Extraction of Polyphenols from Cydonia Oblonga Leaves and Their Effect on Mitochondrial Membrane Stability

Bakhtishod Matmurotov[1*], *Sakhiba* Madrakhimova[2], *Nuriddin* Soliyev[3], and *Alimjan* Matchanov[2]

[1]Tashkent Medical Academy, Tashkent, Uzbekistan
[2]Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan
[3]Namangan State University, Namangan, Uzbekitsan

Abstract. *Cydonia oblonga Mill. (quince)* is a fruit tree native to Azerbaijan, Dagestan, Turkmenistan, and Iran, known by various names including Baher Dana in Urdu and Behi in Hindi. Widely cultivated in Portugal, Turkey, Central Asia, India, and Europe, quince cultivars vary in flowering time and yield. High-yielding cultivars such as Aurora, Zoloto Skif, and Sophia show strong environmental adaptation. New cultivars such as Zolotistaya (28.4 mg/100 g vitamin C) and Padarok (32.3 mg/100 g) are rich in vitamin C and catechins. Phenolic compounds in quince leaves, particularly tannins (11%), have antidiarrheal properties. Extraction methods showed high phenolic content when the leaves were dried at optimal temperatures and extracted using ethanol systems. Quince polyphenols exhibit antioxidant and membrane stabilizing effects, which were observed in mitochondria under Fe2+/ascorbate stress, with inhibition values up to 90.3% at 30 μg/mL PF-4 extract. The study concludes that quince leaf extracts have significant antioxidant potential for pharmaceutical applications.

Keywords: Polyphenol, Cydonia oblonga Miller (COM), mitochondria, membrane, extract, differential centrifugation, concentration, extraction, suspension, Ethylenediaminetetraoxusnaya acid (EDTA)

1 Introduction

Cydonia oblonga Mill. (Quince) is a fruit tree native to several regions, including Azerbaijan, Dagestan, Turkmenistan, and Iran, and is widely cultivated for its medicinal properties [1]. The primary problem addressed in this study is the need for optimizing extraction methods to maximize the yield of bioactive phenolic compounds from quince leaves. Previous research has highlighted the therapeutic potential of quince due to its antioxidant and anti-

* Corresponding author: b.matmurotov@mail.ru

inflammatory properties [2]. Studies show that polyphenols in fruits and vegetables have beneficial health effects, including cardiovascular protection [3], anti-diabetic properties [4], and anticancer activities [5]. Furthermore, quince leaf extracts are known for their high tannin content, which contributes to their antidiarrheal and antimicrobial effects [6]. By optimizing extraction methods, this study offers a promising solution, enhancing the potential of quince leaf extracts as therapeutic agents, particularly in the field of medical sciences where antioxidants and bioactive compounds are in high demand for developing new treatments (Fiigure 1).

Fig. 1. Cydonia oblonga Mill. fruit and flower.

2 Materials and Methodology

In this study, Cydonia oblonga Miller (COM) leaves were dried to a constant mass and treated with pure chloroform to remove lipophilic compounds. The leaves were then extracted using 70% acetone, followed by the complete removal of the solvent. The resulting aqueous extract was treated with ethyl acetate, and the solvent was evaporated using a rotary evaporator. The dried extract was further processed by immersion in pure chloroform. Mitochondria from rat livers were isolated using the Schneider differential centrifugation method. The separation medium contained 250 mM sucrose, 10 mM Tris-chloride, and 1 mM EDTA at pH 7.4. Lipid peroxidation (LPO) in mitochondrial membranes was studied using the Fe2+/ascorbate system with specific concentrations of Fe2SO4 (20 μM) and ascorbate (400 μM). Mitochondrial permeability transition pore (mPTP) permeability was measured based on mitochondrial swelling kinetics at 28°C using a UV-5000 spectrophotometer at 540 nm.
The study excluded certain phenolic compounds that were not recoverable under the employed solvent system and focused on isolating tannins and flavonoids from quince leaves, which are known for their high bioactivity.

3 Results and Discussion

In order to determine the presence of polyphenolic compounds in the leaves of Cydonia oblonga Miller (COM), several color qualitative reactions specific to them were first carried out:

1. ***Qualitative reaction with $FeCl_3$.*** 1 gram of leaves was taken and placed in test tubes and a 1% yellow solution of iron(III) chloride in alcohol was added to them. The solution turned dark blue (reaction characteristic of phenolic compounds, (Fig.2).

Fig. 2. Qualitative reaction with $FeCl_3$.

2. ***Qualitative reaction with concentrated NaOH.*** This was followed by extraction for 15 min with stirring using a magnetic stirrer and the solution turned dark brown in color. (reaction typical of polyphenols (Fig.3).

Fig. 3. Reaction of polyphenols.

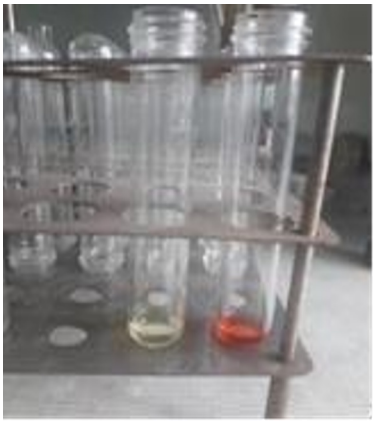

Fig. 4. Flavan-3-ol reaction.

3. ***Qualitative reaction with vanillin solution.*** Boiling water was poured over the dried quince leaf and left for 5 minutes, then a 1% solution of vanillin in concentrated HCl was added to it, the solution turned bright red (flavan-3-ol reaction) Fig. 4. Quince leaves were collected and dried in the shade in October to extract polyphenols. First, 100 g of crushed leaves were extracted with chloroform (1:10 ratio) three times for 2 hours to remove lipophilic compounds. The material was dried, and 70% aqueous acetone (1:6 ratio) was used for extraction at 55-60°C for 2 hours. The acetone was removed with a rotary evaporator, and the aqueous fraction was further extracted with ethyl acetate (1:4 ratio). The ethyl acetate extract was concentrated, precipitated with hexane, filtered, washed, and dried to isolate the polyphenols Fig.5.

Medicinal properties of BFMs obtained from local medicinal plants were studied in vitro. The study of the effect of plant BFMs on the LPO process in the liver mitochondria of laboratory animals provides preliminary information about their antioxidant properties. Convenient experiments for in vitro studies in this regard use the Fe2+/ascorbate inducer system. This allows to directly determine the effectiveness of lipophilic antioxidants by inducing the LPO process under the influence of the system. In the scientific literature, there is information about the disruption of the antioxidant system in membranes.

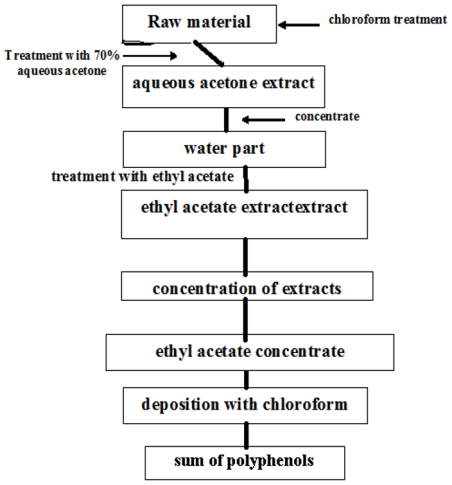

Fig. 5. Flowchart of the extraction process for polyphenols from Cydonia oblonga Miller (Quince) leaves.

Therefore, in the experiments, the correcting effect of polyphenol extract (PF-4) isolated from *Cydonia Oblonga* (Behi) leaves on the LPO process observed as a result of induction of mitochondrial membranes under the influence of Fe^{2+}/ascorbate was studied and its antioxidant properties were evaluated. Initially, 10 μM Fe2SO4 and 600 μM ascorbate were used to induce LPO in mitochondrial membranes. Adding a fixed amount of Fe^{2+}/ascorbate to the incubation medium has a direct inducing effect on mitochondrial membranes and stimulates the LPO process. In this case, the permeability of mitochondrial membranes increases, and the membrane potential decreases, and the permeablization process is observed. The induced POL process in mitochondria under the influence of Fe^{2+}/ascorbate was designated as the control group (100%). The effects of Sydonia Oblong (Bexi) leaf extract (PF-4) on the LPO process in mitochondrial membranes were studied in the research.

Table 1. Inhibitory effect of PF-4 extract isolated from *Cydonia Oblonga Miller* leaves on LPO process in mitochondria.

№	PF-4 extract μg/ml	Control, Fe2+/ascorbate	Experiment, Fe2+/ascorbate+PF-4	Inhibition %
		(ΔA_{540}nm/min)	(ΔA_{540}nm/min)	
1	**3**	100	66.3±1.75*	33.7%
2	**10**	100	45.8±1.40**	54.12%
3	**20**	100	19.6±0.83**	80.4%
4	**30**	100	10.7±0.89 ***	90.3%

*(IM: KSI - 125 mM, tris-NSI - 10 mM, pH 7.4. Concentrations: Fe_2SO_4 – 10 μM, ascorbate 600 μM; mitochondrial protein 0.5 mg/ml. Control - Fe^{2+}/ascorbate. ***R<0.001)*

The antioxidant effect of PF-4 was observed according to increasing concentrations added to the medium (3,10,20, 30 μg/ml). In studies, 3 μg/ml of PF-4 induced Fe^{2+}/ascorbate-induced mitochondrial membrane permeability was 66.3±1.75 compared to the control, and the LPO process was inhibited by 33.6%. So, this indicator is clearly PF-4 means that it is a substance with antioxidant properties. Increasing the concentration of PF-4 in the incubation medium also increased its LPO inhibitory activity. That is, 10 μg/ml μl of PF-4 in the

incubation medium addition, mitochondrial permeability was 45.8±1.40, and the LPO process was inhibited by 54.12%. Also, mitochondrial membrane permeability under the influence of PF-4 at 10 μg/ml was 19.6±0.83, and under the influence of 30 μg/ml it was 10.7±0.89 did, the LPO process in mitochondria was inhibited by 80.4% and 80.2%, respectively (Tab.1). These indicators prove that the PF-4 extract isolated from the leaves of *Cydonia Oblonga* is a substance.

In our experiments, Ca^{2+} ions were chosen to induce mPTP induction, and 30 μM Ca^{2+}we used concentration. In initial screening experiments, adding Ca^{2+} to the incubation medium increased mPTP permeability (t-300 sec) increased compared to the control and this indicator was defined as 100% (Table. 2).

Table 2. Effect of PF-4 extract isolated from Cydonia oblonga Miller leaves on mPTP status.

№	PF-4 extract μg/ml	Control, $CaCl_2$ (ΔA_{540}nm/min)	Experience, PF-4 (ΔA_{540}nm/min)	Inhibition%
1	**2**	100	65,4±1,58*	34,6%
2	**5**	100	45,1±1,12**	54,9%
3	**10**	100	35,4±1,27**	64,6%
4	**15**	100	17,6±0,81**	82,3%

*(IM: 200 mM sucrose, 20 μM EGTA, 5 mM succinate, 2 μM rotenone, 20 mM Tris, 20 mM HEPES, and 1 mM KH2PO4, pH 7.4. Control-10 μM Ca^{2+}. *P<0.05; ***P<0.001.)*

In *in vitro* studies, PF-4 extract from the leaves of Cydonia Oblonga (COM) at concentrations (2, 5, 10, 15 μg/ml) had an inhibitory effect on mPTP induced by Ca^{2+} ions. The inhibitory activity was evident from the first concentration of 2 μg/ml added to the incubation medium (34.6%). The inhibitory activity of PF-4 extract against mPTP according to the concentrations added to the incubation medium was as follows: 5 μg/ml (45.1±1.12) 54.8%; 10 μg/ml μl (35.4±1.27) 64.5%; 15 μg/ml (17.6±0.81) was 82.3%.

4 Conclusion

This study focuses on optimizing the extraction of bioactive polyphenolic compounds from *Cydonia oblonga* (quince) leaves, known for their antioxidant, anti-inflammatory and medicinal properties. Quince leaves were dried, treated with chloroform and subjected to a multi-step extraction using 70% aqueous acetone and ethyl acetate. The extracts were concentrated and analyzed for phenolic content. Qualitative tests including reactions with $FeCl_3$, NaOH and vanillin confirmed the presence of polyphenols. In vitro studies on rat liver mitochondria demonstrated the antioxidant activity of PF-4 extract with inhibition rates up to 90.3% against lipid peroxidation and mitochondrial permeability transition. The results of this study suggest the potential application of quince leaf polyphenols in the development of therapeutic agents, especially due to their antioxidant and membrane stabilizing properties. Future research may explore broader pharmacological applications of these compounds and optimize extraction for large-scale applications.

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