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Evaluating the Effect of *Gontscharovia popovii* Essence and Extract on Eight Bacterial Strains and Comparison of the Antimicrobial Effect with Some Antibiotics

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The position of traditional medicine in our country and availability of a rich source of plants in Iran on the one hand, and the problems present in treatment of infections resulted from antibiotic-resistant microbes on the other hand, prompts more careful investigation of medicinal plants. Thyme is a plant, belonging to *Labiatae* family. *Gontscharovia Popovii* is a type of thyme found in southern regions of Iran. The aim of the study is to evaluate the effect of the essence and extract of *Gontscharovia Popovii* on eight bacterial strains. In this study, *Gontscharovia Popovii* was collected from the southern regions of Iran, and confirmed by the Herbarium Department, Iranian Institute of Medicinal Plants, Jahad Daneshgahi. The plant extract was prepared using percolation method, and the essence was obtained by hydrodistillation using Clevenger apparatus. Qualitative and quantitative analysis of chemical compounds of *Gontscharovia Popovii* was performed using GC/Mass chromatography. Then, the effects of various concentrations of the essence and extract of the plant on the bacterial species were evaluated and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured. The results of evaluation of the essence indicated that there are 49 compounds in the plant essence, among which thymol is the major one. Moreover, it was demonstrated that the essence and extract of *Gontscharovia Popovii* have a high anti-bacterial potential, and inhibits the growth of the bacteria studied. However, among the strains studied, *Pseudomonas aeruginosa* had the highest resistance against the essence and extract; such that the MIC of *Gontscharovia Popovii* essence and extract for *Pseudomonas aeruginosa* was 2 and 5 μl, respectively. This is while lower concentrations were effective on other bacterial strains. Essence and methanol extract of *Gontscharovia Popovii* showed a considerable inhibitory effect on growth of pathogenic bacteria, particularly gram-positive ones.

Key Words: Essence; Extract; *Gontscharovia popovii*; Pathogenic bacteria.

Infectious diseases are among the main causes of mortality throughout the world and each year many people die from the infections. According to statistics, infectious diseases are the second cause of death around the world[1, 2]. The discovery of antibiotics in the 20th century played an important role in treatment of the diseases, and opened new horizons for definite treatment of these

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infections. When antibiotics were first discovered, it was supposed that their use would eradicate different infections. However, soon it was found that some bacteria are naturally resistant against some groups of antibiotics, or have become resistant owing to antibiotic misuse[3,4]. As the number of antibiotic-resistant bacteria increased, various attempts have been carried out to employ the anti-microbial potential of plants. Employment of medicinal plants in treatment of microbial infections was common since long times ago[5,6]. The antimicrobial agents obtained from plants, eliminate the bacteria with mechanisms different from those employed by antibiotics. This is of clinical importance in treatment of infections caused by microbial resistant species[7,8]. Thyme is a perennial plant and is one of the most commonly used medicinal plants. Since the 16th century, it has been introduced as a valuable medicinal plant[9,10]. Essence and extract of the plant has a high disinfectant and antimicrobial potential[11,12]. Thyme has many different strains, with a widespread extension in different regions of the world, particularly in Mediterranean areas and Asia. In the current study, we used *Gontscharovia Popovii*. The plant belongs to *Labiatae* family. It is a herbaceous fragrant plant with hardwood roots, a thick stem, and brown skin. Stem leaves are of 10-18 × 4-10 mm size. The plant is found in Afghanistan, Pakistan, middle Asia (Tajikistan), and southern regions of Iran[13,14].

MATERIALS AND METHODS

Collection and identification of the plant

Gontscharovia Popovii was collected from southern regions of Iran, and the specie was identified and confirmed in the Herbarium Department, Iranian Institute of Medicinal Plants. To dry the plant, its aerial parts were kept in shadow with appropriate ventilation at 25-30 ºC for one week.

Essence preparation and its analysis

First, the dried aerial parts of the plant were ground, and then its essence was extracted using hydrodistillation with Clevenger apparatus. The essence was analyzed using gas chromatography (GC/Mass) (Agilent 6890) equipped with the column dimension of 30 m × 0.25 mm with HP-5MS film of 0.25 µm thickness.

Extract preparation

The extract was prepared using percolation method.

Bacteria studied

Preparation of microbial suspension

First, all bacterial samples were dilutes. Twenty four hours before performing each step of the experiment, using stock culture media, new 24-hour cultures were prepared. To this end, using a sterile swab and aseptic culture medium and next to the flame, samples were obtained from the stock culture. The samples were added to test tubes containing sterile distilled water to prepare the suspensions. In fact, some colonies on the surface of BHI culture medium were transferred to nutrient broth test tubes. We used Mc Farland method to determine the concentration of bacterial suspension.

Evaluation of antimicrobial effect of the essence and extract

The antimicrobial effects of the *Gontscharovia Popovii* extract and essence were evaluated by the disc method. To this end, we used nutrient agar plates. For bacterial culture, a sample of 100 µl was obtained from each microbial suspension and the sample was placed on solid

Table 1. The names and features of bacteria studied

Name	ATCC No.	PTCC	Classification
Staphylococcus aureus	6538	-	G+
Streptococcus pyogenes	-	1447	G+
Pseudomonas aeruginosa	9027	-	G-
Salmonella typhi	-	1639	G-
Shigella flexneri	-	1234	G-
Escherichia coli	8739	-	G-
Bacillus subtilis	12711	-	G+
Proteus mirabilis	49565	-	G-

media prepared before. The process was the same for all strains. Then, discs containing the extract and essence with the diameter of 6 mm were prepared and placed in a small becher, and the becher was covered with aluminum foil. The becher containing the discs was autoclaved at 15 Psi and 121 ºC for 15 minutes. Then, for each strain on each disc 0.05, 0.1, 1, 2, and 5µl of the essence and extract were added. The discs were placed on plates and fixed on the culture media with mild pressure. The plates were incubated at 35-37 ºC for 24 hours, and then the results were evaluated. To this end, the plates were evaluated using colony counter with magnifying lens, and the diameter of no-growth halos were measured in millimeter. To determine the antimicrobial potential of the essence and extract, we used positive control disc containing pre-defined concentrations of antibiotics (gentamycin, penicillin, and ampicillin with the concentration of 10 µg and amoxicillin with concentration of 25 µg).

Determination of MIC and MBC values

Considering the dry weight in each milliliter of the essence and extract, the concentrations obtained as the relative Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were transformed into weight values of the extracts. Then, according to the serial dilution method, weight values corresponding to MIC and MBC of the extract and essence were solved in 1 ml of the Mueller-Hinton broth medium. Similar to what was mentioned before, 5×10^5 bacteria were added to each test tube, and after 24-hour of incubation at 35 ºC, the MIC and MBC values for each strain was determined in µg/ml.

RESULTS

Identification of the compounds present in the essence of *Gontscharovia Popovii*

The results obtained demonstrated that the amount of essence obtained from *G. popovii* was 1% of the dry weight (1 ml essence from 100 g dry plant). In the essence evaluated, 49 compounds (99.76%) were identified, among which thymol (24.43%) was the main one. Other compounds identified are presented in Table 2.

Determination of the zone of inhibition diameter

Evaluation of the zone of inhibition

Table 2. The compounds present in the essence of *Gontscharovia popovii*

Row	Componds' name	Retention index	Percent
1	alpha-Thujene	931	1.53
2	alpha-Pinene	941	5.29
3	alpha-Fenchene	949	0.11
4	Camphene	955	2.41
5	Thuja-2,4(10)-diene	959	0.16
6	beta-Pinene	982	0.89
7	3-Octanone	987	0.55
8	Myrcene	995	2.52
9	3-Octanol	1003	0.15
10	alpha-Phellandrene	1009	0.52
11	delta-3-Carene	1015	0.13
12	alpha-Terpinene	1024	3.07
13	para-Cymene	1036	10.09
14	Limonene	1031	1.34
15	1,8-Cineole	1043	2.73
16	trans-Ocimene	1053	0.56
17	gamma-Terpinene	1071	7.78
18	cis-Sabinene hydrate	1077	2.58
19	Terpinolene	1094	0.56
20	trans-Sabinene hydrate	1105	0.49
21	cis-Verbenol	1150	0.12
22	Camphor	1154	1.43
23	Borneol	1187	11.36
24	Terpinen-4-ol	1206	0.66
25	para-Cymen-8-ol	1217	0.33
26	alpha-Terpineol	1227	0.78
27	Methyl Ether Thymol	1243	2.99
28	Methyl Ether Carvacrol	1251	0.35
29	NI	1295	0.24
30	Thymol	1312	24.43
31	Carvacrol	1335	5.07
32	Eugenol	1375	0.10
33	alpha-Copaene	1392	0.13
34	beta-Bourbonene	1403	0.32
35	trans-Caryophyllene	1441	2.51
36	beta-Gurjunene	1446	0.17
37	Aromadendrene	1458	0.24
38	Clovene	1462	0.15
39	alpha-Humulene	1472	0.14
40	cis-Muurola-4(14),5-diene	1480	0.11
41	Valencene	1491	0.44
42	trans-beta-Guaiene	1512	0.52
43	beta-Bisabolene	1520	0.99
44	gamma-Cadinene	1531	0.35
45	delta-Cadinene	1538	0.68
46	Spathulenol	1599	0.36
47	Caryophyllene oxide	1606	0.89
48	1,10-di-epi-Cubenol	1634	0.20
49	Epi-alpha-Cadinol	1659	0.29
50	alpha-Cadinol	1673	0.16

Table 3. The zone of inhibition diameter in various concentrations of the essence (mm)

Bacteria	0/05µL	0/1µL	0/5µL	1 µL	2 µL	5 µL
Staphylococcus aureus	14	15	16	18		
Streptococcus pyogenes	13	15	17	19		
Pseudomonas aeruginosa	-	-	-	-	8	9
Salmonella typhi	12	14	17	19		
Shigella flexneri	14	16	18	21		
Escherichia coli	12	13	15	18		
Bacillus subtilis	12	14	17	20		
Proteus mirabilis	12	14	16	19		

Table 4. The zone of inhibition diameter in various concentrations of the extract (mm)

Bacteria	0/05µL	0/1µL	0/5µL	1 µL	2 µL	5 µL
Staphylococcus aureus	10	11	13	15	18	23
Streptococcus pyogenes	9	12	14	16	21	26
Pseudomonas aeruginosa	-	-	-	-	-	5
Salmonella typhi	6	9	11	14	16	21
Shigella flexneri	8	11	13	16	19	23
Escherichia coli	5	6	9	11	14	19
Bacillus subtilis	6	8	12	15	17	23
Proteus mirabilis	5	7	10	12	15	20

Table 5. The zone of inhibition diameter of various antibiotics (mm)

Bacteria	Gentamicin	Amoxicillin	Penicillin	Amoxicillin
Staphylococcus aureus	20	31	36	33
Streptococcus pyogenes	16	-	-	-
Pseudomonas aeruginosa	19	-	-	-
Salmonella typhi	18	10	10	20
Shigella flexneri	18	13	10	16
Escherichia coli	18	11	-	14
Bacillus subtilis	23	12	11	9
Proteus mirabilis	15	10	11	18

diameter indicated that gram-positive bacteria are more sensitive to the essence and extract of *Gontscharovia Popovii* compared with gram-negative bacteria. Comparison of the mean zone of inhibition diameter in various concentrations of the extract and essence demonstrated that by increasing the concentration of the essence and extract, the diameter of the zone of inhibition increased. Considering the essence, at the lowest concentration (0.05 µl), *Staphylococcus aureus* showed the highest sensitivity, while as the concentration increased, *Shigella flexneri* was the most sensitive strain. At concentration 2 and 5 µl, for all bacteria except *Pseudomonas aeruginosa*, the zone of inhibition covered all the plate, which showed the high sensitivity of the strains to the *Gontscharovia Popovii* essence. However, the most resistant strain to the essence was *Pseudomonas aeruginosa*. The strain was completely resistant at 1µl and lower concentrations of the essence, and did not show any the zone of inhibition. With regard to the extract of *Gontscharovia Popovii*, as the extract concentration increased, the bacteria sensitivity increased. The most sensitive strain to the extract was *Streptococcus pyogenes*. This is while the most resistant strain was again *Pseudomonas aeruginosa*, that did not have any sensitivity at the extract concentration less than 2 µl, and only at the concentration of 2 µl, a no-growth halo of 5mm diameter was formed. The MIC and MBC values are provided in Tables 6 and 7.

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the essence of *Gontscharovia Popovii* (µg/ml)

Bacteria	MIC	MBC
Staphylococcus aureus	300	600
Streptococcus pyogenes	150	400
Pseudomonas aeruginosa	> 1500	> 2500
Salmonella typhi	350	800
Shigella flexneri	250	800
Escherichia coli	400	900
Bacillus subtilis	325	700
Proteus mirabilis	400	900

Table 7. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract of *Gontscharovia Popovii* (µg/ml)

Bacteria	MIC	MBC
Staphylococcus aureus	725	800
Streptococcus pyogenes	500	800
Pseudomonas aeruginosa	> 2000	> 3000
Salmonella typhi	800	1000
Shigella flexneri	550	1000
Escherichia coli	850	> 1100
Bacillus subtilis	700	900
Proteus mirabilis	850	> 1100

DISCUSSION

Since thousands of years ago, traditional medicine has been used for therapeutic purposes. In this respect, the essences and extracts obtained from medicinal plants have a considerable position. Owing to emergence of drug resistance, higher attention has been recently attracted toward plants as natural sources[15].

In this study, it was demonstrated that the essence and extract of *Gontscharovia Popovii* have a high anti-bacterial potential, and in lower concentrations can inhibit bacterial growth. Moreover, it was observed that *Streptococcus pyogenes* was the most sensitive strain to the essence and extract. The most resistant strain to the extract and essence was *Pseudomonas aeruginosa*; such that the strain was almost resistant to the extract, and only higher concentrations of the essence inhibited the growth of the bacterium. The essence analysis demonstrated that thymol was the main component.

Some studies have been performed on other strains of thyme. Nickavar et al (2005) analyzed the volatile oils obtained from the aerial parts of *Thymus daenensis* subsp. doenensis and *Thymus kotschyanus* using GC and GC/MS. Twenty six components were identified in the essence of *T. daenensis* subsp. daenensis, among which the major ones were thymol (74.7%), *P*-cymene (6.5%), β- caryophyllene (3.8%), and *methyl carvacrol* (3.6%). In the *T. kotschyanus* essence, 31 components were determined, with the major components being thymol (38.6%), carvacrol (33.9%), γ-terpinene (8.2%), and P-cymene (7.3%). Both essences were rich in monoterpene phenolic compounds, particularly thymol and carvacrol (16). In another study, the antibacterial effect of volatile oils of *Thymus pubescens* and *Thymus serpyllum* before and after flowering were evaluated on *E. coli*, *B. subtilis*, and *P. aeruginosa*. The results confirmed the antibacterial effect of the plants, and showed that even at low concentrations; except for *P. aeruginosa*, the essences had a high bactericidal effect on the bacteria evaluated[17]. Alzoreky *et al*. studied the effects of extracts obtained from plants collected from China, Japan, Thailand, and Yemen on *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella infantis*. Using agar dilution method, the MICs of extarcts were determined to be in the range of 165-2640 mg/l. The most sensitive microorganism to the extract of *Thymus serpyllum*, *Azadirachta indica*, *Ruta graveolens*, *Rumex nervosus*, and *Zingiber officinalis* was *B. cereus* with the MIC in the range of 165-660 mg/l[18]. In the study carried out by Sokmen *et al*. (2004), the antimicrobial and antioxidant effects of the essence and methanol extract of *Thymus spathulifolius* were evaluated *in vitro*. Results of antimicrobial tests indicated that the essence of *T. spathulifolius* has a strong inhibitory effect on the growth of microorganisms studied, except on four strains of fungi. However, the methanol extract had a moderate antibacterial effect, with no antifungal and antibacterial effect. Furthermore, the chemical components of the essence were analyzed using GC and GC/MS methods and 28 components were identified in it. The main components were thymol (36.5%), carvacrol (29.8%), P-cymene (10%), and γ-terpinene (6.3%)[19].

CONCLUSION

The results obtained in the study showed that the use of *Gontscharovia Popovii* in treatment of infections caused by pathogenic bacteria is promising.

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