ESTIMATION OF QUERCETIN CONTENT IN THREE DIFFERENT SPECIES OF EUPATORIUM BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

V. Nithya and M. Kamalam *

Department of Botany, PSGR Krishnammal College for Women, Coimbatore - 641004, Tamil Nadu, India.

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ABSTRACT: Three different species of Eupatorium namely E. glandulosum, E. odoratum and E. triplinerve belongs to the family Asteraceae are selected for the analysis of quercetin content. Leaves are extracted with ethanol and water and used for the analysis of quercetin by HPTLC technique using the mobile phase containing toluene: ethyl acetate: formic acid: methanol (5.5:4:1:0.5). Determination of quercetin content was performed by densitometric scanning under 254 nm, and the quercetin was detected at the Rf value of 0.54. The quantity of the quercetin content in plant extract was estimated by the calibration curve obtained from the standard quercetin. The result showed that the ethanolic extracts of E. glandulosum showed a high amount of quercetin (17.44 mg/g) followed by E. odoratum (13.4 mg/g) and E. triplinerve (9.29 mg/g).

INTRODUCTION: Plants produce a high diversity of secondary metabolites which are helpful in the defense mechanism of plants played against abiotic stress, and many of them have some medicinal importance. Quercetin is a flavonol, proved to be a potent antioxidant among polyphenols [1, 2, 3]. It possesses antiviral, antibacterial, anticarcinogenic and anti-inflammatory effects [4, 5]. As per the literature, quercetin was reported in E. glandulosum [6], E. perfoliatum [7] and E. cannabinum [8]. In the present study, three different species of Eupatorium such as E. glandulosum, E. odoratum and E. triplinerve have been selected for the estimation of quercetin using High-Performance Thin Layer Chromatography.

E. glandulosum belongs to the family Asteraceae is a native of Mexico, introduced as an ornamental shrub in several countries. In India, the tribes of Nilgiris use the leaves of this plant to heal wounds and small injuries [9]. E. odoratum is a fast growing perennial shrub, native of Central and South America has spread in tropical and subtropical regions of the world. The tribes of Indonesia used the leaf extract to cure skin diseases, poison bites, wounds, burns, cough, diabetes, diarrhea, fever, inflammation, and rheumatism. The boiled roots are used to cure urinary disorders [10, 11, 12].

E. triplinerve commonly called as Ayapana is an ornamental, erect, perennial herb having aromatic leaves. In tribal medicine, it is used to cure a fever with convulsions, pneumonia, indigestion, and cough [13]. Hence, to consider the medicinal importance of the above-said plants, the present study is undertaken with the objective of estimation of the quantity of quercetin content in three different species of Eupatorium using HPTLC technique.
MATERIALS AND METHODS:
Collection of Plant Material: The leaves of *E. glandulosum*, *E. odoratum*, and *E. triplinerve* were collected from Nilgiri Hills of Western Ghats, Coimbatore and Kanjikode Kerela respectively and certified by Botanical Survey of India, Coimbatore, Tamil Nadu. The plant materials are maintained in BSI Coimbatore under Voucher no: BSIR/RC/5/23/2017/Tech/338, BSIR/RC/5/23/2017/Tech/339 and BSIR/RC/5/23/2017/Tech/340. The leaves were washed thoroughly, dried, powdered and stored in air tight container for the study.

Preparation of Standard Solution: Quercetin (1mg/10ml) was prepared by dissolving 1 mg of quercetin in 10 ml of methanol in a standard flask.

Preparation of Plant Extracts: The leaf powder was defatted with petroleum ether and extracted with Ethanol (70 °C) and water (100 °C) in a Soxhlet apparatus. The extract was then dried and dissolved in a required amount of methanol.

Chromatography and Detection of Quercetin: Chromatography was performed on a 10 × 20 cm precoated HPTLC Silica gel 60 F254 plates (E-Merck, Mumbai, India). Aliquots of each of the extracts were separately applied (Samples and standard) to the plate as 6 mm wide band with an automatic TLC applicator Linomat-5 applicator (CAMAG, Switzerland), 5 mm from the bottom. The mobile phase consisted of Toluene: Ethyl acetate: Formic acid: methanol (5.5:4:1:0.5) and dried in air and scanned densitometrically Fig. 1, 2a and 2b. The calibration curve of quercetin was found to be linear in the range of 4 to 32 mg/spot. A good linear relationship of standard quercetin was found to be R² = 0.998 concerning the concentration and peak area Fig. 3 and 4. The regression equation was found to be Y = 1268x + 1578 with respective to concentration.

![Fig. 1: Densitometric Chromatogram of Quercetin and Leaf Extracts (3D View)](image)

The total number of peaks was found to be 10, 12 and 12 in ethanol extracts of *E. glandulosum, E. odoratum*, and *E. triplinerve* respectively Fig. 5, 6 and 7. The water extracts of *E. glandulosum, E. odoratum* and *E. triplinerve* showed 9, 7 and 10 peaks respectively Fig. 8, 9 and 10. The Rf value of standard quercetin was determined as 0.54 Fig. 3. In the plant samples, the ethanol and water extracts showed the peak at the Rf values of 0.54 and 0.55 Fig. 5, 6, 7, 8, 9 and 10.
A- Ethanol extract of *E. glandulosum*; B- Aqueous extract of *E. glandulosum*; C- Ethanol extract of *E. odoratum*; D- Aqueous extract of *E. odoratum*; E- Ethanol extract of *E. triplinerve*; F- Aqueous extract of *E. triplinerve*; G-N- Different concentration of quercetin
Among the selected three plants, the ethanol extract of *E. glandulosum* showed the maximum amount of quercetin (17.44 mg/g) followed by *E. odoratum* (13.40 mg/g) and *Eupatorium triplinerve* (9.29 mg/g). Water extracts showed a minimum amount of quercetin (2.67 mg/g) in *E. triplinerve* Table 1 and 2.

Apart from quercetin, all the leaf extracts showed many numbers of peaks at different $R_f$ values which shows the presence of various other compounds.
Similarly, the ethanolic extract of *Calamus rotang* showed the Rf value of quercetin at 0.54 but in contrast, a polyherbal syrup zymodyne and methanolic leaf and flower extract of *Moringa oleifera* showed the presence of quercetin at the Rf value of 0.86 and 0.35 respectively. The HPTLC analysis of an aqueous extract of *Eruca sativa* was found to be 17.94 which is equal to *E. glandulosum*.

### Table 1: Peak Table of Quercetin in Leaf Extracts

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Plant name</th>
<th>Solvent name</th>
<th>Total number of peaks</th>
<th>Peak no.</th>
<th>Rf</th>
<th>Peak area</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Eupatorium glandulosum</em></td>
<td>Ethanol</td>
<td>10</td>
<td>8</td>
<td>0.55</td>
<td>21395</td>
<td>37.74</td>
</tr>
<tr>
<td>2</td>
<td><em>Eupatorium glandulosum</em></td>
<td>Aqueous</td>
<td>9</td>
<td>5</td>
<td>0.54</td>
<td>6258</td>
<td>13.57</td>
</tr>
<tr>
<td>3</td>
<td><em>Eupatorium odoratum</em></td>
<td>Ethanol</td>
<td>12</td>
<td>8</td>
<td>0.55</td>
<td>14157</td>
<td>23.42</td>
</tr>
<tr>
<td>4</td>
<td><em>Eupatorium odoratum</em></td>
<td>Aqueous</td>
<td>7</td>
<td>6</td>
<td>0.55</td>
<td>4411</td>
<td>7.85</td>
</tr>
<tr>
<td>5</td>
<td><em>Eupatorium triplinerve</em></td>
<td>Ethanol</td>
<td>12</td>
<td>9</td>
<td>0.55</td>
<td>10655</td>
<td>19.42</td>
</tr>
<tr>
<td>6</td>
<td><em>Eupatorium triplinerve</em></td>
<td>Aqueous</td>
<td>10</td>
<td>8</td>
<td>0.55</td>
<td>5013</td>
<td>10.02</td>
</tr>
</tbody>
</table>

As per the literature, quercetin possesses biological and therapeutic effects including anti-cancer, anti-oxidative, anti-microbial and anti-inflammatory, cardioprotective and hepatoprotective activities. Hence, from the above findings, it is confirmed that the leaf possesses a good amount of quercetin content.

### Table 2: Quercetin Content in Plant Extracts

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Name of the plant extract</th>
<th>Amount of Quercetin (mg/g)</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. glandulosum</em></td>
<td>17.44</td>
<td>2.92</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>E. odoratum</em></td>
<td>13.40</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>E. triplinerve</em></td>
<td>9.29</td>
<td>2.67</td>
<td></td>
</tr>
</tbody>
</table>

### Conclusion:

The quantitative estimation of quercetin was analyzed in three different species of *Eupatorium* leaves by HPTLC fingerprinting technique. The quercetin content was found to be maximum in the ethanolic extract of all the three plants than water extract. The ethanolic extract of *E. glandulosum* leaves showed maximum quercetin content than other two species studied. Since, the leaves possess the promising amount of quercetin, it can be used for curing various ailments.

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### Conflict of Interest:

The authors declare no conflicts of interest.

### References:


