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THE ELLAGITANNIN GERANIIN AND ITS HYDROLYSIS PRODUCTS ISOLATED AS INSECT GROWTH INHIBITORS FROM SEMI-ARID LAND PLANTS*

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Key Word Index—*Geranium viscosissimum* var. *viscosissimum*; *Erodium cicutarium*; Geraniaceae; *Tamarix chinensis*; Tamaricaceae; *Quercus gambelii*; Fagaceae; *Cistus villosus*; Cistaceae; *Heliothis virescens*; Noctuidae; insect growth inhibitors; semi-arid land plants; phenolics; ellagic acid; geraniin.

Abstract—The phenolic dilactone, ellagic acid, was isolated and identified from the hot methanolic extracts of five species of semi-arid land plants as an insect growth inhibitor active against the polyphagous herbivore, *Heliothis virescens* (tobacco budworm). Ellagic acid bound as its hexahydroxydiphenoyl ester in the ellagitannin, geraniin, also was isolated (using milder extraction procedures) and spectrally identified from one of the five species (i.e. *Geranium viscosissimum* var. *viscosissimum*) as a growth inhibitor of *H. virescens*. Evidence is given for the hypothesis that the labile geraniin is a protoxin which releases insect growth inhibitors, particularly ellagic acid, upon hydrolytic cleavage. The free and bound forms of ellagic acid may explain the insect growth inhibitory activity detected in the methanolic extracts of many species in the Hamamelididae–Dilleniidae stock.

INTRODUCTION

In order to identify compounds which may be useful as sources and models of new insect control agents, we have been screening over 800 species of xerophytic plants, mostly from arid and semi-arid regions of the western U.S.A., for activity against insects. For example, we have isolated mosquito larvicidal chromenes from *Hemizonia fitchii* (Compositae) [1] and insecticidal linear furanocoumarins from *Thamnosma montana* (Rutaceae) [2].

The availability and biological activity of some xerophytic plants make them a promising area of research for the discovery of new insect control agents. For instance, xerophytic plants are available on a massive scale from marginal regions which generally cannot be used economically for food production [3]. In addition, a biologically active chemical constituency against insect pests has been discovered in several species of xerophytic plants [4, 5].

In the present paper, we report on the isolation, identification, quantitation and bioassay of free ellagic acid (1) from the biologically active hot methanolic extracts of two species of semi-arid land plants, *Tamarix chinensis* Lour. (Tamaricaceae) and *Geranium viscosissimum* Fisch. and Meyer var. *viscosissimum* (Geraniaceae), and on the detection and quantitation of ellagic acid (1) from an additional three species, *Quercus gambelii* Nutt. (Fagaceae), *Erodium cicutarium* (L.) L'Her. (Geraniaceae) and *Cistus villosus* L. (Cistaceae). In addition, we report herein on the isolation, identification and bioassay of ellagic acid bound as its hexahydroxydiphenoyl ester in the ellagitannin, geraniin (2). Geraniin (2), and at least two of its hydrolysis products, ellagic acid (1) and gallic acid (3), exhibited growth inhibitory activity against larvae of the polyphagous insect pest, *Heliothis virescens* (Fabr.) (Noctuidae).

RESULTS AND DISCUSSION

The hot methanolic extracts of the aerial parts of *G. viscosissimum* var. *viscosissimum*, *T. chinensis*, *Q. gambelii*, *E. cicutarium* and *C. villosus* caused growth inhibition when fed in an artificial diet to first instar larvae of *H. virescens* (tobacco budworm). The same bioassay was used to monitor the fractionation of the active extracts of *G. viscosissimum* var. *viscosissimum* and *T. chinensis*. This resulted in the isolation and identification of the phenolic dilactone, ellagic acid (1), as the active constituent in both plant extracts. Subsequently, ellagic acid (1) was detected in the growth inhibitory extracts of *Q. gambelii*, *E. cicutarium* and *C. villosus* (Table 1).

Following its isolation, the potency of ellagic acid (1) as an insect growth inhibitor was determined. Thus, the EC_{50}, or effective concentration for 50% growth inhibition, was found to be 147 ppm or 0.49 mmol/kg diet wet weight (Table 2). At least a part of this growth inhibition may be attributed to a lowering of the nutritional quality of the diet. For example, even though dietary ellagic acid (1) at 400 ppm actually stimulated the assimilation of material across the gut wall (approximate digestibility, AD) of third instar larvae, both the ECI (efficiency of conversion of ingested food) and ECD (efficiency of conversion of digested food) were depressed, being about one half of control values (Table 3). The

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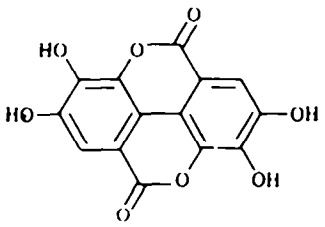

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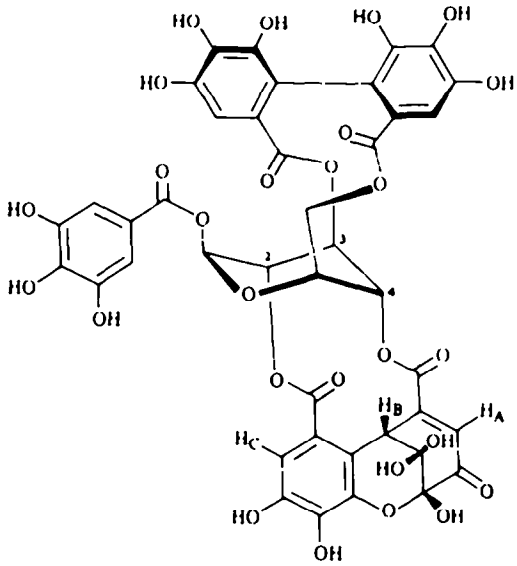

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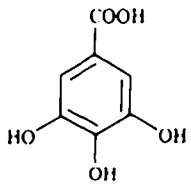

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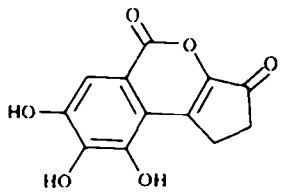

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lower dietary concentration of 200 ppm ellagic acid (1) only slightly affected the nutritional physiology of the third instar larvae.

Although the mode of action of ellagic acid (1) as an insect growth inhibitor is unknown, other activities of ellagic acid (1) are associated with the ability of its four phenolic groups to readily complex with metals such as magnesium and calcium. For example, in the wood pulping process, troublesome deposits of ellagic acid–metallic complexes can cause corrosion and scale formation [6, 7] and can adversely affect the wet strength of bonds of phenol–formaldehyde adhesives with wood [8]. On the other hand, such complexes are hemostatic and can control bleeding in animals [9, 10] and in humans [11, 12]. The folklore of North American Indians records that ellagitannin-containing plants, such as those in the genera *Arctostaphylos*, *Geranium*, *Erodium*, *Rhus* and *Quercus* were used for preparing hemostatic, astringent and antiseptic teas. These teas, prepared by boiling appropriate plant parts (e.g. roots) in water, were used to control bleeding, both topically and systemically, in oral infections and inflammations (e.g. moniliasis, candidiasis, thrush, pyorrhea, periodontal disease), as mild uterine hemostats (to control excessive menstrual bleeding and post-partum hemorrhaging), to treat skin burns and abrasions, and in the treatment of hemorrhoids, diarrhea and dysentery [13–15]. The effects of ellagic acid on the mammalian coagulation mechanism are well known

Table 1. Plant species determined to contain the insect growth inhibitor ellagic acid in hot methanolic extracts of their aerial parts

Genus-species (Family)	Common name	Locale of collection	Percentage yield from aerial parts (dry weight)			
			Hexane extractables	Methanol extractables	Total extractables	Ellagic acid
Quercus gambelii Nutt. (Fagaceae)	Gambel oak	Pinyon Mesa near Brial Canyon, Mesa Co., CO	1.42	21.40	22.82	1.32
Erodium cicutarium (L.) L'Her. (Geraniaceae)	Storksbill	Wawawai Landing, Whitman Co., WA	2.42	18.16	20.58	0.77
Geranium viscosissimum Fisch. and Meyer var. *viscosissimum* (Geraniaceae)	Sticky geranium	Kramer Strip, Whitman Co., WA	4.10	30.03	34.13	1.50
Tamarix chinensis Lour. (Tamaricaceae)	Salt cedar	West of Orla, Culberson Co., TX	2.23	17.18	19.41	1.25
Cistus villosus L. (Cistaceae)	Rock rose	South of Mount Laguna, near Live Oak Springs, San Diego Co., CA	1.99	18.12	20.11	0.86

[16, 17]. Apparently, ellagic acid–metallic complexes form insoluble aggregates which bind to factor XII (Hageman factor) of the intrinsic blood coagulation pathway [18]. The resulting induced hypercoagulation state is characterized by a shortening of the clotting time.

Ellagic acid (1) can also form adducts with some electrophilic molecules. For example, ellagic acid (1) has been observed to inactivate the mutagenic bay-region diol epoxides of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons via adduct formation [19, 20]. Presently, ellagic acid (1) is being evaluated as a prototype of a new class of cancer-preventing drugs [21], and may prove useful in modulating the risk of cutaneous cancer from environmental chemicals [22]. Various other activities ascribed to ellagic acid (1) include sedation and ataxia in mice [23] and the inhibition of enzymatic activities [22, 24], inhibition of nitrification by *Nitrosomonas* [25] and inhibition of plant germination and growth (i.e. allelopathy) [26–30].

We detected ellagic acid (1), as its pertrimethylsilyl derivative, by GC at levels between 0.77% (in *E. cicutarium*) and 1.50% (in *G. viscosissimum* var. *viscosissimum*) of the total dry weight of the aerial parts of the five plant species (Table 1). Varying amounts of free ellagic acid (1) have been reported previously in several species in the genera *Geranium* [31–34], *Tamarix* [31, 35–37], *Quercus* [31, 38–40] and *Cistus* [41]. No previous report of ellagic acid (1) in any species of *Erodium* was found. However, there are folklore reports concerning the hemostatic and antiseptic properties of aqueous *Erodium cicutarium* extracts [15].

Although the large amounts (0.77–1.50%) of free ellagic acid (1) could explain, for the most part, the activity of the hot methanolic extracts against *H. virescens* (EC_{50} 0.0147%), in the fresh plant very little ellagic acid (1) is in the free form, but is instead bound into more complex molecules such as the ellagitannins [34, 42]. For instance, in a number of species of *Geranium*, ellagic acid (1) is predominantly bound in the ellagitannin, geraniin (2) [33, 34, 43]. A milder extraction method, that is, one excluding heat, revealed this to also be the case for *G. viscosissimum* var. *viscosissimum*. Geraniin (2) was easily detected in tissue which had been extracted at room temperature, while no geraniin (2) was detected in heated samples of similar tissue (see Experimental). Apparently, the large amounts of ellagic acid (1) detected, at least for *G. viscosissimum* var. *viscosissimum*, were artifacts of the extraction method used.

Since ellagic acid (1) is probably not encountered free in large amounts in fresh plant tissues, but rather as one or more bound forms, we isolated and bioassayed geraniin (2). On a mmol/kg diet basis, geraniin (2) was almost twice as active as a growth inhibitor of *H. virescens* larvae than was ellagic acid (1) (EC_{50} 0.26 and 0.49, respectively) (Table 2). An increased activity of geraniin (2) might be expected since complete hydrolysis of geraniin (2) *in vivo* would yield, besides ellagic acid (1), equimolar amounts of brevifolin (4) (or other phenolic lactones) and gallic acid (3) [44, 45]. Although the biological activity of brevifolin (4) as an insect growth inhibitor is unknown, gallic acid (3) exhibited some growth inhibitory activity against *H. virescens* larvae (EC_{50} 7.40 mmol/kg diet) (Table 2).

Ellagitannins are characterized by the tanning property of forming hydrogen bonds between the phenolic hydroxyls of the tannin and the free amino and amido groups of proteins [46, 47]. In addition to being used for

Table 2. Growth inhibitory activity of some bioactive constituents derived from *Geranium viscosissimum* var. *viscosissimum* fed in an artificial diet to first instar larvae of *Heliothis virescens*

Test compound	EC_{50}* (mmol/kg diet)	EC_{50}* (ppm)	Confidence† limits	Slope
Geraniin	0.26	250	195–320	1.68
Ellagic acid	0.49	147	103–140	1.78
Gallic acid	7.40	1262	931–3843	2.03

* EC_{50} is the effective concentration of additive necessary to reduce larval growth to 50% of the control values. Units are given in both mmol/kg diet wet weight and ppm of diet wet weight.

† Confidence limits and slope were determined using the method of Litchfield and Wilcoxon [70].

Table 3. The nutritional indices (± s.e.) of third instar *H. virescens* larvae grown for 5 days on an artificial diet containing ellagic acid

	Control	200 ppm ellagic acid	400 ppm ellagic acid
AD*	43.17 ± 3.88%	39.96 ± 7.06%	61.75 ± 12.19%
ECD†	37.47 ± 4.73%	40.40 ± 3.60%	15.41 ± 8.36%
ECI‡	15.90 ± 1.08%	15.74 ± 1.96%	8.28 ± 1.90%

*AD refers to approximate digestibility = (amount ingested − feces/amount ingested) × 100%.

†ECD refers to efficiency of conversion of digested food = (weight gain/amount ingested − feces) × 100%.

‡ECI refers to efficiency of conversion of ingested food = (weight gain/amount ingested) × 100%.

the commercial conversion of hides and skins into nonputrescible leather [48, 49], this tanning property has also been hypothesized to be responsible for the biological activities of ellagitannins including enzymatic inhibition [50], allelopathy [51], fungitoxicity [52, 53] and feeding deterrent activity against reptiles [54], mammals [46, 55] and insects [38, 46, 55–57].

The latter activity, that of feeding deterrency, has been considered to be due to the protein binding capacity of the tannins which renders potential food sources unpalatable (hence deterring feeding). Recently, however, this view has come under criticism, especially in the light of the lack of "explicit physicochemical information relating to the ability of polyphenols to bind protein" [58]. In the case of the polyphenol geraniin (2), it seems unlikely that its insect growth inhibitory activity is due to a feeding deterrent effect since in a cotton leaf disk 'choice' bioassay [59] it was found to be inactive against *H. virescens* larvae at levels as high as 200 $\mu g/cm^2$ disk (unpublished observations). Ellagitannins, although more active as protein precipitants than are the condensed tannins [60], are nevertheless fairly labile, especially to heat, enzymatic hydrolysis and acidic and alkaline conditions [48, 61, 62]. Although geraniin is a fairly stable ellagitannin due to its 2,4-linked dehydrohexahydroxydiphenoyl ester group [63–65], we have shown that it is hydrolysed by hot methanolic extraction of leaves containing it. For instance, no geraniin (2) was detected when aerial parts of *G. viscosissimum* var. *viscosissimum* were extracted with hot methanol in a Soxhlet apparatus, while easily detectable quantities of geraniin (2) (over 1% of the total dry weight of the aerial parts) were evident in aerial parts extracted with methanol at ambient temperature (see Experimental). In addition, when geraniin (2) was incubated with a larval gut homogenate of *H. virescens* at pH 8.0 (the pH of the gut), an increase in the UV absorbance at 360 nm was observed, possibly indicating the release of ellagic acid (1) (see Experimental). No changes in absorption were observed with geraniin (2) in buffer alone (unpublished observations). The labile geraniin (2) therefore possibly acts as a protoxin by releasing insect growth inhibitory hydrolytic products such as ellagic acid (1) *in vivo* following ingestion.

Other workers have been interested in the chemosystematic significance of free and bound forms of ellagic acid [31, 42, 66, 67]. For example, the occurrence of ellagic acid (1) in the dicots was found to be confined to 15 out of 39 orders [31]. Subsequent work with ellagic acid (1) bound as its hexahydroxydiphenoyl ester in ellagitannins has shown a fundamental cleavage between the subclasses Magnoliidae, Caryophyllidae, Asteridae and Ranunculidae, from which they are virtually absent, and the subclasses Hamamelididae, Dilleniidae and Rosidae, all of which contain a high percentage of genera containing these compounds [42, 46]. The possession of ellagitannins apparently is a primitive condition of Hamamelididae–Dilleniidae (H–D) stock [60].

Knowledge of the distribution of the free (1) and bound (2) forms of ellagic acid may be useful for explaining the insect growth inhibitory activity we have identified in the hot methanolic extracts of the aerial parts of many species in the H–D stock (Table 4). We are presently investigating the possibility that the active constituencies of these plants at least include the insect growth inhibitor ellagic acid (1).

In the light of the susceptibility of at least one species of insect (i.e. *H. virescens*) to ingestion of either the free (1) or bound (2) forms of ellagic acid, coupled with the large amounts of free (1) or bound (2) ellagic acid found in a number of plant species, ellagic acid (1) is probably a constituent of the natural chemical defenses of certain plants against some insects. As such, it could become a candidate model for the total synthesis or semisynthesis of new insecticides, especially if its solubility and potency can be enhanced. Fortunately, ellagic acid (1) is neither mutagenic [19] nor acutely toxic in tests with experimental animals [11, 68] and humans [12]. Of course, more active compounds modelled after ellagic acid (1) would also have to be tested for mutagenicity, mammalian

Table 4. Plant species found to contain insect growth inhibitory activity in the hot methanolic extracts of their aerial parts

Order*	Family	Species
Ericales	Ericaceae	*Arbutus arizonica* (Gray) Sarg., *Arctostaphylos patula* Greene, *A. pungens* H.B.K.
Euphorbiales	Euphorbiaceae	*Croton texensis* (Klotzsch) Muell.-Arg. ex DC., *Euphorbia esula* L., *E. robusta* (Engelm.) Small, *Jatropha dioica* Sesse ex Cerv.
Fagales	Fagaceae	*Quercus gambelii* Nutt., *Q. havardii* Rydb., *Q. hypoleucoides* A. Camus
Geraniales	Geraniaceae	*Erodium cicutarium* (L.) L'Her., *Geranium viscosissimum* Fisch. and Meyer var. *viscosissimum*
Rosales	Rosaceae	*Amelanchier utahensis* Koehne, *Cercocarpus ledifolius* Nutt. ex T. and G. var. *intricatus* (S. Wats.) M. E. Jones, *C. montanus* Raf., *Cowania mexicana* D. Don, *Fallugia paradoxa* (D. Don) Endl., *Peraphyllum ramosissimum* Nutt., *Purshia tridentata* (Pursh) DC., *Rosa woodsii* Lindl. var. *woodsii*, *Vauquelinia californica* (Torr.) Sarg.
Sapindales	Anacardiaceae	*Rhus glabra* L., *R. trilobata* (Nutt.) Gray, *R. trilobata* Nutt. var. *simplicifolia* (Greene) Barkl., *R. virens* Lindheimer ex Gray
Tamaricales	Tamaricaceae	*Tamarix aphylla* (L.) Karst., *T. chinensis* Lour.
Violales	Cistaceae	*Cistus villosus* L.

*Ellagic acid has been found in these orders [31].

toxicity, etc. Hopefully, such hypothetical compounds would prove not only to be effective insect control agents, but also to be environmentally acceptable in terms of biodegradability and health safety.

EXPERIMENTAL

Collection and extraction. Aerial whole plant parts of *Q. gambelii*, *E. cicutarium*, *G. viscosissimum* var. *viscosissimum*, *T. chinensis* and *C. villosus* were collected in May and June in various locations in Colorado, Washington, Texas and California (Table 1). Following collection, the plant parts were air-dried and mailed along with a voucher specimen to Salt Lake City. The voucher specimens have been deposited in the herbarium of the Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois, U.S.A. The remainder of the plant materials were oven-dried upon arrival (70°), and then ground to pass a 20 mesh screen in a Wiley mill. Approximately 20 g of each sample were sequentially Soxhlet-extracted for 20 hr, first with hexane and then with MeOH. (Hexane defatted the plant materials by removing non-polar extractables.) The residues (marcs) were oven-dried (4 hr at 70°) following hexane extraction to remove traces of solvent before extracting with MeOH. Following extraction with MeOH to remove extractable carbohydrates, polyphenolics and other polar and semi-polar constituents, the residues (marcs) were again oven-dried (48 hr at 70°) and weighed. The hexane and MeOH extracts were concd by evaporation and weighed, and yields were then calculated gravimetrically (Table 1).

Bioassays. As previously described [69], an artificial diet bioassay with newly-hatched larvae of the tobacco budworm, *H. virescens*, was used to detect insect growth inhibitory activity in the plant extracts and to monitor the fractionation of the extracts. The same bioassay was used to determine the potency of the isolated constituents. Potency was determined as the effective concentration (EC_{50}) of compound added to the diet necessary to cause a 50% reduction in weight. EC_{50} values were determined from log probit paper and analysed statistically by the method of Litchfield and Wilcoxon [70].

The same artificial diet bioassay was used to determine the nutritional indices of ellagic acid (1) following the method of Reese and Beck [71]. The nutritional indices included the approximate digestibility (AD), efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI).

An *in vitro* assay was utilized to determine the hydrolysis of geraniin (2) by a gut homogenate of third instar *H. virescens* larvae. The assay consisted of the monitoring of the incubation of geraniin (2) in gut homogenate at 25° and 1 M Tris buffer at pH 8.0 (the pH of the larval gut) for an increase in the UV absorbance at 360 nm [a maximum of free ellagic acid (1)].

Isolation of ellagic acid (1). Since only the hot methanolic extracts of the five plant species were found to be active in the bioassays with *H. virescens*, further fractionation was confined to them. For example, the hot methanolic extract of *G. viscosissimum* var. *viscosissimum* was washed with hexane (× 3) and partitioned between EtOAc and water. The biologically active EtOAc fraction was then subjected to paper partition chromatography (Whatman 0.16 mm No. 1 Chr) in *n*-BuOH–HOAc–H_2O (BAW 4:1:5, upper phase, solvent system A), 6% v/v aq. HOAc (solvent system B), and H_2O (solvent system C). Bands were cut from the chromatograms, extracted with warm MeOH and bioassayed against *H. virescens*. The bands causing growth inhibition were combined and precipitated from cold EtOAc. The precipitate that formed was recrystallized from pyridine to yield biologically active pale yellow needles. Spectrophotometric (UV, IR) and chromatographic (thin-layer, paper, gas–liquid) comparisons of the needles with an authentic sample (as well as chemical reaction with nitrous acid) established the identity of the active constituent as ellagic acid (1). Ellagic acid (1) was subsequently isolated or detected in the active hot methanolic extracts of all five species of plants.

Quantitation of free ellagic acid (1). Free ellagic acid (1) in the hot methanolic extracts of *T. chinensis*, *G. viscosissimum* var. *viscosissimum*, *Q. gambelii*, *E. cicutarium* and *C. villosus* was pertrimethylsilylated (in Tri-Sil Z) and analysed by GC [8]. Quantitation of the peak eluting at the same retention time (26.1 min under the conditions described below) as an authentic sample of pertrimethylsilylated ellagic acid was accomplished by comparison of either the peak height plotted on a linear calibration curve, or the peak area calculated by integration, with that of the authentic sample.

Glass capillary gas chromatography. GC analyses were performed using a Varian Vista 6000 Series gas chromatograph equipped with a split/splitless capillary injector and a flame ionization detector (FID, 350°). Separations were achieved with a fused silica wall-coated open tubular (WCOT) capillary column (30 m × 0.32 mm i.d.) coated with bonded phase DB-5 (0.25 μm film thickness; J & W Scientific, Inc., Rancho Cordova, CA) using nitrogen as the carrier gas [column head pressure, 10 psi

(0.70 kg/cm²)]. GC analyses were carried out in the split mode (split ratio, 1:25) with the injector (all-glass-lined inlet system) temperature at 275°. The oven temperature was programmed from 120 to 325° at 8°/min and held at 325° for 5 min.

Ellagic acid (1) was dissolved in Tri-Sil Z (Pierce Chemical Co., Rockford, IL) to ensure complete silylation of all free hydroxyl groups. Samples (0.1–1.0 μl) were delivered with a Hamilton Microliter Model 7001 1.0 μl syringe fitted with a needle spacer. Peaks were recorded and peak areas were calculated using a Varian (Spectra-Physics) model SP4270 computing recorder/integrator (chart speed, 10 mm/min).

Isolation of geraniin (2). Fresh aerial parts of *G. viscosissimum* var. *viscosissimum* were cut into small pieces and soaked in MeOH at ambient temperature. The resulting aq. MeOH extracts were subsequently combined, flash evaporated *in vacuo* (at ambient temperature) followed by freeze-drying, washed with hexane and partitioned between EtOAc and H_2O. The EtOAc fraction, which was found to be active in bioassays with *H. virescens*, was then subjected to liquid chromatography on Sephadex LH-20-100 (25–100 μ, Sigma) in 100% MeOH either on a gravity or a low pressure (1.2 ml/min, 4 psi) column. Eluted fractions were combined according to their relative retention values on cellulose thin-layer plates (Eastman Kodak) in BAW (4:1:5, upper) and their colour reaction to the Procter–Paessler reagent (nitrous acid in the absence of oxygen) [66, 72]. The bioassay indicated that the majority of the activity resided in two fractions. Crystals of one of these fractions, giving a yellow colour in the Procter–Paessler reagent, were identified (UV, IR) as ellagic acid (1). The other active fraction yielded crystals which, after an initial reddening, rapidly turned blue, and finally slowly turned yellow in the Procter–Paessler reagent, indicating the presence of esters of hexahydroxydipbenic acid [42]. These latter biologically active crystals, which represented over 1% of the dry weight of the aerial parts, were subsequently identified (UV, IR, ^{1}H NMR, FAB-MS) as geraniin (2) by comparison with literature values [33, 45, 73].

Fast atom bombardment mass spectrometry of geraniin (2). A fast atom bombardment (FAB) mass spectrum of geraniin (2) was obtained on a Varian MAT 731 high-resolution double-focusing mass spectrometer operated at an accelerating potential of 8 kV. The instrument was modified to accept a FAB gun (Ion Tech, Ltd., FAB 11 N saddle field-type ion source) closely following the configuration of Martin *et al.* [74]. A neutral xenon (Xe^0) beam of 6 kV energy and a neutral current of approximately 5×10^{-7} A were employed. Ion source pressure was 1×10^{-5} torr. Conditions for mass spectrometry and the FAB gun were the same in both the positive and negative ion modes. No positive ion FAB spectrum was obtainable with either glycerol solution or with glycerol containing 5% HOAc. The sample dissolved in neat glycerol did, however, afford a spectrum under negative ion detection. The major ion observed (*m/z* 951) corresponds to $[M-H]^-$, with additional ions observed at *m/z* 965 (M′) and *m/z* 935. The corresponding glycerol adducts were seen shifted by +92 amu. M′, which is 14 amu greater than the ion corresponding to the expected molecular weight (at *m/z* 951), may indicate the presence of a molecular species containing an additional methylene equivalent (*O*-methyl group) compared to geraniin (2). The ion observed at *m/z* 935 could be $[M-O]^-$ or $[M'-CH_2O]^-$.

Ellagic acid (1). R_f (A) 0.39 (streak), R_f (B) 0.01, R_f (C) 0.05 (streak) (mauve under longwave UV light; red-brown after spraying with 6% nitrous acid reagent and heating); UV λ_{max}^{MeOH}: 253, 365 nm; UV λ_{max} (1 M Tris buffer, pH 8.0): 219, 254, 279, 360 nm; IR ν_{max}^{KBr}: 3420, 3080, 1695, 1620, 1585, 1505, 1425, 1395, 1335, 1260, 1200, 1115, 1050, 925, 880, 815, 760 cm^{-1}.

Geraniin (2). R_f (A) 0.58 (streak), R_f (B) 0.15, R_f (C) 0.12 (streak) (dark blue under longwave UV light; red-brown after spraying with 6% nitrous acid reagent and heating); UV λ_{max}^{MeOH}: 223, 283 nm; UV λ_{max} (1 M Tris buffer, pH 8.0): 224, 240, 328 nm; IR ν_{max}^{KBr}: 3400, 1740 (sh), 1730, 1710, 1700 (sh), 1615, 1345, 1210 cm^{-1}; ^{1}H NMR (JEOL 270 MHz, acetone-d_6): δ4.32 (*m*, 1H, glu-H-5), 4.80–4.93 (*m*, 2H, glu-H-3, H-6_B) 5.17 (*s*, 1H, H_B), 5.40–5.57 (*m*, 3H, glu-H-2, H-4, H-6_A), 6.53 (*s*, 1H, H_A), 6.56 (*br s*, 1H, glu-H-1), 6.67 [*s*, 1H, hexahydroxydiphenoyl (HHDP)], 7.15 (*s*, 1H, HHDP), 7.19 (*s*, 2H, galloyl), 7.20 (*s*, 1H, H_C); FAB-MS (negative ion mode), *m/z* 951 $[M-H]^-$ ($C_{41}H_{27}O_{27}$), corresponding to a molecular formula of $C_{41}H_{28}O_{27}$ (M 952).

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