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CHEMICAL ANALYSIS OF THE COMPOSITION OF HERBAL PLANTS OF THE FAMILY ASTERACEAE

Mokshin D.S.[1*], Polyakov V.V.[2]
[*]Non-profit limited company "M. Kozybayev North Kazakhstan University", Petropavlovsk, Republic of Kazakhstan
[*]E-mail: menwolf94@mail.ru

Abstract

An analysis of scientific data on the botanical description, distribution area and biological properties of herbaceous plants of the Asteraceae family is given: chamomile, common yarrow, and calendula officinalis. The possibilities of practical application of plant materials for medical purposes are shown. A comparative analysis of the chemical composition of chamomile, common yarrow and calendula officinalis is given. Lipids, amino acids, flavonoids, phenolic acids, and vitamins were identified in the composition of plants. Quantitative analysis of lipids and flavonoids was carried out. As a result of the study, the lipid content in vegetable raw materials was found to be from 2.09 to 3.24%, as well as the content of flavonoids in terms of quercetin ranging from 3.52 to 4.08%.

Keywords: *Asteraceae* family, *Matricaria chamomilla L., Achillea millefolium L., Calendula officinalis*, thin layer chromatography, circulation extraction, working standard sample.

АСТРОВ ТҰҚЫМДАСЫНЫҢ ШӨПТЕСІН ӨСІМДІКТЕРІНІҢ ҚҰРАМЫН ХИМИЯЛЫҚ ТАЛДАУ

Мокшин Д.С.[1*], Поляков В.В.[2]
[*]КЕАҚ «М. Қозыбаев атындағы Солтүстік Қазақстан университеті», Петропавл, Қазақстан Республикасы
[*]E-mail: menwolf94@mail.ru

Аңдатпа

Астровтар тұқымдасының шөптесін өсімдіктерінің ботаникалық сипаттамасы, таралу аймағы және биологиялық қасиеттері туралы ғылыми мәліметтерге талдау келтірілген: түймедақ дәріханасы, кәдімгі мыңжапырақ, дәрілік календула. Өсімдік шикізатын медициналық мақсатта практикалық қолдану мүмкіндіктері көрсетілген. Дәріхана түймедақының, кәдімгі мыңжапырақ пен дәрілік календуланың химиялық құрамына салыстырмалы талдау берілген. Өсімдіктердің құрамында липидтер, аминқышқылдары, флавоноидтар, фенол қышқылдары, дәрумендер анықталды. Липидтер мен флавоноидтардың сандық талдауы жүргізілді. Зерттеу нәтижесінде өсімдік шикізатындағы липидтердің мөлшері 2,09-дан 3,24% - ға дейін, сондай-ақ кверцетинге есептегенде флавоноидтардың мөлшері 3,52-ден 4,08% - ға дейін анықталды.

Түйін сөздер: Астровтар тұқымдасы, *Matricaria chamomilla L., Achillea millefolium L., Calendula officinalis*, жұқа қабатты хроматография, айналым экстракциясы, стандартты жұмыс үлгісі.

ХИМИЧЕСКИЙ АНАЛИЗ СОСТАВА ТРАВЯНИСТЫХ РАСТЕНИЙ СЕМЕЙСТВА АСТРОВЫЕ

Мокшин Д.С.[1*], Поляков В.В.[2]

[*]Северо-Казахстанский университет имени М. Козыбаева, Петропавловск, Республика Казахстан

[*]E-mail: menwolf94@mail.ru

Аннотация

Приведен анализ научных данных по ботаническому описанию, ареалу распространения и биологическим свойствам травянистых растений семейства Астровые: ромашка аптечная, тысячелистник обыкновенный, календула лекарственная. Показаны возможности практического применения растительного сырья в медицинских целях. Приведен сравнительный анализ химического состава ромашки аптечной, тысячелистника обыкновенного и календулы лекарственной. В составе растений были идентифицированы липиды, аминокислоты, флавоноиды, фенолокислоты, витамины. Проведен количественный анализ липидов и флавоноидов. В результате исследования выявили содержание липидов в растительном сырье от 2,09 до 3,24%, а также содержание флавоноидов в пересчете на кверцетин в пределах от 3,52 до 4,08%.

Ключевые слова: семейство Астровые, *Matricaria chamomilla L., Achillea millefolium L., Calendula officinalis*, тонкослойная хроматография, циркуляционная экстракция, рабочий стандартный образец.

Introduction

Comparative chemical analysis of herbaceous plants of the Asteraceae family was considered on the example of such plants as chamomile, common yarrow, calendula officinalis.

Chamomile – *Matricaria chamomilla L.* is an annual herbaceous plant from the Compositae family. It has strong erect branched stems, the length of which reaches up to 35-40 cm, the leaves are alternate, with a single basket at the ends of the stems and branches. Flowers in baskets with a conical receptacle. The flowers on the edges are white, female reed, the flowers in the middle are bisexual, tubular yellow. The fruits of the achene are from 0.8-1 mm long and 0.25 mm wide with several thin ribs.

The finished raw material is a flower basket of a conical shape without pedicels or with their remains, but not longer than 3 cm. Each basket bears 12-18 marginal reed white pistillate flowers with three cloves at the end and many median tubular yellow bisexual flowers with a five-toothed corolla. All flowers - with a bare lower ovary, without volutes and without a calyx. Receptacle glabrous, hemispherical or conical, pitted, hollow. The wrapper of the basket is tiled, 2-3-row. The smell is fragrant, the taste is bitter-spicy [1-2].

The range of chamomile covers the whole of Europe, Kazakhstan, the Caucasus, Asia, Siberia, Mongolia. Chamomile grows throughout Northern Kazakhstan, grows in fields and weedy places, but due to intensive plowing of land, large thickets are rare, so chamomile is taken into cultivation in the villages. Chamomile also grows in gardens, near dwellings in all areas.

Chamomile is used internally in infusions and decoctions as a diaphoretic, antispasmodic and carminative; externally - for rinses, enemas and as an emollient, anti-inflammatory and antiseptic agent. Chamomile is included in various fees. Chamomile infusion is used for colds, severe pain in the abdomen, cramps, hot flashes, etc. Chamomile decoction, drunk on an empty stomach, cleanses the skin. Chamomile is often used in combination with other plants and individual substances.

Chamomile has disinfectant and anti-inflammatory properties, binds an increase in the secretion of the gastrointestinal tract, enhances bile secretion and stimulates appetite. It has a weak atropine-like effect, relaxes smooth muscles, eliminates spasms of the abdominal organs.

Common yarrow, trees, bloodwort – *Achillea millefolium L.* from the Compositae family. This is a perennial herbaceous plant with a creeping rhizome, from which shoots with rosettes of basal leaves and flower-bearing non-branched stems 20-50 cm high depart. A perennial herb 20-80 cm high. The leaves are lanceolate or linear-lanceolate. The flowers are white, yellow, pink, red, collected in baskets, forming complex corymbs 2-15 cm in diameter. The fruit is a seed. Blooms from July to September [3].

The plant is fragrant. Grows along roads, fields, dry meadows, bushes and forest edges throughout Northern Kazakhstan. Distributed in the forest, forest-steppe, steppe zones on the meadow slopes of the mountains.

Yarrow is an old folk remedy. Its juice was used in Kazakhstan in the 15th century to stop nosebleeds. Later they began to be used as a means of enhancing digestive activity. Yarrow herb infusion significantly increases the rate of blood clotting. In addition, its preparations have anti-inflammatory and bactericidal properties.

An infusion of herbs and a liquid extract - Extractum Millefolii fluidum - are used as a hemostatic agent for internal, mainly uterine bleeding. How bitterness is included in pharmacy tea. A decoction is recommended for washing hair. Raw materials are used in collections and for the preparation of herbal preparations. The shelf life of raw materials is 5 years [4].

Calendula officinalis (marigold) *Calendula officinalis (L.)* is an annual herbaceous plant from the Compositae family. Calendula officinalis - a plant with a branched stem about 60 cm high. The leaves are alternate, the lower ones are elongated, rounded at the end; upper - sessile, lanceolate. The entire green part is pubescent with small hairs. The flowers are yellow, orange, collected in large, single baskets located at the ends of the stems. Marginal flowers are reed, pistillate, arranged in 2-3 rows, and in double varieties - up to 15 rows. Median flowers are tubular, staminate.

The fruits of the achenes are formed from reed flowers and are arranged in 3 rows, the outer, the largest, crescent-shaped, the middle ones are annular, the inner ones are hooked, small, all have tubercles on the outer surface, but when sown they give the same plants. Blooms in July - August. Seeds ripen in August [5].

The area of calendula officinalis covers Central and Southern Europe, Central Asia. In Northern Kazakhstan, calendula officinalis is cultivated everywhere as very popular ornamental flowers; do not meet wildly.

The most famous areas of application of calendula in traditional medicine are diseases of the gastrointestinal tract.

Research methods

The study was carried out using theoretical and experimental methods. The theoretical study consisted in a comparative analysis of scientific sources, generalization of information from similar studies in science. Practical research consisted in the analysis of the chemical composition of the plant using circulation extraction, paper chromatography, thin layer chromatography, qualitative reactions, photometric research methods.

Results and discussion

For a comparative analysis of the chemical composition of the studied plants, the following classes of compounds were determined: lipids, amino acids, flavonoids, phenolic acids, and vitamins.

Qualitative analysis.

1) Determination of lipids.

The investigated fractions were obtained by circulation extraction with 70% concentration of ethyl alcohol on a Soxhlet apparatus. Lipid analysis was carried out by TLC on Silufol plates using the solvent system: petroleum ether - diethyl ether - acetic acid - 80:20:1. Chromatograms were developed in iodine vapor to enhance the color of spots [6].

The lipid composition of the aerial parts of the studied plants includes the following classes of lipids: hydrocarbons, carotenoids, sterol esters, fatty acids, triglycerides, monoglycerides, diglycerides, glycerol 1-O-monoalkyl esters, and phospholipids. Sterols have been found in marigold and chamomile extracts. Waxes were not identified in the submitted samples.

2) Determination of amino acids.

Identification of amino acids was carried out by paper chromatography, in the solvent system butanol-acetic acid-water (15:3:7), developer - 1% solution of ninhydrin in 95% acetone. The analysis of amino acids is carried out by coincidence on the chromatogram of the position of the amino acids of the test mixture with the amino acids of the standard. Amino acids of the standard and test mixture are detected as blue-violet spots (proline with ninhydrin gives a yellow compound)

All samples contained alanine, proline, valine, and aspargine. Cysteine is found only in chamomile and calendula extracts. Histidine is found everywhere except for calendula extract. Leucine is not found in yarrow extract.

3) Determination of flavonoids

To analyze the flavonoid composition, alcohol extracts of the aerial parts of the studied plants were used. Flavonoids were detected using thin layer chromatography on Silufol plates. The system of solvents butanol - acetic acid - water in the ratio 4:1:5 was used. Points were identified using a marker – rutin and quercetin.

To determine flavonoids, the following developers are used: 5% aqueous solution of Na_2CO_3 , 1% solution of $AlCl_3$ (alcohol), 1% solution of $Pb(CH_3COO)_2$.

Spot R_f with a value of 0.69, as a representative of the classes of flavonols - routine; similarly identified spot R_f with a value of 0.79, as a representative of the flavonol classes - quercetin.

The test results showed the same qualitative composition in the studied raw materials. The following classes were identified in the tested samples: flavonols, chalcones, flavonones.

4) Determination of phenolic acids

As a test product for the content of phenolic acids, alcohol solutions of the aerial parts of the studied raw materials were taken. The studies were carried out by TLC on Silufol plates. Chromatography was carried out in the solvent system benzene – dioxane – acetic acid (90:25:4). To develop the chromatogram, an aqueous solution of iron ammonium alum was used, the spots turned green and blue, which also proves the presence of phenolic acids in the analyzed mixture [6].

Cinnamon, salicylic, ferulic, aconitic, caffeic, and gallic acids were found in all samples. Protokachetic acid is found everywhere except for chamomile extract, p-hydroxybenzoic acid is found only in chamomile extract.

5) Determination of vitamins

The presence of quercetin and other flavonoids indicates the presence of P-vitamin activity.

Determination of vitamin A. Dissolve 2-3 ml of water extract in 4-5 ml of chloroform and add concentrated sulfuric acid. A blue color appears, quickly turning into a brown-red.

Determination of vitamin B_1. To 2-3 ml of aqueous extract in a test tube, add 5-10 drops of a 5% solution of potassium hexacyanoferrate (III) and mix the contents thoroughly. When heated, the liquid turns yellow due to the conversion of thiamine to thiochrome.

Next, add 1 ml of isobutyl alcohol to the test tube and shake the contents vigorously. The upper alcohol layer is transferred to another test tube and the fluorescence of this solution is observed in ultraviolet rays [7].

Determination of vitamin B_2. To 2-3 ml of aqueous extract in a test tube, add 5-10 drops of concentrated HCl. Drop 1 pea of metallic zinc into a test tube. The release of hydrogen bubbles begins, the liquid gradually turns pink, then discolors.

Determination of vitamin C. To 1-2 ml of water extract add 2 drops of potassium hydroxide solution, 2 drops of potassium hexacyanoferrate (III) and vigorously shake the contents of the test tube. Then add 6-8 drops of a 10% hydrochloric acid solution and 1-2 drops of a solution of iron chloride (III) to the test tube. We observe the precipitation of a blue (greenish-blue) precipitate of Prussian blue [8].

Determination of vitamin E. To 2-3 ml of alcohol extract add 10 drops of concentrated nitric acid and shake the contents of the tube. An emulsion is formed, which gradually exfoliates: the upper oily layer becomes red in color. Staining is due to the oxidation of α-tocopherol to α-tocopherylquinone, which is red or yellowish red.

Determination of vitamin P. Vitamin P was determined using 2 methods [8]:

- To 1-2 ml of a saturated aqueous solution of the investigated raw material (crushed), carefully add 1 ml of concentrated sulfuric acid along the wall of the test tube. A yellow-colored ring appears at the boundary of two liquids.

- To 1 ml of the extract of the investigated raw material (crushed) was added a piece of magnesium tape or magnesium metal powder and 3-4 drops of concentrated hydrochloric acid. The liquid acquires a shade of pink, but upon standing, the intensity of the color increases.

Vitamins A, B_1, B_2, E, C, P were found in all studied samples (chamomile, calendula officinalis, common yarrow).

Quantitative analysis.

Quantitative determination of lipids.

The isolation of lipids must be carried out quickly, under conditions that maximally exclude the influence of such factors as elevated temperature, air oxygen, and light [9].

2.0-2.5 g of the crushed aerial parts of the studied plants are transferred into dried (at 105°C) and weighed on an analytical balance bags of thick filter paper. The bag with the sample was placed in a 250 ml conical flask, filled with 35-40 ml of acetone, and then 3-4 ml of chloroform was poured into it.

Mix the contents of the flask, close the cork and leave for a week in a dark place. We remove the bag with the fat-free material from the flask, wash it 2-3 times with chloroform, then place it in a wide crystallizer and put it in a fume hood to evaporate the solvent. Then dry for 2.5 hours in an oven at 100-105°C. Then the package is placed in a bottle, cooled in a desiccator for 45 minutes and weighed. If, after drying, yellow or brown streaks appear on the bag, this is due to the oxidation of the oil, which was poorly extracted. In this case, the analysis is repeated, increasing the volume of the solvent and the duration of the extraction.

The percentage of lipids in absolutely dry raw materials is calculated by the formula (1):

$$X = \frac{(m - m_1) \cdot 100 \cdot 100\%}{m \cdot (100 - \omega)} \quad (1)$$

where m – weight of the sample of raw materials before extraction, g
m_1 – weight of the sample of raw materials after extraction, g
ω – raw material moisture content, %

During the experiment, the following experimental data were determined:

- chamomile: $m_1 = 1,96$ g, $m = 2,00$ g, $\omega = 4,47\%$.
- calendula officinalis: $m_1 = 1,94$ g, $m = 2,00$ g, $\omega = 7,45\%$.
- common yarrow: $m_1 = 1,95$ g, $m = 2,00$ g, $\omega = 5,13\%$.

Based on the experimental data, the percentage of lipids was calculated using the formula (1):

- chamomile: $X = \frac{(2,00 - 1,96) \cdot 100}{2,00 \cdot (100 - 4,47)} \cdot 100\% = 2,09\%$;
- calendula officinalis: $X = \frac{(2,00 - 1,94) \cdot 100}{2,00 \cdot (100 - 7,45)} \cdot 100\% = 3,24\%$;
- common yarrow: $X = \frac{(2,00 - 1,95) \cdot 100}{2,00 \cdot (100 - 5,13)} \cdot 100\% = 2,64\%$.

Thus, the content of total lipids in the aerial part of chamomile is 2.09%, calendula officinalis is 3.24%, common yarrow is 2.64%.

Quantitative determination of flavonoids.

For the quantitative determination of flavonoids, alcohol solutions of the aerial part of the studied plants are taken [9].

Preparation of solutions. Solution A: Place 1 g of raw material in a 50 ml volumetric flask, add 20-30 ml of 95% ethyl alcohol, mix and bring to the mark (50 ml). Solution B: Dissolve 20-50 mg of quercetin standard in 50 ml of 90% ethanol. Solution C: 5 g of $AlCl_3 \cdot 6H_2O$ are dissolved in 20 ml of ethyl alcohol.

Analysis.

Test solution: put 5 ml of solution A and 1 ml of solution C into a 10 ml test tube and dilute to 10 ml with 95% ethyl alcohol.

Reference solution for the test solution: put 5 ml of solution A into a 10 ml test tube and dilute to 10 ml with 95% ethyl alcohol.

Quercetin standard solution: put 1 ml of solution B and 1 ml of solution C into a 10 ml test tube, dilute to 10 ml with 95% ethanol.

Reference solution for the standard solution: put 1 ml of solution B into a 10 ml test tube and dilute to 10 ml with 95% ethanol.

After 40 minutes, we measure the optical density of the test and standard solutions at a wavelength of 425 nm in a cuvette 1 cm thick.

The content of the sum of flavonoids in terms of quercetin is determined by the formula (2):

$$X = \frac{D \cdot m_0 \cdot 100 \cdot 100\%}{D_0 \cdot m \cdot (100 - \omega)} \quad (2)$$

where D – optical density of the test solution
D_0 – optical density of standard quercetin raw material
m – weight of raw materials, g
m_0 – weight of quercetin, g
ω - raw material moisture content, %

During the experiment, the following experimental data were determined:

- pharmacy chamomile : D = 0,050, D_0 = 0,027, m_0 = 0,02 g, m = 1,00 g, ω = 4,47%.
- calendula officinalis: D = 0,044, D_0 = 0,027, m_0 = 0,02 g, m = 1,00 g, ω = 7,45%.
- common yarrow: D = 0,051, D_0 = 0,027, m_0 = 0,02 g, m = 1,00 g, ω = 5,13%.

Based on the experimental data, the content of the sum of flavonoids in terms of quercetin was calculated using the formula (2):

- chamomile: $X = \frac{0,050 \cdot 0,02 \cdot 100}{0,027 \cdot 1,00 \cdot (100 - 4,47)} \cdot 100\% = 3,88\%$;
- calendula officinalis: $X = \frac{0,044 \cdot 0,02 \cdot 100}{0,027 \cdot 1,00 \cdot (100 - 7,45)} \cdot 100\% = 3,52\%$;
- common yarrow: $X = \frac{0,051 \cdot 0,02 \cdot 100}{0,027 \cdot 1,00 \cdot (100 - 7,45)} \cdot 100\% = 4,08\%$.

Thus, the quantitative content of flavonoids in terms of quercetin in the aerial part of chamomile is 3.88%, calendula officinalis is 3.52%, common yarrow is 4.08%.

Conclusion

Thus, the qualitative chemical composition of the extracts of the aerial part of the plants of chamomile, calendula officinalis and common yarrow has been studied. The following classes of compounds have been identified in plants: lipids, phenolic acids, amino acids, flavonoids, tannins, and vitamins.

The quantitative content of lipids and flavonoids in the composition of the aerial part of the plants of chamomile, calendula officinalis and common yarrow was studied. The content of lipids and flavonoids in the studied raw materials is approximately the same, lipids in the range of 2,0-3,3%, flavonoids in the range of 3,5-4,1%.

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