

RESEARCH ARTICLE

## Antimicrobial Effect of *Cyclamen persicum* Tuber Extracts Against Bacteria and *Candida* Species

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### Abstract

The antimicrobial resistant microorganisms will take us again to an era where a simple infection could lead to serious illness and death, interest in using medicinal plants as a source of new antimicrobials has increased. *Cyclamen persicum* is a traditional medicinal plant that showed to have some medicinal effects. The aim of the present study was to evaluate the antimicrobial effect of *C. persicum* tuber water, acetone, ethanol and methanol extracts on different pathogenic bacteria and candida. It was found that *C. persicum* tuber acetone, ethanol and methanol extracts have antibacterial effect on many Gram positive and Gram negative bacteria specially *S. pyogenes*, *S. aureus*, *E. faecalis*, *P. mirabilis*, *K. pneumoniae*, *E. cloacae*, *P. aeruginosa* and *S. flexneri*. *C. persicum* tuber acetone, ethanol and methanol extracts also showed great antifungal effect against all *Candida* used in this study. The study also reported extracting saponin from *C. persicum* tubers methanol extract.

**Keywords:** *Cyclamen persicum* tubers, Antimicrobial effect, Saponin.

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## INTRODUCTION

Extracts of medicinal plants were used traditionally in healing infectious diseases all over the world, but since the discovery of antibiotics from fungal and bacterial sources in the mid of the 20<sup>th</sup> century the use of plant extracts almost demolished<sup>1</sup>. The overuse and the misuse of antimicrobials particularly in developing countries led to increase the prevalence of antibiotic and antifungal drug resistant worldwide<sup>2,3</sup>. The antimicrobial resistant bacteria and fungus are raising attention as a worldwide serious medical problem as shown in the world health organization (WHO) report that was published in 2014<sup>3</sup>.

The antimicrobial resistance of the common pathogenic Gram-positive bacteria (*Staphylococcus*, *Streptococcus* and *Enterococcus* species), Gram-negative bacteria (*Enterobacter*, *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Salmonella* and *Shigella* species)<sup>3,4</sup> and *Candida*<sup>5</sup>, is taking us over again to the pre- antimicrobial era where a simple infection could lead to serious illness and death<sup>6</sup>. Interest in using medicinal plants as a source of new antimicrobial compounds has increased recently, especially after the fail in discovering novel antimicrobials since late 1980s<sup>7,8</sup>.

One of the traditional medicinal plants here in Jordan is *Cyclamen persicum*. *C. persicum* is used traditionally in relieving spasm pain<sup>9</sup> and as an anticancer medication agent, but no reports of using *C. persicum* tubers as a traditional antimicrobial agent. Jaradat et al. in 2015 found that *C. persicum* is rich with antioxidant compounds<sup>10</sup>. A study by Mahasneh et al. in 1999 found that the aerial plant parts of *C. persicum* have antimicrobial effects on four bacteria species, the study was on the aerial parts and reported the antimicrobial activity only<sup>9</sup>.

The aim of the present study was to evaluate the antimicrobial effect of *C. persicum* tuber water, acetone, ethanol and methanol extracts on different pathogenic bacteria and candida. The study measured the antimicrobial activity, the minimum inhibition concentration (MIC) and the minimum bactericidal/ fungicidal concentration (MBC/ MFC). The study also reported extracting saponin from *C. persicum* tubers.

## MATERIALS AND METHODS

### Tested microorganisms

Microorganisms used in this study were five Gram positive bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Methicillin-resistant Staphylococcus aureus*, *Staphylococcus aureus*, *Streptococcus pyogenes*), Seven Gram negative bacteria (*Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Shigella sonnei*) and Four *Candida* species (*Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Candida krusei*). Microorganisms were obtained from microbiology laboratory, Zarqa University College- Al-Balqa Applied University, Jordan. All microorganism stocks were preserved in nutrient broth with 15% glycerol and kept in -80°C freezer.

### Plant material collection and identification

Wild *C. persicum* tubers were collected during the spring (March and April) of 2017 from the North West areas of Jordan (mainly from Salt and Irbid), and were identified by the botanist Hussein Alhrout MSc, PhD (Department of Allied Medical Sciences, Al-Zarqa University College, Al-Balqa Applied University, Al-Salt, Jordan). A voucher specimen (NO. 10002) has been deposited at the Laboratory of Botany- Zarqa University College- Al-Balqa Applied University, Jordan.

### Plant extracts preparation

Wild *C. persicum* tubers were washed to remove soil. Tubers were then homogenized using either sterile distilled water, acetone, ethanol or methanol as solvents. The homogenized mixtures were then incubated in a rotating water bath (50°C, 70 rpm) for 24 hours. Mixtures were then filtered three times using Whatman No. 1 filter papers. Mixtures were then centrifuged twice for 15 minute at 10000 RCF, supernatant of the water extract was then lyophilized while the acetone, ethanol and methanol extracts were evaporated in an incubator at 25°C. Dried extracts were kept in air tight bottles and frozen at -20°C till use<sup>11</sup>. For measuring the antimicrobial effect of the *C. persicum* tubers crude extracts, a stock concentration of 400 mg/ml were prepared by using DMSO as a solvent for the acetone, ethanol and methanol extracts and distilled water for the water extract. The stocks were then diluted to the concentration of 100mg/ml using distilled water

and sterilized using a 0.22 µm membrane filter. The sterile 100 mg/ml stock solutions were then kept at 4°C till use (not more than 24 hours).

### Antimicrobial effect measurements

#### Antimicrobial activity assay

The antimicrobial activity of the extracts were measured by the agar well diffusion method. Microorganism being tested was grown in Mueller Hinton broth (MHB, Thermo Scientific) at 37°C overnight on a rotary shaker, the growth was then diluted using MHB to a turbidity equivalent to the density of 0.5 McFarland standard, 250 µl of the microorganism growth was then inoculated into 15 ml of molten Mueller Hinton Agar (MHA, Thermo Scientific) and poured into petri dish. 6 mm diameter wells were made on the solidified MHA, plant extract (100 µl) at the designated concentration (50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml) and the negative control (solvent without plant extract) were placed separately in each well. Plates were then left at room temperature for 1 hour to allow the extracts to diffuse, plates were then incubated at 37°C overnight. Inhibition zones were measured from the base of the plate resting 5-7 cm above black flat surface and illuminated by reflecting light source<sup>11</sup>, inhibition zones above 9mm (including the wells diameter) were only reported. Experiments were performed in duplicate and repeated independently three times.

In this study, the antimicrobial activity results <6.25 mg/ml were reported to have a strong antimicrobial activity, while results 6.25-25 mg/ml were reported to have a moderate antimicrobial activity, results >25 mg/ml were reported to have weak antimicrobial activity and results >50 mg/ml were reported to have no antimicrobial activity.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) determination

This study measured the MIC of *C. persicum* tuber extracts at the concentrations 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml. MIC were measured as follow, using sterile 96-Well microplates (Thermo Fisher) 150 µl of double strength extract (100, 50, 25, 12.5, 6.25 and 3.13 mg/ml), 140 µl of MHB and 10 µl of tested microorganism cultured in MHB to concentration equal to 0.5 McFarland standard were added into

each well. 300 µl of MHB were used as negative control, 290 µl of MHB with 10 µl of microorganism cultured in MHB were used as positive control. We included a blank for each concentration of the bacterial plant extract mixture, which contained 150 µl of the designed double strength extract and 150 µl of MHB. Plates were then incubated at 37°C overnight. After incubation, turbidity of each well was measured using a microplate reader (Bio-Rad). The MIC is the lowest concentration of the extract agent that inhibited the growth of a microorganism (no change in turbidity compared to the blank). Each MIC experiment was made in duplicate and repeated three times.

To measure the MBC and MFC, 25 µl of each concentration of the bacterial plant extract mixture obtained in the MIC wells was subculture (in triplicates) on MHA, the subcultures were then incubated at 37°C overnight. MBC and MFC is the lowest concentration at which no microorganism growth (colonies) was seen on the subculture. Each MBC and MFC experiment was made in duplicate and repeated three times.

In this study, the MIC, MBC and MFC values <6.25 mg/ml were reported to have a strong value, while results 6.25-25 mg/ml were reported to have a moderate value, results >25 mg/ml were reported to have weak value and results >50 mg/ml were reported to have no MIC, MBC or MFC value.

#### Characterization of the *C. persicum* methanol extract

Dry *C. persicum* tuber methanol extract was dissolved in a mixture of methanol and hexane solvents, and left at 4°C overnight. The materials dissolved in the hexane layer and the precipitate portions of the extract were discarded. The material dissolved in the methanol layer was dried and further analyzed by Fourier Transform Infrared and NMR (1H and 13C) spectra using Bruker FTIR vertex 70 and Bruker Avance 400MHz spectrometers, respectively. The NMR chemical shifts were given in ppm relative to solvent peaks (deuterated methanol).

## RESULTS AND DISCUSSION

### *C. persicum* tuber water extract antimicrobial effect

*C. persicum* tuber water extract did not show any antimicrobial effect at the Gram

positive bacteria, Gram negative bacteria and the *Candida* species used in this study. Using water extraction techniques such as boiling, soaking or chowing is the traditional way of extracting active compounds from medicinal plants, this way of extraction seems to be ineffective in extracting antimicrobial compounds from *C. persicum*. This result may explain why *C. persicum* is not been used traditionally as an antimicrobial agent.

#### ***C. persicum* tuber extracts effect at Gram positive bacteria**

Results of the antibacterial activity, MIC and MBC of *C. persicum* extracts on Gram positive bacteria are shown in table 1.

One of the best antibacterial effect of *C. persicum* on Gram positive bacteria was found against *S. pyogenes*. *C. persicum* tuber acetone, ethanol and methanol extracts showed a moderate antibacterial activity and MIC value to *S. pyogenes*, the extracts also showed a weak MBC value. Another promising result were found against *E.*

*faecalis*, *C. persicum* tuber ethanol and methanol extracts showed a moderate antibacterial activity and MIC value to *E. faecalis*, and also showed a weak MBC effect to *E. faecalis*, a study by Okmen et al. in 2014 found that *Cyclamen mirabile* tuber (another specie of the genus *Cyclamen*) had an antimicrobial effect on *E. faecalis*, the study found a strong and moderate MIC value of the ethanol and the methanol extracts, respectively<sup>12</sup>. This is the first study that showed the antibacterial effect of *C. persicum* to *S. pyogenes* and *E. faecalis*.

*C. persicum* tuber acetone, ethanol and methanol extracts had moderate antibacterial activity and MIC value to *S. aureus*, but none of the extracts showed a MBC value to *S. aureus*. A study by Mahasneh et al. in 1999 found a good effect (inhibition zone >8 mm) of the *C. persicum* whole aerial plant parts ethanol extract, while the *C. persicum* whole aerial plant parts butanol and petroleum ether extracts showed a weak effect<sup>9</sup>. It seems that *C. persicum* tuber and the

**Table 1.** Antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration of acetone, ethanol, methanol and water extracts of *C. persicum* tubers on different pathogenic Gram positive bacteria.

	Microorganism	Extraction solvent	Activity <sup>a</sup> (mg/ ml)	MIC (mg/ ml)	MBC (mg/ ml)
1.	<i>B. subtilis</i>	Acetone	Nil	50	Nil
	<i>B. subtilis</i>	Ethanol	Nil	Nil	Nil
	<i>B. subtilis</i>	Methanol	50	12.5	Nil
	<i>B. subtilis</i>	Water	Nil	Nil	Nil
2.	<i>E. faecalis</i>	Acetone	Nil	Nil	Nil
	<i>E. faecalis</i>	Ethanol	25	6.25	50
	<i>E. faecalis</i>	Methanol	25	6.25	50
	<i>E. faecalis</i>	Water	Nil	Nil	Nil
3.	MRSA	Acetone	Nil	Nil	Nil
	MRSA	Ethanol	Nil	Nil	Nil
	MRSA	Methanol	Nil	Nil	Nil
	MRSA	Water	Nil	Nil	Nil
4.	<i>S. aureus</i>	Acetone	12.5	25	Nil
	<i>S. aureus</i>	Ethanol	12.5	25	Nil
	<i>S. aureus</i>	Methanol	25	12.5	Nil
	<i>S. aureus</i>	Water	Nil	Nil	Nil
5.	<i>S. pyogenes</i>	Acetone	25	12.5	50
	<i>S. pyogenes</i>	Ethanol	12.5	25	50
	<i>S. pyogenes</i>	Methanol	25	12.5	50
	<i>S. pyogenes</i>	Water	Nil	Nil	Nil

<sup>a</sup>The antibacterial activity shown is the lowest extract concentration that showed a  $\geq 9$  mm inhibition zone; Nil: No result (activity, MIC or MBC) was found; Results presented are the most common result of the three independent experiments, experiments were performed in duplicate and repeated independently three times.

whole aerial plant parts have similar effects on *S. aureus*. Another study by Okmen *et al.* in 2014 found that *C. mirabile* tuber ethanol and methanol extracts had antibacterial activity to *S. aureus* and Coagulase-negative staphylococci<sup>13</sup>. The fact that *C. persicum* and *C. mirabile* tubers have similar effects to *S. aureus* may indicate that both plant have a similar antimicrobial compound.

The study showed that *B. subtilis* was weakly effected by the *C. persicum* tuber methanol and acetone extract, while the ethanol extract did not show any antimicrobial effect. The methanol

extract showed a moderate MIC value and a weak antibacterial activity while the acetone extract only showed a weak MIC value. A study by Mahasneh *et al.* in 1999 found a weak effect of the whole aerial plant parts *C. persicum* ethanol and petroleum ether extract on *Bacillus cereus* a close specie to *B. subtilis*, the study also showed no effect of the *C. persicum* butanol extract on *B. cereus*<sup>9</sup>. It seems that, the active antimicrobial compound in *C. persicum* only have an inhibitory effects on *Bacillus* bacteria. A study by Okmen *et al.* in 2014 found that *C. mirabile* tuber ethanol and

**Table 2.** Antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of acetone, ethanol, methanol and water extracts of *Cyclamen persicum* tubers on different pathogenic Gram negative bacteria.

S. No.	Microorganism	Extraction solvent	Activity <sup>a</sup> (mg/ ml)	MIC (mg/ ml)	MBC (mg/ ml)
1.	<i>E. cloacae</i>	Acetone	6.25	12.5	50
	<i>E. cloacae</i>	Ethanol	3.125	12.5	50
	<i>E. cloacae</i>	Methanol	6.25	12.5	50
	<i>E. cloacae</i>	Water	Nil	Nil	Nil
2.	<i>E. coli</i>	Acetone	Nil	Nil	Nil
	<i>E. coli</i>	Ethanol	100	50	Nil
	<i>E. coli</i>	Methanol	100	25	Nil
	<i>E. coli</i>	Water	Nil	Nil	Nil
3.	<i>K. pneumoniae</i>	Acetone	6.25	6.25	12.5
	<i>K. pneumoniae</i>	Ethanol	6.25	6.25	12.5
	<i>K. pneumoniae</i>	Methanol	1.56	1.56	6.25
	<i>K. pneumoniae</i>	Water	Nil	Nil	Nil
4.	<i>P. mirabilis</i>	Acetone	3.125	12.5	50
	<i>P. mirabilis</i>	Ethanol	3.125	12.5	25
	<i>P. mirabilis</i>	Methanol	3.125	12.5	25
	<i>P. mirabilis</i>	Water	Nil	Nil	Nil
5.	<i>P. aeruginosa</i>	Acetone	12.5	12.5	50
	<i>P. aeruginosa</i>	Ethanol	12.5	12.5	50
	<i>P. aeruginosa</i>	Methanol	6.25	12.5	50
	<i>P. aeruginosa</i>	Water	Nil	Nil	Nil
6.	<i>S. enterica</i>	Acetone	Nil	Nil	Nil
	<i>S. enterica</i>	Ethanol	Nil	Nil	Nil
	<i>S. enterica</i>	Methanol	Nil	Nil	Nil
	<i>S. enterica</i>	Water	Nil	Nil	Nil
7.	<i>S. flexneri</i>	Acetone	25	12.5	50
	<i>S. flexneri</i>	Ethanol	25	12.5	50
	<i>S. flexneri</i>	Methanol	12.5	6.25	50
	<i>S. flexneri</i>	Water	Nil	Nil	Nil

<sup>a</sup>The antibacterial activity shown is the lowest extract concentration that showed a  $\geq 9$  mm inhibition zone; Nil: No result (activity, MIC or MBC) was found; Results presented are the most common result of the three independent experiments, experiments were performed in duplicate and repeated independently three times.

methanol extracts had an antibacterial activity on *B. subtilis* at a 60 mg/ml concentration<sup>12</sup>.

Finally, no antibacterial effect was shown against MRSA using any of the *C. persicum* tuber extracts. A study by Quave *et al.* in 2008 found that *Cyclamen hederifolium* tuber ethanol extract had an inhibitory effect on biofilm formation of MRSA but the study did not find a MIC value to MRSA growth<sup>14</sup>. The antibacterial effect of *C. persicum* crude extracts on MRSA has not been previously reported.

#### **C. persicum tuber extracts effect at Gram negative bacteria**

Results of the antibacterial activity, MIC and MBC of *C. persicum* extracts on Gram negative bacteria are shown in table 2.

Table 2 shows that *C. persicum* acetone, ethanol and methanol extracts had an outstanding antibacterial effect on *P. mirabilis* and *K. pneumoniae* which may have a potential use in the future. As shown in table 2, *C. persicum* acetone, ethanol and methanol extracts had a strong antibacterial activity and a moderate MIC and MBC values on *P. mirabilis*. Multidrug-resistant *P. mirabilis* have been reported and recovered worldwide, making it a major emerging problem in antimicrobial resistance<sup>15</sup>, our results show that *C. persicum* acetone, ethanol and methanol extract may have a potential solution to this problem. Another promising result was found in the *C. persicum* acetone, ethanol and methanol extract on *K. pneumoniae*. *C. persicum* methanol extract showed a strong antibacterial activity and MIC, and showed a moderate MBC value on *K. pneumoniae*. While *C. persicum* acetone and ethanol extracts showed a moderate antibacterial activity, MIC and MBC value on *K. pneumoniae*. Carbapenem-resistant *K. pneumoniae* is a recent high concern antibiotic resistant bacteria. Scientists considers Carbapenem-resistant as the most worrying evolution in the antibiotic resistance crisis, and the fact that carbapenem resistance has a transferable mechanism makes it even a more serious matter<sup>16</sup>. Our results show that *C. persicum* methanol extract may have a potential solution to this problem.

As shown in table 2, *C. persicum* acetone, ethanol and methanol extracts showed a moderate antibacterial activity (exception: ethanol extract showed a strong antibacterial activity) and MIC

value, and showed a weak MBC value on *E. cloacae*, *P. aeruginosa* and *S. flexneri*. *E. cloacae* is the most clinically isolated *Enterobacter* specie and is raising high concerns because of their ability of expressing new  $\lambda$ -lactamases and carbapenemases<sup>17</sup>, *P. aeruginosa* is resistant to many antimicrobials and can develop resistance to any antimicrobial compound, recently the emergence of the *P. aeruginosa* carbapenem resistant strains have increased their danger<sup>18,19</sup>. *S. flexneri* is also resistant to broad-spectrum  $\beta$ -lactam ampicillin and it was found that 2% of *Shigella* isolates are resistant to azithromycin which is a treatment solution to carbapenem-resistant bacteria when combines with colistin<sup>20</sup>. Here again *C. persicum* acetone, ethanol and methanol extracts may have potential solution to these antimicrobial drug resistant bacteria.

*E. cloacae*, *P. aeruginosa* and *S. flexneri* are equipped with resistance-nodulation-cell division (RND) type efflux pump which plays an important part in antibiotic resistance<sup>21-23</sup>. Chevalier *et al.* in 2008 found that 40% of the multidrug resistant *E. cloacae* has an antibiotic efflux pump making it even a more serious problem<sup>24</sup>. It seems that the antimicrobial compound in *C. persicum* are minimally effected by these efflux pumps.

*C. persicum* acetone extract did not show any antibacterial effect on *E. coli*, while *C. persicum* ethanol and methanol extracts showed week antibacterial activity and MIC value (exception: methanol extract showed a moderate MIC value), but did not show MBC value. It was found that saponins isolated from *C. mirabile* and *Cyclamen coum* had a very weak antibacterial activity and MIC values to both *E. coli* and *P. aeruginosa*<sup>25</sup>. Finally, *C. persicum* acetone, ethanol and methanol extracts did not show any antibacterial effect on *S. enterica*.

#### **C. persicum tuber extracts effect at Candida species**

Generally, *C. persicum* tuber acetone, ethanol and methanol extracts had a stronger antimicrobial effect on candida species than on bacteria species. Results of the antifungal activity, MIC and MFC of *C. persicum* extracts on Candida species are shown in table 3.

*C. persicum* tuber acetone, ethanol and methanol extracts has a promising antifungal results against Candida species, especially in the

case of *C. glabrata* which the extracts (acetone, ethanol and methanol) showed a strong MIC value (3.125 mg/ml). *C. persicum* tuber acetone, ethanol and methanol extracts showed a moderate antifungal activity, MIC and MFC values to all other *Candida* species studied in this paper, exceptions is in *C. persicum* tuber acetone extract and *C. persicum* tuber ethanol extract which showed a weak MBC values to *C. albicans* and *C. krusei*, respectively. A study by Sajjadi et al. in 2016 found that triterpenoid saponins extracted from *C. coum* had antifungal effect on *C. albicans* strains and *C. tropicalis* strains<sup>26</sup>.

*Candida* causes infection called candidiasis which is recognized as the most fungal infection in the world. Invasive candidiasis is most commonly (90%) caused by *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. Invasive candidiasis is a major cause of death in intensive care unit patients and patients receiving immunosuppressive drugs, patients who usually get intensive antibacterial therapy<sup>3,27</sup>. A recent publication by the WHO in 2014 reported

that resistance to fluconazole (common antifungal) and echinocandins (newest antifungal agent) by *Candida* is increasing worldwide, this increase will complicate the control of candidiasis in the health care system<sup>3</sup>. *C. persicum* tuber acetone, ethanol and methanol extracts have the potential to play a role in controlling candidiasis.

### Saponin extraction from *C. persicum* tuber methanol extract

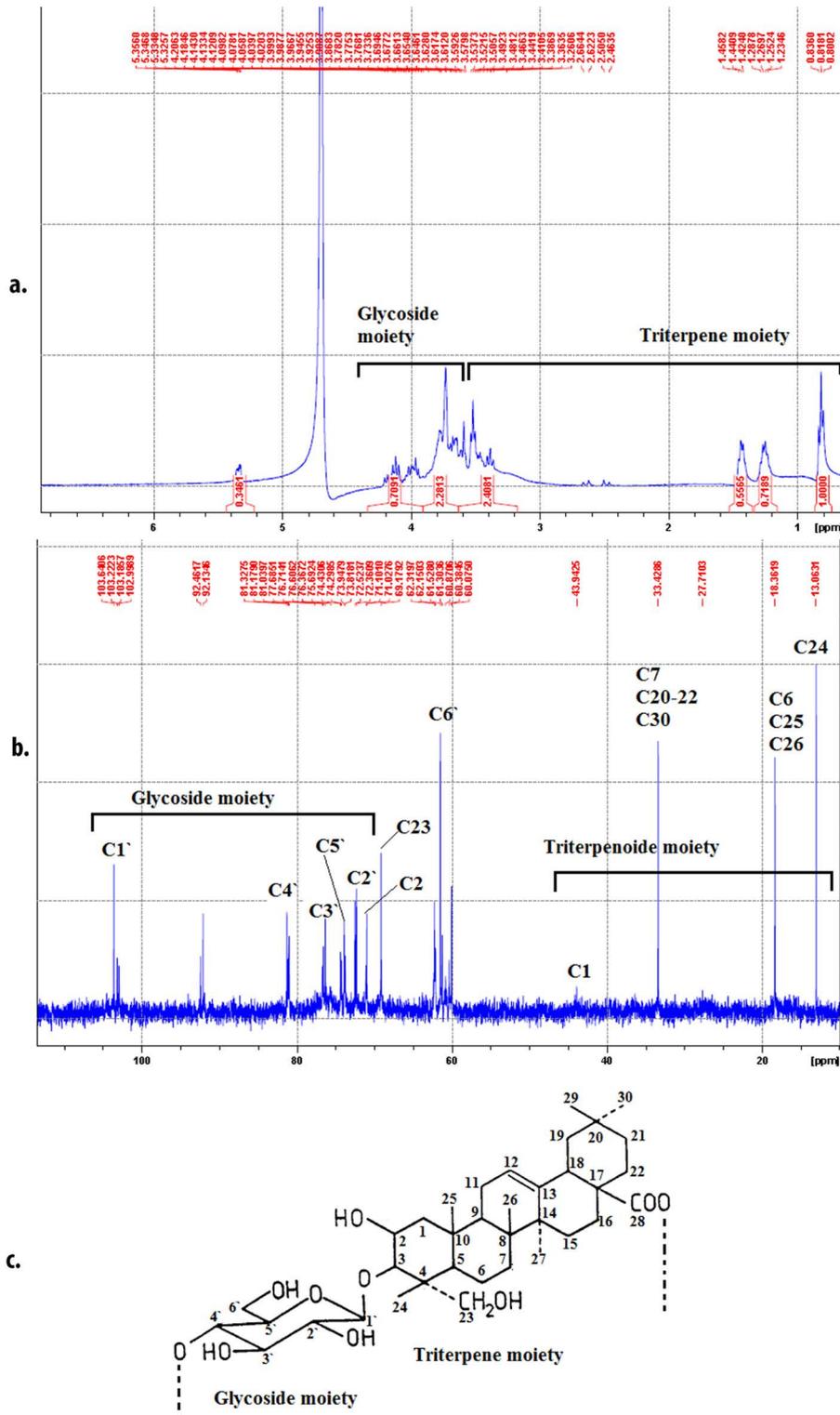
#### Characterization of methanol extract

<sup>1</sup>H and <sup>13</sup>C-NMR analysis of the methanol extract revealed that the extract is composed mainly from saponins with triterpene and sugar moieties Figure 1. The assignments of <sup>1</sup>H and <sup>13</sup>C-NMR peaks are shown in Figure 1 and were done according to reported assignments of saponins<sup>28,29</sup>. The FTIR spectrum of methanol extract showed strong bands at 3324 cm<sup>-1</sup> due to O-H stretching, 1039 and 992 cm<sup>-1</sup> due to C-O stretching. Furthermore, weak absorption bands were observed at 2953 and 2872 cm<sup>-1</sup> due to C-H stretching, 1715 cm<sup>-1</sup> due to C=O stretching and 1622 cm<sup>-1</sup> due to C=C stretching.

**Table 3.** Antifungal activity, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of acetone, ethanol, methanol and water extracts of *Cyclamen persicum* tubers on different pathogenic *Candida* species

	Microorganism	Extraction solvent	Activity <sup>a</sup> (mg/ ml)	MIC (mg/ ml)	MFC (mg/ ml)
1.	<i>C. albicans</i>	Acetone	12.5	12.5	50
	<i>C. albicans</i>	Ethanol	12.5	12.5	25
	<i>C. albicans</i>	Methanol	12.5	12.5	25
	<i>C. albicans</i>	Water	Nil	Nil	Nil
2.	<i>C. tropicalis</i>	Acetone	6.25	6.25	12.5
	<i>C. tropicalis</i>	Ethanol	6.25	12.5	25
	<i>C. tropicalis</i>	Methanol	12.5	12.5	25
	<i>C. tropicalis</i>	Water	Nil	Nil	Nil
3.	<i>C. glabrata</i>	Acetone	6.25	3.125	12.5
	<i>C. glabrata</i>	Ethanol	6.25	3.125	12.5
	<i>C. glabrata</i>	Methanol	6.25	3.125	12.5
	<i>C. glabrata</i>	Water	Nil	Nil	Nil
4.	<i>C. krusei</i>	Acetone	12.5	6.25	25
	<i>C. krusei</i>	Ethanol	12.5	6.25	50
	<i>C. krusei</i>	Methanol	6.25	6.25	12.5
	<i>C. krusei</i>	Water	Nil	Nil	Nil

a The antifungal activity shown is the lowest extract concentration that showed a  $\geq 9$  mm inhibition zone; Nil: No result (activity, MIC or MFC) was found; Results presented are the most common result of the three independent experiments, experiments were performed in duplicate and repeated independently three times.



**Fig. 1.** <sup>1</sup>H-NMR (a) and <sup>13</sup>C-NMR (b) of methanol extract, and general structure of saponins with carbon numbers (c)

### **C. persicum tuber saponin**

Saponin is a secondary metabolite produced by different high plant species which showed antibacterial and antifungal activities against different microorganisms<sup>25</sup>. *C. mirabile* and *C. coum* tubers have shown to produce different saponins<sup>25</sup>. Extraction of saponin can be carried out in many ways<sup>25,30,31</sup>, in this paper we have shown a simple and safe way of extracting saponin, the key point was in homogenizing *C. persicum* tubers with methanol but without drying the tuber, then further purifying the crude tuber methanol extract by hexane as explained in the methodology section. The isolation of saponins from *C. persicum* was also reported by Mihci-Gaidi et al in 2010<sup>32</sup> and El Hosry et al. in 2014<sup>33</sup>.

A study by Calis et al. in 1997 showed that saponins extracted from *C. mirabile* and *C. coum* had antibacterial effects on some Gram positive bacteria (*S. aureus* and *E. faecalis*), Gram negative bacteria (*E. coli* and *P. aeruginosa*) and showed significant antifungal effect on many *Candida* species (*C. albicans*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea* and *C. tropicalis*)<sup>25</sup>. Other studies also showed that, saponin extracted from *C. coum* tubers can markedly reduce the production of pyocyanin from *P. aeruginosa*<sup>34</sup> and significantly inhibit *P. aeruginosa* biofilm formation when combined with ciprofloxacin<sup>35</sup>. Saponin extracted from *C. coum* tubers have also showed antifungal effects to *C. albicans*, *C. tropicalis* and *C. krusei*<sup>26</sup>. As our results are in coherence with the above studies it is mostly possibly that the main antimicrobial compound found in *C. persicum* tuber is saponin.

### **CONCLUSION**

It is concluded that *C. persicum* has antibacterial effect against many Gram positive bacteria (best shown on *S. pyogenes* and *E. faecalis*) and Gram negative bacteria (outstanding antibacterial effect on *P. mirabilis* and *K. pneumoniae*), and a high antifungal effect against different *Candida* species. It seems that saponin plays a major role in these antimicrobial effects.

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### **CONFLICT OF INTERESTS**

The author declares that there are no conflict of interest.

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