



Ethnopharmacology, Ecological Requirements, Antioxidant and Antimicrobial activities of *Perovskia abrotanoides* Karel. Extract for Vaginal Infections From Semnan Province

Mohammad Ghafourian¹, Masoumeh Mazandarani^{2*}

Abstract

Objectives: *Perovskia abrotanoides* Karel. is an aromatic plant cultivated in North of Iran, which has been used as anti-inflammatory, sedative, and anti-infective. This work intended to determine the ecological requirements, antioxidant and antimicrobial activities of *P. abrotanoides* Karel. extract from Semnan province (North of Iran).

Materials and Methods: Aerial parts of plant in bloom were collected from Tash Mountain (2120 m) in September, 2014. Some ecological requirements and traditional data were obtained from rural people. Ethanol extracts of plant were obtained by maceration method; total flavonoid (TF) and total phenols (TP) were measured by spectrophotometry; antioxidant capacity was measured by TAC, RP, and DPPH; and the antimicrobial activity was studied against some microorganisms using well method and minimum inhibitory concentration (MIC) assay.

Results: Field observations showed that the *P. abrotanoides* Karel. is one of the wild growing herbs in the feet of the mountain in Semnan province (2000-2700 m). This area has dry cold climate type, with annual raining of 280 mm, temperature rate of 15.5 °C, and sandy clay loam soil (Ec=0.9 and pH= 7.1). Plant extract was rich source of TF (84.2±0.4 mg QUE/g) and TP contents (143.4±0.2 mgGAE/g) and had good antioxidant activity (IC₅₀= 15.03±1.2 mg/mL) especially in DPPH method. *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, and *Enterococcus faecalis* were the most sensitive microorganisms with inhibition zones of 34, 32, 28, 21, 19 mm and MIC values of 58, 45, 53, 63, and 85 mg/mL, respectively.

Conclusion: *Perovskia abrotanoides* Karel. has the potential productivity of TP and TF constituents with suitable anti-Candida, antibacterial and antioxidant activities. Therefore it can be used as natural anti-infective to treat many infectious diseases such as vaginal infections.

Keywords: Antioxidant, Antimicrobial, Vaginal infection, Ecology, Ethnopharmacology, *Perovskia abrotanoides* Karel., North of Iran

Introduction

Reactive oxygen species (ROS) can induce many current inflammatory and infectious disorders (atherosclerosis, infection, cancer, arthritis, hypertension, diabetes, Alzheimer and Parkinson diseases). Therefore natural antioxidant and antimicrobial byproducts of medicinal plants are very useful in the prevention and treatment of many infections. Some of these byproducts are classified as antioxidants, anti-fungal, and antibacterial, because they can produce many ranges of secondary metabolites (terpenoids, flavonoid, and phenolic components), and can play a main role in inhibiting and scavenging the free radicals as anti-pathogen, anti-inflammatory and anticancer. Thus, the global trend is toward investigation and screening of natural antioxidants from wild medicinal plants to prevent and treat many current infectious diseases (1).

Aromatic plants like many Lamiaceae species have been used for centuries as the source of valuable natural

antioxidants in vegetable, food, and drugs in traditional medicine. Therefore, many recent researchers have been interested in chemical extraction, antioxidant and their anti-microorganism properties (2-4).

Perovskia abrotanoides Karel. with local name “wisk”, belonging to Lamiaceae family, grows in mountainous regions of Semnan, Golestan, Isfahan, Khorasan, and Mazandran provinces (5), as well as Afghanistan, Pakistan, and Turkmenistan (5,6). The essential oil composition, antifungal, antibacterial, anthelmintic, antinociceptive and antioxidant activities of *P. abrotanoides* have been studied previously (7-10). They have reported phenolic, flavonoid, and terpenoid components (thymol, menthol, carvacrol, γ -terpinene 4-ol, and p-cymene) in essential oil of *perovskia*, which can induce high antioxidant and antimicrobial activities against microorganisms (3,11,12). In this regard, this work intended to determine the ecological requirements, antioxidant and antibacterial activities of *P. abrotanoides* Karel. extracts from Tash mountainous



region in Semnan province.

Ecological Requirements

The main ecological requirements of *P. abrotanoides* Karel. and its ethnopharmacological knowledge were obtained from many field observations, and natural habitats of this plant (Tash village - 2020 m). This region is located in Southeast of Semnan province, in the latitude of 36° 37' 24" to 36° 34' 28" and longitude of 54° 35' 26" to 54° 24' 32" bordering the Alborz area with sandy clay loam soils. Its average height is 600 to 2750 m above sea level in dry cold climate with average rainfall of 305 mm/y and a mean temperature of -2/8°C (January to February) and 17/3°C (July to August).

Plant Material and Extract Preparation

The aerial parts of *P. abrotanoides* Karel. in bloom were collected in September 2013 from Tash Mountain (2020 m). A voucher specimen of plant was identified and preserved (No. HRCMP:129), and deposited at the Herbarium of RCMP (Research Center of Medicinal plants, Islamic Azad University, Gorgan branch, Golestan province, Iran). The aerial parts of plant in bloom was shade-dried, powdered, and stored at 4°C until in vitro studies. One gram of plant parts with 100 mL ethanol 80% were extracted by maceration. Extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained from extracts were evaporated in dry rotary evaporator at 40°C and were stored at 4°C (13).

Chemicals

2,2'-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St., Louis, USA). BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), and methanol were purchased from Merck Co. (Germany).

Antioxidant Activity Tests

Reducing Power Assay

A dried extract (12.5–1000 µg) in 1 mL of the corresponding solvent was combined with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (K₃Fe(CN)₆; 10 g/L). Then the above mixture was incubated at 50°C for 30 minutes. After that, 2.5 mL of trichloroacetic acid (100 g/L) was added and then the mixture was centrifuged at 1650 g for 10 minutes. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (1 g/L), and the samples' absorbance was measured at 700 nm (14).

1,1-diphenyl-2-picryl hydrazyl radical scavenging capacity assay

The ability of the extracts for free radical scavenging was assessed by the method suggested by Arabshahi et al (14). Briefly, 1 mL of a 1 mM methanolic solution of DPPH was mixed with 3 mL of extract solution in

methanol (containing 12.5–1000 µg of dried extract). The mixture was then vortexed vigorously and left for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Total Antioxidant Capacity

Total antioxidant capacity of the extracts was evaluated according to the procedure described by Arabshahi et al (14). A 0.1 mL of sample (plant extract) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated in a thermal block at 95°C for 90 minutes. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (UVmini-1240) against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The total antioxidant activity was expressed as ascorbic acid equivalent. The calibration curve was prepared by mixing ascorbic acid (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) with ethanol (80%) (14).

Bacterial Strains

Ten microbial species (1 yeast and 8 gram-positive and 1 gram-negative bacteria) were analyzed. The bacteria (numbers 1 to 9) were taken from international collections (they were sensitive to the antibiotics listed below). The yeast sample (number 10) and the bacteria (numbers 1 to 9), obtained from the Microbiology Laboratory, Golestan University of Medical Sciences, were: *Shigella dysenteriae* (PTCC1188), *Pseudomonas aeruginosa* (PTCC1430), *Escherichia coli* (PTCC1399), *Staphylococcus aureus* (PTCC1431), *Bacillus cereus* (PTCC1015), *Salmonella typhimurium* (PTCC1596), *Staphylococcus epidermidis* (PTCC1114), *Enterococcus faecalis* (PTCC1393), and *Klebsiella pneumoniae* (PTCC1291), and one fungal isolate was *Candida albicans* (PTCC5027).

Antimicrobial Potential of the Plant Extracts

The bacterial cultures were grown in Brain Heart Infusion liquid medium at 37°C. After 8 hours of growth, each microorganism was inoculated on the surface of Mueller-Hinton agar (Pronadisa, Madrid) plates. Subsequently, filter paper discs (6 mm in diameter) saturated either with plant extract (50 µL) were placed on surface of each inoculated plate. The plates were incubated at 37°C for 24 hours. After this period, their inhibition zone could be observed. Overall, cultured bacteria with halos equal to or greater than 7 mm were considered susceptible to either the tested extract. DMSO (dimethyl sulfoxide) was used to dissolve the extracts in the culture media when necessary.

The controls were the solvents used for each extract and they showed no inhibitions in preliminary studies.

An amount of 0.01 mL of each bacterial suspension, equivalent to McFarland tube No. 0.5 (108 CFU/mL), was inoculated on the agar of every well. The culture plates were then incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which no visible growth was observed (15). The Mueller-Hinton agar containing DMSO without plant extract was used as negative control while gentamycin was used as positive control.

Statistical Analysis

The statistical analyses were carried out in triplicate and given as the mean \pm standard deviation (SD). The data of all experiments were analyzed using Analysis of variance (ANOVA) which was used for comparison of the effectiveness of the anti-bacterial activities. Differences were considered significant at $P < 0.05$.

Results

Results of many field observations showed that in Semnan province, the vegetative growth of *P. abrotanoides* Karel. starts from late of May to early September. It blooms from July to late of August and the ethnopharmacological survey of plant showed that the *P. abrotanoides* Karel. is the most wild aromatic perennial herb, which often grows in inclined and sunny places around Tash grassland mountain (2000-2700 m). This grassland mountain is 70 km far from Shahrud city, with annual raining of 280 mm, annual temperature of 15.5°C in dry cold climate, and sandy clay loam soil, with $E_c=0.9$ and $pH = 7.1$. This plant with local name of “Wisk”, has been used traditionally lonely or in combination with other medicinal herbs (*Thymus carmanicus*, *Artemisia sieberi*, *Mentha longifolia*, and *Ferula gummosa*) as an antispasmodic, sedative, anti-inflammatory, antihelmintic, antifungal and anti-infective to treat rheumatism, migraine, UTI, leishmaniasis, dysmenorrhoea, and vaginal and dermal infections. There are some examples as below:

To treat dermal infection: Preliminary data showed that the ointment which was produced by compounds of some medicinal plants such as *T. carmanicus*, *M. longifolia*, *Onosma dichroanthum*, *Malva neglecta*, and *Hypericum perforatum* as with the extract of *P. abrotanoides* has a strong potential as anti-inflammatory, antifungal, antibacterial, and anti-infective to treat dermal infections and heal wounds such as burned skin.

Sedative and anti-inflammatory: The massage of the oil of *P. abrotanoides*, *Stachys inflata*, *A. sieberi*, and *Capsicum annuum* as anti-spasmodic, anti-inflammatory and sedative is to treat rheumatism, backache, sciatica, and gout.

Vaginal infections: The suppository tampon (the decoction of *P. abrotanoides* with the fruit of *Juniperus sabina*,

M. longifolia, and the fruit of *Cupressus* and *Quercus*) injected to vagina is consumed for the treatment of vaginal infections.

Anti-helmentic: consumption of the drug combined with pomegranate extract, *F. gummosa*, *Artemisia annua*, *P. abrotanoides*, and *Peganum harmala* in morning fasting.

According to (Tables 1 and 2, phytochemical and antioxidant assays showed that the content of total phenols (TP = 143.4 ± 0.2 mg GAE/g) and total flavonoids (TF = 84.2 ± 0.4 mg QUE/g) in aerial parts of *P. abrotanoides* Karel., had good antioxidant activity with $IC_{50} = 15.03 \pm 1.2$ mg/mL, especially in DPPH method, which had lower content of IC_{50} in free radical scavenging.

Inhibition zones (IZ) and MIC values of the ethanol extract of *P. abrotanoides* Karel. in comparison with 2 standard antibiotics (BHT and BHA) were presented in Table 1. The results indicated that the ethanol extract of plant exhibited moderate to high level of antibacterial activity on tested bacteria with IZ (12.4 ± 0.5

$- 34.1 \pm 0.4$ mm), respectively. *C. albicans*, *S. aureus*, *S. epidermidis*, *B. cereus*, *E. faecalis*, and *E. coli* were the most sensitive tested microorganisms with inhibition zones of 34, 32, 28, 21, and 19 mm and MIC values of 58, 45, 53, 63, and 85 mg/mL, respectively, and other bacteria (*P. aeruginosa*, *E. faecalis*, *K. pneumonia*, *Salmonella* and *Shigella*) showed moderate effects against the plant extract, respectively.

Discussion

The resistance of pathogenic fungi, including *C. albicans*

Table 1. Antioxidant Activity of *Perovskia abrotanoides* Karel. in Tash Mountain in Semnan Province (2020 m)

| Antioxidant Activity, IC_{50} (μ g/mL) | Aerial Parts | BHA | BHT |
|---|-----------------|------------------|------------------|
| TAC | 45.3 \pm 0.1 | | |
| RP | 32.3 \pm 0.31 | 3.85 \pm 0.351 | 3.13 \pm 0.404 |
| DPPH | 15.03 \pm 1.2 | | |

Table 2. In Vitro Antimicrobial Activity and MIC Values in Ethanol Extract of *Perovskia abrotanoides* Karel. From Tash Mountainous Region (2020 m), Semnan Province (North of Iran)

| Microorganisms | IZ (Mean \pm SD) | MIC (μ g/mL) | Gentamycin |
|-----------------------------------|--------------------|-------------------|------------|
| <i>Staphylococcus aureus</i> | 32.1 \pm 0.4 | 45.1 | 16.7 |
| <i>Staphylococcus epidermidis</i> | 28.4 \pm 0.3 | 53.2 | 14.7 |
| <i>Bacillus cereus</i> | 21.6 \pm 0.5 | 63.7 | 16.5 |
| <i>Enterococcus faecalis</i> | 19.1 \pm 0.1 | 85.5 | 9.6 |
| <i>Escherichia coli</i> | 17.4 \pm 0.2 | 102.1 | 11 |
| <i>Pseudomonas aeruginosa</i> | 15.2 \pm 0.3 | 124.1 | 9 |
| <i>Klebsiella pneumonia</i> | 12.5 \pm 0.1 | 132.9 | -- |
| <i>Salmonella typhimurium</i> | 12.7 \pm 0.1 | 172 | 11 |
| <i>Shigella dysenteriae</i> | 12.4 \pm 0.5 | 134.3 | 11 |
| <i>Candida albicans</i> | 34.1 \pm 0.4 | 58.3 | 18 |

Abbreviations: IZ, Inhibition zones; MIC, minimum inhibitory concentration.

and other species isolated from patients, against antifungal agents has increased (16). Around 75% of adult women have at least one episode of vaginal infection during their life, with prevalence of *C. albicans* from 70% to 90%). Many traditional medications derived from plant sources including the wild aromatic medicinal plants, especially from mountainous regions, are still being used; therefore, they can be renewable candidates in nature and major sources of new anti-oxidant and anti-pathogenic compounds (1).

According to Tables 1 and 2, it was shown that the content of total phenols (TP= 81.7±0.3 GAE/ g) and total flavonoids (TF= 34.2±0.8 QUE/g) in aerial parts of *P. abrotanoides* Karel. had good antioxidant activity with $IC_{50} = 21.8 \pm 0.1 \mu\text{g/mL}$, especially in DPPH method, which had higher content of IC_{50} in free radical scavenging. The ethanol extract of plant exhibited moderate to high level of antibacterial activity against tested bacteria with IZ (12.1±0.8 – 27.7±0.8 mm) respectively, except for *Shigella dysenteriae*, which is the gram-negative resistant bacterium. But other bacteria (*S. aureus*, *S. epidermidis*, *B. cereus*, and *E. faecalis*) and *Candida albicans* were the most sensitive with inhibition zones of 27, 22, 16, and 15 mm, and MIC values of 60, 68, 53, and 83 mg/mL, respectively.

In many similar researches, it was reported that the natural antioxidants (terpenoides, phenolic compounds, and flavonoids) in many aromatic mountainous plants, which can play a main role in free radical scavenging, positively reduce risk of developing infectious, inflammatory, diabetic, cancer, hypertension, and other current diseases. Therefore, there is a growing interest to identify natural antioxidants in wild aromatic plants with strong radical scavenging activities (1).

In relation to these results, to confirm the traditional uses of these plants in Tash village as anti-inflammatory, antihelminthic, and anti-infective in the treatment of vaginal infections and leishmaniasis, other researches showed that the terpenoides and polyphenols present in the extracts of *P. abrotanoides* Karel., *M. longifolia*, *F. gummosa*, *T. carmanicus*, *A. sieberi*, *A. annua*, *P. harmala*, and *J. sabina* can be used as anti-infectious, anti-inflammatory, antifungal, and sedative (1,7-10). Hence, the current study was conducted on rich secondary metabolites of above-mentioned plants and their antioxidant activity due to their traditional medicinal uses in Golestan and Semnan provinces as sedative, antispasmodic, anti-infectious, and anti-inflammatory in the treatment of many vaginal and dermal infections.

According to the reports, in Iranian traditional medicine, traditional system of medicine in Pakistan, and traditional medicine in Turkey, applying a poultice of *P. abrotanoides*, *A. sieberi*, *J. sabina*, and *M. longifolia* with sesame oil has been attended in the treatment of many dermal wounds and leishmaniasis (8,17). The extracts of Leaves of *P. abrotanoides* with the black fruit of *J.*

sabina and the gums of *F. gummosa* have been used as pain killer, anti-inflammatory and anti-infective to treat UTI and urinary and vaginal infections in Golestan and Mazandaran provinces (1,10).

In similar reports, essential oils rich in phenolic compounds and terpenoides such as carvacrol, γ -terpinene, 1,8-cineole, terpinene 4-ol, α and β -pinene, camphor and thymol were reported to possess high levels of antimicrobial activity. In fact, other constituents such as camphen, 1,8-cineol, γ -terpinene, and terpinene 4-ol have been considered to display relatively good activity due to their possible antagonistic effects, anti-inflammatory, anti-infective, and sedative to treat cold, flu, arthritis, rheumatism, vaginal infection, UTI, and leishmaniasis (12).

In confirmation of our data, many similar researches have shown that the antioxidant and antimicrobial activities of other plant species such as *P. abrotanoides*, *M. longifolia*, *Thymus* spp., *A. annua*, *A. sieberi*, *J. sabina*, *P. harmala*, and *Punica granatum* have been previously studied and many of them have reported that the antimicrobial activity of essential oil and the extracts, relative to their main phenolic components (thymol, menthol, carvacrol, γ -terpinene terpinene 4-ol and p-cymene), has shown a high inhibitory effect against a wide range of microorganisms (3,11,18-20).

Antihelminthic and anti-fungal properties of *P. abrotanoides*, *J. sabina*, *F. gummosa*, and *T. carmanicus* is due to the presence of many phenolic, flavonoid, and terpinene compounds such as thymol, menthol, sabinene, camphor, 1,8-cineole, verbenone, alpha terpineol, terpinolene, and gamma terpinene have been found in many essential oils of these plants as anti-inflammatory, anti-infective, anti-fungal, antioxidant, anti-tumor, antihelminthic, and anti-pathogenic to treat many infectious diseases such as dermal, vaginal, and urinary infections, arthritis muscle spasm and dysmenorrhea (20-26). Therefore, our results in this paper and other researches can confirm the traditional consumption of these medicinal plants in North provinces of Iran (Golestan and Semnan) as anti-spasmodic, anti-inflammatory, antihelminthic, antifungal, and anti-infective to treat UTI, vaginal infection, depression, and stress of arthritis and muscle spasm in pregnant women.

These data indicate that the *P. abrotanoides* Karel. extract and its constituents (terpinen-4-ol, γ -terpinene, carvacrol, verbenone, terpinolene, and sabinene) may be applied as antibacterial, antifungal, and antioxidant agents and moreover as an anticancer agent. Thus the literature has described that the antibacterial and antioxidant activities of *P. abrotanoides* can depend on its terpenoids and flavonoids (terpinen-4-ol, γ -terpinene, and carvacrol) in essential oil or extract (21,23,24,27).

In the report of Tolossa et al (15), the γ -terpinene, thymol, p-cymene, carvacrol, pulegone, isomenthone,

and piperitone oxide were respectively shown as the major constituents of *Satureja thymbra*, *P. abrotanoides*, *T. carmanicus*, *A. sieberi*, and *F. gummosa* which grow in wild mountainous regions and have strong antioxidant and antimicrobial activities against fungi and tested bacteria, respectively (1,15).

Conclusion

Our results in this study, first, can confirm the traditional uses of *P. abrotanoides* Karel. as antispasmodic, sedative, and antinfected in treating rheumatism, vaginal infection, UTI, and dysmenorrhoea and then prove that the ethanol extract of plant like other mentioned *Thymus*, *juniperus*, and *Ferula* species can have great potential of antimicrobial, antifungal and antioxidant to be used as natural antimicrobial and antioxidant drugs and additives in the preservation of processed food and drug industry. These data indicate the possibility that *Perovskia abrotanoides* extracts and its constituents may be applied as antibacterial and antifungal agents and moreover as an antivaginal agent for novel anti-inflammatory and antioxidant activities.

Acknowledgments

The authors are grateful to RCMP (Research Center of Medicinal plants), Islamic Azad University, Gorgan branch. We also thank prof. Ghaemi for his assistance in methodology section and for his comments which greatly improved the manuscript.

Ethical Issues

The local ethics committee approved the study.

Conflict of Interests

None.

Financial Support

Self-funded.

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