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Flavonoid content of *Eupatorium glandulosum* and *Coolebroke oppositifolia*

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Abstract

Objective: Estimation of quercetin content of *Eupatorium glandulosum* and *Coolebroke oppositifolia* by HPLC. Materials and methods: The presence of quercetin (3, 3^{l}, 4^{l}, 5, 7 - pentahydroxyflavone) present in the leaves of *Eupatorium glandulosum* (Family- Asteraceae) and *Coolebroke oppositifolia* (Family : Labiatae) was quantitatively estimated by high performance liquid chromatography using reversed phase (RP-HPLC) with stepwise gradient elution on a RP C18 column. A two step elution with: acetonitrile- water- phosphoric acid (85%): from 16-83-1 to 32-67-1(v/v) was made. Results: The leaf extracts of *E. glandulosum* and *C. oppositifolia* contained 4.96 and 2.10 % and the respective powdered leaf contained 0.19 % (w/w) and 0.09 % (w/w) of quercetin . Conclusion: The method developed is useful to obtain quantitative values for quercetin present in these plant species, which may be useful for standardization of the same.

Keywords: *Eupatorium glandulosum, Coolebroke oppositifolia*, Flavonoid, Quercetin, RP-HPLC.

1. Introduction

Eupatorium glandulosum (Asteraceae) is a profusely branching undershrub upto 90-120 cm in height with a few ascending branches; leaves simple, opposite, subsessile, lanceolate, subentire and glabrous type. The leaves are used as astringent, thermogenic and stimulant in folklore medicine in India [1]. Presence of flavonoid glycosides in the leaves has been reported [2-3].

Coolebroke oppositifolia Smith (Labiatae) is distributed in hilly parts of India upto an altitude of 4000m [1]. It is a functionally dioecious, much branched shrub; leaves are light green, crowded towards the end of branches. The leaves are having potential therapeutic activity in wound healing and inflammation [4]. Different flavonoids such as 5,6,7-trimethoxyflavones and 5,6,7,4^{l}-tetramethoxyflavones etc have been reported to be present in different parts of this plant [5,6].

In the present investigation we propose an RP-HPLC method for the estimation of quercetin present in the leaf extract of these two plant species.

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2. Materials and methods

2.1 Plant Material

Fresh leaves of *E. glandulosum* and *C. oppositifolia* were collected from Ootacamund, a hill station of the district Nilgiri of Tamilnadu, India during January-February, 1999. It was identified by the Botanical Survey of India, southern circle, Coimbatore. A reference specimen of all the samples collected have been kept in our laboratory. The leaves were dried in the shade (25°C), and pulverized separately in a mechanical grinder, passed through a 40-mesh sieve and stored in a closed vessel for future use.

2.2 Extracts and Standard Used

To remove chlorophyll and other polar materials, the powdered leaves of both the plants used were extracted with diethyl ether using a soxhlet extraction apparatus. The marc obtained thereafter was extracted with ethanol-water (9:1 v/v) through maceration for one week. After the maceration was over it was filtered and concentrated in a rotavapour under reduced pressure. The semisolid mass thus obtained was lyophilized. This lyophilized ethanol extract was used for further study. The yield of different extracts from various plant species has been shown in Table 1. The standard sample, quercetin was obtained from the sample bank of the Dept of Pharmacognosy, Leiden/Amsterdam Center for drug Research, Leiden, The Netherlands.

2.3 Chemicals Used

All solvents used were of HPLC grade for the HPLC eluent and for extraction p.a. quality. Acetonitrile was obtained from Rathburn Chemicals Ltd., Scotland. Methanol was obtained from JT Baker, Deventure, The Netherlands. Water was obtained using Milli-Q water purification system (Millipore, Bedford, USA). All other reagents and solvents were from Merck, Darmstadt, Germany. Mobile phase was filtered over a 0.45 μm nylon membrane filter (type NL17, Schleicher & Schull, Dussel, Germany) and degassed by vacuum.

2.4 Preparation of Sample

The freeze dried extract of the leaves of both the plants (1 g) was diluted with 10 ml solvent system 'A' containing - acetonitrile : water : phosphoric acid (16:83:1). Solubilisation was improved by using an

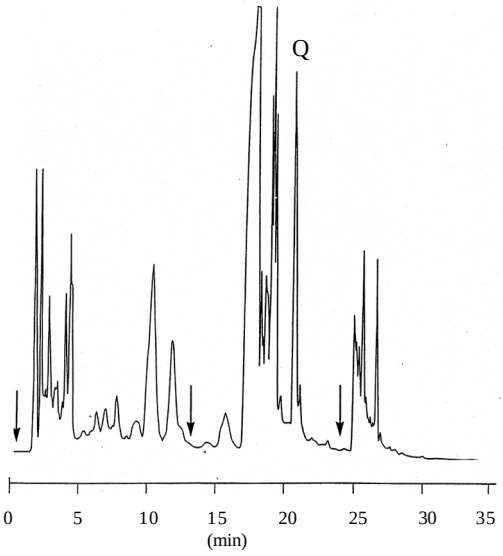

Fig. 1. Chromatogram of *Eupatorium glandulosum* extract. Column: C18, 250 X 4.6 mm; flow rate 2.0 ml/min; Q = Quercetin; UV: 254 nm

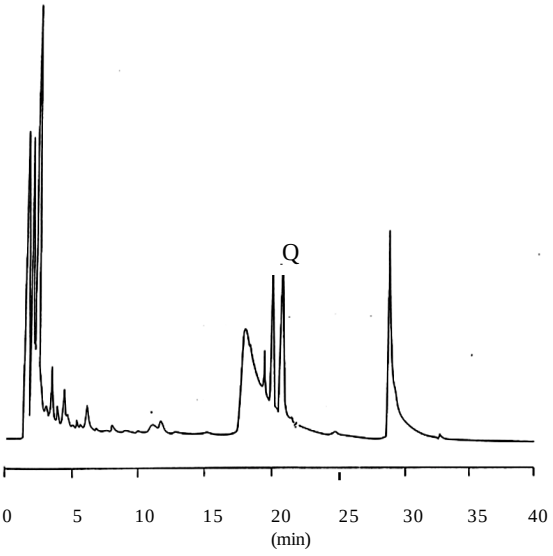

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Fig. 2. Chromatogram of *Coolebroke oppositifolia* extract. Column: C18, 250 X 4.6 mm; flow rate 2.0 ml/min; Q = Quercetin; UV: 254 nm.

Table 1. Extractive values with concentration of quercetin present in leaf extract of different plant species [Each data represent average of three determination ± SE for quercetin content].

Plant Name	Extractive values (% w/w)	Quercetin content (% w/w)[a]	
		Extract	Powdered drug
E. glandulosum	3.9	4.96 ± 0.21	0.19 ± 0.02
C. oppositifolia	4.3	2.10 ± 0.09	0.09 ± 0.04

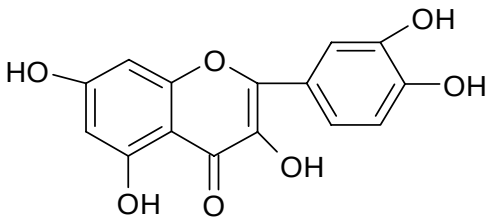
[a] n = 3

ultra sonicator (Sonocor model SC-50-22 of Sonicol Instrument Corporation, NY, USA). Each sample was centrifuged for 2 min at 15500 g (14000 rpm) in an eppendorf using centrifuge (Hermle Z 231M, Germany) to remove the undissolved components. The quercetin standard solution was made with methanol and from there further dilution was done with the respective solvent system used and in every case 50µl of these solution were injected.

2.5 Chromatographic Analysis

The HPLC system consisted of LKB pump, type 2150 (Bromma, Sweden), an autosampler, type WISP 710B (Waters) a photodiode array detector type 990 (Waters) and a Waters 5200 printer plotter was used. Columns used were Phenomenex Hypersil 5 C18, 250 X 4.6 mm (Phenomenex, Torrance, CA, USA). For elution two different mixtures of mobile phases with varying concentration of solvents were used. Mobile phase 'A' containing - acetonitrile : water : phosphoric acid (16:83:1); 'B' was- acetonitrile:water:phosphoric acid (32:67:1).

The presence of quercetin (Rf 0.80) in the plant extracts were confirmed by TLC co-elution with the authentic sample using the solvent system ethyl acetate:formic acid :water (85:10:5) over silica gel G plates (Merck) [7]. Quantification of these flavonoids was performed by using the method reported by Kartnig *et al* [8] with some modification [9]. Before starting every measurement the column was equilibrated to normal starting condition by running the solvent system 'A' for 10 min at a flow rate of 2 ml/min.



QUERCETIN

Throughout the analysis the flow rate was 2 ml/ min, run time was 1-15 min with eluent A then changed with eluent B for 15-35 min with eluent B respectively. 50µl of each sample was injected and the specific solvent system was changed accordingly- starting with eluent 'A' then changed to eluent 'B' at 15th minute. Detection was carried out at 254 nm.

Quantitative analysis of quercetin (t^R 20.9 min) was made by injecting known amounts of this compound. The calibration curve in duplicate for quercetin was made using HPLC peak area (at 254 nm) *vs* concentration. The peak areas of quercetin in the plant extracts were with in the straight part of the curve. From this curve the concentrations of quercetin in different extracts were calculated using the area under curve (AUC) at the specific retention time of the compound.

3. Results and Discussion

In this study an attempt has been made to develop an HPLC system for the quantitative analysis of quercetin in the aerial parts extracts of two different plant species (*Coolebroke oppositifolia* and *Eupatorium glandulosum*) available in India. A stepwise gradient with different eluent in several concentrations was used to obtain sufficient resolution and suitable retention. Fig.

1 and Fig. 2 show the HPLC chromatograms of *E. glandulosum* and *C. oppositifolia* respectively, where the presence of quercetin was detected.

The presence of quercetin was confirmed by comparing the chromatographic behavior of the individual extracts with that of the standard solution of quercetin. Amount of the extract (% yield of extractives corresponding to dry powdered materials) is shown in Table 1. From the standard curve the amount of quercetin present in the extracts as well as in the powdered plant materials has been calculated and shown in Table 1. Both the plant species contain quercetin in varying amounts, the concentration of the same in *E. glandulosum* was higher than *C. oppositifolia.*

Owing to the high separation power of the liquid chromatographic system used in this investigation and to the selectivity of the detector, this method allowed us to obtain quantitative values for the quercetin present in these plant species, which may be useful for the standardization of these plants species.

4. Acknowledgements

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