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CONTENTS

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PHYTOCHEMICAL COMPOSITION OF POLYPHENOLIC COMPOUNDS OF ACHILLEA COLLINA (BECKER EX RCHB.)

Summary

Achillea L. genus include about 150 species and there is widely distributed on Europe, Asia, North Africa, North America. Up to 30 Achillea species grow in Ukraine. Achillea millefolium L. and Achillea collina (Becker ex Rchb.) (syn. Achillea millefolium ssp. collina (Becker ex Rchb.) is perspective for use in official medicine. A scheme for analysis of etanolic and methanolic extracts of Achillea collina (Becker ex rchb.) herbal raw material including of flavonoids and hydroxycinnamic acids by TLC and HPLC and their determination by high-performance liquid chromatography was proposed. The aim of the research was study the accumulation,

qualitative and quantitative content of polyphenolic compounds in flowers and leaves of Achillea collina (Becker ex Rchb.). The 12 flavonoids are identified in Achillea collina (Becker ex Rchb.) flowers (1.303±0.110%) and leaves (0.862±0.091%). Contents of the apigenin-7.4'-bi-O-glucoside (0.304±0.004%), luteolin-7.3′-bi-glucoside (0.300±0.005%) and luteolin-7-O-glucoside (0.154±0.004%) were significant and quercitin 3-0-rutinosid yielded the lowest amount (0.019±0.002%) in study of flowers Achillea collina (Becker ex Rchb.). The 10 hydroxycinnamic acids are identified in Achillea collina (Becker ex Rchb.) leaves (0.521±0.058%) and flowers (0.478±0.051%). Contents of the neo-chlorogenic acid (0.174±0.004%) and crypto-chlorogenic acid (0.088 ± 0.007%) were the highest and P-coumaric acid yielded the lowest amount (0.010 ± 0.001%) in study of flowers Achillea collina (Becker ex Rchb.). The study proved the presence of biologically and pharmacologically important flavonoids and hydroxycinnamic acids making the plant Achillea collina (Becker ex Rchb.) beneficial for the preparation of phytodrugs.A combination of two methods (TLC and HPLC) may be useful in a quality control as it used allow qualitative analysis of herbal raw material while maintaining accurate quantification of extract composition.

Introduction

Asteraceae family has about 23000 species, 1620 genera, 30 tribes and 5 subfamiles throughout the world. The Achillea L. genus is included in the Anthemidiae Cass. Tribe and is represented by about 115 taxa in the world [4; 11; 20; 26]. Achillea collina (Becker ex Rchb.) commonly known as Achillea millefolium ssp. collina (Becker ex Rchb.).

This is a flowering plant in the family Asteraceae. Achillea L. genus include about 150 species. It is native to temperate regions of the Northern Hemisphere in Asia, Europe and North America [5; 7; 8].

The herbal raw material of species Achillea L. genus contains: essential oils, monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, flavonoids, tannins, vitamin K_1, coumarins, lignans, aminoacids, organic acids, chemical elements, sterols, isovaleric acid et all. [15; 16; 17; 18].

With the increasing tendency for the use of essential oils in both food and pharmaceutical industries, more and more scholars investigate the Achillea millefolium subspecies.

The essential oils composition of the Achillea millefolium subspecies can have different quality and quantity in different geographical and environmental conditions and during different periods of the plant growth.

Qualitative and quantitative content of the essential oils of Achillea collina (Becker ex Rchb.) is studied the most.

Essential oils of Achillea millefolium subsp. millefolium contain the following compounds: α-pinene, β-pinene, sabinene, 1.8-cineole (22.83%), 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 [2; 23; 27].

K. Baczek, O. Kosakowska, J. L. Przybyl et al. was determined the variability of 20 Achillea millefolium L. populations, introduced into ex situ conditions. Total content of essential oil ranged from 0.10 to 1.00 %.

Among 24 identified compounds β-pinene, 1.8-cineole, terpinen-4-ol, nerolidol and chamazulene were dominants [20].

M. M. Nadim, A. A. Malik, J. Ahmad et al. determined the essential oil contents of Achillea millefolium cultures growing in India under tropical conditions and at the end of the study, 30 different components were obtained.

Eleven of the obtained compounds were common: α-pinene (6.28%), β-pinene (6.26%), sabinene (17.58%), 1.8-cineole (13.04%), p-cymene (1.11%), γ-terpinene (1.19%), bornyl acetat (7.98%), β-caryophyllene (2.31%), terpinen-4-ol (6.17%), α-terpineol (1.04%), borneol (12.41%) [25].

N. A. Vernikovskaya, Z. A. Temerdashew were determined the phenolic compounds of aqueous extracts of Achillea millefolium L. herb including the identification by gas and liquid chromatography mass spectrometry and determination by HPLC.

On the basis of the scheme phenolic compounds (phenolcarbonic, cimmaric and caffeoylquinic acid) were identified in Achillea millefolium L. herb by GC-MS. Six caffeoylquinic acids were identified by LC-MS.

The content of eight phenolic compounds (protocatechuic, trans-caffeic, 3-O-caffeoylquinic, 5-O-caffeoylquinic, 4-O-caffeoyquinic, 3.4-O-caffeoy-lquinic, 3.5-O-caffeoylquinic and 4.5-O-caffeoylquinic acids) were determined in Achillea millefolium L. herb using HPLC-UV-DAD [1].

K. Baczek, O. Kosakowska, J.L. Przybyl et al. were determined four flavonoids (luteolin, luteolin $3^{/}$.7–diglucoside, apigenin, apigenin-7-glucoside) in herb of Achillea millefolium L. Apigenin-7-glucoside was present in the highest. In addition phenolic acids (caffeic acid derivatives, rosmarinic, cichoric) also were identified [20].

O. A. Kyslychenko was determined the flavonoids in the overground part of Achillea millefolium L. The results of the studies is found of qualitative composition and quantitative content of 12 flavonoids (vicenin, luteolin-$3^{/}$.7-O-diglucoside, apigenin glycoside, luteolin-7-O-glucoside, rutin, apigenin-7-O-rutinoside, luteolin, apigenin, chrysoeriol, diosmetin, genkwanin, acacetin). It was found out that the highest amount of flavonoids is accumulated in flours, the least – in stems [22].

The species of Achillea L. genus historically used in folk medicine. Traditionally herb is used as a styptic and vulnerary remedy. The herb is possessed of diaphoretic, astringent, tonic, coagulant, stimulant, antimicrobial, anti-inflammatory and mild aromatic activities.

The species of Achillea L. genus contains a variety of antioxidant constituents such as flavonoids and sesquiterpene lactones. These compounds scavenge free radicals throughout the body, which reduces the cellular damage and degradation [6]. The sesquiterpene lactone achilloline A was studied more closely to investigate its antioxidant actions more specifically in the astrocytes of the nervous system.

It was found that achilloline A was able to act by inhibiting microglial activation, modulate MAPK activity and reduce reactive oxygen species levels in the microglial cells. This shows a significant ability for achilloline A to protect the astrocytes of the nervous system, mainly thought antioxidant and free radical preventive action. The extracts of herb of Achillea L. were shown to protect the pancreatic β-cells from damage. The β-cells of the pancreas are the source of insulin for the human body. Damage to these cells will result in diabetes, which has a wide range of negative health implications. Protecting this cells or reducing the damage through the use of herbal raw material species of Achillea L. genus could be an important control method and treatment option for this widespread and fatal disease [9; 10; 12; 14; 21].

It is interesting to note, that usually the hydroxycinnamic acids accumulate in herbal raw material and are determined by method HPLC in conjunction with flavonoids. Hydroxycinnamic acids is added to food for technological purposes including manufacturing, processing, preparation, treatment, packaging, transportation or storage, and also as food additives. This compounds provide flavoring for ice cream, bakery and confectionery [13; 21; 26].

There are up to 20 remedies at Ukrainian market (Bittner Balsam, Vitaon Balsam, Gastrovitol, Gepaliv, Liv 52, Menodoron, Rotokanum, Somaton Balsam, Tonsilgon H, Cholaflux, Wundahyl et all.) which contain biologically active compounds of herbal raw material of Achillea millefolium L.

The herbal raw material of Achillea collina (Becker ex Rchb.)) is perspective for use in official medicine.

The aim of the research was study the accumulation, qualitative and quantitative content of polyphenolic compounds in flowers and leaves of Achillea collina (Becker ex Rchb.).

1. Materials & Methods

The leaves and flowers of Achillea collina (Becker ex Rchb.) were collected as samples in the different areas of Ukraine in the period of flowering during Jule to September 2016. The plants are identified by a taxonomist from National University of Pharmacy, Botany department, Kharkiw, Ukraine.

Collected samples were dried for 15 hours at temperature (35±2°C) in drying chamber «Termolab СНОЛ 24/350» (Ukraine). Electric comminuting machine was used to grind dried samples and sieved with 0.1 mm mesh.

2. Qualitative and Quantitative Control Method

Analysis of the herbal raw material was carried out according to the requirements of the State Pharmacopoeia of Ukraine [3].

Samples (0.1 g) were weighed and 5 mL of 96% ethanol was added. Then samples were vortexed and placed in a water bath at 80°C for 20 min.

Samples were centrifuged at 3000 rpm for 3 min and supernatant was decanted. The residue was re-extracted with 5 mL of 96% ethanol. Both supernatants were pooled and stored at – 20°C prior to analysis.

Herbal raw material of Achillea collina (Becker ex Rchb.) for HPLC (0.02 g) were weighed into a 5 mL graduated tube and 5 mL of 90% methanol was added. Then samples were stood for 30 minutes in ultrasonic bath and infused at room temperature for 24 hours.

Samples were centrifuged at 4.000 rpm for 5 min and filtered through a Teflon membrane filter into tube for analysis.

Thin Layer Chromatography (TLC) was performed according to the method described in of the State Pharmacopoeia of Ukraine with slight modifications.

5 μL of extract and standard simples were spotted in TLC plates («Aluminium oxide 150 F 254 (0.20 mm) (MERCK, Germany)», «Sorbfil АФ-А», «Sorbfil UF-254»).

Samples were researched on contain flavonoids in different mobile phases: 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 TLC Hydroxycinnamic Acids used 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 30^0C, and then observed under UV 366 nm and after spraying with Vanillin-Sulfuric acid. The Rf values and color of the spots were recorded.

The polyphenolic compounds were defined by method HPLC using liquid chromatographs Agilent technology 1100 (1) and «Shimadzu LC–20 Prominence» (2).

The separation 1 was achieved by the chromatographic column «ZORBAX-SB C-18» (3.5 x 210 mm).

The mobile phase was contained 0.6% aq. trifluoroacetic acid solution (Solvent A); 70% methanol with 0.6% aq. trifluoroacetic acid solution (Solvent B) and 100% methanol (Solvent C) the flow rate was adjusted to 0.25 ml/min, the column was thermostatically controlled at 35^0C and the injection volume was kept at 5 µl.

The separation 2 was achieved by the chromatographic column «Phenomenex Luna C18» (4.6 x 250 mm).

The mobile phase was contained 0.1% aq. trifluoroacetic acid solution (Solvent A); 0.1% aq. trifluoroacetic acid solution in acetonitrile (Solvent B) the flow rate was adjusted to 1.00 ml/min, the column was thermostatically controlled at 35^0C and the injection volume was kept at 5 µl.

HPLC chromatograms were detected using a photo diode array UV detector at different wavelengths according to absorption maxima of analyzed compounds.

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.

2. Results and discussion

Achillea collina (Becker ex Rchb.) is an erect, herbaceous, perennial plant that produces one to several stems 0.9-1.3 m in height (Im. 1).

Leaves are evenly distributed along the stem, with the leaves near the middle and bottom of the stem being the largest. The leaves have varying degrees of pubescence.

The leaves are 3–11 cm long, bipinnate and arranged spirally on the stems. The leaves are cauline, and more or less claspring.

The inflorescens has 4 to 9 bract and contains ray disk flowers which are white to pink. The generally 3 to 8 ray flowers are ovate to round. Disk flowers range from 15 to 40 mm.

The inflorescence is produced in a flat-topped capitulurn cluster and the inflorescences are visited by many insects, featuring a generalized pollination system. The small achene-like fruits are called cypsela.

Image 1. An outward appearance of Achillea collina (Becker ex Rchb.)

The plant commonly flowers from Jule to September [5; 7].

Thin layer chromatography is the widely used analytical technique in herbal raw material standardization process due to its simplicity, rapidness and cost effectiveness.

In the present study, we compared the TLC patterns of Achillea collina (Becker ex Rchb.) with standard samples. It was revealed that tested samples showed common spots.

Use of TLC finger prints has been established as a reliable tool for identification and authentication of medicinal and aromatic plants.

High-performance liquid chromatography is found suitable for the detection and the determination of flavonoids present in various plants.

As the mobile phase was used to methanol and aq. trifluoroacetic acid solution using the conditions as described in experimental section.

With this optimum separation of the analytes was achieved enabling the quantification in the sample extract. HPLC chromatogram of the mixture is presented in Im. 2.

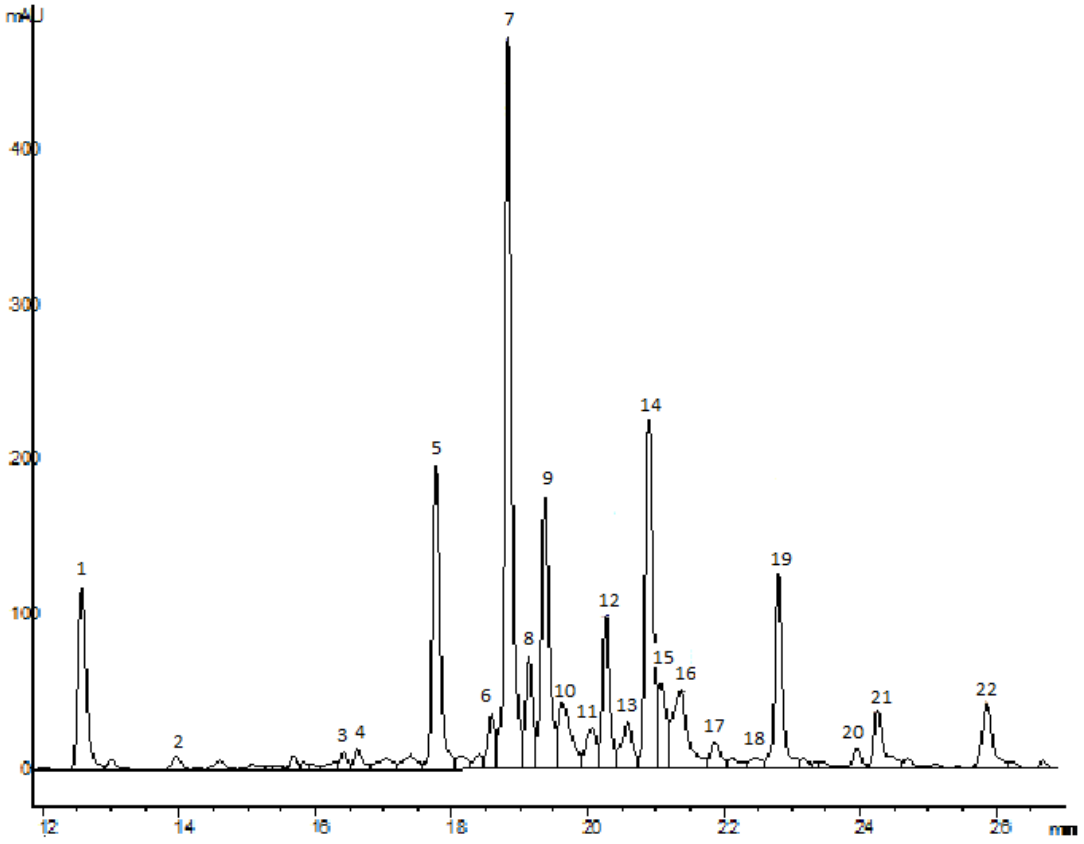

Image. 2. The polyphenolic compounds of Achillea collina (Becker ex Rchb.) flowers. 1. Chlorogenic acid; 2. P-coumaric acid; 3. Ferulic acid; 4. Caffeic acid; 5. Crypto-chlorogenic acid; 6. Isovitexin; 7. Neo-chlorogenic acid; 8. Saponarin; 9. Luteolin-7, 3/-bi-glucoside; 10. Luteolin 6 C-glucoside; 11. Quercitin 3-0-rutinoside; 12. Iso-chlorogenic acid; 13. Rutin; 14. Apigenin-7, 4/-bi-O-glucoside; 15. Isoramnetin O-acetylhehoside; 16. Luteolin-7-O-glucoside; 17. 3.4-O-dicaffeoylquinic acid; 18. 3.5-O-dicaffeoylquinic acid; 19. Apigenin-7-O-glucoside; 20. Rosmarinic acid; 21. Luteolin; 22. Apigenin

As shown in the chromatogram, all investigated compounds were successfully separated. The constituents under investigation were identified by the recorded absorption spectra, which were comparable both for extracts of Achillea collina (Becker ex Rchb.) and standard substances. Using the chromatographic conditions calibration curves the four flavonoids and five hydroxicinnamic acids were established through analyzing working standard solutions in two samples (Image 3, 4, 5, 6, 7, 8, 9, 10).

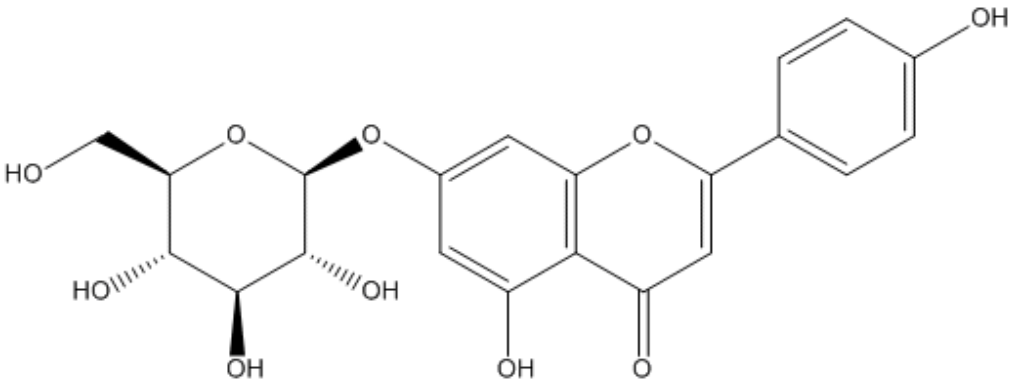

Image 3. The structure of Apigenin-7-O-glucoside

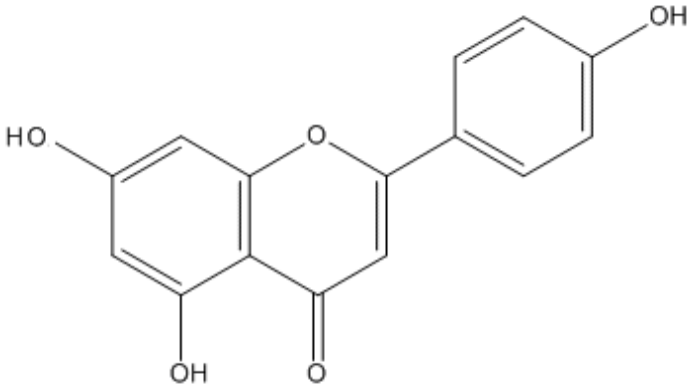

Image 4. The structure of Apigenin

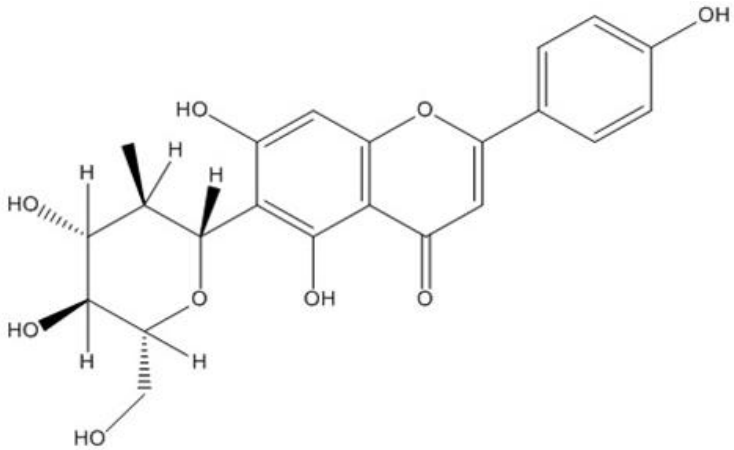

Image 5. The structure of Isovitexin (apigenin-7-O-glucoside)

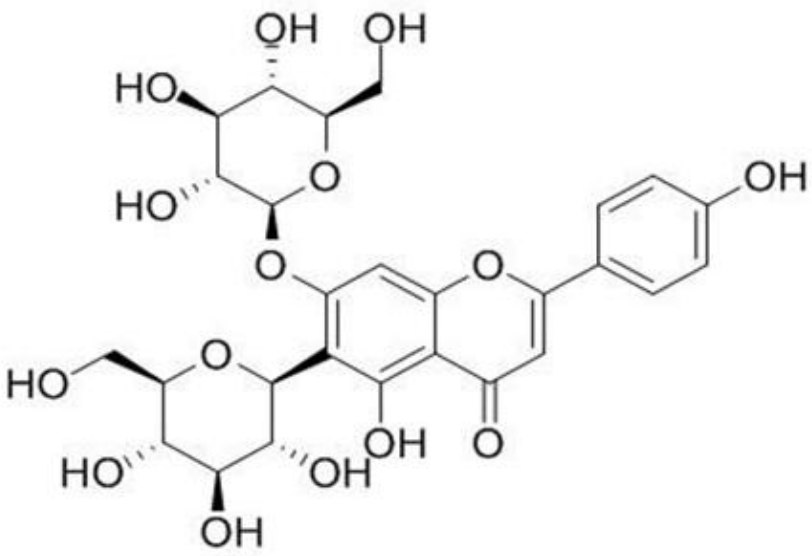

Image 6. The structure of Saponarin (apigenin-6.7-O-bi-glucoside)

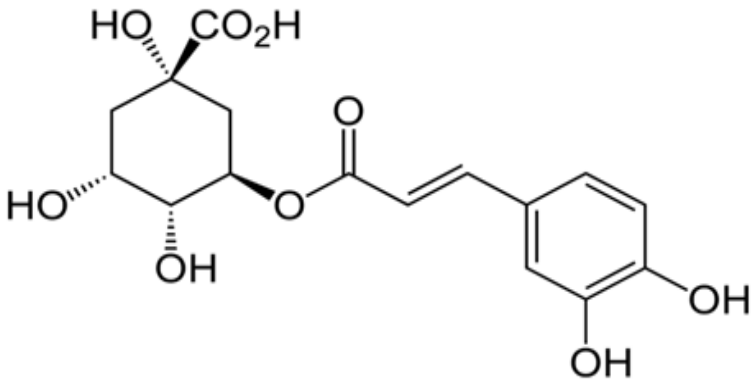

Image 7. The structure of Chlorogenic acid

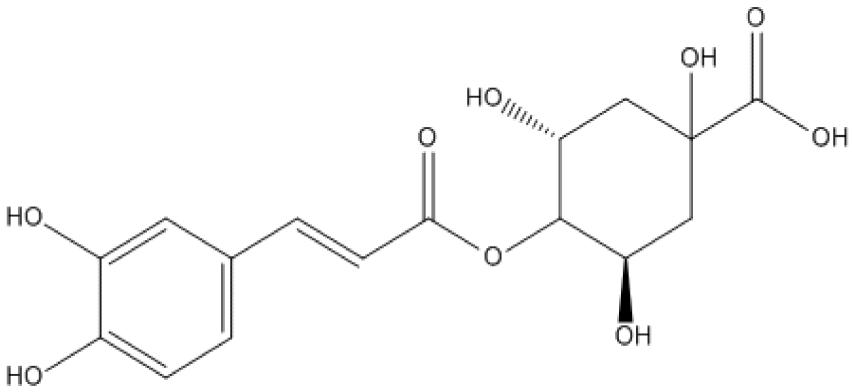

Image 8. The structure of Crypto-Chlorogenic acid

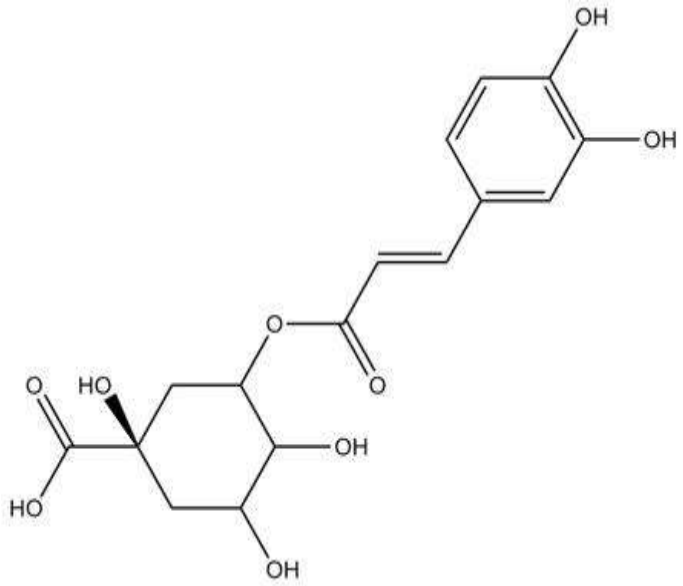

Image 9. The structure of Neo-Chlorogenic acid

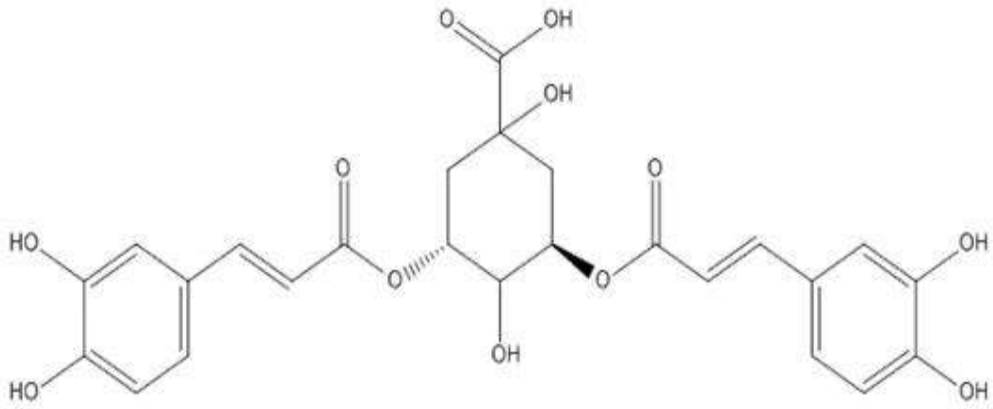

Image 10. The structure of Iso-Chlorogenic acid

Values and percentage concentrations of polyphenolic compounds in the flowers and leaves of Achillea collina (Becker ex Rchb.) (flavonoids and hydroxicinnamic acids) in the plant extract obtained from these analyses are tabulated in Table 1, 2.

As a result of chemical analysis by HPLC methods content of flavonoids and hydroxicinnamic acids were determined.

The 12 flavonoids are identified in Achillea collina (Becker ex Rchb.) flowers (1.303±0.110%) and leaves (0.862±0.091%).

There are isovitexin, saponarin, luteolin-7.3′-bi-glucoside, luteolin 6-C-glucoside, quercitin 3-0-rutinosid, rutin, apigenin-7.4′-bi-O-glucoside, isoramnetin O-acetylhehoside, luteolin-7-O-glucoside, luteolin, apigenin.

Apigenin, luteolin and glucosides are exhibit significant pharmacological activities, including anti-oxidation, anti-inflammation, anti-diabetic etc.

Their also used for the treatment of cancer, vericose veins, haemorrhoids, haemorrhagic stroke.

Contents of the apigenin-7.4′-bi-O-glucoside (0.304±0.004%), luteolin-7.3′-bi-glucoside (0.300±0.005%) and luteolin-7-O-glucoside (0.154±0.004%) were significant and quercitin 3-0-rutinosid yielded the lowest amount (0.019±0.002%) in study of flowers Achillea collina (Becker ex Rchb.).

The content of flavonoids in the leaves was lower than in the flowers: apigenin-7.4′-bi-O-glucoside (0.210 ± 0.011%), luteolin-7.3′-bi-glucoside (0.200 ± 0.011%) and luteolin-7-O-glucoside (0.112 ± 0.010%).

The 10 hydroxycinnamic acids are identified in Achillea collina (Becker ex Rchb.) leaves (0.521±0.058%) and flowers (0.478±0.051%).

There are chlorogenic acid, p-coumaric acid, ferulic acid, caffeic acid, crypto-chlorogenic acid, neo-chlorogenic acid, iso-chlorogenic acid, 3.4-O-dicaffeoylquinic acid, 3.5-O-dicaffeoylquinic acid, rosmarinic acid.

Contents of the neo-chlorogenic acid (0.174±0.004%) and crypto-chlorogenic acid (0.088±0.007%) were the highest and P-coumaric acid yielded the lowest amount (0.010±0.001%) in study of flowers Achillea collina (Becker ex Rchb.).

The content of hydroxycinnamic acids in the leaves was a bit higher than in the flowers: neo-chlorogenic acid (0.178±0.021%) and crypto-chlorogenic acid (0.092±0.008).

The results were treated by the method of mathematical statistics with the use of program «Statistica 6 for Windows» (Stat. Soft. Inc., № AXXR712D-833214FANS). The described HPLC method could be use for the qualitative and quantitative analysis of polyphenol compounds in plant materials.

The study proved the presence of biologically and pharmacologically important flavonoids and hydroxycinnamic acids making the plant Achillea collina (Becker ex Rchb.) beneficial for the preparation of phytodrugs. A combination of two methods (TLC and HPLC) may be useful in a quality control as it used allow qualitative analysis of herbal raw material while maintaining accurate quantification of extract composition. The results of the study can be used for developing the quality control profile by the pharmaceutical and phytopharmaceutical industries.

Table 1

The quantitative contents of polyphenolic compounds in the flowers of Achillea collina (Becker ex Rchb.), ($\bar{x}\pm\Delta\bar{x}$)%, n=6

№	Compound	Quantitative content, %	Retention time, min	$\lambda_{max.}$, nm
1	Chlorogenic acid	0.070±0,005	12.56	218; 245; 300; 326
2	P-coumaric acid	0.010±0.001	13.57	228; 310
3	Ferulic acid	0.011±0.001	16.34	235, 295, 325
4	Caffeic acid	0.012±0.001	16.56	326
5	Crypto-chlorogenic acid	0.088±0.007	17.54	326
6	Isovitexin	0.069±0.007	18.55	340
7	Neo-chlorogenic acid	0.174±0.004	18.84	218; 247; 304; 328
8	Saponarin	0.031±0,003	19.20	271, 336
9	Luteolin-7,3'-bi-glucoside	0.300±0,005	19.36	255; 266; 349
10	Luteolin 6-C-glucoside	0.038±0.004	19.85	350
11	Quercitin 3-0-rutinoside	0.019±0.002	20.12	352
12	Iso-chlorogenic acid	0.038±0.002	20.27	219; 235; 245; 300; 329
13	Rutin	0.042±0.005	20.53	259; 362,5
14	Apigenin-7,4'-bi-O-glucoside	0.304±0,004	20.89	267; 339
15	Isoramnetin O-acetylhehoside	0.075±0.008	21.20	362
16	Luteolin-7-O-glucoside	0.154±0.004	21.36	257; 268; 348
17	3,4-O-dicaffeoylquinic acid	0,028±0,003	21,88	328
18	3,5-O-dicaffeoylquinic acid	0.0210.003	22.30	330
19	Apigenin-7-O-glucoside	0.201±0.019	22.79	268; 339
20	Rosmarinic acid	0.026±0,003	23.97	328
21	Luteolin	0.070±0.008	24.25	242; 254; 266; 291, 350
22	Apigenin	0.034±0.004	25.86	267, 338
23	Flavonoids, %	1.303±0.110	–	–
24	Hydroxycinnamic acids,%	0.478±0.051	–	–

Table 2

The quantitative contents of polyphenolic compounds in the leaves of Achillea collina (Becker ex Rchb.), ($\bar{x}\pm\Delta\bar{x}$)%, n=6

№	Compound	Quantitative content, %	Retention time, min	$\lambda_{max.}$, nm
1	Chlorogenic acid	0.075±0.006	12.56	218; 245; 300; 326
2	P-coumaric acid	0.013±0.001	13.57	228; 310
3	Ferulic acid	0.015±0,001	16.34	235, 295, 325
4	Caffeic acid	0.016±0,001	16.56	326
5	Crypto-chlorogenic acid	0.092±0.008	17.54	326
6	Isovitexin	0.030±0.002	18.55	340
7	Neo-chlorogenic acid	0.178±0.021	18.84	218; 247; 304; 328
8	Saponarin	0.020±0.002	19.20	271, 336
9	Luteolin-7,3'-bi-glucoside	0.200±0.011	19.36	255; 266; 349
10	Luteolin 6-C-glucoside	0.011±0.001	19.85	350
11	Quercitin 3-0-rutinoside	0.008±0.001	20.12	352
12	Iso-chlorogenic acid	0.042±0,003	20.27	219; 235; 245; 300; 329
13	Rutin	0.021±0.001	20.53	259; 362,5
14	Apigenin-7,4'-bi-O-glucoside	0.210±0.011	20.89	267; 339
15	Isoramnetin O-acetylhehoside	0.050±0.004	21.20	362
16	Luteolin-7-O-glucoside	0.112±0.010	21.36	257; 268; 348
17	3,4-O-dicaffeoylquinic acid	0.032±0.002	21.88	328
18	3,5-O-dicaffeoylquinic acid	0.029±0.002	22.30	330
19	Apigenin-7-O-glucoside	0.140±0.010	22.79	268; 339
20	Rosmarinic acid	0.029±0.003	23.97	328
21	Luteolin	0.040±0.005	24.25	242; 254; 266; 291, 350
22	Apigenin	0.020±0.002	25.86	267, 338
23	Flavonoids, %	0.862±0.091	–	–
24	Hydroxycinnamic acids,%	0.521±0.058	–	–

Conclusions

1. The study of qualitative and quantitative content of phenolic compounds in the flowers and leaves Achillea collina (Becker ex Rchb.) by

TLC and HPLC methods were conducted. In herbal raw material of Achillea collina (Becker ex Rchb.) 12 flavonoids and 10 hydroxycinnamic acids were identified.

2. The flavonoids in Achillea collina (Becker ex Rchb.) flowers (1.303±0.110%) and leaves (0.862±0.091%) were detected. Contents of the apigenin-7.4'-bi-O-glucoside (0.304±0.004%), luteolin-7.3/-bi-glucoside (0.300±0.005%) and luteolin-7-O-glucoside (0.154±0.004%) were the most significant.

3. The hydroxycinnamic acids in the leaves of Achillea collina (Becker ex Rchb.) (0.521+0.058%) and in the flowers (0.478+0.051%) are defined. The high amount of neo-chlorogenic acid were typically for both leaves and flowers.

4. Standardization of herbal raw material of Achillea collina (Becker ex Rchb.) must carry out about biologically active polyphenolic compounds.

5. The results found TLC to be effective for the qualitative analysis, however, HPLC was found to be more accurate for quantitative analysis.

6. The Achillea collina (Becker ex Rchb.) herbal raw material is widely widespread in Ukraine and perspective for phyto drugs with anti-inflammatory, coagulant and antioxidant activities.

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