Physico-chemical properties of calendula flower extract in bitter almond oil elemental composition and microbiological purity

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Abstract. The study used bitter almonds (Amygdalus communis L. Varietas amara D.C.) obtained by cold pressing medicinal nails grown in Navoi region using oil (Calendula officinalis L.) - with the participation of flowers, the extraction process was carried out by maceration (in a ratio of 1:10). The resulting oil extract was studied by its elemental composition, chromatographic analysis of its fatty acids, microbiological purity, chemical and physical constants. **Key words:** Calendula officinalis L, gingvitis, piorrhea, paradontosis, vitamins, extraction, cold pressing method, chromatographic, element analysis, microbiological purity, physical and chemical constants.

1 Introduction

Calendula flower (Calendula officinalis L.)- the plant is a cultivated annual herbaceous plant, stored during flowering by flavonoids, carotenoids, saponins, excipients and organic acids contained in its collected and dried flowers [1-3]. That is why crushed nail flowers are used for angina, pharyngitis, inflammatory-dystrophic form of oral cavity (gingvitis, piorrhea, paradontosis) and inflammatory diseases of the upper respiratory tract [4-7]. Especially in this area, oil extracts based on medicinal plants occupy a special place. At the moment, oil extracts have a number of advantages (naturalness, simple technology, effective effect, hooliganism) that the demand for such drugs is high. That's why, due to the low content of flavonoids in flanovonoid glycosides and the low melting of quartetine in oil, there is little compared to alcohol separation and clarification [8]. Bitter almond oil obtained by the cold pressing method is widely used in folk medicine [10-14]. It contains vitamins A,E,F, D, linoleic and oleic acid from unsaturated fatty acids [9]. Such oil is recommended for skin rejuvenation, moisturizing, wrinkle removal in body, foot skin, face and hair care. It acts against skin inflammation by increasing skin porosity [15-17]. The constant application of almond oil rejuvenates the skin of the face and protects it from the effects of sunlight.

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2 Research purposes

Bitter almonds obtained by cold pressing method (Amygdalus communis L. Varietas amara D.C.) medicinal calendula flower is grown in Navoi regions using oil (Calendula officinalis L.) prepare an oil extract with the participation of and determine the results on its chromatographic, element analysis, microbiological purity, physical and chemical constants.

3 Experimental part

Oil was extracted from its core using the bitter almond variety grown in the khatirchi District of Navoi region. In the extraction of bitter almond oil by cold pressing method, the almond core was first separated from the shell. Then the bitter almond kernels were placed in gauze and kept in boiling water for about a minute. The maggot of a soaked red color was easily separated, and the maggots of white color were spread out until they dried well. If not in a dry state, the water it contains breaks down the amygdalin contained in the maggot during the pressing process to form benzaldehyde and tsianidic acid, which are toxic. Therefore, the sample was pulled out after dressing the confidence that it was dry. The resulting sample was separated by oil by cold pressing method in the pressing equipment “Akita jp”.

From the crushed part of the dried calendula flowers were pulled out on 10 g scales. The sample was placed in a 100 ml flask and mixed with 20 ml of 96% ethyl alcohol on top. In the wet state of the calendula flower, 100 ml of bitter almond oil was placed on it and sterilized the flask in water for 5-7 minutes. After 24 hours, the extract was filtered.

Getting of fatty acids from the extract: for this, a solution of 10% potassium hydroxide in methanol, thoroughly mixing the extract, and in a state of mixing the examined sample in a ratio of 1:10, hydrolyzed in a boiling water bath for 1 hour. To decompose the resulting soaps, an aqueous solution of 50% sulfuric acid was used. Fatty acids were extracted three times with diethyl ether. After that, the ether separation was washed with water and brought to a neutral environment, dried with sodium sulfate, and the ether separation was driven out. Fatty acids were methylated with diazomethane. To clean the formed methylphyrs, a thin layer of silica gel was passed through the following system of solvents: hexane: diethylephyr (in a 4:1 ratio). Iodine vapors were used to open the spots from which the oil was formed. And chloroform was used to desorption of methylphyrs from the content of silicagel. After the loss of chloroform in methylefires, they were dissolved in hexane and analyzed using a flame-ionization detector on Agilent Technologies 6890 n chromatograph. In this case, the length of the chromatograph capillary column was 30 m, the inner diameter was 0.32 mm, and the deposited phase was nr-5, and the temperature was taken equal to 150-270 OS. Helium was used as a chromatographic gas.

4 Quantitative determination of micro and macro elements from the extract

Element analysis was carried out on an optical instrument (ISPMS) of an emission spectrometer, which is an argon plasma linked by induction. When conducting an analysis of the element, 0.5 g of the extract was pulled out in an analytical torose and transferred it to Teflon autoclaves. It was poured into autoclaves 7 ml 65% Li HNO_3 and 1 ml 30% $LiNO_3$. Autoclaves were closed and Berghofc placed in a microwave decomposition. The decomposition program was determined by the type of test substance and the degree of decomposition, the number of autoclaves (12 pieces) was indicated. After decomposition, the structural content in the autoclave was marked with 50 or 100 ml and the volume 0.5% nitric acid. The method of determination was determined by the detected wavelength of micro or

macro elements with maximum emission. When compiling the sequence of taxlil, showed the amount of MG and the degree of dilution in ML. After receiving the actual quantitative data of the substance in the test sample, the device was automatically detected and entered % with RSD with an error restriction in the form of mg /kg (or mkg/G).

The refraction number of the extract was determined in the LIECA MARK refractometer (Germany) equipment. The density of bitter almond oil extract was measured in the pycnometer and electronic pycnometer - DTNSITY ME ners DE-40 (Japan) equipment. The number of acids, the number of saponification, the number of iodine was determined on the basis of DF-X1.

Determination of microbiological purity of the extract: microbiological cleaning of the oil based on DF XI was checked, taking into account the possibility of damage by microorganisms. Microbiological purity of the oil was carried out using the agar method with two layers of gauze in petri bowls of 90-100 mm. 10 g of the oil sample was taken and dispersed in a phosphate buffer (rn 7.0) until the total size came 100 ml. This emulsion was placed in two 4 ml test tubes, bringing the temperature from 45os to 50os, and placed in the # 1 correspondent. 15-20 ml were taken from the same sample and placed in a nutrient medium. Agarni top floor uniformity was ensured by shaking petri bowls. After cooling the bowls, they were placed in an inverted Holt and left for an incubation period of 5 days at a temperature of 35os. The plantings were observed every day. After 48 hours and after passing 5 days, colonies of bacteria in both cups were calculated, the average value of which was calculated and the number of microorganisms in 1 gr of the sample was determined.

5 Results and discussion

The fatty acids contained in the extract were isolated and analyzed. The composition and quantitative description of fatty acids is given in Table 1. According to the results in the picture, it was found that the content of unsaturated acids in the extract is much higher than saturated acids. Of the main unsaturated acids in bitter almond oil, oleic acid was found to be 71.28%, while the extract found an increase of 73.37. In the case of linolenic acid, it showed a decrease from 19.86% to 18.71. From saturated acids, it was found that the content of palmitic acid in bitter almond oil is 5.91%, while the extract contains 5.61, and palmitolein acid has changed from 0.47% to 0.32%. According to the results of the analysis, it was found that the total amount of unsaturated acids in the oil extract of bitter almonds increased by 92.08%, the total amount of saturated acids decreased by 7.92%.

Table 1. Comparative composition of oils of bitter almond oil and extract.

№	Fatty acids	Peak capture time. minutes.	Bitter almond oil	Extract
1.	Myristic	14:0	0.04	0.05
2.	Palmitine	16:0	5.91	5.61
3.	palmitoleic	16:1	0.47	0.32
4.	Stearin	18:0	2.23	1.71
5.	olein +linolene	18:1+18:3	71.28	73.37
6.	Linoleum	18:2	19.86	18.71
7.	Arachin	20:0	0.09	0.09
8.	Eikosen	20:1	0.07	0.09
∑ saturated fatty acids			**8.27**	**7.92**
∑ unsaturated fatty acids			**91.73**	**92.08**

The element composition of the extract is estimated by the method of inductively coupled plasma mass spectrometry, in which it is determined that there are 20 different elements, the description of which is given in Table 1 below.

Table 2. Results of element analysis of the extract.

Elements	Extract mg/kg	Elements	Extract mg/kg
Ag	0.000144	Na	0.173953
Al	0.508611	Mn	0.013770
As	0.002840	Ni	0.012331
Ba	0.062201	Rb	0.000631
Ca	8.865830	Se	0.000281
Cd	0.000078	Sr	0.026977
Co	0.000304	U	0.000131
Cr	0.036114	V	0.001235
Cu	0.016674	Zn	0.144480
Fe	0.894853	Pb	0.038221

The analysis of the metals contained in the extract comes in handy in the interpretation of its healing properties [9]. The element analysis of the extract showed its richness in macro- and microelements. The following series showed a decrease in the amount of elements contained in the extract:

Ca>Fe> Al> Na > Zn> Ba > Pb> Cr> Sr >Cu> Mn > Ni> As> V> Rb> Co> Se> Ag> U > Cd

The results showed that the extract contains a significant amount of Ca, Na, Al, Ba from macronutrients. It is important that the extract contains Fe, Cu, Zn, Mn, Cr, Ni, Se and other elements from trace elements. It is also worth noting that the content of Biometals Fe, Ni, Mn, Cr, Cu, Zn in the extract is high enough, increasing its healing properties, affects the course of biochemical processes in the body (the exchange of proteins, carbohydrates, lipids, the formation of blood and bones, etc.) [10]. In particular, chromium determines the sugar balance in the body and is involved in the carbon exchange process. Regulates the amount of cholisterin in the blood and the formation of chromium nucleic acids in the cell. Helps in the breakdown of fats, normalizes energy metabolism. Participates in the synthesis of Harmons by replacing iodine in the activity of the thyroid gland. The constant entry of 6 mg of chromium in the body is the norm, it is necessary to note that this extract contains 0.036114 mg of chromium. Zinc is a metal that is part of all enzymes and participates in many substance exchange processes, ensuring the division, growth and normal functioning of all cells in the body. It is important for the growth of skin, nails and hair in moderation, healing wounds, increasing body weight, increasing immunity. Strengthens the brain's ability to remember and prevents anemia by participating in blood production. Zinc is a powerful natural antioxidant and iron is one of the most essential micronutrients in the body. Iron is present in the blood and is part of the hemoglobin component. Hemoglobin carries oxygen from the lungs to tissues and cells, in short, with the help of hemoglobin, the body breathes. The most common cause of anemia, namely anemia, is iron deficiency.

In the composition of the extract, the vital necessary metals are fully preserved, and the content of biomethals Fe, Ni, Mn, Cr, Cu, Zn is high enough, and their presence in the choline of Lyophilic compounds ensures easy assimilation by the human body. The combination of the bioavailability of these elements with Bioorganic substances in bitter almond oil increases the pharmacological value of the oil. From heavy metals Pb (0.00063 mg/kg), Cd (0.000078 mg/kg), quantities were found to be relatively low.

The extract is soluble in hexane, diethyl ether, isopropyl alcohol, benzene, chloroform, acetonitril. Ethyl alcohol, practically insoluble in purified water. The main physical and chemical indicators of the extract are presented in Table 2.

Table 3. Extract determination number indicators.

Number indicators	extract
Acids number. mg KOH/g	2.96
Iodine number. mg/100 g	99.87
Number of refraction. nd^{20}	1.4458
Density g/cm^3	0.950
Number of saponification. mgKOH/g	191.72

As you can see from the table, the acid number for the oil extract is 2.96, the iodine number is 99.87, the refraction number is 1.4458, and the density of the oil extract is 0.950. The number of saponification for the oil extract was 191.72 mg/kg.

The total number of fungi is also calculated by the agar method, the results of which are presented in Table 4.

Table 4. Results of determining the microbiological purity of the extract.

Indicators	Requirements by regulatory document	Results of the analyses	Compatibility of MH request
Total number of aerobic bacteria (in 1g or 1ml)	No more than 10^4	< 10	Fits
Total number of fungi (in 1g or 1ml)	No more than 2×10^2	< 10	Fits
Escherichia coli (in 1g or 1ml)	Should not be	not	Fits
Enterobacteriacae (in 1g or 1ml)	No more than 10^2	not	Fits
Salmonella (in 1g or 1ml)	Should not be	not	Fits

The results show that the extract fully complies with the requirements of the regulatory document on microbiological purity

6 Conclusion

An extract of medicinal nail flowers in bitter almond oil was obtained. Its chemical composition has been studied. It was found that the main part of the extract is made up of unsaturated oleic and linoleic acids. Quantitative determination of micro - and Macroelements in the composition of the extract was determined using the method of inductively coupled plasma mass spectrometry. It was found that the extract has a high content of micro and Macroelements necessary for the human body. The chemical constants of the extract were determined.

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