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Chemical Composition and Antibacterial Activity of Wild Rose (Rosa canina L.) Gall Extracts against Gram-Negative Pathogenic Bacteria

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Abstract

Background & Objectives: Treating infections due to antibiotic-resistant bacteria is a challenge and researchers are looking for new antimicrobial compounds. Galls are abnormal plant growths caused by biological agents and have active compounds. The present study was designed to examine the antibacterial effects of different extracts of wild rose gall (Rosa canina L.) against some pathogenic Gram-negative bacilli.

Materials & Methods: Methanol, acetone, and aqueous extracts from galls were prepared using Soxhlet apparatus. The antibacterial activity of the extracts was determined by agar well diffusion method, and the minimum inhibitory concentration and the minimum bactericidal concentration were assessed by the microdilution method. The phytochemical composition of galls was investigated by Gas Chromatography-Mass Spectrometry (GC-MS) method.

Results: The inhibition zones of 500 mg/mL methanol extract of wild rose gall against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Acinetobacter bummani*, *Shigella sonei*, and *Salmonella typhi* were 26.7, 25.0, 25.7, 25.7, 26.7, 23.7 and 18.3, respectively. The antimicrobial activity of the extracts was directly related to their concentration. The extracts were active against all the pathogenic bacteria with average MICs ranging from 15.6 to 31.3 mg/mL. The methanol extract of wild rose gall showed the highest bactericidal effect on P. aeruginosa and A. bummani at 62.5 mg/mL, respectively. Moreover, oleic acid, palmitic acid, and octadecenoic acid were composed 36.66%, 14.40%, and 13.21% of total active compounds in wild rose gall.

Conclusion: All of the wild rose gall extracts showed significant antibacterial activities against Gram-negative bacilli. The antibacterial effects may be related to the high amounts of organic acids in wild rose gall extracts.

Keywords: Anti-bacterial compound, Phytochemicals, Plant extracts, Rosa

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Introduction

The increased resistance of pathogenic bacteria to conventional antibiotics as well as the side effects of synthetic chemical medicines have brought about a trend to find effective natural compounds against the infections caused by emerging antibiotic-resistant bacteria (1-3). Traditional unexplored herbal sources have largely been used for the development of effective new drugs for chemotherapy against antibiotic resistant bacterial infections. In addition to their constitutive effective compounds, which are accompanied by other therapeutic materials, medicinal plants have some advantageous antimicrobial effects.

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The effective plant compounds are not accumulated in the human body and their side effects are lower than many antibiotics (4, 5).

Galls are clumsy structures in some parts of plants. A gall is created by an unnatural growth of plant tissues in a response to the presence of an external creature. Galls are induced by viruses, bacteria, fungi, nematodes, and arthropods (6). Galls feed and protect their guest insect against physical damage and improper environmental situations. Many studies have indicated that the plant galls are valuable treating agents because they have many effective active compounds including tannins and antibacterial organic acids. High tannin concentrations are found in the galls of different plants. Tannins are widely used in different industries including leather, textile and mineral separation industries (7-9).

Wild Rose (Rosa canina L.) which is originated from the Rosacea family creates galls on its stem and leaves. The wild rose galls are created due to the gall-maker wasp (cynipidae) activities, especially the sexual activities of the Diplolepis mayri (10). The rose galls are attractive resources of effective compounds and, therefore, are economically valuable (8, 11). Many studies have been carried out on the antimicrobial activity of oak galls. Ardestani et al. (2019) reported the activity of ethanol extract of Quercus infectoria gall against pathogens with MIC and MBC in the range between 0.125 mg/mL and 16 mg/mL. The most inhibitory and bactericidal activity was observed against *Streptococcus agalactiae* and *Staphylococcus aureus*. The ethanolic extract of Quercus infectoria gall inhibited Trichomonas vaginalis at 800 µg/ml after 24 h (12). Zibaei et al. (2021) revealed that the extract of Ghalghaf Gall has an antimicrobial effect on the gram-positive bacterium, *Staphylococcus aureus* compared to the gram-negative bacterium, *Escherichia coli* (13). Unfortunately, not many studies are available on the antibacterial effects of rose galls. We found in our previous study that the rose methanol extract of this gall has the highest antibacterial effect. The MIC and MBC of methanol extract against *Staphylococcus aureus* and *Enterococcus faecalis* were 62.5, 31.3 mg/mL, respectively (14). Most galls are rich in active ingredients such as flavonoids, tannins, alkaloids, phenolic compounds, anthocyanins, and triterpenes. Over the years, humans have learned to use the galls to treat infectious and non-infectious diseases (9).

The present study aimed to investigate the antibacterial effects of methanol, acetone and aqueous extracts of wild rose galls against numerous Gram-negative bacilli and the evaluation of the extract's composition.

Material & Methods

Collection of the Rosa canina L.galls

Genus of Rosa canina L. identified by Dr. Majid Tavakoli of Agricultural Research, Education and Extension Organization, Khorramabad, Iran and with herbarium code: 00001331 was preserved in the Agricultural & Natural Resources Research Center Collection, Khorramabad, Iran.



Figure 1. The wild rose galls

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The Rosa canina L. galls were collected from meadow and forest areas in the slopes of Zagros Mountains (Iran) in summer period of 2020. The galls were verified and identified in terms of their shapes and type of the wasp that had created them (Figure 1). Galls were initially disinfected in a 10% Sodium hypochlorite solution for 20 min and then, they were washed by sterile distilled water 3-4 times in order to remove the hypocrite residues. Finally, the galls were dried and subsequently powdered in sterile conditions (15).

Preparation of the rose gall extracts

The extracts of rose galls were prepared using a Soxhlet apparatus. Fifty g of powdered galls of R. canina were extracted with 250 mL of solvent (aqueous, methanol and, acetone) for 5 hr. The extracts were then dried using a rotary evaporator in sterile conditions and stored at 4 ºC (13, 16).

Bacterial strains

Antibacterial effects of the wild rose galls extracts were tested against standard strains of Gram-negative bacilli including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 9997), *Acinetobacter baummani* (ATCC 23055), *Shigella sonnei* (ATCC 9290), and *Salmonella typhi* (PTCC 1609). All strains were obtained from the microbial collection of the Iranian Research Organization for Science and Technology (IROST).

Antibacterial activity screening

Agar well diffusion method was employed to determine the antibacterial activity of the rose gall extracts. For this purpose, a suspension of each bacterial strain (1.5×10^8 cfu/mL) was uniformly spread on the surface of a Muller Hinton agar (MHA) medium, using a sterile cotton swab; then wells with 6 mm diameter were cut in the medium. Then, six concentrations of each extract (31.25, 62.5, 125, 250, 500 and 15.6 mg/mL) were prepared, and 100 µL of each concentration was poured into its relevant well. After incubation for 18 h at 37 ºC, all the media were examined for any zone of growth inhibition. The diameter of zones was measured in millimeters and the mean diameters were calculated for each experiment in triplicate. The antibiotic imipenem was used as a positive control and sterile distilled water was used as a negative control (17).

Determination of MICs and MBCs

A broth microdilution susceptibility assay was used to determine the MIC of rose galls. For this purpose, serial dilutions of the extracts, ranging from 15.6 to 500 mg/mL, were prepared in the wells of a 96-well microtiter plate for each tested bacterium. Then, each well was inoculated with 100 µL of bacterial suspension (1.5×10^6 cfu/mL) and then the plate was incubated for 18 h at 37 ºC. After that, the lowest concentration of extract in which opacity was not observed, was detected as the MIC level. In order to determine the MBCs, 20 µLfrom each test well which was determined for MIC was streaked on the surface of a MHA medium. The media were incubated at 37 ºC for 24 h and then the lowest concentration that yielded no single bacterial colony on the medium was regarded as MBC (18, 19).

GC-MS analysis of the wild rose galls

The composition of the wild rose galls was determined using Gas chromatography-mass spectroscopy (GC-MS) method. The device was equipped by an Agilent 5975C mass detector with the electron ionization source (EI) coupled to an Agilent 7890 gas chromatography device that was comprised of a HP-SMS. The thirty-meter length column with 0.25 mm inner diameter and 0.25 µm film thickness was used. The temperatures of the inlet ionization, source of mass detector, the analyzer (quadruple), and the GC/MS interface were regulated at 280 ºC, 150 ºC, 230 ºC and 280 ºC respectively.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using SPSS software version 20. Mean comparisons

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were done by ANOVA and the comparisons between groups were done by Kruskal-Wallis and Mann-Whitney tests. A difference was considered statistically significant when $p < 0.05$.

Results

The results of methanol, acetone, and aqueous wild rose gall extracts on the Gram-negative bacilli are illustrated in Tables 1-3. The diameters of the growth inhibition zones were ranged from 8 to 27 mm. Based on the result obtained by Kruskal-Wallis test for the comparison of the effects of three extracts in different concentrations, a significant difference was observed between the three extracts in all concentrations ($p<0.05$). In more detail, in the concentrations of 62.5-500 mg/mL, the inhibition zones obtained by using the aqueous extract were significantly lower than those of the acetone extract, and the inhibition zones obtained by using the acetone extract were significantly lower than those resulted using the methanol extract. However, there was no significant difference between the diameter of inhibition zones obtained by using the aqueous extract and those observed by using the acetone extract. In all experiments, the diameters of the inhibition zones were significantly decreased by reducing the extracts concentrations ($p<0.05$).

Table 1. The growth inhibition zones (mm) by the wild rose gall methanol extract against Gram-negative bacilli

Bacterial strain	Concentrations of the Methanol Extract (mg/mL)						Imipenem (10 µg/mL)
	500	250	125	62.5	31.3	15.6	
Escherichia coli	26.7 ± 2.9^{a}	23.7 ± 1.2^{b}	22.7 ± 2.5^{b}	22.0 ± 1.7^{b}	20.0 ± 0.0^{c}	18.0 ± 1.0^{d}	35 ± 0.7^{ab}
Enterobacter aerogenes	25.7 ± 1.2^{a}	22.0 ± 1.7^{c}	20.7 ± 2.5^{c}	19.3 ± 1.2^{d}	18.7 ± 1.2^{d}	16.3 ± 1.2^{e}	27 ± 0.2^{ac}
Klebsiella pneumoniae	25.7 ± 1.2^{a}	22.3 ± 2.5^{b}	21.3 ± 1.5^{c}	18.3 ± 1.5^{d}	17.3 ± 2.5^{e}	15.3 ± 1.5^{e}	28 ± 0.5^{ac}
Salmonella typhi	18.3 ± 1.5^{d}	16.0 ± 1.0^{e}	14.0 ± 1.7^{f}	14.7 ± 2.5^{f}	11.3 ± 1.2^{g}	8.7 ± 1.2^{h}	30 ± 0.8^{ad}
Shigella sonnei	23.7 ± 1.2^{b}	18.3 ± 2.9^{d}	16.0 ± 3.6^{e}	15.7 ± 1.2^{e}	12.7 ± 0.6^{g}	9.3 ± 0.6^{h}	27 ± 0.4^{ac}
Pseudomonas aeruginosa	25.0 ± 0.0^{a}	22.3 ± 2.5^{c}	22.3 ± 2.5^{c}	19.3 ± 1.2^{d}	16.0 ± 3.5^{e}	11.0 ± 1.7^{g}	26 ± 1.3^{a}
Acinteobacter baumanii	26.7 ± 2.9^{a}	23.7 ± 1.2^{b}	22.7 ± 0.6^{c}	21.0 ± 1.7^{c}	19.3 ± 1.2^{d}	16.0 ± 1.7^{e}	30 ± 1.1^{ad}

Values are mean±SD

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Table 2. The growth inhibition zones (mm) by the wild rose gall acetone extract against Gram-negative bacilli

Bacterial strain	Concentrations of the Acetone Extract (mg/mL)						Imipenem (10 µg/mL)
	500	250	125	62.5	31.3	15.6	
Escherichia coli	24.7 ± 0.6^{b}	22.3 ± 1.2^{c}	18.3 ± 0.6^{d}	14.7 ± 0.6^{f}	10.3 ± 1.5^{g}	7.7 ± 0.6^{k}	35 ± 0.4^{ab}
Enterobacter aerogenes	24.3 ± 1.2^{b}	20.3 ± 2.5^{c}	16.0 ± 1.7^{e}	15.00 ± 0.0^{f}	11.7 ± 0.6^{g}	7.3 ± 2.0^{k}	27 ± 0.6^{ac}
Klebsiella pneumoniae	22.0 ± 3.6^{c}	16.7 ± 2.9^{e}	14.3 ± 1.2^{f}	12.3 ± 0.6^{g}	9.3 ± 1.2^{h}	11.3 ± 1.2^{g}	28 ± 1.1^{ac}
Salmonella typhi	20.0 ± 0.0^{c}	18.3 ± 1.5^{d}	15.7 ± 1.2^{e}	15.0 ± 0.0^{f}	10.3 ± 0.6^{g}	11.3 ± 1.2^{g}	30 ± 0.9^{ad}
Shigella sonnei	23.3 ± 1.5^{b}	20.0 ± 0.0^{c}	17.0 ± 1.0^{e}	13.3 ± 0.6^{g}	10.3 ± 2.1^{g}	10.7 ± 1.2^{g}	27 ± 0.5^{ac}
Pseudomonas aeruginosa	20.0 ± 0.0^{c}	17.0 ± 1.7^{e}	16.7 ± 1.5^{e}	12.0 ± 1.0^{g}	9.0 ± 2.7^{h}	10.7 ± 1.2^{g}	26 ± 0.5^{a}
Acinteobacter baumanii	22.7 ± 0.6^{b}	19.3 ± 1.2^{d}	15.7 ± 1.2^{e}	12.7 ± 1.2^{g}	9.3 ± 1.2^{h}	11.0 ± 1.7^{g}	30 ± 0.5^{ad}

Values are mean±SD

Table 3. The growth inhibition zones (mm) by the wild rose gall aqueous extract against Gram-negative bacilli

Bacterial strain	Concentrations of the Acetone Extract (mg/mL)						Imipenem (10 µg/mL)
	500	250	125	62.5	31.3	15.6	
Escherichia coli	20.7 ± 1.2^{c}	15.0 ± 0.0^{f}	16.3 ± 1.2^{e}	11.7 ± 1.5^{g}	10.7 ± 1.6^{g}	7.3 ± 2.6^{k}	35 ± 0.5^{ab}
Enterobacter aerogenes	21.0 ± 1.7^{c}	19.3 ± 1.2^{d}	16.0 ± 1.7^{e}	14.0 ± 1.7^{f}	7. 7 ± 2.6^{k}	-	27 ± 0.3^{ac}
Klebsiella pneumoniae	21.0 ± 1.7^{c}	19.3 ± 1.2^{d}	18.3 ± 1.5^{d}	14.0 ± 1.7^{f}	8.7 ± 1.2^{h}	7.0 ± 0.0^{k}	28 ± 0.4^{ac}
Salmonella typhi	18.3 ± 2.9^{d}	14.0 ± 1.7^{f}	12.0 ± 0.0^{g}	11.3 ± 1.2^{g}	9.3 ± 1.2^{h}	7.0 ± 2.4^{k}	30 ± 0.2^{ad}
Shigella sonnei	22.0 ± 1.7^{c}	18.3 ± 2.9^{d}	15. 7 ± 2.0^{e}	13.0 ± 1.7^{g}	10.0 ± 0.0^{h}	7.0 ± 0.0^{k}	27 ± 0.6^{ac}
Pseudomonas aeruginosa	20.0 ± 0.0^{c}	16.0 ± 1.7^{e}	13.0 ± 1.7^{g}	12.3 ± 0.6^{g}	9.3 ± 1.2^{h}	7.0 ± 0.0^{k}	20 ± 1.0^{c}
Acinteobacter baumanii	22.0 ± 1.7^{c}	17.7 ± 2.5^{d}	14.3 ± 1.2^{f}	10.7 ± 1.2^{g}	7.7 ± 0.6^{k}	7.0 ± 0.0^{k}	30 ± 1.0^{ad}

Values are mean±SD

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The highest diameter of growth inhibition zone was observed in the same concentrations in methanolic extract, followed by acetone and aqueous extract. The antimicrobial activity of the extracts and the diameter of the growth inhibition zone were directly related to the decrease in the concentration of the extracts. The highest growth inhibition zone was observed in methanolic extract on E. coli and A. baumannii (26.7 mm).

The MIC and MBC values obtained against each tested bacterium using the wild rose gall methanol, acetone and aqueous extracts are shown in the Table 4.

Table 4. The MIC and MBC values (mg/mL) of wild rose gall methanol, acetone and aqueous extracts

Bacteria	Methanol Extract		Aqueous Extract		Acetone Extract	
	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	31.3	62.5	15.6	31.3	15.6	31.3
Enterobacter aerogenes	15.6	31.3	31.3	62.5	15.6	31.3
Klebsiella pneumoniae	15.6	62.5	31.3	62.5	15.6	31.3
Salmonella typhi	31.3	62.5	31.3	62.5	15.6	62.5
Shigella sonnei	31.3	62.5	31.3	62.5	15.6	62.5
Pseudomonas aeruginosa	31.3	62.5	15.6	31.3	15.6	31.3
Acinetobacter baumanii	15.6	62.5	15.6	31.3	15.6	31.3

The results from the detection of wild rose gall composition are presented in Table 5.

Oleic acid (36.66%) and palmitic acid (14.40%) constitute more than a third of the wild rose gall components.

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Table 5. The compounds detected in wild rose gall by GC-MS

Compounds detected	Compound (%)	Retention time	Indices
Oleic Acid	36.66	(min)	Kovat,s
Palmitic acid	14.40	32.722	2162
Octadecenoic acid , Red oil	13.21	31.680	2071
Benzenetriol	2.46	32.824	2195
Valeric acid	2.21	25.103	-
Methyl palmitate	2.09	6.553	881.7
Pyrogallol	1.92	31.339	1931
Valeric Acid	1.63	23.934	-
Stearic acid	0.59	6.436	877.1

Discussion

The results of agar well diffusion experiments obtained in the present study for detection of the antibacterial effects revealed that the methanol, acetone and aqueous extracts of wild rose galls had antibacterial activities against gram-negative bacilli. Results showed that the diameters of growth inhibition zones increased by increasing the concentration of the extracts and a significant difference was observed between the diameter of inhibition zone and different extract concentrations ($p < 0.05$). In this study, the inhibition zones of the methanol extract in all concentrations were significantly higher than those of aqueous and acetone extracts with the same concentrations. Farzaei et al. (2014) examined the antibacterial effect of plant extracts and reported that methanol 85% had more capability for extracting the active compounds from the plants compared to other solvents. Selecting the appropriate method and using suitable solvents are important factors affecting the biological effectiveness of plant extracts. Plant extracts are usually dissolved in organic solvents such as ethanol, methanol, acetone, and hexane. The extracts obtained by these solvents have been more effective than aqueous extracts (20). Basri et al. extracted oak (Quercus infectoria) gall antibacterial compound by using acetone and methanol as solvents.

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The extraction resulted in the release of compounds such as tannin, tannic acid, and gallic acid while in the present study, aqueous extract was also effective and had antibacterial activity (15). Jahaniyan-Najafabadi (2013) examined the antibacterial characteristics of Andricus mayri and Andricus moreae galls of oak on some prevalent infectious bacteria. They concluded that the type of the extracting solvent, the type of the gall, the method of extraction, and the utilized extract concentration had affected the results obtained. Therefore, their results were not comparable to the results obtained via using other plants galls (21). However, the common point from all related research studies has been the antibacterial characteristics of oak galls because of its tannin content. Tannins are phenolic compounds with the antibacterial effects, which have been detected along with other effective compounds such as organic acids in different galls (22, 23). The antibacterial characteristics of the various extracts of the oak galls can be related to the existence of tannins in them. They proved that there are many types of tannin in various plant extracts which can be extracted (24, 25). Although there was no tannin detected in the rose gall in the present study, the chemical analysis by GC/MS revealed that there was a vast spectrum of the organic acids such as oleic acid and palmitic acid. The high amounts of these organic acids can be a reason for the antibacterial effects of the wild rose gall extracts. Studies have shown that fatty acids can kill gram-negative bacteria by interfering with and altering the structure of the cytoplasmic membrane and outer membrane of gram-negative bacteria (26). Oleic acid is capable of preventing growth of various ocular pathogenic gram-negative and gram-positive bacteria (27).

There are few available studies about the identification of effective compounds and antibacterial effect of the wild rose gall and therefore there is no published information about it. Baseri et al. studied the antibacterial effects of aqueous and acetone extracts of the oak galls produced by the gall maker wasp from the Cynips tinctoria against *S. aureus*, *S. epidermis*, *B. subtilis*, *S. typhimurium*, *E. coli*, and *P. aeruginosa*. The MICs of the acetone and aqueous oak gall extracts were similar and equal to 0.078 mg/mL. In the present study, the MICs of acetone and aqueous extracts of the wild rose gall on *E. coli* and *P. aeruginosa* were also obtained similar and equal to 31.3 mg/mL. The MIC values of acetone and aqueous extracts on *S. typhi* were 31.3, and 15.6 mg/mL, respectively. The results of Zarei et al.'s study also demonstrated that the antibacterial activities of the methanol and ethanol extracts of the oak gall were highest among the other extracts. They examined antibacterial effects of extracts against *P. aeruginosa*, *A. baummani*, *E. coli*, *S. sonnei* and *Klebsiella pneumonia*. The MIC and MBC values by the methanol and ethanol extracts of the oak gall were reported to be 12.5 mg/ml and 25 mg/mL, respectively (28). The obtained value of MIC by the wild rose gall ranged from 15.6 to 31.3 mg/mL, which is close to the MIC and MBC values of oak galls. The probable difference was due to the type of the gall and the existing active compounds in them. The results of statistical analysis revealed that in the majority of cases, there is a direct relationship between the diameters of inhibition zones and the galls extract concentration. This trend shows that galls have specific antibacterial effects, which increases when the concentration of their effective material has been increased. The antibacterial effect of methanol extract of the wild rose gall in the concentration of 500 mg/mL was similar to that of the antibiotic, Imipenem used in the present study. The methanol, acetone and aqueous extracts of the wild rose gall were effective on gram-negative bacilli and had similar antibacterial activity. The results of this study differ in some respects from other studies. These differences may be due to different geolocation of galls, type, and species of the wasps that induced the gall production and various extracting methods.

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Conclusion

It was concluded that methanol, acetone and aqueous extracts of the wild rose gall have significant antibacterial activities against pathogenic gram-negative bacilli. The antimicrobial activity of methanol and acetone extracts of wild rose was comparable to that of imipenem. The constituent compounds of this type of gall were detected in this study for the first time. Oleic acid, palmitic acid, and octadecanoic acid were the main constituents of wild rose.

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Conflict of interest

The authors declared no conflict of interest.

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