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Antibacterial effects of aqueous and organic quince leaf extracts on gram–positive and gram–negative bacteria

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Abstract. Gram–positive and gram–negative bacteria are the most common/prevalent human infectious agents. With increased bacterial resistance to the chemical agents, finding herbal combinations with no side effects seems necessary. The aim of this study was to investigate the effect of aqueous, ethanolic and acetonic Quince leaf extracts on the following bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium, Enterobacter aerogenes* and *Klebsiella pneumonia*. Leaves were collected and dried and aqueous, ethanolic, and acetonic extracts were prepared using percolation method. Antibacterial effect of the extracts was determined using agar–well diffusion test and the average diameter of the inhibition zone was measured. Then, using broth macro–dilution test, bacterial minimum inhibitory concentration and minimum bactericidal concentration were determined as well. The results showed that ethanolic Quince leaf extract had the greatest effect on gram–negative and gram–positive bacteria. The aqueous extract showed the weakest effect on tested bacteria. Aqueous, ethanolic and acetonic Quince leaf extracts not only had the inhibitory effect on the bacteria, but also had antibacterial properties. According to the results, Quince leaf extracts can be used against the bacterial infections of the study.
Keyword: Antibacterial effect, Quince leaf extract, Gram positive bacteria, Gram negative bacteria.

Introduction

Long–term use of antibiotics has led to the emergence of bacterial resistant to these medicines and poses significant clinical problems in the treatment of infectious diseases.

Thus, some extensive research seems necessary to discover new antimicrobial substances from other sources, including plants It is known that plants have a variety of antimicrobial agents with no or very few side effects [DERAKHSHAN, *et al.*, 2011].

Different species of Quince (*Cydonia oblonga*) are good natural resources of flavonoids and phenolic acids which are considered as effective treating ingredients of plants. Quince is a potential antioxidant and microbial source for human health [ALAA, *et al.*, 2011].

Quince is a shrub or a tree of the rose family. It is mostly distributed in the northern and west northern parts of Iran, especially East and west Azerbaijan, Guilan, Kermanshah and Lorestan [IRAN DICTIONARY OF PLANT NAMES, 1997, SALEHI, 2008]. On the other hand, Iran supplies 75 % of the world Quince production [PHARMACOGENOSY, 1996, BUTNARIU and GIUCHICI, 2011, RASHED and BUTNARIU. 2014a, BUTNARIU and CORADINI, 2012].

Quince fruit belongs to the family of apple and pear, but the color is yellow and the seeds are usually larger than that of apple and pear [ALESIAN, *et al.*, 2010].

Escherichia coli are the normal flora of the colon and to a lesser extent of the small intestine in all warm–blooded animals. The gram–negative bacterium remains in water, dust and feces for weeks to months.

The importance of *E. coli* is due to its pathogenicity, causing food poisoning and intestinal diseases in humans, especially infants [MEDICAL MICROBIOLOGY, 2010].

Staphylococcus aureus is one of the main fever–causing gram–positive coccids in humans and is accounted for over 80 % of bacterial infections.

These bacteria can cause very different side effects such as acne, folliculitis and impetigo [MIRNEZAD and RAZAVI

2010, BUTNARIU, *et al.,* 2012, RASHED and BUTNARIU, 2014b, BUTNARIU, 2014, PETRACHE, *et al.,* 2014].

Pseudomonas aeruginosa, which is often found in human skin and intestine in a few numbers and in the affected areas of the body, is a major pathogen.

The gram–negative bacterium is resistant to many antimicrobial agents.

This bacterium is responsible for wound and burn infections [MEDICAL MICROBIOLOGY, 2001]. *Salmonella typhimurium* causes gastroenteritis in humans and other mammals. *Entrobacter aerogenes* causes urinary tract infections and septicemia and *Klebsiella pneumoniae* also causes pneumonia, urinary tract infections and septicemia. *Salmonella*, *Entrobacter* and *Klebsiella* are all gram–negative bacteria [MEDICAL MICROBIOLOGY, 2010].

The current study aims at investigating the antimicrobial effect of aqueous, ethanolic and acetonic Quince leaf extracts on *E. aerogenes*, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, and *K. pneumoniae*.

Material and methods

Quince leaves were collected in early September of 2012 in Hashtrood, East Azerbaijan and then confirmed by a botanist. After drying these leaves in shade, extraction was performed by percolation method. Bacterial strains of *E. coli, E. aerogenes*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, and *K. pneumoniae* were prepared from Reference Centre of Ayatollah Mousavi Hospital in Zanjan.

To prepare aqueous, ethanolic and acetonic Quince leaf extracts, 50 g of leaf powder and 500 mL sterile distilled water, 50 g of leaf powder and 500 mL of ethanol 80 % (Merck, Germany), and 50 g of leaf powder and 500 mL of pure acetone (Sigma) were used, respectively.

After the plant remained in solvents for 24 hours, extracts were passed through the filter cloth, the filtered solutions were centrifuged at 2500 rpm for 20 minutes using centrifuge model ROTIXDA 50 SA manufactured by HETTICH.

Sediments were discarded and the extracts supernatant solutions were concentrated by vacuum distillation where the extracts were separated from the solvents. The prepared extracts were frozen at –80 °C until they were used.

To evaluate the antimicrobial effects of the extracts prepared, agar–well diffusion test was used. In this method, in addition to pure extracts, dilutions of 1:2, 1:4, 1:8, 1:16 and 1:32 were prepared in sterile distilled water.

On Mueller Hinton Agar Medium (MHA), wells with diameter of 5 mm were drilled using a sterile Pasteur pipette.

Then the bacteria with a standard turbidity of a 0.5 McFarland were picked up by a sterile swab and cultured in dense in three different directions on areas of wells.

Wells were filled with certain concentrations of extracts and then, the prepared media were incubated at 37 °C for 24 h in incubator [ELLEN and SRYDNEY, 1990].

Then the diameter of inhibitory zones was measured with a ruler.

To avoid the possibility of making errors in the procedure, tests were repeated three times.

The bacterial Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by broth macro dilution.

Firstly, double serial dilutions of 1:2, 1:4, 1:8, 1:16 and 1:32 were prepared for each extract in tubes containing 1 mL of Mueller Hinton Broth medium, and then a series of prepared serial dilutions was used for each bacterium.

For each dilution, 5×10^5 bacteria per mL of fluid in the tubes were added.

Coupled with each serial dilution, a positive control tube (culture medium + bacteria + 1 % of solvent without extract) and a negative control tube (culture medium without bacteria) were used.

The samples were incubated for 24 h at 37 °C in incubator. 0.5 mL of every tube lacking turbidity was cultured on MHA medium.

The first tube of low extract concentrations lacking the turbidity due to bacterial growth was considered as MIC concentration and the first tube of extract concentrations in which 99.9 % of the initial amounts of bacteria added were

missed and only 0/1 % of bacteria were grown in the subculture was considered as MBC concentration for all extracts.

All above steps were performed 3 times [IZADI, *et al.*, 2009, ALIZADEH, *et al.*, 2013].

Statistical analysis of data

In this study, data were analyzed statistically using SPSS software version 18 and One–Way ANOVA test.

The significant amount was considered ($p < 0.05$).

Results and discussion

Among the extracts prepared from the Quince leaves, pure ethanolic extract with an inhibition zone of the average diameter of 24±4 mm, showed a stronger antibacterial effect on *S. typhimurium* in well diffusion method (Figure 1).

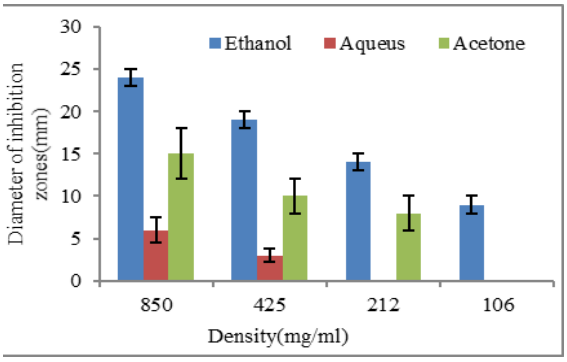

Figure 1. Mean ± SD inhibition of zone diameter of *S. typhimurium* in millimetres.

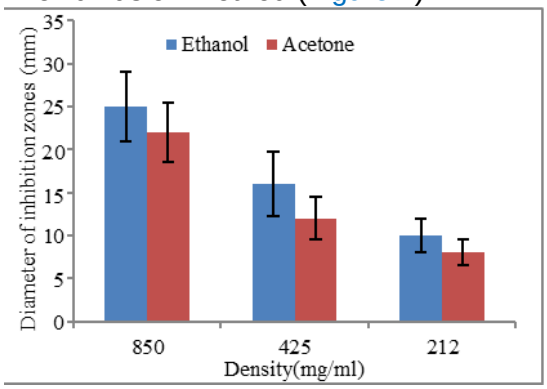

Figure 2. Mean ± SD inhibition of zone diameter of *E. coli* in millimetres.

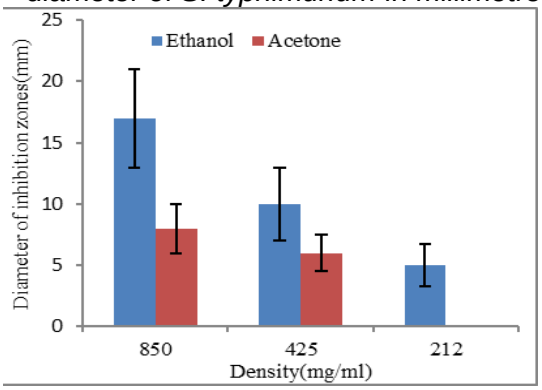

Figure 3. Mean ± SD inhibition of zone diameter of *K. pneumoniae* in millimetres.

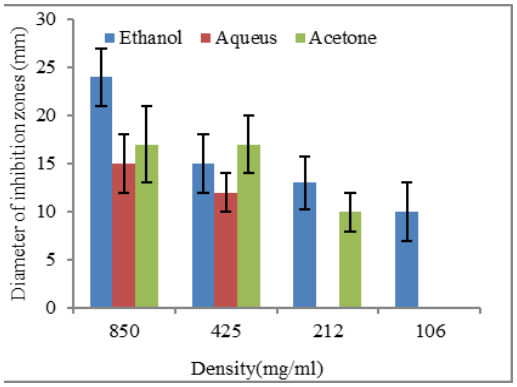

Figure 4. Mean ± SD inhibition of zone diameter of *E. aerogenes* in millimetres.

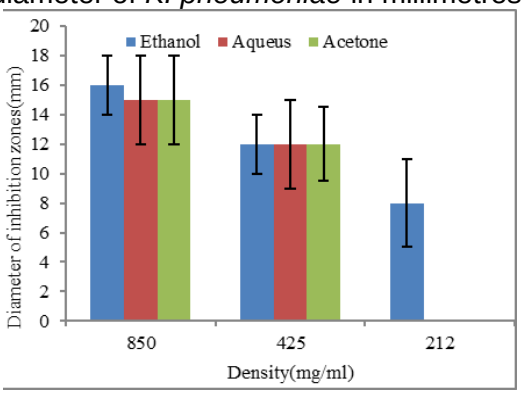

Figure 5. Mean ± SD inhibition of zone diameter of *P. aeruginosa* in millimetres.

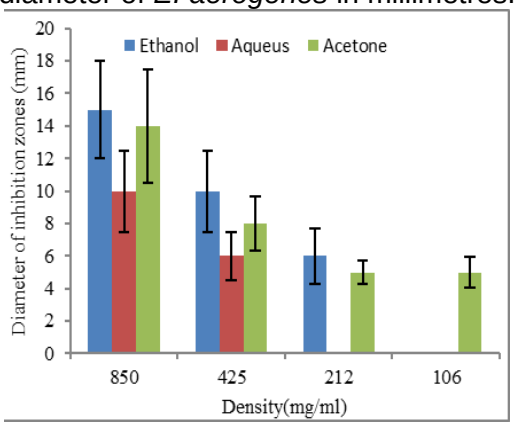

Figure 6. Mean ± SD inhibition of zone diameter of S. aureus in millimetres.

The more dilution of the prepared extracts resulted in reduced antimicrobial effects. So, in dilutions of 1:16 and 1:32 no bacterial susceptibility was observed in

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the well diffusion method. According to Figure 2, the water extract did not show any antimicrobial effect on *E. coli*.

Ethanolic extract (25 ± 2 mm), had more antimicrobial activity than the alcoholic extract on *E. coli*. Among the extracts prepared from Quince leaves, the aqueous extract did not show any antimicrobial effects on *K. pneumoniae*.

Acetonic extract compared to aqueous extract showed a weaker antimicrobial activity on *K. pneumoniae* (Figure 3). In dilutions of 1:8, 1:16 and 1:32, no bacterial susceptibility was observed in the well diffusion method.

All three extracts had antimicrobial effects on *E. aerogenes*, the ethanolic extract with an average diameter of 24 ± 3 mm, had more antimicrobial effect than the other two extracts (Figure 4).

As can be seen in Figure 5, all three extracts in the pure form and in a dilution of 1:2 show almost similar antimicrobial activity on *P. aeruginosa* (aqueous extract 16 ± 2, acetonic and the ethanolic extract 15 ± 3) and as the concentration of the extracts were reduced the antimicrobial activity decreased.

Ethanolic (15 ± 2) and acetonic (14 ± 3) extracts showed a stronger antimicrobial activity on *S. aureus* than the aqueous extract (Figure 6).

Table 1 shows the amounts of MIC and MBC of extracts for every six bacteria in mg/liter. As can be seen, *S. typhimurium*, *K. pneumoniae* and *P. aeruginosa* showed complete resistance to aqueous Quince leaf extract in macro dilution method and in the case of *E. coli*, high doses of aqueous extract were required to kill the bacterium.

In macro dilution method, the minimum bactericidal dose of the extract for all bacteria was that of ethanolic extract and, the results show the great power of ethanolic extract in compared to other extracts especially aqueous extract.

Table 1.

MIC and MBC of the extracts (mg/mL)

Row	Organ Extract / Bacteria	Quince Leaf Acetonic		Ethanolic		Aqueous	
		MBC	MIC	MBC	MIC	MBC	MIC
1	*S. typhimurium*	98	98	375	188	–	–
2	*E. coli*	196	196	94	94	800	800
3	*K. pneumoniae*	392	196	196	196	–	–
4	*E. aerogenes*	98	98	49	24	400	400
5	*P. aeruginosa*	196	196	98	49	–	–
6	*S. aureus*	196	98	47	23	534	534

According to the World Health Organization (WHO), about 80 % of the population in developed countries uses medicinal plants for treatment of diseases.

Meanwhile the increasing microbial resistance to available antibiotics has led to study medicinal plants to find new antimicrobial compounds [MOSHAFI, *et al.*, 2012].

There are several medicinal plants that have been used to treat infections in traditional medicine [COWAN, 1999].Due to bioavailability, reduced side effects and more biodegradability in compared to existing antibiotics, medications obtained from these plants are being considered seriously [MEHRU *et al.*, 2008, BUTNARIU, and BOSTAN, 2011, BARBAT, *et al.*, 2013, BUTNARIU, 2012].

During studies on various parts of Quince, this fruit have been introduced as a good, cheap and natural source of antioxidants such as phenolic acids and flavonoids [BRANCA, *et al.*, 2004, IANCULOV, *et al.*, 2004, BUTU, *et al.*, 2015, CAUNII, *et al.*, 2015, BUTNARIU, *et al.*, 2016]. Quince leaves take part in removing free radicals, and their anti–hemolytic activity has also been demonstrated [ALAA, *et al.*, 2010, BUTNARIU, 2006, BUTU, *et al.*, 2014a, BUTNARIU, *et al.*, 2005].

Agar well diffusion method is used to determine the dose of an antimicrobial agent and according to the results, as the concentration of the extracts are reduced, the antimicrobial activity is also reduced.

These findings are in agreement with the results of the study conducted by

Alizadeh and collab. in 2013, the antimicrobial effect of Quince fruit and seed was examined [ALIZADEH *et al.*, 2013].

The research conducted by Hosseini and collab. studied the antimicrobial effect of aqueous extract of wood sorrel (oxalidaceae) [HOSSEINI *et al.*, 2013] on *S. aureus* and *E. coli.*

Also, the research conducted by Tajik and collab. in 2008 examined the effect of aqueous garlic extract on pathogenic microorganisms [TAJIK and SHOKUHI SABET JALALI, 2008, HOSSEINI, *et al.*, 2010, ALIZADEH, *et al.*, 2013].

The antimicrobial activities of three aqueous, acetonic and ethanolic extracts were not observed in dilutions of 1:16 and 1:32 against any of the bacteria [BUTU, *et al.*, 2014b, BUTNARIU, *et al.*, 2015a, RODINO, *et al.*, 2014, BUTNARIU, *et al.*, 2015b].

Acetonic and ethanolic Quince (*Cydonia oblonga*) leaf extracts had stronger antimicrobial effect on *S. typhimurium* that was in agreement with the study of Alipour and collab. [ALIPOUR YEGANE, *et al.*, 2008].

Ethanolic and acetonic Quince leaf extract had stronger antimicrobial effects on the gram–negative bacterium, *E. coli*, in compared to other bacteria, that is in consistent with the study of Nezamabadi and collab. using hydro–alcoholic and aqueous extracts of sumac on *E. coli* [NEZAMABADI, *et al.*, 2011].

Another study by Chaleshtary and collab. showed that the ethanolic extract of snapdragon (*Scrophulariastriata*) has a significant antimicrobial effect on *E. coli* in vitro, which is compatible with the present study [SHARAFATI–CHALESHTORI, *et al.*, 2009].

Aqueous and acetonic Quince leaf extracts showed a weaker antimicrobial activity against *K. pneumoniae* in compared to *S. typhimurium* and *E, coli*.

In a study conducted by Moshefi and collab. in 2004, using the extracts of Azerbaijani sage (*Saliva atropatana*) no significant antimicrobial activity on *K. pneumoniae* was observed [MOSHAFI, *et al.*, 2012] that it is likely that the bacteria are highly resistant to antimicrobial agents.

Ethanolic Quince leaf extract, has more antimicrobial effect than aqueous extract against *E. aerogenes,* which is consistent with the study of Abdullahzadeh and collab.

They found that ethanolic eucalyptus extract of (*Eucalyptus globulus*) compared to aqueous eucalyptus extract has greater antimicrobial activity against *Brucella melitensis* and *Brucella abortus* [ABDULLAHZADEH, *et al.*, 2011].

Pseudomonas has been more resistant to medicinal plants and even to antibiotics in compared to other bacteria and these substances have less effect on it [BRANCA, *et al.*, 2004].

Quince leaf extracts showed weaker antimicrobial activity against *P. aeruginosa* than *S. typhimurium*, *E. coli* and *E. aerogenes* that is consistent with the study results of Tajik and collab. they also found the lowest antimicrobial effect against pathogenic bacteria tested for *P. aeruginosa* [TAJIK and SHOKUHI SABET JALALI, 2008].

In another research, Moghtader and collab. examined the antimicrobial effects of cumin seeds (*Bunium persicum Boiss*) on some gram–positive and gram–negative species of bacteria. Results indicate minimal antimicrobial effect against *P. aeruginosa* [MOGHTADER, *et al.*, 2010].

In 2009 Majnouny and collab. studying the antimicrobial effects of alcoholic extract of fenugreek (*Trigonella foenum*) on different bacterial strains, found that extracts of the plant had stronger antimicrobial effect against *S. aureus* in compared to gram–negative bacteria [MAJNOUNY *et al.*, 2009, ABIRI, *et al.*, 2009].

Another study by Doost Mohammadi and collab. showed that extracts of mallow (*Malva lavatera*) showed no antimicrobial effect on *S. aureus* [DOOST MOHAMMADI, *et al.*, 2011], but in the present study, the acetonic Quince leaf extract showed antimicrobial activity against *S. aureus*.

Thus, it can be said that the acetonic Quince leaf extract is stronger antibacterial agent than the acetonic extracts of mallow.

Conclusions

Ethanolic leaf extract showed the highest antimicrobial effect on *E. aerogenes* and *S. aureus*.

The aqueous extract in compared to the other extracts showed a weaker antimicrobial effect, on *K. pneumoniae, P. aeruginosa*, and *S. typhimurium*.

Antimicrobial effect of Quince leaf extract has a wide range; this means that it is effective on gram–negative bacteria and also on gram–positive bacteria.

According to the results, we can say that the *Quince* leaf extracts have antibacterial properties and can be used as a therapeutic agent against the studied bacterial infections.

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