

Antioxidant and Antimicrobial activity of *Pedicularis sibthorpii* Boiss. And *Pedicularis wilhelmsiana* Fisch ex.

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ABSTRACT

Purpose: This research paper presents antioxidant and antimicrobial activities of *Pedicularis sibthorpii* and *Pedicularis wilhelmsiana* which grow in Azerbaijan/Iran with claimed a lot of therapeutic effects. **Methods:** DPPH assay and agar well diffusion method were carried out to determine antioxidant and antimicrobial activities respectively. **Results:** Methanolic extract showed better antioxidant activity compared to other crude extracts (n-hexane and dichloromethane). Methanolic extracts of both *Pedicularis sibthorpii* and *Pedicularis wilhelmsiana* were found to have antibacterial activity especially against gram positive strains of *S. ureus*, *S. epidermidis*. No antifungal activity was observed in the tested extracts. **Conclusion:** Existence of some phenolic compounds in methanolic extracts, such as phenylethanoids and flavonoids (found in other species of *Pedicularis*), which cause both antioxidant and antibacterial activities, is probable. Antimicrobial and antioxidant activity of the methanolic extracts supports further studies related to phytochemical investigation and bioassay of different fractions to isolate pure compounds of plants.

Introduction

As a folk medicinal herb *Pedicularis* is one of the most widely used groups of medicinal plants which have different therapeutic effects on cardiac problems, exhaustion, spontaneous sweating and digestion problems.¹ There has been no biological research on *Pedicularis sibthorpii* and *Pedicularis wilhelmsiana* which grow in Azerbaijan/Iran. *Pedicularis* species are also traditionally used in India for some clinical disorders such as cold, cough and fever.² Since these traditional applications can be attributed in part to the antimicrobial and antioxidant activity of the plant, in this study we intended to evaluate experimentally the invitro antibacterial effects of *P. sibthorpii* and *P. wilhelmsiana* against some clinically important species and antioxidant activity of these two plants were evaluated by DPPH test.

Materials and Methods

Plant material and extraction

The arial parts of *Pedicularis sibthorpii* Boiss and *Pedicularis wilhelmsiana* Fisch ex. were collected from Lighvan and Arasbaran region respectively in East Azerbaijan province, Iran in 2009. Voucher specimens for this collection (TBZ FPI 701, TBZ FPI 700) have been deposited in the Herbarium of the Faculty of Pharmacy, Tabriz, Iran.

Arial parts of plant were air dried and powdered. A 200g amount of powder of sample was extracted by n-hexane (8hours), dichloromethane (10hours) and methanol (8hours) in soxhlet extractor. Solvents removed by rotary evaporator and resulting extracts were used for further examinations.

DPPH assay

For determining antioxidant activity of the extracts, bleaching of purple colored methanol solution of 1, 1-diphenylpicryl hydrazyl (DPPH) was measured by spectrophotometric assay. In order to obtain different sample concentrations dilutions series were prepared. 50 µl of each concentration of methanolic extract were added to 5ml of 0.004% methanolic solution of DPPH. After incubation of solutions at room temperature for 30 min, bleaching of DPPH was monitored at 517 nm against a blank. Inhibition of DPPH was calculated as RC_{50} , extrapolated from dose-response curve. Tests were carried out in duplicate.^{3,4}

Antimicrobial assay

Bacterial cultures of gram negative species *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Salmonella paratyphi* (ATCC 4420), as well as gram positive species namely

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Staphylococcus epidermidis (ATCC 12228), *Bacillus cereus* (ATCC 9372), *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240) and a fungi (*Candida albicans*) were used to evaluate antimicrobial properties of the methanolic extract. The bacterial strains in lyophilized form purchased from institute of pasture, Iran. Centrifuged pallets of bacteria from 24 hours cultures were mixed with distilled water, and the turbidity was corrected by adding sterile distilled water until 0.5 McFarland's turbidity standard [10^8 colony forming units (CFUs) per ml] was obtained. Then these inoculums were used for seeding the Muller Hinton agar (MERCK). Autoclaved Muller Hinton agar medium was allowed to cool up. Then it was seeded with 10 ml of prepared inoculums (10^6 CFUs per ml). The antimicrobial activity of tested extract was monitored using agar diffusion method, which is highly recommended method for routine assessment of preliminary antimicrobial screening. Using Muller Hinton plates, inoculated with a 0.5McFarland's standard of selected bacteria, 5 wells for test samples, two for solutions of extract and different fractions, and one for vehicle control (DMSO), were applied to each Petri plate. For incubation and analysis, by micropipette 100 μ l of test solution was poured in respective well. Petri plate was incubated at 37°C. After 24 hours of incubation, diameter of the clear zones, showing no bacterial growth, around each well (excluding well diameter) was measured with the help

of venire calipers. Triplet plates were prepared for each sample.⁵⁻⁷ Compounds that have shown significant antibacterial activity, at this concentration were further assayed for their minimum inhibitory concentration. Serial two-fold dilutions of fractions were prepared in broth. Cultures containing only sterile nutrient broth, which did not influence bacterial growth, were used as controls. To each test tube an equal volume of the adjusted inoculums was added. After incubation at 37 °C for 24 h the MIC was read. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of a fraction which was able to completely inhibit the growth of each bacterial strain.⁸

Results

Results of antioxidant test

Table 1 demonstrated antioxidant activities of n-hexane, dichloromethane and methanolic extracts of *P.sibthorpii* and *P.wilhelmsiana* by DPPH test.

Results of antimicrobial test

The results for antibacterial activity of the methanolic extract as Mean Inhibition Zone diameters (MIZD) as well as the MIC values against susceptible strains have been shown in table 2. N-hexan and dichloromethane esextracts of two plants did not show any antimicrobial activity.

Table 1: antioxidant activities of the extracts of *Pedicularis sibthorpii* and *Pedicularis wilhelmsiana*

Extracts of P.s.*	RC ₅₀ (mg/ml)	Extracts of P.w.*	RC ₅₀ (mg/ml)
hexan	2.098	hexan	1.26
dichloromethane	No activity	dichloromethane	No activity
methanol	0.033	methanol	0.159

*P.s.: *Pedicularis sibthorpii*, *P.w.: *Pedicularis wilhelmsiana*

Table 2: Antibacterial activity of the methanolic extracts as Mean Inhibition Zone Diameter \pm SD (MIZD) and Minimum Inhibitory Concentration (MIC) of the extract against different strains (n=3).

MO*		<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S. paratyphi</i>	<i>S. epidermidis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>M. luteus</i>	<i>Candida albicans</i>
M.E. of P.S*	MIZD \pm SD (mm)	-	11.6 \pm 0.28	-	15.6 \pm 0.57	-	8.5 \pm 0.5	-	-
	MIC (mg/ml)	-	200	-	200	-	200	-	-
M.E. of P.W*	MIZD \pm SD (mm)	-	14.3 \pm 0.47	-	15.6 \pm 0.6	-	8.5 \pm 0.4	15.6 \pm 0.53	-
	MIC (mg/ml)	-	200	-	200	-	200	200	-

MO*: microorganisms, M.E. of P.S*: Methanolic Extract of *Pedicularis sibthorpii*, M.E. of P.W*: Methanolic Extract of *Pedicularis wilhelmsiana*

As it can be seen from table 2, the total methanolic extract of *P.sibthorpii* demonstrated inhibitory activity against some strains (*P. aeruginosa*, *S. epidermidis*, *S. aureus*), and methanolic extract of *P.wilhelmsiana* showed inhibitory activity against *P. aeruginosa*, *S. epidermidis*, *S. Aureus* and *M.luteus*. Methanolic

extracts did not show any inhibitory activity against gram-negative strains (*E.coli*, *S. Paratyphi* and *B.cereus*) except *P.aeruginosa*. Moreover methanolic extracts of *P.sibthorpii* and *P.wilhelmsina* were not active against *C.albicans* as well.

Discussion

The results of DPPH test as it can be seen in table 1 indicate that RC_{50} of methanolic extracts of two plants are lower than n-hexane and dichloromethane extracts, which shows methanolic extracts are potent antioxidants. These results reveals existence of phenolic compounds such as phenylethanoids and flavonoids (found in other species of *Pedicularis*) in both methanolic extracts, which are powerful antioxidants and have good radical scavenging activities.^{9,10} Moreover, the current study found that methanolic extract of *P.sibthorpii* is more potent antioxidant than methanolic extract of *P.wilhelmsiana*. This finding suggests that compounds with antioxidant activity (phenolic compounds) are found more in methanolic extract of *P.sibthorpii* than methanolic extract of *P.wilhelmsiana*.

The results of in vitro antimicrobial assay of methanolic extract of *P.sibthorpii*, shown in table 2, demonstrates that methanolic extract of this plant is active against *P.aeruginosa*, *S.epidermidis* and *S.aureus*. Methanolic extract of *P.sibthorpii* is more active against *S.epidermidis* than other susceptible species. The results of well diffusion assay of methanolic extract of *P.wilhelmsiana* shown in table 2, indicates that the extract is active against *P.aeruginosa*, *S.epidermidis*, *S.aureus* and *M.luteus*. This extract was more active against *S.epidermidis* and *M.luteus* than other strains. None of these two extracts were active against *C.albicans* by well diffusion method. According to results shown in table 2, methanolic extract of *Pedicularis wilhelmsiana* is more potent than methanolic extract of *Pedicularis sibthorpii* against susceptible strains. Furthermore the results of this study is in agreement with previous results which show that, the most susceptible strains, were gram-positive microorganisms (*S.aureus*, *S.epidermidis* and *M.luteus*).^{5,11,12} It could be due to several possible reasons; one is the presence of multilayer structure of membrane surrounding each gram-negative bacteria cell, whereas, gram-positive bacteria consist of a single layer, accounting for why gram-negative bacteria are more resistant to antibiotics than other gram-positive bacteria.¹³⁻¹⁵ Among them *S.epidermidis* which cause serious infections in human and other animals, was more susceptible to both methanolic extracts than other susceptible strains. Contrary to expectations, methanolic extracts of *P.sibthorpii* and *P.wilhelmsiana* were active against *P.aeruginosa* a gram-negative strain and methanolic extract of *P.wilhelmsiana* was more active than methanolic extract of *P.sibthorpii*. Due to multi resistance feature of *P.aeruginosa* finding an effective antimicrobial agent against this microorganism is a difficult task.¹⁶ Insusceptibility of *Candida albicans* to the tested extracts may be Due to lipophylic characteristic of fungi structure such as *C.Albicans*. Methanolic extracts of these two plants containing hydrophilic compounds were not active against this strain.¹⁷⁻¹⁹

According to the findings, it can be concluded that existence of some phenolic compounds in methanolic extracts, such as phenylethanoids and flavonoids (found in other species of *Pedicularis*), which cause both antioxidant and antibacterial activity, is probable. Antibacterial and antioxidant activity of methanolic extracts supports further studies like isolation and purification of phenolic compounds, which will be carried out in further investigations and the relationship between biological activities of the extracts with isolated compounds will be revealed.

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This article was written based on a dataset of PhD thesis, registered with the number of 43 at pharmacy faculty in Tabriz University of Medical Sciences.

Conflict of interest

The authors report no conflicts of interest.

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