

# Phenolic Acids and Depsides from Some Species of the *Erodium* Genera

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Z. Naturforsch. **56c**, 943–950 (2001); received July 26/August 8, 2001

*Erodium*, Phenolic Acids, Plant Polyphenols

Six natural polyphenolic compounds, brevifolin carboxylic acid, brevifolin, ellagic acid, methyl gallate, gallic acid and protocatechuic acid have been isolated from the methanol extract of the whole plant of *Erodium cicutarium* (L.) L'Hérit. (*Geraniaceae*). Structures were determined by conventional methods of analysis and confirmed by MS and NMR spectral analysis. The distribution of these compounds in the other species of the *Erodium* genera (*E. botrys*, *E. chium*, *E. ciconium*, *E. cicutarium*, *E. glutinosum* subsp. *dunense*, *E. gruinum*, *E. manescavi*, *E. pelargoniflorum*, *E. petraeum*) were examined by HPLC with a RP-18 column, and MGD-TLC methods on unmodified silica gel and silica gel chemically modified with polar and nonpolar groups (HPTLC-Si 60 LiChrospher, HPTLC-NH<sub>2</sub>, HPTLC-DIOL, HPTLC RP-18W).

## Introduction

*E. cicutarium* (Common stork's bill) is a one- or two-year herb growing up to about 50 cm and indigenous to Europe, Asia and North America. Other species of the *Erodium* genera are Mediterranean plants. Decoction from the aerial parts of *E. cicutarium* plant has been recommended for treatment of dysentery, fever, wounds and worm infections as a traditional medicine (Lis-Balchin, 1993; 1994). In Middle Europe it was employed as antihaemorrhagic drug in gynecology to stop the uterine bleeding and as general haemostipticum. Ellagitannin-containing plants, such as these from the genera *Erodium* are used for preparing astringent and antiseptic teas announced in stomatitis (Klocke *et al.*, 1986). Both water and methanol extract from *E. cicutarium* demonstrated antiviral effect in relation to *Herpes virus* type 1, *Vesicular stomatitis*, *Vaccinia virus* and myxoviruses (Zielinska-Janczylik *et al.*, 1987). Non of these extracts did induce interferon in suspension of human leukocytes. However, the methanol extract exerted a stimulatory effect on the synthesis of interferon induced with Newcastle disease virus in cell cultures. The strongest viricidal fractions contain some polyphenols, excluding flavonoid glycosides (Zielinska-Janczylik *et al.*, 1988). In immunological tests the low concentrations of methanol extract from *E. cicutarium* stimulated whereas the high

concentrations inhibited free radicals activity of human granulocytes *in vitro* (Fecka *et al.*, 1997). This observation was in agreement with previously described antioxidant properties of several species from *Erodium*. The polyphenolic fraction from flowering aerial parts of these species exerted *in vitro* a free radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Lamaison *et al.*, 1993) and an inhibitory effect on the Fe<sup>+2</sup>- induced lipid oxidation (Sroka *et al.*, 1994). Previous papers reported the presence of ellagitannins, flavonoid glycosides, phenolic acids and essential oil (Lis-Balchin, 1993). Salech *et al.* (1983) studied the flavonoids of fourteen *Erodium* from two sections: *Pulmosa* Boiss. and *Erodium* (= *Barbata* Boiss.). Ellagic acid (0.77%) was detected in hot methanol extract of *E. cicutarium* by GC (Klocke *et al.*, 1986). The total concentration of flavonoids, tannins and geraniin in *E. chium*, *E. ciconium* and *E. cicutarium* were determined using both colorimetric and chromatographic methods (Lamaison *et al.*, 1993). In this work we have defined the polyphenols content of methanol extracts from nine species of *Erodium*. Phenolic acids and depsides have been examined with help of qualitative and quantitative analytical techniques.

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## Material and Methods

### Plant material

The following species from the *Erodium* genera (*Geraniaceae*) were analyzed: *E. botrys* (Cav.) Bertol., *E. ciconium* (L.) L'Hérit., *E. cicutarium* (L.) L'Hérit., *E. chium* Sibth. et Sm. non Willd., *E. glutinosum* subsp. *dunense* (Andreas) Rothm., *E. gruinum* (L.) L'Hérit., *E. manescavi* Cosson, *E. pelargoniiflorum* Boiss. et Heldreich, *E. petraeum* (Gouan) Willd.. All herbs at the flowering stage were collected in Poland in September 1996–1998. *E. cicutarium* (L.) L'Hérit. was obtained from a natural habitat (a voucher specimen is deposited in our Department). Another species were cultivated from selected seeds (Jardin Botanique Nantes, France) at the herbarium of the University of Medicine in Wrocław.

### Preparation of extracts

Phenolic compounds, derivatives of phenol carboxylic acid and hydroxycinnamic acids were prepared from 5 g dried and powdered herbs of each species from the *Erodium* genus according to the method described by Van Sumere *et al.*, (1972). Free phenolic acids and phenolic acids obtained after the hydrolytic cleavage of *O*-glycosides or esters were designated in diethyl ether extracts. Enzyme hydrolysis:  $\beta$ -glucosidase (Koch-Light, Colnbrook Bucks, England); the acetic buffer; pH 4.5; 37 °C; 10 h. Alkaline hydrolysis: 2 M NaOH; pH 12; room temperature; 120 min. Methanol extracts were prepared by a well-known procedure. The second portions (5 g) of powdered row materials were first exhaustively extracted (Soxhlet) with  $\text{CH}_2\text{Cl}_2$  and next with methanol on the waterbath. After filtration crude methanol extracts were concentrated under reduce pressure at 40 °C, and the 0.1 g from each obtained residues dissolved in 10 ml of HPLC grade methanol (Merck, Darmstadt, Germany).

### Isolation of polyphenols

The isolation of compounds A-F (Fig. 1) was performed only for *Erodium cicutarium* herb. The other species were analyzed with help of chromatographic techniques. Powdered and dried aerial parts (800 g) of *E. cicutarium* were extracted successively with *n*-hexane,  $\text{CH}_2\text{Cl}_2$  and methanol in

a Soxhlet apparatus. The crude methanol extract was filtered, dryness under reduce pressure at 40 °C, dissolved in water (300 ml) and partitioned with diethyl ether (liquid-liquid extractor). The diethyl ether fraction of the methanol extract from *E. cicutarium* (6 g from total ratio 22.5 g) was separated on silica gel column (Si 60, 0.15–0.30 mm; Macherey-Nagel, Düren, Germany) using stepwise gradient elution with  $\text{CH}_2\text{Cl}_2$  containing increasing concentration of methanol – 10%, 20%, 30% to 60%. Seven fractions were collected (Fr-1→Fr-7). The fractions consisting of polyphenols were finally purified on octadecyl (RP-18, 40  $\mu\text{m}$ , 60 A, LC J.T.Baker, Phillipsburg, USA) with solvent systems consisted of methanol and water. Subsequent purification by a combination of silica gel and octadecyl chromatographies afforded 4 individual compounds: compound **A** (115 mg) from Fr-2 using 10% methanol as the eluent, compound **B** (85 mg) from Fr-3 using 10% methanol, compound **C** (800 mg) from Fr-5 using 10% methanol, compound **D** (30 mg) from Fr-6 using 20% methanol. Compound **E** (65 mg) was separated from compound **F** (31 mg) on the  $\text{NH}_2$  microcolumn (500 mg, 3 ml J.T.Baker, Phillipsburg, USA). In these conditions constituent **F** was selectively absorbed from the methanol solution of Fr-7 on a propylamine bad and washed with methanol-formic acid (99.5:0.5; v/v). Compound **E** was crystallized from the methanol eluate. Structure of isolated constituents were elucidated based on chemical evidences and spectral analyses.

### Standard solutions

Polyphenolic stock standard solutions (1 mg/ml) were prepared by dissolving 5 mg of each standards in 5 ml of HPLC grade methanol (Merck, Darmstadt, Germany). Working standard solutions were prepared by dilution with methanol and filtered through membrane filters (Millipore, 0.22  $\mu\text{m}$ ). All standards of phenolic acids were purchased from Extrasynthèse (Lyon, France).

### Apparatus and conditions

IR and UV spectra were measurement on a Unicam SP 1000 spectrometer and a Perkin Elmer UV/VIS Lambda 20 spectrophotometer. ESIMS were recorded on a AMD 604 mass spectrometer and ESMS on a AMD 404 mass spectrometer.  $^1\text{H}$ -,

$^{13}\text{C}$ -NMR spectra were obtained on a Bruker WM 52 spectrometer.

In TLC were used following HPTLC precoated plates (Merck, Darmstadt, Germany),  $20 \times 10$  cm: silica gel Si 60, LiChrospher Si 60, aminopropyl silica, octadecyl RP-18W and HPTLC DIOL  $10 \times 10$  cm. They were spotted with 10–20  $\mu\text{l}$  samples of diethyl ether extracts, 5–15  $\mu\text{l}$  solutions of individual standards and their mixture as 8–10 mm bands. Chromatograms were developed in horizontal teflon DS-chambers (Chromdes, Lublin, Poland) with the gradient mobile phase program I, II, III (Table I) or two time with eluent IV - water-methanol-formic acid (89:10:1; v/v/v), over a path of 9 cm. Plates were dried in a stream of warm air for 5 min after each step. Colored compounds were detected under UV light (254, 365 nm) and by spraying with 1% methanol  $\text{FeCl}_3$  (Fig. 2), or with *bis*-diazotized sulfanilamide (Fig. 3).

The HPLC system consisted of two pumps (Knauer, Berlin, Germany), a sample injector and a variable wave length UV detector (Knauer variable wavelength monitor type 87.00) connected to a personal computer (HPLC Software/Hardware Package Version 2.21A). Separations were achieved using a LiChroCART® 250–4 100 RP-18 (5  $\mu\text{m}$ ) column with a LiChroCART® 4–4 100 RP-18 (5  $\mu\text{m}$ ) pre-column (Merck, Darmstadt, Germany). Detection was carried out by on-column measurement of UV absorption at 280 nm. The sensitivity was set at 0.04 a.u.f.s. The flow rate was adjusted to 1.0 ml/min. Phenolic acids and depsides were separated using an acetonitrile-water gradient with formic acid addition according to program V (Table II). A 20  $\mu\text{l}$  volume of analyzed samples was injected. The calibration graphs of polyphenols were prepared by measuring the peak area and they were linear in the examined range (0.02–0.10 mg/ml). For the HPLC analyses

Table I. Gradient elution programs for the MGD-TLC method when using various plates. Plates were dried after each step.

Program I. Four-step gradient program for silica gel plates.

Eluent:	% (v/v)			Volume	Development	Time
Step No	Cyclohexane	Diisopropyl ether	Formic acid	[ml]	distance [mm]	[min]
1	–	80	20	1	20	3
2	20	78	2	5	90	20
3	20	78	2	5	90	20
4	20	79	1	5	90	20

Program II. Two-step gradient program for propylamine plates.

Eluent:	% (v/v)		Volume	Development	Time
Step No	Acetone	Acetic acid	[ml]	distance [mm]	[min]
1	85	15	5	90	15
2	90	10	5	90	15

Program III. Four-step gradient program for diol plates.

Eluent:	% (v/v)		Volume	Development	Time
Step No	<i>n</i> -Hexane	Ethyl-methyl ketone	[ml]	distance [mm]	[min]
1	30	70	1	20	2
2	60	40	2	90	15
3	70	30	2	90	15
4	80	20	2	90	15

Table II. The gradient elution program V for the HPLC method with the RP-18 column. The composition of mobile phase (v/v); solvent A: acetonitrile-formic acid (95:5), and solvent B: water-formic acid (95:5).

Step No	Time		Eluent		Type of elution
	(min)		(v/v)		
1	0–2	10%	A in B		isocratic elution
2	2–17	10%–30%	A in B		linear gradient
3	17–20	30%–70%	A in B		linear gradient

gradient grade acetonitrile was used. Water was glass distilled and deionized. Solvent solutions were vacuum degassed with sonication prior to usage. All experiments were performed at room temperature (20 °C).

## Results and Discussion

Phenolic acids and depsides in the *Erodium* genus were investigated by chromatographic techniques. We carried out an isolation and an identification of main constituents of diethyl ether fraction from the methanol extract of *E. cicutarium*. The composition of polyphenols from the other species of *Erodium* was established using HPLC and MGD-TLC methods. Dried and powdered aerial parts of plants were extracted with methanol under reflux. Polyphenolic compounds were partitioned between Et<sub>2</sub>O and water. Phenolic acids and depsides were examined before and after hydrolysis of tannins (Van Sumere *et al.*, 1972). The diethyl ether fraction from *E. cicutarium* was chromatographed on silica gel by the CC method. Elution with CH<sub>2</sub>Cl<sub>2</sub> containing increasing proportions of methanol separated polyphenols according to their polarity. Eluates consisting of phenolic acid or depsides were finally separated on octadecyl with a solvent system containing methanol and water. Subsequent purification by combination of octadecyl and silica gel chromatographies afforded 6 individual compounds (A–F). Structures of these principles (Fig. 1) were elucidated by chemical methods (mainly co-chromatography, hydrolytic degradation, melting point) and spectroscopic methods (UV, IR, MS, NMR). These separated constituents were identified as methyl gallate (A), protocatechuic acid (B), gallic acid (C), brevifolin carboxylic acid (D), brevifolin (E) and ellagic acid (F) by di-

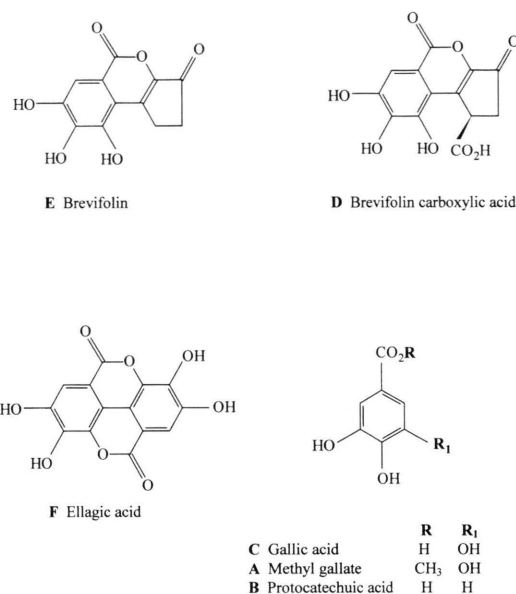


Fig. 1. Structures of phenolic acids and depsides identified in the *Erodium* species.

rect comparison of their physical and spectral profiles with literature values (Klocke *et al.*, 1986; Shimizu *et al.*, 1989; Nawwar *et al.*, 1994; Lin and Lin, 1997). We have also examined the distribution of phenolic acids and depsides in the other species of *Erodium*.

Unmodified silica gel and silica gel chemically modified with polar (amino, diol) and nonpolar (*n*-octadecyl) groups were employed for analysis of polyphenols by TLC. The best separation of phenolic acids was achieved on silica gel Si 60

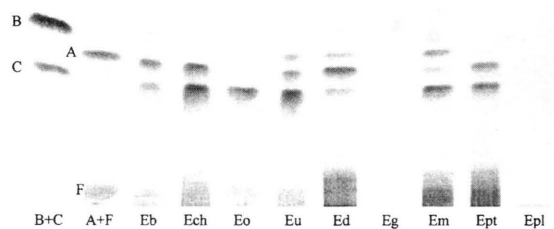


Fig. 2. Chromatogram of methanol extracts from analyzed *Erodium* species and standards developed according with program I on the HPTLC LiChrospher silica gel plate. Polyphenols were visualized by spraying with FeCl<sub>2</sub> reagent. Tracks: **A** methyl gallate, **B** protocatechuic acid, **C** gallic acid, **F** ellagic acid, **Eb** *E. botrys*, **Ech** *E. chium*, **Eo** *E. ciconium*, **Eu** *E. cicutarium*, **Ed** *E. glutinosum* subsp. *dunense*, **Eg** *E. gruinum*, **Em** *E. manscavi*, **Ept** *E. petraeum*, **Epl** *E. pelargoniflorum*.



LiChrospher and octadecyl plates using program I (Fig. 1) and mobile phase IV relatively. Satisfied results were observed for diol and program III. Depsides were determined on propylamine plates at program II (Fig. 3). HPLC-RP 18 separation with acetonitrile-water gradient was characterized good resolution for both phenolic acids and depsides. The optimal separation of the standard mixture was recorded for 5% concentration of formic acid as pH modifier (Fig. 4). The described MGD-TLC method on the silica gel and chemically modified stationary phases is selective for separation of phenolic acids and depsides compared with commonly used unmodified layers. Because of, analyzed compounds are well separated from a complex mixture, the MGD-TLC method can be used for densitometric evaluations. The HPLC gradient for the characterization of polyphenolic com-

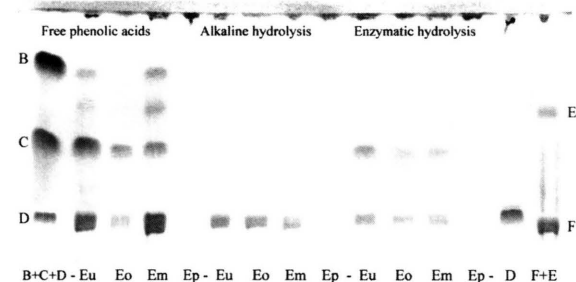


Fig. 3. Chromatogram of diethyl ether fractions from analyzed *Erodium* species and standards developed according with program II on the HPTL NH<sub>2</sub> plate. Polyphenols were visualized by spraying with both FeCl<sub>2</sub> and bis-diazotized sulfanilamide reagent. Tracks: **B** protococatechuic acid, **C** gallic acid, **D** brevifolin carboxylic acid, **E** brevifolin, **F** ellagic acid, **Eo** *E. ciconium*, **Eu** *E. cicutarium*, **Em** *E. manescavi*, **Ep** *E. pelargoniflorum*.

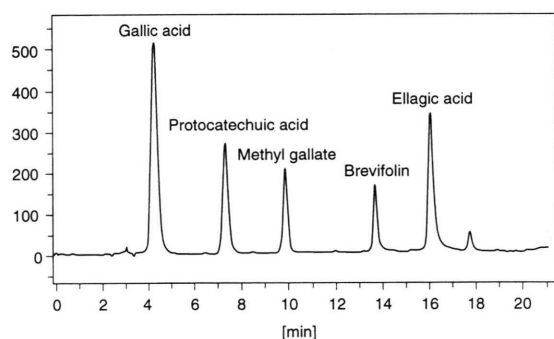


Fig. 4. HPLC chromatogram of diethyl ether fraction from *E. cicutarium* (RP-18, program V,  $\lambda=280$  nm).

pounds in an analyzed material can be applied for a chemosystematic study of other species from the *Erodium* genera in the polyphenols range.

Phenolic acids and depsides are secondary metabolites derived by hydrolysis of tannins. The *Geraniaceae* family afford ellagitannins with geraniin as a main constituent (Okuda *et al.*, 1983; 1989b). Geraniin are hydrolyzed with dilute HCl yielding ellagic acid, brevifolin carboxylic acid, brevifolin, gallic acid and glucose (Okuda *et al.*, 1989a). Investigated methanol extracts and diethyl ether fractions yield phenolic compounds in high concentration (Table III). Obtained results indicate that the major constituents of free phenolic acids are gallic acid, gallic acid methyl ester and protococatechuic acid. The second polyphenolic group are depsides: ellagic acid, brevifolin and its precursor brevifolin carboxylic acid. In low concentration appear following phenol carboxylic acids: salicylic; *m*- and *p*-hydroxybenzoic; 2,3-dihydroxybenzoic; gentisic; vanillic and hydroxycinnamic acid derivatives: caffeic; *p*-coumaric; ferulic. Gallic acid and gentisic acid are present mainly as free or as  $\beta$ -glucosides. Protocatechuic acid and depsides form ester bonds. *E. botrys*, *E. chium*, *E. cicutarium*, *E. glutinosum* subsp. *dunense*, *E. manescavi* and *E. petraeum* have the similar qualitative composition of non-glycosylated polyphenols but they reveal quantitative differences. The highest content of depsides have been observed in *E. manescavi* and *E. petraeum*. The mean value for gallic acid is about 0.48% of dried weight of extracts and for ellagic acids 2.0% respectively. Methyl gallate has been detected in a high concentration in *E. chium* and *E. petraeum*. *E. ciconium* does not synthesize the methyl ester of gallic acid. It is interesting that *E. gruinum* and *E. pelargoniflorum* possess only traces of phenolic acids with no presence of methyl gallate. The presence of gallic acid, protococatechuic acid, brevifolin carboxylic acid and caffeic acid were also described in *E. moschatum* (L.) L'Hérit by Jer-Huei Lin and Mei-Fan Lin (1997). Because, phenolic acids and depsides have been detected in the majority of tested methanol extracts their biologic significance may be discussed. These identified polyphenols possess antioxidant (Kimura *et al.*, 1983a, 1983b; Hatano *et al.*, 1989, 1990), antibacterial (Kolodziej *et al.*, 1999), antiviral (Kane *et al.*, 1988a; 1988b) and antiphlogistic actions (Okuda *et al.*, 1983; 1989b). El-

Table III. The contents of major phenolic acids and depsides in methanol extracts (mg per 1 g of dry weight) from nine species of the *Erodium* genera.

POLYPHENOLS	<i>E. botrys</i>	<i>E. ciconium</i>	<i>E. cicutarium</i>	<i>E. chium</i>	<i>E. glutinosum</i> subsp. <i>dunense</i>	<i>E. gruinum</i>	<i>E. manescavi</i>	<i>E. pelargo-</i> <i>niiflorum</i>	<i>E. petraeum</i>
	[mg/g]								
Gallic acid	2.00	7.39	12.40	7.07	0.92	0.10	8.29	0.45	10.76
Protocatechuic acid	–	0.65	3.93	0.67	2.28	0.39	0.08	0.98	1.46
Gallic acid methyl ester	5.30	–	18.38	27.95	14.73	–	11.47	–	37.87
Brevifolin	3.62	8.41	25.95	7.12	6.79	0.45	50.74	–	13.99
Ellagic acid	17.49	5.19	11.88	8.33	23.43	6.81	26.41	0.51	80.28

(–) absent.

lagic acid, gallic acid and methyl gallate are also know as antimutagenic and anticarcinogenic plant agents. They prevent of a carcinogenesis process by inhibition of initiation or promotion stages (Perchellet *et al.*, 1992). Additionally, gallic acid and its metabolites induce the death signal (apoptosis) via their prooxidant action in some cancer cell lines (Nogaki *et al.*, 1998; Sakagami *et al.*, 1997a, 1997b; Sakaguchi *et al.*, 1998, 1999). The other studies show that ellagic acid significantly reduces acid secretion and H<sup>+</sup>,K<sup>+</sup>ATPase activity on gastric ulcers (Murakami *et al.*, 1991). To sum up, we can conclude that isolated polyphenols might be considered as a potential substances of promising previously detected properties of methanol extracts from *E. cicutarium* and other species of *Erodium*. Also, one might speculate that dietary intake of such polyphenols reduces the risk of some forms of cancers and other various degenerative diseases.

#### Compound A: Methyl gallate

Pale needles, recrystallized from MeOH-H<sub>2</sub>O, mp. >202 °C;

M<sub>r</sub> 184.14, EI-MS *m/z*: 184, 153, 125, 107, 97, 79, 51, 39;

TLC R<sub>f</sub>: 0.51 (HPTLC Si 60 L, program I), 0.55 (HPTLC DIOL, program III), 0.57 (HPTLC RP-18, eluent IV); brawn under UV<sub>365</sub>, dark blue after spraying with FeCl<sub>3</sub> reagent, yellow with *bis*-diazotized sulfanilamide; HPLC R<sub>t</sub>: 9:51 (program V); normal acid hydrolysis (2 M aq. HCl, 100 °C, 2h) yielded gallic acid; UV λ max (MeOH): 273, 365 nm. IR ν max (KBr): 3500–2800, 2850, 1700, 1625, 1540, 1450, 1375, 1335, 1270, 1220, 1100, 1060, 1015, 920, 870, 780, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR

(CD<sub>3</sub>OD) δ: 7.42 (s, 2H, H-2,6), 3.82 (s, 3H, OCH<sub>3</sub>).

#### Compound B: Protocatechuic acid

Colorless needles, recrystallized from MeOH-H<sub>2</sub>O, mp 214–216 °C;

TLC R<sub>f</sub>: 0.58 (HPTLC Si 60 L, program I), 0.76 (HPTLC NH<sub>2</sub>, program II), 0.51 (HPTLC DIOL, program III), 0.74 (HPTLC RP-18, eluent IV); brawn under UV<sub>365</sub>, dark blue after spraying with FeCl<sub>3</sub> reagent, pink-gray with *bis*-diazotized sulfanilamide; HPLC R<sub>t</sub>: 7:17 (program V).

#### Compound C: Gallic acid

Colorless needles, recrystallized from MeOH-H<sub>2</sub>O, mp 240 °C;

TLC R<sub>f</sub>: 0.44 (HPTLC Si 60 L, program I), 0.46 (HPTLC NH<sub>2</sub>, program II), 0.37 (HPTLC DIOL, program III), 0.89 (HPTLC RP-18, eluent IV); brawn under UV<sub>365</sub>, dark blue after spraying with FeCl<sub>3</sub> reagent, yellow-brown with *bis*-diazotized sulfanilamide; HPLC R<sub>t</sub>: 4:14 (program V).

#### Compound D: Brevifolin carboxylic acid

Yellow powder, recrystallized from MeOH, mp. > 310 °C;

M<sub>r</sub> 292.18, ESI-MS *m/z*: neg. ion. 291.1[M-H]<sup>-</sup>, pos. ion 293[M+H]<sup>+</sup>;

TLC R<sub>f</sub>: 0.20 (HPTLC NH<sub>2</sub>, program II), 0.36 (HPTLC RP-18, eluent IV); violet under UV<sub>365</sub>, yellow-green after spraying with FeCl<sub>3</sub> reagent, pink with *bis*-diazotized sulfanilamide; HPLC R<sub>t</sub>: 15:25 (program V); a solution of D (5 mg) in distilled H<sub>2</sub>O (3 ml) was heated in a fused glass tube at 140 °C for 1h, and the precipitate was recrystal-

lized from MeOH to afford brevifolin, which was identified by comparison (TLC, HPLC) with compound E;

UV  $\lambda_{\max}$  (MeOH): 279, 350, 362p nm; IR  $\nu_{\max}$  (KBr): 3200, 1708, 1648, 1620, 1598, 1550, 1526, 1484, 1390, 1260, 1204, 1142, 1096, 930  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{COCD}_3+\text{D}_2\text{O}$ )  $\delta$ : 7.76 (s, 1H, H-6), 4.88–4.86 (t, 1H, H-1), 3.01 (s, 2H, H-2);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{COCD}_3+\text{D}_2\text{O}$ )  $\delta$ : 195.68 (C-3), 174.73 ( $\text{CO}_2\text{H}$ ) 161.68 (C-5), 149.54 (C-3a), 145.62 (C-9), 143.94 (C-7), 141.18 (C-8), 140.59 (C-9a), 115.98 (C-9b), 113.89 (C-5a), 109.13 (C-6), 42.06 (C-1), 37.67 (C-2).

#### Compound E: Brevifolin

Yellowish powder, recrystallized from MeOH, mp. > 310 °C;

$M_r$  248.18, LSI-MS  $m/z$ : pos. ion. 249  $[\text{M}+\text{H}]^+$ ; TLC  $R_f$ : 0.60 (HPTLC  $\text{NH}_2$ , program II), 0.22 (HPTLC DIOL, program III), 0.16 (HPTLC RP-18, eluent IV); w UV<sub>365</sub> violet under UV<sub>365</sub>, gray after spraying with  $\text{FeCl}_3$  reagent, pink with *bis*-diazotized sulfanilamide; HPLC  $R_t$ : 13:43 (program V);

UV  $\lambda_{\max}$  (MeOH): 276, 348, 366p nm; IR  $\nu_{\max}$  (KBr): 3600–2600, 1680, 1620, 1580, 1510, 1400, 1300, 1180 1125, 1090, 1040, 1000, 920, 800, 770  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d$ )  $\delta$ : 7.28 (s, 1H, H-6), 3.18 (m, 2H, H-1), 2.4 (d, 2H, H-2);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d$ )  $\delta$ : 195.50 (C-3), 160.55 (C-5), 149.32 (C-3a), 144.97 (C-9), 144.24 (C-7), 141.43 (C-8), 140.31 (C-9a), 115.44 (C-9a), 113.09 (C-5a), 107.97 (C-6), 33.00 (C-2), 23.82 (C-1).

#### Compound F: Ellagic acid

Yellowish powder, recrystallized from MeOH, mp. > 310 °C;

$M_r$  302, LSI-MS  $m/z$ : neg. ion. 300.8  $[\text{M}-\text{H}]^-$ ; TLC  $R_f$ : 0.17 (HPTLC  $\text{NH}_2$ , program II), 0.11 (HPTLC DIOL, program III), 0.15 (HPTLC RP-18, eluent IV) violet under UV<sub>365</sub>, yellow-green after spraying with  $\text{FeCl}_3$  reagent, yellow with *bis*-diazotized sulfanilamide; HPLC  $R_t$ : 16:04 (program V); UV  $\lambda_{\max}$  (MeOH): 253, 365 nm; IR  $\nu_{\max}$  (KBr): 3420–2400, 1710, 1625, 1590, 1505, 1435, 1400, 1345, 1260, 1200, 1115, 1060, 930, 890, 815, 760  $\text{cm}^{-1}$ .

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