Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Antibacterial and anti-fungal effects of water and alcoholic extracts of *Portulaca oleracea* L.

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ABSTRACT

Portulaca oleracea is a folk medicine in various countries. This study aimed to consider the anti-microbial effects of the water and alcoholic extracts of *P. oleracea* and its orange leaf extracts against Staphylococcus aureus, *Streptococcus salivarius*, Klebsiella, *Pseudomonas aeruginosa* and *Candida albicans* strains. Plant extracts were obtained using the maceration method. The antimicrobial activity of the extracts was measured using the Disc agar diffusion (DAD) test method. The minimum inhibitory (MIC) and bactericidal (MBC) concentrations for each extract were considered by the macro dilution method. Both aqueous and methanolic extracts showed acceptable antibacterial and anti-fungal activities at all concentrations (from 30 mg mL^{-1} to pure extract. However, pure extracts showed significantly higher inhibitory effects compared to the other concentrations ($p < 0.01$). The inhibitory effect of both aqueous and methanolic extracts was significantly increased in proportion to their concentrations ($p < 0.05$); however, methanolic extract was slightly more effective than aqueous extract. Compared to ampicillin antibiotics, pure methanolic and aqueous extracts showed significantly higher inhibitory effects against *S. aureus* and *Klebsiella* ($p < 0.05$). *S. aureus* was a more sensitive strain to methanolic (showing the MIC of 30 mg mL^{-1} and MBC of 50 mg mL^{-1}) and aqueous (showing the MIC of 50 mg mL^{-1} and MBC of 70 mg mL^{-1}). *P. oleracea* extracts, especially alcoholic extract, exhibited acceptable antibacterial and anti-fungal properties against various microorganisms. However, further clinical research is needed to obtain information about the effectiveness of the plant extracts such as Antibacterial, Anti-fungal, Water extract, Alcoholic extract, MIC, MBC.

Keywords: *Portulaca Oleracea*, Antibacterial, Anti-fungal, Water extract, Alcoholic extract, MIC, MBC.
Article type: Research Article.

INTRODUCTION

Portulaca oleracea is a member of Portulacaceae family which is widely distributed in many parts of the world, including in the United States, Asia, Europe, and the Mediterranean countries (Uddin *et al.* 2014). This plant is a major source of essential nutrients and antioxidants such as β-carotene, ascorbic acid, and α-linolenic acid (omega-3 fatty acid; Liu *et al.* 2000; Chengcheng *et al.* 2019). In traditional medicine, *P. oleracea* has been used for the treatment of headache, cough, shortness of breath, burns, septic, liver, and gastric diseases (Zhou *et al.* 2015; Li *et al.* 2020). Many studies showed the anti-inflammatory, antibacterial, antiulcerogenic, and antioxidant properties of the *P. oleracea* (Zhou *et al.* 2015; Dosumu *et al.* 2019). Recent studies have demonstrated that *P. oleracea* has also anticancer, neuroprotective, and antidiabetic activities (Abdel Moneim 2013; Uddin *et al.* 2015; Iranshahy *et al.* 2017; Butnariu *et al.* 2018; Li *et al.* 2020). It has also a good anti-bacterial, anti-parasite, anti-virus, and anti-fungal activities against a wide range of isolates. For instance, Oh *et al.* (2000) reported that *P. oleracea* displays acceptable anti-fungal effects against the genera Trichophyton. Dong *et al.* (2010) reported that *P. oleracea* exhibits anti-virus activity against simplex virus type 2. Some studies showed the anti-bacterial effect of this plant extract against a wide range of gram-negative and gram-positive bacteria such as *E. coli*, *Neisseria*

gonorrhea, *P. aeruginosa*, *S. aureus*, *S. faecalis*, and *Bacillus subtilis* (Elkhayat *et al*. 2008; Zhou *et al*. 2015). The high antimicrobial effect of *P. oleracea* is likely due to its chemical composition and antioxidant activity. Phytochemical studies revealed that this plant contains various compounds such as flavonoids, alkaloids, vitamins, trace elements, terpenoids, and proteins (Zhu *et al*. 2010; Zhou *et al*. 2015; Chengcheng *et al*. 2019; Li *et al*. 2020). Flavonoids are the most biologically active compound present in *P. oleracea* which has a wide range of pharmacological properties, including anti-inflammation, antibacterial, antivirus, and antioxidation properties (Zhu *et al*. 2010; Butnariu *et al*. 2018; Rahimi *et al*. 2019). In recent years, most bacterial and fungal isolates have been resistant to different types of antibiotics (Friães *et al*. 2015).

For this reason, microbial-based diseases have become one of the most common complications in hospitals and public health throughout the world. Therefore, many attempts have been made to identify, control, and treatment of microbial diseases and their resistance to antibiotics (Fakih *et al*. 2018). This is very important for some bacterial strains such as *P. aeruginosa* and *S. aureus* which are now considered as one of the most significant infectious strains in humans and hospitals (Dibah *et al*. 2014; Afshar *et al*. 2016). Since medicinal plants contain various chemical compounds such as flavonoids and alkaloids, they can be used as anti-microbial natural compounds without any adverse effects (Iranshahy *et al*. 2017; Chengcheng *et al*. 2019). Therefore, due to the antioxidative and antimicrobial properties of *P. oleracea*, this study aimed to assess the antimicrobial effects of the both alcoholic and water extracts of this plant against *S. aureus*, *S. salivarius*, *Klebsiella*, *P. aeruginosa*, and *C. Albicans.*

MATERIALS AND METHODS

Sampling and analysis of samples

Healthy *Portulaca oleracea* plant was collected from Mazandaran, Province North, and identified by the Department of Botany, Agricultural University. A voucher specimen was deposited in the herbarium of the Faculty of Agriculture. Lyophilized *S. aureus*, *S. Salivarius*, *Klebsiella*, *P. aeruginosa*, and *C. Albicans* were provided by the microbial bank of Standard Research Organization and Industrial Research, Tehran- Iran. Ampicillin, erythromycin, and chloramphenicol were selected as standard antibiotics in this study. Miconazole and fluconazole were used as standard antifungal antibiotics.

Isolation of plant extracts

Air-dried plant leaves (5 g) were powdered and the extracts were obtained by maceration method. Plant powders were mixed with 70 mL methanol (for methanolic extract) or water (for aqueous extract). The resulting mixtures were placed on a rotary magnet and stored for 48 h at room temperature, then the contents of the container were filtered and the filtered solution was left in a rotary at 30 ^{o}C so that its water and alcohol evaporated. The extraction yield was measured by the division of extract weight to the total mass of applied plant leaves. Finally, different concentrations of plant extracts were prepared by dissolving the appropriate amount of extract in normal saline.

Antibacterial activity test

Disc agar diffusion (DAD) test using Kirby-Bauer method according to the CLSI procedure was applied for the assessment of the antibacterial effects of different antibiotics against bacterial strains. The test bacteria (0.5 McFarland) were spread onto the surface of the medium with a sterile swab. Sterile disks were paced in a bacterial culture medium and then 20 µL of each extract at different concentrations (30, 50, 70, 100 mg mL^{-1}, and pure extract) were added to each disk. Each test was done in triplicate. Ampicillin, erythromycin, and chloramphenicol disks were used as an antibacterial positive control, while miconazole and fluconazole as an antifungal positive control. The agar plates were incubated for 24h at 37 ^{o}C and the diameter of the zone of inhibition for each microorganism was measured.

Determination of MIC and MBC/MFC concentrations

The MIC of the plant extracts against the examined bacteria or fungus was determined by the broth macro dilution method. About 20 µL of the extracts at each concentration were added to 1 mL MHA medium containing 1×10^{7} CFU mL^{-1} bacteria or fungous. Two test tubes served as a positive and negative control, respectively. The test tubes were incubated at 37 ^{o}C for 24 h. The concentration at which complete inhibition of the growth was observed was recorded as MIC. To determine MBC or MFC, the broth was taken from each well and inoculated in Mueller Hinton agar for 24 h at 37 ^{o}C.

Statistical analysis

Data were reported as means ±SD. Comparison of the mean of inhibition zones between each treatment group was considered using the One-Way ANOVA: Post Hoc-Tukey test. Data were analyzed using SPSS software (IBM, version 19). A p-value of less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

The antimicrobial activity of *P. oleracea* aqueous and methanolic extracts as well as antibiotics against *P. aeruginosa*, *S. salivarius*, *S. aureus*, *Klebsiella*, and *C. albicans* are summarized in Table 1. Antimicrobial activities of aqueous and methanolic extracts were found for examined bacteria at all concentrations (from 30 mg mL^{-1} to pure extract); however, pure extracts showed significantly higher inhibitory effects on them compared to the other concentrations (30-100 mg).

Table 1. Antibacterial activity of *P. oleracea* extracts and antibiotics.

	Inhibition zone (mm)				
Treatments	***P. aeruginosa***	***S. salivarius***	***S. aureus***	***Klebsiella***	***C. albicans***
Ampicillin	25 ± 1	26.33 ± 1.52	22.67 ± 1.52	14.6 ± 0.57	-
Erythromycin	23 ± 2.64	29.67 ± 1.52	31 ± 1	25 ± 1	-
Chloramphenicol	38.33 ± 1.52	30 ± 1	37.67 ± 1.52	27 ± 1	-
Methanolic extract (mg mL^{-1})					
30	6 ± 0	7 ± 0	8 ± 1	6 ± 0	6 ± 0
50	7.33 ± 0.57	9 ± 1	11 ± 1	7.33 ± 0.57	7 ± 0
70	8.33 ± 0.57	13 ± 1	18 ± 1	9 ± 1	7.33 ± 0.57
100	10.67 ± 0.57	20 ± 1	24 ± 1	12.33 ± 0.57	10.33 ± 0.57
Pure	17 ± 1	25.33 ± 1.52	29 ± 1	17 ± 1	13.33 ± 0.57
Aqueous extract (mg mL^{-1})					
30	6 ± 0	6 ± 0	8.67 ± 0.57	8.33 ± 0.57	6 ± 0
50	6.67 ± 0.57	8 ± 0	10.33 ± 0.57	7.33 ± 0.57	6.67 ± 0.57
70	7.67 ± 0.57	12.33 ± 1.52	16 ± 1	6.33 ± 0.57	6.67 ± 0.57
100	9.33 ± 0.57	19 ± 1	21 ± 1	11 ± 1	8.33 ± 0.57
Pure	15 ± 1	22.67 ± 0.57	23.67 ± 1.52	15 ± 1	11 ± 1

There was a significant difference in the mean diameter of the inhibition zone of microorganisms at each plant extract concentration ($p < 0.001$; Table 1). At 30 mg mL^{-1}, the extracts showed the lowest inhibition zone diameter for all studied strains (Figs. 1-5; $p < 0.05$). Chloramphenicol exhibited significantly the highest inhibitory effect against all bacterial strains than other antibiotics and also *P. oleracea* extracts. Erythromycin was the second most effective antibiotic against all bacterial strains. Miconazole showed significantly higher inhibition zone diameter against *C. albicans* (23 ± 2 mm) compared to fluconazole (16.33 ± 1.51 mm) and also the plant extracts ($p < 0.001$; Fig. 5). The inhibitory effects of both aqueous and methanolic extracts were significantly increased in proportion to their concentrations ($p < 0.05$; Figs. 1-5); however, methanolic extract was slightly more effective than aqueous one. Compared to ampicillin, pure methanolic and aqueous extracts showed significantly higher inhibitory effects against *S. aureus* and *Klebsiella* ($p < 0.05$; Figs. 3-4). The MIC and MBC of extracts are presented in Table 2. The methanolic extract showed significantly lower MIC and MBC values compared to aqueous extract ($p < 0.05$). *S. Aureus* was found to be more sensitive to methanolic extract showing the MIC of 30 mg mL^{-1} and MBC of 50 mg mL^{-1}, while the MIC and MBC values of aqueous extract for *S. aureus* were 50 mg mL^{-1} and 70 mg mL^{-1}, respectively.

Table 2. The MIC and MBC of *P. oleracea* extracts.

	Methanolic extract		Aqueous extract	
	MIC (mg mL^{-1})	**MBC/MFC (mg mL^{-1})**	**MIC (mg mL^{-1})**	**MBC/MFC (mg mL^{-1})**
P. aeruginosa	50	70	70	100
S. aalivarius	50	70	50	100
S. aureus	30	50	50	70
Klebsiella	50	70	50	100
C. albicans	50	70	70	100

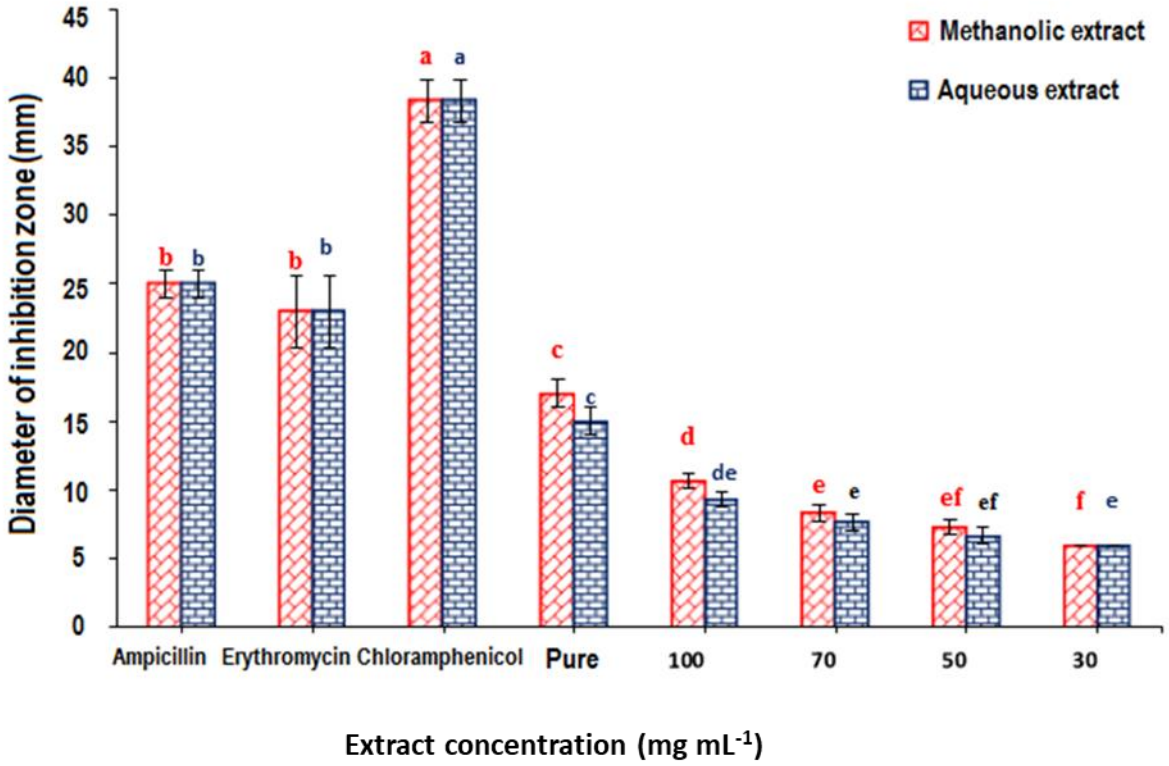

Fig. 1. Comparison of the effect of *P. oleracea* aqueous and methanolic extracts at different concentrations on *P. aeruginosa.*

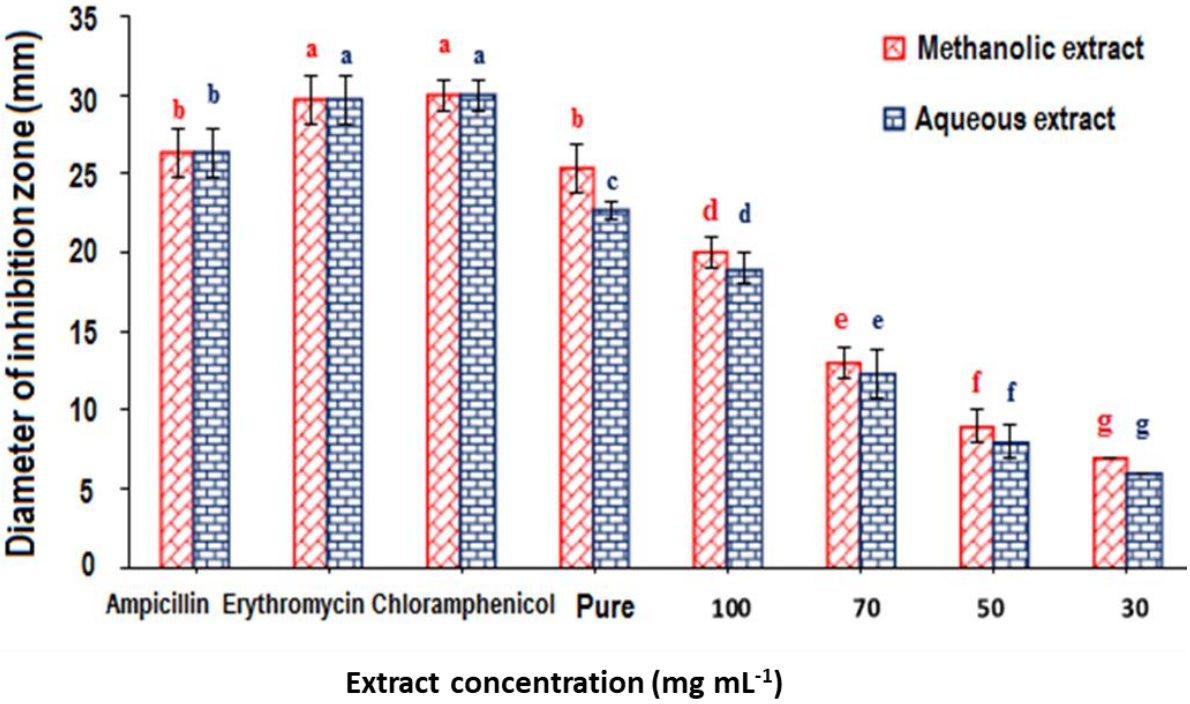

Fig. 2. Comparison of the effect of *P. oleracea* aqueous and methanolic extracts at different concentrations on *S. salivarius*.

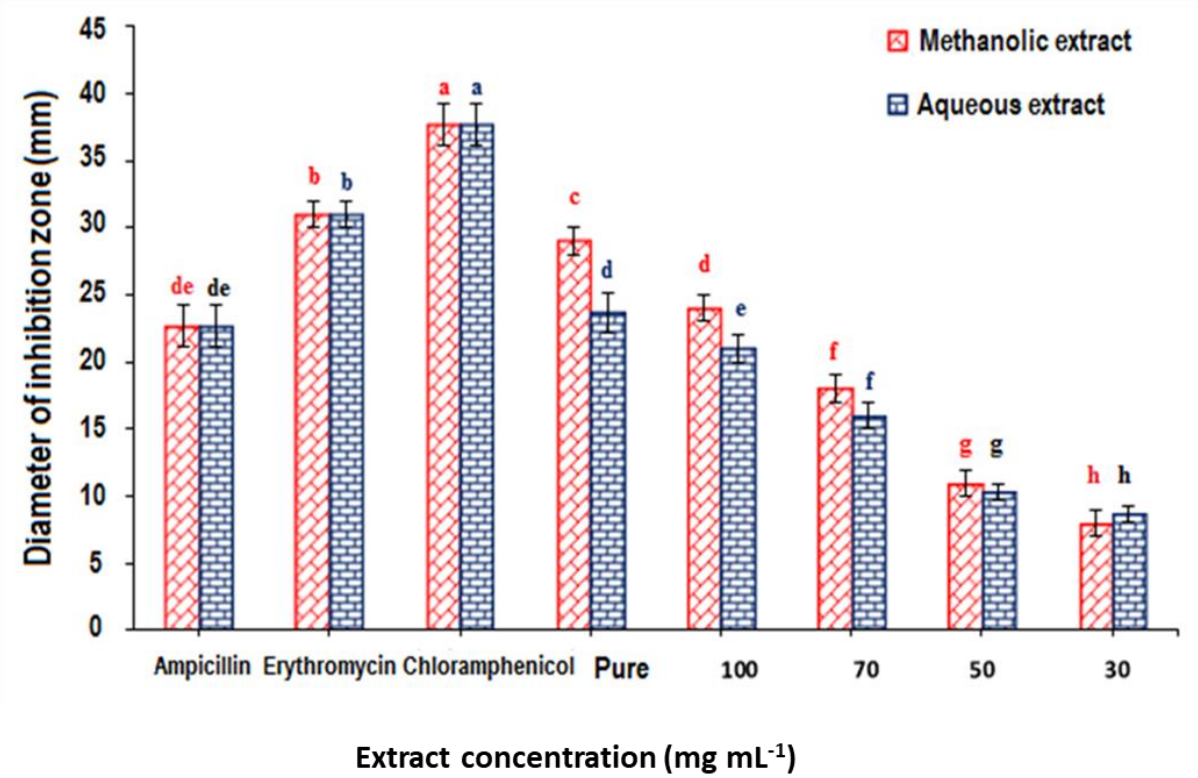

Fig. 3. Comparison of the effect of *P. oleracea* aqueous and methanolic extracts at different concentrations on *S. aureus*.

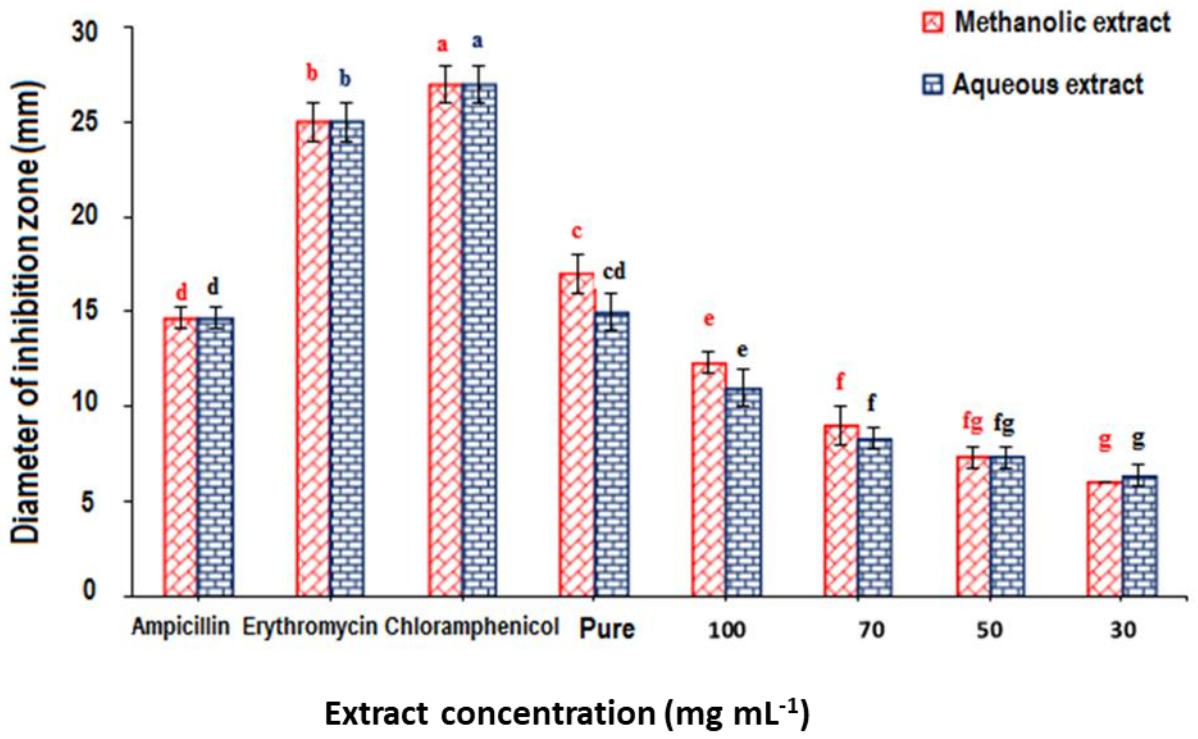

Fig. 4. Comparison of the effect of *P. oleracea* aqueous and methanolic extracts at different concentrations on *Klebsiella*.

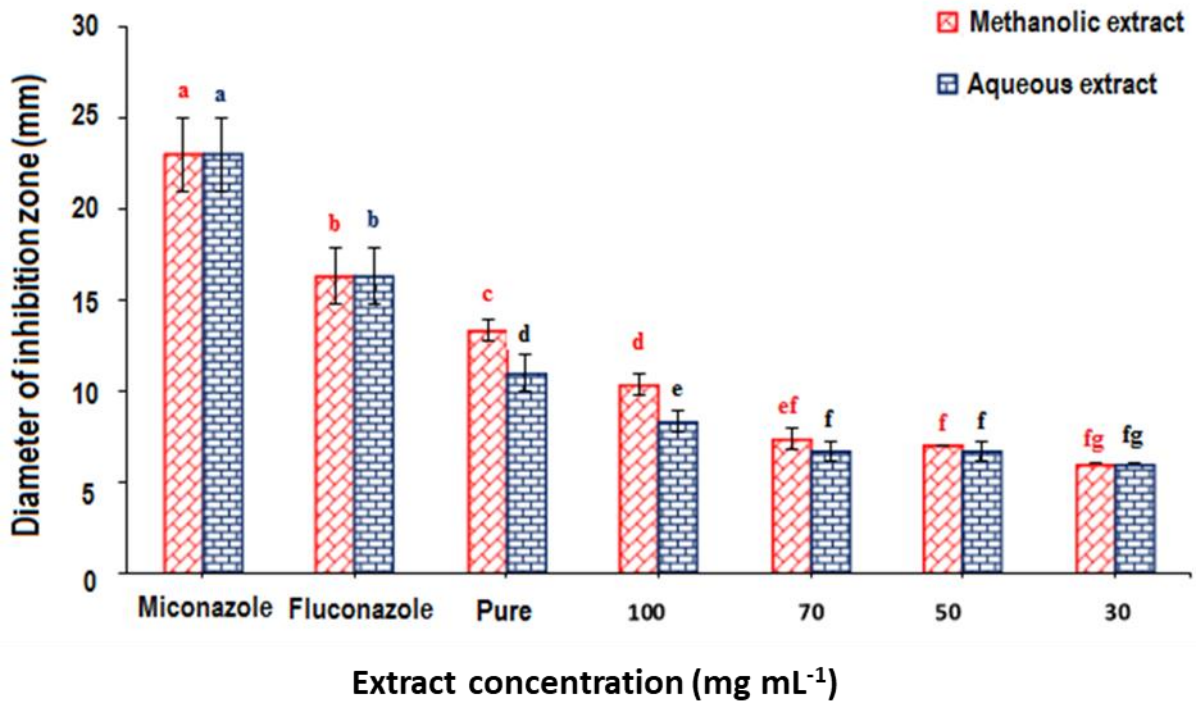

Fig. 5. Comparison of the effect of *P. oleracea* aqueous and methanolic extracts at different concentrations on *C. albicans.*

DISCUSSION

In recent years, the development of microbial resistance to various antibiotics has increased the use of traditional medicine as an alternative form of health care system (Peterson E & Kaur 2018; Zhu *et al*.2019). For this reason, many investigations have focused on investigating the antimicrobial activity of medicinal plants against various human pathogenic bacterial and fungal strains (Atef *et al*. 2019; Abu El-Wafa *et al*. 2020 ; da Costa Júnior *et al*. 2020). Plant extracts have now been extensively used in Iranian traditional medicine. They are not only used as flavoring agents in foods and beverages but also considered as potential natural products against various microbial isolates due to the existence of antimicrobial compounds such as phenolic compounds (Khameneh *et al*.2019). Therefore, many studies have been focused on biologically active compounds isolated from different plant species for the reduction of pathogenic microorganisms because of their resistance to antibiotics (Cheesman *et al*. 2017; Ramona *et al*. 2017; Nwonuma *et al*.2019; Manandhar *et al*.2019). In this study, we evaluated the antimicrobial effects of *P. oleracea* aqueous and methanolic extracts at different concentrations against *S. aureus*, *S. salivarius*, *Klebsiella*, *P. aeruginosa*, and *C. albicans*. Our findings have revealed that both aqueous and methanolic extracts have acceptable antibacterial and anti-fungal effects in a dose-dependent manner; however, the methanolic extract was slightly more effective than aqueous one. The antimicrobial effects of plant extracts were increased in proportion to their concentrations. Pure extracts showed significantly higher antibacterial and anti-fungal activities compared to the other concentrations. Although antibiotics showed significantly higher antibacterial and anti-fungal effects than the plant extracts, pure methanolic and aqueous extracts showed significantly higher inhibitory effects against *S. aureus* and *Klebsiella* compared to ampicillin antibiotics. We also found that *S. aureus* was a more sensitive strain to methanolic (showing the MIC of 30 mg mL^{-1} and MBC of 50 mg mL^{-1}) and aqueous (showing the MIC of 50 mg mL^{-1} and MBC of 70 mg mL^{-1}). Several lines of studies showed that *P. oleracea* extract possesses antibacterial, antifungal, anti-parasite, and antiviral activities (Oraibi *et al*. 2017; Dosumu *et al*. 2019). For instance, Nayaka et al. (2014) considered the antibacterial effect of flavonoid apigenin isolated from *P. oleracea* against several pathogenic bacterial strains such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*. The apigenin has acceptable antibacterial properties against all strains (MIC > 4 mg mL^{-1}), especially *S. typhimurium* (17.36 ± 0.18 mm) and *P. mirabilis* (19.12 ± 0.01 mm). In another study, Gharirvand Eskandari et al. (2016) investigated the effect of hydroalcoholic extract of *P. oleracea* on *Leishmania major* promastigotes at different times. Although the IC50 of standard glucantime drug against clinical isolates of promastigotes after 24, 48, and 72h (IC50: 26, 19, and µg mL^{-1}) was significantly lower than that of alcoholic extract (IC50: 1160, 385, and 140 µg mL^{-1}); plant

extract showed a considerable anti-leishmanial effect. In another study, Cho *et al.* (2008) found that the *P. oleracea* water and alcoholic extracts possess high antioxidant and antimicrobial activities against Helicobacter pylori, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *E. coli*, and *Streptococcus mutans*. The MICs of plant extract on *H. pylori*, *S. epidermidis*, *S. aureus*, *E. coli*, and *S. mutans* were 200, 50, 100, 100, and 150 mg mL^{-1}, respectively. Similarly, our findings showed MICs between 30-100 mg mL^{-1} against various strains. A previous study showed that *P. oleracea* has antifungal activity against dermatophytes of the genera Trichophyton (Oh *et al*. 2000). Dong *et al.* (2010) found that a pectic polysaccharide of *P. oleracea* exhibited an antiviral effect against simplex virus type II which was due to the inhibition of virus penetration and not virus adsorption. A previous study reported that 70% methyl alcohol extract of *P. oleracea* has acceptable antibacterial and antifungal activities against *E. coli*, *P. aeruginosa*, *Neisseria gonorrhea*, *S. aureus*, *Bacillus subtilis*, *Streptococcus faecalis* and *C. albicans* (with inhibition zones of 14, 15, 15, 13, 14, 15 and 12 mm, respectively ; Elkhayat *et al*. 2008), in accordance with the results of the present study. Based on these accomplished data and our findings, both water and alcoholic *P. oleracea* extracts possess acceptable antibacterial and antifungal effects against a wide range of microorganisms; however, the exact mechanism is unclear. The presence of flavonoids at high concentrations is likely a major mechanism for the antibacterial, antivirus, anti-inflammation, and antioxidant properties of *P. oleracea*. Previous studies indicated that *P. oleracea* contains high levels of flavonoids (Nayaka *et al*. 2014 ; Gatea *et al*. 2017). Flavonoids such as kaempferol and genistin are biologically active compounds that are present in this plant extract at high concentration (Alu'datt *et al*. 2019). Therefore, further studies are needed to consider its diverse chemical compounds against a wide spectrum of antibiotic-resistant microorganisms.

CONCLUSION

Our findings have revealed that the *P. oleracea* extracts, especially alcoholic ones, possess acceptable antibacterial and anti-fungal properties against various microorganisms. Although the mechanisms of its action have not been addressed, the antimicrobial property of the *P. oleracea* extracts is probably attributable to the flavonoids and phenolic compounds. Further studies are needed to obtain information regarding the practical effectiveness of these plant extracts to prevent the growth of various human pathogenic bacteria as antimicrobial agents in new drugs for the therapy of infectious diseases in humans.

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