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Flavonoid and Galloyl Glycosides Isolated from *Saxifraga spinulosa* and Their Antioxidative and Inhibitory Activities against Species That Cause Piroplasmosis

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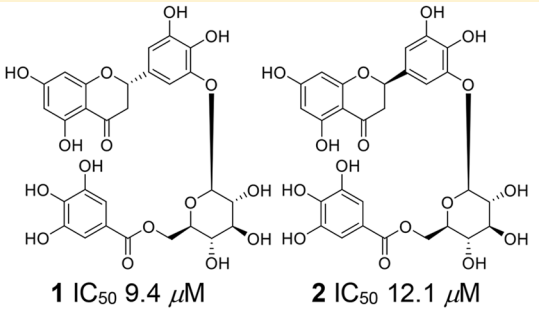
Supporting Information

Saxifraga spinulosa



Babesia bovis growth inhibition assay

ABSTRACT: Eight new flavonoid-based 3′-*O*-*β*-D-glucopyranosides (**1**−**8**) and three new galloyl glucosides (**9**, **11**, **12**), were isolated from the aerial parts of *Saxifraga spinulosa*, along with 25 known compounds. The structures of the new compounds were elucidated by spectroscopic methods. Most of the isolated compounds exhibited potent DPPH radical-scavenging activities. Further, their inhibitory activities were evaluated against *Babesia bovis*, *Babesia bigemina*, *Babesia caballi*, and *Theileria equi*, protozoan parasites that cause piroplasmosis in livestock. The results indicated that several of these compounds showed growth-inhibitory effects on such organisms that cause piroplasmosis.

The genus *Saxifraga*, one of the largest genera of the Saxifragaceae family, comprises 540 species and is distributed widely in mountainous and rocky regions, with 12 species being found in Mongolia.[1] *Saxifraga spinulosa* Adams is a perennial herbaceous plant common in mainland China, Russia, and Mongolia, inhabiting stony marginal terrain.[2] In Asian folk medicine, numerous wild-collected *Saxifraga* species are used to treat different disorders, including cancer, headache, hemorrhoids, influenza, liver diseases, neuralgia, phthisis bulbi, and tympanitis.[3,4] Moreover, the aerial parts of *S. spinulosa* are used in Russia and the Tibetan plateau to treat conjunctivitis, gynecopathy, and diseases of the larynx.[5]

Flavonoids, phenolic acids, terpenoids, and phytosterols have been reported as chemical constituents of *S. montana*,[6] *S. melanocentra*,[7] *S. stolonifera*,[8] *S. tangutica*,[9] *S. hirculus*,[10] *S. cernua*, *S. micranthidifolia*, *S. tolmiei*, *S. tricuspidanta*, *S. melanocentra*,[11] and *S. cuneifolia*.[12] Although detailed chemical structures of its active compounds have not been identified to date, phytochemical investigations have revealed that extracts of *S. spinulosa* exhibit inhibitory activities against acetylcholinesterase and Gram-negative bacteria.[13] Thus, among the 52 Mongolian medicinal plant extracts tested for their DPPH

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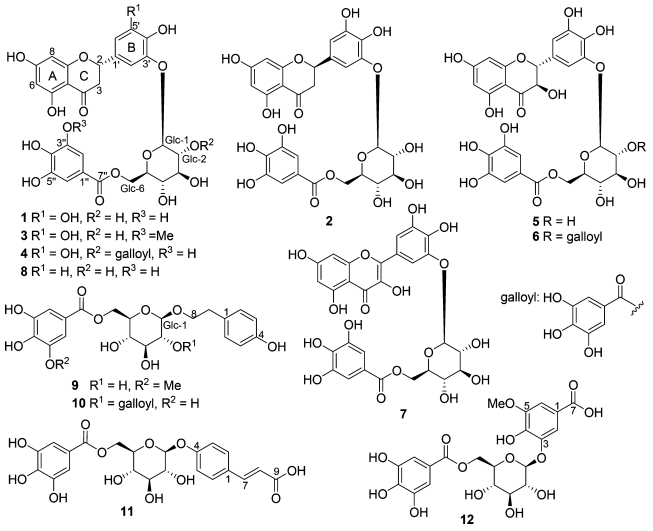
radical-scavenging activity, the *n*-butanol extract of the aerial parts of *S. spinulosa* displayed the greatest activity (IC_{50} 28.7 ± 0.1 μg/mL).[14]

Piroplasmosis is caused by pathogenic protozoa of the genera *Babesia* and *Theileria*, [*Babesia bovis*, *B. bigemina*, *B. caballi*, and *Theileria equi* (synonym: *Babesia equi*)].[15] *B. bovis* and *B. bigemina* infect cattle and destroy their red blood cells, leading to an increased risk of mortality. Clinical symptoms include hemolytic anemia, fever, hemoglobinuria, and marked splenomegaly. Similarly, equine piroplasmosis is caused by *B. caballi* and *T. equi*. All these protozoal diseases negatively affect animal husbandry and, hence, the economy of developing countries that have a considerable dependence on livestock farming. In Mongolia, the cattle industry is an important sector of the national economy, with bovine babesiosis caused by *B. bovis* or *B. bigemina* being a serious problem.[16] Therefore, the identification of babesiacidal compounds from native Mongolian plants may bring significant veterinary benefits to local livestock farming.



In this study, eight new flavonoid glucosides (**1**–**8**) and three new galloyl glucosides (**9**, **11**, and **12**) were isolated from the aerial parts of *S. spinulosa* together with 25 known compounds. The new compounds display the rare structural feature of having a glucosyl moiety connected to the C-3′ carbon of a pyrogallol B-ring. The isolated compounds were screened for their DPPH radical-scavenging and inhibitory activities against *Babesia* and *Theileria* parasites.

RESULTS AND DISCUSSION

An acetone–H_2O extract of the aerial parts of *S. spinulosa* was dissolved in H_2O and the more lipophilic constituents were partitioned into Et_2O. The aqueous extract was separated by HPLC using an ODS column to afford 36 compounds. Compounds of previously known structure were identified by comparing their chemical and spectroscopic characteristics with reported data. These were identified as gallocatechin,[17] catechin,[17] gallocatechin-3-*O*-gallate (**13**),[18] epigallocatechin-3-*O*-gallate,[18] 3-*O*-(6″-*O*-galloyl-β-D-glucopyranosyl)-gallocatechin,[12] quercetin,[19] luteolin,[19] myricetin (**14**),[20] rutin (**15**),[21] quercetin 3-*O*-β-D-glucopyranoside (**16**),[21] quercetin 3-*O*-α-L-rhamnopyranoside,[22] quercetin 3-*O*-β-D-(6″-*O*-galloyl)-glucopyranoside (**17**),[23] quercetin 3-*O*-β-D-(6″-*O*-galloyl)-galactopyranoside (**18**),[23] myricetin 3-*O*-β-D-galactopyranoside (**19**),[24] eriodictyol (**20**),[25] taxifolin (**21**),[26] dihydromyricetin,[27] (2*R*,3*R*)-dihydromyricetin 3′-*O*-β-D-glucopyranoside,[28] naringenin 7-*O*-β-D-(6″-*O*-galloyl)glucopyranoside,[29] tyrosol,[30] 6″-*O*-galloyl salidroside,[31] 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(2″,6″-di-*O*-galloyl)glucopyranoside (**22**),[32] phenethyl *O*-α-L-rhamnopyranosyl-(1–6)-*O*-β-D-glucopyranoside,[33] and gallic acid.[23] Analysis of their ^1H and ^{13}C NMR spectra revealed that compounds **1**–**9** and **11**–**12** exhibit a common structural feature of having an acylated glucopyranosyl moiety. The glucopyranosyl D-configurations of these new compounds were determined by HPLC analysis based on the detection of D-glucose derivative peaks after acid hydrolysis and derivatization;[34] the anomeric proton coupling constants (J = 7.0–8.0 Hz, H-1″) were indicative of their β-orientation.[28]

The ^1H and ^{13}C NMR spectra of **1** and **2** were similar, with a molecular formula of $C_{28}H_{26}O_{16}$ determined for both compounds on the basis of the HRFABMS data (**1**: m/z 641.1113 and **2**: m/z 641.1127 $[M + Na]^+$, calcd for $C_{28}H_{26}O_{16}Na$, 641.1117). The ^1H NMR spectrum of compound **1** displayed an oxymethine proton resonance (δ_H 5.10, 1H, dd, J = 11.5, 3.5 Hz, H-2) coupled to methylene protons (δ_H 2.94, 1H, dd, J = 17.5, 11.5 Hz, H-3ax; 2.65, 1H, dd, J = 17.5, 3.5 Hz, H-3eq), with coupling constants of 11.5 and 3.5 Hz indicating the presence of axial and equatorial protons[29] in the flavonoid C-ring. Additionally, two sets of aromatic proton doublets (δ_H 5.86, 1H, d, J = 2.5 Hz, H-6; and 5.85, 1H, d, J = 2.5 Hz, H-8 and δ_H 6.75, 1H, d, J = 1.0 Hz, H-2′; and 6.67, 1H, d, J = 1.0 Hz, H-6′) were observed, with the latter assigned to the aglycone B-ring based on NOE correlations between H-2 and H-2′ and -6′ and with the former assigned to the A-ring based on their HMBC correlation with C-7 (δ_C 168.3). The NOE correlation between H-2′ and the anomeric proton of the glycosyl moiety (δ_H 4.79, 1H, d, J = 7.0 Hz, H-1″) together with the long-range HMBC correlation between H-1″ and C-3′ (δ_C 147.1) revealed that the glycosyl moiety is bonded to C-3′ (Figure 1). The phenolic proton

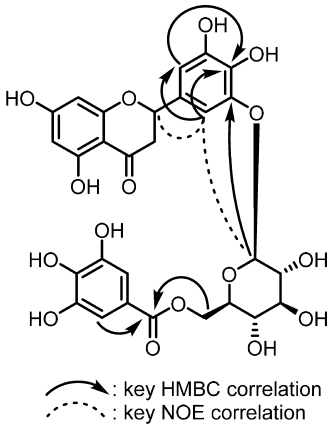


Figure 1. Structures and key HMBC and NOE correlations of **1** and **2**.

singlet at δ_H 7.06 (2H, s, H-2‴ and H-6‴) and the signals of aromatic (δ_C 121.2, C-1‴; 110.1, C-2‴ and C-6‴; 146.5, C-3‴ and C-5‴; 139.9, C-4‴) and carbonyl carbons (δ_C 168.2, C-7‴) indicated the presence of an acylated galloyl moiety in **1** and **2**. The H-6″ proton (δ_H 4.58, 1H, dd, J = 12.0, 2.0 Hz; 4.47, 1H, dd, J = 12.0, 5.0 Hz) and carbon (δ_C 64.5) resonances were downfield-shifted when compared to those of the nonacylated form,[28] suggesting that the acyl moiety is bonded to C-6″. HMBC experiments revealed the H-6″ proton to exhibit long-

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range coupling with the C-7‴ carbonyl carbon, supporting this conclusion. The negative Cotton effect of **1** at ca. 289 nm in the ECD spectrum indicated its 2*S*-configuration (Figure 2).[35]

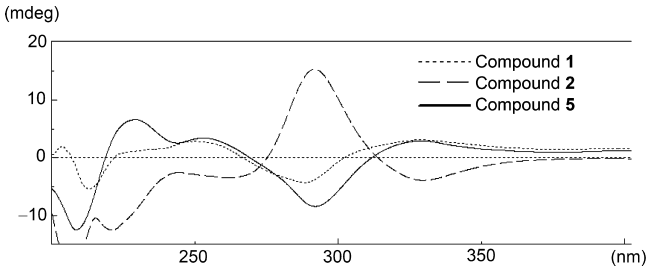

Figure 2. ECD spectra of compounds **1**, **2**, and **5**.

Based on these data, the structure of **1** was determined as (2*S*)-3′-*O*-*β*-D-(6″-*O*-galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone. In turn, the positive Cotton effect of **2** at ca. 291 nm in the ECD spectrum (Figure 2) revealed the structure of **2** as having a 2*R*-configuration (Figure 2);[35] thus, this compound was assigned as (2*R*)-3′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone.

Compound **3** was assigned the molecular formula, $C_{29}H_{28}O_{16}$, on the basis of its HRFABMS data (*m*/*z* 655.1261 $[M + Na]^+$, calcd for $C_{29}H_{28}O_{16}Na$, 655.1273); this compound comprised one more CH_2 moiety than **1**. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **1**, except for the presence of resonances from a galloyl aromatic ring (δ_H 7.11, d, *J* = 2.0 Hz, H-2‴; 7.17, d, *J* = 2.0 Hz, H-6‴; δ_C 121.1, C-1‴; 106.1, C-2‴; 149.1, C-3‴; 140.8, C-4‴; 146.2, C-5‴; 112.0, C-6‴, 168.1, C-7‴) and an *O*-methyl group (δ_H 3.79, 3H, s; δ_C 56.6). The long-range HMBC coupling between the *O*-methyl proton and C-3‴ and the NOE correlation between the *O*-methyl proton and H-2‴ indicated that **3** features a 3‴-*O*-methylgalloyl moiety instead of the galloyl moiety observed in **1**. The negative Cotton effect at 288 nm implied a 2*S* absolute configuration,[35] thus, **3** was determined as (2*S*)-3′-*O*-*β*-D-[6″-*O*-(3‴-*O*-methyl)galloyl]glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone.

On the basis of the HRFABMS data (*m*/*z* 793.1236 $[M + Na]^+$, calcd for $C_{35}H_{30}O_{20}Na$, 793.1226), the molecular formula of **4** was determined as $C_{35}H_{30}O_{20}$, suggesting the presence of an additional galloyl moiety ($C_7H_4O_4$) when compared to **1**. In the corresponding ^1H and ^{13}C NMR spectra, two sets of galloyl group resonances were observed (δ_H 7.13, 2H, s, H-2‴ and H-6‴; δ_C 121.2, C-1‴; 110.5, C-2‴ and C-6‴; 146.5, C-3‴ and C-5‴; 140.1, C-4‴; 168.1, C-7‴ and δ_H 7.09, 2H, s, H-2⁗ and H-6⁗; δ_C 121.3, C-1⁗; 110.2, C-2⁗ and C-6⁗; 146.5, C-3⁗ and C-5⁗; 139.9, C-4⁗; 168.2, C-7⁗). The long-range HMBC correlations of H-2″ (δ_H 5.20, overlapping) with C-7‴ and those of H-6″ (δ_H 4.63, 1H, dd, *J* = 12.0, 2.0 Hz; 4.48, 1H, dd, *J* = 12.0, 5.0 Hz) with C-7⁗ indicated the presence of a 2,6-digalloylglucopyranosyl moiety, while the negative Cotton effect at 288 nm implied a 2*S*-configuration of the flavanone moiety.[35] Therefore, the structure of **4** was determined as (2*S*)-3′-*O*-*β*-D-(2″,6″-di-*O*-galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone.

The molecular formulas of **5** and **6** were determined as $C_{28}H_{26}O_{17}$ (*m*/*z* 657.1058 $[M + Na]^+$, calcd for $C_{28}H_{26}O_{17}Na$, 657.1066) and $C_{35}H_{30}O_{21}$ (*m*/*z* 809.1153 $[M + Na]^+$, calcd for $C_{35}H_{30}O_{21}Na$, 809.1175), respectively, based on their molecular ions observed in the HRFABMS. Compounds **5** and **6** comprised one oxygen atom more than **1** and **4**, respectively, corresponding to the presence of hydroxy groups. Moreover, many ^1H and ^{13}C NMR spectroscopic features observed for **1** and **5** were also observed in **4** and **6**, respectively. Thus, **5** and **6** included an oxymethine proton (**5**: δ_H 4.39, 1H, d, *J* = 11.5 Hz, H-3; **6**: δ_H 4.34, 1H, d, *J* = 11.5 Hz, H-3) and a carbon (**5**: δ_C 73.6, C-3; **6**: δ_C 73.7, C-3) instead of the aliphatic methylene of the corresponding **1** and **4**. This suggested the presence of a flavanonol unit instead of a flavanone core. The coupling constants between H-2 and H-3 (*J* = 11.5 Hz) and the negative Cotton effects at about 292 nm in the ECD spectra of **5** (Figure 2) and **6** were both indicative of a 2*R*,3*R*-configuration.[17,26,28] Based on these data, the structure of **5** was determined as (2*R*,3*R*)-3′-*O*-*β*-D-(6″-*O*-galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanonol, and that of **6** as (2*R*,3*R*)-3′-*O*-*β*-D-(2″,6″-di-*O*-galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanonol.

Compound **7** was assigned the molecular formula $C_{28}H_{24}O_{17}$ (*m*/*z* 655.0937 $[M + Na]^+$, calcd for $C_{28}H_{24}O_{17}Na$, 655.0909), with two hydrogen atoms less than that of **5**. The ^{13}C NMR spectrum of **7** displayed two oxygenated olefinic carbon resonances (δ_C 147.0, C-2; 137.6, C-3) instead of the two oxygenated sp^3 carbons observed in **5**. This suggested that **7** comprised a flavonol myricetin core[20] instead of the flavanonol core in **5**. Nonequivalent proton resonances (δ_H 7.69, 1H, d, *J* = 2.0 Hz, H-2′; 7.50, 1H, d, *J* = 2.0 Hz, H-6′) were observed in the ^1H NMR spectrum of **7**. These supported the conclusion that the glycosyl moiety is bonded to C-3′. Therefore, the structure of **7** was determined as 3′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavonol.

In the ^1H NMR spectrum of **8**, a set of coupled proton resonances (δ_H 7.17, 1H, d, *J* = 2.0 Hz, H-2′; 6.85, 1H, d, *J* = 8.5 Hz, H-5′; 7.01, 1H, dd, *J* = 8.5, 2.0 Hz, H-6′), indicative of a catechol B-ring, was observed. The molecular formula $C_{28}H_{26}O_{15}$, as determined from the HRFABMS data (*m*/*z* 625.1169 $[M + Na]^+$, calcd for $C_{28}H_{26}O_{15}Na$, 625.1168), demonstrated that **8** contains one oxygen atom less than **1**. This suggested that the aglycone moiety of **8** to be eriodictyol.[17] The long-range HMBC correlation of the anomeric proton (δ_H 4.85, 1H, d, *J* = 7.5 Hz, H-1″) with C-3′ (δ_C 144.9) established that the glucosyl moiety is bonded to C-3′, whereas the negative Cotton effect at 287 nm suggested a 2*S*-absolute configuration.[35] Collectively, these data were used to assign **8** as (2*S*)-3′-*O*-*β*-D-(6″-*O*-galloyl)glucopyranosyloxy-5,7,4′-trihydroxyflavanone.

While the ^1H and ^{13}C NMR spectra indicated that **1**−**12** all share a 6″-*O*-galloylglucopyranosyl unit, compounds **9**−**12** were found to contain different aglycone moieties rather than a flavonoid core as in **1**−**8**. The ^1H and ^{13}C NMR data of the [6′-*O*-(3″-*O*-methyl)galloyl]glucopyranosyl moiety in **9** were similar to those observed for **3**. Further, resonances from a *p*-phenyl (δ_H 6.96, 2H, d, *J* = 9.0 Hz, H-2 and H-6; 6.65, 2H, d, *J* = 9.0 Hz, H-3 and 5; δ_C 130.5, C-1; 130.9, C-2 and C-6; 116.1, C-3 and C-5; 156.7, C-4) and an ethoxy (δ_H 2.80, 2H, m, H-7; 3.72, 1H, m, H-8; 3.92, 1H, m, H-8; δ_C 36.5, C-7; 72.4, C-8) unit were observed in the spectra of **9** instead of the flavanone signals observed in the spectra of **3**. In the HMBC spectrum, the anomeric proton (δ_H 4.34, 1H, d, *J* = 8.0 Hz, H-1′) exhibited long-range coupling with C-8, while H-2 and H-6 were coupled with C-7 and H-8 was coupled with C-1, suggesting *p*-tyrosol as the aglycone moiety in **9**.[31] Based on these data and a molecular formula of $C_{22}H_{26}O_{11}$ as determined by the HRFABMS data (*m*/*z* 489.1385 $[M + Na]^+$, calcd for

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$C_{22}H_{26}O_{11}Na$, 489.1372), the structure of **9** was assigned as 8-*O*-*β*-D-[6′-*O*-(3″-*O*-methyl)galloyl]glucopyranosyl-*p*-tyrosol.

The closely related compound **10** exhibited spectroscopic features similar to those displayed by the *p*-tyrosol moiety in **9** and 3′-*O*-*β*-D-(2″,6″-di-*O*-galloyl)glucopyranosyl moieties in **6**, with a molecular formula of $C_{28}H_{28}O_{15}$ determined by HRFABMS (*m*/*z* 627.1319 $[M + Na]^+$, calcd for $C_{28}H_{28}O_{15}Na$, 627.1324). In the HMBC spectrum, long-range coupling was observed between H-1′ (δ_H 4.61, 1H, d, *J* = 8.0 Hz) and C-8 (δ_C 72.1), H-2′ (δ_H 4.93, 1H, dd, *J* = 9.5, 8.0 Hz) and C-7″ (δ_C 167.7), and H-6′ (δ_H 4.55, 1H, dd, *J* = 12.0, 2.5 Hz; 4.47, 1H, dd, *J* = 12.0, 5.5 Hz) and C-7‴ (δ_C 168.4). Therefore, the structure of **10** was identified as the previously known compound 8-*O*-*β*-D-(2′,6′-di-*O*-galloyl)glucopyranosyl-*p*-tyrosol.[36]

For **11**, two sets of coupling proton resonances (δ_H 7.48, 2H, d, *J* = 9.0 Hz, H-2 and H-6; 7.09, 2H, d, *J* = 9.0 Hz, H-3 and H-5 and δ_H 7.59, 1H, d, *J* = 16.0 Hz, H-7; 6.35, 1H, d, *J* = 16.0 Hz, H-8) and a carbonyl carbon at δ_C 170.9 (C-9) suggested the presence of a *p*-coumaroyl moiety, with a coupling constant between that of H-7 and H-8 (16.0 Hz) indicative of a *trans*-orientation.[32] The molecular formula of **11** ($C_{22}H_{22}O_{12}$) was determined by the HRFABMS data (*m*/*z* 501.0996 $[M + Na]^+$, calcd for $C_{22}H_{22}O_{12}Na$, 501.1008). Furthermore, the NOE correlations between H-1′ (δ_H 4.94, 1H, d, *J* = 7.5 Hz) and H-3 and H-5 supported the structure of **11** as being 4-*O*-*β*-D-(6′-*O*-galloyl)glucopyranosyl-(*E*)-*p*-coumaroyl acid.

Resonances attributed to six aromatic (δ_C 122.3, C-1; 113.2, C-2; 146.5, C-3; 142.6, C-4; 149.3, C-5; 109.7, C-6), one carbonyl (δ_C 170.3, C-7), and one methoxy (δ_C 56.8) carbon were observed in the ^{13}C NMR spectrum of **12**. The corresponding HMBC spectrum displayed long-range coupling between a set of proton doublets (δ_H 7.57, 1H, d, *J* = 2.0 Hz, H-2; 7.39, 1H, d, *J* = 2.0 Hz, H-6) and C-7, the methoxy proton (δ_H 3.89, 3H, s) and C-5, and H-1′ (δ_H 4.92, 1H, d, *J* = 7.5 Hz) and C-3. The molecular formula $C_{21}H_{22}O_{14}$ was assigned to compound **12** on the basis of its HRFABMS data [*m*/*z* 521.0888 $[M + Na]^+$, calcd for $C_{21}H_{22}O_{14}Na$, 521.0906). Thus, its structure was determined as 3-*O*-*β*-D-(6′-galloyl)-glucopyranosyl-5-*O*-methylgallic acid.

The isolated compounds were screened for DPPH radical-scavenging activity (Table 1), with trolox as a positive control (IC_{50} 23.3 *μ*M). All the new glucosides exhibited potent activities (IC_{50} 19.0−72.9 *μ*M). A crude extract of *S. spinulosa* has been reported to display the highest DPPH radical-scavenging activity among numerous Mongolian medicinal plants,[13,14] which may now be attributed, at least in part, to the presence of the new flavonoid and galloyl group-containing isolated compounds.

Inhibitory activities of the isolated compounds also were determined against *Babesia bovis*, *B. bigemina*, *B. caballi*, and *Theileria equi* (Table 2). Diminazene aceturate was used as a positive control.[37] *B. bovis* was inhibited by **1**, **2**, **10**, **13**, and **22** (IC_{50} 3.8−12.1 *μ*M). Compounds **1**, **2**, **10**, **13**, **17**, and **20**−**22** displayed inhibitory activity against *B. bigemina* (IC_{50} 11.0−46.7 *μ*M), while *B. caballi* was inhibited by **10**, **14**−**19**, **21**, and **22** (IC_{50} 2.5−51.3 *μ*M). In turn, compounds **14**, **19**, and **21** inhibited *T. equi* (IC_{50} 41.7, 40.6, and 51.6 *μ*M, respectively). Compounds **1**, **2**, **10**, **13**, **17**, **18**, and **22** are glucosides bearing galloyl groups, whereas **15**−**18** are quercetin glycosides. A quercetin glucoside and a phenolic glucoside bearing a galloyl group were reported as antibabesial compounds against *B. gibsoni*,[38] and the findings of the present study are consistent with this report.

Table 1. DPPH Radical Scavenging Activities of Compounds Isolated from *Saxifraga spinulosa*

compound	IC_{50} (*μ*M)[a]
1	53.1 ± 0.3
2	58.8 ± 0.4
3	64.9 ± 0.6
4	42.3 ± 0.6
5	29.3 ± 0.3
6	42.5 ± 0.2
7	44.7 ± 0.2
8	72.9 ± 0.6
9	49.1 ± 0.2
10	31.4 ± 0.2
11	35.1 ± 0.4
12	19.0 ± 0.3
trolox	23.3 ± 0.3

[a]Treatments were performed in triplicate.

Table 2. Inhibitory Activity of Compounds Isolated from *Saxifraga spinulosa* against Three *Babesia* and One *Theileria* Species

compound	*B. bovis*[a,b]	*B. bigemina*[a,b]	*B. caballi*[a,b]	*T. equi*[a,b]
1	9.4	19.9	-	-
2	12.1	22.7	-	-
10	5.9	27.1	15.6	-
13	3.8	11.0	-	-
14	-	-	51.3	41.7
15	-	-	20.3	-
16	-	-	23.7	-
17	-	27.2	9.7	-
18	-	-	4.4	-
19	-	-	36.7	40.6
20	-	46.7	-	-
21	-	15.0	41.5	51.6
22	6.4	15.3	2.5	-
diminazene aceturate[39]	0.41	0.18	0.03	0.67

[a]Other isolated compounds were inactive at 20 *μ*g/mL; the structures of compounds **13**−**22** are included in this Supporting Information. [b]IC_{50} (*μ*M), treatment was replicated three times for each concentration.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2300 polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded on a Shimadzu MPS-2450 (Shimadzu, Kyoto, Japan). ECD spectra were recorded on a JASCO J-700 spectropolarimeter (JASCO). ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a JEOL JNM-AL400 FT-NMR spectrometer (JEOL, Tokyo, Japan), and the chemical shifts were reported as *δ* values with TMS as an internal standard (measured in methanol-d_4 and DMSO-d_6). Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulsed-field gradient. HRFABMS data were obtained by a JEOL JMS700 mass spectrometer (JEOL), with a glycerol matrix. Preparative HPLC was performed applying a JASCO 2089 with UV detection at 210 nm (JASCO), using the following columns: Ultra Pack ODS-SM-50C-M (Yamazen, Osaka, Japan, 37 × 100 mm), TSKgel ODS-120T

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(Tosoh, Tokyo, Japan, 21.5 × 300 mm), Cosmosil 5C_{18}-AR-II (Nacalai Tesque, Kyoto, Japan, 20 × 250 mm), Mightysil RP-18 GP (Kanto Chemical, Tokyo, Japan, 10 × 250 mm), and Develosil C_{30}-UG-5 (Nomura Chemical, Aichi, Japan, 20 × 250 mm), Capcell Pak C_8 (Shiseido, Japan, 20 × 250 mm).

Plant Material. The aerial parts of *Saxifraga spinulosa* were collected near Melkhii Khad (N 47°56.230′; E 107°24.964′; H 1972 m), Tuv Province, Mongolia, in June 2015. Dr. Ch. Sanchir, Institute of General and Experimental Biology, Mongolian Academy of Sciences, identified the plant species. A voucher specimen (47.02.07.15A) was deposited at the herbarium of the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia.

Extraction and Isolation. The collected plant material was dried, powdered (630 g), and extracted by maceration with an acetone–water mixture (4:1) (3 × 10 L) at 60 °C. The dried extract (139.6 g) was suspended in H_2O (1 L) and extracted with Et_2O (1 L). The Et_2O and aqueous extracts were separately combined (repeated twice) and evaporated to dryness under reduced pressure. The aqueous extract (96.1 g) was subjected to column chromatography (Mitsubishi HP-20, 70 × 470 mm), with elution performed using 4 L H_2O (fr 1A, 45.4 g), 2 L MeOH–H_2O (1:4, v/v) (fr 1B, 8.3 g), 2 L MeOH–H_2O (2:3, v/v) (fr 1C, 19.4 g), 2 L MeOH–H_2O (3:2, v/v) (fr 1D, 17.2 g), 2 L MeOH–H_2O (9:1, v/v) (fr 1E, 3.4 g), 2 L MeOH (fr 1F, 219 mg). Fraction 1E was loaded on a reversed-phase ODS column (ODS-SM-50C-M; Yamazen, 37 × 100 mm) and eluted with MeOH–H_2O (1:1, v/v) (frs 2A–2E), MeOH–H_2O (3:2, v/v) (frs 2F–2H), and MeOH–H_2O (4:1, v/v) (frs 2I–2J). Fraction 2B was subjected to preparative HPLC to yield compounds **1** (7.1 mg), **2** (9.1 mg) [Capcell Pak C_8, CH_3CN–H_2O (7:33, v/v) containing 0.2% TFA as the mobile phase], **3** (4.6 mg), **4** (9.8 mg), **8** (7.8 mg), **20** (11.0 mg), **21** (2.5 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:3) containing 0.2% TFA], **16** (1.5 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:4, v/v) containing 0.2% TFA], quercetin 3-*O*-α-L-rhamnopyranoside (2.8 mg) [Cosmosil 5C_{18}-AR-II, CH_3CN–H_2O (1:4, v/v) containing 0.2% TFA], **22** (4.0 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA], gallic acid (10.4 mg) [TSKgel ODS-120T, CH_3CN–H_2O (1:4, v/v) containing 0.2% TFA], and naringenin 7-*O*-β-D-(6″-*O*-galloyl)glucopyranoside (3.1 mg) [Mightysil RP-18 GP, CH_3CN–H_2O (1:3, v/v) containing 0.2% TFA]. Quercetin (9.2 mg) and luteolin (0.9 mg) were obtained by purification of fractions 2D [Develosil C_{30}-UG-5, CH_3CN–H_2O (7:13, v/v) containing 0.2% TFA] and 2E [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:7, v/v) containing 0.2% TFA], respectively. Fraction 1D was loaded on to a reversed-phase ODS column (ODS-SM-50C-M) and eluted with MeOH–H_2O (3:7, v/v) (frs 3A–3H), MeOH-H_2O (2:3, v/v) (frs 3I–3K), and MeOH-H_2O (1:1, v/v) (frs 3L–3M). Compounds **6** (8.0 mg), **7** (2.1 mg), and **11** (2.9 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] were obtained by purification of fraction 3E; **9** (7.9 mg), **10** (12.1 mg), phenethyl *O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside (1.5 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:4, v/v) containing 0.2% TFA], **14** (6.2 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:3, v/v) containing 0.2% TFA], **17** (2.5 mg), **18** (15.6 mg) [Capcell Pak C_8, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA], and **19** (4.6 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:4, v/v) containing 0.2% TFA] were obtained by purification of fractions 3F and 3G; **15** (8.4 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:4, v/v) containing 0.2% TFA] was obtained by purification of fr 3H. Fraction 1C was loaded on a reversed-phase ODS column (ODS-SM-50C-M) and eluted with MeOH–H_2O (1:9, v/v) (frs 4A–4F), MeOH–H_2O (1:4, v/v) (frs 4G–4K), MeOH–H_2O (3:7, v/v) (frs 4L–4P), and MeOH–H_2O (2:3, v/v) (frs 4Q–4R). Fractions 4C and 4D were subjected to preparative HPLC to yield catechin (2.3 mg), and 3-*O*-(6″-*O*-galloyl-β-D-glucopyranosyl)-gallocatechin (21.5 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:9, v/v) containing 0.2% TFA]. (2*R*,3*R*)-Dihydromyricetin 3′-*O*-β-D-glucopyranoside (3.2 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (1:7, v/v) containing 0.2% TFA], and tyrosol (1.6 mg) [Mightysil RP-18 GP, CH_3CN–H_2O (1:9, v/v) containing 0.2% TFA] were obtained by purification of fraction 4E; epigallocatechin-3-*O*-gallate (4.8 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] and dihydromyricetin (2.3 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] were obtained by purification of fraction 4F; **5** (64.5 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] was obtained by purification of fraction 4G; **13** (25.3 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] was obtained by purification of fraction 4H; gallocatechin (55.8 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] was obtained by purification of fraction 4I; **12** (2.6 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] was obtained by purification of fraction 4J; 6″-*O*-galloyl salidroside (25.7 mg) [Develosil C_{30}-UG-5, CH_3CN–H_2O (3:17, v/v) containing 0.2% TFA] was obtained by purification of fraction 4L.

*(2S)-3′-O-β-D-(6″-O-Galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone (**1**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 82.3 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.34), 249 (3.49), 286 (3.96) nm; ECD (*c* 0.00024, MeOH) ([θ]) 250 (+9900), 289 (−14900), 329 (+10900) nm; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.06 (2H, s, H-2‴ and H-6‴), 6.75 (1H, d, *J* = 1.0, Hz, H-2′), 6.67 (1H, d, *J* = 1.0, Hz, H-6′), 5.86 (1H, d, *J* = 2.5, Hz, H-6), 5.85 (1H, d, *J* = 2.5, Hz, H-8), 5.10 (1H, dd, *J* = 11.5, 3.5 Hz, H-2), 4.79 (1H, d, *J* = 7.0 Hz, H-1″), 4.58 (1H, dd, *J* = 12.0, 2.0 Hz, H-6″), 4.47 (1H, dd, *J* = 12.0, 5.0 Hz, H-6″), 3.73 (1H, m, H-5″), 3.52 (overlapping, H-2″, H-3″, and H-4″), 2.94 (1H, dd, *J* = 17.5, 11.5 Hz, H-3ax), 2.65 (1H, dd, *J* = 17.5, 3.5 Hz, H-3eq); ^{13}C NMR (methanol-d_4, 100 MHz) δ 197.2 (C-4), 168.3 (C-7), 168.2 (C-7‴), 165.4 (C-5), 164.4 (C-8a), 147.2 (C-5′), 147.1 (C-3′), 146.5 (C-3‴ and C-5‴), 139.9 (C-4‴), 136.8 (C-4′), 131.2 (C-1′), 121.2 (C-1‴), 110.1 (C-2‴ and C-6‴), 109.9 (C-6′), 108.8 (C-2′), 104.4 (C-1″), 103.4 (C-4a), 97.1 (C-6), 96.1 (C-8), 79.9 (C-2), 77.4 (C-3″), 75.8 (C-5″), 74.9 (C-2″), 71.5 (C-4″), 64.5 (C-6″), 43.5 (C-3); HRFABMS (positive-ion mode) *m*/*z* 641.1113 [M + Na]$^+$ (calcd for $C_{28}H_{26}O_{16}Na$, 641.1117).

*(2R)-3′-O-β-D-(6″-O-Galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone (**2**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 40 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.42), 249 (3.54), 286 (4.01) nm; ECD (*c* 0.00018, MeOH) ([θ]) 261 (−8700), 291 (+38900), 326 (−9900) nm; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.06 (2H, s, H-2‴ and H-6‴), 6.75 (1H, d, *J* = 1.0, Hz, H-2′), 6.67 (1H, d, *J* = 1.0, Hz, H-6′), 5.86 (1H, d, *J* = 2.5, Hz, H-6), 5.85 (1H, d, *J* = 2.5, Hz, H-8), 5.10 (1H, dd, *J* = 11.5, 3.5 Hz, H-2), 4.79 (1H, d, *J* = 7.0 Hz, H-1″), 4.58 (1H, dd, *J* = 12.0, 2.0 Hz, H-6″), 4.47 (1H, dd, *J* = 12.0, 5.0 Hz, H-6″), 3.73 (1H, m, H-5″), 3.52 (overlapping, H-2″, H-3″, and H-4″), 2.94 (1H, dd, *J* = 17.5, 11.5 Hz, H-3ax), 2.65 (1H, dd, *J* = 17.5, 3.5 Hz, H-3eq); ^{13}C NMR (methanol-d_4, 100 MHz) δ 197.2 (C-4), 168.3 (C-7), 168.2 (C-7‴), 165.4 (C-5), 164.4 (C-8a), 147.2 (C-5′), 147.1 (C-3′), 146.5 (C-3‴ and C-5‴), 139.9 (C-4‴), 136.8 (C-4′), 131.2 (C-1′), 121.2 (C-1‴), 110.1 (C-2‴ and C-6‴), 109.9 (C-6′), 108.8 (C-2′), 104.4 (C-1″), 103.4 (C-4a), 97.1 (C-6), 96.1 (C-8), 79.9 (C-2), 77.4 (C-3″), 75.8 (C-5″), 74.9 (C-2″), 71.5 (C-4″), 64.5 (C-6″), 43.5 (C-3); HRFABMS (positive-ion mode) *m*/*z* 641.1127 [M + Na]$^+$ (calcd for $C_{28}H_{26}O_{16}Na$, 641.1117).

*(2S)-3′-O-β-D-[6″-O-(3‴-O-Methyl)galloyl]glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone (**3**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 53 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.33), 249 (3.47), 287 (3.97) nm; ECD (*c* 0.00023, MeOH) ([θ]) 251 (+6600), 288 (−22200), 328 (8600) nm; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.17 (1H, d, *J* = 2.0 Hz, H-6‴), 7.11 (1H, d, *J* = 2.0 Hz, H-2‴), 6.72 (1H, d, *J* = 1.5 Hz, H-6′), 6.66 (1H, d, *J* = 1.5 Hz, H-2′), 5.86 (1H, d, *J* = 2.0 Hz, H-6), 5.82 (1H, d, *J* = 2.0 Hz, H-8), 5.00 (1H, dd, *J* = 12.5, 3.0 Hz, H-2), 4.80 (1H, d, *J* = 7.5 Hz, H-1″), 4.67 (1H, dd, *J* = 12.0, 2.0 Hz, H-6″), 4.45 (1H, dd, *J* = 12.0, 6.5 Hz, H-6″), 3.79 (3H, s, OMe-3‴), 3.76 (1H, m, H-5″), 3.50 (overlapping, H-2″ and H-3″), 3.48 (1H, m, H-4″), 2.85 (1H, dd, *J* = 17.0, 12.5 Hz, H-3ax), 2.58 (1H, dd, *J* = 17.0, 3.0 Hz, H-3eq); ^{13}C NMR (methanol-d_4, 100 MHz) δ 197.3 (C-4), 168.2 (C-7), 168.1 (C-7‴), 165.4 (C-5), 164.4 (C-8a), 149.1 (C-3‴), 147.3 (C-5′), 147.1 (C-3′), 146.2 (C-5‴), 140.8 (C-4‴), 136.7 (C-4′), 131.4 (C-1′), 121.1 (C-1‴), 112.0 (C-6‴), 109.9 (C-2′), 108.2 (C-6′), 106.1 (C-2‴), 104.3 (C-4a), 104.3 (C-1″), 97.2 (C-6), 96.2 (C-8), 79.9 (C-2), 77.5 (C-3″), 75.9 (C-2″), 74.9 (C-5″),

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71.8 (C-4″), 64.9 (C-6″), 56.6 (OMe-3‴), 43.9 (C-3); HRFABMS (positive-ion mode) m/z 655.1261 $[M + Na]^+$ (calcd for $C_{29}H_{28}O_{16}Na$, 655.1273).

*(2S)-3′-O-β-D-(2″,6″-di-O-Galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanone (**4**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 30 (c 0.9, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.45), 247 (3.72), 285 (4.12) nm; ECD (c 0.00018 MeOH) ($[\theta]$) 257 (+7800), 288 (−11500), 329 (+7800) nm; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.13 (2H, s, H-2‴ and H-6‴), 7.09 (2H, s, H-2‴ and H-6‴), 6.64 (1H, d, J = 2.0 Hz, H-6′), 6.61 (1H, d, J = 2.0 Hz, H-2′), 5.87 (1H, d, J = 2.0 Hz, H-6), 5.85 (1H, d, J = 2.0 Hz, H-8), 5.20 (1H, d, J = 7.0 Hz, H-1″), 5.20 (overlapping, H-2″), 5.09 (1H, dd, J = 12.0, 3.5 Hz, H-2), 4.63 (1H, dd, J = 12.0, 2.0 Hz, H-6″), 4.48 (1H, dd, J = 12.0, 5.0 Hz, H-6″), 3.81 (overlapping, H-3″ and H-5″), 3.67 (1H, 9.5, 9.0, H-4″), 2.85 (1H, dd, J = 17.0, 12.0 Hz, H-3ax), 2.59 (1H, dd, J = 17.0, 3.5 Hz, H-3eq); ^{13}C NMR (methanol-d_4, 100 MHz) δ 197.3 (C-4), 168.2 (C-7), 168.2 (C-7‴), 168.1 (C-7‴), 165.4 (C-5), 164.5 (C-8a), 147.3 (C-5′), 146.7 (C-3′), 146.5 (C-3‴ and C-5‴), 146.5 (C-3‴ and C-5‴), 140.1 (C-4‴), 139.9 (C-4‴), 137.1 (C-4′), 131.3 (C-1′), 121.3 (C-1‴), 121.2 (C-1‴), 110.5 (C-2‴ and 6‴), 110.2 (C-6′), 110.2 (C-2‴ and C-6‴), 108.6 (C-2′), 103.3 (C-4a), 102.5 (C-1″), 97.1 (C-6), 96.2 (C-8), 79.9 (C-2), 76.0 (C-3″), 76.0 (C-5″), 75.5 (C-2″), 71.5 (C-4″), 64.3 (C-6″), 43.8 (C-3); HRFABMS (positive-ion mode) m/z 793.1236 $[M + Na]^+$ (calcd for $C_{35}H_{30}O_{20}Na$, 793.1226).

*(2R,3R)-3′-O-β-D-(6″-O-Galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanonol (**5**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 58 (c 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.38), 249 (3.35), 289 (3.97), 412 (2.95) nm; ECD (c 0.00018, MeOH) ($[\theta]$) 253 (+12000), 292 (−29300), 328 (+10200) nm; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.04 (2H, s, H-2‴ and H-6‴), 6.84 (1H, d, J = 2.0 Hz, H-2′), 6.75 (1H, d, J = 2.0 Hz, H-6′), 5.92 (1H, d, J = 2.5 Hz, H-6), 5.84 (1H, d, J = 2.5 Hz, H-8), 4.84 (1H, d, J = 7.0 Hz, H-1″), 4.78 (1H, d, J = 11.5 Hz, H-2), 4.58 (1H, dd, J = 12.5, 2.5 Hz, H-6″), 4.46 (1H, dd, J = 12.5, 5.0 Hz, H-6″), 4.39 (1H, d, J = 11.5 Hz, H-3), 3.72 (1H, m, H-5″), 3.53 (overlapping, H-2″, H-3″, and H-4″); ^{13}C NMR (methanol-d_4, 100 MHz) δ 197.9 (C-4), 168.6 (C-7), 168.3 (C-7‴), 165.2 (C-5), 164.2 (C-8a), 147.0 (C-3′), 147.0 (C-5′), 146.4 (C-3‴ and C-5‴), 139.8 (C-4‴), 137.1 (C-4′), 129.4 (C-1′), 121.2 (C-1‴), 111.3 (C-6′), 110.1 (C-2‴ and C-6‴), 109.9 (C-2′), 104.5 (C-1″), 101.7 (C-4a), 97.4 (C-6), 96.2 (C-8), 84.7 (C-2), 77.4 (C-3″), 75.8 (C-5″), 74.9 (C-4″), 73.6 (C-3), 71.3 (C-2″), 64.4 (C-6″); HRFABMS (positive-ion mode) m/z 657.1058 $[M + Na]^+$ (calcd for $C_{28}H_{26}O_{17}Na$, 657.1066).

*(2R,3R)-3′-O-β-D-(2″,6″-di-O-Galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavanonol (**6**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 17 (c 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.43), 249 (3.60), 285 (4.02) nm; ECD (c 0.00018, MeOH) ($[\theta]$) 232 (+18500), 292 (−16000), 329 (+9600) nm; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.13 (2H, s, H-2‴ and H-6‴), 7.09 (2H, s, H-2‴ and H-6‴), 6.71 (2H, s, H-2′ and H-6′), 5.92 (1H, d, J = 1.5 Hz, H-6), 5.84 (1H, d, J = 1.5 Hz, H-8), 5.22 (overlapping, H-1″), 5.21 (overlapping, H-2″), 4.76 (1H, d, J = 11.5 Hz, H-2), 4.60 (1H, br d, J = 12.0, Hz, H-6″), 4.48 (1H, dd, J = 12.0, 5.0 Hz, H-6″), 4.34 (1H, d, J = 11.5 Hz, H-3), 3.80 (overlapping, H-3″ and H-5″), 3.69 (1H, dd, J = 9.5, 9.0 Hz, H-4″); ^{13}C NMR (methanol-d_4, 100 MHz) δ 198.0 (C-4), 168.7 (C-7), 168.3 (C-7‴), 168.2 (C-7‴), 165.3 (C-5), 164.3 (C-8a), 147.1 (C-3′), 147.1 (C-5′), 146.6 (C-3‴ and C-5‴), 146.5 (C-3‴ and C-5‴), 140.1 (C-4‴), 139.9 (C-4‴), 137.6 (C-4′), 129.4 (C-1′), 121.3 (C-1‴), 121.2 (C-1‴), 110.5 (C-2‴ and 6‴), 110.2 (C-6′), 110.2 (C-2‴ and 6‴), 110.0 (C-2′), 102.7 (C-1″), 101.8 (C-4a), 97.5 (C-6), 96.4 (C-8), 84.7 (C-2), 76.0 (C-3″), 76.0 (C-5″), 75.5 (C-2″), 73.7 (C-3), 71.4 (C-4″), 64.3 (C-6″); HRFABMS (positive-ion mode) m/z 809.1153 $[M + Na]^+$ (calcd for $C_{35}H_{30}O_{21}Na$, 809.1175).

*3′-O-β-D-(6″-O-Galloyl)glucopyranosyloxy-5,7,4′,5′-tetrahydroxyflavonol (**7**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 84 (c 0.2, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.69 (1H, d, J = 2.0 Hz, H-2′), 7.50 (1H, d, J = 2.0 Hz, H-6′), 7.00 (2H, s, H-2‴ and H-6‴), 6.28 (1H, d, J = 2.0 Hz, H-8), 6.15 (1H, d, J = 2.0 Hz, H-6), 4.85 (overlapping, H-1″), 4.70 (1H, br d, J = 12.5 Hz, H-6″), 4.42 (1H, dd, J = 12.0, 5.0 Hz, H-6″), 3.75 (1H, m, H-5″), 3.57 (overlapping, H-2″ and H-3″), 3.54 (overlapping, H-4″); ^{13}C NMR (methanol-d_4, 100 MHz) δ 177.3 (C-4), 168.4 (C-7‴), 165.5 (C-7), 162.5 (C-5), 158.1 (C-8a), 147.0 (C-2), 147.0 (C-3′), 147.0 (C-5′), 146.3 (C-3‴ and C-5‴), 139.7 (C-4‴), 138.5 (C-4′), 137.6 (C-3), 123.6 (C-1′), 121.2 (C-1‴), 111.5 (C-6′), 110.2 (C-2′), 110.1 (C-2‴ and C-6‴), 104.6 (C-4a), 104.2 (C-1″), 99.3 (C-6), 94.4 (C-8), 77.6 (C-3″), 76.0 (C-5″), 74.8 (C-2″), 71.5 (C-4″), 64.4 (C-6″); HRFABMS (positive-ion mode) m/z 655.0937 $[M + Na]^+$ (calcd for $C_{28}H_{24}O_{17}Na$, 655.0909).

*(2S)-3′-O-β-D-(6″-O-Galloyl)glucopyranosyloxy-5,7,4′-trihydroxyflavanone (**8**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 28.2 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.04), 285 (3.71) nm; ECD (c 0.00021, MeOH) ($[\theta]$) 249 (+4700), 287 (−13500), 328 (+3600) nm; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.13 (1H, s, OH-5), 7.17 (1H, d, J = 2.0 Hz, H-2′), 7.01 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 6.95 (2H, s, H-2‴ and H-6‴), 6.85 (1H, d, J = 8.5 Hz, H-5′), 5.89 (1H, d, J = 2.0 Hz, H-8), 5.87 (1H, d, J = 2.0 Hz, H-6), 5.42 (1H, dd, J = 12.5, 3.0 Hz, H-2), 4.85 (1H, d, J = 7.5 Hz, H-1″), 4.37 (2H, m, H-6″), 3.69 (1H, m, H-5″), 3.45 (overlapping, H-4″), 3.35 (overlapping, H-2″ and H-3″), 3.22 (1H, dd, J = 17.5, 12.5 Hz, H-3ax), 2.71 (1H, dd, J = 17.5, 3.0 Hz, H-3eq); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 196.2 (C-4), 166.6 (C-7), 165.7 (C-7‴), 163.4 (C-8a), 162.7 (C-5), 147.0 (C-4′), 145.5 (C-3‴ and 5‴), 144.9 (C-3′), 139.5 (C-4‴), 129.6 (C-1′), 121.4 (C-6′), 119.3 (C-1‴), 116.0 (C-5′), 114.8 (C-2′), 108.6 (C-2‴ and C-6‴), 101.8 (C-1″), 101.6 (C-4a), 95.8 (C-6), 95.0 (C-8), 78.1 (C-2), 75.7 (C-3″), 73.7 (C-5″), 73.3 (C-2″), 69.0 (C-4″), 62.7 (C-6″), 41.8 (C-3); HRFABMS (positive-ion mode) m/z 625.1169 $[M + Na]^+$ (calcd for $C_{28}H_{26}O_{15}Na$, 625.1168).

*8-O-β-D-[6′-O-(3″-O-Methyl)galloyl]glucopyranosyl-p-tyrosol (**9**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 20 (c 0.4, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.22 (1H, d, J = 2.0 Hz, H-6″), 7.17 (1H, d, J = 2.0 Hz, H-2″), 6.96 (2H, d, J = 9.0 Hz, H-2 and H-6), 6.65 (2H, d, J = 9.0 Hz, H-3 and H-5), 4.60 (1H, dd, J = 11.5, 2.5 Hz, H-6′), 4.40 (1H, dd, J = 11.5, 6.5 Hz, H-6′), 4.34 (1H, d, J = 8.0 Hz, H-1′), 3.92 (1H, m, H-8), 3.77 (3H, s, OMe-3″), 3.72 (1H, m, H-8), 3.58 (1H, m, H-5′), 3.42 (overlapping, H-3′ and H-4′), 3.22 (1H, dd, J = 9.5, 8.0 Hz, H-2′), 2.80 (2H, m, H-7); ^{13}C NMR (methanol-d_4, 100 MHz) δ 168.2 (C-7″), 156.7 (C-4), 149.1 (C-3″), 146.3 (C-5″), 140.7 (C-4″), 130.9 (C-2 and C-6), 130.5 (C-1), 121.4 (C-1″), 116.1 (C-3 and C-5), 112.0 (C-6″), 106.1 (C-2″), 104.5 (C-1′), 78.0 (C-3′), 75.5 (C-5′), 75.1 (C-2′), 72.4 (C-8), 72.0 (C-4′), 65.0 (C-6′), 56.6 (OMe-3″), 36.5 (C-7); HRFABMS (positive-ion mode) m/z 489.1385 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{11}Na$, 489.1372).

*8-O-β-D-(2′,6′-Di-O-galloyl)glucopyranosyl-p-tyrosol (**10**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 32 (c 1.1, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.11 (2H, s, H-2″ and H-6″), 7.11 (2H, s, H-2‴ and 6‴), 6.85 (2H, br d, J = 8.5 Hz, H-2 and H-6), 6.49 (2H, br d, J = 8.5 Hz, H-3 and H-5), 4.93 (1H, dd, J = 9.5, 8.0 Hz, H-2′), 4.61 (1H, d, J = 8.0 Hz, H-1′), 4.55 (1H, dd, J = 12.0, 2.5 Hz, H-6′), 4.47 (1H, dd, J = 12.0, 5.5 Hz, H-6′), 3.92 (1H, m, H-8), 3.67 (1H, dd, J = 9.5, 9.0 Hz, H-3′), 3.63 (overlapping, H-8), 3.63 (overlapping, H-5′), 3.56 (1H, t, J = 9.0, H-4′), 2.66 (1H, m, H-7); ^{13}C NMR (methanol-d_4, 100 MHz) δ 168.4 (C-7‴), 167.7 (C-7″), 156.6 (C-4), 146.6 (C-3‴ and C-5‴), 146.5 (C-3″ and C-5″), 139.9 (C-4″), 139.9 (C-4‴), 130.9 (C-2 and C-6), 130.8 (C-1), 121.7 (C-1‴), 121.4 (C-1″), 116.1 (C-3 and C-5), 110.5 (C-2‴ and C-6‴), 110.2 (C-2″ and C-6″), 102.5 (C-1′), 76.2 (C-3′), 75.6 (C-5′), 75.4 (C-2′), 72.1 (C-8), 71.9 (C-4′), 64.6 (C-6′), 36.3 (C-7); HRFABMS (positive-ion mode) m/z 627.1319 $[M + Na]^+$ (calcd for $C_{28}H_{28}O_{15}Na$, 627.1324).

*4-O-β-D-(6′-O-Galloyl)glucopyranosyl-(E)-p-coumaroyl acid (**11**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 90 (c 0.1, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.59 (1H, d, J = 16.0 Hz, H-7), 7.48 (2H, d, J = 9.0 Hz, H-2 and H-6), 7.12 (2H, s, H-2″ and H-6″), 7.09 (2H, d, J = 9.0 Hz, H-3 and H-5), 6.35 (1H, d, J = 16.0 Hz, H-8), 4.94 (1H, d, J = 7.5 Hz, H-1′), 4.64 (1H, dd, J = 12.0, 2.5 Hz, H-6′), 4.34 (1H, dd, J = 12.0, 7.5 Hz, H-6′), 3.78 (1H, m, H-5′), 3.51 (overlapping, H-2′ and H-3′), 3.42 (1H, m, H-4′); ^{13}C NMR (methanol-d_4, 100 MHz) δ 170.9 (C-9), 168.1 (C-7″), 160.6 (C-4), 146.6 (C-3″ and C-5″), 146.1 (C-7), 140.0 (C-4″), 130.8 (C-2 and C-6), 130.0 (C-1), 121.4 (C-1″), 118.0 (C-5 and C-3), 117.4 (C-8), 110.3 (C-2″ and C-6″), 101.9 (C-1′), 78.0 (C-3′), 75.8 (C-5′), 74.9 (C-2′), 71.9 (C-4′), 65.0 (C-6′);

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HRFABMS (positive-ion mode) m/z 501.0996 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_{12}Na$, 501.1008).

*3-O-β-D-(6′-Galloyl)glucopyranosyl-5-O-methylgallic acid (**12**).* Colorless amorphous solid; $[\alpha]^{22}_D$ − 41 (*c* 0.2, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.57 (1H, d, *J* = 2.0 Hz, H-2), 7.39 (1H, d, *J* = 2.0 Hz, H-6), 7.13 (2H, s, H-2″ and H-6″), 4.92 (1H, d, *J* = 7.5 Hz, H-1′), 4.61 (1H, dd, *J* = 12.0, 2.0 Hz, H-6′), 4.36 (1H, dd, *J* = 12.0, 6.0 Hz, H-6′), 3.89 (3H, s, OMe-5), 3.78 (1H, m, H-5′), 3.55 (1H, m, H-2′ and H-3′), 3.50 (1H, m, H-4′); ^{13}C NMR (methanol-d_4, 100 MHz) δ 170.3 (C-7), 168.4 (C-7″), 149.3 (C-5), 146.5 (C-3), 146.5 (C-3″ and C-5″), 142.6 (C-4), 139.8 (C-4″), 122.3 (C-1), 121.3 (C-1″), 113.2 (C-2), 110.2 (C-2″ and C-6″), 109.7 (C-6), 103.9 (C-1′), 77.3 (C-3′), 76.0 (C-5′), 74.8 (C-2′), 71.4 (C-4′), 65.0 (C-6′), 56.8 (OMe-5); HRFABMS (positive) m/z 521.0888 $[M + Na]^+$ (calcd for $C_{21}H_{22}O_{14}Na$, 521.0906).

Sugar Identification. Glucosidic moiety identification was carried out according to a previously described method.[34] Compounds **1** (1.0 mg), **2** (1.0 mg), **3** (1.0 mg), **4** (2.0 mg), **5** (4.0 mg), **6** (1.0 mg), **7** (0.6 mg), **8** (0.6 mg), **9** (1.0 mg), **10** (1.0 mg), **11** (1.0 mg), and **12** (1.0 mg) were separately hydrolyzed with 7% HCl (1 mL) at 60 °C for 2 h; each residue was stirred with L-cysteine methyl ester (5 mg) and *o*-tolyl isothiocyanate (10 μL) in pyridine (0.5 mL). The reaction mixtures were analyzed by HPLC (Cosmosil 5C_{18}-AR-II, 4.6 × 250 mm; CH_3CN−H_2O (1:3, v/v) containing 0.2% TFA, 1.0 mL/min; detection at 250 nm). The peaks of authentic L-glucose (t_R = 15.5 min) and D-glucose (t_R = 16.9 min) derivatives were used to identify the glucosidic moieties in **1**−**12** as D-glucose based on the corresponding retention times of 16.9 min.

DPPH Radical-Scavenging Activity. The DPPH radical-scavenging activities of the compounds isolated from *S. spinulosa* were measured as described in a previous procedure.[39] Briefly, solutions of each compound (0.1−200 μg/mL) were prepared in MeOH. An aliquot (100 μL) of the sample solution was added into each well of a 96-well microplate, containing 100 μL of 0.06 mM DPPH in methanol. The combined solutions were gently mixed for 30 min at 20 °C. Inhibition activity was determined by measuring the optical density at 510 nm (ImmunoMini NJ-2300 microplate reader, Cosmo Bio, Carlsbad, CA, USA) and expressed as the sample concentration required to scavenge 50% of the DPPH free radicals (IC_{50}). All samples were analyzed in triplicate, with trolox (Wako Pure Chemical Industry, Ltd., Osaka, Japan) used as a positive control.

Inhibitory Effects of Compounds on the Growth of *Babesia* and *Theileria*. The Texas strain of *B. bovis* and the Argentine strain of *B. bigemina* were obtained from Washington State University (Pullman, WA, USA). The USDA strains of *B. caballi* and *Theileria equi* were obtained from the Equine Research Institute, Japan Racing Association (Tochigi, Japan). These four strains were used to evaluate the inhibitory effects of the isolated compounds on the growth of *Babesia* and *Theileria*. These were maintained in bovine or equine red blood cells (RBCs) using a microaerophilic stationary-phase culture system, with Medium 199 used for *B. bovis*, *B. bigemina*, and *T. equi* and RPMI-1640 used for *B. caballi* (both from Sigma-Aldrich, Tokyo, Japan), as previously reported.[15,37] Tests with parasites were performed using a previously described assay with slight modifications.[15,37] Briefly, parasite-infected RBCs (iRBCs) were diluted with uninfected RBCs to afford a parasitemia of 1%. From this stock, 2.5 (bovine *Babesia*) or 5 (equine piroplasms) μL of iRBCs were dispensed into a 96-well culture plate (Nunc, Roskilde, Denmark) with 97.5 or 95 μL of the culture medium containing five different concentrations of the inhibitors (1, 2, 5, 10, and 20 μg/mL) of the four strains. Cultures were incubated at 37 °C for 4 days without changing the medium. On day 4 of culture, 100 μL of lysis buffer with SYBR Green I nucleic acid stain (2×, Lonza, Walkersville, MD, USA: 10,000×) were added and incubated for 8 h in the dark place at room temperature. Subsequent, relative fluorescent values were determined by a fluorescent plate reader (Fluoroskan Ascent, Thermo Labsystems, Philadelphia, PA, USA), and the IC_{50} values of compound against growths of parasites were calculated by curve fitting.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00142.

NMR spectroscopy data for **1**−**12**, including ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}COSY, HMQC, and HMBC (PDF)

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Notes

The authors declare no competing financial interest.

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