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PROPOLIS OIL EXTRACT: QUALITATIVE AND QUANTITATIVE ASPECTS

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Abstract

In this work, we studied the extraction of propolis with oils. For the experiment, we used propolis samples collected in different regions of Ukraine (Kharkiv, Sumy, Poltava, Kyiv, and Dnipropetrovsk regions) and determined their compliance with DSTU 4662:2006 "Propolis". All the samples we collected met the requirements of this standard, and a combined batch of propolis was used to obtain oil extracts. The most common vegetable oils (corn, sunflower, and olive oils) were chosen as extragents, and their quality was confirmed. The technological approach to obtaining oil extracts was based on the data of previous studies; the extraction was carried out at 60C and constant stirring for 1 hour. The obtained oil extracts of propolis were evaluated by appearance, acid and peroxide numbers, density, identification and quantification of phenolic substances. Presence of substances of phenolic and flavonoid structure was confirmed by specific reactions. As a result of the quantitative determination of the TPC in the obtained extracts it was established that the total phenolic content (TPC) in the studied oil extracts is about 4.95±0.15 %, which corresponds to the quantitative content of these biologically active substances in the propolis substance (not less than 25.0 %).

Keywords: Propolis; oily extract; technology; quality indexes; phenolic compounds.

МАСЛЯНИЙ ЕКСТРАКТ ПРОПОЛІСУ: ЯКІСНІ ТА КІЛЬКІСНІ АСПЕКТИ

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Анотація

У даній роботі ми вивчали екстракцію прополісу оліями. Для експерименту використовували зразки прополісу, зібрані в різних регіонах України (Харківська, Сумська, Полтавська, Київська, Дніпропетровська області) та визначали їх відповідність ДСТУ 4662:2006 «Прополіс». Усі відібрані зразки відповідали вимогам цього стандарту, а для отримання олійних екстрактів використовували комбіновану партію прополісу. Екстрагентами були обрані найпоширеніші рослинні олії (кукурудзяна, соняшникова, оливкова), якість яких була підтверджена. Технологічний підхід до отримання олійних екстрактів базувався на даних попередніх досліджень; екстракцію проводили за 60 °С і постійному перемішуванні протягом 1 години. Отримані масляні екстракти прополісу оцінювали за зовнішнім виглядом, кислотним і перекисним числами, густиною, ідентифікацією та кількісним визначенням фенольних речовин. Наявність речовин фенольного та флавоноїдного складу підтверджено специфічними реакціями. У результаті кількісного визначення загального вмісту фенольних сполук (ТРС) в отриманих екстрактах встановлено, що ТРС в досліджуваних олійних екстрактах становить близько 4.95±0.15 %, що відповідає кількісному вмісту цих біологічно активних речовин у субстанції прополісу (не менше, ніж 25.0 %).

Ключові слова: прополіс; масляний екстракт; технологія; показники якості; фенольні сполуки.

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Introduction

An urgent task of modern medicine and pharmacy is the development and introduction of new highly effective medicines for the treatment of wound processes of various etiologies and musculoskeletal injuries. Beekeeping products, in particular propolis, have long been used in medical and pharmaceutical formulations [4; 5; 15; 17; 18; 32].

Propolis is a sticky resinous substance that bees collect from the buds and bark of trees, also known as "bee glue" because bees use it to cover surfaces, seal holes and crevices in their hives, thus creating a sterile environment that protects them from microorganisms and spore-forming organisms, including fungi and mold [1–7]. For the production of propolis, bees use materials obtained as a result of various phytobiochemical processes occurring in different parts of plants. The chemical composition of propolis is variable and there are many types of propolis, which are determined by the botanical features of its place of origin. Depending on the plants common in the area, the color of propolis can vary from dark green to brown; the taste is bitter with pungent or astringent tones, with a pungent odor or almost odorless [9; 21].

Chemical composition of propolis is relatively well studied. More than 180 compounds have been identified. Propolis is rich in plant phytoncides, it contains many organic acids and terpene compounds. Propolis contains resin acids and alcohols, artypilin, phenols, tannins, balms (cinnamic alcohol, cinnamic acid), waxes, essential oils, flavonoids, amino acids and a small number of B vitamins. Typical components of propolis are acacetin, apigenin, alpha-acetooxy-betulenol, kaempferid, rhamnosintrin, ermanin [2; 3].

Phenolic compounds are dominant in the composition of propolis. It has a stable content of flavones and flavonols which includes such components as quercetin, isorhamnetin, kaempferol, 3,4'-dimethoxykaempferol, kaempferide, rhamnetin, penduletin, rhamnocitrin, galangin, chrysin and methoxygalangin-I, etc. In addition were isolated and identified flavonoids from the groups of flavones, isoflavones, isohydroflavones, chalcones, dihydrochalcones and other polyphenols - phenylpropanoids, chlorganenic acids, stilbenes, lignans. [2; 12; 13; 21; 29].

The biological activity of propolis is determined by the interaction of all its components. It has been found to have anti-inflammatory, immune-modulatory, wound healing, anesthetic, anticarcinogenic, antimicrobial, antiprotozoal, antiviral, antioxidant, antitumor and antiulcer pharmacological activity [4; 18; 20; 30]. Published studies have shown that propolis and its derivatives contain many natural antimicrobial compounds with a broad spectrum of action against various types of bacteria and that it increases the effectiveness of common antibiotics. Scientific studies have shown that propolis has an effect on more than 100 strains of microorganisms. On gram-positive bacteria – it inhibits the growth of white and golden staphylococci, hemolytic streptococcus, on gram-negative bacteria – it inhibits the growth of pathogens of paratyphoid, toxic infections and fungi of the genus Candida [1; 6; 8; 9; 11]. The antiviral activity of propolis against many strains of influenza has been revealed, and the anti-inflammatory and bronchodilator effect of its preparations in the treatment of chronic lung diseases has been proven [1; 6–8; 11; 13; 14; 19; 23; 31].

Ukraine has abundant sources of this valuable raw material, with an estimated annual production of 5–6 tons of propolis. The growing interest in propolis in the healthcare and medical sector, as well as the growing awareness of its therapeutic properties, are considered to be the main driving forces for the propolis market. These factors are expected to play a significant role in the growth and development of the propolis industry in the coming years.

Due to its wide range of pharmacological effects, propolis, mainly in the form of water-alcohol extracts, is included in pharmaceuticals used as antimicrobial, antifungal, antiviral, anti-inflammatory, wound healing and reparative agents. This ingredient is widely used in cosmetics and in the food industry. [5; 18].

Although ethanol extraction of propolis is a simple and effective method, it has disadvantages such as a strong aftertaste, limited use in the cosmetic and pharmaceutical industries, as it can cause an irritating effect, and has limitations or unsuitability for the treatment of certain diseases in ophthalmology, otorhinolaryngology, pediatrics, or in case of alcohol intolerance.

The nature of the extractant can be different, and studies show that its polarity and extractivity determine the qualitative and quantitative composition of the final product, which determines the pharmacological activity. Extractants such as water, water-propylene glycol solutions, and vegetable oils can also be used in

the extraction of propolis [1; 5; 10]. There is little data on the study of aqueous solutions of propolis. Biologically active substances are mostly poorly soluble in water, and the content of phenolic compounds in aqueous extracts is 10 times lower than in alcohol extracts [10]. However, it is known that aqueous propolis extract has a wider spectrum of antimicrobial activity than alcohol extract [1; 23].

Only a few articles have been devoted to the preparation of propolis oil extracts, where vegetable oils were used as extractants [8; 10; 19]. Microbiological studies have shown the high activity of propolis oil extracts against gram-positive bacteria, yeasts and dermatophytes [19].

Oil extracts (medicinal oils) are one of the oldest dosage forms, but have not found wide application in modern medical and pharmaceutical practice. They are non-toxic, capable of extracting and dissolving a wide range of biologically active compounds, which makes it possible to produce phytochemicals with a high content of active ingredients. In the formulation of semi-solid dosage forms, medical oils, in addition to their pharmacological effects, affect the rheological properties of the base, spreading and penetration of the drug. It should be noted that oils, as lipophilic solvents, promote the release of a whole group of valuable fat-soluble components contained in plant materials, such as carotenoids, steroids, tocopherols, retinol, chlorophylls, organic and fatty acids, as well as a number of unsaturated fatty acids, K vitamins, D vitamins, essential oils and other compounds. Unlike other extracts (decoctions, alcoholic tinctures, dry concentrates), the absorption of an oil extract occurs not only through the blood but also through the lymphatic system. Secondly, for many medicinal substances and vitamins, oil is a good preservative, it protects substances from direct access of oxygen and other oxidants, and there are practically no chemical reactions between plant ingredients in oil, since there is no dissociation (plant extracts contain a number of components that act synergistically). The oil restores vascular microcirculation, dissolves Peyer's plaques and removes toxins.

Such extracts may be the optimal form of propolis for inclusion in topical medicinal and cosmetic preparations, so in our research we focused on the oil extraction of propolis and the evaluation of quality indexes of these extracts.

Materials and methods

Samples and materials. Propolis samples were collected in 2021 in Kharkiv, Sumy, Poltava, Kyiv and Dnipro regions (Ukraine).

The quality indexes of propolis samples were evaluated according to DSTU "Propolis" 4662:2006 [24]. From each sample 5-6 point samples were selected. The weight of the combined sample should be at least 25.0 g. The combined sample was cooled at minus 10 °C, grinded and mixed. Then the combined sample was divided into two parts, placed in clean, dry jars, one of which was sent for testing, the other was hermetically sealed and stored in case of repeated testing.

The study of microbiological purity and antibacterial activity of propolis samples was performed according to the methods of the State Pharmacopoeia of Ukraine [25] at the Department of Clinical Laboratory Diagnostics and Immunology of NTU "KhPI".

Sunflower oil (DSTU 4492:2017), corn oil (DSTU 8808:2003), and olive oil (DSTU 5065:2008) were used for extraction [28]. Samples of vegetable oils were prepared in accordance with the requirements of the State Standards of Ukraine (DSTU 2575-94; DSTU 4349:2004) [28]. The following main quality indexes of vegetable oils were determined: acid number - by titration (neutralization) of free fatty acids with alkali in the presence of an indicator (phenolphthalein) and peroxide number – by titration of isolated iodine with sodium thiosulfate solution (DSTU ISO 660:2006; DSTU 4570:2006) [26; 27].

A Specord 200 spectrophotometer (Germany), Sartorius analytical balance (SARTORIUS, Germany), and class A glassware that meets the requirements of the State Pharmacopoeia of Ukraine [25] were used in the experiment.

Methods.

Extracts preparation. Propolis was ground to a particle size of 3–5 mm and weighed 10.00 g. 90 g of sunflower, olive, or corn oil was poured into a porcelain mortar, placed in a water bath, and heated to a temperature of 60 °C. Grinded propolis was added to the heated oil in portions. The extraction process was carried out for 1 hour with constant stirring at a temperature of 60 °C. After this time, the extract was cooled to 45 °C and filtered through a double layer of gauze. The biological activity of propolis is mainly related to phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives. The standardization of propolis preparations by the total content of phenolic compounds is accepted [16; 22; 24; 25].

Identification. **A.** The ultraviolet absorption spectrum of the studied propolis solution

prepared for quantitative determination in the region from 220 nm to 320 nm has a maximum absorption at a wavelength of (290 ± 2) nm.

B. *Hydroxyl group in the phenolic ring.* A 1.0 g weight of propolis solution was placed in a 50 mL conical flask, 20 mL of ethanol (96 %) P was added and stirred for 10 min or placed in an ultrasonic bath for 10 min. The solution was filtered through a paper filter (ethanol extract).

To 2 ml of the alcohol extract of propolis was added 0.2 ml of *iron (III) chloride* P3 solution, and a brownish-green color was appeared.

C. *Phenolic compounds*. To 2 mL of ethanol extract was added 0.25 mL of lead (II) acetate of basic solution P, and a bright yellow precipitate was formed.

D. *Cyanidine reaction*. To 1 ml of ethanol extract were added 2-3 drops of concentrated hydrochloric acid and 1–2 parts of magnesium metal powder. A pink coloration was observed.

Quantitative determination of total phenolics compound (TPC). The determination was carried out by absorption spectrophotometry in the ultraviolet and visible regions in accordance with the requirements of the State Pharmacopoeia of Ukraine 2.0, 2.2.25.

The test solution. About 0.05 g of propolis oil solution (exact weight) was placed in a 50 ml conical flask, 25 ml of 96 % alcohol was added and heated with reflux condenser for 30 minutes, then cooled and filtered through a paper filter into a 25.0 ml volumetric flask and brought to the mark with 96 % alcohol. 1.0 ml of the resulting solution was placed in a 10.0 ml volumetric flask, brought to the mark with 96 % alcohol and stirred.

Potassium dichromate standard sample solution. Place about 0.06 g (exact weight) of potassium dichromate P in a 1000.0 mL volumetric flask, dissolve in 100 mL of water R, add 5 mL of sulfuric acid diluted with P, bring the volume of the solution to the mark with water and stir.

Compensating solution. 96 % ethyl alcohol.

The optical density of the test solution was measured on a spectrophotometer at a wavelength of 290 nm in a cuvette with a layer thickness of 10 mm in comparison with the compensation solution.

In parallel, the optical density of the standard sample of potassium dichromate solution was measured using water R as a compensation solution.

The TPC (X), in %, was calculated by the formula (1):

$$x = \frac{A_1 \cdot 25 \cdot 10 \cdot m_0 \cdot 0{,}1715 \cdot 1000}{A_0 \cdot m \cdot 1 \cdot 1000}, \quad (1)$$

A_1 is the optical density of the test solution;
A_0 is the optical density of the solution of the standard sample of potassium dichromate;
m is the weight of 10% propolis solution in oil, g;
m_0 is the weight of potassium dichromate, g;
0.1715 is the conversion factor for the absorption of potassium dichromate to the sum of phenolic compounds at a wavelength of 290 nm.

The TPC in the oil solution of propolis should be from 4.75 % to 5.25 %.

The determination of organoleptic indexes, extract density, acid and peroxide numbers was carried out according to the methods of the State Pharmacopoeia of Ukraine [25].

In accordance with the requirements of the State Pharmacopoeia of Ukraine (vol. 1, p. 77), article "5.3. N.1. Statistical analysis of the results of a chemical experiment" [25], was determined the metrological characteristics of the methodology (calculation of measurement uncertainty),

where $\bar{X}$ – average value obtained from the study of six samples,
S 2 –variance,
S – standard deviation,
$S_{\bar{x}}$ – standard deviation of the mean result;
Δx - confidence interval of the result of a single determination,
$\Delta\bar{x}$ – confidence interval of the mean result,
ε – relative uncertainty of the result of a single determination,
$\bar{\varepsilon}$ – relative uncertainty of the mean result.

Results and discussion

The selected raw material was analyzed by its appearance – it is a resinous amorphous or brittle mass, heterogeneous in structure. The color depends on the geographical origin and place of deposition in the hive, on contamination and storage conditions and varies from gray to brownish-green. The smell of propolis resembles the spicy aroma of vegetable resins and essential oils or may be absent altogether. The taste is bitter, burning, astringent [5; 24]. Consistency depends on temperature. At temperatures below 15 °C, propolis is hard and brittle, at 20–30 °C and above, propolis becomes soft and plastic. Freshly collected propolis is soft and sticky, and during storage and exposure to sunlight it hardens and becomes brittle. Propolis becomes liquid at a temperature of 64–69 °C. Its density depends on the wax content and ranges from 1.11 to 1.27 g/cm^3.

The quality of propolis as a commercial product and raw material for the pharmaceutical industry is regulated by DSTU-4662:2006 "Propolis. Technical conditions". The results of the study of propolis samples collected in different regions of Ukraine for organoleptic and physicochemical parameters are shown in Table 1. All data are the average of 3 measurements.

Table 1

Quality indexes of propolis collected in different regions of Ukraine according to DSTU-4662:2006 "Propolis. Technical conditions"

Index	Requirements	Propolis sample				
		Kharkiv region	Sumy region	Poltava region	Kyiv region	Dnipro Region
Description	Dark gray with a greenish, yellowish or brownish tint, heterogeneous at the fracture with a specific odor	Dense lumps of grayish-brown color , non-uniform size, with a weak specific smell	A wax-like mass of yellow-brown color, heterogeneous on fracture with a specific smell	Wax-like mass of brownish-green non-uniform in color with a specific smell	A wax-like mass of grayish-yellow color, heterogeneous on fracture with a weak specific smell	A brownish-yellow wax -like mass non-uniform in color with a specific smell
Solubility	practically not soluble in water, alcohol, ether, chloroform, acetone	responds	responds	responds	responds	responds
Identification :						
with lead (II) acetate solution	form yellow precipitate (phenol compounds)	responds	responds	responds	responds	responds
with iron (III) chloride solution	appears brown – green coloring (phenolic compounds)	responds	responds	responds	responds	responds
Cyanidine reaction	solution colored from orange to red color (flavonoids)	responds	responds	responds	responds	responds
Loss in mass at drying, %	should not exceed 3 %	1.4	2.1	2.7	1.8	2.4
Mechanical impurities, %	no more than 15 %	7.3	8.4	9.7	12.3	8.5
Wax, %	no more than 20 %	10.7	14.6	12.8	13.5	16.6
Extractive substances, %	no more than 35 %	31.4 + 0.1	33.2 + 0.1	29.9 + 0.2	34.2 + 0.1	32.5 + 0.1
Antimicrobial activity	should suppress growth of the test microorganism in a concentration of no more than 0.08 %	responds	responds	responds	responds	responds
Microbiological purity	is allowed the presence of no more than 100 micro-organisms, incl. fungi in 1 g of the sample. Not allowed availability bacteria family *Enterobacteriaceae , Staphylococcus aureus, Pseudomonas aeruginosa*	responds	responds	responds	responds	responds
Phenolic compounds, %	Sum of phenolic compounds should be at least 25 %	27.1 + 0.3	29.4 + 0.1	27.9 + 0.2	32.2 + 0.2	30.5 + 0.1

Despite the differences in appearance, which may be due to the peculiarities of the geographical region, the type of flora and the conditions of bees keeping, the samples were almost identical in terms of physical, chemical and microbiological parameters. All selected samples of propolis met the requirements of DSTU "Propolis" 4662:2006 [24], did not differ significantly in the content of phenolic compounds and were used to prepare oil extracts. Given that the samples of propolis we selected met the DSTU "Propolis" 4662:2006 they were combined for further research. This is reasonable from the point of view that about 100 g of

propolis can be collected from one bee colony per season and the industry uses pooled batches of propolis that meet the requirements of the above standard.

Sunflower, olive and corn oils were chosen as extractants. Their composition is dominated by monounsaturated oleic acids with a small amount of di- and triunsaturated acids. As natural antioxidants, they reduce oil oxidation and increase its shelf life. Oil quality indexes are shown in Table 2. All data are the average of 3 measurements.

Table 2

Quality indexes of vegetable oils

Index	Refined sunflower oil	Refined olive oil	Refined corn oil
Transparency:	Transparent, without sediment	Transparent, without sediment	Transparent, without sediment
Taste and smell	The taste is rich, without bitterness Light aroma, inherent sunflower oil	The taste is rich with the aftertaste of a ripe olive, the aroma is pleasant of a ripe olive	light yellow in color with a weak characteristic smell, mild piquant and slightly sweet taste
Acid number (mg KOH /g)	0.23 ± 0.17	0.22 ± 0.27	0.25 ± 0.18
Peroxide number (1/2 O mmol /kg)	5.80 ± 0.26	5.28 ± 0.30	5.67 ± 0.15

The results of our research showed that the acid number and peroxide number in the oil samples met the requirements of the DSTU [26–29] for this type of oil, which confirms the quality of the oils and compliance with the requirements during production, processing and transportation.

Based on the literature, we chose the concentration of propolis in the oil extract – 10 %, as the most optimal for providing antimicrobial properties [3; 5; 7; 8; 11; 14; 17].

To reduce viscosity and increase extractivity the extractant was heated to 60 °C. This temperature regime is also considered optimal for the extraction of thermolabile substances that can be destroyed at higher values [5; 10; 13]. At elevated temperatures propolis forms a viscous, sticky mass that adheres to the walls of the extractor, so the extraction was carried out with constant stirring. After one hour the propolis was almost completely dissolved in the oil, leaving a slight insoluble dark brown precipitate. Longer extraction times do not affect the completeness of the extraction.

After cooling all obtained extracts formed a thick ointment-like mass with a characteristic smell of propolis, the color varied from yellowish-brown to greenish-brown, depending on the type of oil used as an extractant.

The results of determining the organoleptic and physicochemical parameters of propolis extracts are shown in Table 3. All data are the average of 3 measurements.

Table 3

Organoleptic and physicochemical parameters of propolis oil extracts

Sample	Description	Acid value (mgKOH /g)	Peroxide value (1/2 O mmol /kg)	Density g/ ml
Propolis extract 10 % in sunflower oil	A thick ointment-like homogeneous mass of yellowish-brown color, with a characteristic smell of propolis	0.19 ± 0.22	5.25 ± 0.21	0.936 ± 0.13
Propolis extract 10 % in olive oil	A thick ointment-like mass of uniform greenish-yellow color, with a characteristic smell of propolis	0.21 ± 0.16	4.67 ± 0.19	0.937 ± 0.23
Propolis extract 10 % in olive oil	A thick ointment-like homogeneous mass of yellowish-brown color, with a characteristic smell of propolis	0.19 ± 0.24	4.86 ± 0.22	0.94 ± 0.18

To identify compounds of phenolic structure identification reactions with solutions of iron (III) chloride and lead (II) acetate were used. As a result of the reaction a specific color or precipitate was formed, which confirmed the presence of polyphenolic substances in the studied propolis oil extracts. Substances of flavonoid structure were confirmed by a specific reaction with a cyanidin sample. The results of the identification of phenolic compounds in the

studied propolis extracts are shown in Table 4. All data are the average of 3 measurements.

Table 4

Results of identification of phenolic compounds in propolis oil extracts

Sample	With iron (III) chloride solutions	With a solution of lead acetate (II)	Cyanidine test
10 % propolis extract in sunflower oil	a brown-green color appears	a bright yellow precipitate is formed	pink color
10 % propolis extract in olive oil	a brown-green color appears	a bright yellow precipitate is formed	pink color
10 % propolis extract in corn oil	a brown-green color appears	a bright yellow precipitate is formed	pink color

The method of absorption spectrophotometry in the ultraviolet region was used to identify polyphenolic compounds in the object of study using a specific absorption maximum of 0.02 % alcohol solution (Fig.).

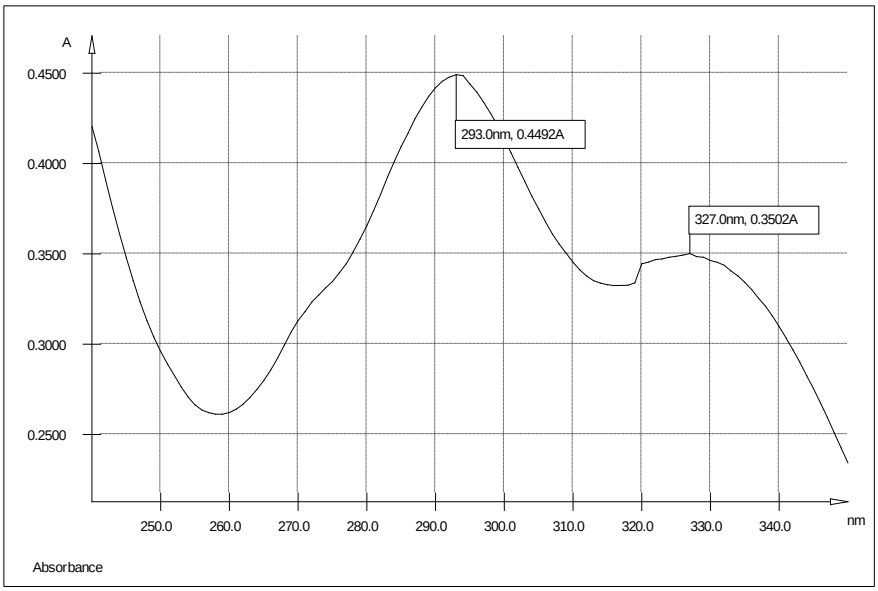

Fig. UV spectrum of 0.02% ethanol propolis solution

The presence of a specific absorption maximum of the ethanol solution obtained from the studied propolis oil solution at a wavelength of 293 nm (Fig.), inherent in flavonoids and aromatic acids containing phenolic groups, led to the use of the method of absorption spectrophotometry in the ultraviolet region for the standardization of propolis [24; 25].

The spectrophotometric method is based on the determination of the optical density of ethanol solutions of propolis at a wavelength of 290±5 nm. The content of TPC in propolis is calculated by the method of specific absorption or by the method of an external standard. As an external standard, a solution of potassium dichromate was used, the linear dependence of which was proved by previous studies [24; 25].

The results of spectrophotometric determination of the TPC in propolis oil extracts are given in Table 5. All data are the average of 3 measurements.

Table 5

TPC in propolis oil extracts

Sample	Weight of propolis solution, g	Optical density	TPC, %	Metrological characteristics
10 % propolis extract in sunflower oil	0.0501	0.4492	4.92	$\bar{X}$= 4.95 S^2 = 0.0208 S = 0.1442 $S_{\bar{x}}$= 0.0589 Δx = 0.3706 $\Delta\bar{x}$= 0.1513
10 % propolis extract in olive oil	0.0553	0.5050	5.01	
10 % propolis extract in corn oil	0.0537	0.4708	4.81	

ε= 3.06 %
$\bar{\varepsilon}$ = 7.50 %

As a result of the quantitative determination, it was established that the TPC in the studied oil extracts is about 4.95±0.15 %, which corresponds to the quantitative content of these biologically active substances in the propolis substance (not less than 25.0 %). For all samples the results of determining the amount of TCP are within the statistical error. The oils we used in the experiment can be applied as extractants for the production of propolis oil extracts with specified quality indexes.

Many scientific works of recent decades are related to the study of propolis extracts and the determination of their quality indicators [2; 9; 12; 16; 21; 22; 29], but only a few of them are devoted to the study of propolis oil extracts [8; 10; 19]. Propolis has a very complex chemical composition, so the generally accepted method of its standardization is the determination of TPC [2; 9; 16; 22; 24; 25]. A commonly used spectrophotometric method for determining the mass fraction of flavonoid and other phenolic compounds in propolis at a wavelength of 290 nm [22; 24; 25]. At the same time in the studies of foreign scientists the Folin-Cocalteu method is most often used to quantify the amount of phenols in terms of the equivalent of gallic acid [2; 16; 29].

Propolis extracts were obtained by Kubiliene et al. using different extractants [10]. The type of solvent and extraction conditions significantly affect the total content of phenolic compounds. Thus, when extracted with olive oil in the ratio of raw material to extractant 1:10 for 5 hours at room temperature, the content of phenolic compounds in the final product was 0.5±0.2 mg/ml GAE, and when extracted with a mixture of polyethylene glycol, olive oil and water when heated to 70 °C, the extraction time was reduced to 15 minutes, and the yield of phenolic compounds was 9.5±1.3 mg/ml GAE.

Propolis extracts obtained using different solvents in a ratio of raw material to extractant of 1 : 10 and heated to 40 °C for 7 days were examined for the total flavonoid content [13]. For the propolis extract in olive oil, the total flavonoid content was 0.20 ± 0.04 %.

According to the results of our studies, the total content of phenolic substances in 10 % propolis oil extract was 4.95±0.15 %, which correlates with the data of previous studies.

The antimicrobial activity of propolis extract in olive oil determined by Tosi et al [19] was slightly higher against gram-positive bacteria, yeasts, and dermatophytes compared to glycerol extracts, and practically did not differ from the antimicrobial activity of ethanol extracts. This confirms the prospects for the introduction of propolis oil extracts into the formulations of semi-solid dosage forms with antimicrobial activity.

Conclusions

This study investigated the extraction of propolis with oils and determined the quality indexes of the starting materials: propolis and oil extragents (corn, sunflower, olive oils), as well as evaluated the qualitative and quantitative indexes of the obtained extracts.

It was determined that all the propolis samples collected in different regions of Ukraine meet the requirements of DSTU 4662:2006 “Propolis”. The quality of the vegetable oils used as extractants was confirmed. Obtained propolis oil extacts were evaluated by appearance, acid and peroxide values, density, identification and quantification of phenolics. Presence of substances of phenolic and flavonoid structure were confirmed by a specific reactions. As a result of the quantitative determination of the TPC in the obtained extracts it was established that the TPC in the studied oil extracts is about 4.95±0.15 %, which corresponds to the quantitative content of these biologically active substances in the propolis substance (not less than 25.0 %).

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