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EXPERIMENTAL PAPER

The antimicrobial activity of *Prunella vulgaris* extracts

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Summary

Prunella vulgaris (*Labiatae* family) or self-heal is traditionally used for different ailments such as eye pain and inflammation, headache, dizziness, sore throat and wound healing. Total phenolic and total flavonoid contents of extracts (methanol, ethanol and aqueous) were determined by a spectrophotometer. The antimicrobial activity was evaluated by micro broth dilution assay. The total phenolic content of *P. vulgaris* extracts were higher in aqueous extract (156.5 mg GAC/g) followed by ethanol extract and methanol extract. The TFC content of *P. vulgaris* methanol extract (82.8 mg QE/g) was higher than ethanol extract (22.7 mg QE/g) and aqueous extract (16.2 mg QE/g). The antimicrobial activity of methanol or ethanol extracts was higher than aqueous extract from *P. vulgaris*. The sensitivity of microorganisms to different extracts is related to type of pathogens. There is no positive relation between total phenolic content and its antimicrobial activity. *Prunella vulgaris* ethanolic extract as a source of phenolic and flavonoid contents can be used as an antimicrobial agent.

Key words: *Prunella vulgaris, antimicrobial activity, phenolic, flavonoid, extract*

INTRODUCTION

Prunella vulgaris, a perennial plant from *Labiatae* family, is widely distributed in Asia, Europe and Iran. It is traditionally used for eye pain, inflammation, headache, dizziness, sore throat and wound healing [1]. *P. vulgaris* is known as self-heal; contains several active components, including oleanolic acid, betulinic acid, ursolic acid, flavonoids and rosmarinic acid [2-4]. Some pharmacological activities such as the immunmodulatory effect [5,6], anti-viral activity against HSV-1, HSV-2 [7, 8], HIV [9, 10], antioxidant activity [1, 11] and anti hyperglycemic action [8] were confirmed. In spite of its traditional uses as an antiseptic agent for treatment of wounds and sore throat, there are a few literatures on its antimicrobial activity. The antimicrobial activity of *P. vulgaris* methanol extract against *Staphylococcus aureus* and *Enterococcus faecalis* was confirmed [1]. The *P. vulgaris* were used in oral preparations for control of gingivitis [12]. In China, the aqueous extract from fruit spikes were used in typical dose of 9–15 g per day for different ailments [13].

The aim of this research was to evaluate the total phenolic and total flavonoid contents of *P. vulgaris* methanol, ethanol, aqueous extracts and their antimicrobial activities against different kind of microorganisms as traditional uses.

MATERIALS AND METHODS

Plant materials and extraction

The dried aerial parts of *Prunella vulgaris* were collected from Rasht (Gilan Province, Iran) in July-August 2012 and identified by the Agriculture Department of Barij Essence Center, Kashan, Iran and authenticated under number 162-1.

The aerial sample was grinded and subjected to extraction by a percolation method with water, methanol and ethanol-water (70:30, v/v). The powdered *P. vulgaris* aerial parts were mixed with solvent at the ratio of 1:10 (w/v) in percolator for 24 h at ambient temperature. Then, the mixture was filtered through Whatman filter paper No. 2, the residue rinsed with the same solvent and the extract dried under vacuum.

Total phenolic content

Total phenolic contents (TPC) of the extracts were determined by a spectrophotometer using the Folin-Ciocalteu's reagent [14]. A concentration of 1 mg/ml of each extract was prepared. 0.1 ml of extract and 0.5 ml of Folin-Ciocalteu's reagent (10%) was added. After 3-8 min, 0.4 ml of 7.5% (w/v) sodium carbonate solution was added and mixed. After 1 h at ambient temperature, the absorbance of

reaction mixture was measured at 765 nm. TPC was calculated using the following equation: W= ((Abs-0.0089)/0.0647)×100, where Abs is absorbance and w is the weight (μg). All tests were conducted in triplicate and averaged. The results were expressed as mg of TPC per g of dry extract as gallic acid (GAC).

Total flavonoid content

The modified aluminum chloride colorimetric method was used [15]. 0.5 ml of diluted standard solution and each extract were separately mixed with 1.5 ml of ethanol (95%), 0.1 ml of aluminum chloride (10%), 0.1 ml of potassium acetate (1 M) and 2.8 ml distilled water. After incubation at a room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. The results were expressed as mg of total flavonoid content (TFC) per g of dry extract as quercetin (QE).

Microbial strains and antimicrobial evaluation by microbroth dilution assay

Staphylococcus aureus ATCC 25923, *Staphylococcus epidermidis* ATCC 14490, *Staphylococcus saprophyticus* ATCC 15305, *Enterococcus faecium* ATCC 25778, *Enterococcus faecalis* ATCC 29212, clinical isolate of *Streptococcus agalactiae*, *Streptococcus pyogenes* ATCC 8668, *Streptococcus pneumoniae* ATCC 33400, *Streptococcus mutans* ATCC 35668, *Streptococcus sobrinus* ATCC 27607, *Streptococcus sanguis* ATCC 10556, *Streptococcus salivarius* ATCC 9222, *Bacillus cereus* ATCC 1247, *Bacillus subtilis* ATCC 6051, *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028, *Shigella dysenteriae* PTCC 1188, *Shigella flexeneri* NCTC 8516, *Enterobacter aerogenes* NCTC 10009, *Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* ATCC 13880 and fungi *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 90030, *Aspergillus flavus*, *Aspergillus niger* ATCC 16404, *Aspergillus parasiticus* ATCC 15517, were used. Bacterial suspensions were prepared in Brain Heart Infusion (BHI) broth to a concentration of approximately 10^8 CFU/ml using standard routine spectrophometric methods. Suspensions of fungi were made with Sabouraud dextrose broth (10^6 CFU/ml). Subsequent dilutions were made from the above mentioned suspensions, which were then used in the tests.

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values of the extracts were determined by micro broth dilution assay. The extracts were twofold serially diluted with 10% DMSO which contains 51.2-0.2 mg/ml of each extract. Antibiotics were used as positive control. These dilutions were prepared in a 96-well microtiter plate. MOPS-buffered RPMI 1640 (fungi), cation adjusted Mueller-Hinton broth (non fastidious bacteria) and Todd Hewitt broth (fastidious bacteria) were used as broth media. After shaking, 100 μl of extract dilutions was added to each well. The above mentioned microbial

suspensions were diluted (1×10^6 CFU/ml for bacteria; 10^4 CFU/ml for fungi) and then 100 μl was added to each well and incubated at 35±2°C. MICs were defined as the lowest concentration of dilution that inhibits bacteria and fungi after 24, 48 h, respectively. MLC values were the first well that showing no growth on solid media [16]. DMSO (10%) was used as a control. The experiments were performed for three times in the same conditions.

RESULTS

TPC and TFC of different extracts were determined as shown in table 1. The TPC of *P. vulgaris* extracts were higher in aqueous extract (156.5 mg GAC/g) followed by ethanol extract (122.1 mg GAC/g) and methanol extract (115.7 mg GAC/g), respectively. The TFC of *P. vulgaris* methanol extract (82.8 mg QE/g) was higher than ethanol extract (22.7 mg QE/g) and aqueous extract (16.2 mg QE/g).

Table 1.

Total phenolic content (TPC), total flavonoid content (TFC) of the extracts

Extract	TPC (mg GAC/g)	TFC (mg QE/g)
Aqueous	156.5	16.2
Methanol	115.7	82.8
Ethanol	122.1	22.7

TPC – Total Phenolic Content; TFC – Total flavonoid Content, GAC – Gallic acid, QE – Quercetin

The antimicrobial activities of methanol, aqueous, ethanol extracts were presented in table 2. The best activity of aqueous extract was against *S. aureus*, *S. sobrinus*, *S. salivarius* (MIC, MLC= 6.4, 12.8 mg/ml), followed by *S. sobrinus* (MIC=MLC=25.6 mg/ml). The best antimicrobial activity of ethanol extract was against *S. aureus*, *S. mutans*, *B. cereus* and *A. niger* (MIC=MLC=3.2, 6.4 mg/ml) followed by *S. epidermidis*, *S. pneumoniae*, *S. pyogenes*, *S. sanguis*, *S. salivarius*, *P. aeruginosa*, *B. subtilis*, *C. albicans* and *S. marscenes* (MIC=MLC=6.4 mg/ml). The MIC and MLC values of ethanol extract against other microorganisms were lower or equal to 12.8 mg/ml. The methanol extract of *P. vulgaris* exhibited the best activity against *St. mutans* (MIC=MLC= 3.2 mg/ml), *S. aureus*, *S. epidermidis*, *S. sobrinus*, *S. sanguis*, *S. salivarius*, *S. dysenteriae*, *S. flexeneri*, *P. aeruginosa* (MIC, MLC= 3.2, 6.4 mg/ml). *S. saprophyticus*, *S. pneumoniae*, *S. pyogenes*, *E. faecalis*, *E. faecium*, *S. agalactiae*, *K. pneumoniae*, *E. aerogenes*, *A. flavus*, *A. niger* and *S. marcescenes* with MIC and MLC 6.4 and 12.8 mg/g had lower sensitivity to methanol extract. Methanol extract had cidal activity against *E. coli*, *B. subtilis*, *B. cereus*, *C. albicans*, and *C. glabrata*. The less sensitive to methanol extract microorganism was *S. typhimurium* (MIC, MLC= 12.8, 25.6 mg/ml).

Table 2.

The antimicrobial activity of *P. vulgaris* extract by microbroth dilution assay

	Aqueous extract [mg/ml]		Ethanol extract [mg/ml]		Methanol extract [mg/ml]		Antibiotic [μg/ml]	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
S. aureus	6.4	12.8	3.2	6.4	3.2	6.4	0.25	0.25
S. saprophyticus	6.4	*	12.8	12.8	6.4	12.8	0.25	0.5
S. epidermidis	6.4	*	6.4	6.4	3.2	6.4	0.5	1
S. pneumoniae	51.2	*	6.4	6.4	6.4	12.8	0.25	0.5
B. subtilis	*	*	6.4	6.4	12.8	12.8	0.125	0.25
B. cereus	*	*	3.2	6.4	12.8	12.8	0.25	8
S. pyogenes	51.2	*	6.4	6.4	6.4	12.8	0.125	0.25
E. faecium	25.6	*	6.4	12.8	6.4	12.8	1	2
E. faecalis	51.2	*	6.4	12.8	6.4	12.8	1	2
S. agalactiae	*	*	6.4	12.8	6.4	12.8	0.5	1
S. mutans	25.6	25.6	3.2	6.4	3.2	3.2	0.25	0.25
S. sobrinus	6.4	12.8	6.4	12.8	3.2	6.4	0.25	0.5
S. sanguis	*	*	6.4	6.4	3.2	6.4	2	4
S. salivarius	6.4	12.8	6.4	6.4	3.2	6.4	0.5	1
S. flexeneri	*	*	6.4	12.8	3.2	6.4	0.25	0.5
E. coli	*	*	6.4	12.8	12.8	12.8	0.25	0.5
S. typhimurium	*	*	6.4	12.8	12.8	25.6	1	2
K. pneumoniae	*	*	6.4	12.8	6.4	12.8	0.25	0.25
S. dysenteriae	51.2	*	6.4	12.8	3.2	6.4	0.25	0.5
P. aeruginosa	*	*	6.4	6.4	3.2	6.4	0.25	0.5
E. aerogenes	*	*	12.8	12.8	6.4	12.8	0.25	0.5
S. marcescens	*	*	6.4	6.4	6.4	12.8	1	2
C. albicans	*	*	6.4	6.4	6.4	6.4	0.125	0.25
C. glabrata	*	*	6.4	12.8	6.4	6.4	0.25	0.5
A. flavus	51.2	*	6.4	12.8	6.4	12.8	0.5	1
A. parasiticus	*	*	6.4	12.8	12.8	12.8	0.5	1
A. niger	*	*	3.2	6.4	6.4	12.8	0.5	0.5

* The value was higher than 51.2; **Antibiotics** – vancomycin (Gram-positive), gentamycin (Gram-negative), amphotericin B (yeast and fungi); **MIC** – Minimal inhibitory concentration; **MLC** – Minimal lethal concentration

DISCUSSION

The present study confirmed that the solvent of plant extraction has an important effect on biologically active content. Although, it is reported that methanol

can extract the higher amount of phenolic compounds than aqueous extract [17], our study showed that the highest amount of phenolic is present in aqueous extract but the methanol extract contains higher amount of total flavonoid content.

Today's, because of microbial resistance to new antibiotics and adverse effects of these drugs, researchers are interested in new natural sources of newer agents with antimicrobial activities. One of these new sources is evaluating the traditional uses of medicinal plants. *P. vulgaris* is a known plant with traditional uses for treatment of infectious diseases such as wounds and sore throat, minor injuries, sores, burns, bruises and can also be used as a mouthwash to treat mouth ulcers. It is known for its antiviral activity against HSV and HIV. The antiviral activity is related to an anionic polysaccharide [7]. In this research, we evaluated the antimicrobial activity of *P. vulgaris* extracts (aqueous, ethanolic and methanolic) in *in vitro* conditions against different kinds of microorganisms, including Gram-positive, Gram-negative bacteria, yeast and filamentous fungi and also their total phenolic and total flavonoid contents, in order to finding the relation between TPC and TFC and their antimicrobial activity. Thus, *P. vulgaris* aqueous extract with higher TPC and lower TFC exhibited the weak antimicrobial activity than that of ethanol and methanol extracts. The amount of TPC in ethanol and methanol extracts were some equal, while TFC of methanol extract was higher than that of ethanol extract. There is not a direct relation between total phenolic content and its antimicrobial activities. Main phenolic acid component of *P. vulgaris* is rosmarinic acid [2, 18]. Rosmarinic acid or α-O-caffeoyl-3-4-dihydroxyphenyllactic acid is the multifunctional caffeic acid ester with antimicrobial activity against *Bacillus cereus*, *B. subtilis* and *B. polymyxa* [19]. Gram-negative bacteria were previously reported to be highly susceptible to rosmarinic acid [19]. Our results exhibited that this antimicrobial activity is not related to phenolic compounds such as rosmarinic acid, because the sensitivity of Gram-positive, Gram-negative ones is depending on type of pathogens. There is a negative relation between total phenolic content and its antimicrobial activity. Among ethanol and methanol extracts from *P. vulgaris*, the sensitivity of each bacterium is depending on the type of pathogens no total flavonoid content.

CONCLUSION

The TPC of *P. vulgaris* extracts were higher in aqueous extract followed by ethanol extract and methanol extract. The TFC of *P. vulgaris* methanol extract was higher than ethanol extract and aqueous extract. Ethanol extracts of *P. vulgaris* showed the best antimicrobial activity against tested microorganisms. There is a negative relation between total phenolic content and its antimicrobial activity.

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DZIAŁANIE ANTYBAKTERYJNE WYCIĄGÓW Z *PRUNELLA VULGARIS*

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S t r e s z c z e n i e

Prunella vulgaris (*Labiatae*), jest tradycyjnie używana w leczeniu różnych schorzeń, takich jak ból i zapalenie oka, ból głowy, zawroty głowy, ból gardła i leczenie ran. Całkowitą zawartość fenoli i flawonoidów w wyciągach (metanolowym, etanolowym i wodnym) określono za pomocą spektrofotometru. Działanie antybakteryjne oszacowano metodą micro broth dilution. Całkowita zawartość fenoli była najwyższa w wyciągu wodnym z *P. vulgaris* (156,5 mg GAC/g), następnie w wyciągu etanolowym i metanolowym. Zawartość TFC w wyciągu metanolowym z *P. vulgaris* (82,8 mg QE/g) była wyższa niż w wyciągu etanolowym (22,7 mg QE/g) i wodnym (16,2 mg QE/g). Działanie antybakteryjne wyciągu metanolowego lub etanolowego z *P. vulgaris* było silniejsze niż wyciągu wodnego. Wrażliwość mikroorganizmów na różne wyciągi zależy od typu patogenów. Nie ma pozytywnej zależności między całkowitą zawartością fenoli i działaniem antybakteryjnym. Wyciągi etanolowe z *Prunella vulgaris* mogą być uważane za źródło fenoli i flawonoidów i stosowane przeciwbakteryjnie.

Słowa kluczowe: *Prunella vulgaris, działanie antybakteryjne, fenole, flawonoidy, wyciąg*