweed.

REFERENCES

1. Christensen LP, Brandt K. Bioactive polyacetylenes food plants of the Apiaceae family: occurrence, bioactivity and analysis. J Pharm Biomed Anal. 2006; 41:683-693.
2. Koyro OO, Stepanova SI, Shtrygol' S Yu. Investigation of mineral composition of leaves and extract of goutweed. Medical Chemistry [in Ukrainian]. 2009; 2:116-119.
3. Koyro OO, Stepanova SI, Shtrygol' S Yu. Quantitative determination of the hydroxycinnamic acids amount in the goutweed raw material. Ukrainian Journal of Clinical and Laboratory Medicine [in Ukrainian]. 2009; 4(2):52-55.
4. Shtrygol' S Yu, Stepanova S I, Tovchiga OV, Koyro OO. Goutweed (*Aegopodium podagraria* L.). The Prospects of Application in Medicine. Provizor [in Russian]. 2008; 7:50-53. Available from: //www.provisor.com.ua/archive/2008/N07/htr_ls78.php?part_code=68&art_code=6511

5. Tovchiga OV. The investigation of the goutweed (*Aegopodium podagraria* L.) diuretic, nephroprotective and hypouricemic action as the basis for the drug development–The manuscript. Kharkiv; 2009; 21. Available from: // www.i-med.tv/index.php?option=com_content&view=article&id=23&Itemid=8&lang=en&it_id=1134&page=114
6. Koyro OO, Tovchiga OV, Shtrygol' S Yu. Experimental substantiation of the gout weed extract application in associated liver and kidneys toxic affection. Ukrainian Biopharmaceutical Journal [in Ukrainian]. 2011; 2(13):24-28.
7. Kapetanosa C, Karioti A, Bojovic S. et al. Chemical and principal-component analyses of the essential oils of *Apioideae taxa* (apiaceae) from Central Balkan. Chemistry and Biodiversity. 2008; 5:101-119.
8. Orav A, Viitak A, Vaher M. Identification of bioactive compounds in the leaves and stems of *Aegopodium podagraria* by various analytical techniques. Procedia Chemistry. 2010; 2:152-160.
9. Paramonov EA, Khalilova AZ, Odinokov VN, Khalilov LM. Identification and biological activity of volatile organic compounds isolated from plants and insects III. Chromatography-mass spectrometry of volatile compounds of *Aegopodium podagraria*. Chemistry of Natural Compounds. 2000; 36(6):584-586.
10. Davides NV. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J Chromatogr. 1990; 503(1):1-24.
11. Berkhin E.B., Diuretics [in Russian]. Moscow. Medicine. 1967; 156.
12. Bonsnes RW, Taussky HA. On the colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem. 1945; 158:581-591.
13. Evans RT. Manual and automated methods for measuring urea based on a modification of its reaction with diacetyl monoxime and thiosemicarbazide. J Clin Pathol. 1968;21:527-529.
14. Eichhorn F, Zelmanowski S, Lew E. Improvement of the uric acid determination by the carbonate method for serum and urine. J Clin Pathol. 1961; 14:450-452.
15. Haloui M, Louedec L, Michel JB, Lyoussi B. Experimental diuretic effects of Rosmarinus officinalis and Centaurium erythraea. J Ethnopharmacol. 2000; 71:465-472.
16. Stanic G, Samaržija I. Diuretic activity of Satureja montana subsp. Montana extracts and oil in rats. Phytother Res. 1993; 7:363-366.
17. Johnson LR. Essential medical physiology. San Diego. Academic Press. 2004; 1008.