ISSN 0974-4169(Print)
0974-4150(Online)

www.ajrconline.org

RESEARCH ARTICLE

Herbal tea for the treatment of Urinary diseases as Potent diuretic and Anti-inflammatory agent

Kateryna Taran, Ayaou Abderrahim, Vera Kravchenko, Olena Novosel, Svitlana Taran
National University of Pharmacy, Kharkiv, Ukraine
*Corresponding Author E-mail: **kate.taran@gmail.com, ayaou@bluewin.ch, Kvn5135@gmail.com, lenanovosel1@ukr.net, svitlana.grig@gmail.com**

ABSTRACT:
The herbal tea of previously patented composition for the treatment of urinary diseases containing twelve plants (St. John's wort, Wild pansy, Horsetail field, Common heather, Elecampane *i.a.*) was analyzed for the qualitative composition and quantitative content of phenolic compounds. The diuretic and anti-inflammatory activity of herbal tea thick extract was assessed. The determination of phenolic compounds was carried out by high performance liquid chromatography using standards of phenolic acids and flavonoids. Diuretic activity was checked by the method of *Berkhin* for the dosage in the range from 100 to 700mg/kg. Level of urination was assessed during 1, 2, and 3 hours of experiment. Edema was induced with 1% carrageenan. The dosage of oral treatment was from 100 to 250mg/kg. Paw volume was checked at time equal to 1, 2, 3, 4, and 5 hours. Several groups of phenolic compounds were detected, and six substances were identified individually. The total content of phenolic compounds was 23.8mg/g, with the content of flavonoids 10.4mg/g and chlorogenic acid 2.7mg/g. The herbal tea showed diuretic activity of 100% growth at the dosage of 200mg/kg. Further increase of dosage led to antidiuretic activity. From the dosage of 500mg/kg we observed the diuretic activity again, which was the highest for the dosage of 700mg/kg showing 271% growth in relation to the control group. The anti-inflammatory activity was the highest at the dosage of 200 and 250mg/kg and was equal to 25% in relation to the control group.

KEYWORDS: Herbal tea; phenolic compounds; diuretic; anti-inflammation.

1. INTRODUCTION:
Phytotherapy plays a big role in the treatment of diseases of urinary system. The herbal medications contribute to the improvement of the functional state of kidneys and urinary tract. The pharmaceutical market of Ukraine suggests a number of plant medications for the treatment of the urinary system diseases. Available herbal medications contain plant extracts or grinded plant material in tea bags.

Received on 18.02.2020 Modified on 08.03.2020
Accepted on 28.03.2020
Asian J. Research Chem. 2020; 13(3):175-179.
DOI: 10.5958/0974-4150.2020.00034.6

Canephron tablets and oral drops contain Centaury herb, Lovage root, and Rosemary leaves[1]. *Nephrophyt* herbal tea in the form of tea bags contains twelve medicinal plants such as Elder flowers, Plantain leaf, Common knotgrass herb, Horsetail field shoots, Shepherd's purse herb, Corn silk, Dandelion root, Burdock root, Bearberry leaves, Peppermint, Chamomile flowers, and Beggar ticks herb[1]. *Urolesan* in the dosage form of capsules, drops or syrup contains Fir oil, Peppermint oil, Wild carrot fruits liquid extract, Hop cones liquid extract, and Pot marjoram herb liquid extract[1,2]. U*rocholum* drops contain the water-alcohol extract from Wild carrot fruits, Orthosiphon leaves, Knotgrass herb, Corn silk, Elder flowers, Horsetail field shoots, Hop cones, Birch buds, St. John's wort herb, and Peppermint[1]. These plant

medications are prescribed for the therapy in combination with antimicrobial and/or diuretic and anti-inflammatory drugs. As a monotherapy, they are prescribed only for the diseases with the low degree of urinary system malfunction.

There are data collected from the folk physician of Sumy region of Ukraine *Vasilii Korchan* (1893-1983) that the herbal tea of the composition patented[3] was successfully prescribed by him for the monotherapy of glomerulonephritis, pyelonephritis, cystitis, and urethritis. The composition patented includes plants which are the source of phenolic compounds and are known for their usage in Ukrainian folk medicine both as diuretics and medications for different inflammatory diseases (St. John's wort, Wild pansy, Horsetail field, Common heather, Coltsfoot, Wild pansy herb, Convallaria, and Elecampane)[4,5].

Therefore, our aim was to evaluate the level of diuretic and anti-inflammatory activity of this plant composition in order to check the possibility of this object usage for the monotherapy of urinary diseases. As phenolic compounds contribute to pharmacological effects needed during the treatment of urinary diseases (such as anti-inflammatory, antimicrobial, diuretic, spasmolytic effects and P-vitamin activity)[6] we wanted also to check the presence and quantity of flavonoids and other groups of phenolic compounds in our object.

2. MATERIALS AND METHODS:

2.1. Composition used:

The medicinal herbal tea had the following composition (mass parts):

1.	St. John's wort herb (*Hyperici herba*)	20
2.	Wild pansy herb (*Violae herba*)	20
3.	Peppermint (*Menthae piperitae folia*)	20
4.	Tansy flowers (*Tanaceti flores*)	20
5.	Horsetail field shoots (*Equiseti arvensis herba*)	20
6.	Coltsfoot leaf (*Farfarae folia*)	20
7.	Wild thyme herb (*Serpylli herba*)	6
8.	Elecampane rhizome and root (*Inulae rhizomata et radices*)	5
9.	Sunflower flowers (*Helianthi flores*)	5
10.	Elder fruits (*Sambuci fructus*)	5
11.	Convallaria leaf (*Convallariae folia*)	5
12.	Common heather herb (*Callunae vulgaris herba*)	3

The dry plant material was weighed and ground separately and mixed till the state of powder with the size of particles not more than 2 mm. The medicinal herbal tea had appearance of coarse yellowish-green powder consisting of particles of dry plant material.

The obtained medicinal herbal tea was used for the determination and quantification of phenolic compounds.

2.2. The dosage form for the evaluation of biological activity:

In order to obtain the more convenient dosage form for the biological investigations we obtained the thick extract from the medicinal herbal tea. The extract was obtained by percolation. The solvent used was distilled water; the ratio of solvent per dry herbal tea was 10:1. The solvent was evaporated at 50-55°C using a rotation vacuum evaporator. The extract obtained was a thick viscous brown mass with a characteristic odor; the yield was 15%.

2.3. Chemicals used:

The reagents for the determination of phenolic compounds and carrageenan for the evaluation of anti-inflammatory activity were purchased from Sigma-Aldrich reagents, Germany.

2.4. Determination of phenolic compounds:

The identification and quantification of polyphenols in the herbal tea was carried out by the method of high-performance liquid chromatography (HPLC).

For the extraction of biologically active substances we used ethyl alcohol added to the sample in the ratio of 20 ml of 60% ethanol per 1g of sample. The extraction was carried out in a sealed container for five days at room temperature with occasional stirring in accordance with the method[7]. Extraction was carried out in the dark to prevent transformation of the extracted substances under the influence of light. Before analysis, the extract was filtered using a Supelco Iso-Disc Filters PTFE 25-4 (25 mm x 0.45μm) syringe filter. We used Prominence LC-20 Shimadzu liquid chromatographic system (Japan) consisting of the following functional modules: DGU-20A3 degasser, LC-20AD pump module, SIL-20AC auto sampler, SPD-20AV photometric detector, CTO-20A column thermostat, Agilent Technologies Microsorb-MV-150 column (reverse phase, silica gel with C_{18}(-(CH_2) 17-CH_3 group attached), length 150mm, diameter 4.6mm, sorbent grain size 5um).

HPLC conditions were:

1) The composition of the mobile phase (eluent) was methanol and 0.9% phosphoric acid solution in deionized water (Sigma-Aldrich reagents, Germany).
2) We used gradient chromatography. This mode is described for the qualitative separation of individual phenolic acids and flavonoids in plant extract[8]. The initial ratio of eluent components was 1:9. The content of methanol in the eluent during the analysis was changed according to the following scheme: the first 13 minutes – the increase from 10 to 40%; from the 13th to the 20th minute – the increase from 40 to 53%; from the 20th to the 26th minute – the increase from 53 to 55%; from the 26th to the 40th minute – the retention of 55%; from 40th to 41st minute – the

decrease from 40% to 10%; from the 41st to the 56th minute – the retention of 10%.
3) The speed of the eluent was 0.5ml/min.
4) The temperature of the column was 40°C.
5) The volume of the injected sample was 5µl.

Identification of substances in the extract was carried out by comparing the retention time and spectral characteristics with similar characteristics of the standards in accordance with the method[9] of the identification of polyphenols, for which chromatography was carried out at a wavelength of 225, 255, 286 and 350 nm[9-12]. For identification of individual substances or groups of polyphenols the following standards were used: chlorogenic acid and caffeic acid (phenolic acids), catechin (catechins), flavanones naringin, naringenin, hesperidin and hesperetin; flavones luteolin and apigenin, flavonols rutin, quercetin and myricetin, anthocyanin cyanidin.

2.5. Animals:

The study of diuretic activity was carried out using male white mongrel rats weighing 160-170g. The anti-inflammatory activity was checked using male white mongrel rats weighing 190-200g. They were fed with standard pellet diet and water *ad libitum*. All animals were acclimatized for at least one week before the experimental session. All the experimental procedures were done following the guidelines of the Institutional Animals Ethics Committee (IEAC).

2.6. Evaluation of diuretic activity:

The diuretic activity of the thick extract was studied by *Berkhin* method[13]. The object was injected with a special catheter *per os* 30 minutes before water load. The water load was calculated as 3% per 100g of animal weight. Animals were divided into eight groups. Control group obtained only water; group A received 100; groups B, C, D, E, F, and G received 200, 300, 400, 500, 600 and 700 mg/kg of herbal tea thick extract, respectively. The amount of urine was recorded after one, two, and three hours after the start of the experiment.

2.6. Evaluation of anti-inflammatory activity:

Anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema assay[14]. For the anti-inflammatory activity against the acute inflammation, animals were divided into five groups. Control group (carrageenan control) did not receive any oral treatment; Group A received 100; Group B, Group C, and Group D received 150, 200, and 250mg/kg of herbal tea thick extract, respectively. Edema was induced by sub plantar injection of 100µL of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat of all the groups except the control group. Animals of groups A, B, C, and D were treated with the single oral dose of herbal tea thick extract an hour prior to carrageenan injection. Paw volume were measured just before the carrageenan injection and then at time equal to 1, 2, 3, 4, and 5 hours after carrageenan injection. The volume of paw was determined by the measurement of the displaced liquid volume by pletismometer Panlab by Harvard Apparatus. Increase in paw volume was measured as the difference of volumes of inflamed and intact paws at the respective hours.

3. RESULTS:

3.1. Determination of phenolic compounds:

The identification characteristics of the listed standards were obtained under the chromatographic conditions described above. The calibration dependences peak area - standard content had a linear form with an accuracy not lower than $r^2 = 0.994$ (Fig. 1).

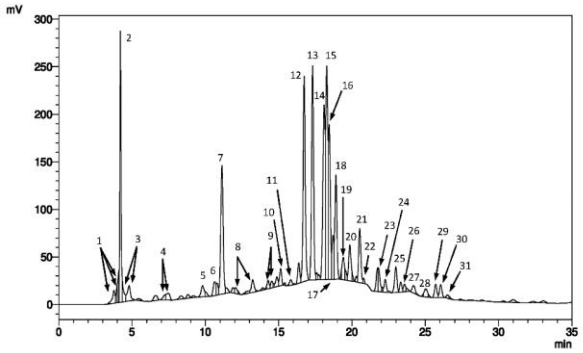

Fig. 1: The chromatogram of ethanolic extract of the sample of the medicinal herbal tea at 255 nm

The read maxima: 1, 2, 3 – catechin-like phenolic compounds (these substances had spectral characteristics of catechines though their peaks were out of areas characteristic for catechins); 4, 6, 8, 11, 12, 13, 18, 24, 30 – phenolic acids; 5 – catechins; 7 – chlorogenic acid; 9, 14 – myricetin glycosides; 10 – hesperetine glycosides; 15 – rutin; 16, 26, 29 – apigenin glycosides; 17, 20, 21, 31 – glycosides of flavonols; 19 – luteolin glycosides; 22 – naringenin; 23 – quercetin; 25 – luteolin; 27 – naringenin glycosides; 28 – apigenin.

The total number of polyphenolic compounds detected by HPLC was 87, and seven substances were identified individually. The data about the content of substances is shown in the table 1.

Table 1. Content of polyphenolic compounds in the herbal tea

Groups of polyphenolic compounds		Individual substances	
Name	**Content, µg/g**	**Name of phenolic compound**	**Content, µg/g**
Phenolic acids	9413.29	Chlorogenic acid	2758.95
		Caffeic acid	0
Catechins	472.25	Catechin	0
Catechin-like compounds	3494.22	-	-
Flavanons	1597.64	Naringin	0
		Naringenin	48
		Hesperidin	0
		Hesperetin	0
Flavones	2293.31	Luteolin glycosides	260.18
		Luteolin	150.08
		Apigenin glycosides	1874.87
		Apigenin	8.18
Flavonols	5834.91	Rutin	2688.44
		Quercetin	114.43
		Myricetin glycosides	1258.73
		Myricetin	0
Isoflavones	68.77	-	-
Total of flavonoids	10384.49	-	-
Unidentified polyphenols	635.04	-	-
Total of phenolic compounds	23809.43	-	-

As it can be seen, six individual substances (chlorogenic acid, naringenin, luteolin, apigenin, rutin, quercetin) were identified. Such groups of phenolic compounds as hydroxycinnamic acids, flavonoids (groups of catechins, flavanons, flavones, flavonols, and isoflavones), and catechin-like compounds were quantified. The total content of phenolic compounds including unidentified polyphenols was 23.8mg/g, with the content of flavonoids 10.4mg/g (rutin 2.7mg/g) and chlorogenic acid 2.8mg/g.

3.2. Evaluation of diuretic activity:

The total amount of urine during three hours of experiment was calculated. The diuretic activity was the increase of urination in comparison to the control group expressed in per cent. The results are given in the fig. 2.

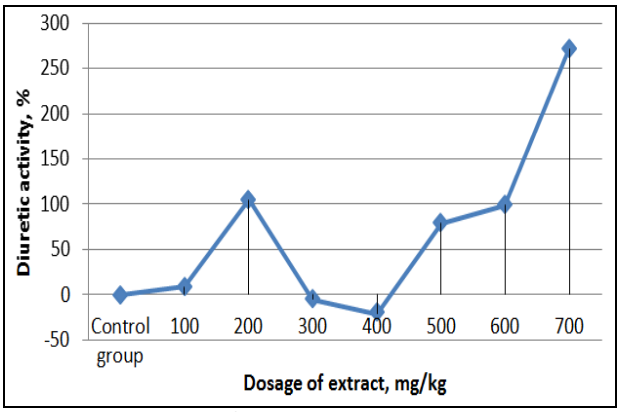

Fig. 2: Diuretic activity of herbal tea thick extract.

We observed the situation when the increase of dosage till 200mg/kg gave the increase of diuretic activity but then dosages of 300 and 400mg/kg produced the antidiuretic effect. The further increase of the dosage produced the increase of the activity again. The diuretic activity in the dosage 700mg/kg was 272% in comparison to the control group.

3.3. Evaluation of anti-inflammatory activity:

The indicator of anti-inflammatory activity was the degree of suppression of edema of rat's paw expressed in per cent. The dosage of herbal tea thick extract checked for anti-inflammatory activity was from 100 to 250 mg/kg. As it can be seen from the diagram (Fig. 3), the activity increased with the increase of the dosage. The highest activity (25%) was registered at the dosages 200 and 250mg/kg.

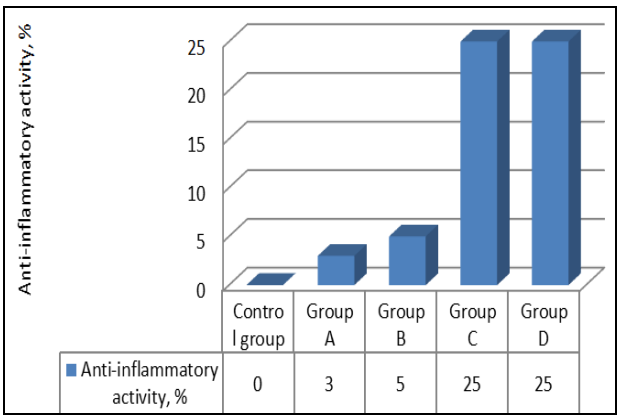

Fig. 3: Anti-inflammatory activity of herbal tea thick extract.

4. DISCUSSION:

As it can be seen from the results of qualitative and quantitative analysis of polyphenols, flavonoids in the medical herbal tea are represented greatly by glycosides. For the most part they are apigenin glycosides, myricetin glycosides, and rutin. Hesperetine glycosides and

naringenin glycosides were identified but they are different from hesperidin and naringin correspondingly.

The experiment showed that the thick extract of the medicinal herbal tea with the increase of the dosage from 100 to 200mg/kg showed the increase of diuretic activity but further increase of the dosage of herbal tea produced the antidiuretic effect. Beginning from the dosage 500 mg/kg we observed the diuretic activity again. The dosage of 700mg/kg had the most pronounced effect on the kidney function, increasing the urination by 272% in relation to the control.

We found that the thick extract of the medicinal herbal tea had the anti-inflammatory activity in relation to the control. The activity was dose-dependent. The highest dosage investigated was 250mg/kg, which showed the anti-inflammatory activity of 25% the same as the activity of the dosage 200mg/kg. The rather high level of anti-inflammatory activity can be explained by the presence of flavonoids in the medicinal herbal tea.

The profile of diuretic activity needs further investigation taking into consideration the data that the renal mechanisms of the diuretic effect of the same plant can vary depending on the dose, dosage form, and experimental conditions[6]. The anti-inflammatory activity of the herbal tea thick extract in higher dosage needs further investigation but the results obtained create good preconditions for possible usage of the herbal tea in the monotherapy of urinary system diseases.

5. CONFLICT OF INTEREST:

None.

6. REFERENCES:

1. Compendium 2011. Medicines. Ed. VN Kovalenko, A.P. Viktorova. MORION, Kiev, 2011, 2320 p.
2. Mashkovsky M.D. Medicines: In 2 volumes. 15th ed., Revised. New Wave, Moscow, 2005, 1216 p. http://padaread.com/?book=17596
3. K.A. Taran, S.G. Ram. Collection for the prevention and treatment of kidney, bladder and urinary tract diseases K-1, UA 117720, 2018. https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=250667
4. A.A. Krylov, etc. Phytotherapy in the complex treatment of diseases of internal organs Health, Kiev, 1991, 238 p.
5. F.I. Machchur. Handbook of Phytotherapy. Health, Kiev, 1984, 264 p.
6. Strigol S.Yu. etc. Medicinal plants, kidneys and uric acid metabolism. Title, Kharkov, 2014, 424 p.
7. A.P. Levitsky, I.V. Khodakov, E.S. Raitseva. Extraction of polyphenols from grape leaves. Kharchova science and technology. 2012; 20 (3): 36–37.
8. E.K. Vertikova, I.V. Khodakov., A.P. Levitsky. Method for the determination of chlorogenic acid. News of dentistry. Specialist. VIP: 2010; 73 (5): 2-5.
9. I.V. Khodakov. A method for identifying polyphenols in plant extracts using HPLC. Determination of the composition of soy isoflavones. Methods and objects of chemical analysis. 2013; 8 (3): 132-142. http://nbuv.gov.ua/UJRN/Moca_2013_8_3_6
10. I.V. Khodakov, A.P. Levitsky, O.A. Makarenko. Method for identifying polyphenols in the old extracts, UA 80597, 2013. https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=187652
11. I.V. Khodakov. A method for identifying polyphenols in plant extracts, Visnik stomatologii. Specialist. VIP. 2012; 80 (7): 42.
12. I.V. Khodakov, O.A. Makarenko. High-performance liquid chromatography in the study of plant polyphenols, Visnik stomatologii. Specialist. VIP. 2010; 735 (2010): 59-60.
13. E.B. Berkhin Methods of studying the effects of new chemical compounds on kidney function, Journal of Pharmaceutical Chemistry. 1977; 1 (5): 3-11.
14. C. A. Winter, E. A. Risley, G. W. Nuss, Carrageenan-induced edema in the hind paw of rat as an assay for anti-inflammatory activity, Proceedings of the Society for Experimental Biology and Medicine. 1962; 111: 544-547. https://doi.org/10.3181/00379727-111-27849