

Further Investigation into the Flavonoid Profile of *Balsamorhiza Macrophylla* (Nutt.) A. Gray (Asteraceae)

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Abstract

This research has identified the vacuolar flavonoids of *Balsamorhiza macrophylla* Nutt., which consist of Quercetin-3-O-galactoside, Quercetin-3-O-rutinoside, and Quercetin-7-O-glucoside. Exudate lipophilic flavonoids were also investigated. Results vary slightly from those of earlier study. This study shows the presence of Quercetin 3 - O - methyl ether, Quercetin 3,6,4'- O - trimethyl ether, Quercetin 3,4'-O-dimethyl ether, 3,3'-O-dimethyl ether and an unknown compound which absorbs at 290nm, possibly a flavanone. The first three compounds agree with earlier results, which also include Tamarexetin, Axillarin and Spinacetin. Workers had reported earlier that flavonoid profiles often vary due to environmental or ecological differences, and whether this applies to *Balsamorhiza macrophylla* is part of this investigation.

Keywords: *Balsamorhiza macrophylla*, Asteraceae, flavonoid, quercetin O-glycosides, quercetin O-methyl ether

1. Introduction

Flavonoids are phenolic compounds with nuclei arranged in a C₆ - C₃ - C₆ configuration (see Figure 1). They are one of the largest groups of naturally occurring phenolic compounds, which are likely to be encountered in any plant extract (Markham, 1982). Most flavonoids are glycosylated, and glycosylation makes them less reactive and more water (sap) soluble (Onyilagha, 1994; Onyilagha and Grotewold, 2004). This in turn helps in the storage of these compounds in the cell vacuole, where they are often found (Grotewold, 2004). Plant flavonoids are generally stored in the vacuoles (Harborne and Turner, 1984). However, some flavonoids are known to accumulate on the surfaces of leaves, flowers, and other tissues. These are known as surface, external, or exudate flavonoids. In some cases, they are simply referred to as lipophilic flavonoids (Williams et al., 1993). It should be noted however, that some vacuolar flavonoids are also lipophilic in nature. For example, the plant species *Vellozia streptophylla* (Velloziaceae) accumulates lipophilic flavonoids such as quercetin 3-methyl ether, and quercetin 3,4'-dimethyl ether in their leaf vacuoles (Williams et al., 1993), but these compounds appear as leaf surface flavonoids in *Balsamorhiza macrophylla* (Asteraceae) (McCormick et al., 1985). Research results are overall consistent in showing that surface flavonoids are predominantly aglycones, although the presence of quercetin 7-O-glucoside was reported in leaf exudates of *Chrysanthemum segetum*, and quercetin 3-O-glucoside, quercetagenin O-3-glucoside and quercetagenin 6-Me-7-O-glucoside in leaf exudates of *Heteranthemis viscidhirta* (Valant-Vetschera et al., 2003).

The plant species, *Balsamorhiza macrophylla* (Nutt.) A. Gray is one of the shrubby members of the family Asteraceae (Heliantheae - Verbesininae). Bremer (1994) has given a detailed account of the family and placed the genus under the subtribe Verbesininae, members of which are widely distributed in North and South America, Africa, tropical Asia and the Pacific. The genus *Balsamorhiza* is however restricted to western United States, southwestern Canada and Mexico (Bremer, 1994). Keil (1993) described *Balsamorhiza macrophylla* as a shrub with erect stems and branching from base. Leaves are short petioled, ovate to round, entire to serrate-lobed, glandular. Inflorescence characteristically a head, 10 - 12mm, 1 - 3 per panicle on short branches.

Earlier studies (McCormick et al., 1985; Bohm and Choy, 1987) show that members of the genus *Balsamorhiza* are rich in both exudate and vacuolar flavonoids. Although the exudate flavonoids of *Balsamorhiza macrophylla* had been studied, the vacuolar flavonoid profile is not yet given equal attention. This study is designed to identify the vacuolar flavonoids of *Balsamorhiza macrophylla* and to determine whether flavonoids in this species vary due to environmental or ecological differences.

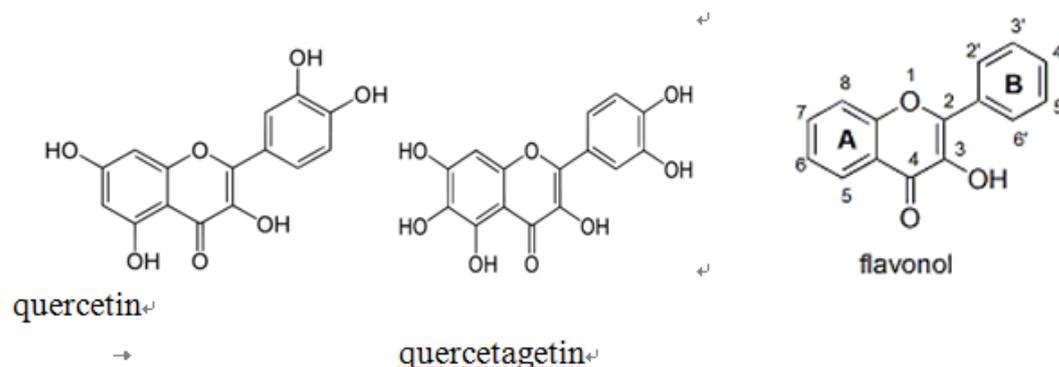


Figure 1. Basic structure of quercetin, quercetagenin and flavonol flavonoids. Quercetin is a flavonol with OH (hydroxy) group at 3, 5, 7, 3', 4' positions. Kaempferol is a flavonol with OH group at 3, 5, 7, 4' positions. Quercetagenin is a flavonol with OH group at 3, 5, 6, 7, 3', 4' positions

2. Materials and Methods

Plant material: Leaves of *Balsamorhiza macrophylla* were collected on the hills east of Hyde Park, about 50 kilometers south of Cache National Forest, Utah, in the United States. A voucher specimen is deposited in our laboratory.

Extraction and separation of surface flavonoids: Flavonoid extractions followed the procedures as detailed in Onyilagha and Grotewold (2004); McCormick et al. (1985). Briefly, dried leaves were washed three times in methylene dichloride to extract the surface flavonoids. The extract was taken to dryness *in-vacuo*. The residue was extracted with MeOH and chromatographed over a polyclar AT column using dichloro-methane : methanol (3 : 1) and with increasing amounts of MeOH. Fractions from the column were further separated on silica gel G plate using toluene-acetic acid (4:1). Flavonoid bands were separately chromatographed in 30% HOAc on cellulose avicel plates and finally resolved on polyamide layer using ethyl formate, cyclohexane, n-butanol, formic acid (50:25:23:2 respectively).

Vacuolar flavonoids: The washed leaf materials were completely air dried prior to determination of the vacuolar flavonoids. About 50 g dried leaves were extracted in 70% ethanol in a boiling water-bath for 10 min (Onyilagha, et al., 2009). The extract was allowed to stand overnight for complete extraction, and then evaporated to dryness using a rotary evaporator. The residue was extracted with boiling water, filtered, and the flavonoids then extracted in saturated n-butanol. The butanol extract was dried *in-vacuo* and the flavonoids taken up in MeOH. Subsequent treatments followed the methods of Onyilagha et al. (2003).

Flavonoid aglycones: About 5 g of macerated dry leaves were hydrolyzed in approximately 20 ml of 2 M HCl in a glass tube, followed by heating in a boiling water-bath for 10 min, and cooled at room temperature. Flavonoid aglycones were extracted in ethyl-acetate, and the ethyl acetate was evaporated in fume-hood. Subsequent treatments of flavonoid residues followed the same process as shown in Poe et al. (2013).

The exudate, vacuolar, and aglycone flavonoids are known compounds. They were readily identified by their colour under the UV light in the presence of ammonia fumes, and by

comparing their *rf* values, retention time in hplc, and spectral data with information from literature (Onyilagha, 1994; Williams, et al., 1993, 1995; McComick, et al., 1985). They were also subjected to co-chromatography with authentic markers in three solvents such as BAW, FORESTAL, and 50% acetic acid in water (Harborne and Harborne, 1998; Onyilagha, 1994).

3. Results

Three vacuolar and five exudate compounds were identified. Quercetin was the only aglycone flavonoid identified from acid hydrolysed plant leaf materials. The vacuolar flavonoids were quercetin 3-O-galactoside, quercetin 3-O-rutinoside (rutin) and quercetin 7-O-glucoside. The exudate flavanoids were quercetin 3-O-methyl ether; quercetin 3,3'-O-dimethyl ether; quercetin 3,4'-O-dimethyl ether; quercetagenin 3,6,4'-O-trimethyl ether and one unknown compound (Table 1). The unknown compound has a single peak, which absorbs at 290nm, possibly a derivative of flavanone.

Table 1. Vacuolar and exudate flavonoids in *Balsamorhiza macrophylla*

Flavonoid Group	Plant Leaf Materials Were Obtained From:		Reference
	Hyde Park, Utah, USA	Tony Grove, Utah, USA	
Vacuolar Flavonoids:			
Qu 3-galactoside	✓		In this study
Qu 3-rutinoside	✓		In this study
Qu 7-glucoside	✓		In this study
Exude Flavonoids:			
Qu 3-methyl ether	✓	✓	In this study & McCormick et al., 1985
Qu 3,3'-dimethyl ether	✓		In this study
Qu 3,4'-dimethyl ether	✓	✓	In this study & McCormick et al., 1985
Quercetagenin 3,6,4'-trimethyl ether (Centaureidin)	✓	✓	In this study & McCormick et al., 1985
Unknown (Flavanone?)	✓		In this study
Quercetagenin 3,6-dimethyl ether (Axillarin)		✓	McCormick et al., 1985
Quercetagenin 3',6-dimethyl ether (Spinacetin)		✓	McCormick et al., 1985
Qu 4'-methyl ether (Tamarexetin)		✓	McCormick et al., 1985

Qu = Quercetin

A comparison of our results with earlier study (McCormick et al., 1985) is shown in Table 1. The absence of surface flavonoids such as axillarin, spinacetin and tamarexetin in our

specimen is very noticeable and same may be said of the absence of vacuolar flavonoids and surface compounds, example, quercetin 3,3'-O-dimethyl ether and flavanone-like (derivative) compound from the specimens of the earlier workers.

4. Discussion

Results point to the dominance in this species of quercetin based flavonoids in both the vacuolar and exudate compounds. This agrees with the results of McCormick et al., (1985). The inability of this species to accumulate kaempferol to a detectable concentration seems to be an important characteristic. However, there is need to study the flavonoid profiles in other species of *Balsamorhiza* to enable an informed statement on the use of quercetin flavonoid as a taxonomic marker in this genus. Accumulation of exudate lipophilic flavonoids in the species is not surprising because the species is a member of the plant family Asteraceae, a family whose species have been known to accumulate varying structures of flavonoids on their leaf surfaces (Onyilagha and Grotewold, 2004).

The lack of agreement in results of accumulated surface flavonoids in this study and that of McCormick et al (1985) is surprising. This study did not detect axillarin, spinacetin and tamarexetin flavonoids as did McCormick and co-workers, and they did not detect quercetin 3,3'-O-dimethyl ether and a flavanone derivative as we did. In a similar situation, Rieseberg et al (1987) observed considerable variation in flavonoid profile in *Helianthus annuus*, but could not show a definable phylogeographic patterns among the compounds detected. Nevertheless, plant materials used by McCormick and co-workers were obtained from Tony Grove, Cache National Forest, Cache Co, Utah, USA. Our specimen was obtained from hills east of Hyde Park, about 50km South of Cache National Forest, Utah. Although environmental or ecological situations influence flavonoid accumulation, but it may be too hasty to suggest that environmental and/or ecological differences between the two sites may have contributed to the variation in flavonoid compounds accumulated because many more species of this genus need to be investigated. However, Bohm (1987) in studying the flavonoids of *Luetkea* species from different populations in North America observed that the southernmost populations showed increased tendency to produce acylated flavonoid monoglucosides, coupled with decreased levels of eriodictyol 7-O-glucoside. Samples from the Queen Charlotte Islands were different apparently due to the absence of eriodictyol 7-O-glucoside. In another study, Wells and Bohm (1988) observed some variations in flavonoid compounds of *Luetkea pectinata* materials from different locations. In relation to the present study, it may not be adequate at this moment to regard the species, *Balsamorhiza macrophylla* or indeed the genus *Balsamorhiza* as a group that exhibits inter-populational flavonoid variation. It may be necessary therefore to study many more specimens from several other locations in order to arrive at a convincing conclusion.

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