SOME PHENOLIC COMPOUNDS OF *SALVIA PLEBEIA* R. BR.

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ABSTRACT

A linear relationship existed between the medicinal potential and phytoconstituents specially phenolic contents of the medicinal herbs. *Salvia plebeia* is one of important plant of high medicinal potential. The present study deals with the HPLC analysis of phenolic compounds of this herb. High-performance liquid chromatography (HPLC) coupled with diode-array detection was used to identify and quantify the phenolic compound. Among the identified phenolic compounds, the quantity of rosmarinic acid was the predominant followed by leuteolin and hispidulin respectively.

Key words: Phytoconstituents, Phenolics, *Salvia plebeia* R. Br., High performance liquid chromatography

INTRODUCTION

Salvia plebeia R. Br. is one of most important herb known for its medicinal potential. It is a small annual herb growing at higher altitudes or on hilly tracks. It has been investigated that, this plant have various medicinal properties. The major medicinal uses of this herb include, antimicrobial activity, antioxidant activity, diuretic, astringent and vermifuge. The plant paste showed antibiotic properties while its seeds are being used to treat diarrhea, gonorrhea, menorrhagia and hemorrhoids (Bhadange, 2011). The present study is focused on the isolation and identification of phenolic content of this plant, as phenolic content is mostly related to the antioxidant activity, astringent property, diuretic property and many others.

MATERIAL AND METHODS

The plant material of *Salvia plebeia* R. Br. Was collected from the Chikhaldara forest range during August- September 2009. The plant was identified taxonomically with the help of flora of Maharashtra state (Singh and Kartikeyan, 2001) and local taxonomist. The collected plant material was preserved in deepfreezer (-20^{o}C) until further use.

Analysis of phenolic compounds

The amount of total phenolics in the herb extracts was determined with the Folin Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard. Samples were introduced into test cuvettes, and then 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using the Shimadzu UV-Vis spectrophotometer after incubating at 30°C for 1.5 h. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight.

HPLC Analysis

The sample (2.0 g) was extracted twice with 15 mL of acetone using a homogenizer. The extract was centrifuged, and the residue was washed and agitated twice with 5 mL of solvent. The combined extract was evaporated to dryness under reduced pressure. The residue was dissolved in 4 mL of methanol, and 20 □ L aliquots were analyzed by HPLC. The plant extract used for HPLC analysis were passed through a 0.45-ím filter before injection into a reverse phase NOVA-PAK C18 column at ambient temperature (20 °C). A photodiode array detector was used. The mobile phase was acetonitrile (A) and acidified water containing 2.5% formic acid (B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/ min and the wavelengths of detection were set at 280, 330, and 350 nm. Scanning between 200 and 450 nm was performed, and the data were collected.

RESULTS AND DISCUSSION

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids (Kahkonen *et al.,* 1999).

Phenolic acids have been repeatedly implicated as natural antioxidants in fruits, vegetables, and other plants.

For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom. Caffeic acid has been found to have high activity comparable to that of the flavonoid, uercetin (Larson, 1988). The most widespread and diverse phenolics are the flavonoids which have the same C15 (C6-C3-C6) skeleton and possess antioxidant capacity toward a variety of easily oxidizable compounds (Robards *et al*, 1999).

In many herbs, the main flavonoid constituents are flavonol aglycones such as quercetin, myricetin, kaempferol, and their glycosides (Kahkonen *et al.,* 1999). In general, flavonoids containing multiple hydroxyl groups have higher antioxidant activities against peroxyl radicals than do phenolic acids. However, the flavonoid glycosides (including rutin, naringin, and hesperidin) usually have low ORAC values (Robards *et al.,* 1999).

Table 1: Phenolic compounds in *Salvia plebeia* (mg/100g fresh weight).

Phenolic compounds	Quantity (mg/100g fresh weight)
Vanilic acid	2.15± 0.52
Caffeic acid	5.32±0.30
Rosemarinic acid	102.5±0.24
Leuteolin	22.6±0.34
Hispidulin	15.4± 0.24
Naringinin	1.8 ±0.09

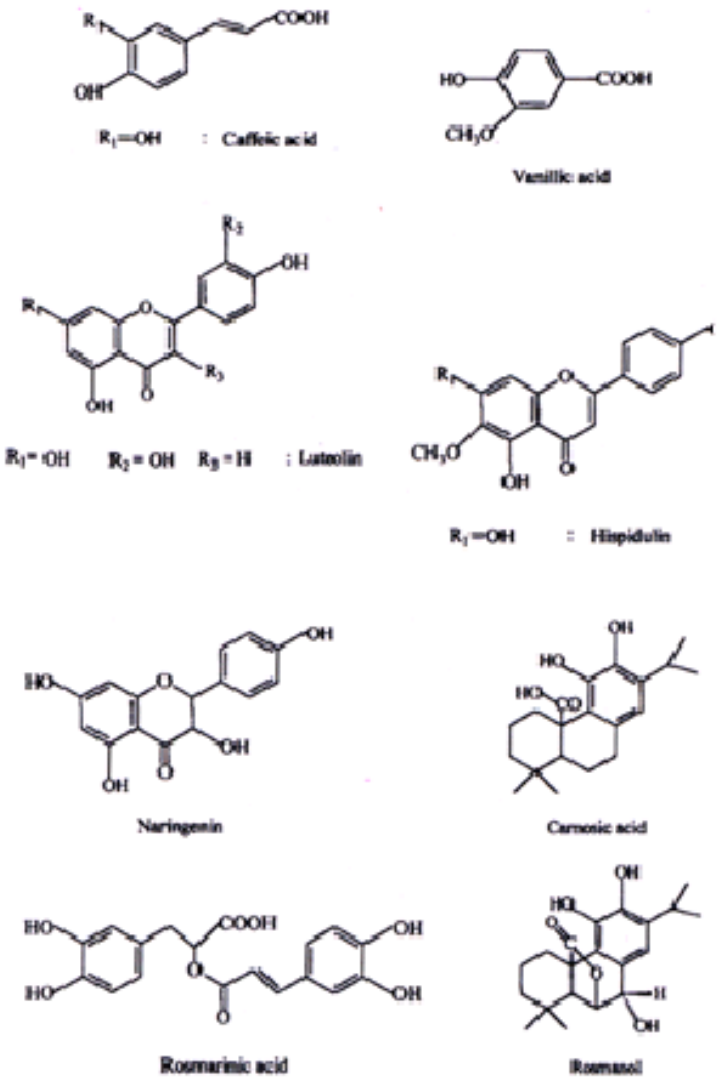

Figure-2: Structure of some phenolic compounds identified in *Salvia plebeia* extract.

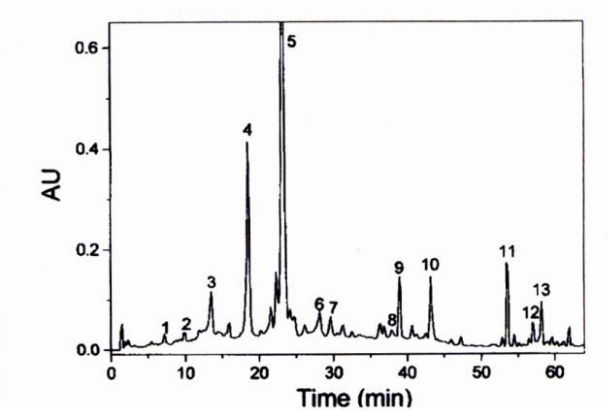

Figure 1: HPLC Chromatogram of *Salvia plebeia* extract

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Salvia plebeia is widely used as a natural source of medicinal properties for the treatment of various diseases. The HPLC analysis of whole plant extracts showed that a large number flavonoids and phenolic acids were present in significant amounts (Table-1, Figure 1). The total phenolic content was found to be 1.24 ± 0.05 mg of GAE/ g fresh weight. Rosmarinic acid (102.5 mg/100 g of fresh weight) and luteolin (22.6 mg/100 g of fresh weight) were the most abundant phenolic constituents in the extracts and were readily identified by comparison with authentic standards. Other compounds with characteristic spectra of vanillic acid, caffeic acid, ferulic acid, luteolin 7-O-glucoside, rosmarinic acid, 4',5,7,8-tetrahydroxyflavone, scutellarein, apigenin, hispidulin, cirsimarin, carnosol, carnosic acid, and methyl carnosate were detected (Figure 2). These results are analogous to earlier published reports of chromatographic and UV spectra data (Okamura *et al.,* 1994; Cuvelier *et al.,* 1996 and Andrade *et al.,* 1998) of allied species of *Salvia*. Thus it can be concluded that the phenolics of Salvia might play vital role in imparting medicinal potential to this herb.

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