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Research Article:

Evaluation of Various *In-vitro* Biological Activities of Crude Quince Seed Ethanolic Extract

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Abstract

Background: for a long time, natural bio-actives have been used to treat, cure, and prevent illnesses. Fruit seed crude extracts have been found to have various beneficial biological activities, such as anti-inflammatory, antioxidant, and antitumoral potential. **Methods:** this study investigated quince seed extract made using absolute ethanol as a solvent regarding flavonoid and phenolic content, as well as antioxidant, anti-inflammatory, and anticancer properties. The extract was obtained using two methods, microwave-facilitated extraction and sequential microwave-ultrasound-assisted extraction. **Results:** the extract from the microwave-facilitated method had higher flavonoid and phenolic content with superior antioxidant activity. Hydroxyl free radical and DPPH assays' IC_{50} were 121.04±1.10 and 112.98±1.02, respectively, compared to the sequential method, which had IC_{50} values of 193.19±0.86 and 187.23±0.95. The microwave method also displayed significantly higher inhibitory activity against inflammatory enzymes and selectivity against COX2 compared to the sequential one. However, the extracts did not exhibit any antiproliferative effect on malignant or healthy cellular lines at the applied concentrations, and they did not enhance the effectiveness of 5-FU (5-Fluorouracil) in preventing the growth of cancerous cells. However, the mutual application in general results in increasing the IC_{50} value of 5-FU toward the healthy cellular line (MCF-A10). Therefore, the mutual application of these extracts with 5-FU can be seen as a protective measure. **Conclusion:** the crude extract obtained through the microwave-facilitated method had better TPC, TFC, antioxidant, anti-inflammatory activity, and protective potential than the sequential method.

1. Introduction

Natural bio-actives have been successfully utilized for treating, curing, and preventing illnesses for a very long time. Today a large number of medications are natural or synthesized from natural ingredients. Roughly 25% of prescribed drugs are produced from plants (1). In addition, the international community nowadays prefers natural components over those of a synthetic origin in items used in daily life (2).

A growing number of the by-products produced by fruit processing, including seeds, are typically discarded but may instead be used as an unusual source of bi-actives. Based on information about the output of fruits worldwide in 2013 and the presence of seeds in various crops fruits, the amount of quince seeds has been estimated at 32–57 thousand tons, with a significant amount of these seeds can be retrieved and used as a natural source of phytocompounds (3).

Quince (*Cydonia oblonga* Mill.) belongs to the Cydonia genus, a *Rosaceae* family member. Quince is well known for its nutritional benefits. The yellowish quince fruit is built with two vertical rows of fixed-focus seeds. This fruit has gained a lot of attention due to its antibacterial, anti-inflammatory, and anti-ulcer properties. It is now considered a cost-effective and easily accessible source of valuable nutrients with biologically active plant chemicals. However, there has been a few studies on its seeds (4,5).

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It is worth mentioning that oxidative stress and chronic inflammation are recognized to play a role in the development of cancer. These factors can lead to the cancerous transformation of cells, promote tumor growth and spread, trigger the formation of new blood vessels, and alter tissue structure (6). In the past few years, numerous studies on fruit seed crude extracts have revealed various beneficial biological activities with promising applications, including, anti-inflammatory, antioxidant, and antitumoral activities as well as antiparasitic, antiviral, and antimicrobial potential (7,8). In this scope, this study had been conducted to measure the amount of phenolic and flavonoid compounds present in quince seed ethanolic extract and to assess its antioxidant, anti-inflammatory, and cytotoxic effects.

2. Materials and Methods

2.1 Reagents and equipment

Sigma-Aldrich and Chemlab were used as sources for the chemicals and solvents used in this study. An Expert from the University of Mosul/College of Agriculture and Forestry taxonomically distinguished the dried quince seeds after they were obtained at a public market in Mosul. The extraction processes were accomplished using a microwave oven (Moulinex, MW531070, France) and a bath of water fitted with an ultrasound generator (40 kHz-Power sonic 410, 350 W).

2.2 Quince seed's extraction

To extract the seeds of *Cydonia oblonga*, the seeds were ground and sifted into a fine powder. Thirty grams of the powder were mixed with 300 ml of absolute ethanol in a beaker, creating a solid-to-solvent ratio of 1:10. Two methods were used for extraction. The first was microwave-facilitated, where the sample was placed in a microwave fixed at 180 W, rotating on a turntable for even heating, and irradiated for 90 seconds. The second was microwave-ultrasound-assisted, where the sample was first irradiated in the microwave for 90 seconds, then transferred to the bath and sonicated at 30°C for a period of 30 minutes. The ethanol-based extracts were coded as Qu-1 and Qu-2, based on the two extraction methods used, with number 1 representing the first method and number 2 representing the second. After extraction, the extracts were vaporized under reduced pressure (9).

Using ethanol as a solvent for extraction offers several benefits. It is non-toxic, has preservative qualities, evaporates quickly at low temperatures, and enables quick absorption of the extract by the human body. Since ethanol is safe for human usage, ethanolic extracts can be ingested or applied directly to the skin. Furthermore, research has shown that the ethanolic extract of quince seeds exhibits superior *in vitro* biological activities compared to extracts from other solvents (10,11).

2.3 Total phenolic content (TPC)

The colorimetric method developed by Folin-Ciocalteu was used to calculate the TPC of each coded ethanol-based extract. Briefly, from a mother solution of aqueous crude solid of concentration 1 mg/ml, a daughter solution of 200 µl was diluted up to 3 ml using distilled water (DW) as a diluent. The investigation was initiated by mixing the daughter solution with 0.5 ml of the reagent (Folin-Ciocalteu). Blending process lasted for a period of 3 minutes and was followed by treating the working blend with a 2 ml volume of 20% aqueous Na_2CO_3. After the lab incubation out of daylight for 1 hr. and at 650 nm, the visible absorbance value was defined. From the gallic acid calibration curve, the TPC was figured, and to certify the results, three independent trials were conducted (12).

2.4 Total flavonoid content (TFC)

The TFC was identified using the colorimetric $AlCl_3$ approach of each coded ethanol-based extract. In brief, from a mother solution of aqueous crude solid of concentration 1 mg/ml, a daughter solution of 50 µl was diluted sequentially to 1 ml using methanol as a diluent and up to 4 ml with DW. The investigation was initiated by mixing the daughter solution with an aqueous $NaNO_2$ solution (0.3 ml, 5%). After a lab incubation period of 5 min, an aqueous $AlCl_3$ solution (0.3 ml, 10%) was added, and the working blend was preserved in a light-free place for 6 min. The resultant combination was treated with an aqueous NaOH solution (2 ml, 1N), and the volume in total was made equal to 10 ml using DW as a diluent. The lab incubation out of daylight for 15 min was before the visible absorbance reading measurement at 510 nm. The TFC was computed from the already prepared calibration curve of rutin, and to confirm the findings, three lab replicates were conducted (13).

2.5 Inspecting the antioxidant attribute

The original solution has a 1 mg/ml concentration, while the diluted solutions have the following concentrations: 1000, 750, 500, 250, 125, 100, 50, and 25 µg/ml. For the reference antioxidant drug, vitamin C (V-C), other concentration levels were employed, comprehending 200, 100, 50, 25, 12.5, and 6.25 µg/ml. The diluents for the tests of DPPH, ·OH, and total reducing potential (TRP) were ethanol, K_3PO_4 buffer (0.2 M, pH 7.8), and K_3PO_4 buffer (0.2 M, pH 6.6), respectively. Various assays for scavenging free radicals, such as DPPH scavenging, Hydroxyl Reactive Moiety-scavenging, and total reducing capacity, have been performed in accordance with the references (4,14).

2.5 Inspecting the anti-inflammatory attribute

5-Lipoxygenase (5-LOX) antagonist assay

The solids afforded from vaporizing the extraction solvent were tested using the 5-LOX antagonizing estimator, bought from Sigma Aldrich and keyed 437996. In brief, daughter concentration measurements ranging from 25 to 1000 µg/ml were prepared from a mother solution (1 mg/ml). The concentration levels of the reference drugs, aspirin (ASA) and celecoxib (CLB), were ranged from 0.25 to 64 µg/ml. The assay blend is comprised of the explored enzyme (90 µl), conventional chromogen solution (100 µl), specific sample concentration (100 µl), and substrate (arachidonic acid, 10 µl, 0.1 mM). The absorbance scores were specified spectrophotometrically at 490 nm after a 10-minute gestation period in relation to the blank. These scores, with their corresponding concentration measurements, were employed to calculate the antagonist percentages, which were then used to quantify the target IC_{50} values (15).

Cyclooxygenase (COX) antagonistic assay

The COX (ovine/human) antagonizing forecasting model, bought from Cayman and keyed at 560131 was employed in this assay. The concentration levels of the mother and daughter solutions are similar to those prepared in the 5-

LOX assay. The researched enzyme (10 µl), Tris-HCl buffer (0.96 ml, 0.1 M), and particular sample concentration (100 µl) make up the biomarker mixture. Following a 10-minute gestation at 37°C, a substrate (10 µl, 0.1 mM) named arachidonic acid was combined to the mix, and the resultant blend was treated after 2 min with Ellman's reagent (50 µl, 1 M). In relation to the blank and at 410 nm, the absorbance score of the test blend was specified, and the collected scores were subsequently employed to calculate the antagonistic percentages and the IC_{50} scores (16).

2.6 Inspecting the anticancer attribute

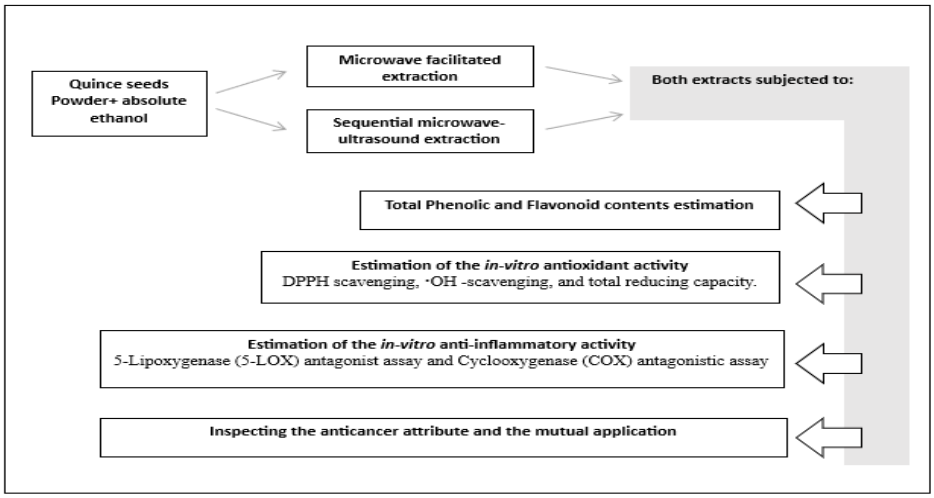
In this inspection, the MTT-based sight approach was used. In a nutshell, eight aqueous daughter solutions with concentration levels ranging between 6.25 µg/ml and 800 µg/ml were created from an aqueous mother solution (1 mg/ml). 10,000 malignant/healthy cells were placed in a welcoming cultural community in each assigned well of a 96-well arrangement and allowed to bioproliferate at 37 °C for a 24-hr incubation time frame. The active cells within each well were bioincubated separately with an assigned concentration of the researched chemical-based entity or reference drug at the same temperature but for a 72-hr time frame when the cells had consistently adhered to the well walls. A sight detector (28 µl, 3.27 mM), MTT, was planted, and the culture medium was extracted out of the well before the bioincubation was kept at 37 °C for 1.5 hrs. The microarray analyzer was used to distinguish between readings from treated ($R_{treated}$) and untreated ($R_{untreated}$) wells by measuring their optical absorbance values at 492 nm. The cell growth inhibition percent (GP%) was calculated using the formula ($R_{untreated}$-$R_{treated}$/$R_{untreated}$) ×100. The non-linear regression equation used to determine the magnitude of IC_{50} outlined the information of the GP% variable against the matching log concentrations. The standard deviation (SD) results across three distinct studies were calculated to confirm the findings (14).

Two malignant cell lines named MCF-7 and HeLa and one healthy cell line known as MCF-A10 were employed in this inspection using 5-fluorouracil (5-FU) as a reference anticancer drug.

3.1 Influence of mutual application

To evaluate quantitatively and qualitatively the effect of individually applying each of the obtained extracts with 5-FU against the two malignant and one healthy cellular line, the previously described MTT-based methodology was employed. The only variation is the application of each 5-FU concentration with its corresponding concentration of a defined extract (17). **Scheme 1** outlines the steps that were used to carry out this study.

release. Calcium channels are composed of 1, 2, and 3 subunits, as demonstrated by molecular biology methods employing skeletal muscle L-type calcium channels. The most important function is performed by the calcium transmission channel, The L subunit specifically produces a compound, and there have been twelve 1 subunits cloned as well have also been cloned, and based on how similar their gene sequences are, they have been grouped into 3 subdivisions called Cav1.x, Cav2.x, and Cav3.x (8).



Scheme 1. An outline of the methodology that was used to conduct this study

3. Results and discussion

The world is currently facing a major health crisis caused by chronic diseases like cancer, heart disease, and diabetes, which have resulted in millions of people suffering from disabilities and even death. To combat these diseases, natural compounds known as phytochemicals, which are non-nutrient compounds found in plants, can provide various health benefits and offer protection against the development of chronic diseases. Plant seeds, which are widely distributed in many regions of the world, contain

high quantities of these compounds, phenolics, in particular, can scavenge endogenous and exogenous free radicals. Additionally, polyphenols have the capacity to influence intracellular signaling and activate endogenous defense mechanisms, offering indirect security. Numerous investigations into the seeds of *Rosaceae* plants have shown a unique phenolic profile as well as a wide range of intriguing phytocompounds (18–21). This led to the present study, which aimed to scrutinize a number of biological activities of quince seeds' ethanolic extract, which is accomplished by two extracting methods.

3.2 Total phenolic and flavonoid contents estimation

Cydonia oblonga, a plant with a high concentration of phenolic compounds, has been found to possess antioxidant properties. These compounds act as hydrogen donors, reducing agents, and effectively neutralizing free radicals. The presence of phenolics in fruit, seeds, and peel extracts of this plant indicates why it has been utilized in traditional herbal remedies (22). Polyphenols, known as flavonoids, are the most common type. Due to their numerous phenolic OH groups, these compounds possess a higher potential for antioxidant activity. Total flavonoids show different trends from the data of total phenolics, as flavonoids belong to the polyphenol compound class (23).

In the present study, the use of microwave extraction technique resulted in a higher total phenolic (TPC) and flavonoid content (TFC) compared to sequential microwave-ultrasound-assisted extraction. This is evident from **Table 1**. An earlier research by Amin et al. also reported an acceptable total phenolic content in the ethanolic extract, which was prepared by mixing seed powder with ethanol and chloroform and extracting it for 24 hours at a temperature below the solvents' boiling points individually (10).

3.3 Estimation of the in-vitro antioxidant activity

As of yet, no successful chemotherapy has been discovered for the chronic illness caused by oxidative stress. However, recent research has revealed that certain (poly)phenolic compounds found in plants possess antioxidative properties. It is known that there are thousands of phytochemicals containing phenol rings with varying structural elements. The phenolic compounds found in seeds have been proven to lessen the danger of diverse ailments such as coronary heart diseases, inflammation, cancer, stroke, atherosclerosis, and neurodegenerative diseases related to oxidative stress (24,25).

Table 2 shows that the crude ethanolic extract obtained through microwave extraction has higher antiradical activity than the sequential microwave-ultrasound-assisted extraction method. Both methods exhibited parallel scavenging capacity to DPPH and hydroxyl free radicals as in our previous study, but ultrasound extraction had superior activity (4). The ethanolic extract of quince seeds in our study has a higher free radical scavenging capacity compared to other studies. For example, Shaida *et al.* found the IC_{50} of DPPH neutralizing for the ethanol based extract of quince seeds to be 299.98 µg/ml, while Amin *et al.* reported IC_{50} values of 361.68 µg/ml for DPPH scavenging, for the ethanolic extract of quince seeds (10,26). Our study's antioxidant activity results are premium to those of earlier research, which may be due to the mechanical, physical, chemical changes resulting from the extraction techniques used in our studies, resulting in better extraction of phytochemicals with radical scavenging potential. Moreover, it is worth noting that the antioxidant response of phenolic compounds can vary depending on their specific chemical structure (7,27).

3.4 Estimation of the in-vitro anti-inflammatory activity

Inflammation is a complex process that occurs when tissues react to irritation, infections, or trauma. This process is employed in diverse ailments, including DM, arthritis, and cancer. However, conventional treatments for these inflammatory diseases, such as steroidal and nonsteroidal anti-inflammatory drugs, can cause many side effects. For this reason, researchers have been exploring natural sources for new anti-inflammatory agents, as they are typically thought to be more palatable and safer than traditional medications. In this work, we assessed the quince seed extract *in vitro* anti-inflammatory properties by measuring its inhibitory activity against two inflammatory enzymes, 5-LOX and COX (24,28,29).

3.5 Inspecting the anticancer attribute and the mutual application

Cancer is a widespread disease that poses a significant threat to human health globally. Traditional chemotherapy treatments come with several side effects such as pain, damage to the heart, liver, and kidneys. One of the most effective drugs used in chemotherapy is 5-fluorouracil (5-FU). However, it has a toxic effect on healthy tissues and can cause tumors to develop resistance, limiting its effectiveness in clinical applications. The use of natural compounds can boost 5-FU's potency against cancerous cells while reducing its negative side effects (30–32).

The results of three independent runs indicated that the applied extracts exhibited an undetected antiproliferative effect on neither malignant nor healthy cellular lines at the applied concentrations. To verify the methodology used, the IC_{50} scores of the utilized 5-FU were compared with those found in the literature. This comparison indicates the validity of the methodology used, where the differences between the obtained scores and those of the literature were negligible. The former scores are 12.43±0.95, 13.39±1.01, and 42.33±0.97 for MCF-7, HeLa, and MCF-A10, respectively. While the findings revealed that the mutual application can't potentiate the antiproliferative activity of 5-FU at the concentrations used against the two malignant cell lines. On the other hand, this mutual application results in increasing the IC_{50} value of 5-FU toward the healthy cellular line (MCF-A10), as recorded in the **Table 4**. The Qu-1 extract can elevate the IC_{50} value of 5-FU more than the other extract. As a result, the effect of the used extracts upon mutual application with 5-FU can be considered protective.

Table 1. Results acquired from the assessment of the TPC and TFC of the quince seeds' extracts

Extract code	TPC (µg GAE/g crude solid)±SD (n=3)	TFC (µg RU/g crude solid)±SD (n=3)
Qu-1	2.2578±1.82	1.4901±1.76
Qu-2	1.0341±2.05	0.7342±1.53

GAE and RU are the abbreviations of the gallic acid and rutin equivalents.

Table 2. Results were acquired from investigating the *in-vitro* free radicals scavenging capacity of quince seeds' extract

Extract/reference code	IC_{50} (µg/ml)±SD (n=3)		
	DPPH	·OH	TRP
Qu-1	112.98±1.02	121.04±1.10	109.45±1.07
Qu-2	187.23±0.95	193.19±0.86	181.29±0.89
V-C	48.52±1.02	50.21±0.98	48.19± 0.96

The results are presented as the average of three individual trials, expressed as IC_{50} (µg/ml)±SD. TRP is the abbreviation of total reducing power.

Table 3. Results were acquired from investigating the *in-vitro* anti-inflammatory capacity of quince seeds' extract

Extract/reference code	COX1	COX2	Selectivity highlighter (SH)	5-LOX
Qu-1	52.16±1.02	45.22±0.92	1.15	62.77±1.01
Qu-2	210.23±1.05	197.34±0.96	1.07	102.84±1.08
ASA	3.57 ± 0.88	29.98 ± 0.98	0.12	23.89±1.02
CLB	7.42 ± 0.93	1.49 ± 0.94	4.98	18.28±1.02

The results are presented as the average of three individual trials, expressed as IC_{50} (µg/ml)±SD. The SH is computed by dividing the tested compound's IC_{50} against COX1 by the IC_{50} against COX2. The higher SH score, the more selectivity against COX2.

Table 4. Results were acquired from investigating the combined application of quince seeds' extract with 5-FU on healthy cellular line

Healthy cellular line	Codes of the reference antiproliferative drug as well as the blends used		
	5-FU+**Qu-1**	5-FU+**Qu-2**	5-FU
MCF-A10	122.17±0.97	56.36±0.89	42.33±0.97

The results recorded as IC_{50} (µg/ml)±SD (n=3)

4. Conclusion

The ethanolic extract of quince seeds in this study was examined for its TPC, TFC, potential antioxidant, anti-inflammatory, and anticancer properties using two methods: Microwave-facilitated and sequential microwave-ultrasound-assisted extraction. The results showed that the crude extract obtained through the microwave-facilitated (Qu-1) method had better TPC, TFC, antioxidant and anti-inflammatory activity than the sequential method (Qu-2). However, the extracts did not exhibit any antiproliferative effect on either malignant or healthy cellular lines at the applied concentrations. Moreover, it did not increase the antiproliferative activity of 5-FU against the two malignant cell lines. Nonetheless, the extract showed a protective effect when used with 5-FU. These findings suggest that quince seed extract might be beneficial in preventing and treating ailments related to oxidative stress and inflammation. To ascertain the ideal dosage and administration strategies and to better

comprehend the underlying mechanisms of these effects, more investigations are required.

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تقييم الأنشطة البيولوجية المختلفة في المختبر للمستخلص الإيثانولي الخام لبذور السفرجل

الخلاصة

المقدمة: لفترة طويلة، تم استخدام العناصر الحيوية الطبيعية لعلاج الأمراض والوقاية منها. تم التوصل الى أن المستخلصات الخام لبذور الفاكهة لديها العديد من الأنشطة البيولوجية المفيدة، وذلك بكونها مضادة للالتهابات، ومضادات الأكسدة، ومضادة للأورام. **الطرائق**: بحثت هذه الدراسة في مستخلص بذور السفرجل المصنوع باستخدام الإيثانول المطلق كمذيب فيما يتعلق بمحتوى الفلافونويد والفينول، وخصائصه مضادة للأكسدة، ومضادة للالتهابات، ومضادة للسرطان. تم الحصول على المستخلص باستخدام طريقتين، الاستخلاص بالميكروويف والاستخلاص المتسلسل بمساعدة الموجات فوق الصوتية. **النتائج**: يحتوي المستخلص من الطريقة الاولى على محتوى أعلى من الفلافونويد والفينول بالضافة لنشاط اعلى كمضاد للأكسدة . كانت فحوصات الجذور الحرة للهيدروكسيل و DPPH معبرا عنها ب IC_{50}كالتالي 121.04 ± 1.10 و112.98 ± 1.02، على التوالي، مقارنة بالطريقة التسلسلية، التي كانت قيم IC_{50} لها 193.19 ± 0.86 و187.23 ± 0.95. أظهرت طريقة الميكروويف أيضًا نشاطًا مثبطًا أعلى بكثير ضد الإنزيمات الالتهابية وانتقائية ضد COX_2 مقارنة بالطريقة المتسلسلة. ومع ذلك، لم تظهر المستخلصات أي تأثير مضاد للتكاثر على الخطوط الخلوية الخبيثة أو السليمة عند التراكيز المطبقة، كما أنها لم تعزز فعالية FU-5 في منع نمو الخلايا السرطانية. ومع ذلك، نتج التطبيق المشترك إلى زيادة قيمة IC_{50} لمركب FU-5 باتجاه الخط الخلوي السليم. لذلك، يمكن اعتبار التطبيق المشترك لهذه المستخلصات مع FU-5 بمثابة إجراء وقائي . **الاستنتاج**: كان للمستخلص الخام الذي تم الحصول عليه من خلال الطريقة الميكروويف محتوى فينولي وفلافينويدي ومضادات الأكسدة ونشاط مضاد للالتهابات وإمكانات وقائية للخلايا الطبيعية أفضل من الطريقة التسلسلية.

الكلمات المفتاحية: بذور السفرجل، مستخلص ايثانولي، مضاد للالتهابات، مضاد للاكسدة، مضاد للاورام