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## LEAF PHENOLICS OF *GUNNERA MANICATA* (GUNNERACEAE)

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### ABSTRACT

Foliar material of *Gunnera manicata* (Gunneraceae) was examined for the presence of phenolics using standard extraction and identification techniques. Two flavonoids, quercetin 3-O-galactoside and quercetin 3-O-glucoside and an unidentified ellagitannin are reported as occurring in *G. manicata*. The presence of quercetin 3-O-galactoside and a high concentration of ellagitannins offers some phytochemical support for a putative relationship between the Gunneraceae and Saxifragales.

Key words: *Gunnera manicata*, Gunneraceae, Saxifragales, leaf phenolics, ellagitannin, flavonoids.

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### INTRODUCTION

The Gunneraceae consist of about 40 species of small to gigantic perennial herbs, primarily distributed disjunctively in the Southern Hemisphere. Unique among the angiosperms, *Gunnera* L. is the only genus known to have an intracellular endosymbiosis with the Cyanophyta (Silvester and Smith 1969). The genus *Gunnera* is phylogenetically problematic and has traditionally been placed in the Haloragaceae (Cronquist 1981). More recent studies emphasizing morphological, anatomical, embryological, palynological, and cytological differences between *Gunnera* and putatively related Haloragalean taxa support the treatment of *Gunnera* as a separate family, the Gunneraceae (Dahlgren 1975, 1983; Cronquist 1981).

Because the Gunneraceae are poorly characterized chemically, an investigation of the leaf phenolics of *Gunnera manicata* Linden was initiated to provide biochemical data for comparative biosystematic purposes.

### MATERIALS AND METHODS

Dried leaf material of *Gunnera manicata* was obtained from plants in cultivation at the Royal Botanical Garden (Edinburgh) (RSA voucher: Doyle VIII-1-86/1).

Leaf phenolics were extracted into 80% MeOH, filtered, concentrated under vacuum and samples used for subsequent two-dimensional paper chromatography (2D-PC) and column chromatography. Initial 2D-PC separation on Whatmann 3MM paper was performed using TBA and 15% HOAc as solvents (Mabry, Markham, and Thomas 1970). Resultant chromatograms were examined first under UV light and then sprayed with 3% aqueous ferric chloride in order to locate phenolic compounds.

Preparative quantities of the phenolic compounds were separated by column chromatography (2 × 25 cm) over Polyclar AT. Fractions containing phenolics were identified by 1D-PC, those containing the same compound were pooled together and concentrated by volume reduction under vacuum. The purified compounds were characterized by acid and enzymatic hydrolysis, paper chromatography, and UV-spectroscopy using standard methods of Mabry, Markham, and Thomas (1970).

Table 1. Chromatographic properties of *Gunnera manicata* leaf phenolics.<sup>1,2</sup>

Compound	$R_f (\times 100)$			
	TBA	HOAc	BAW	H <sub>2</sub> O
1 (Q 3-glu)	43	51	58	14
2 (Q 3-gala)	36	41	59	08
3 (Ellagitannin)	04	48	16	11

<sup>1</sup> Solvents: TBA, t-BuOH-HOAc-H<sub>2</sub>O (3:1:1); 15% HOAc; BAW, BuOH-HOAc-H<sub>2</sub>O (4:1:5).

<sup>2</sup> Uncorrected values.

A separate sample (0.1 g) of dried leaf material was examined for ellagitannins following the procedure of Bate-Smith (1972).

## RESULTS

### Flavonoids

Initial examination of methanolic extracts of *Gunnera manicata* foliage by 2D-PC revealed one major (compound 1) and one minor (compound 2) flavonoid constituent. The fluorescence color of both glycosides on paper under UV light was dark, changing to bright yellow with ammonia vapor, which suggested that their structure was a 3-OH substituted flavonol with a free 4'-OH. Larger quantities of the two flavonoids were purified by column chromatography. Both of these compounds yielded quercetin upon acid hydrolysis (identified by co-chromatography in four solvents: BAW, Forestal, 15% HOAc, and TBA).

Chromatographic and spectroscopic properties of the two quercetin glycosides are presented in Tables 1 and 2.

Both compounds yielded quercetin after enzyme hydrolysis. Compound 1 was completely hydrolyzed by  $\beta$ -glucosidase treatment, but compound 2 was only partially hydrolyzed.

Chromatographic and spectroscopic properties of compounds 1 and 2 agree with published values of quercetin 3-O-glucoside (Q 3-glu) and quercetin 3-O-galactoside (Q 3-gala), respectively (Harborne 1967; Mabry et al. 1970). The identification of quercetin 3-galactoside is based mainly on chromatographic properties and must remain provisional at present. The relatively small amount of this constituent precluded a more complete characterization of this compound.

### Ellagic Acid

The presence of ellagic acid was noted based on its chromatographic properties in TBA, BAW, 15% HOAc, and H<sub>2</sub>O and its characteristic fluorescence color under UV illumination. Ellagic acid is present in easily detectable amounts in the original methanolic extract, but is present in much greater quantities in acid-hydrolyzed samples. This observation suggested the presence in leaf tissues of substantial amounts of ellagitannins.

### Ellagitannins

An aqueous methanolic extract of *Gunnera manicata* was subjected to a modified Hoepfner reaction (Bate-Smith 1972) and produced a dark blue reaction, characteristic of ellagitannins. One-dimensional paper chromatograms of the

Table 2. Spectroscopic properties of *Gunnera manicata* flavonoids. Shoulders are enclosed in parentheses and absorption maxima are expressed in nanometers.

	Compound	
	1	2
MeOH	258, (268), (298), 360	257, (267), 294, 358
+NaOMe	274, 328, 410	272, 325, 409
+AlCl <sub>3</sub>	275, (301), (320), 429	276, (302), (320), 434
+AlCl <sub>3</sub> /HCl	269, (301), 365, 400	274, (301), 363, 401
+NaOAc	274, 325, 380	273, 331, 372

methanolic leaf extract in several solvents were sprayed with FeCl<sub>3</sub> and revealed one major (compound 3) and several minor dark grey spots, also verifying the presence of ellagitannins (Block, Durrum, and Zweig 1958). The chromatographic properties of the major ellagitannin constituent (compound 3) are shown in Table 1. These chromatographic properties do not correspond closely with those of any of the few presently well-defined ellagitannins (Hathaway 1969).

#### DISCUSSION

Like many other taxa with highly reduced flowers, the phylogenetic relationships of *Gunnera* remain obscure. Various phylogenetic affinities have been suggested including the Haloragales s.l. (e.g., Bentham and Hooker 1865; Schindler 1905; Thorne 1968; Takhtajan 1969; Hutchinson 1973; Cronquist 1981), Myrtales (Onagraceae) (Gibbs 1974; Moore 1978), Saxifragales s.l. (Huber 1963; Dahlgren 1975; Takhtajan 1980), Umbellales (Gibbs 1974), Urticales (Jussieu 1789; Endlicher 1837), and even the Balanophoraceae (Mabberley 1978; Hansen 1980).

Many putative phylogenetic associates of the Gunneraceae are as poorly characterized as *Gunnera* itself; thus, little comparative phytochemical analysis is possible. The results from this study offer some new evidence for an alliance between the Gunneraceae and Saxifragales. Support for this postulate could be drawn from the occurrence of quercetin 3-galactoside in some members of the Saxifragaceae (e.g., *Tellima*, *Tiarella*, and *Sullivantia*) (Collins and Bohm 1974; Soltis 1980; Soltis and Bohm 1984) and its apparent occurrence in *Gunnera manicata*. The presence and abundance of ellagitannins in *G. manicata* is also notable; high concentrations of tannins are characteristic of many perennial herbaceous taxa within the Saxifragaceae (Bate-Smith 1973). Bate-Smith (1973) has suggested the use of tannins as a major character in the systematic classification of the dicots. Identification and quantification of tannins from *Gunnera* and potentially allied taxa may shed additional light on the systematic position of the Gunneraceae.

Clearly, more phytochemical data are required on the constituents of *Gunnera* and its possible phylogenetic associates before substantive systematic conclusions can be made.

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