Phytochemical analysis and antioxidant activity of methanolic extract of *Plectranthus hadiensis* (Forssk.) Schweinf. ex Spreng. aerial parts

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Herbal medicine has been in use by diverse civilizations in different parts of the world for centuries. Present study investigates the phytochemical constitution of the methanolic extract of the aerial part of *Plectranthus hadiensis* (Forssk.) Schweinf. ex Spreng. The preliminary phytochemical screening of the methanolic extract had revealed high content of phenolics which was confirmed by TLC, HPTLC and HPLC studies. The antioxidant activity was checked using DPPH assay, nitric oxide radical scavenging activity assay and reducing power assay. The methanolic extract showed considerably high antioxidant activity compared to the standards used, viz. ascorbic acid and BHT (Butylated Hydroxy Toluene). The presence of phenolics supported medicinal uses of the species and active biomolecules could be isolated for pharmaceutical applications.

**Keywords:** *Plectranthus hadiensis*, TLC, HPTLC, HPLC, DPPH, Radical scavenging activity, Antioxidant, Phenols.

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**Introduction**

Plants have been used in traditional medicines throughout the world for thousands of years and continue to provide new remedies to human kind. The adverse effects of oxidative stress on human health have become a serious issue, therefore, a great deal of effort is focused on using available experimental techniques to identify natural antioxidants from plants. The World Health Organization (WHO) has estimated that about 80% of the earth’s inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components³. Lack of antioxidants, which can quench the reactive free radicals, facilitates the development of inflammatory diseases². One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources³. These natural plant antioxidants can therefore, protect us like preventive medicine.

*Plectranthus hadiensis* (Forssk.) Schweinf. ex Spreng. (Family-Lamiaceae) is reportedly found in Tamil Nadu on river banks and sandy loams and in the wild, in some parts of Kerala. It is used in Ayurvedic formulations for treating inflammation related ailments⁴. This study focuses on the phytochemical profiling of the plant using chromatographic techniques; TLC, HPLC and HPTLC and study of its antioxidant property. The antioxidant activity of the methanolic extract of the plant was measured using DPPH, reducing power assay and nitric oxide scavenging activity assays.

**Materials and Methods**

Shoot part of *P. hadiensis* was collected from Kerala, India and authenticated by Dr. Kunhikannan, Scientist E, IFGTB, Coimbatore, India. The shoot part was pulverized and 100 g was refluxed with methanol in the ratio 1:10(w/v). The extract was evaporated to dryness using rotary flash evaporator (Buchi type). The crude extract was used for the chromatographic profiling and different concentrations of the extract were prepared from the resultant crude methanolic extract for assessing the antioxidant activity.

**TLC and HPTLC**

Thin layer chromatography was performed to generate a chromatographic profile of the extract. Toluene-Ethyl acetate (1:1) was used as the solvent system and viewed under UV light at 365 nm⁵.
A densitometric HPTLC analysis was performed for the development of characteristic fingerprinting profile. The *P. hadiensis* methanolic extract (PV) of shoot was dissolved with HPLC grade methanol (100 mg/0.5 ml). The solution was centrifuged at 3000 rpm for 5 minutes. Then, 2 µl of the sample solution and 2 µl of standard solution were loaded as 5 mm bands on the 3 × 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. Quercetin was taken as the standard. The sample loaded plates were kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (polyphenol) and the plate was developed in the respective mobile phase upto 90 mm. The developed plates were dried by hot air to evaporate solvents from the plates. The plates were kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV366 nm. The plates were fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500 nm. The Peak table, Peak display and Peak densitogram were noted.

**Qualitative analysis by HPLC**

HPLC analysis was performed to separate and identify the marker constituent in the methanolic extract. Polyphenolic standards including cinnamic acid, coumarin and Quercetin were purchased from Sigma-Aldrich. All the standards were prepared freshly by dissolving in methanol to get a concentration of 1 mg/ml. HPLC was carried out according to modified method of Irene et al. (2004) in Tiger LC isocratic system. Acetonitrile and water (7:3) with 0.1% formic acid was used as solvent system. Flow rate was maintained at 1 ml/min throughout the run. Luna 5µ C18 100 A column (250 × 4.6 mm) was used for separation and peaks were detected at 330 nm.

**Antioxidant assays**

**DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay:** The modified method of Blois (1984) was adopted for DPPH assay. The extracts were dried in vacuum oven and redissolved in 50% methanol. Different concentrations of the extract was prepared from 50 µg/ml to 1 mg/ml in methanol and used for the assay. 1 ml of DPPH (1 mm) was added to the final volume of the extract. The mixture was left for 20 minutes in dark at room temperature. Same procedure was followed with the standard; ascorbic acid. Absorbance was measured after 20 minutes at 517 nm. Control was taken without the extract. The decrease in absorbance was then converted to percentage antioxidant activity (% AA) using the formula:

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AA\% = 100 - \frac{[\text{Absblank} - \text{Abssample}]}{\text{Abscontrol}} \times 100
\]

**Nitric oxide radical scavenging activity:** The compound sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH (7.2) producing nitric oxide radicals (NO). Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. The scavenging effect of the plant extract on nitric oxide was measured according to the modified method of Marcocci et al. (2004) in comparison with ascorbic acid. An aliquot of 1 ml of the incubation solution was removed and diluted with 1 ml of Griess reagent. Ascorbic acid was taken as standard and control was taken without the extract. The absorbance of the chromophore that formed with Naphthylethylenediamine dihydrochloride was immediately read at 540 nm. The percentage scavenging activity was calculated using the formula described above.

**Reducing power:** The ability of the extracts to reduce Fe³⁺–Fe²⁺ was assessed by the method of Yildirim et al. (2004). Extracts at different concentrations (20, 40, 60, 80 and 100 µg) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 min. 2.5 ml of 10% trichloroacetic acid was later added and the tubes were centrifuged at 3000 rpm for 10 min. 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride was added. BHT was taken as standard and control was taken without the extract. Absorbance was measured at 700 nm. Increasing absorbance values of the reaction mixture indicated increasing reducing power of the extract.

**Results and Discussion**

The phytochemical analysis of the extract was performed as there was no literature supporting the studies on the phytochemical composition of *P. hadiensis*. The species is a common ingredient of Ayurvedic formulations given for treating patients
with any type of carcinoma, ailments related to chronic inflammation and possess significant anti-fungal and anti-bacterial activity. Our studies have already shown potential anti-inflammatory and cytotoxic activity of the methanolic extract at lower concentrations. Chromatographic fingerprint symbolizes the active chemical constituents of the herbal medicines. This study would help in providing a chromatographic fingerprint of the plant, which could be used to determine authenticity and reliability of the chemical constituents of herbal drugs prepared with this plant.

**TLC and HPTLC:** Thin layer chromatography was used to separate individual chemical constituents of the methanolic extract. From a diverse range of solvent systems used for performing TLC, Toluene-Ethyl acetate (1:1) was used, which presented good separation of the bands on the TLC plate with good-quality resolution. 2 major and 2 minor bands were visualized (Violet and Blue-major and light green and light violet-minor). The coloured bands were visible in daylight and the R_f values were recorded (Table 1). This TLC chromatogram could be used as the reference standard for further separation and isolation of the compounds from the plant (Figure 1).

Initial screening of the methanolic extract had revealed the presence of high phenolic content. Chromatograms of the extract and standard when compared showed that the methanolic extract is rich in phenol acids (Figure 2 and Figure 3). Peak 7 in the extract chromatogram confirmed the presence of high phenolic content which matched with the peak present in the chromatogram of the standard Quercetin used. Further purification of the extract could yield specific phenolic compounds.

**HPLC:** Analysis of the methanolic extract was carried out using reverse phase HPLC and the chromatographic profile was compared with the retention times of the reference standards (cinnamic acid, coumarin and quercetin) (Figures 4-7). The UV-spectra of the eluted compounds revealed that the methanolic extract of *P. hadiensis* had high content of phenolic acids. The chromatographic profile of the methanolic extract showed the peaks corresponding

<table>
<thead>
<tr>
<th>Rf value</th>
<th>Colour of the bands</th>
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<tbody>
<tr>
<td>0.562</td>
<td>Violet</td>
</tr>
<tr>
<td>0.587</td>
<td>Light green</td>
</tr>
<tr>
<td>0.621</td>
<td>Blue</td>
</tr>
<tr>
<td>0.759</td>
<td>Light violet</td>
</tr>
</tbody>
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![Fig. 1—TLC chromatogram of methanolic extract](image1)

![Fig. 2—HPTLC chromatogram of the extract](image2)
to standard antioxidative polyphenols used, viz. cinnamic acid, coumarin and quercetin. This substantiated the results obtained from the TLC and HPTLC results.

**Antioxidant assays**

*DPPH assay:* The DPPH assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products. The methanolic shoot extract exhibited a dose dependent higher radical quenching activity (Table 2). The 100 µg/ml concentration showed higher radical scavenging activity (79.45%) than Ascorbic acid (76.98%) and thereafter the pattern continued till 1 mg/ml. The results of this study infer that the methanol extract reduces the radical to the corresponding hydrazine when it reacts with the hydrogen ions released from the samples, which contain antioxidant principles.

*Nitric oxide radical scavenging activity:* Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial
and antitumor activities\textsuperscript{14}. Suppression of released NO may be partially attributed to direct NO scavenging, as the methanolic extract of \textit{P. hadiensis} decreased the amount of nitrite generated from the decomposition of SNP \textit{in vitro}. The scavenging of NO by the extract increased in dose dependent manner. A significant decrease in the NO radical due to the scavenging ability of extract and Ascorbic acid can be observed (Table 3). The activity at 1 mg/ml was found to be 88.88\% for the methanolic extract and 92.74\% for ascorbic acid.

\textbf{Reducing power}: Reducing capacity of the extract components may serve as a significant indicator of its potential antioxidant activity\textsuperscript{15}. Different studies have indicated that the electron donation capacity of bioactive compounds is associated with antioxidant activity\textsuperscript{16,17}. Phenolic compounds are reported to be the major phytochemicals in plants responsible for

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**Fig. 5**—HPLC chromatogram of cinnamic acid

**Fig. 6**—HPLC chromatogram of coumarin
antioxidant activity of plant materials. Table 4 shows the reducing activity of the extract and the standard BHT. Reducing power of both extract and BHT increased with increasing concentration. At 100 µg/ml concentration, the absorbance of extract and BHT were found to be 1.341 and 1.507, respectively.

**Conclusion**

Recent years have seen an exponential increase in research of antioxidant properties of medicinal plants. If it is accepted that higher intake of natural antioxidants containing phenolic acids are associated with long term health benefits, then the results presented in this paper offer possible avenues toward health promotion by identifying those compounds. The health promoting properties of *P. hadiensis* shoot may be due to its antioxidant properties and is also attributed to its multitherapeutic characteristics. The methanolic extract showed high content of phenol acids, which was confirmed using HPTLC and HPLC. Thus, *P. hadiensis* might be useful in the development of raw materials of medicine. Further isolation and characterization and *in vitro* and *in vivo* studies on the shoot of *P. hadiensis* are necessary to substantiate the use of this plant in the traditional system of medicine.
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References