HPLC DETERMINATION OF FLAVONOIDS IN THE METHANOL EXTRACTS OF MALACHRA CAPITATA (L) L

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ABSTRACT

Methanol extracts of Malachra capitata root, stem and leaf samples were analyzed for the quantification flavonoid profile using HPLC. The results of HPLC analysis indicate the presence of flavonoid compounds -gallic acid, caffeic acid, rutin, quercetin and ferulic acid in the root sample methanol extracts of Malachra capitata while the methanol extract of leaf sample shows only two compounds (rutin and ferulic acid) and one compound (gallic acid) in the stem sample methanol extract. Maximum amount of flavonoid compound (gallic acid -30mg/g) noted in the stem sample as compared to other compounds among the plant samples tested.

KEY-WORD: HPLC analysis, Malachra capitata, Flavonoid compound, Methanol extracts.

INTRODUCTION

Phytochemical evaluation is one of the tools for quality assessment of plants. In order to discover new bioactive compounds, extract are simultaneously evaluated by chemical screening.[1] The qualitative analysis which produces a fingerprint chromatogram obtained under standard conditions can be very useful for quality control of phytochemicals. Phenolic compounds and flavonoids are widely distributed in plants which have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic, etc.[2- 3] Malachra capitata (L.) is an herb belongs to Malvaceae family. Reports indicate that the root of M. capitata is used in traditional remedies for many disease conditions –pain, hepatic cirrhosis, inflammation, diarrhea, convulsion, dementia, pyrexia, ulcer and healing of wounds.[4-7] The present study was performed to
examine the flavonoid profile in the methanol extracts of *Malachra capitata* (L.) by HPLC analysis.

**MATERIALS AND METHODS**

**Plant material**

*Malachra capitata* was collected from Komaneri village of Thoothukudi district, Tamil Nadu, India and identified by Dr. Chelladurai, Research Officer, Central Council for Research in Ayurveda and Siddha, Palayamkottai (Figure 1).

**Preparation of plant material extracts for HPLC**

The dried plant materials of different parts of *M. capitata* were separately shade dried and pulverized to powder in a mechanical grinder (Figure 1). Powdered samples of different parts of (root, stem and leaf) *M. capitata* was extracted for 6h with 10mL of methanol in glass-stopped vessels on a hotplate with magnetic stirring at 40°C. After centrifugation at 3000g for 10 min, the extracted sample was decanted and the remaining solid residue was extracted three times with 5mL of methanol. The extract was evaporated in vacuum to dryness at 40°C.
The solid residue was reconstituted with 5mL of methanol and 20µL was injected into the HPLC system.

**HPLC Analysis of Flavonoid Compounds**

The HPLC system consisted of a HPLC Shimadzu Class-VP V6.14 SP2; coupled to a variable UV absorbance detector (model SPD-10A, Shimadzu, Tokyo, Japan) operated at wavelength 280nm. The chromatogram was recorded using an electronic integrator (chromate- Integrator, Model Hitachi, D-2500, Hitachi, Tokyo, Japan). Chromatographic separation was carried out using a column-C18 reversed-phase (RP) analytical HPLC column (Phenomenex 100a, Nucleosil, 25044.6mmi.d. 5µm particle size) (Phenomenex, Torrance, CA). The mobile phase consisted of solvent-A water-ascetic acid (25:1), solvent-B methanol. The flow rate was held constant at 1.0mL/min. Pumps (Binary gradient): B. Conc. 0.0; B. curve -0.0; P. Max -400.0 kgf/cm²; P. Min. -0.0 kgf/cm²; CTO-10ASvp: temperature was maintained at 40°C; SPD-10Avp (Det.A): Lamp –D2; Polarity (+); wave length Ch. 1: 280nm. Gallic acid, caffeic acid, rutin, quercetin and ferulic acid standards were used.[8]

**RESULTS AND DISCUSSION**

HPLC analysis of *M. capitata* root, stem and leaf methanol extracts are presented in Table 1 to 3 and Figure 2 to 4. The results indicate the presence of flavonoids in *M. capitata* plant samples at varied concentrations. The methanol extract of *M. capitata* root sample indicate the presence of five flavonoid compounds (gallic acid, caffeic acid, rutin, quercetin and ferulic acid) in different concentrations. Among the flavonoid compounds detected, gallic acid found in maximum concentration (20mg/g) and is followed by quercetin (10mg/g) while other three compounds are less in amount (Table 1; Figure 2).

<table>
<thead>
<tr>
<th>Flavonoid compounds detected (Detector A -280nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the compound*</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Gallic acid</td>
</tr>
<tr>
<td>Caffeic acid</td>
</tr>
<tr>
<td>Rutin</td>
</tr>
<tr>
<td>Quercetin</td>
</tr>
<tr>
<td>Ferulic acid</td>
</tr>
</tbody>
</table>
Table 2: Flavonoid content in stem of *Malachra capitata* identified by HPLC analysis

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Concentration (mg/g)</th>
<th>Retention Time</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>30.0</td>
<td>5.458</td>
<td>1787</td>
<td>18781</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>Below Detection Limit</td>
<td>9.325</td>
<td>0</td>
<td>179</td>
</tr>
<tr>
<td>Rutin</td>
<td>Below Detection Limit</td>
<td>10.367</td>
<td>0</td>
<td>269</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Below Detection Limit</td>
<td>12.017</td>
<td>0</td>
<td>256</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>Below Detection Limit</td>
<td>24.458</td>
<td>2</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 2 and Figure 3 reveal the outcome of HPLC analysis of flavonoids in *M. capitata* stem sample methanol extract. Among the flavonoid compounds noted, gallic acid content found more in amount (30mg/g) while all other flavonoids are noted below the detectable limit.

HPLC analysis in the methanol extract of *M. capitata* leaf sample shows two compounds – rutin (10mg/g) and ferulic acid (10mg/g) while other three flavonoids are below the detectable limit (Table 3; Figure 4).
Table 3: Flavonoid content identified in leaf of *Malachra capitata* by HPLC analysis

<table>
<thead>
<tr>
<th>Name of the compound*</th>
<th>Concentration (mg/g)</th>
<th>Retention Time</th>
<th>Height</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>Below Detection Limit</td>
<td>5.750</td>
<td>829</td>
<td>1511</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>Below Detection Limit</td>
<td>9.325</td>
<td>0</td>
<td>578</td>
</tr>
<tr>
<td>Rutin</td>
<td>10.0</td>
<td>10.092</td>
<td>0</td>
<td>4054</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Below Detection Limit</td>
<td>12.067</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>10.0</td>
<td>24.625</td>
<td>3</td>
<td>883</td>
</tr>
</tbody>
</table>
Several workers have investigated the effects of natural flavonoids. Flavonoids can be derived from any part of the plant and the medicinal values of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. Some studies suggest that flavonoids may be useful in the treatment of many impaired conditions. It has been found that flavonoids possess antioxidant and free radical scavenging activity and capable of both preventing and eliminating the effects of reactive oxygen species.

Gallic acid is known to have anti-inflammatory, antimutagenic, anticancer and antioxidant activity. It also seems to have antifungal, antiviral and antibacterial properties. Gallic acid was found to show cytotoxicity against cancer cells without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage. It has been found very beneficial in uterine, pulmonary and nephritic haemorrhages. It has given benefit in purpura and used to treat albuminuria and diabetes. It is a known matrix-metalloproteinase inhibitor. All these properties make gallic acid a pharmacologically important compound.

The flavonol quercetin has shown much promise as an antioxidant agent, imparting a protective effect in reducing the risk of developing cancer and cardiovascular disease. Quercetin is frequently used therapeutically in allergic conditions, including asthma and hayfever, eczema and hives. Additional clinical uses include treatment of gout, pancreatitis and prostatitis, which are also, in part, inflammatory conditions.

Ferulic acid is a phenolic acid of low toxicity; it can be absorbed and easily metabolized in the human body. Ferulic acid has been reported to have many physiological functions, including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anti-cancer activities. It also protects against coronary disease, lowers cholesterol and increases sperm viability. Because of these properties and its low toxicity, ferulic acid is now widely used in the food and cosmetic industries. Ferulic acid exhibits a wide range of therapeutic properties like anti-inflammatory, antiatherogenic, antidiabetic, antiageing, neuroprotective, radioprotective and hepatoprotective effects. Ferulic acid also exhibits wide variety of biological activities such as antioxidant, antiinflammatory, antimicrobial, antiallergic, hepatoprotective, anticarcinogenic, antithrombotic, increase sperm viability, antiviral and vasodilatory actions, metal chelation and modulation of enzyme activity, activation of transcriptional factors, gene expression and signal transduction. The HPLC studies
conducted with the methanol extracts of M. capitata root, stem and leaf samples revealed an appreciable amount of flavonoid gallic acids (root and stem), quercetin (root), rutin and ferulic acid (leaf), which conforms their medicinal potential.

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