



Original Article

Phytochemical Analysis and Antimicrobial Evaluation of Quince Seeds' Extracts

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ABSTRACT

Microbial infection and microbial resistance to the classical antibiotics have been immense challenges which portend the health of societies. This has directed researchers to straight their attention onto the plants to discover new possible antimicrobial compounds. In this work, 5 solvents were employed to extract the dried seeds powder of quince by ultrasound including ethanol, ethyl-acetate, chloroform, n-hexane, and diethyl-ether. Each extract was subjected to three in vitro antimicrobial tests using the broth-dilution method. The antibacterial effect against the following aerobic bacteria which are *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603, *Haemophilus influenzae* ATCC 49247, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 6539, *Shigella dysenteriae* ATCC 13313, and non-pathogenic *Escherichia coli* BAA-1427 applying ciprofloxacin antibiotic as a standard reference, antibacterial study against anaerobic bacteria which include *Bacteroides fragilis* ATCC 25285, *Clostridium perfringens* ATCC 13124, *Fusobacterium necrophorum* ATCC 25286, and *Prevotella melaninogenica* ATCC 25845 employing metronidazole as a reference, and antifungal study versus *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16888, nystatin was the reference. The toxicity against the normal bacterial flora was evaluated to verify the safety profile of the extracts. The results revealed that the extracts have antimicrobial activities with supremacy linked to ethanol extract. In accordance to the activity evidence values, the extracts displayed bactericidal and fungicidal activities, with ethanol extract having the least harmful effect against non-pathogenic microbial strain.

GRAPHICAL ABSTRACT



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Introduction

Long-standing, evolving, and resurging infectious diseases concerns continue to assault the world. These menaces extensively diversify in terms of probability and severity. In addition, they have a variety of effects on morbidity and mortality, and wide range of the economic and social repercussions [1,2]. Globally, microbial infections are accountable for millions of deaths every year and annually, bacterial infections kill about 700,000 people [3,4]. The problem is complicated by the infelicitous use of antimicrobial agents and the development of pathogenic protection mechanisms [5,6].

Recently, many studies have been conducted with the aim of finding auspicious solutions to devastate these problems. The exploration of new antimicrobials derived from the natural sources is of the utmost interest and demand [7].

Natural products play a vital role in drug discovery, expressively for cancer and infectious diseases. About one third of all Food and Drug Administration approved drugs are obtained from natural origin [8,9]. Natural products have unique characteristics as compared to synthetic compounds, including prodigious scaffold diversity and structural complexity [10]. Besides, they keep a higher molecular mass, a larger number of sp³ carbon atoms (representing a more complex 3D structure) and oxygen atoms, higher numbers of H- bond donors and acceptors, and greater molecular rigidity, all of which could be treasured in drug discovery tackling protein-protein interaction [11–13].

Those products obtained from natural sources are optimized structurally to serve specific biological functions such as the endogenous defense mechanism regulation and interaction with other organisms; this explains their high significance for infectious diseases. Additionally, their usage in traditional medicine may provide insights related to their efficacy and safety [14].

One of the most noteworthy sources of biologically active natural products are medicinal plants which are the fundamental unit of traditional medicine system in many nations [15,16]. The plant extracts and active phytochemicals have been employed in folk medicine for the treatment

of a variety of ailments since the dawn of time because they are enriched with complex bioactive compounds serving as botanical pesticides, or as bactericidal and fungicidal agents active against pathogens of human diseases [9,17].

Evidence suggested that a diet rich in fruits is contemplated as healthy and beneficial in many of conditions such as cardiovascular and infectious diseases. The dietary guidelines advocated that at least five servings of vegetables and fruits have to be consumed per day [18,19].

Cydonia oblonga, or quince is a Rosaceae family and Spiraeoideae subfamily plant that originated in Asia Minor and Southeast Europe and has since spread to the other regions of the world. It is native to Iran, and its distribution centers are northern Iran's woodland and middle elevations. The tree can grow up to 8 meters tall in the shape of a shrub. The ripe fruits are golden yellow and contain numerous seeds within a mucilaginous material structure [20]. Quince is highly popular for its nutritional and medicinal uses. It exerts strong anti-oxidant properties which imputed to its plentiful polyphenolic and vitamin C contents. It is also abundant with calcium, phosphorus, potassium, and sodium, among other minerals [21]. It is a prosperous source of proteins, carbohydrates, vitamins, fiber, diverse organic acids, and minerals. Quince is said to be nutritionally superior to apples in terms of nutritional value. Its content of vitamin C and minerals (Na, K, Ca, Mg, Fe, and P) was reported to be twofold as that of apples. Hence, this underrated fruit has a potential of a super fruit [22].

Seeds of quince are employed conventionally for the treatment of cough, diarrhea, dysentery, bronchitis and sore throat which attributed to its rich contents of phytochemicals such as tannins, sterols, and terpenoids contents [21]. Quince seeds extracts have been used in cosmetics for a long time for their hydrating properties. Furthermore, the seeds is a source of mucilage which has beneficial effects on skin fibroblast by enhancing its proliferation [23].

Antimicrobial activity of several aerial sections of the quince plant has been indicated to be effective against different pathogenic microbial strains. The

experiments on seeds, on the other hand, are limited [24].

In this regard, the current work aimed at the estimation of the phytochemical and antimicrobial properties of quince seeds extracts utilizing broth dilution method.

Materials and Methods

Solvents, reagents, and culture media were attained from Sigma-Aldrich. The standard fungal and bacterial strains were earned from microbiologicals. The seeds were obtained from local herbal medicine shop in Mosul. Ultrasonic bath (40 kHz, 350 W, Power sonic 410, Korea) was the instrument used for the extraction process.

Preparations of Seeds Extracts

The seeds were demolished in an electrical blender, then sifted affording fine powder [25,26]. Twenty-five grams of seeds powder was discretely submersed in conical flasks that each one contained 250 mL of solvent which are ethanol, ethyl acetate, chloroform, ether, and n-hexane. Then, the flasks were settled in an ultrasonic bath for 30 minutes at 30 °C. The mixtures were filtered using Whitman No. 1 filter paper once the extraction process was completed. The resulting extracts were concentrated under low pressure. The left material was refrigerated for coming tests [27,28].

Phyto-nutrient Tests

The resultant five extracts were evaluated for the presence of different phyto-nutrients including carbohydrates, proteins, emodins, glycosides, flavonoids, phenols, coumarins, saponins, terpenoids, tannins, anthocyanins, betacyanins, alkaloids, amino acids, and proteins. The methods endorsed by Harborne were used to conduct these tests [29].

Microbiological Evaluation

The attained five extracts, including **UE**, **UA**, **UC**, **UD**, and **UH**, were appraised for their activity to frustrate the growth of the pathogenic aerobic Gram (-) bacteria, anaerobic bacteria, and fungi. To discern the safety of these extracts toward non-pathogenic bacterium, *Escherichia coli* (BAA-1427), and their toxicity versus this normal bacterial flora was scrutinized.

Assessment of the Activity versus Aerobic Gram (-) Bacteria

In this study, the antibacterial activities toward the relevant Gram (-) bacteria were figured out using broth dilution modus, with Mueller-Hinton broth (MHB) serving as a growth-promoting environment, ciprofloxacin (CP) serving as a standard, and sulfinylbismethane (DMSO) serving as a negative qualifier. The quantity of residue was determined after drying 2 mL of the defined extract with a concentration of 100 mg/mL.

Concisely, the inceptive concentration of the extract was prepared as 7.5 mg/ 5 mL of residue in DMSO as a solvent. As a solution-thinner, the autoclaved distill water was used to prepare successive dilutions from 1024 to 0.25 µg/mL. To a tagged test, tubes containing 3 mL (MHB), 0.2 mL of bacterial suspension were adjusted at 0.5 McFarland using autoclaved distill water, and 1 mL specified extract concentration were poured. After 24-hour incubation time at 37 °C, bacterial growth was visually investigated, on the basis of which concentration displayed the imperceptible bacterial proliferation, the above-outlined methodological strategies were repeated using diluted quantities created based on 4, 1, 0.5, or 0.05. Eventually, the first microbiological variable named Minimum Inhibitory Concentration (Measure I) was verified [30].

By mixing 3 mL of MHB with 0.5 mL of 2nd diluted concentrations, the second microbiological variable (Measure II) was examined. The concentration which revealed no turbidity was regarded as Measure II [31]. By dividing the values of Measure II and Measure I over each other, a third variable denominated as activity evidence (Measure III) was calculated for individual extract versus every employed bacterial type. To meliorate the results, the utilized methodology was repeated for three times [32].

Assessment of the Activity versus Anaerobic Bacteria

To delve the activity of the procured extracts against anaerobic infectious bacteria, a technique similar to that used for the activity estimation toward the aerobic ones was adopted with some conspicuous differences, including the growth environment and conditions. As a growth-

promoting environment, Brucella-agar enriched with sheep blood (5%) was employed, using Metronidazole (MZ) as a standard and DMSO as a qualifier. In addition, the incubation was carried out for 48 hours at 37 °C in a container supported with an anaerobic milieu (80% N₂, 10% CO₂, and 10% H₂), anaerobe marker, and palladium metal as a catalyst [33].

Assessment of the Activity versus Pathogenic Fungi

The obtained extracts were assessed against 2 fungal strains using a fairly modified approach from that used to evaluate their effectiveness versus aerobic bacteria. The amended parameters included the use of Sabouraud-dextrose broth as a growth-promoting environment, nystatin (NY) as a standard, and a 48-hour incubation at 30 °C [30].

Results and Discussion

Phytochemical Analysis

The phytochemical analysis study of quince seeds extracts confirmed the presence of key medicinal components in the sample as presented in Table 1. In comparison to the other extracts, the ethanolic extract had higher phytoconstituent content including tannins, carbohydrates, alkaloids, steroids, betacyanins, proteins, and coumarins. Ethyl acetate extract revealed positive results for carbohydrates, alkaloids, steroids, betacyanins, proteins, and coumarins. Positive results of chloroform extract for phenols, steroids, betacyanins, proteins, and coumarins was indicated, while diethyl-ether extract showed positive results for carbohydrates, steroids, betacyanins, proteins, and coumarins. N-hexane extract contains the least number of phytochemicals which include steroids, proteins, and coumarins.

Table1: Phytochemical screening assay of quince seeds extracts

Test name	Phytoconstituent	UE	UA	UC	UH	UD
Pew's test	Flavonoids	χ	χ	χ	χ	χ
Lead acetate test		χ	χ	χ	χ	χ
Braymer's test	Tannins	⊘	χ	χ	χ	χ
Liebermann-Burchard test	Terpenoids	χ	χ	χ	χ	χ
Molisch's test	Carbohydrates	⊘	⊘	χ	χ	⊘
Mayer's test	Alkaloids	⊘	⊘	χ	χ	χ
Ammonium hydroxide test	Emodins	χ	χ	χ	χ	χ
Ferric chloride test	Phenols	χ	χ	⊘	χ	χ
Salkowski test	Steroids	⊘	⊘	⊘	⊘	⊘
Pigment-dependent test	Anthocyanins	χ	χ	χ	χ	χ
Pigment-dependent test	Betacyanins	⊘	⊘	⊘	χ	⊘
Ninhydrin test	Amino acids	χ	χ	χ	χ	χ
Xanthoproteic test	Proteins	⊘	⊘	⊘	⊘	⊘
Olive oil test	Saponins	χ	χ	χ	χ	χ
NaOH test	Coumarins	⊘	⊘	⊘	⊘	⊘
Fluorescence test		⊘	⊘	⊘	⊘	⊘
Liebermann's	Glycosides	χ	χ	χ	χ	χ

The symbols **UE**, **UA**, **UC**, **UD**, and **UH** are referred to as the ultrasound-utilized solvent ethanol, ethyl acetate, chloroform, diethyl ether, and hexane, respectively. The symbols χ and ⊘ are referred to as negative and positive results, respectively.

Investigation of Microbiological Activities

In spite of the availability of a large spectrum of antimicrobials, the continual development of microbial multi-drug resistance to currently accessible antimicrobial agents has become one of the major public health challenges [34]. The

development of the innovative therapeutic techniques for treating different infectious diseases is a worldwide health priority today [35]. Numerous studies proved that the plant extracts possess antimicrobial properties against different pathogens [36].

The successful extraction of biologically active compounds from plants are based largely on election of an appropriate solvent and extraction protocol. The solvents for extraction are selected in accordance to the their polarity and therefore, the capacity to isolate definite types of compounds with diverse structures and physicochemical properties [37].

Antibacterial Activities

The antibacterial activities against six aerobic bacterial strains, as demonstrated in Table 2, and Figures 1-3, revealed that the 5 extracts have a weaker antibacterial impact than conventional ciprofloxacin against the tested bacterial strains. Ethanol extraction has yielded a virtuous extract with better antimicrobial activities against all tested pathogenic organisms. This result may be explained by the highest number of phytochemicals in UE [38-41]. Most of these phytochemicals are related to polyphenolic compounds such as tannins and coumarins that are known to have antimicrobial activity [42-45].

In addition, more polar solvents are more effective at extracting organic and inorganic compounds than solvents of low polarity [40]. Among these compounds, tannins have the ability to inhibit extracellular enzymes and to enter the cell wall in sufficient proportions to react with other ultra-structural components, causing cell wall production to be inhibited [46]. EU revealed varying degrees of antibacterial activities against aerobic pathogenic bacteria with the Measure I and Measure II values of (12-15.50 µg/mL), (13.5-17.50 µg/mL), respectively while Measure III value is ranged between (1.13 and 1.24). The most sensitive aerobic bacterial strain to EU was *Pseudomonas aeruginosa* ATCC 27853 followed by *Haemophilus influenzae* ATCC 49247. The lowest inhibition level against pathogenic aerobic bacterial strains was related to hexane extract, as evidenced by the low number of phytochemicals present in it. The remaining 3 extracts including UA, UC, and UD antimicrobial activities were fluctuated between that of ethanolic and n-hexane extract.

Table 2: Measure I, Measure II, and Measure III values of quince seeds extracts versus aerobic bacteria

Aerobic Gram (-) bacteria	Microbiological variable	Symbols of the standard and tested extracts					
		CP	UE	UA	UC	UD	UH
<i>Pseudomonas aeruginosa</i> ATCC 27853	Measure II	0.85	13.50	20.50	22.00	22.00	89.00
	Measure I	0.75	12.00	18.00	18.50	58.50	67.50
	Measure III	1.13	1.13	1.14	1.19	1.23	1.32
<i>Klebsiella pneumonia</i> ATCC 700603	Measure II	0.45	15.50	21.00	23.50	76.00	98.00
	Measure I	0.40	13.50	19.50	20.00	62.50	69.00
	Measure III	1.13	1.15	1.08	1.18	1.22	1.42
<i>Haemophilus influenzae</i> ATCC 49247	Measure II	0.65	15.50	23.00	24.00	68.00	76.00
	Measure I	0.60	12.50	19.00	22.50	60.00	69.50
	Measure III	1.08	1.24	1.21	1.07	1.13	1.09
<i>Escherichia coli</i> ATCC 25922	Measure II	0.95	16.00	21.00	26.00	72.00	80.00
	Measure I	0.85	14.00	17.50	18.00	60.50	66.00
	Measure III	1.31	1.14	1.20	1.44	1.19	1.21
<i>Salmonella typhi</i> ATCC 6539	Measure II	0.95	17.50	25.00	25.00	72.00	80.00
	Measure I	0.80	15.50	21.00	21.50	63.00	67.50
	Measure III	1.19	1.13	1.19	1.16	1.14	1.19
<i>Shigella dysenteriae</i> ATCC 13313	Measure II	0.70	16.00	26.00	30.00	69.50	69.50
	Measure I	0.55	13.50	21.00	22.00	63.50	65.00
	Measure III	1.27	1.19	1.24	1.36	1.09	1.07
<i>Escherichia coli</i> BAA-1427	Measure II	0.95	80.00	32.00	36.50	18.50	12.00
	Measure I	0.85	64.00	24.50	24.50	12.00	9.50
	Measure III	1.31	1.25	1.31	1.49	1.54	1.26

The data were demonstrated in terms of µg/mL.

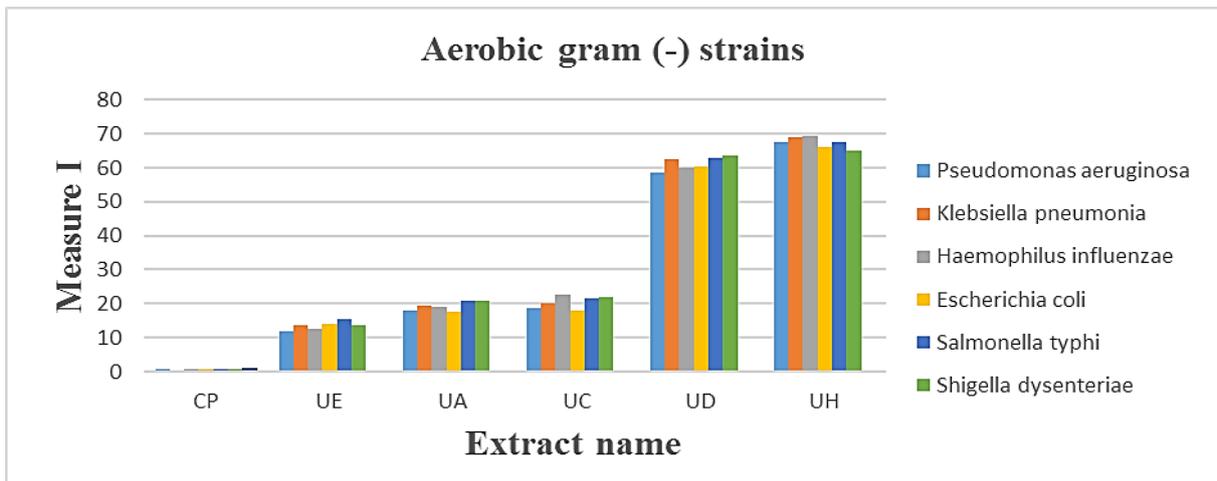


Figure 1: Measure I values of quince seeds extracts against aerobic bacterial strains

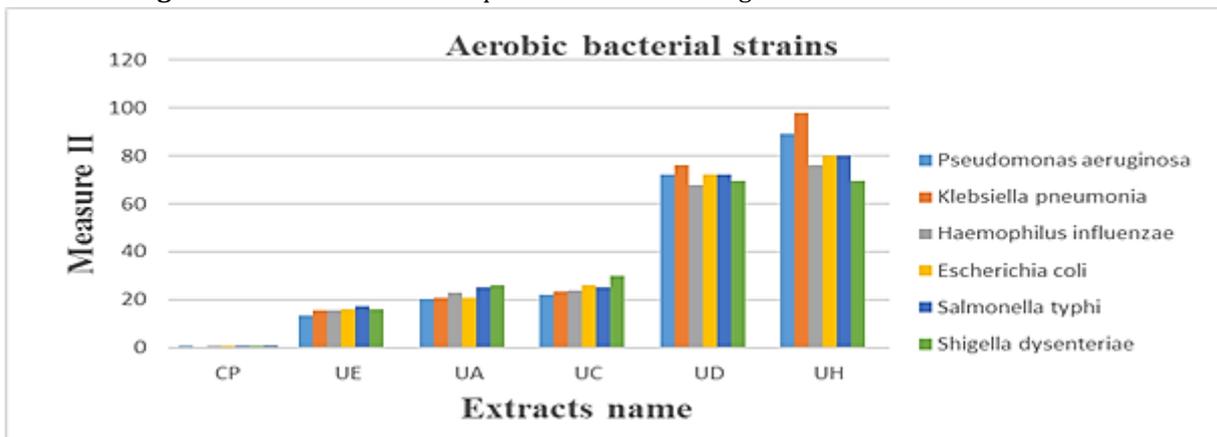


Figure 2: Measure II values (µg/mL) of quince seeds extracts against aerobic bacterial strains

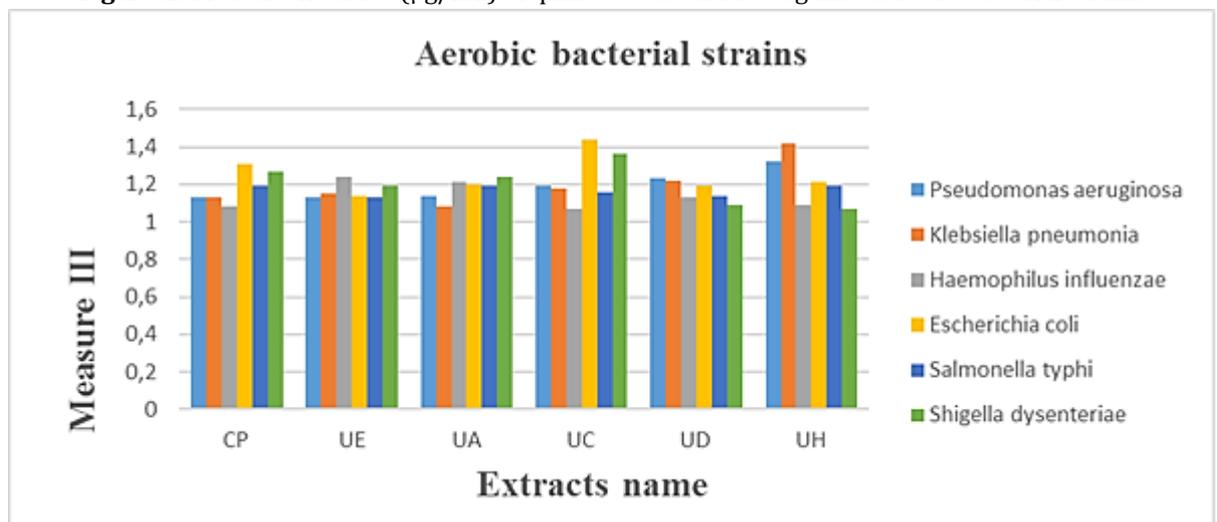


Figure 3: Activity evidence (Measure III) values of quince seeds extracts against aerobic bacteria strains

Furthermore, when compared to ciprofloxacin, the extracts exhibit a lower level of toxicity against nonpathogenic *Escherichia coli* BAA-1427, with the least effects connected to UE, with Measure I

and Measure II values of 64 µg/mL and 80 µg/mL, respectively. UH, on the other hand, has the highest level of toxicity against this bacterial flora as depicted in Figures 4 and 5.

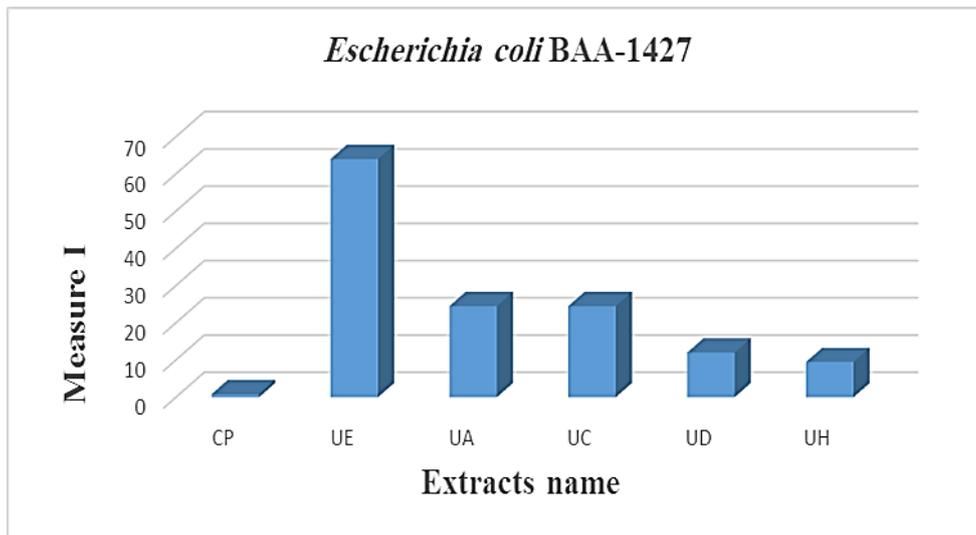


Figure 4: Measure I value of quince seeds extracts against non-pathogenic *E. coli*

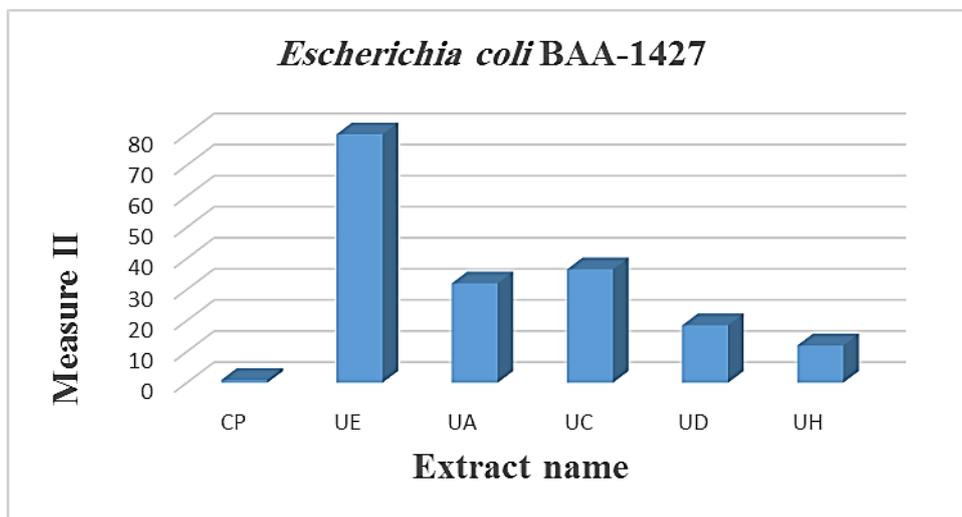


Figure 5: Measure II values of quince seeds extracts against non-pathogenic *E. coli*

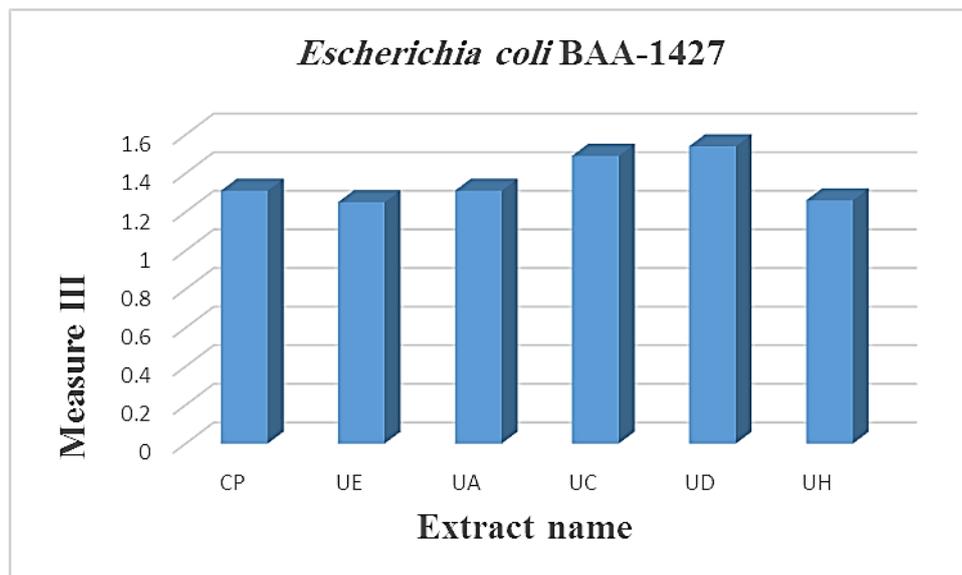


Figure 6: Measure III values of quince seeds extracts against non-pathogenic *E. coli*

Regarding the anaerobic strains as listed in Table 3, the extracts possess effects that did not exceed the inhibitory effect elicited by the standard antibiotic MZ. Comparatively, as with pathogenic

aerobic bacteria, ethanol and n-hexane extracts demonstrated the highest and lowest level of inhibition [47]. *Bacteroides fragilis* ATCC 25285

was the most sensitive anaerobic bacteria. The variables Measure I, Measure II, and Measure III are presented in Figures 7, 8, and 9.

Table 3: Measure I, Measure II, and Measure III values of quince seeds extracts versus anaerobic bacteria

Anaerobic bacteria	Microbiological variable	Symbols of the standard and tested extracts					
		MZ	UE	UA	UC	UD	UH
<i>Bacteroides fragilis</i> ATCC 25285	Measure II	3.50	280.00	296.00	298.00	463.00	484.00
	Measure I	3.00	256.00	278.00	280.00	394.00	402.00
	Measure III	1.17	1.09	1.06	1.06	1.18	2.00
<i>Clostridium perfringens</i> ATCC 13124	Measure II	0.95	285.00	302.00	336.00	481.00	512.00
	Measure I	0.80	257.00	282.00	288.00	398.00	430.00
	Measure III	1.19	1.11	1.07	1.17	1.21	1.19
<i>Fusobacterium necrophorum</i> ATCC 25286	Measure II	1.80	289.00	326.00	347.00	467.00	516.00
	Measure I	1.70	260.00	284.00	288.00	394.00	436.00
	Measure III	1.06	1.11	1.15	1.20	1.19	1.18
<i>Prevotella melaninogenica</i> ATCC 25845	Measure II	0.85	292.00	355.00	356.00	495.00	538.00
	Measure I	0.75	260.00	287.00	290.00	402.00	444.00
	Measure III	1.13	1.12	1.24	1.23	1.23	1.21

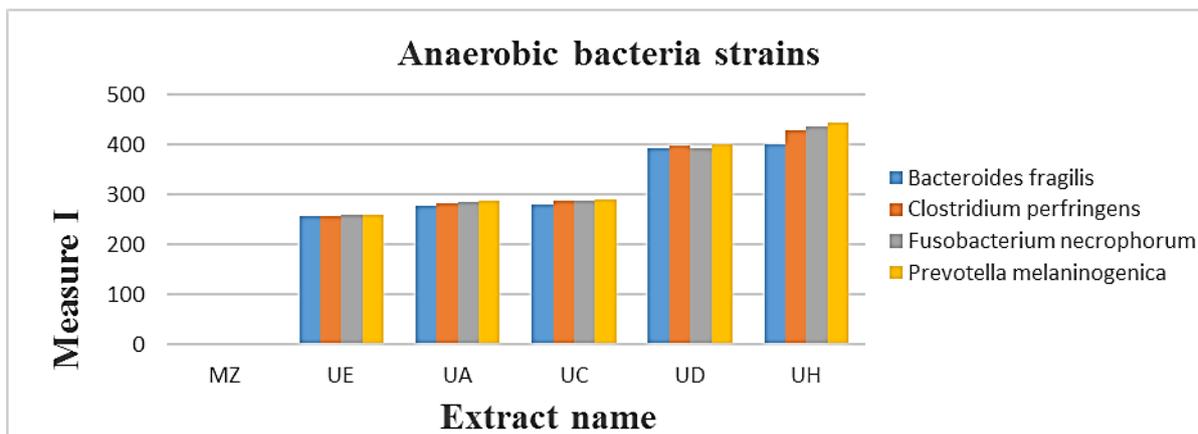


Figure 7: Measure I values of quince seeds extracts against anaerobic bacterial strains

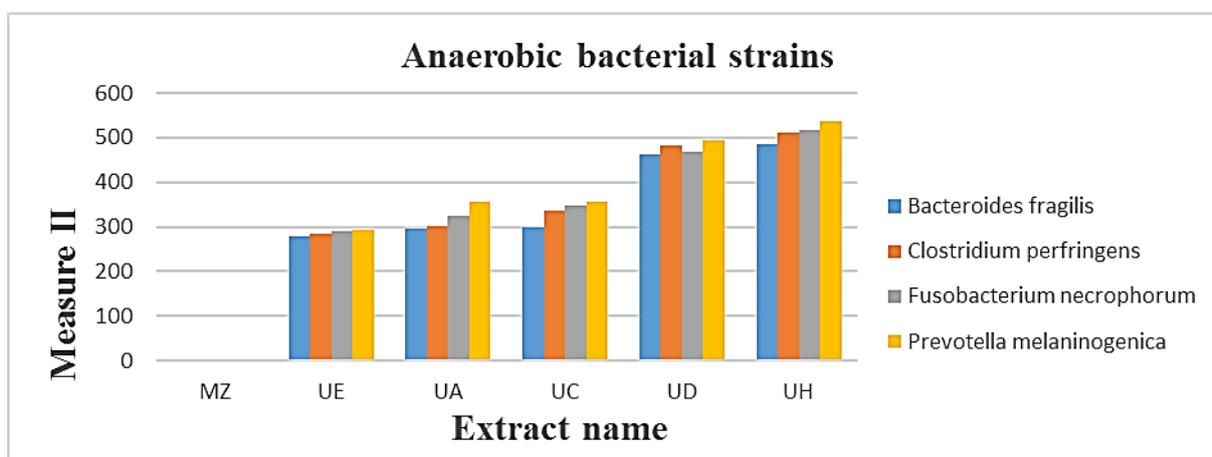


Figure 8: Measure II values (µg/mL) of quince seeds extracts against anaerobic bacterial strains

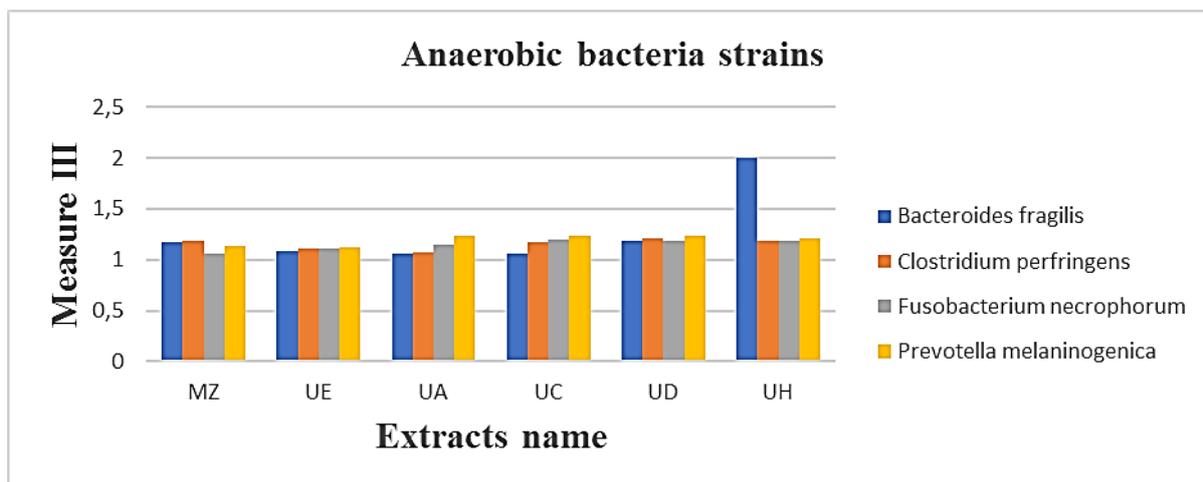


Figure 9: Measure III values of quince seeds extracts against anaerobic bacterial strains

Antifungal Activity

Two fungal strains were examined for their susceptibility to quince seeds extracts. These pathogenic strains include *Candida albicans*, ATCC 10231 and *Aspergillus niger*, ATCC 16888.

The results are demonstrated in Table 4. Figures 10-12 indicated that the five extracts had weaker inhibitory action against the tested fungi than nystatin, with UE having the topmost activity, with

Measure I and Measure II values of (18-28 µg/mL) and (24-38 µg/mL) respectively [48]. The sensitivity of *Candida albicans* ATCC 10231 to UE was greater than that of *Aspergillus niger* ATCC 16888. Hexane extract had the lowest fungicidal activity, as revealed by the highest Measure I and Measure II values of (58-76 µg/mL) and (70-94 µg/mL), respectively [49]. The extracts of chloroform and ethyl-acetate were about equally effective against the fungi tested [50].

Table 4: Measure I, Measure II, and Measure III of quince seeds extracts against two pathogenic fungi

Pathogenic fungi	Microbiological variable	Symbols of the standard and tested extracts					
		NY	UE	UA	UC	UD	UH
<i>Candida albicans</i> ATCC 10231	Measure II	6.00	24.00	46.00	48.00	56.00	70.00
	Measure I	4.00	18.00	32.00	32.00	44.00	58.00
	Measure III	1.50	1.33	1.44	1.50	1.27	1.21
<i>Aspergillus niger</i> ATCC 16888	Measure II	12.00	38.00	56.00	56.00	70.00	94.00
	Measure I	8.00	28.00	42.00	42.00	44.00	76.00
	Measure III	1.50	1.36	1.33	1.33	1.59	1.24

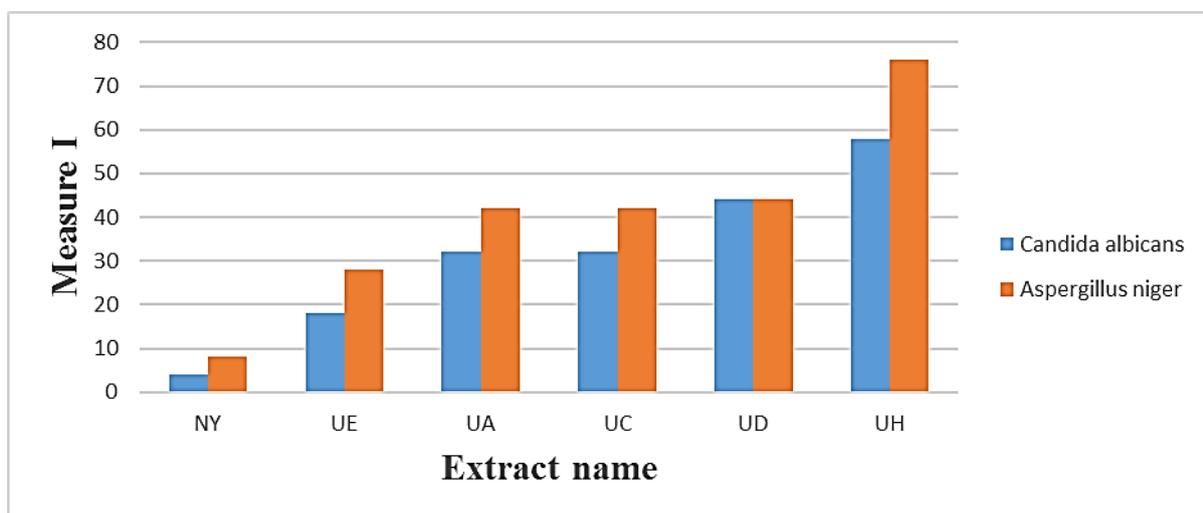


Figure 10: Measure I values (µg/mL) of quince seeds extracts against two fungal strains

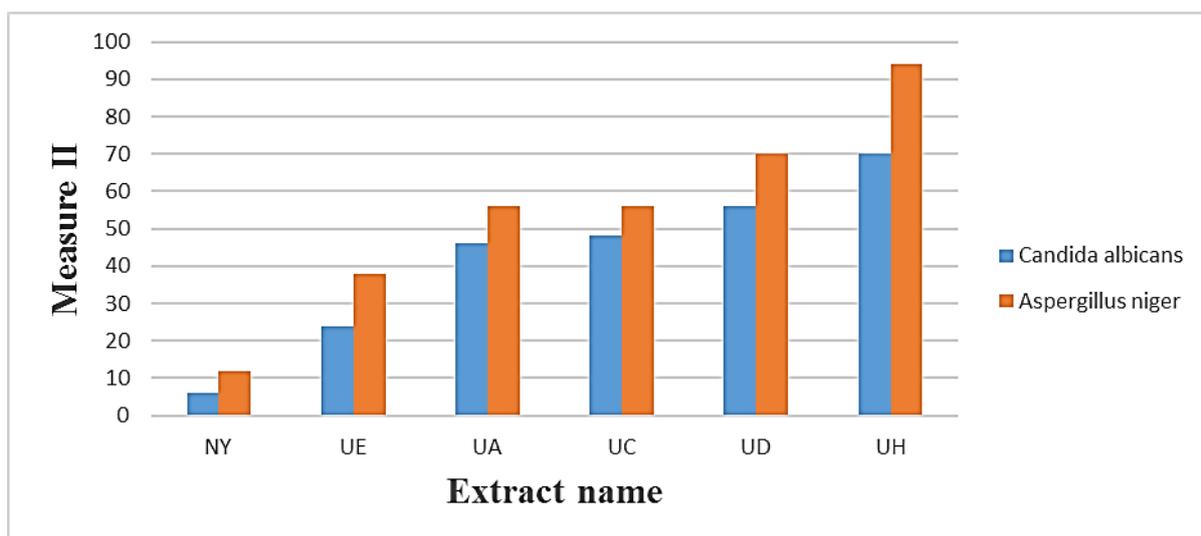


Figure 11: Measure II values (µg/mL) of quince seeds extracts against two fungal strains

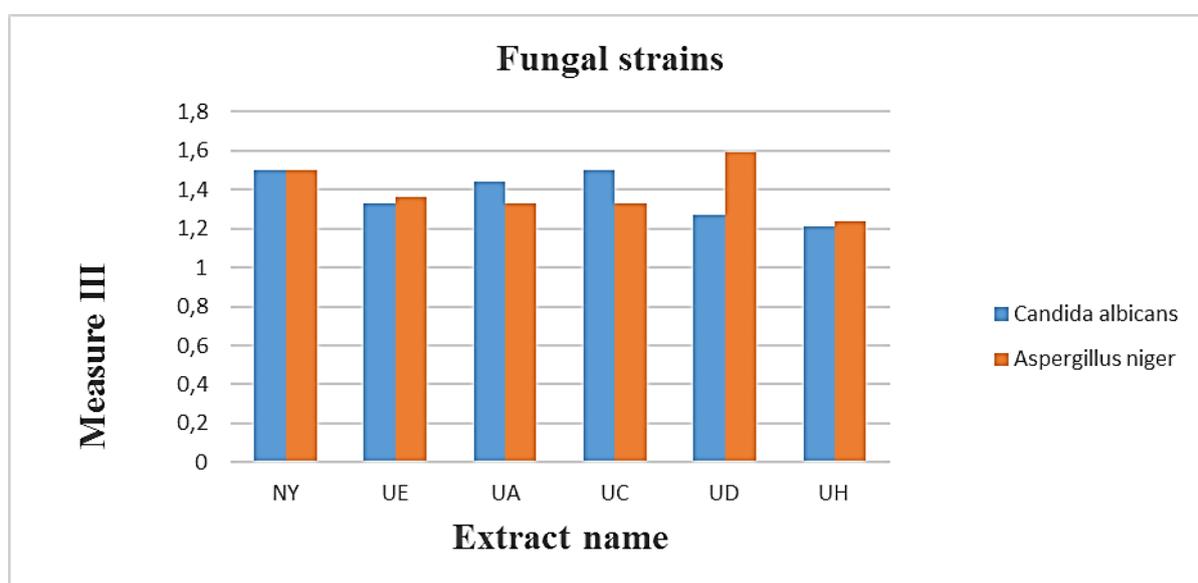


Figure 12: Measure III values of quince seeds extracts against 2 fungal strains

To assess the type of activity, the Measure II / Measure I ratios, also known as the activity evidence [51], were determined against each fungal and bacterial strain. The seeds extracts were found to be fungicidal and bactericidal against the tested strains based on this ratio of less than 4 [52].

Conclusion

In the current study, the obtained results verified that the quince seeds extract revealed antibacterial and antifungal activities with prepotency attained to ethanolic extract, despite lower than the activity of reference drugs ciprofloxacin, metronidazole, and nystatin. This could be owing to the wide range of phytochemicals present in the plant, along with the type of solvent utilized to fully extract these

bioactive components. In addition, it has minimum activity against nonpathogenic *E. coli*. The lowest activity was linked to n-hexane extract. In addition, the extracts exhibited bactericidal and fungicidal effects against tested pathogenic microorganisms. It might be possible to assert that the extracts of this plant seeds encompass compounds having antimicrobial properties that can be used as antimicrobial agents in innovative medications which could be used as a treatment for various infectious diseases in human with minimum side effects compared with well-known currently available antibiotics.

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Authors' contributions

All the authors met the criteria of authorship based on the recommendations of the international Committee of Medical Journal Editors.

Conflict of Interest

There are no conflicts of interest in this study.

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