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Extraction of Mucilage from Quince (Cydonia Oblonga M.) Seed and Investigation of its Biological Activities

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Abstract: Quince seed mucilage has been traditionally used as a cough remedy, but little has been reported about its extraction and biological activities. This study investigated the extraction of mucilage from quince seed through various methods. Single-step aqueous maceration, ultrasonic pretreatment followed by aqueous maceration, and microwave pretreatment followed by aqueous maceration were used for mucilage extraction, while Soxhlet was applied as a reference method. The antitussive and expectorant activity of the mucilage was evaluated using the ammonia-induced cough model and phenol red secretion model in mice, respectively. Results indicated that the highest mucilage (191.2±0.5 mg/g) and saponin (94.8±0.6 mg/g) extraction yields were obtained when 2 min microwave irradiation was used before aqueous maceration (70 °C, 2 h). At the 400 mg/kg dose, the mucilage significantly lengthened the cough incubation period and reduced the cough frequency in mice. Results also showed the considerable expectorant activity of the mucilage ($p < 0.01$) at the dose of 400 mg/kg compared to the control and positive control groups. The mucilage showed significant antibacterial activity against *Streptococcus pyogenes* with MIC and MBC values of 62.5 and 125 µg/mL, respectively. The quince seed mucilage was curatively effective on coughing and expectoration, providing significant evidence for the traditional use of this mucilage as a cough remedy.

Keywords: quince seed mucilage; extraction method; antitussive agent; expectorant activity; antibacterial property.

1. Introduction

Quince (*Cydonia oblonga* M.) is one of the medicinal plants native to Western Asia, from Iran to Turkestan, but nowadays cultivated in many parts of the world [1]. The yellow color fruit and its seeds, as well as the buds, leaves, and bark of the tree, have been used for various therapeutic purposes. Especially noteworthy is quince seed, which has been used in traditional remedies to treat cough, dryness of the throat, and malignant ulcers [2].

Quince seed mucilage is a water-soluble polysaccharide consisting of numerous sugar units connected to each other to form a macromolecule; it is mainly composed of glucuronic acid and glucuronoxylan-based biomaterials [1, 3]. This biological macromolecule has shown several curative properties, primarily associated with its antioxidant, anti-inflammatory, and

antimicrobial activities [3]. It has been used for therapeutic and medicinal applications such as wound healing [4], drug delivery [5], and the development of scaffolds [1, 6]. Due to its biocompatible, non-toxic, and biodegradable nature [7], quince seed mucilage has also found application in wastewater treatment as a natural coagulant [8], in the food industry as a packaging material [9], edible film [10, 11], coating material [12], and emulsifier [13, 14], in cosmetic products as a moisturizer [15], electrochemical sensor development [16] and as a surfactant [17, 18].

A literature survey portends that numerous investigations have been done to extract mucilage from quince seed to investigate its therapeutic and food applications, most of which have been traditional and low-yield extraction methods. For example, in a study on the fabrication of quince seed mucilage–derived 3D scaffolds for the proliferation of human amniotic mesenchymal stromal cells, Şimşek *et al*. [19] extracted mucilage from quince seed using the maceration method. The mucilage was obtained by macerating the quince seed in Milli-Q water for 24 h at 30 ˚C, which had an extraction yield of 62.8 mg mucilage/g quince seed. An almost similar extraction method was used by Ghadermazi *et al*. [20], who used the extracted mucilage for the coacervation of whey protein isolate. The mucilage was extracted by soaking the quince seeds in water at 60 ˚C for 2 h, then keeping the solution at room temperature for about 12 h. The obtained mucilage had an extraction yield of 142 mg mucilage/g quince seed. In another study undertaken by Kawahara *et al*. [15], the effect of quince seed mucilage on ameliorating the atopic symptoms of keratinocyte-associated skin inflammation was investigated. Quince seeds were immersed in ethanol at room temperature for one week; the obtained mucilage had an extraction yield of 3 mg mucilage/g quince seed. Such conventional extraction methods, notwithstanding the long extraction time, have low extraction efficiencies. In contrast, advanced ultrasonic and microwave-assisted extraction techniques require shorter extraction times while providing higher efficiency than conventional extraction methods. Despite these merits, reports on using these methods to extract mucilage from quince seeds are scarce.

Hitherto, some investigations have been carried out to evaluate the antibacterial activity of quince seeds mucilage. For example, Alizadeh *et al*. [21] investigated the antimicrobial activity of quince seed extracts against *Enterobacter aerogenes*, *Escherichia coli*, and *Klebsiella pneumoniae*. The results showed that the obtained mucilage had a bactericidal effect on the studied bacteria, and *Escherichia coli* compared to other bacteria, was the most sensitive. In another study undertaken by Al-Noamy [22], the antibacterial activity of the aerial part of the quince plant (leaves, seeds, fruits) on some Gram-negative and Gram-positive bacteria isolated from Otitis media and stool samples from gastroenteritis cases were investigated. The aqueous seed extract showed was most effective against *Enterococcus faecalis* bacterium.

This study implements microwave and ultrasound pretreatments to enhance the extraction yield before the maceration step. Due to the traditional use of quince seed mucilage as a cough remedy, the antitussive and expectorant effects of the extracted mucilage are studied in an experimental animal study. In addition, the antibacterial effect of the mucilage on the pathogenic bacteria, *Streptococcus pyogenes*, is also studied *in vitro*. To the best of our knowledge, little has been reported about the antitussive and expectorant activity of quince seed mucilage in the literature. This study can help provide insightful information in these regards.

2. Materials and Methods

2.1. Materials.

Quince (Cydonia oblonga M.) seeds were obtained from a herbal medicine shop located in the central market of Tehran, Iran. The seeds were washed to remove impurities, dried in an oven, and stored for mucilage extraction. The solvents and chemicals used for the phytochemical characterization of the extracted mucilage were chloroform and hydrochloric acid (Scharlau, Spain), lead acetate, ferric chloride, sulfuric acid, and Mayer's reagent (Merck, Germany). Ursolic acid (Sigma Aldrich, USA) was used as a standard for determining saponin concentration in the extracted mucilage. As a cough suppressant drug, Dextromethorphan was obtained from Pursina Pharmaceutical Co., Iran. Phenol red (Sigma Aldrich, USA) was applied to evaluate the expectorant activity. Vancomycin (Pursina Pharmaceutical Co., Iran) was used as a positive control for an antibacterial test.

2.2. Preparation of quince seed mucilage.

Several extraction methods were used for the extraction of mucilage from quince seed. The influential parameters in each method were optimized using the one-factor-at-a-time (OFAT) approach to achieving a high extraction yield. The implemented extraction methods were single-step aqueous maceration, ultrasonic pretreatment followed by aqueous maceration, and microwave pretreatment followed by aqueous maceration. Soxhlet extraction was also employed as a reference technique to assess the efficiency of the used extraction methods. In all extraction tests, water was used as the extraction solvent. Water was chosen as the extraction solvent since it is regarded as one of the best solvents in conventional medicine from a pharmacological standpoint.

For the extraction of mucilage from quince seed through single-step maceration, 1 g of the seeds was soaked in a specified amount of distilled water used as the extraction solvent and heated in a water bath. The solvent-to-solid mass ratio was in the range of 10:1 to 30:1, the maceration duration was between 1 to 4 h, and the temperature was varied from 50 to 80 ˚C.

As another extraction method, ultrasound pretreatment followed by aqueous maceration was used. For this purpose, 1 g of quince seed was mixed with 25 mL distilled water and sonicated using a probe ultrasonic homogenizer (Hielscher, UP400S) for 2 to 8 min at different ultrasonication powers (160 to 280 W). This was considered a pretreatment step, and the sample was then subjected to aqueous maceration at 70 ˚C for 2 h, as described.

Alternatively, microwave pretreatment followed by aqueous maceration was employed to extract the mucilage from the seeds. For this, 1 g of quince seed was added to 25 mL distilled water and irradiated in a microwave oven (Samsung, CQ4250) at 100 to 450 W for 1 to 4 min. After microwave pretreatment, the sample was transferred to a hot water bath and heated at 70 ˚C for 2 h.

For Soxhlet extraction, which was carried out at a fixed condition, 12 g of quince seed was embedded into a cellulose thimble (33 × 100 mm, thickness 1.5 mm) and extracted using 300 mL of distilled water at 70 ˚C for 12 h. Then, the solvent was evaporated using a rotary evaporator to a volume of 50 mL.

The extracted solutions, obtained from different extraction methods, were centrifuged at 7000 rpm for 5 min to separate the mucilage from the seeds. The separated mucilage was

dried in an oven at 60 ˚C. All extraction experiments were replicated three times to ensure the accuracy of the results.

2.3. Characterization of mucilage.

The mucilage powder obtained from the optimized extraction method was analyzed using a Fourier transform infrared (FTIR) spectrometer (WQF-510A, China) to investigate its functional groups. The amorphous nature of the mucilage was characterized using an X-ray diffractometer (XRD, Panalytical, X′ PertPro, Netherlands).

The porosity of the mucilage was analyzed using the hexane infiltration method [19]. Quince seed mucilage (1 g) was immersed into a graduated cylinder containing a known volume of n-hexane as the displacement liquid (V_1) for 10 min. Afterward, the total volume (n-hexane with n-hexane-impregnated mucilage) was noted (V_2). Finally, n-hexane-impregnated mucilage was meticulously removed from the cylinder, and the remaining n-hexane volume was recorded (V_3). The porosity (%) of mucilage was calculated as:

$$\text{Porosity (\%)} = \frac{V_1 - V_3}{V_2 - V_3} \times 100 \quad (1)$$

The swelling behavior of mucilage was analyzed by conducting a phosphate-buffered saline (PBS) uptake test, according to Yilmaz *et al*. [23]. The mucilage powder was weighted (m_{dry}) and soaked in PBS solution (pH ~ 7.4) at 37 °C for 24 h. Then, the wet form of mucilage, taken out from the PBS, was weighed (m_{wet}). The swelling ratio (%) of mucilage was calculated using the following equation:

$$\text{Swelling ratio (\%)} = \left[\frac{(m_{wet} - m_{dry})}{m_{dry}}\right] \times 100 \quad (2)$$

A 1% w/v solution was prepared by dissolving 1 g of mucilage powder in distilled water to determine the pH, density, and viscosity. The density of the solution was measured using a glass pycnometer, the pH was measured by a digital pH meter (pH212, Hanna, USA), and the viscosity was determined using a viscometer (DV-E, AMETEK Brookfield, USA). Moreover, 2 g of the mucilage powder was analyzed to estimate its food value in ash, moisture, protein, carbohydrate, and fat [24, 25].

2.4. Determination of saponins.

The saponins content of the mucilage was determined by high-performance liquid chromatography analysis (HPLC, Smartline, Knauer, Germany). The HPLC has equipped with Eurospher II 100-5 C18 column (250 mm × 4.6 mm; 5 µm, particle size) with a pre-column and UV detector 2500 series. The mobile phase was composed of acetonitrile: methanol (80:20 v/v) and was used at a flow rate of 0.5 mL/min at room temperature. Ursolic acid, a pentacyclic triterpenoid compound, widely occurs in nature as an aglycone precursor for triterpenoid saponins [26]. Accordingly, ursolic acid was used as a standard saponin and detected at the wavelength of 210 nm; the saponin content was expressed as ursolic acid equivalent [27, 28]. To quantify the triterpenoid saponin content of the extracted mucilage, a calibration curve was first developed by injecting 100 µL of several dilutions of ursolic acid dissolved in methanol (31.25, 62.5, 125, 250, and 500 ppm) into the HPLC (standard curve equation: y = 39305.58x + 5.27767; R^2 = 0.9812). Then, the mucilage solution was injected into the system to determine the triterpenoid saponin content; the peak area at the specific retention time and the standard calibration curve were used to determine saponins concentration.

2.5. Antibacterial activity.

The antibacterial activity of the extracted mucilage was tested against *Streptococcus pyogenes* PTCC 9702 provided by the Pasteur Institute of Iran (Tehran, Iran). The microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A bacterial inoculum was prepared in a brain heart infusion (BHI) by adjusting its turbidity to 0.5 McFarland standard. The extracted mucilage was dissolved in distilled water (2000 µg/mL) and then diluted with dimethyl sulfoxide (DMSO, 10%) to obtain several concentrations in the range of 0.97 to 1000 µg/mL. Thereafter, 95 µl of BHI culture medium, 5 µL of bacterial suspension, and 100 µL of different concentrations of the mucilage were poured into each well of sterile 96-well plates and incubated at 37 °C overnight. Three wells without mucilage were considered negative controls. The lowest concentration of mucilage inhibiting the visible growth of bacteria in the wells was demonstrated as MIC. About 5 µL suspension was harvested from the wells with no sign of bacterial growth and then spotted cultured on solid agar plates. MBC is defined as the concentration that completely prevents bacterial growth [29].

The agar disc diffusion method (the National Committee for Clinical Laboratory Standards or NCCLS, 1997) was also employed to measure the inhibition zone diameter [30]. In this procedure, the bacterial inoculant (~100 µL; 0.5 McFarland) was firstly cultured on BHI agar plates. Then, the discs impregnated with 10, 25, and 50 mg mucilage/ mL were inserted into the cultured agar plates, and all plates were incubated at 37 °C. Solvent (DMSO, 10%) was used as a negative control, and the antibiotic Vancomycin was applied as a positive control (25 mg/ mL).

2.6. Animals.

Male ICR mice weighing 25 ± 3 g were used for the study. The mice were healthy and were housed at room temperature (20 to 25 ˚C) at a 12-h light-dark cycle. The animal study was performed strictly following the procedure approved by the Institutional Animal Care and Use Committee, Babol University of Medical Sciences.

2.7. Antitussive activity.

The procedure for testing the antitussive activity of the extracted mucilage was adopted from the literature [31, 32]. After 3 days of adaptation, 15 ICR mice were randomly divided into 5 groups. Group 1 served as the control group and was treated with distilled water, Group 2 served as the positive group and was treated with Dextromethorphan (200 mg/kg), Groups 3, 4, and 5 served as test groups and were respectively treated with quince seed mucilage of 100, 200 and 400 mg/kg. The oral administration of mice was performed once a day at 10:00 am for 5 consecutive days. After 1 h of the last administration, the mice were exposed to an ammonium hydroxide (1 mL) cotton ball in a 1000 mL special chamber, and the time of the first cough (cough incubation period) was recorded. After 1 min, the mice were taken out of the exposure chamber, and their coughing time within 2 min was recorded.

2.8. Expectorant activity.

To evaluate the expectorant activity of the quince seed mucilage, a phenol red excretion experiment was carried out as described in the literature [31, 32]. After 7 days of adaptation, 15 ICR mice were randomly divided into 5 groups. Group 1 served as the control group and

was treated with distilled water, Group 2 served as the positive group and was treated with ammonium chloride (200 mg/kg), Groups 3, 4, and 5 served as test groups and were respectively treated with quince seed mucilage of 100, 200 and 400 mg/kg. The oral administration of mice was performed once a day at 10:00 am for 5 consecutive days. After 30 min of the last administration, phenol-red solution (5% in saline w/v) was injected intraperitoneally in a single dose of 0.1 mL/10 g into the mice. After 45 min of usage of the phenol-red solution, the mice were sacrificed. The trachea was then separated from the thyroid cartilage and immediately put into 2 mL of normal saline. Then, it was ultrasonicated for 15 min; afterward, 2 ml of $NaHCO_3$ solution (5% w/v) was added to the saline solution, and the absorbance of the mixture was measured at the wavelength of 558 nm using Spekol 1500 UV-Vis spectrophotometer (Analytic Jena Instruments Co., Ltd., Germany).

2.9. Statistical analysis.

All tests were carried out in triplicate, and mean values ± SD were reported. Analysis of variance (ANOVA) was carried out by Tukey's multiple range test using SPSS software version 18.0 (SPSS Inc., IBM Corp.). The difference between the data was considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Mucilage extraction.

3.1.1. Extraction yield by single-step aqueous maceration.

The maceration method was used for mucilage extraction, and the effect of 3 parameters, including extraction time, temperature, and solvent-to-solid ratio, on the extraction yields of mucilage and saponin was investigated. The effect of extraction time on the mucilage and saponin extraction yields over the range of 1–4 h is shown in Figure 1A; in this set of experiments, other parameters were constant (temperature: 70 ˚C and solvent-to-solid ratio: 25:1). As observed, by increasing the extraction time from 1 to 2, the mucilage and saponin extraction yields enhanced from 163.12±0.7 and 68.3±0.5 to 169.6±1.1 and 72.4±0.9 mg/ g quince seed, respectively. When the extraction time was prolonged to above 2 h, the mucilage and saponin extraction yields reduced to 160±0.6 and 63.1±0.7 mg/ g quince seed at 4 h, respectively. This indicates that the increased extraction time was not adequate to enhance the extraction yields. Generally, based on Fick's second law of diffusion, the extension of the extraction time leads to an increased extraction yield [33]. Over time, more water molecules penetrate the seeds, dissolve mucilage, and diffuse out from the seeds. The relative adsorption of compounds could explain the decrease in the yield of mucilage and saponin over time in the quince seeds during the maceration process [34]. However, the extraction time has an optimum value; the extended extraction time at a high temperature (70 ˚C) would lead to thermal instability and degradation of some compounds [35]; therefore, the extraction yields mucilage and saponin reduced. Therefore, 2 h was chosen as the optimal extraction time for further studies.

The effect of temperature on mucilage and saponin extraction yield was investigated in the range of 50 to 80 ˚C; the results are illustrated in Figure 1B. Results showed that with an increase in the temperature from 50 to 70 ˚C, the mucilage and saponin extraction yield demonstrated an increasing trend from 161.4± 1.1 and 67.1± 0.6 mg/ g quince seed to 169.6±

0.7 and 72.4± 0.9 mg/ g quince seed, respectively. However, when the temperature was further increased to 80 °C, the mucilage and saponin extraction yields decreased to 161.3± 0.9 and 65.6± 0.8 mg/ g quince seed, respectively. High temperature could disrupt the cell structure and lead to increased cell membrane permeability, enhancing the target component solubility and mass transfer. An increment of solvent temperature could reduce the surface tension, thus increasing the wettability of the solid material and consequently resulting in a more efficient extraction.

Moreover, at high temperatures, the solvent's viscosity reduces, thus allowing better penetration of solvent into the solid matrix; hence, the extraction process is accelerated and improved [36]. However, high extraction temperatures can cause the decomposition of some compounds, especially the depolymerization of some polysaccharides [19]. According to the results, the extraction temperature of 70 °C was the optimum temperature used for further experimentation.

The influence of the solvent-to-solid ratio on the extraction yields was investigated by varying this ratio from 10:1 to 30:1; the results are depicted in Figure 1C. As can be seen, by increasing the solvent-to-solid ratio from 10:1 to 25:1, the extraction yield of mucilage and saponin increased from 154.1± 0.9 and 61.3± 1.1 mg/ g quince seed to 169.6± 1.2 and 72.4± 0.6 mg/ g quince seed, respectively, while, by increasing this ratio to 30:1, the extraction yields decreased to 165.6± 0.9 and 69.2± 0.8 mg/ g quince seed, respectively. Based on the mass transfer principles, the concentration gradient generated between the bulk of the solvent and the solid is the driving force for mass transfer. This concentration gradient will increase by increasing the amount of solvent, and thus, a higher extraction efficiency could be expected. Nevertheless, there should be an equipoise between the amount of solid and the extraction solvent. At low solid-to-solvent ratios, an inadequate amount of solvent may reduce the solvent's ability to penetrate the plant matrix; thus, the extraction yield decreases. Conversely, at high solvent-to-solid ratios, upon a further increase in solvent amount, the extraction of undesired compounds from the plant's matrix may be encouraged; therefore, the purity of the targeted components and their extraction yields decrease [37]. According to the obtained results and the presented discussion, the solvent-to-solid ratio of 25:1 was the most suitable in the studied range.

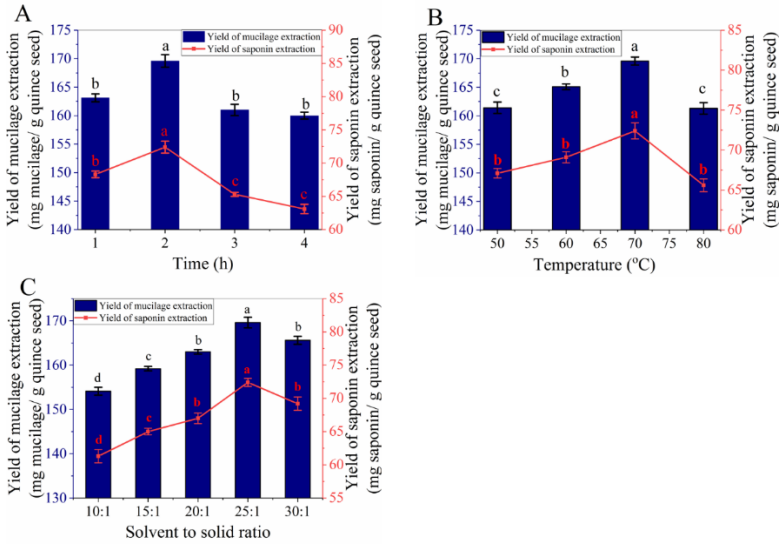

Figure 1. Effect of **(A)** maceration time (at 70 °C and solvent-to-solid ratio 25:1), **(B)** temperature (for 2 min and the solvent-to-solid ratio of 25:1), and **(C)** solvent-to-solid ratio (for 2 min at 70 °C) on the extraction yields of mucilage and saponin from quince seeds. Different letters (a–d) indicate a significant difference between examined parameters by comparison in Tukey's test ($P < 0.05$).

3.1.2. Extraction yields by ultrasonic pretreatment followed by aqueous maceration.

The effect of ultrasonic pretreatment before aqueous maceration at the optimized condition (2 h, 70 °C, and solvent-to-solid ratio of 25:1) on the mucilage and saponin extraction yields was studied. In this study, ultrasonication time (2, 4, 6, and 8 min) and ultrasonication power (160, 200, 240, and 280 W) were the investigated parameters. Figure 2A exhibits the influence of ultrasonic treatment time on extraction yields. According to the results, by increasing the time from 2 to 6 min, extraction yields of mucilage and saponin increased from 174.9± 0.9 and 62.8± 0.6 mg/ g quince seed to 190.1± 0.7 and 90.6± 0.5 mg/g quince seed, respectively, while by extending the extraction time to 8 min, the extraction yields respectively decreased to 186.2± 0.9 and 47.5± 1.1 mg/g quince seed. The ultrasound waves disrupt the solid cell wall and reduce the particle size, providing a large contact area in the extraction medium between the solid and the solvent, which improves the extraction yield. However, at prolonged extraction time, with a decrease in the concentration gradient, the tendency of solvent for penetration into the solid matrix decreases, and the extraction yield reduces [38]. Accordingly, the ultrasonic pretreatment time of 6 min was appropriate to achieve a good extraction yield.

Figure 2B shows the effect of different ultrasonic powers on extraction yields. With an increase in ultrasonic power from 160 to 200 W, the extraction yield of mucilage and saponin enhanced from 185.2± 0.9 and 60.2± 0.8 mg/g quince seed to 190.1± 0.7 and 90.6± 0.5 mg/g quince seed, respectively. By further increase of the power beyond 200 W, the extraction yields decreased. Ultrasound-assisted extraction is based on two fundamental principles, acoustic cavitation, and microstreaming [39]. The cavitation phenomenon can induce cavitation bubbles to generate, resonate, and collapse in the extraction solvent; therefore, it causes intensive shear forces on the solid matrix. Cavitation development and microstream augmentation during ultrasonic treatment can cooperatively enhance the extraction yield. By propagating the ultrasonic field in the solvent, the acoustic pressure of the liquid increases, and when it exceeds the cavitation threshold, the maximum effect of ultrasound can be expected. Applying ultrasonic power beyond the optimum value weakens the cavitation effect, where the cavitation bubbles are most likely to grow too large to collapse. Moreover, excessive generation of cavitation bubbles at high ultrasonic power hinders the scattering of the ultrasound waves and mass transfer, thus reducing the effect of ultrasonication on the extraction [40]. Therefore, the ultrasonic power of 200 W was considered the optimum.

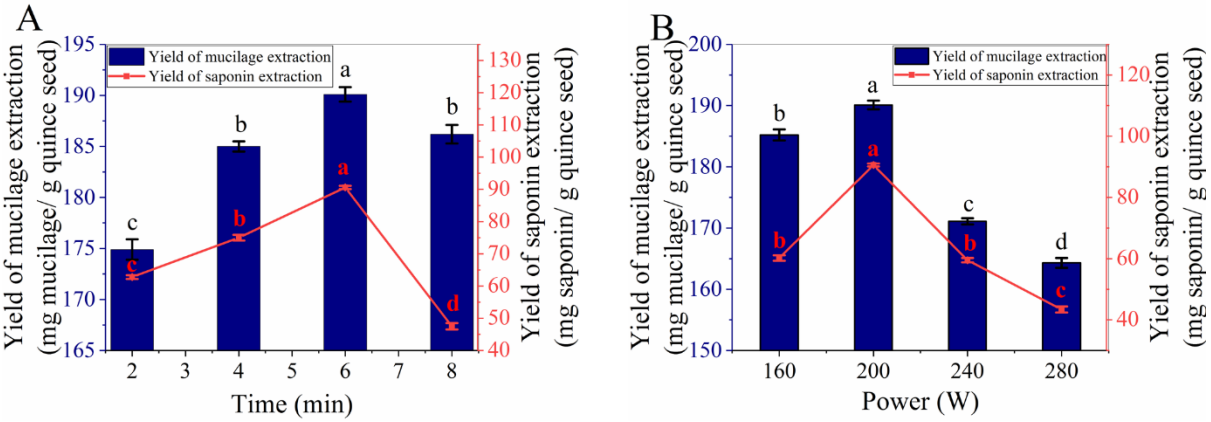

Figure 2. Effect of **(A)** sonication time (at 200 W) and **(B)** power (for 6 min) on the extraction yields of mucilage and saponin from quince seeds; maceration temperature: 70 °C, maceration time: 2 h, solvent-to-solid ratio: 25:1 and sonication time: 6 min; different letters (a–d) indicate a significant difference between examined parameters by comparison in Tukey's test ($P < 0.05$).

3.1.3. Extraction yields by microwave pretreatment followed by aqueous maceration

As another extraction method, microwave pretreatment was used before maceration at the optimized condition (extraction at a solvent-to-solid ratio of 25:1, 70 °C for 2 h) to investigate its influence on mucilage and saponin extraction yields. The effect of microwave irradiation time on the extraction yields was investigated at different exposure times, from 1 to 4 min, and the results are illustrated in Figure 3A. By increasing the microwave irradiation time, the extraction yields increased and at 2 min reached the maximum amount (191.2± 0.5 mg mucilage/g quince seed and 94.8± 0.6 mg saponin/g quince seed). However, when the microwave irradiation time was prolonged to above 2 min, both extraction yields were reduced (169± 0.5 mg mucilage/g quince seed and 58.9± 0.5 mg saponin/g quince seed at 4 min). Microwave irradiation offers fast solvent penetration into the cellular matrix through *in situ* molecular interaction and, thus, leads to the quick dissolution of the available compounds to be extracted [41]. Generally, increasing the extraction time could enhance the amounts of extracted compounds, but excessive exposure to radiation causes a significant increase in the temperature and results in the thermal degradation of compounds [37, 38]. Therefore, the irradiation time of 2 min was selected as the optimum microwave extraction time.

Figure 3B illustrates the effect of different levels of microwave power (100-450 W) on mucilage and saponin extraction yields. Results indicated that increasing microwave power enhanced the extraction yields so that maximum yields were acquired at 180 W (191.2± 0.5 mg mucilage/g quince seed and 94.8± 0.6 mg saponin/g quince seed), while by further increasing the power, the extraction yields decreased. The microwave power and temperature are interrelated, so increasing the power will increase the medium temperature [42]. The increase in temperature enhances the solvent's capability for penetration into the solid matrix, and because of the fall in the solvent viscosity and surface tension, the solubilization of targeted compounds increased. Nevertheless, at high temperatures, the extraction yield might be adversely affected due to the thermal degradation of some compounds [43]. Since at 180 W, the highest extraction yields were obtained, this microwave power was chosen as the optimum.

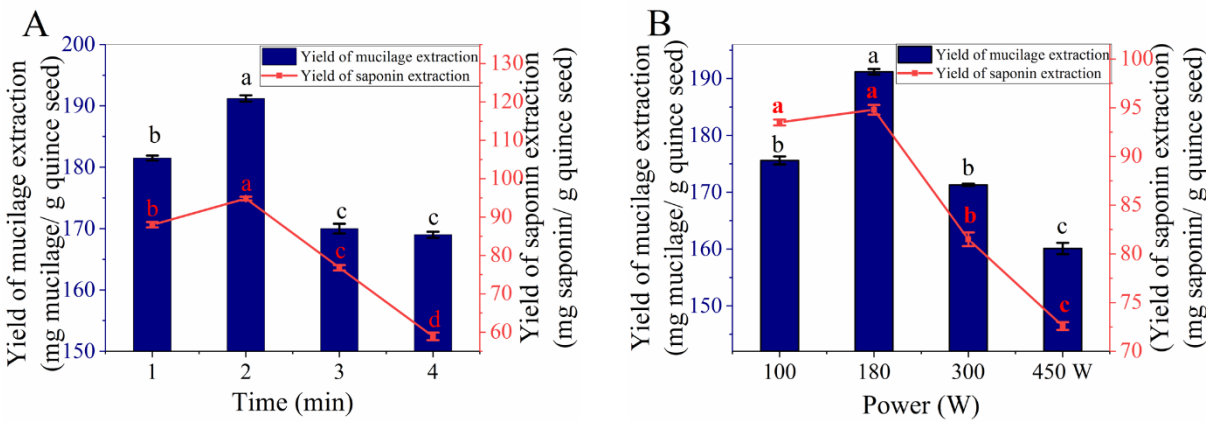

Figure 3. Effect of **(A)** microwave irradiation time (at 180 W) and **(B)** microwave power (for 2 min) on the extraction yields of mucilage and saponin from quince seeds; maceration temperature: 70 ˚C, maceration time: 2 h, solvent-to-solid ratio: 25:1 and microwave irradiation time: 2 min; different letters (a–d) indicate a significant difference between examined parameters by comparison in Tukey's test ($P < 0.05$)

Table 1 summarizes the results for mucilage and saponin extraction yields from quince seed using all four methods. As can be seen, microwave pretreatment followed by the aqueous maceration method with extraction yields of 191.2± 0.5 mg mucilage/g quince seed and 94.8± 0.6 mg saponin/g quince seed had a better performance than other extraction methods. The

yields obtained from this method were higher than those obtained from Soxhlet extraction, which is usually used as a reference method to evaluate the efficiency of other extraction techniques, while the extraction time was considerably shorter. Therefore, the extracted mucilage from this method was characterized to determine its physicochemical and nutritional attributes and evaluate its therapeutic properties.

Table 1. Mucilage and saponin extraction yields from the quince seed obtained by different methods at the optimum conditions.

Extraction method	Extraction condition	Mucilage extraction yield (mg /g quince seed)	Saponin extraction yield (mg /g quince seed)
Soxhlet	Temperature: 70 ˚C Time: 12 h	175.2 ± 7.2	63.8 ± 5.0
Single-step aqueous maceration	Temperature: 70 ˚C Time: 2 h	169.1 ± 6.2	72.4 ± 5.1
Ultrasonic pretreatment followed by aqueous maceration	Sonication time: 6 min Sonication power: 200 W Maceration time: 2 h Maceration temperature: 70 ˚C	190.1 ± 1.8	90.6 ± 1.9
Microwave pretreatment followed by aqueous maceration	Microwave irradiation time: 2 min Microwave power: 180 W Maceration time: 2 h Maceration temperature: 70 ˚C	191.2 ± 0.5	94.8 ± 0.6

3.2. Characterization of the extracted mucilage

Microwave pretreatment of quince seeds followed by aqueous maceration for mucilage extraction resulted in 191.2± 0.5 mg dried mucilage per gram of quince seed. The nutritional value of the extracted mucilage was identified, and the results are summarized in Table 2. The results show that carbohydrates are the major constituents (77.80%) of the mucilage. This result could be expected as the literature suggests that quince seed mucilage is a complex of cellulose microfibrils associated with glucuronoxylans with a high portion of readily hydrolyzable polysaccharides [44, 45]. Particularly saponins, also known as sapogenins, are present in significant amounts in the extracted mucilage and are known to have expectorant and antitussive activities [46]. Saponins are amphipathic glycosides with one or more hydrophilic glycone moieties on a lipophilic aglycone backbone (either a triterpene or steroid derivative).

Some physicochemical properties of the mucilage extracted from quince seeds were determined, and the results are summarized in Table 2. The pH of mucilage was found to be 5.5± 0.4, which indicates the naturally obtained mucilage does not irritate the oral cavity mucous membrane, so is suitable for use in natural form and uncoated [47]. The viscosity of the mucilage solution was found to be 1472± 21 cP, which might be due to the high molecular weight of acquired mucilage with a molecular weight of about 200,000 [48], and its density was 0.32± 0.15 g/ml. The porosity of mucilage was measured as 78.6± 3.1%; the presence of high porosity in mucilage structure could enhance liquid absorption, produce a large surface area, and facilitate mucilage dissolution [49]. Finally, the swelling ratio of mucilage was found to be 13100± 282%, representing the high ability of mucilage to absorb liquids and exchange nutrients [49].

Table 2. Nutritional value, phytochemical analysis, and physicochemical properties of quince seed mucilage.

	Nutritional value				
Parameter	Ash	Moisture	Carbohydrate	Protein	Fat
Percentage (%)	7.33	7.75	77.80	5.09	2.03*

	Phytochemical analysis				
Phytochemicals	Alkaloids	Steroids	Tannins	Flavonoids	Saponins
Results	+	++	–	+	+++
	Physicochemical properties				
Parameter	Density (g/ml)	Viscosity (cP)	Swelling ratio (%)	Porosity (%)	pH
Value	0.32± 0.15	1472± 21	13100± 282	78.6± 3.1	5.5± 0.4

*By difference, + trace amount, ++ detectable, +++ substantially detectable, – not detectable.

Figure 4A shows the FTIR analysis of quince seed mucilage. The characteristic absorbance peak of the hydroxyl group (OH) appeared at 3418 cm^{-1}, which generally constitutes the gross structure of carbohydrates. The bands at 2932 and 2375 cm^{-1} can be attributed to the symmetric stretching vibration of CH_2 and CH vibrational stretching, respectively [50, 51]. The weak absorption peak at 1722 cm^{-1} is caused by C=O [52], and a strong absorption peak can be observed at around 1621 cm^{-1}, which is assigned to COO- asymmetric stretching. The peaks located at 1425 cm^{-1} could be attributed to C–OO symmetric stretching [48, 53]. The absorption band corresponding to oligosaccharide linkage to sapogenins (C-O-C) appeared at 1046 cm^{-1} [54, 55]. These characteristic peaks in the spectrum of quince seed mucilage have been reported previously [1, 56]. Figure 4B presents the XRD pattern of quince seed mucilage powder. The diffraction pattern showed broad peaks at 2θ= 15.1° and 22.8°, indicating the mucilage has a dominant amorphous structure [57]. Due to their structure, the crystalline compounds have poor water solubility and, as a result, have low bioavailability. In contrast, the solubilities of amorphous drugs are about 1,600 times that of the crystalline form [58]. Therefore, the appropriate solubility of quince seed mucilage in water could be attributed to its amorphous state, which offers a lower thermodynamic barrier to dissolution and has led to its high bioavailability [59]. Quantitative determination of saponins in the extracted mucilage was carried out by HPLC analysis. Figure 4C exhibits the HPLC chromatograms of (i) ursolic acid, used as a standard to quantify triterpenoid saponins, and (ii) the extracted mucilage from quince seed. From the analysis, the triterpenoid saponin content of the extracted mucilage, expressed as ursolic acid equivalent, was determined to be 9.48%.

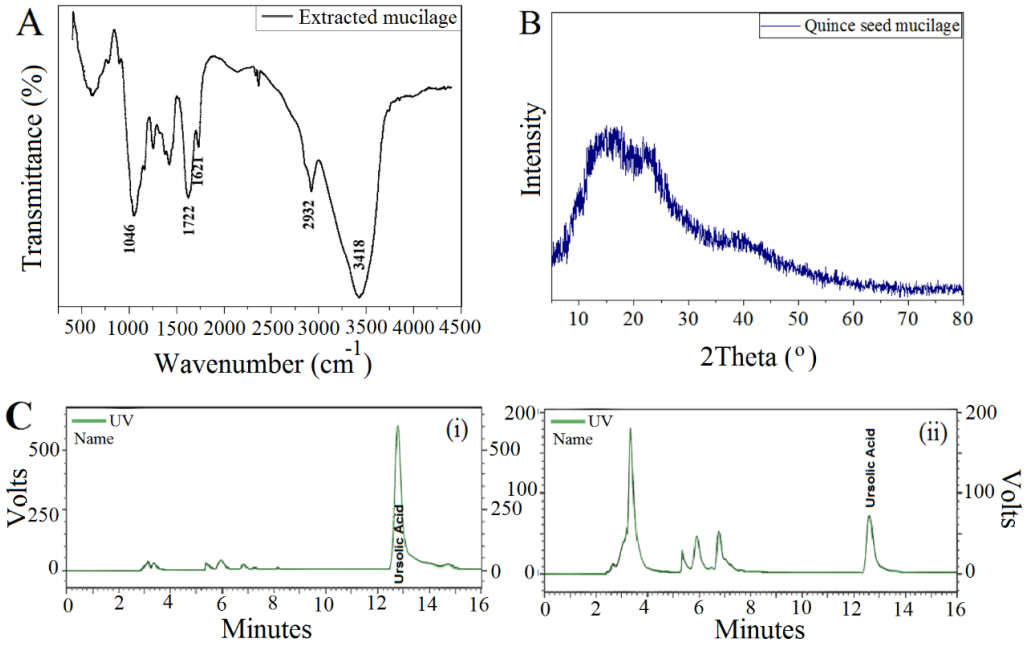

Figure 4. (A) FTIR spectrum of quince seed mucilage, **(B)** X-ray diffractogram of quince seed mucilage, and **(C)** HPLC chromatograms of ***(i)*** standard ursolic acid (100 ppm) and ***(ii)*** aqueous extract of quince seed

3.3. Antibacterial activity.

Streptococcus pyogenes is a pathogenic bacteria responsible for many diseases, specially pharyngotonsillitis. This bacterium has shown resistance against drugs used to treat the corresponding infection. In this study, *in vitro* antibacterial activity of the mucilage extracted from quince seed against *S. pyogenese* was assessed by determining the inhibition zone diameter. The results were compared to those obtained using a commercial antibiotic, Vancomycin (positive control). The results of this test are presented in Figure 5. The quice seed mucilage revealed considerable antibacterial activity against *S. pyogenese* at all examined concentrations. With an increase in mucilage and, thus, saponin concentration, the diameter of the inhibition zone increased. This antibacterial activity was even superior to that of Vancomycin, as could be inferred from comparing the inhibition zone diameter. At mucilage concentrations of 25 and 50 mg/mL, the inhibition zone diameters were 6.73±0.16 and 7.51±0.34 mm, respectively, which were significantly ($P<0.05$) higher than that observed for Vancomycin, 5.31±0.11 mm. According to the results of the microdilution test, the MIC and MBC values of the aqueous extract were found to be 62.5 and 125 µg/mL, respectively. The antibacterial activity of the quince seed mucilage is most probably associated with its phytochemical compounds, including saponins, alkaloids, and steroids, whose antimicrobial activities have been numerously reported in the literature [60, 61]. However, among these secondary metabolites, saponins might present a superior antimicrobial activity owing to their surfactant properties which can interact with cell membrane cholesterol and induce bacterial cell lysis [62].

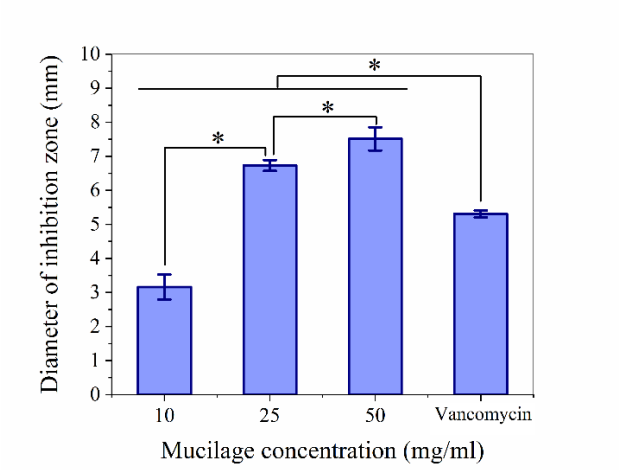

Figure 5. *S. pyogenes* growth inhibition zone caused by different concentrations of the quince seed extract compared to Vancomycin (positive control, 25 mg/ml); mean ± SD; n = 3, * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

3.4. Antitussive and expectorant activity.

In folk medicine in China, quince seed is used as a peripheral or central antitussive that suppresses cough [63]. Cough is generally classified as either nonproductive (dry) or productive (producing mucus commonly with expectoration). Non-narcotic antitussive agents obtained naturally from some plants and fruits anesthetize the stretch receptor located in the pleura, lungs, and respiratory passages by dampening their activity, thus, leading to a decrease in the reflex of cough at its source [64]. The mucilage from quince seed is used via oral or topical routes to treat such disorders, and so far, in traditional cures, it has been mostly used as a non-narcotic antitussive agent. With this background, the antitussive and expectorant

activities of quince seed mucilage, which have been rarely studied, were investigated in this work.

The antitussive activity of the quince seed mucilage was assessed *in vivo* using a cough model induced by ammonium hydroxide in mice. The potency of the mucilage in delaying the cough was evaluated by considering the cough incubation period, defined as the time interval from exposure to ammonium hydroxide to the commencement of the cough. Prolonging the incubation period and reduction of the coughing time in 2 min were indications of antitussive activity of the mucilage. In fact, the longer cough latency period specified the stronger cough-relieving effect of the mucilage, and the lower cough frequency indicated its potent antitussive activity. The antitussive effect of the quince seed mucilage at different concentrations of 100, 200, and 400 mg mucilage/kg in the ammonia-induced cough test is depicted in Figure 6. Results indicated that in mucilage-administered mice, the cough incubation period increased in a dose-dependent manner; with an increase of mucilage dose from 100 to 400 mg/kg, the incubation period was prolonged from 13.7 to 20.7 s (Figure 6B). The commercial antitussive agent, Dextromethorphan (200 mg/kg), could provide an incubation period of 15.7 s. Investigations on the cough frequency in mice (Figure 6C) showed that the highest inhibition of cough (51.80%, compared to control) was achieved in Group 5, treated with 400 mg mucilage/kg. Also, the latent cough period was prolonged by 121.4% in this group. These results were comparable to what was obtained from Group 2, which was treated with Dextromethorphan, where the number of coughs was reduced by 50.4%, and the incubation period was lengthened by 67.8%. Treatment of mice with the quince seed mucilage pronouncedly prolonged the cough incubation period and reduced the cough frequency, especially at the dose of 400 mg/kg. This highlights the cough-curative effect of the aqueous extract of quince seed and its antitussive activity.

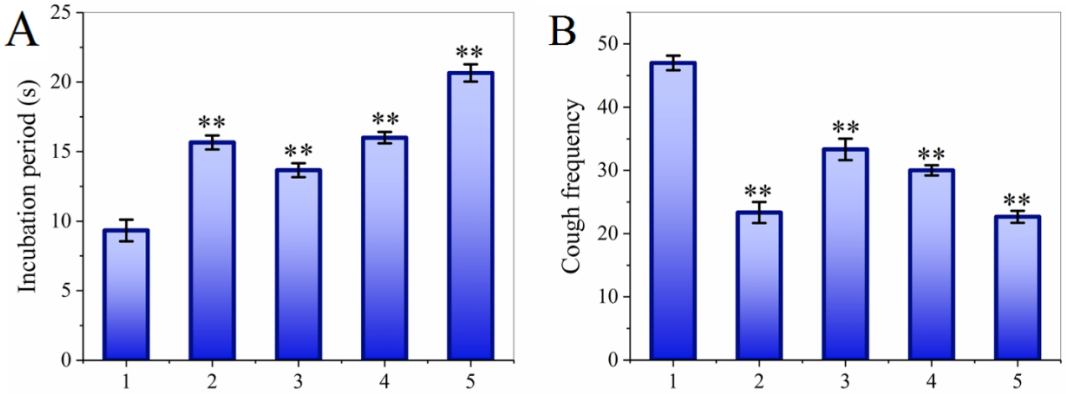

Figure 6. Antitussive activity of the quince seed mucilage on the ammonia-induced cough in mice: **(A)** incubation period and **(B)** cough frequency within 2 min. 1: Control group, mice without any treatment, 2: positive group, mice were given Dextromethorphan (200 mg/kg body weight) and 3-5: test groups, mice were administered doses of 100, 200, and 400 mg mucilage/ kg body weight, respectively. The asterisks (**) indicate statistical significance ($p<0.01$) as compared to the control.

The expectorant activity of the quince seed mucilage was tested by measuring the phenol-red secretion in mice, and the results are illustrated in Figure 7. Results showed that all doses of mucilage could increase the tracheal phenol red secretion compared to the control in a dose-dependent manner. At doses 200 and 400 mg/kg, the phenol-red secretion enhanced by 109.4 and 182.8%, respectively, which was better than that of ammonium chloride at the dose of 200 mg/kg. Several studies have reported the presence of saponins, alkaloids, and phenolic compounds in plants traditionally used to treat cough [32, 46, 65]. As evidenced by our results, quince seed mucilage's antitussive and expectorant activities can be attributed to the presence

of these bioactive constituents. Especially well-known compounds in this regard are saponins, whose antitussive and expectorant properties have been reported [31, 46]. However, these compounds should be isolated and characterized individually to define each chemical constituent's role and clarify the curative mechanism of quince seed mucilage. Their pharmacological effects need to be further clarified in future studies.

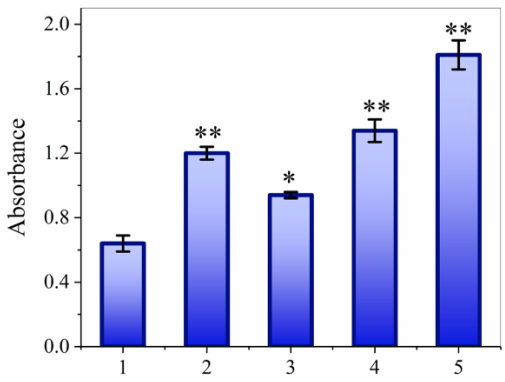

Figure 7. Effect of quince seed mucilage administration on the phenol-red excretion in mice to show the expectorant activity. 1: Control group, mice without any treatment, 2: positive group, mice were given ammonium chloride (200 mg/kg body weight) and 3-5: test groups, mice were administered doses of 100, 200, and 400 mg mucilage/ kg body weight, respectively. The asterisks (** and *) indicate statistical significance ($p<0.01$ and $p<0.05$, respectively) as compared to the control.

4. Conclusions

In the present study, the extraction of mucilage from quince seed was carried out using different methods. Results indicated that using only 2 min microwave treatment before aqueous maceration (70 ^{o}C, 2h) was effective in increasing the mucilage and saponin extraction yields by 13 and 31%, respectively. In addition, the antibacterial, antitussive, and expectorant activities of quince seed mucilage were evaluated. Results revealed that quince seed mucilage had an antibacterial effect against the clinical strain of *S. pyogenes*, with an inhibition effect comparable to that of Vancomycin. In addition, the mucilage showed significant antitussive and expectorant effects, which are important evidence for the traditional use of quince seed mucilage as a cough remedy. These effects are assumed to be associated with saponins and other bioactive compounds in the mucilage. Yet, further investigations are required to confirm the premise and clarify the mechanism of action.

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Conflicts of Interest

The authors declare no conflict of interest.

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