Preliminary Analysis of Phenolic Acid Composition of *Phlomis syriaca*

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Abstract: *Phlomis* genus is one of the genera of *Lamiacea* family with various pharmaceutical activity. Although phythochemical constituents and pharmacological activities of most of the *Phlomis* genus are well documented, there is a scarce of research on *Phlomis syriaca*. This study was performed to reveal phenolic acids of *P. syriaca* inorder to encourage more studies based on its phytochemicals to elucidate the pharmacalogical activity of this plant. Total phenolic compound amount of *P. syriaca* was detected as 48.46 ± 4.30 µg GAEs/mg extract according to Folin–Ciocalteu method. An HPLC method was developed to quantify the amounts of nine phenolic compounds in the methanol extract of *P.syriaca*. The developed analytical system led to the separation, identification and the quantification of nine phenolic compounds most frequently found in plants belonging to the *Lamiaceae* family. The phenolic acids with determined concentrations in descending order were as follows, respectively; p-coumaric acid (5.334 mg/L), caffeic acid (2.367 mg/L), 4-hydroxybenzoic acid (1.978 mg/L), ferulic acid (1. 052 mg/L), chlorogenic acid (0.581 mg/L), rosmarinic acid (0.546 mg/L), protocautechic acid (0.287 mg/L), gallic acid (0.186 mg/L). cinamic acid (0.064 mg/L).

Keywords: *Phlomis syriaca*, Phenolic acid, *Lamiacea*, P-coumaric acid, Caffeic acid.

INTRODUCTION

Lamiaceae, the largest family of the order Lamiales, contains more than 245 genera and 7886 species that are distributed nearly worldwide. It is represented by 46 genera and 782 taxa with a high endemism ratio (44%) in Turkey [1].

The genus *Phlomis* L., a large genus in Lamiaceae, is represented by 34 species in Turkish flora [2]. The flowered parts of *Phlomis* sp. are generally used as an herbal tea known as “çalba” or “şalba” to treat gastrointestinal disorders and to promote good health by protecting the liver, kidney, bone and cardiovascular system [2, 3]. Considering the high economical and medicinal value of the plants in this genus, many studies have focused on the phytochemicals with high pharmaceutical activity present in *Phlomis* sp. Phytochemical studies of the *Phlomis* genus revealed the presence of iridoids, flavonoids, phenylpropanoids, phenylethanoids, lignans, neolignans, diterpenoids, alkaloids and essential oils [4-8]. Literature survey indicated that antidiabetic [9], antinociceptive [10], antiulcerogenic [11, 12], protection of the vascular system [13], anti-inflammatory [14, 15], antiallergic [16], anticancer [17], antimicrobial [18, 19], wound healing [20] and antioxidant [21] properties of *Phlomis* genus were well documented. However, there is a limited information about phythochemicals and pharmacological activity of *P. syriaca.*

To the best of our knowledge, there has been no information available about phenolic acid content of *P. syriaca* in detail. Therefore, this study was undertaken to reveal the phenolic acid composition of *P. syriaca* and to shed light to future studies related to pharmacological activity of this plant.

MATERIAL AND METHOD

All chemicals and reagents used in this study belong to the analytical grade category were purchased from Sigma-Aldrich (Steinheim, Germany).

Fresh aerial parts of *P. syriaca* was collected from Gaziantep, Turkey and authenticated by taxonomist, Dr. Mustafa Pehlivan. A voucher specimen has been deposited in the Herbarium of the Biology Department, Gaziantep University, Gaziantep, Turkey (MPH2017-8). The aerial parts of the plant were dried under shade at 25 ˚C. The air-dried aerial parts of the plant were ground into powder and extracted to exhaustion with methanol using a soxhlet apparatus. The obtained extract was dried using rotary evaporator and stored at – 20 ˚C for further analysis.

Dried methanol extract of the plant was dissolved in methanol to obtain the concentration of 1 mg mL^{-1}. The amount of the phenolic compounds was determined by using a spectrophotometric Folin–Ciocalteu method [22]. Gallic Acid was used as a standard phenolic compound in drawing the standard curve of the absorbance values obtained by this method. The results were expressed as µg Gallic Acid Equivalent (GAE) mg^{-1} dry weight extract ± SD. Each determination was done as replicates.

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The separation of phenolic compounds was performed on a Shimadzu HPLC (Shimadzu, Kyoto, Japan) equipped with LC solution software, a degasser (DGU-20A), a binary gradient pump (LC-20AD), an auto sampler (SIL-20AHT), a column oven (CTO-20AC) and a diode array detection system (SPD-M20A). The column used was a reversed phase Inertsil C_{18} ODS (GL Sciences Inc., Tokyo Japan) column (5µm particle size, 250X4.6 mm i.d., GL Sciences Inc., Tokyo Japan) from GL Sciences (Tokyo, Japan). The column was operated at a temperature of 25 ˚C. The separation was carried out with gradient elution procedure. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows; 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100%B to 10%B (5 min), %10 B (5 min). The injection volume was 10 µl. Simultaneous monitoring was performed at 280 nm. The flow rate was 1ml/min. Spectra were recorded from 190 to 800 nm. Samples and mobile phases were filtered through a 0.22 µm membrane filter, prior to HPLC injection. Each fraction was analyzed in duplicate.

The formerly identified standard phenolic acids from the same botanical family have been utilized in the identification. Stock solutions of the standards were prepared at 6 different concentrations ranging from 40 to 1.25 ppm and injected into HPLC for the establishment of calibration curves.

RESULTS

The total phenolic content of *P. syriaca* methanol extract was expressed as Gallic acid equivalents (µg GAE/mg extract) by reference to the equation (y = 0.0849x + 0.2978, R^2 = 0.9979, n=3) which was obtained from the calibration curve of standard Gallic acid. Total amount of phenolic compounds was found as 48.46 ± 4.30 µg GAEs/mg extract. The phenolic profile of the extract was investigated by HPLC/DAD.

Phenolic compounds were identified and quantified by comparing their retention time and UV–Vis spectral data to known previously injected standards. The linearity range of responses of the standards was determined on six concentration levels. Calibration plots with correlation coefficient r^2 > 0.99 were obtained by peak areas as a function of analyte concentration. HPLC/DAD chromatograms of the extract and the standards at 280 nm were presented (Figure **1**).

The developed analytical system led to the separation, identification and the quantification of nine phenolic compounds most frequently found in plants belonging to the *Lamiaceae* family. The phenolic acids with determined concentrations in descending order were as follows, respectively; p-coumaric acid (5.334 mg/L), cafeic acid (2.367 mg/L), 4-hydroxybenzoic acid (1.978 mg/L), ferulic acid (1. 052 mg/L), chlorogenic acid (0.581 mg/L), rosmarinic acid (0.546 mg/L), protocatechuic acid (0.287 mg/L), gallic acid (0.186 mg/L). cinamic acid (0.064 mg/L).

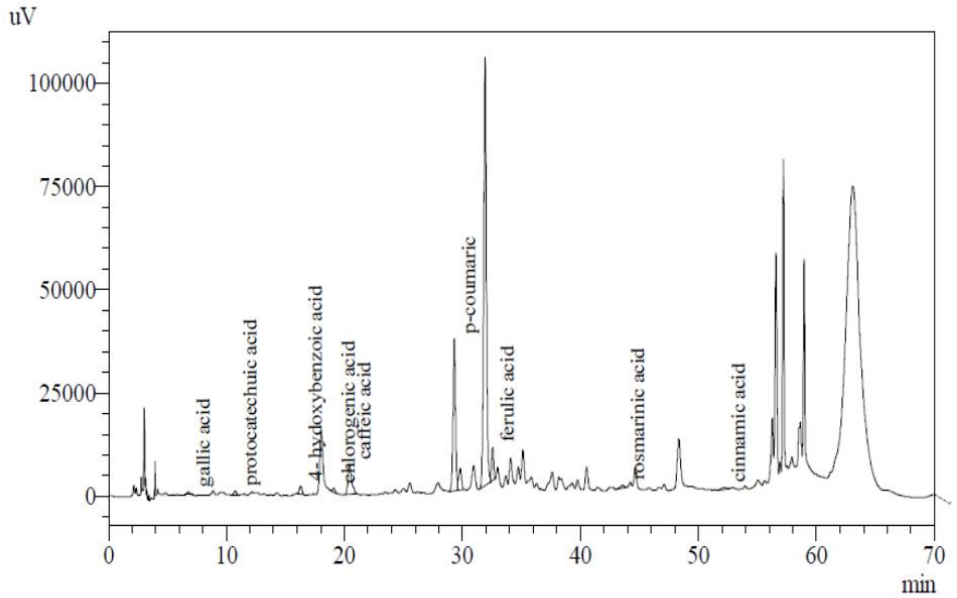

Figure 1: HPLC chromatogram of methanolic extract of *P. syriaca.*

DISCUSSION

Medicinal plants containing therapeutic phytochemicals are all potential reservoirs for new drugs [23]. Among the phytochemicals present in medicinal plants, especially phenolic compounds have gained much interest of researchers because of their various pharmaceutical activities. Although there are many studies on pharmacological activities of the *Phlomis* genus, there is a scarcity of detailed examinations of their phenolic acid composition. Literature survey indicated that there is a limited research on *P. syriaca.* Besides the knowledge on its ethnobotanical usage, data on phytochemical composition of a medicinal plant will make great contribution to reveal its pharmacological activity. This study is the first to report on the phenolic acid composition of *P. syriaca*.

Total phenolic compound amount was detected as 48.46 ± 4.30 µg GAEs/mg extract in methanol extract of *P.syriaca*. Zhan and Whang [8] also detected 39.43± 0.44 and 55.20 ± 0.88 µg GAEs/mg extract in methanolic extract of *P. umbrosa* and *P. megalantha* respectively.

According to results, p-coumaric acid (5.334 mg/L), cafeic acid (2.367 mg/L), 4-hydroxybenzoic acid (1.978 mg/L) and ferulic acid (1. 052 mg/L) were determined as higher in concentration as compared to other phenolic acids in the extract of *P.syriaca*.

Zhan and Whang [8] also determined protocatechuic, chlorogenic, caffeic, rosmarinic acid in methanolic extract of *P. umbrosa* and *P. megalantha*.

Senol *et al*. [24] also analyzed the phenolic acid composition of methanol and ethanol extracts of the aerial parts of *P.grandiflora* var. *grandiflora* by HPLC. They detected protocatechuic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, o-coumaric acid, rosmarinic acid, trans-cinnamic acid in the extracts. According to their results, ferulic acid was the most abundant phenolic acid in the ethanol extract whereas trans-cinnamic acid was the highest one in the methanol extract.

Among the analyzed phenolic compounds, para coumaric acid was found in the highest amount in our study. Many current studies reported that para coumaric acid elevates the level or the activity of enzymes that decrease oxidative stress and inflammation [25-27]. By inhibiting NF-kB gene which act as transcription factors of pro-inflammatory cytokines, para coumaric acid acts as an anti-inflammatory agent [28]. This could explain the ethnobotanical usage of *P. syriaca* against many inflammatory diseases. Presence of phenyl hydroxyl group in the structure of ca provides it free radical scavenging activity by donation of H+ or electron to free radicals [29, 30]. Literature survey indicated various bioactivities of para coumaric acid such as antioxidant, anti-inflammatory, anticancer, antidiabetic, and anti-melanogenic properties [28].

Caffeic acid was detected as the second highest abundant phenolic acid in the extract of *P.syriaca*. Besides its high antioxidant activity, caffeic acid has demonstrated antimicrobial activity against various microorganisms [31].

4-Hydroxy benzoic acid is reported to have antibacterial, antifungal, antimutagenic and antioxidant activity. It has been used as preservative in many drugs, cosmetic products, pharmaceuticals, food and beverages [32, 33].

Ferulic acid is a phenolic compound with multiple functions. In addition to its remarkable antioxidant activity, it has antiapoptotic, anti-inflammatory, antifibrosis, antiplatelet properties [34]. The phosphatidylcholine is a major constituent of the lipid bilayers of cell membranes. It was reported that the ferulic and caffeic acid protected phosphatidylcholine peroxidation induced by UV radiation [35]. This could suggest that these phenolic acids could be promising therapeutic agents in the treatment of dermal diseases.

Taken together, the present study reports the phenolic acid composition of *P. syriaca*, revealing potential pharmacological activities of this plant. This study could pave the way for the potential usage of *P. syriaca* in cosmetics and pharmaceutical preparations.

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