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## The study of biological active compounds of lipophilic fraction of *Schisandra chinensis* (Turcz.) Baill seeds

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**Abstract:** *Schisandra chinensis* (Turcz.) Baill - Chinese magnolia - a well-known fruit plant in traditional Chinese and Korean medicine. The fruits and seeds of *S. chinensis* are rich in biologically active substances. According to the literature (Irfan M. etc, 2020; Liu G-Z. etc, 2020; Sun W. etc, 2021; Zhao T. etc., 2021; Bodrevich B.B. etc, 2017) the following therapeutic effects of *S. chinensis* fruits and seeds can be called: immunomodulatory, tonic, adaptogenic, anti-inflammatory, hepatoprotective, nephroprotective, tonic. The study of the *S. chinensis* raw materials continues in the future, to reveal a wider range of therapeutic actions. The study of *S. chinensis* seeds is quite relevant to expand the range of standardized medicinal raw materials, which is not included in the monographs of the State Pharmacopoeia of Ukraine. The main purpose of this study is to obtain the lipophilic fraction of *S. chinensis* seeds and to study the composition of different groups of lipophilic substances in the resulting fraction. The main method of obtaining the lipophilic fraction of *S. chinensis* seeds is a comprehensive extraction in the Soxhlet apparatus. The chemical reaction was used to determine the presence of tocopherols. The presence of the main groups of biologically active substances in the lipophilic fraction of *S. chinensis* seeds was established by the method of two-dimensional thin-layer chromatography. The study of fatty acid composition was performed by gas chromatography/mass spectrometry (Su L. etc, 2020). The yield of lipophilic fraction of *S. chinensis* seeds was 35.79±0.7%. The obtained lipophilic fraction of *S. chinensis* seeds is an oily liquid of deep yellow color with a characteristic specific odor. The presence of tocopherols was established. Thin layer chromatography in the solvent system: I direction - hexane-acetone (8: 2), II direction - hexane-acetone (8: 4), development reagent - Stahl reagent, identified at least 14 compounds of terpene nature. As a result of studies of fatty acids by gas chromatography/mass spectrometry, the quantitative content of 6 fatty acids was identified and established: palmitic, linoleic, oleic, stearic, gondoic and nonadecanoic. The quantitative content of unsaturated fatty acids significantly exceeded the quantitative content of saturated fatty acids. The results of the study give grounds to draw the following conclusions: the studies confirm the rich composition of the lipophilic fraction of *S. chinensis* seeds: tocopherols, 14 terpenes, 6 fatty acids, of which saturated fatty acids (palmitic, stearic, nonadecanoic) and unsaturated fatty acids, oleic and gondoic); The results of the study suggest the anti-inflammatory, antioxidant, regenerating effect of the lipophilic fraction of *S. chinensis* seeds.

**Keywords.** [chromatography](#), [fatty acids](#), [mass spectrometry](#), [schisandra](#), [seeds](#)

## Introduction

Current researches are increasingly focused on the study and expansion of knowledge about the fruit plant called Chinese magnolia - *Schisandra chinensis* (Turcz.) Baill. The fruits and seeds of this plant are known for their healing properties (Skrypchenko N.V. etc, 2020). From the literature (Kortesoja M. etc, 2019; Xu M. etc, 2020; Skrypchenko N. and others, 2017) we know about the main therapeutic activities of *S. chinensis*: adaptogenic, tonic, reparative, anti-inflammatory, immunomodulatory. Medicinal plant raw materials of Chinese magnolia - fruits and seeds - are rich in the following groups of biologically active substances: lignans, fatty acids, organic acids, macro- and micronutrients, polysaccharides, essential oils, tannins, saponins (Kortesoja M. etc, 2019).

In official medicine, the fruits and seeds of Chinese magnolia are used as a tinctures, tablets, capsules and powders. The pharmaceutical market of Ukraine distinguishes functional food products as dosage forms. *S. chinensis* fruits are a part of them. These functional products exhibit adaptogenic and tonic properties, prevent infectious diseases, strengthen the general immunity (Instructions for use of Chinese magnolia Energy). The study of the raw materials of Chinese magnolia continues in the future, to reveal a wider range of therapeutic actions. The State Pharmacopoeia of Ukraine includes a monograph on *S. chinensis* fruits. Seeds are not pharmacopoeial raw materials. Therefore, the study of biologically active substances of Chinese magnolia seeds is a relevant.

Among the important substances that need to be studied are lipophilic compounds. These include, for example, fatty acids. Fatty acids are carboxylic acids with an aliphatic chain. They are usually saturated or unsaturated. There are two main types of the unsaturated fats: monounsaturated and polyunsaturated. Fatty acids are important dietary sources of fuel for human body and important structural components for cells. They are involved in fat biosynthesis. Fatty acids are contained in lipophilic fractions of medicinal plant raw materials and determine their therapeutic effect: anti-inflammatory, reparative, antibacterial, etc. (He M. etc, 2020, Melnyk I.I. and others, 2021).

Fatty acids are replenished by food. When insufficient amounts of fatty acids enter the human body, atherosclerosis develops rapidly, which causes a number of cardiovascular diseases. Fatty acids reduce the aggregation capacity of platelets, blood cholesterol levels, blood pressure, increase the elasticity of the walls of blood vessels. The lack of fatty acids can lead to stroke or myocardial infarction (Fedosov A.I. etc., 2018).

## Aim

The purpose of our study is to obtain the lipophilic fraction of Chinese magnolia seeds and to study the different groups of biologically active substances there.

Raw materials were collected during flowering in the department of acclimatization of fruit plants of Hryshko Kyiv National Botanical Garden NAS of Ukraine.

## Methods

*Extraction.* The lipophilic fraction from crushed Chinese magnolia seeds (1.0-1.5 mm) was obtained by the method of exhaustive extraction in the Soxhlet apparatus. In this method, chloroform was used as an extractant (Shao. S. etc., 2020). Calculation of the yield of lipophilic substances (X) of Chinese magnolia seeds in percent was performed according to the following formula 1:

$$X = \frac{(m_1 - m_0) \times 100}{m} \quad (1),$$

where X – the content of lipophilic fraction, (%);  
 $m_1$  – the weight of the receiver with lipophilic extract, (g);

$m_0$  – the weight of empty receiver, (g);

$m$  – the weight of Chinese magnolia seeds, (g).

We studied the organoleptic and physicochemical parameters of the lipophilic fraction after obtaining.

*The presence of tocopherols.* The presence of tocopherols was determined by a chemical reaction. 0.05 g of lipophilic fraction was dissolved in 1 ml of chloroform in a test tube with a ground stopper. 2 ml of 0.2% solution of phosphorus-molybdenic acid in glacial acetic acid were added. Observed the presence of the corresponding color (Marchyshyn SM and others, 2020).

*Thin layer chromatography.* The study by two-dimensional thin layer chromatography were performed on plates Silufol UV-254 (Kavelier,

Czech Republic), size 150\*150 mm in solvent systems hexane-acetone: I direction - hexane-acetone (8:2) II direction - hexane-acetone (8:4). Solvents for the preparation of the system used ch.d.a. or h.h. 1% n-dimethylaminobenzaldehyde (Stahl's reagent) was used to develop the chromatogram. After treatment of the chromatographic plate with freshly prepared reagent, it was dried in a fume hood at a temperature of 80-90 ° C for 2-5 min (Nowak A. etc, 2019). Then observed on the chromatogram showed spots that had different colors. The chromatographic plate was viewed in UV light, both before and after treatment with the reagent.

*The research of fatty acid composition.* Fatty acid analysis was performed by gas chromatography/mass spectrometry. A portion of the lipophilic fraction was placed in a glass vial. 2.0 ml of a 2% solution of acetyl chloride in methanol and a solution of nonadecanoic acid (as an inner standard) were added. The mixture was stirred and placed on an ultrasonic bath at 80 °C. Fatty acid methylation was performed for 2 h, after which the obtained fatty acid methyl esters were extracted with hexane. An aliquot of the extract was used for the chromatographic study.

Chromatographic separation was performed on a gas chromatographic mass spectrometric system Agilent 6890N/5973 inert (Agilent technologies, USA). Capillary column HP-5ms (30 m × 0.25 mm × 0.25 μm, Agilent technologies, USA). The separation conditions were as follows: evaporator temperature - 250 °C; interface temperature - 280 °C; temperature programming mode: initial temperature - 150 °C / 4 min, with gradient 5 °C / min, final temperature - 300 °C / 6 min. A sample volume of 1 μl was administered in a flow separation mode of 1:50. SCAN detection mode in the range of 38-400 m/z. The carrier gas flow rate is 1.0 ml/min (Marchyshyn S.M. et al., 2020).

The identification of methyl esters of fatty acids was performed using data from the library of mass spectra NIST 02. The calculation of the quantitative content was performed by adding a solution of internal standard (20 μg / sample) in the test sample according to formula 2:

$$X = \frac{S_x \times m_{is} \times 1000}{S_{is} \times m} \quad (2),$$

where  $X$  – fatty acid content, mg/g;

$m_{is}$  – the mass of the internal standard for the test;

$m$  – the mass of the sample, g;

$S_x$  – the area of the identified compound;

$S_{is}$  – area of internal standard.

## Results

The yield of lipophilic fraction of Chinese magnolia seeds was 35.79±0.7%. The lipophilic fraction of Chinese magnolia seeds is an oily liquid with rich yellow color and a characteristic inherent odor. The solubility of lipophilic fraction of *S. chinensis* seeds: partially soluble in alcohol (with the formation of a white precipitate); completely soluble in chloroform; insoluble in water.

As a result of studying the presence of tocopherols in the lipophilic fraction of Chinese magnolia seeds, an intense emerald green color was observed in vitro with the test solution and reagent. This confirms the presence of tocopherols in the studied lipophilic fraction.

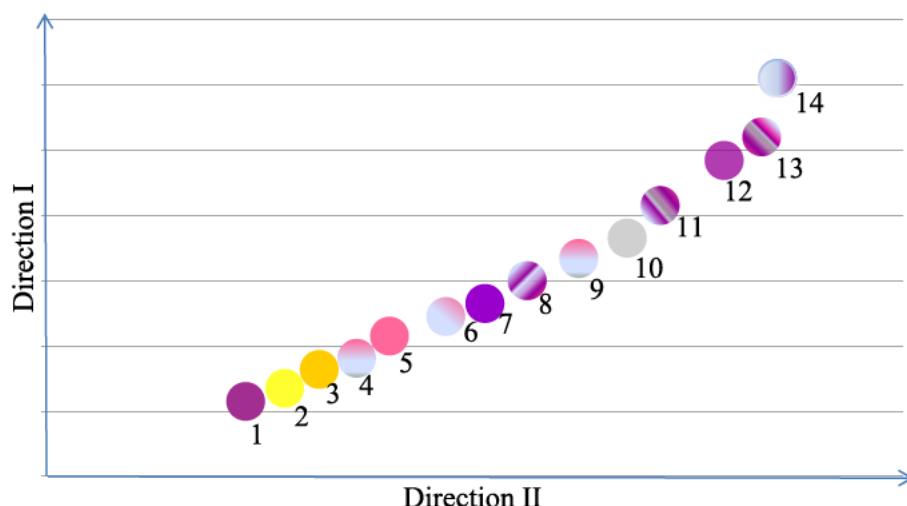
At least 14 compounds were identified in the lipophilic fraction of Chinese magnolia seeds by thin layer chromatography. The scheme of thin-layer chromatography of the lipophilic fraction of Chinese magnolia seeds is shown in Fig. 1.

Spots with the following color were found on the chromatogram in visible light: 1) purple; 2) yellow; 3) yellow; 4) pink-gray; 5) roses; 6) pink-gray; 7) purple; 8) pink-purple; 9) gray-purple; 10) gray; 11) gray-purple; 12) lilacs; 13) pink-purple; 14) gray-purple.

The color of the spots generally corresponded to pink-brown when viewing the chromatograms in UV light at a wavelength of 254 nm, only spot 3 corresponded to a dark yellow color; in UV light at a wavelength of 365 nm, the color of the spots changed to yellow, dark yellow, dark brown, white-blue.

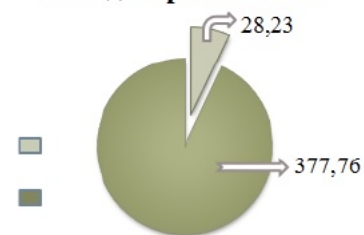
After treatment with Stahl's reagent in visible light, the yellow spots remained unchanged, the blue and brown spots turned pink and purple, new spots and purple spots appeared (Fig. 1); in UV light at a wavelength of 365 nm, four yellow spots were visible. All of the above indicates the presence of terpene compounds..

The quantitative content of 6 fatty acids (table 1) was identified and determined by gas chromatography/mass spectrometry: palmitic, linoleic, oleic, stearic, gondoic and nonadecanoic. The



**Figure 1.** Chromatogram of the lipophilic fraction of Chinese magnolia seeds in visible light. Solvent system: I direction - hexane-acetone (8:2), II direction - hexane-acetone (8:4); detection: Stahl's reagent.

Склад жирних кислот



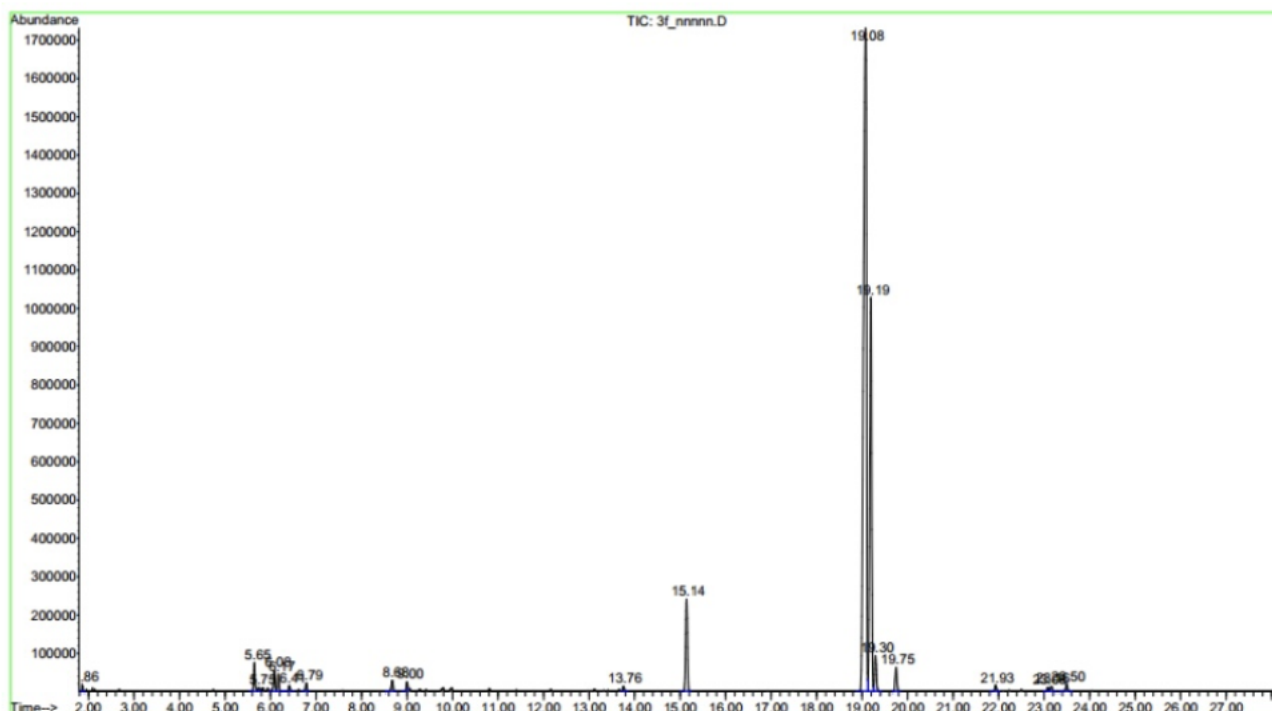
The sum of unsaturated fatty acids 377.76 mg/g  
 The sum of saturated fatty acids is 28.23 mg/g

**Figure 3.** Comparison of the sum of saturated and unsaturated fatty acids in the lipophilic fraction of Chinese magnolia seeds

chromatogram of fatty acids of the lipophilic fraction of Chinese magnolia seeds is shown in Figure 2.

The sum of unsaturated fatty acids of the lipophilic fraction of Chinese magnolia seeds exceeds the sum of saturated fatty acids and is equal to 377.76 mg/g and 28.23 mg/g, respectively, as illustrated in Figure 3.

The lipophilic fraction of Chinese magnolia seeds contains linoleic acid in a big amount ( $267.56 \pm 1.4$  mg/g), which is an essential fatty acid and has the following therapeutic effects: antioxidant, anti-inflammatory, reparative (Melnyk I.I. and others, 2021). Linoleic acid is used in the food industry: baby formula and sports nutrition. It is becoming more popular in cosmetology and



**Figure 2.** Chromatogram of fatty acids of the lipophilic fraction of Chinese magnolia seeds

Number	The trivial name of fatty acids	Systematic name of fatty acids	Chemical formula	Content, mg/g
<b>Saturated fatty acids</b>				
1	Nonadecanoic acid	Honadecyl acid	C19H38O2	Inner standard
2	Palmitic acid	Hexadecanoic acid	C16H32O2	22.57±0.72
3	Stearic acid	Octadecanoic acid	C18H36O2	5.66±0.22
<b>Unsaturated fatty acids</b>				
4	Linoleic acid	CIS, CIS-9,12-octadecadienoic acid	C18H32O2	267.56±1.4
5	Oleic acid	Cis-9-octadecenoic acid	C18H34O2	108.26±4.4
6	Gondoic acid	11-eicosenic acid	C20H38O2	1.94±0.074
<b>The total amount of fatty acids</b>				<b>405.99</b>

**Table 1.** Quantitative content of fatty acids in the lipophilic fraction of Chinese magnolia seeds

is part of a number of cosmetics (Jason Y. etc, 2021).

A rather large component of the lipophilic fraction of *S. chinensis* seeds is oleic acid (108.26±4.4 mg/g), which is part of many cosmetics and has regenerating properties (Melnyk I.I. and others, 2021). Oleic acid is also used as a dietary supplement (Jason Y. etc, 2021).

Gondoic acid (1.94±0.074 mg/g) is an unsaturated fatty acid. It has the lowest content in the lipophilic fraction.

#### Discussions and Conclusions

The lipophilic fraction of Chinese magnolia seeds was obtained the yield of 35.79±0.7%. At least 14 compounds of terpene nature were identified using two-dimensional thin layer chromatography. Qualitative composition and quantitative content of fatty acids were identified and established by gas chromatography/mass spectrometry. The highest content of low linoleic acid - 267.56±1.4 mg/g. The total amount of fatty acids was 405.99 mg/g. Unsaturated fatty acids predominated - 377.76 mg/g.

Thus, the studies confirm the rich composition of the lipophilic fraction of Chinese magnolia seeds and allow to predict the anti-inflammatory, antioxidant, regenerating effect of the lipophilic

fraction of Chinese magnolia seeds. The obtained data can also be used in the development of quality control methods for Chinese magnolia seeds.

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This study did not receive external funding.

#### Conflict of interest

There is no conflict of interest in this article. No rewards received.

#### Consent to publication

All authors of the article are acquainted with the final version of the manuscript and have no objections to its publication. The article does not use personal data and information about patients.

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article.

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## Вивчення біологічно активних речовин ліпофільної фракції насіння лимонника китайського *Schisandra chinensis* (Turcz.) Baill

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**Анотація:** *Schisandra chinensis* (Turcz.) Baill – лимонник китайський – добре відома плодова рослина у традиційній китайській та корейській медицині. Плоди та насіння лимонника китайського багаті на біологічно активні речовини. На даний момент, із проведеного аналізу літератури (Irfan M. etc, 2020; Liu G-Z etc., 2020; Sun W., Shahrajabian etc, 2021 ; Zhao T.etc, 2021); Бодревич Б.Б та ін., 2017) можна назвати наступні терапевтичні дії плодів та насіння лимонника китайського: імуномодулююча, тонізуюча, адаптогенна, протизапальна, гепатопротекторна, нефропротекторна, загальнозміцнювальна. Вивчення сировини лимонника китайського продовжується й надалі, для розкриття ширшого діапазону терапевтичних дій. Дослідження саме насіння лимонника китайського є досить актуальним, для розширення асортименту стандартизованої лікарської сировини, яка не входить до монографій Державної фармакопеї України. Основною метою даного дослідження є одержання ліпофільної фракції насіння лимонника китайського та дослідження складу різних груп ліпофільних речовин у одержаній фракції. Основним методом одержання ліпофільної фракції насіння лимонника китайського є вичерпна екстракція у апараті Сокслета. Хімічну реакцію використано для встановлення наявності токоферолів. Методом двовимірної тонкошарової хроматографії встановлено наявність основних груп біологічно активних речовин у ліпофільній фракції насіння лимонника китайського. Вивчення жирнокислотного складу проводили методом газової хроматографія/мас-спектрометрії (Su L. etc, 2020). Вихід ліпофільної фракції насіння лимонника китайського складав 35,79±0,7%. Отримана ліпофільна фракція насіння лимонника китайського являє собою маслянисту рідину насиченого жовтого кольору із характерним специфічним запахом. Встановлено наявність токоферолів. Методом тонкошарової хроматографії у системі розчинників: I напрямом – гексан-ацетон (8:2), II напрямом – гексан-ацетон (8:4), реактив проявлення – реактив Штала, ідентифіковано не менше 14 сполук терпенової природи. В результаті проведених досліджень жирних кислот методом газової хроматографія/мас-спектрометрії ідентифіковано та встановлено кількісний вміст 6 жирних кислот: пальмітинова, лінолева, олеїнова, стеаринова, гондоїнова та нонадеканова. Кількісний вміст ненасичених жирних кислот значно переважав кількісний вміст насичених жирних кислот. Результати дослідження дають підстави зробити наступні висновки: проведені дослідження підтверджують багатий склад ліпофільної фракції насіння лимонника китайського: наявні токофероли, 14 речовин терпенової природи, 6 жирних кислот, з яких ідентифіковано насичені жирні кислоти (пальмітинова, стеаринова, нонадеканова) та ненасичені жирні кислоти (лінолева, олеїнова та гондоїнова); одержані результати дослідження дають змогу припускати протизапальну, антиоксидантну, регенеруючу дію ліпофільної фракції насіння лимонника китайського.

**Ключові слова.** Газова хроматографія, жирні кислоти, лимонник китайський, мас-спектрометрія, насіння.