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Article

Three Phenolic Glycosides from *Gastrodia elata*

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Three phenolic glycosides, gastrodin (1), parishin (2), and parishin B (3) were isolated from the *n*-BuOH fraction of *Gastrodia elata* (Orchidaseae) by medium pressure liquid chromatography. Their chemical structures were identified by the interpretation of their spectral data and direct comparison with literature values. Gastrodin and parishin were isolated as the major constituents in *G. elata*. Gastrodin may be a suitable index component for quality control of *G. elata*.

Key words: Gastrodia elata, *gastrodin, index component, parishin, parishin B*

Gastrodia elata Blume (Orchidaceae), whose Chinese name is Tianma, grows in the woods of the central provinces of China, Korea, and Japan and is a perennial parasitic herbaceous plant. The rhizome of *G. elata* has been traditionally used as a tonic, a sedative, and an antispasmodic [Soka, 1985], and is also listed officially in the Chinese Pharmacopoeia as an important Chinese herbal medicine called Rhizome *Gastrodia*, which is used for the treatments of headache, migraine, dizziness, epilepsy, rheumatism, neuralgia, paralysis, and other neuralgic and nervous disorders [Bensky *et al.*, 2004; Tang and Eisenbrand, 1992]. *G. elata*, a famous foodstuff in China, has long been consumed raw or steamed with chicken as a delicacy [Yang *et al.*, 2007].

Phytochemical studies of *G. elata* revealed the presence of several phenolic compounds including 4-hydroxy-benzylaldehyde, 4-hydroxybenzylmehtylether, 4-hydroxy-benzylalcohol, 4,4'-dihydroxy-dibenzylether, 4,4'-dihydroxy-diphenyl methan, 4,4'-dihydroxybenzyl sulfoxide, 4,4'-dihydroxybenzyl sulfone, 4-[4'-(4''-hydroxybenzyloxy) benzyloxy]benzyl methyl ether, 3-*O*-(4-hydroxybenzyl)-β-sitosterol, gastrodin, parishins, *n*-butyl-β-D-fructopyranoside and gastrol [Hayashi *et al.*, 2002; Li *et al.*, 2007; Lin *et al.*, 1996; Noda *et al.*, 1995; Pyo *et al.*, 2004; Pyo *et al.*, 2006; Taguchi *et al.*, 1981; Yang *et al.*, 2007; Yun-Choi and Pyo, 1997; Yun-Choi *et al.*, 1998; Zhou *et al.*, 1980]. Among the chemical constituents, a phenolic glycoside, namely gastrodin, was isolated as a major constituent and is officially listed as an index component in the Chinese Pharmacopoeia [Yang *et al.*, 2007]. However, to the best of our knowledge, no report has yet been published on whether gastrodin is a major component of *G. elata* native in Korea, and the index component of *G. elata* is not mentioned in the Korean Pharmacopoeia. Therefore, the present work was designed to isolate and identify the major phenolic glycosides in *G. elata*.

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Abbreviations: EIMS, electron impact mass spectrometry; FABMS, fast atom bombardment mass spectrometry; HPLC, high pressure liquid chromatography; MPLC, medium pressure liquid chromatography; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; TMS, tetramethylsilane

Materials and Methods

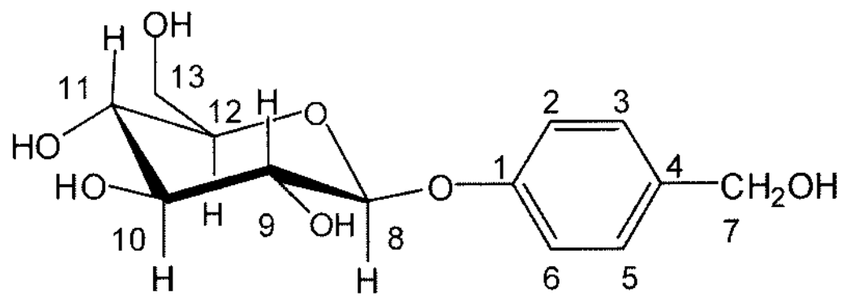
Plant material. The tubers of *G. elata* were collected in Ansung, Muju, Korea, in November 2006, and authenticated by Prof. Young-Bae Seo of the Department of Herbology, College of Oriental Medicine, Daejeon University, Daejeon, Korea. A voucher specimen (KIOM 0079005) has been deposited at the herbarium of Department of Herbal Resources Research, Korea Institute of Oriental Medicine, Korea.

Instruments. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini 2000 instrument (Varian, Palo Alto, CA) using TMS as an internal standard. TMS is a reference for the comparison of all NMR signals generated by the molecule, and NMR signals will be identified based on an internal standard NMR signal. MPLC was performed using a YFLC-GR II equipment (Yamazen equipped with Pump 540, Prep · UV-10 UV detector and GR-202 gradient solvent supporter, Tokyo, Japan) with YFLC 30 cm-long glass columns

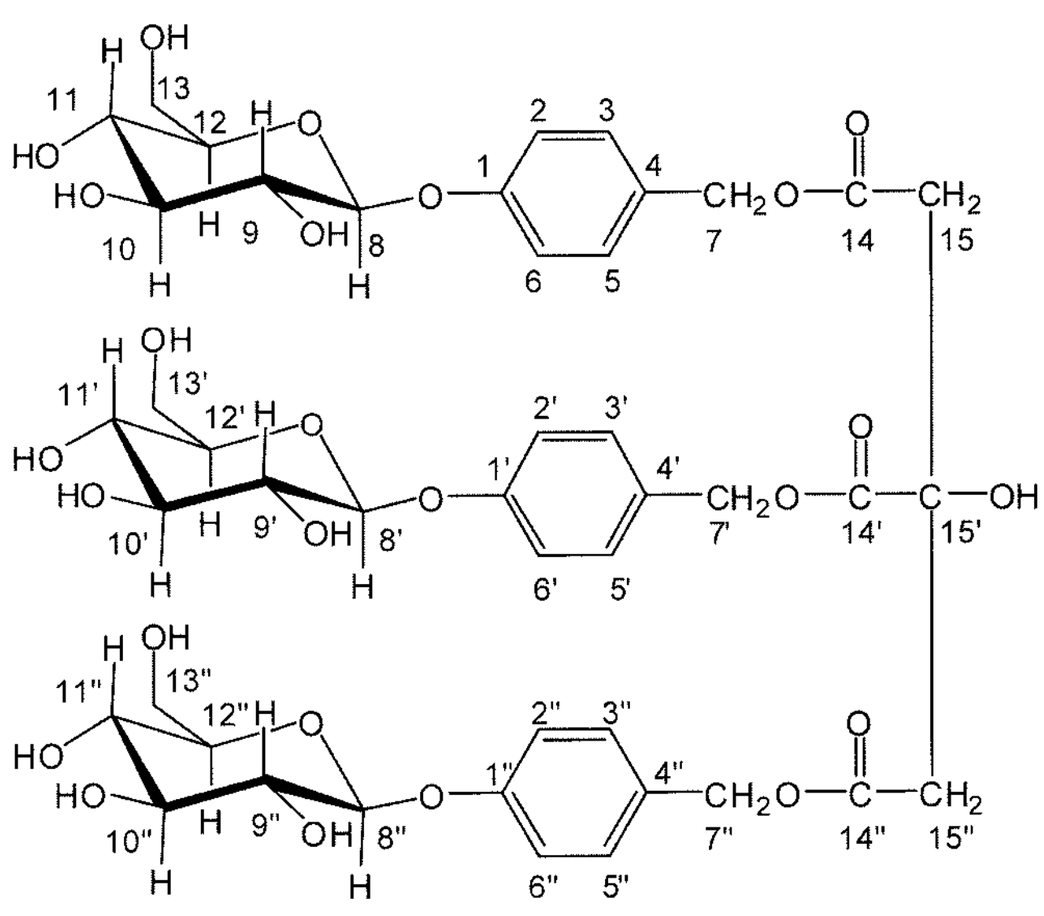
(ϕ = 1.1 or 2.6 cm) packed with YFLC Gel (dp, 40 μm; pore size, 60Å). TLC was performed on silica gel $60F_{254}$ (Merck, Darmstadt, Germany), visualized under a UV lamp or by heating after spraying with 20% aqueous H_2SO_4. All reagent-grade chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC was performed using a Shimadzu SCL-10A VP HPLC system (Kyoto, Japan), consisting of an LC-10AT pump and an SPD-10A VP UV-vis spectrophotometric detector (Shimadzu). Data collection and integration were accomplished using a Chromato-Solution (Shimadzu). For chromatographic analysis, a Grom-SIL120 ODS-5 column (5 μm, 4.6 mm × 150 mm, Grom, Herrenberg, Germany) was used. The mobile phase consisted of methanol (A) and water (B) using a gradient program of 5% A for 0-10 min, 5-60% A for 10-45 min, and 60% A for 45-80 min. The flow rate was 1.0 mL/min, and column temperature was maintained at 30°C. The detection wavelength was set at 224 nm.

Extraction and isolation. Dried tubers of *G. elata* (3 kg) were cut into small pieces and refluxed three times with MeOH. The MeOH extract was suspended in water and partitioned sequentially with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The *n*-BuOH fraction was divided into three fractions (Fr. A~Fr. C) by MPLC using the MeOH : H_2O (40 : 60) solvent system. Fr. A (1.2 g) was chromatographed repeatedly on MPLC to give five fractions (Fr. A-1~Fr. A-5) using the MeOH : H_2O (15 : 85) solvent system. Fr. A-4 (800 mg) was further purified by MPLC [MeOH : H_2O = 15 : 85]. The solvent was delivered at 2 mL/min, and 50-mL fractions were collected using a fraction collector (FR-50N; Yamazen, Tokyo, Japan) to give compound **1** (102 mg). Fr. B (1.1 g) was chromatographed repeatedly on MPLC to yield five fractions (Fr. B1~Fr. B5) using the MeOH : H_2O (30 : 70) solvent system. Fr. B5 (400 mg) was further separated by MPLC [MeOH : H_2O = 25 : 75], with the solvent was delivered at 2 mL/min. Subsequently, 50-mL fractions were collected using a fraction collector (FR-50N) to give compounds **2** (61 mg) and **3** (3.1 mg). The chemical structures of compounds **1**, **2**, and **3** are shown in Fig. 1.

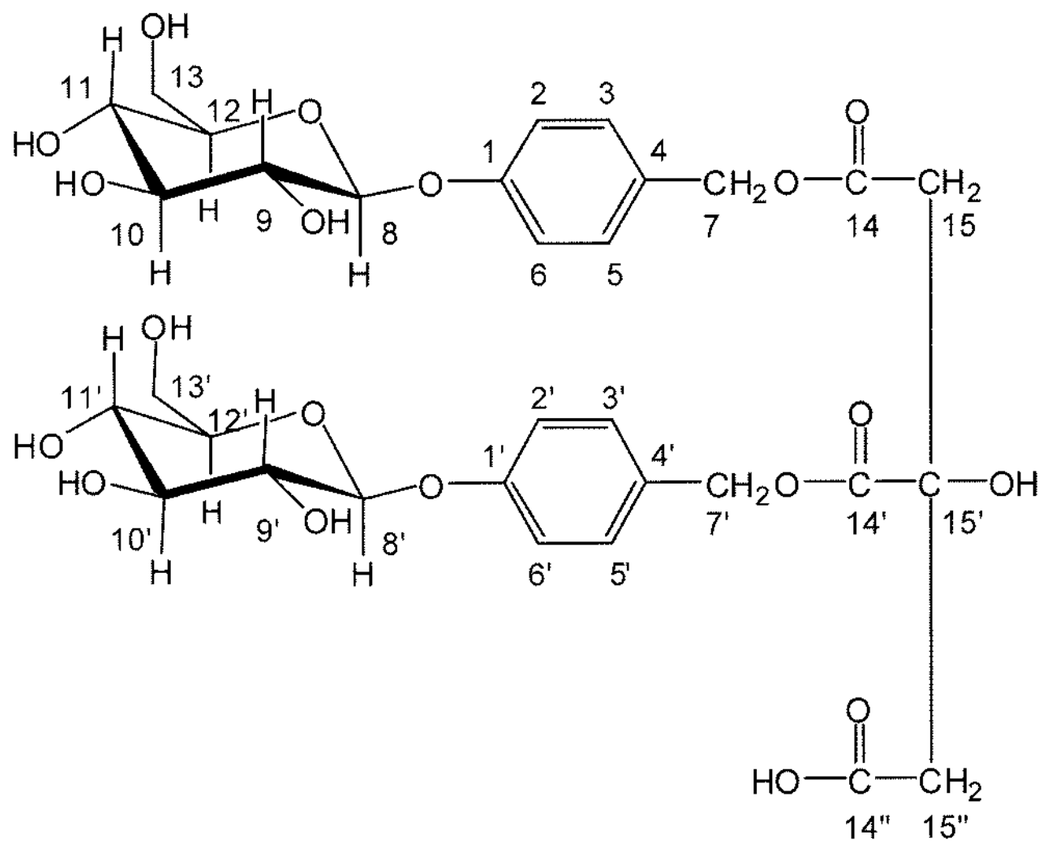
Compound 1: White amorphous powder; m.p. 154~157°C; IR (KBr) ν_{max} (cm^{-1}): 3470, 1874, 1491; UV (EtOH) λ_{max} nm: 269, 221; EIMS *m/z*: 286 $[M]^+$; ^1H NMR (CD_3OD, 300 MHz) δ: 7.28 (2H, d, *J* = 8.7 Hz, H-2, 6), 7.08 (2H, d, *J* = 8.7 Hz, H-3, 5), 4.89 (1H, d, *J* = 7.5 Hz, H-8), 4.54 (1H, s, H-7), 3.90 (1H, dd, *J* = 11.7, 1.5 Hz, H-13a), 3.71 (1H, dd, *J* = 11.7, 4.8 Hz, H-13b), 3.55~3.35 (4H, m, H-9, 10, 11, 12); ^{13}C-NMR (CD_3OD, 75 MHz) δ: 158.65 (C-4), 136.81 (C-1), 129.54 (C-2, 6), 117.84 (C-3, 5), 102.61 (C-8), 78.29 (C-10), 78.17 (C-12), 75.09 (C-9), 71.56 (C-11), 64.97 (C-7), 62.69 (C-13).



gastrodin (**1**)



parishin (**2**)



parishin B (**3**)

Fig. 1. Chemical structures of gastrodin (1), parishin (2), and parishin B (3).

Compound 2: White amorphous powder; m.p. 175~177°C; IR (KBr) ν_{max} (cm^{-1}): 3375, 2931, 1733; UV (EtOH) λ_{max} nm: 277, 271, 221; (-)-FABMS *m/z*: 995 $[M-H]^-$; ^1H NMR (CD_3OD, 300 MHz) δ: 7.26 (4H, d, *J* = 8.7 Hz, H-2, 2", 6, 6"), 7.16 (2H, d, *J* = 8.7 Hz, H-2', 6'), 7.07

(4H, d, J = 8.7 Hz, H-3, 3", 5, 5"), 7.04 (2H, d, J = 8.7 Hz, H-3', 5'), 4.98 (4H, s, H-7a, 7b, 7"a, 7"b), 4.91 (2H, s, H-7'), 4.88 (3H, d, J = 7.5 Hz, H-8, 8', 8"), 3.88 (3H, dd, J = 11.7, 1.2 Hz, H-13a, 13'a, 13"a), 3.69 (3H, dd, J = 11.7, 4.8 Hz, H-13b, 13'b, 13"b), 3.35-3.50 (12H, m, H-9~12, 9'~12', 9"~12"), 2.95 (2H, d, J = 15.3 Hz, H-15a, 15"a), 2.78 (2H, d, J = 15.3 Hz, H-15b, 15"b); ^{13}C NMR (CD_3OD, 75 MHz) δ: 173.30 (C-14'), 169.93 (C-14, 14"), 158.15 (C-4, 4', 4"), 130.04 (C-2, 2', 2", 6, 6', 6"), 129.69 (C-1, 1', 1"), 116.82 (C-3, 3', 3", 5, 5', 5"), 101.29 (C-8, 8', 8"), 77.11 (C-10, 10', 10"), 76.93 (C-12, 12', 12"), 73.89 (C-9, 9', 9"), 73.69 (C-15'), 70.36 (C-11, 11', 11"), 67.22 (C-7'), 66.32 (C-7, 7"), 61.51 (C-13, 13', 13"), 43.71 (C-15, 15").

Compound 3: White amorphous powder; m.p. 170~172°C; IR (KBr) ν_{max} (cm^{-1}): 3387, 2928, 1737, 1069; UV (EtOH) λ_{max} nm: 277, 271, 221; (-)-FABMS *m/z*: 727 $[M-H]^-$; ^{1}H NMR (CD_3OD, 300 MHz) δ: 7.26[a] (2H, d, J = 8.1 Hz, H-2, 6), 7.23[a] (2H, d, J = 8.1 Hz, H-2', 6'), 7.07[b] (2H, d, J = 8.1 Hz, H-3, 5), 7.05[b] (2H, d, J = 8.1 Hz, H-3', 5'), 5.02[c] (2H, br. s, H-7a, 7b), 4.98[c] (2H, br. s, H-7'a, 7'b), 4.89[d] (1H, d, J = 7.5 Hz, H-8), 4.88[d] (1H, d, J = 7.5 Hz, H-8'), 3.88 (2H, dd, J = 11.7, 1.5 Hz, H-13a, 13'a), 3.69 (2H, dd, J = 11.7, 4.8 Hz, H-13b, 13'b), 2.97[e] (1H, d, J = 15.3 Hz, H-15a), 2.91[e] (1H, d, J = 15.3 Hz, H-15"a), 2.80[e] (1H, d, J = 15.3 Hz, H-15b), 2.76[e] (1H, d, J = 15.3 Hz, H-15"b); ^{13}C NMR (CD_3OD, 75 MHz) δ: 173.34 (C-14'), 171.01 (C-14"), 169.96 (C-14), 158.20 (C-4, 4'), 130.12 (C-2, 2', 6, 6'), 130.03 (C-1, 1'), 116.80 (C-3, 3', 5, 5'), 101.32 (C-8, 8'), 77.13[f] (C-10, 10'), 76.94[f] (C-12, 12'), 73.88[f] (C-9, 9'), 70.36[f] (C-11, 11'), 73.63 (C-15'), 67.22[g] (C-7), 66.31[g] (C-7'), 61.50 (C-13, 13'), 43.57[h] (C-15), 43.34[h] (C-15"). The assignment of the chemical shifts with the same superscript may be exchangeable.

Sample extraction for HPLC. Two grams of *G. elata* powder was accurately weighed, soaked with 20 mL water, refluxed twice for 2 h, and filtered through a filter paper. Additional water was added to the extract in a volumetric flask to adjust the final volume to 50 mL. The solution was filtered through a 0.45-μm filter prior to the injection into the HPLC system.

Results and Discussion

The *n*-BuOH extract of *G. elata* was subjected to sequential column chromatography on MPLC to yield gastrodin (**1**), parishin (**2**), and parishin B (**3**). They were obtained as white amorphous powder. All proton and carbon chemical shifts were assigned and confirmed by direct comparison with the spectral data reported in the literature [Baek *et al.*, 1999; Lin *et al.*, 1996; Liu *et al.*, 2002].

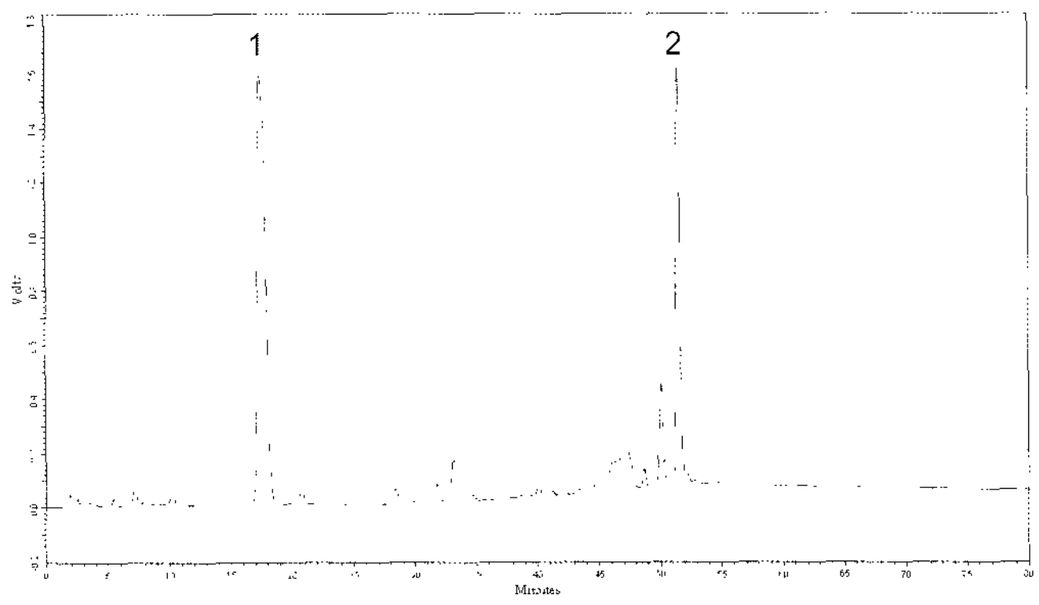


Fig. 2. HPLC chromatogram of *G. elata*. Peak 1, gastrodin (R_t = 17.48 min); peak 2, parishin (R_t = 51.54 min).

Compound **1** showed the molecular ion peak (M^+) at *m/z* 288 in the EIMS. IR spectrum showed the characteristic absorption of hydroxy group (3470 cm^{-1}). In the ^{1}H NMR spectrum, two doublet signals observed at δ 7.28 (2H) and δ 7.08 (2H) were coupled to each other (J = 8.7 Hz), indicating the presence of A_2B_2 system of the 1,4-disubstituted aromatic ring. An anomeric proton at δ 4.89, two oxy-methylene signals at δ 3.90 and 3.71, respectively, and four oxy-methine signals at δ 3.55~3.35 suggested compound **1** to be a glycoside. Two doublet of doublet signals at δ 3.90 (1H, dd, J = 11.7, 1.5 Hz, H-13a) and 3.71 (1H, dd, J = 11.7, 4.8 Hz, H-13b) are indicative of the presence of an oxy-methylene signal in the glucose moiety. ^{13}C-NMR spectrum exhibited thirteen carbon signals including six aromatic carbon signals at δ 158.65 (C-4), 136.81 (C-1), 129.54 (C-2, 6), 117.84 (C-3, 5), *p*-hydroxy benzyl signal at δ 64.97 (C-7), and six glucose signals. An anomeric carbon signal at δ 102.61 and the chemical shifts of other glycosidic carbon signals at δ 78.29, 78.17, 75.09, 71.56, and 62.69 were consistent with the presence of the glucopyranosyl group. The anomeric configuration was elucidated to be β based on the coupling constant (J = 7.5 Hz), which indicated an axial-axial coupling between H-8 and H-9. Comparison of the above data with those in the literature [Baek *et al.*, 1999; Liu *et al.*, 2002] led to the identification of compound **1** as gastrodin (4-β-D-glucopyranosyloxybenzyl alcohol).

Compound **2** showed the pseudomolecular ion peak at *m/z* 995 $[M-H]^-$ in the negative FABMS. IR spectrum showed the characteristic absorption of hydroxy (3375 cm^{-1}), C-H stretching of sp^3 hybridization (2931 cm^{-1}), and carbonyl (1733 cm^{-1}) group. ^{1}H NMR spectrum revealed the presence of the signals of three *p*-disubstituted aromatic ring signals at δ 7.26 (4H, d, J = 8.7 Hz, H-2, 2", 6, 6"), 7.16 (2H, d, J = 8.7 Hz, H-2', 6'), 7.07 (4H, d, J = 8.7 Hz, H-3, 3", 5, 5"), and 7.04 (2H, d,

J = 8.7 Hz, H-3', 5'), three sets of methylene protons in glucopyranosyloxybenzyl alcohol units at δ 4.98 (4H, s, H-7, 7") and 4.91 (2H, s, H-7'), and three sets of methylene protons in the glucose moieties at δ 3.88 (3H, dd, J = 11.7, 1.2 Hz, H-13a, 13'a, 13"a) and 3.69 (3H, dd, J = 11.7, 4.8 Hz, H-13b, 13'b, 13"b). Three anomeric proton signals were shown at δ 4.88 (3H, d, J = 7.5 Hz, H-8, 8', 8"), and the configuration was elucidated to be β based on the coupling constant (J = 7.5 Hz). In addition, two sets of methylene protons arising from the citrate moiety were observed at δ 2.95 (2H, d, J = 15.3 Hz, H-15a, 15"a) and 2.78 (2H, d, J = 15.3 Hz, H-15b, 15"b). In the ^{13}C NMR spectrum, three carbonyl signals at δ 173.30 (C-14') and 169.93 (C-14, 14"), one oxygenated quaternary carbon at δ 73.69 (C-15'), and two sets of the methylene carbon signals of the citrate moiety at δ 43.71 (C-15, 15") suggested the presence of a citrate unit [Lin *et al.*, 1996]. The citrate moiety signals as well as the three glucopyranosyloxybenzyl alcohol signals led to the postulation that the compound **2** was connected by means of the ester bond. The conjunction of the three glucopyranosyloxybenzyl alcohol units and the citrate moiety was determined by interpretation of ^{1}H and ^{13}C NMR spectra. The chemical shift of two carbonyl carbons (C-14, 14") appeared at δ 169.93, and two methylene groups of the citrate moiety in ^{1}H and ^{13}C NMR were shown at δ 2.95 (2H, d, J = 15.3 Hz, H-15a, 15"a), 2.78 (2H, d, J = 15.3 Hz, H-15b, 15"b), and 43.71 (C-15, 15"), indicating the citrate moiety existed as a symmetrical linkage of three glucopyranosyloxybenzyl alcohol moieties. Comparison of the ^{13}C NMR data of the benzylic carbon in gastrodin with that of compound **2** showed that the signals for C-7 and C-7", and C-7' of compound **2** were shifted downfield by 1.35 and 2.25 ppm, respectively, due to the ester bond between the citrate moiety and the glucopyranosyloxybenzyl alcohol units. This is consistent with the negative FABMS of $[M-H]^-$ at *m/z* 995, arising from the conjunction between the three glucopyranosyloxybenzyl alcohols and the citrate moiety. Because of the symmetric distribution of the glucopyranosyloxybenzyl alcohol units, ^{13}C NMR showed symmetrical carbon signals including three symmetrical glucose carbon signals at δ 101.29 (C-8, 8', 8"), 73.89 (C-9, 9', 9"), 77.11 (C-10, 10', 10"), 70.36 (C-11, 11', 11"), 76.93 (C-12, 12', 12"), and 61.51 (C-13, 13', 13"), three aromatic carbon signals at δ 158.15 (C-4, 4', 4"), 130.04 (C-2, 2', 2", 6, 6', 6"), 129.69 (C-1, 1', 1"), and 116.82 (C-3, 3', 3", 5, 5', 5"), two methylene carbon signals of the citrate moiety at δ 43.71 (C-15, 15"), two benzylic carbon signals at δ 66.32 (C-7, 7"), and two carbonyl carbon signals at δ 169.93 (C-14, 14"). On the basis of the above results and the comparison with those in the literature [Baek *et al.*, 1999; Lin *et al.*, 1996], the structure of compound **2** was identified as tris [4-(β-D-glucopyranosyl)benzyloxy] citrate (parishin).

Compound **3** showed the pseudomolecular ion peak at *m/z* 727 $[M-H]^-$ in the negative FABMS. IR spectrum showed the characteristic absorption of hydroxy (3387 cm^{-1}), C-H stretching of sp^3 hybridization (2928 cm^{-1}), and a carbonyl (1737 cm^{-1}) group. NMR data showed close similarity between compounds **2** and **3** except for lack of the signals of one glucopyranosyloxybenzyl moiety. ^{1}H NMR spectrum revealed the presence of the signals of two *p*-disubstituted aromatic rings at δ 7.26 (2H, d, J = 8.1 Hz, H-2, 6), 7.23 (2H, d, J = 8.1 Hz, H-2', 6'), 7.07 (2H, d, J = 8.1 Hz, H-3, 5), and 7.05 (2H, d, J = 8.1 Hz, H-3', 5'), two sets of methylene protons in the glucose moieties at δ 3.88 (2H, dd, J = 11.7, 1.5 Hz, H-13a, 13'a) and 3.69 (2H, dd, J = 11.7, 4.8 Hz, H-13b, 13'b). Two anomeric proton signals were observed at δ 4.89 (1H, d, J = 7.5 Hz, H-8) and 4.88 (1H, d, J = 7.5 Hz, H-8'), and the configuration was elucidated to be β based on the coupling constant (J = 7.5 Hz). In addition, two sets of the methylene protons of the citrate moiety were observed at δ 2.97 (1H, d, J = 15.3 Hz, H-15a), 2.91 (1H, d, J = 15.3 Hz, H-15"a), 2.80 (1H, d, J = 15.3 Hz, H-15b), and 2.76 (1H, d, J = 15.3 Hz, H-15"b). In the ^{13}C NMR spectrum, three carbonyl signals at δ 173.34 (C-14'), 171.0 (C-14"), and 169.96 (C-14), one oxygenated quaternary carbon signal at δ 73.63 (C-15'), and two sets of methylene signals of the citrate moiety at δ 43.57 (C-15) and 43.34 (C-15") suggested the presence of a citrate unit [Lin *et al.*, 1996]. The citrate moiety signals as well as the two glucopyranosyloxybenzyl signals led to the postulation that the compound **3** was connected by means of the ester bond. Comparison of the ^{13}C NMR data of the benzylic carbon in gastrodin with that of compound **3** showed that the signals for C-7 and C-7' of compound **3** were shifted downfield by 2.25 and 1.34 ppm, respectively, due to the ester bond between the citrate moiety and glucopyranosyloxybenzyl alcohols. This is consistent with the negative FABMS of $[M-H]^-$ at *m/z* 727 as compared to that of compound **2** at *m/z* 995, suggesting the absence of a glucopyranosyloxybenzyl unit. Considering the formation of an ester bond between the two gastrodin units and the citrate moiety, two possibilities of the bond formation could be deduced; One being a symmetric distribution between two methylene carbons of the citrate moiety and the other an asymmetrical distribution between methylene carbon and the oxygenated quaternary carbon in the citrate moiety. Unlike compound **2**, ^{13}C NMR spectrum showed split patterns of two methylene carbon signals of the citrate moiety at δ 43.57 (C-15) and 43.34 (C-15"), separated presentation of three carbonyl carbon signals at δ 171.34 (C-14'), 171.0 (C-14"), and 169.96 (C-14), and

two bezylic carbon signals at δ 67.22 (C-7) and 66.31 (C-7'), indicating that the citrate moiety existed in an asymmetrical situation. The spectroscopic data of compound **3** were consistent with those of 1,2-bis[(4-β-D-glucopyranosyloxy)benzyl]citrate (parishin B) in the literature [Lin *et al.*, 1996].

In summary, the contents of gastrodin and parishin, respectively 0.3 and 0.16% as determined by HPLC, were the major chemical constituents of *G. elata* with gastrodin being the most abundant, and parishin B was a minor component (Fig. 2). The assessment of gastrodin as an index component in *G. elata* according to different regions in Korea is remained for further study. However, quantitative analysis of the phenolic glycoside marker could be applied to the quality control of *G. elata*.

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