

STUDY OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *PHOUPSIS STYLOSA*

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ABSTRACT

The aim of this study was to evaluate the antioxidant and antibacterial activity of the leaf and flower extract in *Phoupsis stylosa* plants. *P. stylosa* (Caucasian Crosswort) is a low-growing aromatic perennial plant with whorls of fragrant pink flowers and belongs to family Rubiaceae. The antioxidant activities of extracts were evaluated by determination of DPPH-Scavenging activity, total phenol content. Results showed that inhibition of DPPH significantly was highest in leaf extract than with flower extract but total phenol content significantly was highest in flower extract ($P < 0.01$).

Three microorganism that use in this research were *Staphylococcus aureus* (ATCC 2785), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 1523). The antibacterial activity of extracts was determined by disc diffusion method. Results showed that the highest measuring of inhibitor for antibacterial activity is in leaf extract related to *Escherichia coli* with $15/07 \pm 1/94$ mm diameter inhibition zone (DIZ).

The findings indicated the potentiality of leaf and flower extract of *P. stylosa* for use as biopre-servatives as they showed high antioxidant and antibacterial activities.

Keywords: *Phoupsis Stylosa*, Extract, Antibacterial, Antioxidant

INTRODUCTION

Biochemical reactions in the body generate reactive oxygen species which can damage important biomolecules, leading to several disease conditions. The harmful action of the free radicals can be blocked by antioxidants which scavenge the free radicals and nullify their damaging effect on cellular constituents. Natural antioxidants from plants have been shown to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). Dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage. In addition they have been used in the food industry to prolong shelf life as they inhibit lipid oxidation. Majority of the antioxidants from plants are secondary metabolites like phenolics and flavonoids that have been reported to be potent free radical scavengers. They are found in different parts of the plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006). Many of these phenolic compounds also possess other functional attributes like antimicrobial, anti-inflammatory, antimutagenic, hypocholesteremic and antiplatelet aggregation properties.

Synthetic antioxidants and antimicrobials in use have been shown to have harmful side effects (Gao *et al.*, 1999; Williams *et al.*, 1999; Osawa and Namiki, 1981) therefore, there is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources. Several medicinal plants with ethno-botanical uses have been used traditionally in the treatment of diseases and have been exploited for these desired traits (Patel *et al.*, 2010; Okoro *et al.*, 2010; Lagnika *et al.*, 2011). Consequently, there has been a growing interest to identify natural antioxidants and antimicrobials from these plants (Rice-Evans, 2004; Chanda and Dave, 2009).

Phuopsis stylosa (Caucasian Crosswort) is a low-growing aromatic perennial plant with whorls of fragrant pink flowers and belongs to family Rubiaceae (Mozaffarian 1996; Pink 2004).

The aim of this research is to assess the antioxidant and antimicrobial activity of leaf and flower extracts in *Phuopsis stylosa*.

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MATERIALS AND METHODS

Plant Materials

Phuopsis stylosa were collected from Dohezar with 1150m elevation in Tonekabon City (mazandaran province of Iran). The plant were identified in the herbarium of College of Science, Islamic Azad University, Tonekabon Branch.

Antioxidant Activity

For Study of antioxidant activity, the leaf and flower of *phuopsis stylosa* were cut into small pieces and shade dried at room temperature for fifteen days, finely powdered plant materials were successively extracted with organic solvent methanol basing on order of polarity using soxhlet apparatus (Ahmed et al, 2006). Determination of Free radical scavenging activity by DPPH method 2ml of 0.33% methanolic solution of DPPH was added to different concentration of methanolic *phuopsis stylosa* (100-500µg/ml) extract. After 30 minutes; absorbance was measured at 517nm using UV-Visible spectrophotometer (Brand-Williams et al., 1995). All the tests were performed in triplicate and averaged. Ascorbic acid was used as standard. Percentage scavenging of DPPH free radical was calculated using following equation.

DPPH radical scavenging activity (%) = $(A_{\text{control}} - A_{\text{Test}}) / A_{\text{control}} \times 100$.

Where A_{control} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the extracts or standard.

Determination of Phenolic Contents

Phenolic contents were determined by a Folin-Ciocalteu reagent using a method described by Spanos and Wrolstad (1990), to 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteu's reagent and 2 ml of Na₂CO₃ (7.5%, w/v) were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm. The values were expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

Antimicrobial Activity

The aerial parts of the selected plants were dried in room temperature (27 ± 3 °C) in the dark, and powdered. The methanolic extracts were obtained by maceration of the crude plant powder with methanol/water 90/10 for 2 days (26 ± 3 °C) in the dark. A Disk Diffusion Method was used to determine antimicrobial activity of extracts (Bauer et al., 1966; Cruickshank, 1968), and microorganisms were cultured at 37 °C for 16–24 h and prepared to turbidity equivalent to McFarland standard No. 0.5. and consequently the suspensions were spread on the test plates (nutrient agar). Sterile discs were impregnated with 0.5, 1, 2 and 4 mg of the plants extract, and placed on surface of test plate. Positive control discs with Gentamicin, (10 µg/disc) for bacteria, each extract and control was tested in triplicate and the experiments were repeated three times. Tested organisms were selected by following bacteria: *Staphylococcus aureus* (ATCC 2785), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 1523).

Statistical Analyses

The analysis of variance (ANOVA) was performed using the SPSS 16.0 software, and the means were compared by Duncan's test. The values are reported as mean ± SD.

RESULTS AND DISCUSSION

The radical scavenging activity of the extracts of *P. stylosa* at different concentrations is shown in Table 1. ANOVA test showed that the differences in Extract, concentration and Extract × concentration interactions were significant at $p < 0.01$ (Table 2). Results show that at 250 µg/ml, the radical scavenging activity was highest for leaf extract (25.2967 ± 1.14 %), while it was least for flower extract (4.7233 ± 1.47 %) at 100µg/ml. The scavenging activity of the extracts was dose dependant. The DPPH radical scavenging activity of the plant extracts was in the order of leaf > flower.

The DPPH radical is a widely used model to evaluate the antioxidant property of plant extracts (Ebrahimzadeh et al., 2008). DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour et al., 2009).

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Table 1: Effects of extract, concentration and interaction of extract and concentration on the inhibition (%) in the *Phuopsis stylosa* on DPPH model

Extract	Concentration($\mu\text{g/ml}$)	%Inhibition
leaf	100	15.9733 \pm 0.91d
	150	18.2933 \pm 1.14cd
	200	22.8333 \pm 2.00ab
	250	25.2967 \pm 1.14a
flower	100	4.7233 \pm 1.47f
	150	7.5033 \pm 2.47e
	200	18.1667 \pm 1.69cd
	250	19.9533 \pm 3.34bc
ANOVA	Extract	**
	concentration	**
	Extract \times concentration	**

The data are expressed as means \pm SD ($n=3$). The means marked with the different letter in the same column are significantly ($P < 0.05$) different. Significant levels; **significant at $p < 0.01$; *significant at $p < 0.05$.

Total phenol content of different extracts of *P.stylosa* was presented in Figure 1. Results showed that the differences in leaf and flower extract was significant. The highest total phenol content (1.3886 mg/g fresh weight) was observed at flower extract and the lowest total phenol content (0.4324 mg/g fresh weight) was observed at leaf extract. The total phenol content of the plant extracts was in the order of leaf > flower.

An adequate intake of natural antioxidants could protect macromolecules against oxidative damage in cells (Riso et al., 2005). The term antioxidant refers to free radical scavengers, inhibitors of lipid peroxidation and chelating agent (Lee et al., 2003). Phenolic compounds possess a wide spectrum of biological effects including antioxidant and free radical scavenging (Pellati et al., 2004). Its reported that there is a significant relationship between the presence of total phenol content and antioxidant activity in many species. Phenolic compounds show significant antioxidant activity (Matkowski and Piotrowska, 2006; Wei and Shibamoto, 2007). It seems that there is a significant relationship between the presence of total phenol and antioxidant activity in many species.

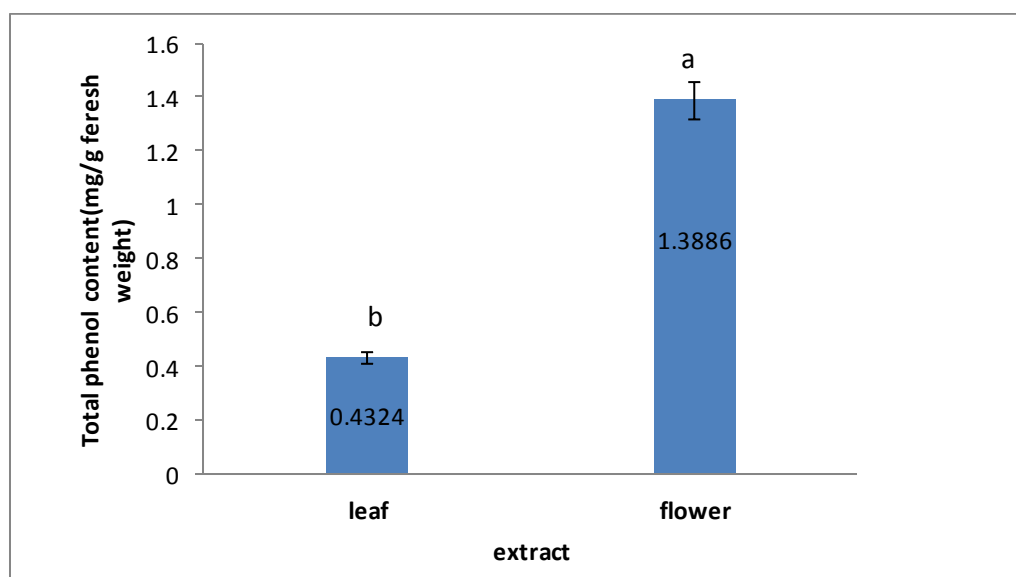


Figure 1: The total phenol contents in different extract of *Phuopsis stylosa*

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Antibacterial activity of different extracts of *Phuopsis stylosa* was presented in Table 2. ANOVA test showed that the differences in diameter of inhibition zone (DIZ) due to Extract, Microorganism and Extract \times Microorganism interactions were significant at $p < 0.01$ (Table 2). Results showed that the highest antibacterial activity of *P. stylosa* was against *E. Coli* with diameter of inhibition zone (DIZ) 15.0667 mm (Table 2). On the other hand, standard antibiotic gentamicine showed significant antibacterial activity against all tested bacteria. The results indicated that standard antibiotic Gentamicine had no comparable activity to some plant extracts as shown in Figure 2.

Bacterial resistance is a growing-problem worldwide (WHO, 2001). One of the measures to combat the increasing rate of resistance in the long run is to have continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with no effective ones. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants were tested and some natural products were approved as new antibacterial drugs. However, there is still an urgent need to identify novel substances active against pathogens with higher resistance (Malika et al., 2004). Lot of works reports antibacterial and phytochemical constituents of medicinal plants and their use for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antibacterial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased astronomically (Hugo and Russell, 1984). Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry (Hostettmann and Hamburger, 1991) and provide natural source of antimicrobial drugs that provides novel compounds that may be employed in controlling some infections globally.

Table 2: Effects of extract, microorganism and interaction of extract and microorganism on the diameter of inhibition zone (DIZ) in the *Phuopsis stylosa* on disc diffusion method

Extract	Microorganism	DIZ
leaf	<i>Staphylococcus aureus</i> (ATCC 2785)	7.4667 \pm 0.99d
	<i>Bacillus cereus</i> (ATCC 11778)	7.8 \pm 1.2d
	<i>Escherichia coli</i> (ATCC 1523)	15.0667 \pm 1.94a
flower	<i>Staphylococcus aureus</i> (ATCC 2785)	9.3333 \pm 1.54c
	<i>Bacillus cereus</i> (ATCC 11778)	11.2667 \pm 2.01b
	<i>Escherichia coli</i> (ATCC 1523)	0.00 \pm 0.00e
ANOVA	Extract	**
	Microorganism	**
	Extract \times Microorganism	**

The data are expressed as means \pm SD ($n = 3$). The means marked with the different letter in the same column are significantly ($P < 0.05$) different. Significant levels; **significant at $p < 0.01$; *significant at $p < 0.05$

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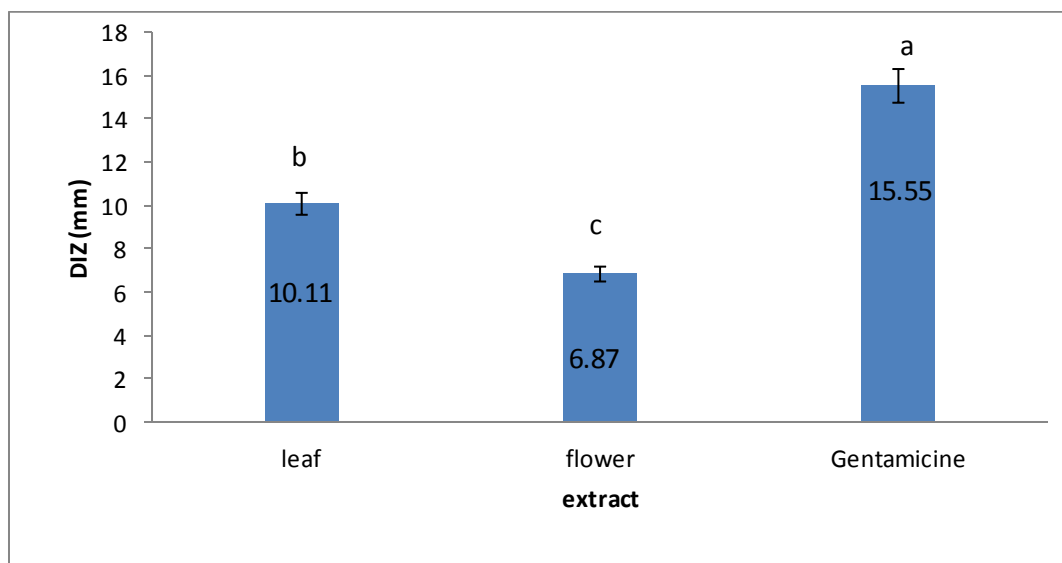


Figure 2: Antibacterial activity of extracts and Gentamicine on the diameter of inhibition zone (DIZ) in the *Phuopsis stylosa* on disc diffusion method

As mentioned earlier, *P. Stylosa* has major medicinal effects and used traditionally. Therefore the potency of these extracts could provide a chemical basis for some of the health benefits claimed for *P. Stylosa* in folk medicine. Further studies are necessary to assess their potential components as effective natural remedies.

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