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ORIGINAL RESEARCH

Antioxidant and antibacterial activity of *Allium saralicum* and *Allium longisepalum* leaves extracts as medicinal plants in Iran

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Abstract

Recently, there has been increasing interest in medicinal plants, due to their content of health-promoting compounds, e.g., phenolics. Hence, this study aims to estimate the antioxidant and antibacterial properties of Soraneh (*Allium saralicum R.M. Fritsch*) and Pichkeh (*Allium longisepalum*) extracts as a Iranian medicinal plants. The study was done by 2,2-diphenyl-2-picrylhydrazyl (DPPH) assays, total phenolic content, total flavonoid content and antibacterial effects of the herbal extracts were determined. According to results, the highest total phenolic content (1.187±0.012mgGAE/g) was obtained in aqueous extract of Pichkeh. The highest total flavonoid content (1.193±0.004mgRE/g) and the antioxidant activity (5.93±0.07%) were found for hydroalcoholic extract of Soraneh and Pichkeh, respectively. In disk-diffusion test, for two extracts, the highest and lowest antibacterial effect was observed for *L. monocytogenes* and *S. aureus,* respectively. Soraneh extract had the highest and the lowest antibacterial effect on *L.monocytogenes* and *P. aeruginosa,* respectively. In the well-diffusion method, Pichkeh extract had the highest antibacterial effect on *S.aureus*. For the Soraneh extract, the highest effect was related to *B. cereus,* and there was also no detectable colony of *S.aureus, P.aeruginosa* and *S. enterica.* The results of present comprehensive analysis demonstrated that Soraneh and Pichkeh leaves possess high phenolic, flavonoid contents and potential antioxidant and antibacterial activity, and could be used as a viable source of bioactive compounds and might be exploited for functional foods and neutraceutical applications.

Keywords: Antioxidant, *A. longisepalum*, *A.saralicum*, Flavonoid, Phenol, Antibacterial

Introduction

The potency of different medicinal plants is related to their individual mechanisms of action in different disorders. Humans consume and use a variety of vegetable materials in the form of leaves, roots, seeds and fruits. Although medicinal plants are widely considered to be of lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effects (De Smet, 2004). Due to increased bacterial drug resistance, it is essential to discover new antimicrobial agents (Bristone et al., 2015).

There can be seen a global tendency towards preservation methods which are both environmental-friendly and healthy in terms of processing, production, and preservation. Nature-oriented methods have been particularly popular in this respect, with a focus on natural additives that have long been applied in different cultures, not only promote palatability of the treated ingredients but also lower the perishability of foodstuff (Berger, 2009). Generally, replacement of medicinal extracts instead of chemical preservatives is so important. It has been proved that this alternative may reduce the adverse effects of chemical preservatives (Prakash et al., 2015). Natural products are defined as natural sources-derived substances having biological activities. These products have long been implemented as alternative health care treatment and in discovery of novel drugs (Dias et al., 2012).

There is a growing interest in natural antioxidants and antimicrobials found in plants because of the worldwide trend toward the use of natural additives in foods. Herbs and spices are one of the most important targets to search for natural antioxidants and antimicrobials from
the point of view of safety (Yanishlieva et al.,2006). Herbs are used in many domains, including medicine, nutrition, flavouring, beverages and cosmetics (Djeridane et al., 2006). Herbal products such as plant essential oils, extracts and their compounds such as phenols and flavonoids have been considered as components with health-promoting ingredients, antioxidant and antimicrobial characteristics (Burt, 2004). Phenolics and flavonoids have a great potential to scavenge free radicals which are found in all parts of the plants, such as leaves, fruits, seeds, roots, and bark (Mathew and Abraham, 2006). The genus *Allium belongs to* the *Amaryllidaceae family* and *Allioideae subfamily*. Different parts of the plants are consumed due to their variety of flavors and textures. Soraneh is an endemic plant of Iran growing widely in the western parts of the country (Figure 1; Right). Pichkeh is the indigenous herbal plant in Iran, which is unknown in scientific sources, therefore, studying its various characteristics can be beneficial (Figure 1; Left).

Figure 1. Pichkeh (left); *Soraneh* (right).

Extraction process of phenolic compounds is a significant factor in determining the antioxidant properties of the extract. Besides, extraction time plays a crucial role in the extract contents (Xia et al., 2006). In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, alkaloids, polyphenolics, carotenoids and coumarins have been identified. For centuries Iran have been using several native herbs for food and for medicinal purposes. Since the western Iran is an indigenous region being rich in native plants such as Pichkeh and Soraneh with numerous antimicrobial and antioxidant properties. This study was aimed at comparing TPC, TFC, and free radical scavenging activity in the extracts obtained from powdered Pichkeh and Soraneh leaves through maceration method to determine the antioxidant and antimicrobial activity of Pichkeh and Soraneh extracts.

Materials and Methods

Soraneh and Pichkeh were collected from Kermanshah province (Iran) in spring. A confirming plant specified by the Herbarium group of the Research Institute of Forests and Rangelands of Iran (Alborz Province, Karaj) and the air-dried plants were milled after being dried in shade, and were transfered to the

extraction section (Laboratory of Science and Technology Park of Tehran University, Karaj). In maceration method, water and ethanol were used as solvents (0:100, 70:30 and 100:0 % and conversely) In order to prepare the extract, after drying the plants in a dark place without any moisture for 7 d, the young leaves of the plants were separated from other parts and then were completely crushed 150 g of powder of both plants was carefully weighed by a digital scale Then aqueous, ethanolic and hydroalcoholic extracts were separately added into erlenmeyer containing 500 ml of distilled water and 96% ethanol. The erlenmeyer was closed with parafilm and placed at ambient temperature on the magnetic mixer for 72 hr until the extraction was completed. Then the solvent and plants mixture were separated by a filter paper (Wattman). Therefore, the residue was compressed to be completely discharged. Finally, the primary extracts were obtained They were centrifuged for 10 min at 3000 rpm Then, the supernatant was collected to evaporate the solvent. The extracts were transferred into a rotary evaporator and evaporated for 1 hr and the concentrated extracts were obtained subsequently. When dried completely, they were scraped with a laboratory knife The extracts were stored in a sterile black bottle at 4^{c} until the next use (Ahmed et al., 2002).

TPC was measured by calorimetry using Foulin-Ciocalteu method (McDonald et al., 2001). Briefly, 20 µl of the extract solution was mixed with 1.160 ml distilled water and 100µl Follin-Ciocalteu in a test tube. After 8 min, 300 µl sodium carbonate (20%)(w/v) was added. The tube tests were agitated and placed in water bath at 40 oC. After that, the absorbance was read at 760 nm by the spectrophotometer. To draw the standard curve of gallic acid, a base 100µg/l solution was prepared. Different concentrations of this solution were prepared, and the absorbance was read as described above. TPC was measured by a linear equation of calibration curve of gallic. Finally, the data were expressed in Eq mg gallic acid/g extract (R^2= 0.9937, Y= 327.44 x- 3.8084) (Figure. 2).

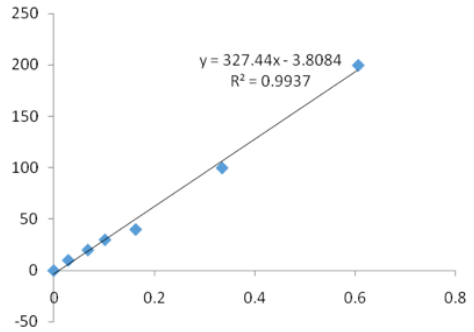

Figure 2. The calibration curve of gallic acid to determine TPC

TFC was meseared by aluminium chloride colorimetry. 0.5 ml of extract, 0.1 ml 10% aluminium chloride, 0.1 potassium acetate and 2.8 ml distilled water were mixed, and after 30 min at room temperature, the absorbance was read at 4.5 nm. To draw calibration curve, Rutin (RE) was used as a standard (Chen et al., 2007) as R^2=0.9989 and Y=673.06 x + 0.6751). Flavonoid content was expressed in Eq mg RE/g extract (Figure 3).

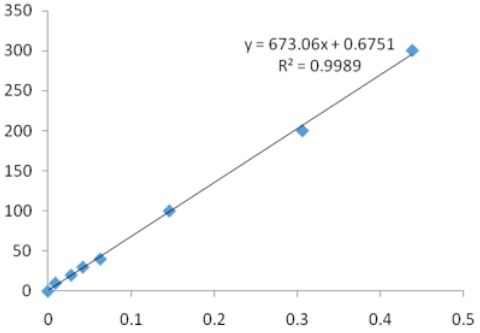

Figure 3. The calibration curve of rutin to TFC determination.

DPPH is a lipophilic radical showing the peak absornance at 517nm. Hydroxil group of antioxidant compounds reduced DPPH by donating hydrogen to free radical DPPH demonstrated by color change of reaction solution from dark purple to bright yellow. Therefore, absorbance at 517 nm decreased (Sousa et al., 2007). In this study, 40 µl of Soraneh and Pichkeh extract were transferred to a test tube to which 1ml of 0.2 mmol DPPH was added. Absorbance declined at 517 nm after 30min. Absorbance of DPPH without extract was considered as control. Radical scavenging was calculated by the following formula:

DPPH scavenging (%)= $((A_0-A_1) / A_0) \times 100$ (Equation 1)

Where A_0 is the absorbance without extract (blank) and A_1 is the absorbance of antioxidative extract. It should be noted that the blank was prepared as the sample, but 40 µl distilled water was used instead of extract. The treatments were ethanolic extract of Pichkeh, aqueous extract of Pichkeh, hydroalcoholic extract of Pichkeh, ethanolic extract of Soraneh, aqueous extract of Soraneh and hydroalcoholic extract of Soraneh. After performing the experiments at different times, the time spent on extraction was 60 sec.

The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish Mueller Hinton Agar (MHA). Nine serial dilutions yielded concentrations of 100, 80, 60, 40, 20, 10, 5, 2.5, and 1.25 mg/mL for extracts and fractions, and four serial dilutions yielded concentrations of 20, 15, 10 and 5 mg/mL for pure substances (NCCLS, 2003a). The treatments were incubated for 24 hr at 36 ± 1°C under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of bacterial growth was measured in mm. Reference commercial discs were used (chloramphenicol, 30 mg purchased from Cecon® and vehicle, 50 mL). The tests were conducted in duplicate (Smania et al., 1999).

Antibacterial activities of medicinal extracts were evaluated using well diffusion method on MHA. The inhibition zones were reported in millimeter. *S.aureus* (ATCC 25923), *B. cereus* (ATCC 11778) , *P. aeruginosa* (10145), *L. monocytogenes* (13932), *S. enterica* (9270), *S. sonnei* (9290) were used as references for the antibacterial assay of Cu-PFHs. Briefly, MHA agar plates were inoculated with bacterial strain under aseptic conditions and the wells (diameter = 6 mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hr. After the incubation, the diameter of the growth inhibition zones was measured. 24 hr single colonies on agar plates were used to prepare the bacterial suspension with the turbidity of 0.5 Mc Farland (equal to 1.5×108 cfu/ml). Turbidity of the bacterial suspension was measured at 600 nm (NCCLS, 2003b).

The results were reported as mean ± standard deviation (SD) (n = 3). The average contents of total phenolic content, total flavonoids and IC_{50} of the extracts prepared by the different extraction methods were statistically investigated using one-way analysis of variance (ANOVA) with Duncan by SPSS for Windows 24.0. A statistical probability (p value) less than 0.05 indicated a statistically significant difference between groups. All graphs were plotted with Microsoft Excel 2013 software (Steel and Torrie, 1980).

Results

Plants are the source of phytochemicals and possess several biological activities. Functional property of a plant relies upon the different secondary metabolites it possesses such as: phenolics, terpenoids, or alkaloids (Shekelle et al., 2003). According to Table 1, the highest total phenolic content belonged to the hydroalcoholic extract of Pichkeh, which had a statistically significant difference from other extracts ($p<0.05$). There was also a significant difference between all the extracts ($p<0.05$)(Table 2).

Table 1. Comparison of the effect of treatments on the TPC of the Soraneh and Pichkeh extract.

Extract Type	TPC (mg/g)
Ethanolic Pichkeh	0.316±0.010^{e}
Aqueous Pichkeh	1.187±0.012^{b}
Hydroalcoholic Pichkeh	1.229±0.014^{a}
Ethanolic Soraneh	0.405±0.017^{d}
Aqueous Soraneh	0.777±0.007^{c}
Hydroalcoholic Soraneh	1.180±0.007^{b}

The hydroalcoholic extract of Soraneh had the highest total phenolic content and the difference from other extracts was significant ($p<0.05$) There was also a significant difference between other extracts ($p<0.05$). The lowest total phenolic content belonged to the ethanolic extract of *A. longisepalum* (Table 2).

Table 2. Comparison of the effect of treatments on the TFC of the Soraneh and *A.longisepalum* extract.

Extract Type	TFC (mg/g)
Ethanolic *Pichkeh*	0.527±0.016^{c}
Aqueous Pichkeh	0.588±0.010^{d}
Hydroalcoholic Pichkeh	1.157±0.030^{b}
Ethanolic Soraneh	0.660±0.012^{c}
Aqueous Soraneh	0.536±0.010^{e}
Hydroalcoholic Soraneh	1.193±0.004^{a}

According to Table 3, hydroalcoholic extract of Pichkeh had the highest antioxidant and radical scavenging activity and was significantly different from other extracts ($p<0.05$). There was also a significant difference between all extracts ($p<0.05$), while the lowest radical scavenging activity

belonged to aqueous extract of Soraneh (Table 6).

Table 3. Comparison of the effect of treatments on the radical scavenging activity of the Soraneh and Pichkeh extract.

Extract Type	IC_{50} (µg/ml)
Ethanolic Pichkeh	17.08 ± 0.62^{b}
Aqueous Pichkeh	12.18 ± 1.92^{c}
Hydroalcoholic Pichkeh	5.93 ± 0.07^{e}
Ethanolic Soraneh	16.15 ± 3.03^{bc}
Aqueous Soraneh	77.04 ± 7.02^{a}
Hydroalcoholic Soraneh	7.87 ± 1.05^{d}

According to Table 4, in the agar diffusion disc method, the highest and the lowest antibacterial effect belonged to *L. monocytogenes* and *P.aeruginosa* respectively (Table 4). Soraneh extract showed the highest antibacterial effect on *L.monocytogenes*, which was not statistically significant from *Sh.sonnei* ($p>0.05$). The lowest effect was foun for *P. aeruginosa,* which was not significantly different from *S. enterica* ($p>0.05$)(Table 4). In Well-diffusion method, Pichkeh extract had the highest antibacterial effect on *S. aureus* (Figure.5) and *P. aeruginoa where* there was a significant difference between these treatments with other bacteria ($P<0.05$). The lowest effect belonged to *L.monocytogenes*, which was significantly different from other bacteria ($p<0.05$). For Soraneh extract, the highest effect was related to *B. cereus* (Figure.6), which was not statistically different from *E. coli* ($p>0.05$) (Figure 7). There was also a relationship between *L. monocytogenes* and *Sh.Sonnei* (Figure.8), while there were no detectable colonies of *S.aureus*, *P. aeruginosa* and *S. enterica* (Figure. 9)(Table 4). According to Table 5, the minimum bacteriocidal concentration (MBC) was negative and both two extracts had a significant effect on inhibiting bacterial growth, but no lethal effect was observed on any of the tested bacteria.

Table 4. Comparison of zone diameters (mm) of different hydroalcohol extracts versus studied bacteria by agar diffusion disc-variant and well diffusion methods (Mean ± SD).*

Extract Type	Disk-diffusion (mm)		Well-diffusion (mm)	
	Pichkeh Extract	Soraneh Extract	Pichkeh Extract	Soraneh Extract
E.coli	1.03 ± 0.06^{c}	0.50 ± 0.00^{b}	2.0 ± 0.00^{bc}	0.5 ± 0.05^{c}
S.aureus	1.03 ± 0.25^{c}	0.08 ± 0.14^{c}	1.00 ± 0.00^{c}	0.02 ± 0.13^{d}
B. cereus	2.03 ± 0.25^{b}	0.47 ± 0.15^{b}	2.0 ± 0.09^{bc}	1.12 ± 0.06^{b}
Ps.aeruginosa	0.03 ± 0.00^{d}	0.03 ± 0.06^{e}	0.01 ± 0.02^{d}	0.01 ± 0.32^{d}
L.monocytogenes	6.10 ± 0.36^{a}	1.07 ± 0.21^{a}	6.00 ± 0.00^{a}	2.27 ± 0.00^{a}
S.enterica	2.03 ± 0.06^{b}	0.03 ± 0.06^{c}	2.18 ± 0.00^{bc}	0.03 ± 0.08^{d}
Sh. sonnei	2.03 ± 0.15^{b}	1.03 ± 0.06^{a}	3.32 ± 0.22^{b}	2.12 ± 0.00^{a}

*Values in the same column, which do not share the same superscript letter, are significantly different ($p<0.05$).

Table 5. Minimum inhibitory concentration (µg/ml) of plant extracts for tested bacteria (Mean ± SD) *

Bacteria	Pichkeh extract	Soraneh extract
E.coli	12.5 ± 0.0^{b}	25.0 ± 0.0^{a}
S.aureus	25.0 ± 0.0^{a}	-
B. cereus	12.5 ± 0.0^{b}	25.0 ± 0.0^{a}
Ps.aeruginosa	25.0 ± 0.0^{a}	-
L.monocytogenes	3.12 ± 0.1^{c}	12.51 ± 0.0^{b}
S.enterica	12.50 ± 0.0^{b}	-
Sh. sonnei	12.50 ± 0.0^{b}	12.50 ± 0.0^{b}

*Values in the same column, which do not share the same superscript letter, are significantly different ($p<0.05$).

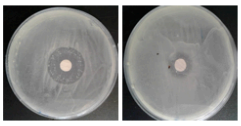

Figure 4. The effect of Pichkeh (left); Soraneh (right) on the *L. monocytogenes*.

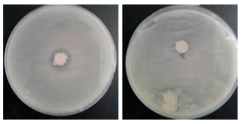

Figure 5. The effect of Pichkeh (left); Soraneh (right) on the *S. aureus*.

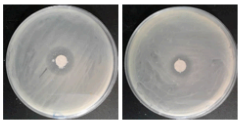

Figure 6. The effect of Pichkeh (left); Soraneh (right) on the *B. cereus*.

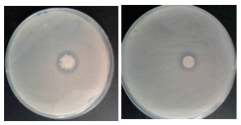

Figure 7. The effect of Pichkeh (left); Soraneh (right) on the *E.coli*.

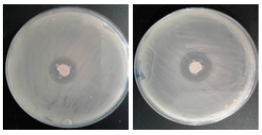

Figure 8. The effect of Pichkeh (left); Soraneh (right) on the *Sh.sonnei*.

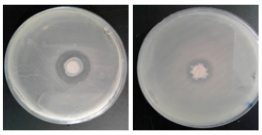

Figure 9. The effect of Pichkeh (left); Soraneh (right) on the *S.enterica*.

Discussion

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom especially in fruits and vegetables (Wojdylo et al., 2007). Besides, the phenolic compounds possess multiple biological properties such as antimutagenic and antibacterial properties, and these activities might be related to their antioxidant activity (Shui and Leong, 2002). Antioxidant activity (AA) of phenolic compounds depends on their reducing characteristics (Forouzani et al., 2013) The phenolics concentration in the produced extracts depends on many parameters such as application method and solvent type, solvent concentration and maceration time (Kasparavicienė et al., 2013) According to the results of present study, the

highest TPC belonged to the hydroalcoholic extract of Pichkeh being significantly different ftom other extracts ($P<0.05$). There was also a significant difference between all the extracts ($P<0.05$)(Table 2).

The results of Falleh et al., (2012) showed that methanolic extract contained higher polyphenolic compounds than ethanolic extract, and ethanolic extract had higher antioxidant activity than methanolic extract

Ying and Han (2011) used three methods of maceration, microwave and ultrasound to extract polysaccharides from the Black Mulbery leaves and reported that among these methods, ultrasound extracted the highest amount of sugar from the Black Mulbery leaves The results of Jacques et al., (2005) contradict the results of this study. They compared the extraction efficiency of quercus leaves with methanol and hexane by ultrasonication and maceration and found that there was no significant difference in extraction efficiency. The results of the research showed that higher phenolic compounds in the extract correlated with its high antioxidant capacity (Do Thi and Hwang 2014).

Increasing the extraction efficiency is probably due to increased water penetration and an increase in the osmosis pressure, which increases the ability to dissolve the phenol, resulting in a higher phenol content. This result contradicts the results of the research conducted by Nirmal et al., (2012). They extracted higher TPC from the extracts of *Solanum nigrum L.* by ethanol than water and petroleum ether. Regarding water as solvent, some studies have shown that water has a higher ability than many other solvents in extraction of phenolic compounds (Vilkhua et al., 2008). The process of extraction of phenolic compounds is an important factor in determining the antioxidant properties of the extract and the extraction time has a significant effect on the extract contents (Xia et al., 2006). The most common factors affecting extraction are characteristics of plant matrix, solvent, temperature, pressure and time (Yurena et al., 2009).

The positive effect of increasing time on the maceration efficiency is justified. Generally, it was concluded that maceration has the ability to achieve the highest TPC in the extract. On the contrary, this conclusion contrasts with the research conducted by Nirmal et al., (2012). It should be noted that different results in analyzing the bioactive compounds of a particular plant can be influenced by different parameters. The type and amount of essential oil or extracts are affected by many parameters such as polyphenols contents (Wang et al., 2002). Often, there is a direct relationship between phenolic and flavonoid contents and the antioxidant properties of these species. Therefore, the quantitative measurement of these compounds is an appropriate way to estimate their antioxidant capacity.

The TFC obtained from the extraction of herbal plants is expressed as rutin or quercetin equivalent in mg/g of the extract Because flavonoids have antioxidant activity, their high levels in the extract indicate that the extract will have high antioxidant activity (Lotito and Frei 2004). The hydroalcoholic extract of Soraneh had the highest TPC. The lowest total phenolic content was found for the ethanolic extract of Pichkeh (Table 4). Stankovic et al., (2011) investigated aqueous and methanolic extracts of different parts of *Tecurium montanum* in terms of phenolic and flavonoid content and reported that the phenolic and flavonoid content of aqueous extract was more than methanolic extract.

The antioxidant capacity of a compound can be measured by the ability of the compound to intercept free radicals by scavenging methods (Huang et al., 2005). Free radicals and reactive oxygen species cause molecules oxidation such as proteins, amino acids, lipids, and nucleic acids, which result in extensive damage to the cell and it's death (Bektas et al., 2005). The antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of samples (Sakanaka et al., 2005).

According to Table 6, hydroalcoholic extract of Pichkeh had the highest radical-scavenging

activity and, the lowest radical scavenging activity belonged to aqueous extract of Soraneh. Peterson et al., (2001) measured antioxidant activity of oat by DPPH and beta-carotene staining methods and determined the TPC of the extract. The researchers found that there was a good correlation between the phenolic compounds and the antioxidant activity.Studies show that high phenolic compounds are the major reason of high antioxidant activity of some extracts, including polar extract as evidenced, and there is a positive relationship between the phenolic compounds content and antioxidant activity of the plants. On the other hand, it seems that plant phenolic compounds with high antioxidant activity can be more extracted through their plant extracts (Chatchawan et al., 2008; Candan et al., 2003).

Alu'datt et al. (2017) reported a positive correlation (r = 0.69) between the content of phenolic compounds and antioxidant activity in *Rosmarinus officinalis* L., while Zhang et al. (2018) reported a positive correlation in methanolic extracts from S. miltiorrhiza. Ru et al. (2019) demonstrated that in the free fraction, the content of each individual phenolic acid (in yellow, white, red and purple fleshed potatoes) was positively correlated with antioxidant activity. Cai et al. (2004) stated that all analysed medicinal herbs exhibited far stronger antioxidant activity and contained significantly higher levels of phenolics than common vegetables and fruits. Peterson et al., (2001) investigated the methanolic extract of some native plants of Mazandaran province in terms of phenolics and flavonoids contents and reported that there is a direct relationship between antioxidant activity and plant polyphenolic compounds. The strong correlations between the results using the two methods of measuring antioxidant capacity and the total phenolic content showed that phenol compounds largely contribute to the antioxidant activities of these plants, and therefore could play an important role in the beneficial effects of these plants. The results were in accordance with other researches (Wong et al., 2006).

Antimicrobial compounds in medicinal herbs are commonly attributed to phenolic components with hydroxyl groups including thymol, carvacrol, carnosol, rutin. Apigenin, terpenoid, and eugenol. Hydroxyl group attaches to active site of enzymes preventing their metabolism (Crocoll et al, 2010). The development of microbial resistance to available antibiotics led the study for new and modern antimicrobial agents (Parekh et al., 2006). Antimicrobial susceptibility test is one of the most important techniques in modern biology. This test is used in pathology to determine the resistance of specific microbial strains to various antimicrobial agents. In pharmacological researchs, it is used to determine the efficacy of antimicrobial agents. In various studies, the antibacterial properties of edible medicinal plants such as garlic, onion, ziziphora, mint, yarrow, watercress and barberry have been reported. These herbal medicines have a long history in traditional medicine in many countries including Iran (Guinoiseau et al., 2010). The antibacterial activity of barberry, galbanum, thymus, onion and kiwifruit extract against *E. coli*, *B. subtilis*, *S. typhi*, *P. aeruginosa*, and *S.aureus* have been studied which mainly attribute these properties to terpenoids, flavonoids, mono-terpenes, alkaloids and some volatile sulfur compounds with a strong odor (Nelson and Regiland, 2007).

In the agar diffusion disc method, the highest and lowest antibacterial effect belonged to *L. monocytogenes* and *P.aeruginosa* respectively. *A. saralicum* extract showed the highest antibacterial effect on *L.monocytogenes*, which was not statistically significant from *Sh.sonnei* (P>0.05). Evaluating the antimicrobial performance of methanolic, aqueous and ethanolic extracts of *Hibiscus sabdariffa L.*, Azizian Shermeh et al., (2017) found that methanolic extract showed the highest antibacterial activity against *S.aureus* (27 ± 0.001mm diameter growth zone). In another report on methanolic, ethanolic and aqueous

extracts of different parts of *W. somnifera* on *B. subtilis*, *S. aureus* and *E. coli*, methanolic and ethanolic extracts showed the most inhibitory effect on *S. aureus* with a growth zone diameter of 22.4 mm, respectively, whereas the aqueous extract showed a relatively moderate effect with a 20.5 % growth zone diameter (Kaur et al., 2015). In a similar study on the antimicrobial effect of acetonic, methanolic, ethanolic and chloroform extracts of different parts of *W. somnifera* on pathogens, methanolic and ethanolic extracts had the most inhibitory effect (Rizwana et al., 2015).

Shan et al., (2009) tested the antibacterial activities of ethanol extracts from five spices and herbs against *L. monocytogenes*, *S. aureus*, and *S. enterica* in raw pork by counting bacterial enumeration. When treated with clove extract, raw pork samples had the fewest colonies of tested bacteria. Cui et al., (2010) tested the antimicrobial activities of 90 plant extracts (water and 99.5% ethanol extracts) against *Clostridium* spp. Clove water extract and found the greatest antimicrobial activity against *Cl.botulinum* in trypticase peptone glucose yeast extract broth (pH 7.0) among all the water extracts, and the MICs of clove extract ranged from 0.1% to 0.2% against *Clostridium* spp. Antimicrobial effects of three extracts (ethyl acetate, acetone, and methanol extracts) of twelve plants were tested on two fungi (*Kluyveromyces marxianus* and *Rhodotorula rubra*) and eight bacteria (*Klebsiella pneumoniae*, *Bacillus megaterium*, *P. aeruginosa*, *S. aureus*, *E. coli*, *Enterobacter cloacae*, *Corynebacterium xerosis*, and *Streptococcus faecalis*) by the disc diffusion method (Keskin and Toroglu, 2011).

Conclusion

The present work has proved that the extracts of leaves of Soraneh and Pichkeh possessed strong antimicrobial and antioxidant properties. This beneficial effect could be attributed to the antioxidant and antimicrobial activities of various compounds. Comparative analysis of different plant parts can be helpful when estimating the benefcial properties of Soraneh and Pichkeh extracts as valuable medicinal raw plant materials to be used for natural antioxidants in phytopharmacy. A more detailed study on the bioactive compound in Soraneh and Pichkeh extracts that contribute to these biological activities as well as their possible mechanism of action are therefore suggested.

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Conflict of interest

Authors declare no conflict of interest.

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