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RELEVANT ISSUES OF MODERN MEDICINE: THE EXPERIENCE OF POLAND AND UKRAINE

Lublin, Republic of Poland
October 20-21, 2017

UNIWERSYTET MEDYCZNY
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International research and practice conference «Relevant issues of modern medicine: the experience of Poland and Ukraine» : Conference proceedings, October 20–21, 2017. Lublin: Izdevnieciba «Baltija Publishing». 156 pages.

Organising Committee:

ISBN 978-9934-571-04-6

STUDY OF POLYPHENOLIC COMPOUNDS OF ACHILLEA MICRANTHOIDES KLOK. ET KRYTZKA HERBS

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The Achillea L. genus is included in the Anthemidiae Cass. Tribe and is represented by about 115 taxa in the world [2, 4]. Achillea L. geus (Asteraceae family) up to 30 species are grows in Ukraine. The herbs of Achillea micranthoides Klok. et Krytzka is perspective for use in official medicine. Achillea micranthoides Klok. et Krytzka is an erect, herbaceous, perentiral plant that produces one to several stems 0,4-0,8 m in height, and has a spreading rhizomatous growth form. The plant commonly flowers from July to August [3]. The herbal raw material of species Achillea L. genus contains: flavonoids, hydroxycinnamic acids, essential oils, tannins, vitamin K_1, coumarins, lignans, aminoacids, organic acids, chemical elements, sterols, isovaleric acid, salicylic acid et all. [5, 6, 7]. The compounds obtained from essential oils of Achillea L. genus species: α-pinene, β-pinene, sabinene, 1,8-cineole, γ-terpinene, p-cymene, β-thujone, camphor, bornyl acetate, β-caryophyllene, terpinen-4-ol, α-terpineol, borneol, caryophyllene oxide, α-elemene, α-selinene, β-selinene were obtained [8, 9]. The polyphenolic compounds is very important components of herbal raw material the species of Achillea L. genus. The species of Achillea L. genus has seen historical use as in traditional medicine, often because of its effects. The herb is purported to be a diaphoretic, astringent, tonic, coagulant, stimulant, antimicrobial, anti-inflammatory and mild aromatic. Usually the hydroxycinnamic acids accumulate in herbal raw material with flavonoids. It can be used to add or restore original color to food. Shelved foods are protected from microorganism by adding hydroxycinnamic acid to prevent deterioration to the food by microorganisms as well as acting as an antioxidant to prolong shelf life foods. The flavonoids and hydroxycinnamic acids by TLC and

HPLC methods determination was proposed. The aims of this study were to compare HPLC and TLC, for qualitative and quantitative analysis of the major constituents of Achillea micranthoides Klok. et Krytzka herbal raw material.

Materials, methods. A research object were flowers and leaves produced in the different regions of Ukraine in the period of flowering (July to August). The Achillea micranthoides Klok. et Krytzka herb, leaves and flowers collected in Zaporozhya region in summer 2016 were object of study. The plants were identified by a taxonomist from National University of Pharmacy, Botany department, Charkiw, Ukraine. Analysis of the herbal raw material was carried out according to the requirements of the State Pharmacopoeia of Ukraine [1]. Drying was conducted in a drying chamber "Termolab CHOJI 24/350" (Ukraine) during 15 hours. The extract of herbal raw material of Achillea micranthoides Klok. et Krytzka herb, leaves and flowers were prepared 0,5% ethanol solution (50%) by Soxhlet method for 12 hour. The extract was cooled, filtered through a Teflon membrane filter, introduced into chromatographic columns. The analyses were conducted by chemical reactions, paper chromatography (PC), thin layer chromatography (TLC) on plates «Aluminium oxide 150 F 254 (0,20 mm) (MERCK, Germany)», «Sorbfil АФ-А», «Sorbfil UF-254». For flavonoids were conducted the systems: benzol:ethyl-acetate: acetic acid:formamide (70:30:2:1), benzol:ethyl-acetate:acetic acid:water distilled (50:50:1:1); ethyl-acetate : methane acid :acetic acid:water distilled (100:11:11:27), ethyl-acetate : acetic acid : water distilled (10:2:3), chloroform:methanol:acetic acid:water distilled (6:2:0,1:0,1); ethyl-acetate : methylethylketon: methane acid : water distilled (50:30:10:10); for hydroxicinnamic acids in the systems: chloroform : ethanol (9:1), chloroform : ethanol : acetic acid : water distilled (6:2:0,1:0,1). The chromatograms were dried in a dryer «USP-2» at a temperature 30^{0}C, viewed in UV light. At the same time conducted chromatographic analysis of standard substances. The polyphenolic compound were conducted by method HPLC with «Shimadzu LC–20 Prominence» The chromatographic column «Phenomenex Luna C18(2)» (l=250 мм), (d=4,6 мм), (t=35°C), (λ =330 nm), (v=1 ml/min), volume 5 mkl. Mobile phase: A: 0,1% trifluoroacetic acid in water distilled; B: 0,1% solution of trifluoroacetic acid in acetonitrile were used. The polyphenol compounds identification of Achillea collina (Becker ex Rchb.) herbal raw material was based on comparison of mass spectra from the Mass Spectral Database with used standard substances (Sigma Chemical Company, USA) and this spectral characteristics.

Results and discussion. The polyphenolic compounds (flavonoids and hydroxylcinnamic acids) were identified in herbal raw material of Achillea micranthoides Klok. et Krytzka by TLC. The content of 13 flavonoids and 10 hydroxycinnamic acids were determined in herbal raw material. The content of flavonoids were found in Achillea micranthoides Klok. et Krytzka flowers (1,441±0,141%) and leaves (0,945±0,090%). In herbal raw material (flowers and leaves) the highest amount of apigenin derivates were determined. The hydroxycinnamic acids were found in Achillea micranthoides Klok. et Krytzka leaves (0,665±0,066%) and flowers (0,515±0,050%). In leaves and flowers of herbal raw material of Achillea micranthoides Klok. et Krytzka, the highest

amount of neo-chlorogenic acid were determined. The results of HPLC method is more effective for the qualitative analysis, however, TLC was found to more accurate for quantitative analysis of polyphenolic compounds. A combination of this methods may be useful in a quality control of herbal raw material of species Achillea L. genus. The 13 flavonoids were identified in flowers and leaves of Achillea micranthoides Klok. et Krytzka: isovitexin, saponarin, luteolin -7, 3′-bi-glucoside, luteolin 6 C-glucoside, quercitin 3-0-rutinosid, quercitin, rutin, apigenin-7,4′-bi-O-glucoside, isoramnetin O-acetylhehoside, luteolin-7-O– glucoside, luteolin, apigenin. In flowers and leaves the highest amount of apigenin derivates were determined (isovitexin, saponarin, apigenin-7,4′-bi-O-glucoside, apigenin-7-O-glucoside, apigenin). The 10 hydroxycinnamic acids were identified in leaves and flowers. There are: chlorogenic acid, p-coumaric acid, ferulic acid, caffeic acid, crypto-chlorogenic acid, neochlorogenic acid, iso-chlorogenic acid, 3,4-O-dicaffeoylquinic acid, rosmarinic acid, In leaves and flowers the highest amount of neo-chlorogenic acid were determined. The results were treated by the method of mathematical statistics with the use of program «Statistica 6 for Windows» (Stat. Soft. Inc., № AXXR712D-833214FANS). Authenticity differences sizes of concentrations was estimated on the t-criterion of Student ($p \geq 95\%$). The Achillea micranthoides Klok. et Krytzka herbal raw material can be used for means with anti-inflammatory. antioxidant, coagulant activities.

Conclusion. The study of Achillea micranthoides Klok. et Krytzka qualitative and quantitative content of phenolic compounds in flowers and leaves by TLC and HPLC methods were conducted. In herbal raw material of Achillea micranthoides Klok. et Krytzka 13 flavonoids and 10 hydroxycinnamic acids were identified. In flowers and leaves of herbal raw material of Achillea micranthoides Klok. et Krytzka the highest amount of apigenin derivates were determined. The hydroxycinnamic acids in Achillea micranthoides Klok. et Krytzka leaves and flowers were contented. In leaves and flowers the highest amount of neo-chlorogenic acid were determined.The Achillea micranthoides Klok. et Krytzka herbal raw material is widely widespread in Ukraine and perspective for means with anti-inflammatory. antioxidant, coagulant activities.

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ОБГРУНТУВАННЯ СКЛАДУ ЛІКАРСЬКОГО ЗАСОБУ ДЛЯ ЛІКУВАННЯ ЗАХВОРЮВАНЬ СЕЧОВИВІДНИХ ШЛЯХІВ

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Запальні захворювання сечовидільної системи займають перше місце серед усіх урологічних захворювань, при цьому частота цих захворювань зростає щороку. Інфекційно-запальні захворювання сечовивідних шляхів є нагальною проблемою не лише за рахунок їх широкого розповсюдження, а й труднощами, які виникають у процесі лікуванні та частими рецидивами. Основним методом лікування інфекційно-запальних процесів сечовидільної системи є етіотропна антибактеріальна терапія. Але використання антибіотикотерапії у хворих з інфекційно-запальними захворюваннями органів сечовидільної системи часто призводить до формування антибіотикорезистентних мікроорганізмів, розвитку токсикоалергічних реакцій, дисбактеріозу, тощо. Тому останнім часом набули широкого застосування фітокомпозиції для лікування інфекційно-запальних процесів сечовивідної системи, як монотерапія так і у складі комплексної терапії [2, 4].