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# ASSESSMENT OF ACUTE TOXICITY (LD50) OF DRY EXTRACTS OBTAINED FROM CYDONIA OBLONGA MILLER LEAVES USING DIFFERENT SOLVENTS

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**Abstract.** The dried leaves of *Cydonia oblonga* Miller (quince) at room temperature were extracted with distilled water and organic solvents: 40% ethanol:water mixture, ethyl acetate. Each step was carried out at 40-60 0C for 3 hours. (Repeated 3 times). In order to increase the concentration of active substances in the extracts, they were concentrated using a rotary evaporator. The obtained extracts were dried in a lyophilizer and dry substances were obtained. The substances were isolated from the extracts with a yield of: aqueous-8%, alcoholic-14%, ethyl acetate-5%, relative to 100 grams of dry plant dry mass. The acute toxicity of the dry substances obtained from the extracts was evaluated in laboratory mice. The substances in the form of substances were administered to the stomach of mice once at a dose of 5000 mg/kg and the condition of the animals was monitored for 14 days. During the entire experiment, no deaths were recorded in animals in all groups receiving all substances in the form of When the results were classified according to OECD, it was determined that these samples belonged to class VI, which is almost non-toxic chemical substances (LD50> 5000 mg/kg)


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**Keywords**: acute poisoning, substance, Cydonia oblonga Miller, rotary evaporator, lyophilizer, lethal dose-$LD_{50,}$ toxicity, extract.

**Annotatsiya.** *Cydonia oblonga* Miller (behi) o‘simligining quritilgan barglari xona haroratida distillangan suv va organik erituvchilar: 40% etanol: suv aralashmasi, etil asetat yordamida ekstraksiya qilindi. Har bir bosqich 40–60 °C da 3 soat davomida amalga oshirildi (3 marta takrorlandi). Ekstraktlardagi faol moddalar konsentratsiyasini oshirish maqsadida ular rotarli bug‘latgich yordamida quyuqlashtirildi. Olingan ekstraktlar liyofilizatorda quritilib, quruq moddalar olindi. Moddalar ekstraktlardan quyidagi unum bilan ajratib olindi: suvli – 8%, spirtli – 14%, etil asetatli – 5% (100 gramm quruq o‘simlik massasiga nisbatan). Ekstraktlardan olingan quruq moddalarning o‘tkir toksikligi laboratoriya sichqonlarida baholandi. Moddalar sichqonlarning oshqozoniga bir marta 5000 mg/kg dozada yuborildi va hayvonlarning holati 14 kun davomida kuzatildi. Butun tajriba davomida barcha moddalarni qabul qilgan guruhlardagi hayvonlarning birortasida ham o‘lim qayd etilmadi. Natijalar OECD tasnifi bo‘yicha baholanganda, ushbu namunalar VI sinfga – deyarli zaharli bo‘lmagan kimyoviy moddalarga mansubligi aniqlandi ($LD_{50}$ > 5000 mg/kg)

**Kalit so‘zlar*:*** o‘tkir zaharlanish, *Cydonia oblonga Miller*, rotorli bug‘latgich, liyofilizator, o‘lim dozasi-$LD_{50}$, zaharlilik, ekstrakt.

**Аннотация.** Высушенные листья *Cydonia oblonga* Miller (айвы) при комнатной температуре экстрагировали дистиллированной водой и органическими растворителями: 40% смесью этанол:вода, этилацетатом. Каждый этап проводили при 40–60 °C в течение 3 часов (повторяли 3 раза). Для увеличения концентрации активных веществ в экстрактах их концентрировали с помощью ротационного испарителя. Полученные экстракты высушивали в лиофилизаторе и получали сухие вещества. Вещества были выделены из экстрактов с выходом: водный – 8%, спиртовой –14%, этилацетатный–5% по отношению к 100 граммам сухой массы растения. Острая токсичность сухих веществ, полученных из экстрактов, была оценена на лабораторных мышах. Вещества вводили в желудок мышей однократно в дозе 5000 мг/кг и наблюдали за состоянием животных в течение 14 дней. В ходе всего эксперимента ни в одной группе животных, получавших все вещества, не было зарегистрировано случаев гибели. При классификации результатов в соответствии с OECD было установлено, что данные образцы относятся к VI классу – практически нетоксичным химическим веществам ($LD_{50}$ > 5000 мг/кг).

**Ключевые слова:** острое токсичность, *Cydonia oblonga Miller*, роторнный испаритель, лиофилизатор, летал доза – $LD_{50}$, токсичность, экстракт.

## 1. INTRODUCTION.


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### 1.1. Summary of *Cydonia oblonga Miller*

Many plants on Earth have healing properties. The World Health Organization has listed over 21,000 plant species used for therapeutic and medicinal purposes worldwide. (M. Zubayr et al.2015). Medicinal plants have been used to treat various diseases since ancient times in human history. Medicinal plants are considered the cheapest and most readily available source of treatment in the primary health care system. (S. Basak et al.,2022). Quince (*Cydonia oblonga*) is a plant with many medicinal properties. It has been widely used to treat various diseases since ancient times. Taking these into account, this scientific research study evaluated the acute toxicity of substances extracted from quince leaves.

The quince tree and its fruit are known by different names in different languages: "Bahee Dana" in Urdu, "Beh" in Persian, "Strythion" in Ancient Greek, and "Bihi" in Hindi. *[Khubnasabjafari et al. 2011, Torkelson, A.R., 2013,.*]. Almost 80% of the quince cultivation areas in the Republic of Uzbekistan fall in the Fergana Valley.. *[Matmuratov et al., 2025)]*

**B. Acute Toxicity**

The toxicity index of drugs is the ability to cause death when administered to an animal in a single dose or at intervals of no more than 6 hours**.** The purpose of studying acute toxicity is to determine the causes of death in animals by analyzing toxic, lethal doses of a pharmacological substance and the clinical manifestations of intoxication. Acute toxicity indicators of drugs can be assessed using various methods.

## 2. MATERIAL AND METHOD.

### 2.1. Extraction of dry matter from leaves of *Cydonia oblonga Miller*

Leaves of *Cydonia oblonga Miller* were collected in September and dried at room temperature. Various solvents were used to extract substances from leaves brought to a constant mass: water and organic solvents. The extraction process was carried out according to methods known in the literature *(Matmuratov B.Ya.et.al. 2024., Madrakhimova S.D., et al.2025).*

### 2.2. Toxicity determination

The experiments used the acute oral toxicity test recommended by OECD (2002) at a strict "fixed dose" (Test No.420.Acute Oral Toxicity-Fixed Dose Procedure (OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070943-enra).

## 3. DISCUSSION OF THE RESULTS

### 3.1. Extraction of substances from quince leaves using various solvents

**A. Extraction of dry aqueous matter from quince leaves**


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The plant leaves were first dried and brought to a constant mass. All analyses were carried out relative to 100 grams of dry plant mass. Then the leaves were extracted with distilled water at a temperature of 50-60$^0$C, in ethyl acetate at a temperature of 40-45$^0$C (the extraction processes with all solvents were repeated 3 times until the solution became colorless). The extracts obtained with all solvents were concentrated in a rotary evaporator and then dried in a lyophilizer. The resulting dry extract was 12% of the total mass. (Figure 1).



**Figure 1. Quince aqueous fraction**

**B. Extraction of dry alcoholic substance from quince leaves**

The plant leaves were first dried and brought to a constant mass. Then the leaves were extracted in a 40% mixture of ethanol and water at 50-60$^0$C (the process was repeated 3 times until the solution became colorless). The ethanol in the plant extract was completely evaporated. It was concentrated. Then the organic solvent was completely removed and dried in a lyophilizer. The resulting dry extract was 14% of the total mass. (Figure 2)



**Figure 2. Quince alcohol fraction**

**C. Extraction of ethyl acetate fraction from quince leaves**

The plant leaves were first dried and brought to constant mass. Then the leaves were extracted in ethyl acetate at 40-45$^0$C (the process was repeated 3 times until the solution became colorless). The plant extract was concentrated. It was dried in a lyophilizer. The resulting dry extract was 7% of the total mass (Figure 3).


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**Figure 3. Quince ethyl acetate fraction**

### 3.2. Determination of acute toxicity

In all tests determining the acute toxicity index, the substances are gradually introduced into the stomachs of animals of the same sex at a fixed dose of 5000 mg/kg. The acute toxicity properties of the substances in the form of ethyl acetate aqueous and alcoholic extracts of *Cydonia oblonga M*. were investigated. All studies were conducted on male, white, healthy, sexually mature laboratory mice with a body weight of 22±2.0 g and a quarantine period of 10-14 days. 5 animals were taken for each group.

Experiments to determine the acute toxicity index were carried out in two stages. In this case, in stage I, 2 mice in the group were introduced into the stomachs of each substance at a dose of 5000 mg/kg, in a volume of 0.4 ml. The general condition of these mice was monitored for 3 days and no deaths were recorded. In the second stage of the experiment: the remaining 3 animals of this group were injected into the stomach with 5000 mg/kg of each substance. The animals taken for control were given distilled water. The general condition of the animals in all groups under study was checked every hour for 14 days. The experiments on all mice were carried out under conditions of unrestricted food. The following conclusions were drawn from the results of the tests: The median lethal dose– LD50 and toxicity class of the substances in the form of ethyl acetate, aqueous and alcoholic extracts of *Cydonia oblonga M*. were determined (https://doi.org/10.1787/9789264070943-enra)., Mironova A.N. 2012) The obtained data were statistically processed by calculating the arithmetic mean (M) and the arithmetic mean error (m). The significance level $p<0.05$ was accepted as a statistically significant change. (Table 1 and Fig.4).

**Table 1**

**Evaluation of acute toxicity properties of ethyl acetate, aqueous and alcoholic extracts of *Cydonia oblonga Miller* leaves in mice (M±m, n=5)**

| Groups | Doza, mg/kg, ml | Number of animals/dead animals in the group | Average mass of animals, g | | | $LD_{50}$, mg/kg |
|---|---|---|---|---|---|---|
| | | | 1 day | 7 day | 14 day | |


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| | | | | | | |
|---|---|---|---|---|---|---|
| Control | 0,5 ml(dis, water) | 5/0 | 22,0± 0,3 | 23,3 ± 0,3 | 23,8± 0,4 | - |
| Ethyl acetate | 5000 mg/kg | 5/0 | 21,0±0,25 | 22,4 ± 0,3 | 23,2± 0,3 | >5000 mg/kg |
| aqueous | 5000 mg/kg | 5/0 | 22,0 ± 0,3 | 23,1 ± 0,3 | 23,9± 0,32 | >5000 mg/kg |
| alcoholic | 5000 mg/kg | 5/0 | 20,0 ± 0,2 | 21,5 ± 0,3 | 23,4± 0,3 | >5000 mg/kg |

*Note: *P<0.05 compared to control*

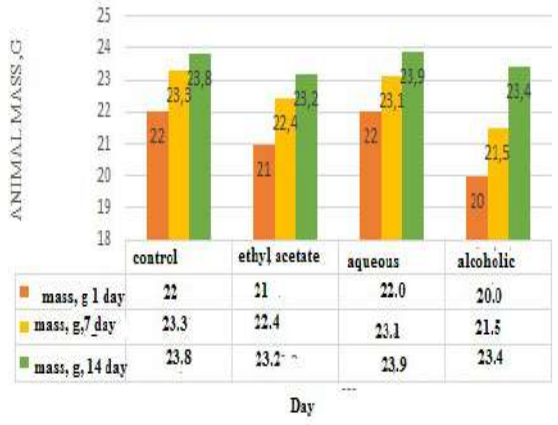



**Figure 4. Evaluation of acute toxicity properties of ethyl acetate, aqueous and alcoholic extracts of *Cydonia oblonga Miller* leaves in mice**

As can be seen from the results in Table 1 and Figure 4, all substances extracted from quince leaves were found to belong to class VI, low-toxic substances.

## CONCLUSION

Quince leaves were extracted with water, alcohol, and ethyl acetate using methods known in the literature. All processes were carried out at certain temperatures. The obtained extracts were concentrated and dried in a lyophilizer. The yield of the substances was 8 % aqueous, 15 % alcoholic, and 7% ethyl acetate per 100 grams of dry plant mass.

The acute toxicity of the isolated substances was studied. From the results of the analysis, it can be concluded that the substances in the form of ethyl acetate, aqueous, and alcoholic extracts of quince leaves were administered to mice once at a dose of 5000 mg/kg. When the results were classified according to OECD, it was found that these samples belong to class VI, which is almost non-toxic chemicals (LD50> 5000 mg/kg).


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