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O‘ZBEKISTONDA O‘SADIGAN BIR YILLIK SHUVOQ O‘SIMLIGIDAN(*ARTEMISIA ANNUA L.*) ARTEMIZININNI AJRATIB OLISHNING TAKOMILLASHTIRILGAN USULI

УСОВЕРШЕНСТВОВАННЫЙ СПОСОБ ВЫДЕЛЕНИЯ АРТЕМИЗИНИНА ИЗ ПОЛЫНИ ОДНОЛЕТНЕЙ (*ARTEMISIA ANNUA L.*), РАСТУЩЕЙ В УЗБЕКИСТАНЕ

AN IMPROVED METHOD FOR THE ISOLATION OF ARTEMISININ FROM ANNUAL WORMWOOD (*ARTEMISIA ANNUA L.*) GROWING IN UZBEKISTAN

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Annotatsiya

Artemisia annua L. o‘simligining turli geografik mintaqalardagi vakillarining kimyoviy tarkibi, xususan, Artemizinin miqdori o‘rganilgan. Lekin, O‘zbekistonda o‘sadigan vakillarining kimyoviy tarkibi o‘rganilmagan. Mazkur tadqiqotda O‘zbekistonda, xususan, Farg‘ona vodiysida o‘sadigan *Artemisia annua L.*o‘simligidan Artemizininni ajratib olish va miqdorini aniqlash maqsad qilingan.*Artemisia annua L.*o‘simligidan Artemizininni ajratib olishda organik erituvchilarda ekstraksiya qilish, ajratib olingan moddaning fizik-kimyoviy ko‘rsatkichlari, kimyoviy tarkibi hamda tuzilishini aniqlashda yupqa qatlamli va ustunli xromatografiya, IQ, mass-spektrometriya usullari qo‘llanilgan. Farg‘ona vodiysida o‘sadigan *Artemisia annua L.*o‘simligidan organik erituvchilarni almashtirish orqali 1,2-1,5 % gacha Artemizininni ajratib olishga erishildi. Petroley efirda ektraksiya jarayoni turli vaqtlarda olib borildi. Ekstraksiya vaqti 2 sutkadan ortganda unum sezilarli o‘zgarmasligi aniqlandi. Olingan moddaning IQ- va mass-spektrlari tahlil qilinganda tuzlishi artemizininning tuzilishiga mos kelishi kuzatildi.Shunday qilib, birinchi marta O‘zbekistonda, Farg’ona vodiysida o‘sadigan *Artemisia annua L.*o‘simligidan Artemizinin biologik faol moddasini toza holda ajratib olindi. Ektraksiya va tozalash metodikasi takomillashtirildi hamda umumiy unumning 1,5 % gacha oshirilishiga erishildi.

Аннотация

Изучен химический состав представителей растения *Artemisia annua L.* в разных географических регионах, в частности количество артемизинина. Однако химический состав его представителей, растущих в Узбекистане, не изучен. Целью данного исследования было выделение и количественное определение артемизинина из растения *Artemisia annua L.* в Узбекистане, особенно в Ферганской долине. Для определения физико-химических показателей, химического состава и структуры выделенного вещества применяли экстракцию артемизинина из растения *Artemisia annua L.* в органических растворителях, методы тонкослойной и колоночной хроматографии, ИК, масс-спектрометрии.Выделение артемизинина из растения *Artemisia annua L.*, растущего в Ферганской долине достигнуто 1,2-1,5% заменой органических растворителей. Процесс экстракции петролейным эфиром проводили в разное время. Было обнаружено, что выход существенно не изменился, когда время экстракции превышало 2 дня. При анализе ИК- и масс-спектров полученного вещества было замечено, что данные соответствует строению полыни.Впервые в Ферганской долине в Узбекистане из растения *Artemisia annua L.* выделено биологически активное вещество артемизинин. Методы экстракции и очистки были усовершенствованы, а общий выход увеличен до 1,5%.

Abstract

The chemical composition of representatives of the plant *Artemisia annua L.* in different geographical regions, in particular the amount of artemisinin, was studied. However, the chemical composition of its representatives growing in Uzbekistan has not been studied. The aim of this study was to isolate and quantify artemisinin from the plant *Artemisia annua L.* in Uzbekistan, especially in the Ferghana Valley.The extraction of artemisinin from the plant *Artemisia annua L.* in organic solvents, thin layer and column chromatography, IR, and mass spectrometry were used to determine the physicochemical parameters, chemical composition, and structure of the isolated substance.Isolation of artemisinin from the plant *Artemisia annua L.,* growing in the Ferghana Valley, was achieved by 1.2-1.5% by replacing organic solvents. The extraction process with petroleum ether was carried out at different times. It was found that the yield did not change significantly when the extraction time exceeded 2 days. When analyzing the IR and mass spectra of the obtained substance, it was noticed that the data corresponds to the structure of artemisinin.For the first time, the biologically active substance artemisinin was isolated from the plant *Artemisia annua L.* growing in the Ferghana Valley in Uzbekistan. Extraction and purification methods have been improved, and the overall yield has been increased to 1.5%.

Калит сўзлар: COVID-19, SARS-2-CoV-2, коронавируслар, фитопрепаратлар, Бир йиллик шувоқ, *Artemisia annua L.*, артемизинин, экстракция, ИҚ-спектр, масс-спектр.

Ключевые слова: COVID-19, SARS-2-CoV-2, коронавирусы, фитопрепараты, полынь однолетняя, *Artemisia annua L.*, артемизинин, экстракция, ИК-спектр, масс-спектр.

Keywords: COVID-19, SARS-2-CoV-2, coronaviruses, phytopreparations, Annual wormwood, *Artemisia annua L.*, artemizinin, extraction, IR spectrum, mass spectrum.

KIMYO

INTRODUCTION. Various natural products and phytopreparations are being studied as potential therapeutic or prophylactic agents with positive mechanisms of action against COVID-19. Some natural products block the binding of SARS-CoV-2 coronary glycoprotein to receptors in human cells. In addition, several natural products have been studied to have immune-boosting, anti-inflammatory effects [1].

LITERATURE REVIEW. Wormwood has long been used in folk medicine to treat various ailments. Bitter and annual wormwood are mainly used in the treatment of vomiting and malaria [2]. *Artemisia annua L.*, an annual wormwood that is widely used in Eastern traditional medicine as an antipyretic, antiemetic, appetite suppressant, and immune-boosting agent, contains anti-inflammatory sesquiterpenoids, including the endoperoxide ring-holding Artemisinin [3]. Chinese scientist Tu Yuyu studied Artemisia and its anti-malarial properties and became one of the winners of the 2015 Nobel Prize in Physiology or Medicine. Artemisinin and its derivatives are usually well absorbed when administered orally. It has been found to be clinically safe in healthy volunteers and patients with malaria [5]. In very rare cases, it also has side effects such as hepatotoxic, mild to moderate headache, nausea, vomiting, fatigue, and anorexia. Based on its general safety, the World Health Organization has recommended artesunate for parenteral treatment of severe malaria [6].

Artemisinin and some of its derivatives are active against a number of pathogenic viruses, such as Human Cytomegalovirus (HCMV), Epstein Barr virus (EBV), and Human Herpes Simplex virus-6 (HHV-6) [7]. The activity of Artemisinin and its derivatives against SARS-2-CoV-2 at micromolar concentrations has also been confirmed. Recent studies by Algerian scientists have shown that Artemisinin and its derivative Artesunate bind to the SARS-CoV-2 corona protein, which binds to Human ACE2 receptor protein, preventing the first step in the process of coronavirus (COVID-19) infection [8].

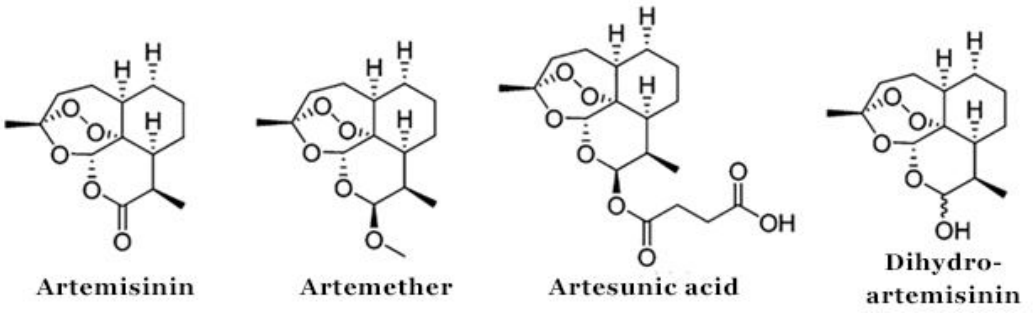

Figure 1. Chemical structure of artemisinin and its derivatives

Chinese scientist Li and others have shown that Artemisinin may contribute to faster recovery in patients with COVID-19. They divided two groups of 41 COVID-19 patients into standard care therapy (control) and Artemisinin and Piperaxin (AP) groups into standard therapy. Patients in the AP group appeared to recover faster from SARS-CoV-2 than patients in the control group [9].

Artemisinin, an ArtemiC medical spray containing frankincense resin from the Boswellia sacrament tree, curcumin, and vitamin C, was administered to patients with COVID-19 in a controlled phase 2 trial in addition to standard care. The results of the study showed that ArtemiC may be more active than standard treatment in contributing to the improvement of patients condition [10]. Similarly, during a recently completed prospective, controlled clinical COVID-19 study, Artemisinin yield significantly improved symptoms in the Artesunate treatment group, transition time to negative SARS-CoV-2 tests, and hospital stay were shorter than in the control group [11].

Currently, many biologically active supplements containing Artemizine and its derivatives, including Artesunate + Artemisinin, Artesunate + amodiachloroquine, Artesunate + pironaridine, Artusunate + Artemisia annua, are considered as additives to the treatment standard in COVID-19 patients with stage 2nd and 3rd research is underway. The efficacy of ArtiVeda™ (Artemisinin) by renowned Indian scientist Wiljay B. Barge and others is being studied in a 4th stage trial in COVID-19 patients with mild to moderate disease [12].

An analysis of the literature [13] revealed that the isolation of Artemisinin from natural sources and its use as a biologically active food additive in the protection and treatment of viral infections is a topical issue. According to the literature, artemisinin 0.3–4% of *Artemisia annua L.* growing in China, 0.4–1.4% of the plant growing in Vietnam, and 0.97% of the plant growing in Iran were isolated. We found 0,6 to 1,7% of artemisinin in plants grown in Uzbekistan. The maximum amount of artemisinin in the plant was found to correspond to the period of full flowering, from the 1st decade of August to the 1st decade of September.

EXPERIMENTAL PART. Extraction of artemisinin. The aerial part of the annual wormwood growing in the Fergana Valley was collected in the first ten days of August during the flowering period, dried and crushed. 200 g of dry raw material was preliminarily boiled in petroleum ether (60-90 ° C), the extract was filtered, and the solvent was evaporated under vacuum. The resulting dark brown syrup was dissolved in 30 ml of chloroform and 170 ml of acetonitrile was added. The formed precipitate was filtered off and by evaporation of the filtrate under high vacuum 5.7 g of gum residue were obtained. The residue was purified by column chromatography on a 2.5x400 column with 60 Å silica gel, size 70-230 mesh, and the addition of ethyl acetate / chloroform (1:15). Column separation was monitored by TLC (R_f = 0.65, EtOAc / $CHCl_3$ 1:15). After recrystallization in hexane, 3 g (1.5 %) of a white crystalline substance was obtained. The boiling point is 152–154 °C, which is consistent with the literature data (152–156 °C) [14, 15].

IR spectroscopic analysis of artemisinin was performed on an American Agilent IR Fourier spectrophotometer using Fourier processing in the range 650–4000 cm^{-1} (Fig. 2).

Mass spectrometric studies were carried out by the method of ionization with molecular nitrogen ions on a Perkin Elmer AxION 2 TOF mass spectrometer (Fig. 3).

DISCUSSION OF THE RESULTS. With the standard method of extracting artemisinin from the aerial part of *Artemisia annua L.* growing in the Fergana Valley, an average yield of 1.2% was obtained. Studies to increase the yield with solvent exchange during extraction have been found to increase the yield to 1.5%. The extraction process with petroleum ether was carried out at different times. It was found that the yield did not change significantly when the extraction time exceeded 2 days. Therefore, 48 hours was chosen as the optimal time to isolate the primary extract.

When the impurity is separated from the resulting dark brown syrup by precipitation with a mixture of chloroform and acetonitrile, the yield of artemisinin decreases with a decrease in the volume ratio of chloroform to acetonitrile from 1: 5 to 1:10. At this stage, the optimal solution is a 15% solution of chloroform in acetonitrile. Chromatographic purification was carried out according to the method described in the literature [16].

The IR spectrum of the isolated artemisinin (Fig. 2) corresponds to the literature data [17]. In particular, intense peaks in the region of 833 cm^{-1}, 931 cm^{-1}, 985 cm^{-1} appear –C–C– stretching vibrations, in the regions of 1010 cm^{-1} and 1018 cm^{-1} –C–O– stretching vibrations appear. It was found that the intensity peak at 1115 cm^{-1} corresponds to the –O–O– stretching vibration. At 1385 cm^{-1}, peaks of moderate intensity are formed, corresponding to the -CH_3 stretching vibration, and at 1456 cm^{-1}, corresponding to the –CH_2– bending vibration. A strong intense peak corresponding to the C=O stretching vibration appears at 1735 cm^{-1}. Weak peaks at 2947 cm^{-1} and 2978 cm^{-1} correspond to stretching vibrations –CH_2.

The mass spectrum of the isolated artemisinin (Fig. 3) mainly shows peaks corresponding to the molecular ions of the dimer (565,3883 m/z, 100%; 566,3920 m/z, 32%) and the monomer (283,2015 m/z, 38 %; 285,206 m/z, 1%).

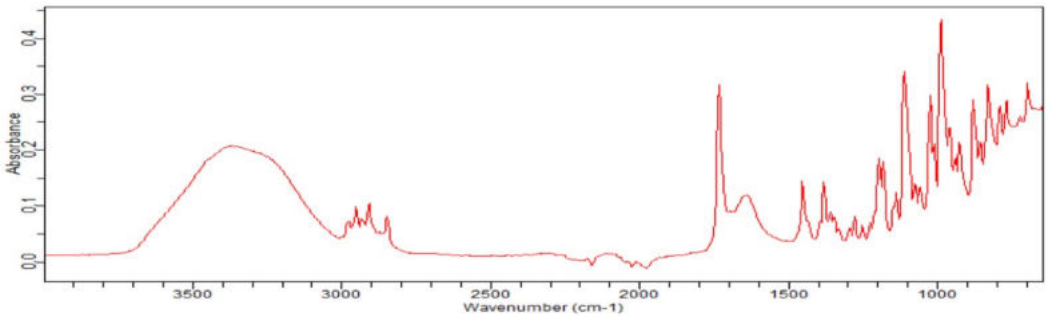

Figure 2. IR spectrum of artemisinin isolated from *Artemisia annua L.* growing in Uzbekistan.

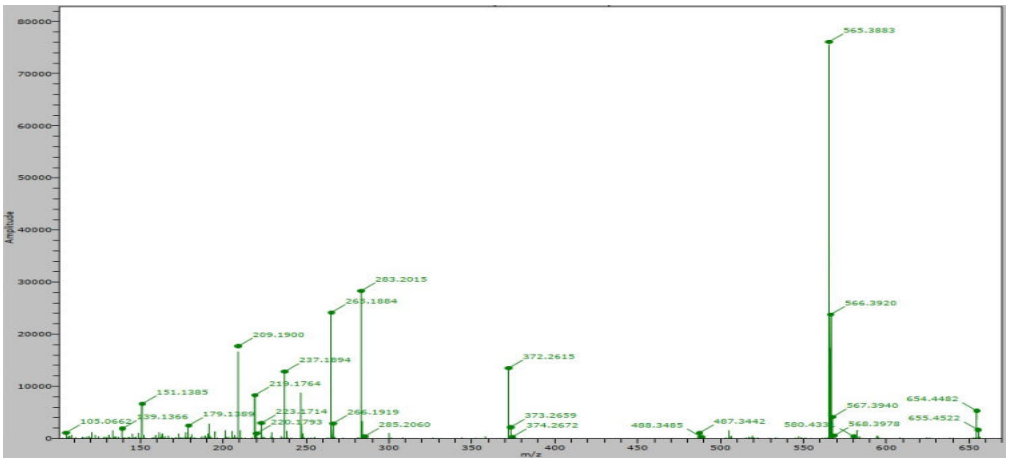

Figure 3. Mass-spectrum of artemisinin isolated from *Artemisia annua L.* growing in Uzbekistan.

CONCLUSIONS. The creation of compositions based on natural remedies isolated from plants used in traditional medicine that have antiviral, anti-inflammatory, immunostimulating properties and their use in modern pandemic conditions as an aid in the prevention and treatment of diseases is of great practical and social importance. importance. For the first time in Uzbekistan, the biologically active substance artemisinin was isolated from the plant *Artemisia annua L.*. Extraction and purification methods have been improved, and the overall yield has been increased to 1.5%.

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