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Short Communication

Analysis of flavonoids in the mature fruit of *Vaccinium uliginosum* L. of China

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SUMMARY

In this study, a new extraction method was developed and two kinds of flavonoids were extracted from the mature fruit of *Vaccinium uliginosum* L. of China. These two kinds of flavonoids were analyzed by spectral and identified by high-performance liquid chromatography (HPLC) and UV/Vis. The extract of the fruit was total acid hydrolyzed. TLC chromatography was subsequently employed to identify the hydrolysate. Two kinds of aglycone flavonoids, quercetin and myricetin, were identified. At the same time PC chromatography was used to identify the monomer sugar in the flavonoids and it was verified as glucose. HPLC, UV/Vis, and Mass spectrum analyses revealed that the flavonoids were quercetin 3-monoglucosides and myricetin 3-monoglucosides.

Key words: Flavonoids; *Vaccinium uliginosum*; Aglycone; Sugar; Fruit

INTRODUCTION

In the present study, a structural analysis is performed on extracted flavonoids from the fruit of *Vaccinium uliginosum* L. of China. The fruit belongs to the Ericaceae genus and is widely distributed in the northern region of China. The mature fruit is dark blue or black in color and is commonly used to make juices, jams, and wines.

Many researchers are interested in flavonoids because of their multiplicity characters including antioxidant, antiinflammatory, antimicrobial, anticancer activities. At present, more than 5000 flavonoids from naturally occurring species have been identified (Hariri *et al.*, 1991; Kater *et al.*, 1996; Ichiyanagi *et al.*, 2004; Mansouri *et al.*, 2005).

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Flavonoids are important secondary polyphenolic metabolite widely distributed in medicinal and edible plants. They also provide fruit and vegetables with flavor and color (Kater *et al.*, 1996).

A new type of extraction method and structural analyses means are developed in the present paper. This study reports in the literature for the first time under the title "Flavonoids in the mature fruit of *Vaccinium.uliginosum*". Recently, various biologic activities in flavonoids have been discovered, including anti-oxidant, anti-fungal and anti-tumor effects. (Hariri *et al.*, 1991; Kater *et al.*, 1996; Ichiyanagi *et al.*, 2004; Mansouri *et al.*, 2005).

MATERIALS AND METHODS

Materials

The mature dark blue fruit of *Vaccinum uliginosum* L. were collected in August 2001 in Hei Long Jiang Province in China. The fruit was stored in a freezer (-20°C).

Extraction and purification of flavonoids

80% methanol was mixed with the desiccated fruit extracted twelve times at room temperature (20°C). After filtration, the extract of the liquid was concentrated and washed with petroleum ether several times. The flavonoids extract was then adsorbed on Polyamide C-200, Sephadex LH20 and Sephadex G-25 column chromatography. Step-wise elution was used to wash the column with H_2O and methanol.

HPLC chromatography

A HPLC chromatography system was used (LiChrospher 100 RP18-5 μm, MERC Kφ4.6×250 mm) at 35°C. Two types of solvent, A-(1.5% phosphoric acid) and B-(1.5% phosphoric acid, 20% acetic acid, 25% acetonitrile) were used for the linear gradient elution. The whole process was completed in 40 min.

RESULTS AND DISCUSSION

In this study, a new extraction method was developed. Two kinds of flavonoids were extracted from the mature fruit of *Vaccinium uliginosum* L. of China. The mature fruit was authenticated, analyzed, extracted and purified, and then adsorbed on Polyamide C-200 column Sephadex LH20 and Sephadex G-25 column chromatography. Later, it was step eluted washed with an admixture of water and methanol.

The result showed that this method is accurate, rapid, and reproducible. The flavonoids were acid hydrolysed and two aglycones and sugar residue were obtained. They were respectively compared with authentic specimens and identified by TLC (Table 2), HPLC (Table 4) and PC chromatography (Table 1). When separated from VF-1 and VF-2, that are quercetin and myricetin of flavonoids regard them as aglycone, as given in Table 2.

The aglycone of the flavonoids was identified by TLC chromatography. The result indicated that the Rf values correspond with two aglycone specimens in Table 2. Spectral shifts (Table 3) of the flavonoids

Table 1. Rg values of sugar of the flavonoids from the *Vaccinium uliginosum* L. of China and the authentic specimens

Sugar	Rg Values on PC (× 100) in BAW II[a]
VF-1	100.0
VF-2	100.0
Glucose	100.0
Galactose	90.0
Arabinose	110.0
Xylose	115.0
Rhamnose	145.0

[a]BAW II: *n*-BuOH: AcOH: H_2O, 4 : 1 : 2, v/v

Table 2. Rf values of aglycone of the flavonoids from the *Vaccinium uliginosum* L. of China and the authentic specimens

Aglycones	Rf Values on TLC(× 100)	
	BAWII[a]	AHW[b]
Aglycone of VF-1	48.0	27.0
Aglycone of VF-2	27.0	16.5
Qurecetin	48.0	27.0
Myrecetin	27.0	16.5
Kaempferol	75.0	38.0

[a]BAW II: *n*-BuOH: AcOH: H_2O, 4 : 1 : 2, v/v; [b]AHW: AcOH : HCl : H_2O, 30 : 3 : 10, v/v

of the *Vaccinium uliginosum* L. of China and the residue of the sugar are glucose, as shown in Table 1. PC chromatography was used to identify the sugar of the flavonoids based on these results. Both the sugars, VF-1 and VF-2, of the flavonoids are monoglucoside, according to their Rg values, as given in Table 1.

Our results from HPLC retention time of aglycone are identical to other researchers (Ferreres et al., 1994; Andrade et al., 1997; Kaneko and Baba, 1999; Senorans et al., 2000). The spectrum analysis outcome indicated that these two kinds of flavonoids are glucoside at 3-hydroxyl group, as shown in Table 3. VF-1 and VF-2 were identified to be quercetin-3glucoside and myricetin-3glucoside, respectively.

After the absorption spectra of the flavonoids were estimated, the two kinds of flavonoids powder

Teble 3. Spectral shifts of the flavonoids of the *Vaccinium uliginosum* L. from China

Flavonoids	Absorption spectrum (nm)			
	MeOH	+ NaMe	+$AlCl_3$ +HCl	+NaAc+H_3PO_3
VF-1	362.6	409.0	418.8, 400.8	402.4, 476.2
	-	-	-	327.2, 376,2
	257.0	271.2	274.0, 269.2	272.6, 261.2
VF-2	366.8	404.8	417.2. 406.2	397.4, 376.0
		327.6	305.0	270.6, 366.0
	259.0	272.0	273.6, 273.0	228.4, 260.6

Table 4. HPLC retention time of aglycone of the flavonoids from the *Vaccinium uliginosum* L. of China and the authentic specimens

Aglycones	tR (min)
Aglycone of VF-1	26.653
Aglycone of VF-2	20.189
Quercetin	26.693
Myricetin	20.400

were identified by UV/Vis spectrum analyses (220 -500 nm) in MeOH, $AlCl_3$, NaOMe, and NaOAc, as outlined in Table 3. HPLC retention time is compared for the two kinds of aglycone of the flavonoids with quercetin and myricetin of aglycone in Table 4. HPLC and TLC analytic results showed that the two kinds of aglycone of flavonoids of the fruit are quercetin and myricetin. The PC chromatography analysis result showed VF-1 and VF-2 in the sugar of flavonoids is glucose. Two 3-monoglucosides of the flavonoids was identified by UV/Vis.

A new extract method is presented in this paper. Two kinds of flavonoids were identified using HPLC, PC, TLC chromatography and UV/Vis in the fruit of *Vaccinium uliginosum* L. of China. The results indicate that this method is accurate, rapid and repeatable.

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