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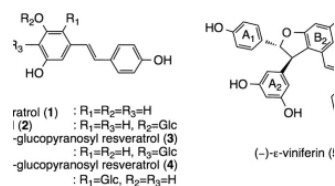
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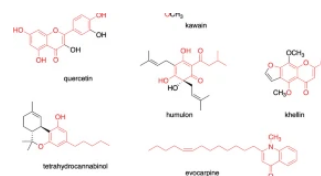
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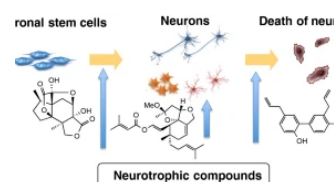
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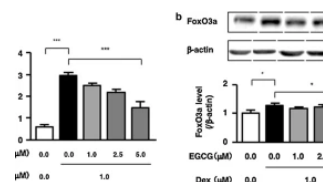
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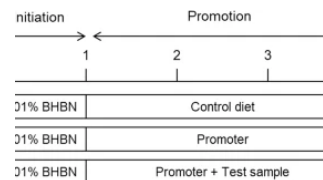


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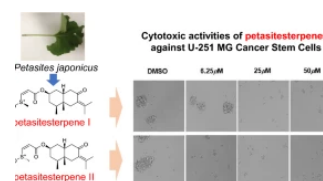


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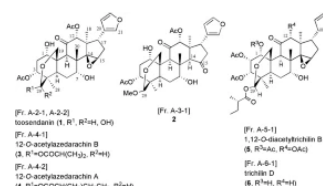


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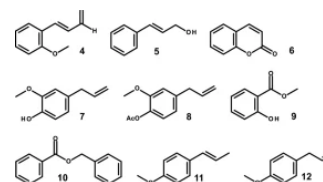


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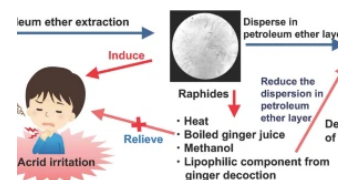


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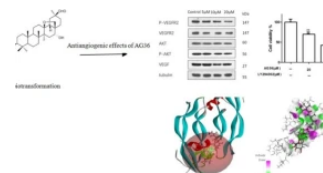


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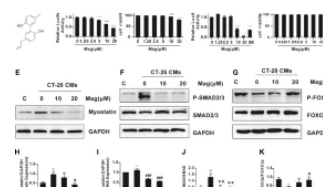


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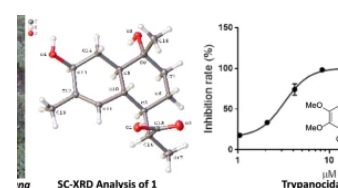


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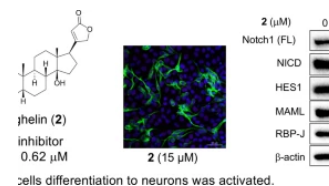


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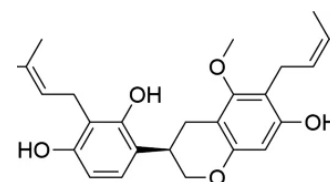


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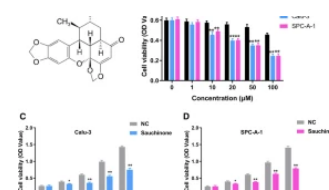


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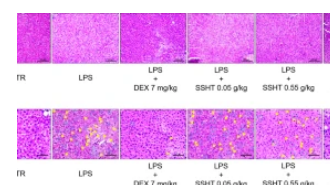
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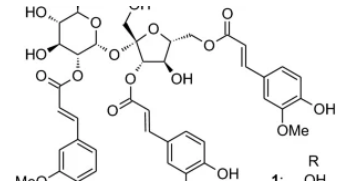
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Nguyen Hoang Uyen, Retno Widyowati ... Katsuyoshi Matsunami

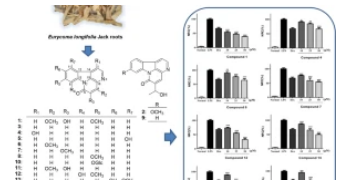
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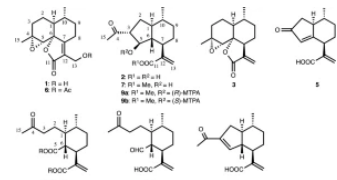
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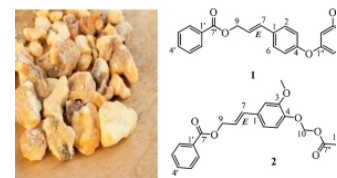
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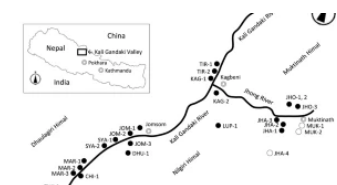


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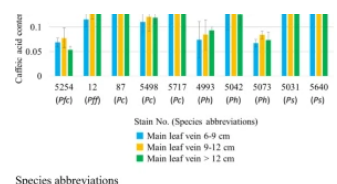
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NOTE

Firmosides A and B: two new sucrose ferulates from the aerial parts of *Silene firma* and evaluation of radical scavenging activities

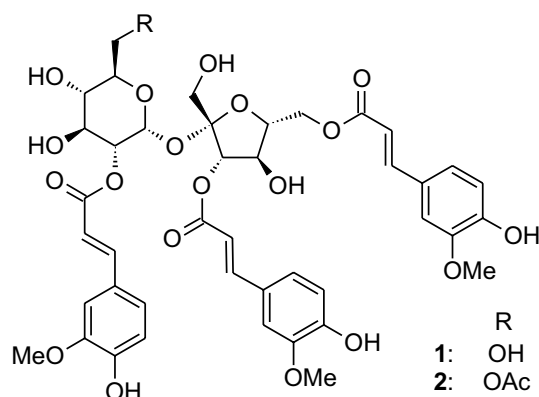
Nguyen Hoang Uyen¹ · Retno Widyowati^{1,2} · Melanny Ika Sulistyowaty^{1,3} · Sachiko Sugimoto¹ · Yoshi Yamano¹ · Susumu Kawakami⁴ · Hideaki Otsuka⁴ · Katsuyoshi Matsunami¹

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Abstract

Two new tri-ferulates of sucrose, firmosides A and B (**1** and **2**, respectively), together with 18 known compounds (**3–20**), were isolated from the aerial parts of *Silene firma*. The structures of the isolated compounds were elucidated by various spectroscopic methods, including 1D, 2D NMR, and high-resolution electro-spray ionization–mass spectrometry (HR-ESI–MS). All the isolated compounds were evaluated for their free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. As a result, two new compounds (**1**, **2**) and **11** demonstrated significant radical scavenging activity, implying the usefulness as antioxidant agents.

Graphic Abstract



Keywords *Silene firma* · Ferulic acid · Radical scavenging activities · DPPH · Sucrose · Firmoside

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11418-020-01426-5>) contains supplementary material, which is available to authorized users.

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Introduction

The genus *Silene* (family: Caryophyllaceae) comprises more than 700 species mainly distributed in the temperate zone of the northern hemisphere. The genus *Silene* includes several taxa previously treated as different genera, such as *Coronaria*, *Cucubalus*, *Lychnis*, *Melandrium*, *Petrocoposis*, and *Viscaria* [1]. *Melandrium firmum* Rohrbach is a synonym of *Silene firma* and is widely distributed in China, Korea, Russia, and Japan. *S. firma* is an annual or biennial herb. The stems erect and reach 30–100 cm in height, and the knots are sometimes dark violet. Leaves are opposite, lanceolate to ovate–lanceolate, 3–10 cm long, 1–3 cm wide, apex acute, and hairs in the margins. Flowers bloom from June to September, and the petals are white. The seeds are black, renal shape, 0.7 to 1 mm, with spines, and ripen from July to August [2, 3]. The dried aerial parts have been used for the treatment of anuria, breast cancer, gonorrhoea, and diseases of lactation in Korea [4], and, as Chinese traditional medicine, of acute nephritis, liver cirrhosis, and ascites in China [5]. The methanolic extract of this plant inhibited the development of benign prostatic hyperplasia using the testosterone propionate induced rat model [6]. Previous phytochemical investigation of this plant reported cytotoxic anthraquinone dimers, triterpenes, β -carboline alkaloids, flavonoids, and mannitol [7–9]

Reactive oxygen species (ROS) play an important role in human physiological processes. However, excessive ROS accumulation leads to oxidative damage of cell membranes, proteins, and DNA, which causes a variety of diseases, including the above-mentioned diseases such as cancer, nephritis, liver cirrhosis, and prostatic hyperplasia [10]. Therefore, the traditional medicinal usage of *Silene firma* may be related to the supplementation of antioxidants as an effective measure for preventing and repairing the damages caused by ROS.

Thus, we aimed to clarify the chemical constituents by the extensive fractionation and purification procedures. Our investigation of the *n*-hexane- and EtOAc-soluble fractions of the aerial parts of *S. firma* revealed the presence of two new ferulic acid sucrose esters named firmosides A and B (**1** and **2**, respectively) (Fig. 1) together with 18 known compounds identified to be vanillin (**3**) [11], (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one (**4**) [12], scopoletin (**5**) [13], (9*S*,12*S*,13*S*)-*E*-9,12,13-trihydroxy-10-octadecaenoic acid (**6**) [14], 20-hydroxy-ecdysone (**7**) [15], luteolin 3'-*O*-methyl- β -*D*-glucopyranoside (**8**) [16], apigenin-6-*C*- β -*D*-glucopyranoside (**9**) [17], maltol β -*D*-glucopyranoside 6'-*O*-benzoate (**10**) [18], helonioside A (**11**) [19], 22-*O*-acetyl 20-hydroxy-ecdysone (**12**) [20], 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl) propan-1-one (**13**) [21], (6*S*,9*R*)-vomifoliol (**14**) [22],

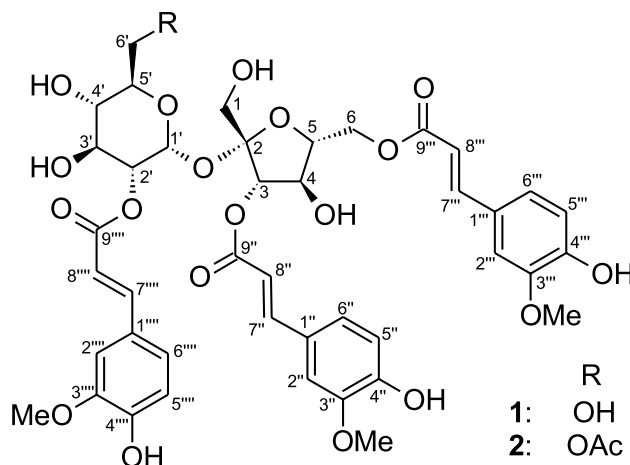


Fig. 1 Structures of new compounds **1** and **2**

4-hydroxybenzaldehyde (**15**) [23], (+)-dehydrovomifoliol (**16**) [24], α,β -dihydroferulic acid (**17**) [25], indole-3-carboxaldehyde (**18**) [26, 27], isovanillic acid (**19**) [28], and grasshopper ketone (**20**) [29] (Fig. 2). These compounds were isolated by performing chromatography such as silica gel, ODS, and HPLC. The chemical structures of the isolated compounds were elucidated by spectroscopic analyses of 1D and 2D NMR spectra (^1H , ^{13}C NMR, DEPT, COSY, HSQC, and HMBC) (Figs S1–S12 in supplementary data) in combination with high-resolution electrospray ionization–mass spectrometry (HR-ESI-MS). This paper mainly deals with the structural elucidation of two new compounds and their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

Results and discussion

The methanolic extract of the aerial parts of *S. firma* was partitioned with *n*-hexane, EtOAc, 1-butanol, and H_2O , successively. The *n*-hexane- and EtOAc-soluble fractions were combined and fractionated by repeated chromatography to afford two new (**1** and **2**) and 18 known compounds (**3**–**20**) (Figs. 1 and 2).

Compound **1** was obtained as a white amorphous powder with a molecular formula of $\text{C}_{42}\text{H}_{46}\text{O}_{20}$ determined from HR-ESI-MS at m/z 869.2501 [$\text{M}-\text{H}$] $^-$ (calcd for $\text{C}_{42}\text{H}_{45}\text{O}_{20}$: 869.2501). The presence of multiple carbonyl and hydroxy groups was estimated from the IR absorptions at 1716, 1697, 1686, and 3344 cm^{-1} , respectively. The UV absorptions at 237 (4.18), 264 (3.69), 300 (4.17), and 327 (4.34) were indicative of the presence of aromatic rings.

The ^1H NMR spectrum displayed three ABX coupling systems [δ_{H} 7.20 (1H, *d*, J = 1.9 Hz, H-2''), 7.13 (1H, *d*, J = 1.9 Hz, H-2'''), 6.98 (1H, *d*, J = 1.9 Hz, H-2'''), 6.73 (1H,

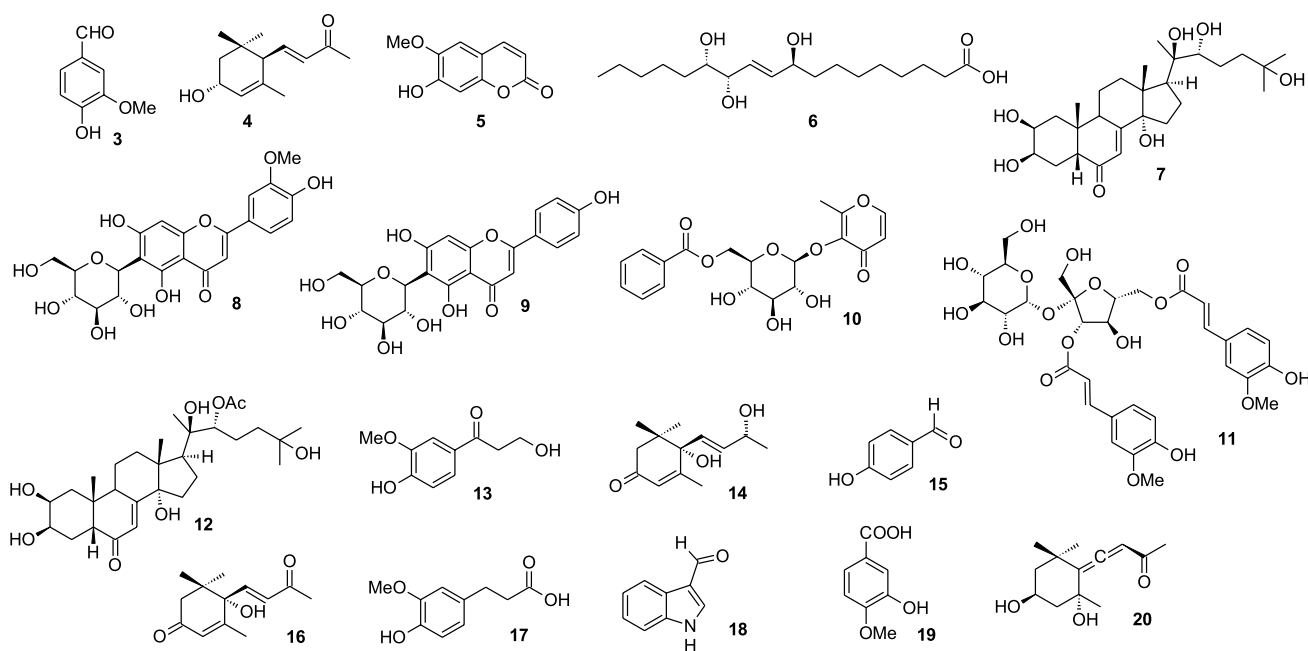


Fig. 2 Structures of known compounds 3–20

d, $J = 8.3$ Hz, H-5''), 6.79 (1H, *d*, $J = 8.2$ Hz, H-5'''), 6.70 (1H, *d*, $J = 8.2$ Hz, H-5'''), 7.08 (1H, *dd*, $J = 8.3, 1.9$ Hz, H-6''), 7.02 (1H, *dd*, $J = 8.2, 1.9$ Hz, H-6'''), 6.92 (1H, *dd*, $J = 8.2, 1.9$ Hz, H-6'''), three methoxy groups at δ_{H} 3.86, 3.75, and 3.84 (each 3H, *s*, 3'', 3''', 3''''-OCH₃, respectively), an anomeric proton at δ_{H} 5.65 (1H, *d*, $J = 3.7$ Hz, H-1'), and three *trans*-olefinic protons pairs with large coupling constants at δ_{H} 7.65 (1H, *d*, $J = 15.9$ Hz, H-8''), 6.45 (1H, *d*, $J = 15.9$ Hz, H-7''), 7.61 (1H, *d*, $J = 15.9$ Hz, H-8'''), 6.34 (1H, *d*, $J = 15.9$ Hz, H-7'''), 7.70 (1H, *d*, $J = 15.9$ Hz, H-8'''), 6.31 (1H, *d*, $J = 15.9$ Hz, H-7'''), which suggested the presence of three feruloyl functions on sugar moiety (Table 1).

The ¹³C NMR spectrum of **1** showed 42 carbon resonances classified by comparing its chemical shift values, DEPT, and HSQC spectra, as three carbonyl carbons at δ_{C} 168.6 (C-9''), 169.2 (C-9'''), and 168.9 (C-9'''), two anomeric carbons at δ_{C} 105.9 (C-2) and 91.4 (C-1'), six olefinic carbons at δ_{C} 115.0 (C-7''), 115.28 and 115.34 (C-7''' or C-7'''), 148.0 (C-8''), 147.3 (C-8'''), 147.6 (C-8'''), three oxygenated methylene carbons at δ_{C} 64.4 (C-1), 66.1 (C-6), and 62.6 (C-6'), seven oxygenated methine carbons at δ_{C} 78.7 (C-3), 74.9 (C-4), 81.2 (C-5), 74.8 (C-2'), 72.4 (C-3'), 71.8 (C-4'), 74.2 (C-5'), three methoxy carbons at δ_{C} 56.5, 56.6, and 56.7, and 18 characteristic carbon signals attributable to three benzene ring (δ_{C} 111.9–150.9) (Table 1). Chemical shift values of feruloyl and isoferuloyl functions have been reported as follows: feruloyl: [δ_{C} ca. 112.0 (C-2), ca. 116.5 (C-5) in CD₃OD] and isoferuloyl: [δ_{C} ca. 115.0 (C-2), ca. 112.5 (C-5) in CD₃OD [30]. The chemical shifts and $\Delta\delta_{\text{C-2,5}}$ values (**1**: $\Delta\delta_{\text{C-2,5}} \approx 4.5$ –4.71 ppm) of three acyl

moieties of **1** were in good agreement with those of feruloyl function (i.e., feruloyl: $\Delta\delta_{\text{C-2,5}} \approx 4.5$ ppm, and isoferuloyl: $\Delta\delta_{\text{C-2,5}} \approx 2.5$ ppm). These findings strongly suggested that the structure of **1** was a disaccharide having three ferulic acid functions.

The ¹H and ¹³C NMR spectroscopic data of **1** were similar to quiquesetinerviiside A [31], except for the presence of *p*-coumaroyl function and chemical shift differences on the sugar moiety probably caused by a difference in the attachment position (Fig. 1 and Table 1). The HMBC spectrum showed the correlations of methoxy protons (3H each, δ_{H} 3.86, 3.75, and 3.84) with C-3'', C-3''' (δ_{C} 149.45/149.48), and C-3'''' (δ_{C} 149.3), respectively. Besides, the correlations of H-2' (δ_{H} 4.68) with C-9'''' (δ_{C} 168.9), H-3 (δ_{H} 5.54) with C-9'' (δ_{C} 168.6), and H-6 (δ_{H} 4.46) with C-9''' (δ_{C} 169.2) revealed the position of three feruloyl groups on C-2', C-3, and C-6, respectively (Fig. 2). The two sugars were connected between C-1' and C-2 determined by the HMBC correlation from H-1' (δ_{H} 5.65) to C-2 (δ_{C} 105.9) (Fig. 3).

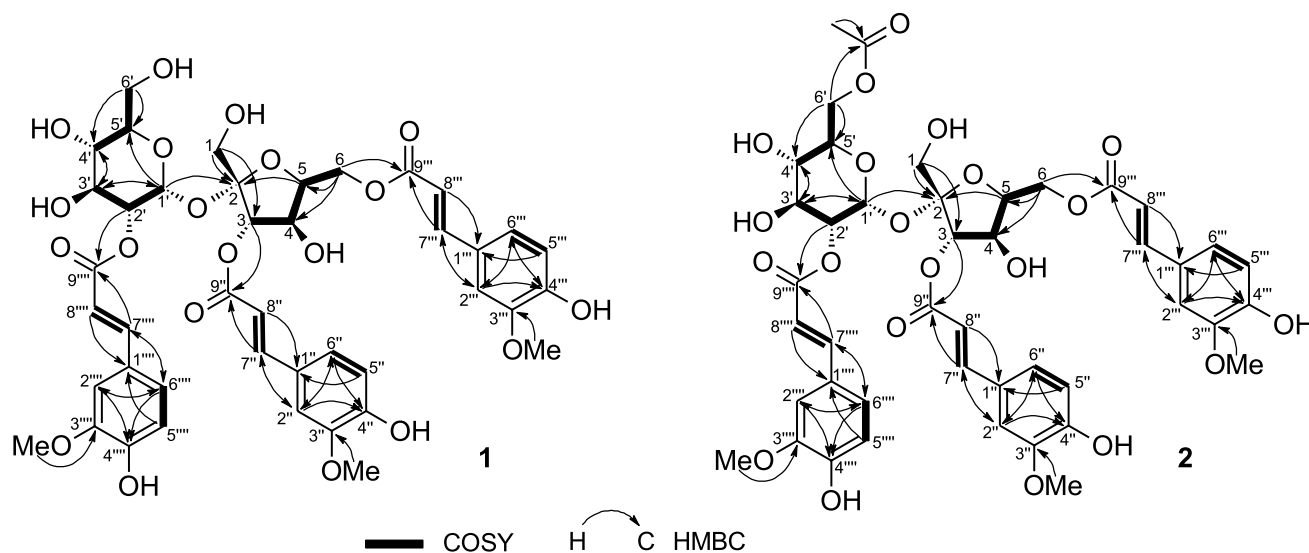
Mild alkaline hydrolysis of **1** with methanolic 100 mM NaOCH₃ liberated D-sucrose and methyl ferulate [32] by HPLC analyses with optical rotation detector and spectroscopic data (HR-ESI-MS and ¹H NMR). Based on these results, the structure of **1** was characterized as (3,6-*O*-diferuloyl)- β -D-fructofuranosyl-(2 \rightarrow 1)-(2-*O*-feruloyl)- α -D-glucopyranoside, designated as firmoside A.

Compound **2** was obtained as a white amorphous powder with a molecular formula of C₄₄H₄₈O₂₁ determined by HR-ESI-MS at m/z 935.2581 [M+Na]⁺ (calcd for C₄₄H₄₈O₂₁Na:

Table 1 ^1H and ^{13}C NMR spectroscopic data for compound **1**

No.	δ_{H} (J in Hz)	δ_{C}	No.	δ_{H} (J in Hz)	δ_{C}
1	3.49 (1H, d, 12.0)	64.4	9''	–	168.6
	3.62 (1H, d, 12.0)		OCH ₃	3.86 (3H, s)	56.5 ^d
2	–	105.9	1'''	–	127.79 ^e
3	5.54 (1H, d, 8.3)	78.7	2'''	7.13 (1H, d, 1.9)	111.9
4	4.42 (1H, t, 8.3)	74.9	3'''	–	149.48 ^a
5	4.12 (1H, m)	81.2	4'''	–	150.7
6	4.46 (2H, d, 5.4)	66.1	5'''	6.79 (1H, d, 8.2)	116.61 ^b
1'	5.65 (1H, d, 3.7)	91.4	6'''	7.02 (1H, dd, 8.2, 1.9)	124.46 ^c
2'	4.68 (1H, dd, 10.0, 3.7)	74.8	7'''	6.34 (1H, d, 15.9)	115.28 ^f
3'	3.94 (1H, m)	72.4	8'''	7.61 (1H, d, 15.9)	147.3
4'	3.48 (1H, t, 9.1)	71.8	9'''	–	169.2
5'	3.95 (1H, m)	74.2	OCH ₃	3.75 (3H, s)	56.6 ^d
6'	3.76 (1H, m)	62.6	1''''	–	127.83 ^e
	3.87 (1H, m)		2''''	6.98 (1H, d, 1.9)	112.0
1''	–	127.7	3''''	–	149.3
2''	7.20 (1H, d, 1.9)	112.1	4''''	–	150.9
3''	–	149.45 ^a	5''''	6.70 (1H, d, 8.2)	116.64 ^b
4''	–	150.7	6''''	6.92 (1H, dd, 8.2, 1.9)	124.2
5''	6.73 (1H, d, 8.3)	116.58 ^b	7''''	6.31 (1H, d, 15.9)	115.34 ^f
6''	7.08 (1H, dd, 8.3, 1.9)	124.37 ^c	8''''	7.70 (1H, d, 15.9)	147.6
7''	6.45 (1H, d, 15.9)	115.0	9''''	–	168.9
8''	7.65 (1H, d, 15.9)	148.0	OCH ₃	3.84 (3H, s)	56.7 ^d

^1H (600 MHz) and ^{13}C NMR (150 MHz) in methanol- d_4 , m: multiplet or overlapped signals, a–f: interchangeable

**Fig. 3** Important HMBC and COSY correlations of **1** and **2**

935.2580), indicating 42 mass unit larger than **1**. The presence of multiple carbonyl and hydroxy groups was suggested from the IR spectrum (1717, 1699, and 1684 cm^{-1} , and the signal at 3344 cm^{-1} , respectively).

The ^1H NMR and ^{13}C NMR spectra (Table 2) were closely similar to **1**, except for a methyl proton at δ_{H} 2.08 (3H, s) and two carbons at δ_{C} 21.0 and 173.0 ppm, suggesting the structure of **2** was an acetyl derivative of **1**.

Table 2 ^1H and ^{13}C NMR spectroscopic data for compound **2**

No.	δ_{H} (J in Hz)	δ_{C}	No.	δ_{H} (J in Hz)	δ_{C}
1	3.45 (1H, d, 12.0)	64.8	8''	7.71 (1H, d, 15.8)	148.1
	3.64 (1H, d, 12.0)		9''		168.6
2		105.6	OCH ₃	3.83 (3H, s)	56.6
3	5.58 (1H, d, 8.5)	78.5	1'''		128.0
4	4.50 (1H, t, 8.5)	74.3	2'''	7.21 (1H, d, 1.8)	112.04 ^c
5	4.12 (1H, m)	81.4	3'''		149.55 ^e
6	4.50 (2H, m)	65.4	4'''		150.8
1'	5.70 (1H, d, 3.8)	90.7	5'''	6.82 (1H, d, 8.2)	116.65 ^d
2'	4.69 (1H, dd, 10.1, 3.8)	74.6	6'''	7.00 (1H, dd, 8.2, 1.8)	124.4
3'	3.92 (1H, m)	72.3	7'''	6.40 (1H, d, 16.0)	115.5
4'	3.38 (1H, t, 9.3)	72.12 ^a	8'''	7.67 (1H, d, 16.0)	147.3
5'	4.19 (1H, m)	72.15 ^a	9'''		169.1
6'	4.16 (1H, m)	65.4	OCH ₃	3.90 (3H, s)	56.7
	4.56 (1H, m)		1''''		127.80 ^b
OAc-6'		173.0	2''''	7.01 (1H, d, 1.8)	112.11 ^c
	2.08 (3H, s)	21.0	3''''		149.57 ^e
1''		127.78 ^b	4''''		150.9
2''	7.27 (1H, d, 1.8)	111.95 ^c	5''''	6.82 (1H, d, 8.2)	116.66 ^d
3''		149.5	6''''	7.10 (1H, m)	124.3
4''		151.0	7''''	6.36 (1H, d, 15.9)	115.2
5''	6.76 (1H, d, 8.2)	116.65 ^d	8''''	7.64 (1H, d, 15.9)	147.7
6''	7.13 (1H, dd, 8.2, 1.8)	124.6	9''''		168.8
7''	6.48 (1H, d, 15.8)	114.9	OCH ₃	3.90 (3H, s)	56.7

^1H (600 MHz) and ^{13}C NMR (150 MHz) in methanol- d_4 , m: multiplet or overlapped signals, a–e: interchangeable

The HMBC correlations of H-2' (δ_{H} 4.69) with C-9'''' (δ_{C} 168.8), H-3 (δ_{H} 5.58) with C-9'' (δ_{C} 168.6), H-6 (δ_{H} 4.50) with C-9''' (δ_{C} 169.1), and H-6' (δ_{H} 4.56 and 4.16) with the acetyl carbonyl (δ_{C} 173.0) suggested three feruloyl groups were on C-2', C-3, and C-6, and the acetyl group on C-6', respectively. The glycosidic linkage was confirmed by the HMBC correlation between H-1' (δ_{H} 5.70) and C-2 (δ_{C} 105.6) to form sucrose.

The absolute stereochemistry was determined by mild alkaline hydrolysis of **2**, as above mentioned, to liberate D-sucrose and methyl ferulate by analyzing with HPLC equipped with the optical rotation detector, HR-ESI-MS, and ^1H NMR. Based on these data, the structure of **2** was determined as (3,6-*O*-diferuloyl)- β -D-fructofuranosyl-(2 \rightarrow 1)-(2-*O*-feruloyl-6-*O*-acetyl)- α -D-glucopyranoside, named firmoside B.

All isolated compounds were examined on DPPH radical scavenging activity (Fig. 4). As a result, firmosides A and B (**1** and **2**, respectively) and known compound **11** showed significant DPPH free radical scavenging activities (IC₅₀ 33.4, 39.1, and 37.9 μM , respectively) comparable to the positive control, Trolox (IC₅₀ 36.4 μM). It is noteworthy that the hexane- and EtOAc-soluble mixture and the first silica gel column chromatographic fractions did not show

any significant activity at 100 $\mu\text{g}/\text{mL}$ (Fig. S13 in supplementary data); however, further fractionation by ODS column chromatography unveiled active components. All compounds (**1**–**20**) were isolated from the active fractions. However, from some active fractions, such as HE-7-3 and 4, no compound was isolated in this study, because of the high complexity and instability of the constituents.

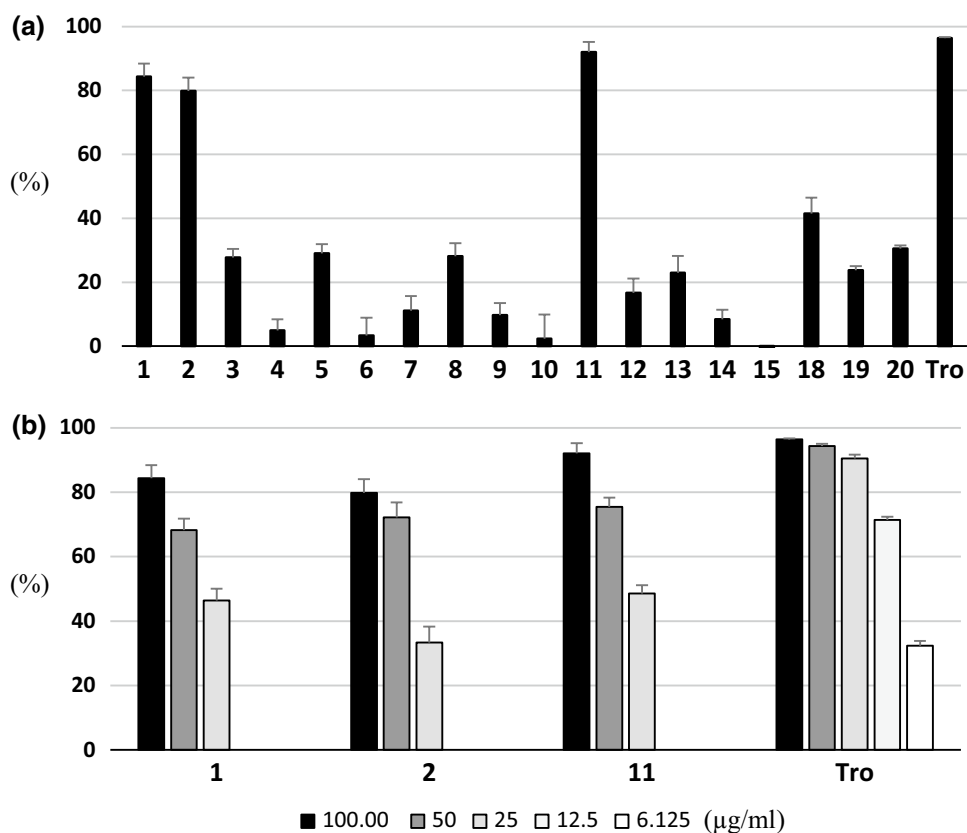
In conclusion, chemical investigation of the *n*-hexane- and ethyl acetate-soluble fractions of the aerial parts of *S. firma* provided 20 compounds (**1**–**20**), including two new ferulic acid esters, firmosides A and B (**1** and **2**), and 18 known compounds (**3**–**20**). The isolated compounds were tested for their DPPH radical scavenging activities, and **1**, **2**, and **11** exhibited strong radical scavenging activity comparable to Trolox, implying the use of them as antioxidant agents.

Experimental

General experimental procedures

^1H and ^{13}C NMR spectra were measured on a Bruker Avance III spectrometer at 600 MHz and 150 MHz, respectively,

Fig. 4 DPPH radical scavenging activity. **a** % inhibition of isolated compounds **1–15**, and **18–20** (100 µg/mL). **b** Concentration-dependent inhibition of **1**, **2**, **11**. **Tro**: Trolox as a positive control



with the residual solvent signal as the reference. IR and UV spectra were recorded on a HORIBA FT-720 and a JASCO V-520 UV/Vis spectrophotometer, respectively. Optical rotations were measured using a JASCO P-1030 spectropolarimeter. Positive- and negative-ion HR-ESI-MS was performed on an LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific).

Silica gel column chromatography (CC) and octadecylsilyl silica gel (ODS) CC were performed on silica gel 60 (E. Merck, Darmstadt, Germany) and Cosmosil 75C18-OPN (Nacalai Tesque, Kyoto, Japan; $\Phi = 35$ mm, $L = 350$ mm), respectively. HPLC was performed on Inertsil ODS-3 column (GL Science, Tokyo, Japan; $\Phi = 10$ mm, $L = 25$ cm, flow rate 2.00 mL/min), and the eluate was monitored with a refractive index monitor. TLC was performed on precoated silica gel 60 F254 plates (E. Merck; 0.25 mm in thickness) by spraying with a 10% solution of H_2SO_4 in ethanol and heated on a hotplate around 150 °C. Sugars were analyzed by HPLC on an amino column using a chiral detector (JASCO OR-2090plus) [Shodex Asahipak NH2P-50, CH_3CN-H_2O (3:1), 1.0 mL/min].

Plant material

Aerial parts of *S. firma* were collected in September 2005 in Imabari, Ehime, Japan, and a voucher specimen was

deposited in the Herbarium of the Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima (Accession No. 05-SR-09-Ehime).

Extraction and isolation

The air-dried plants (10.6 kg) were extracted three times with methanol (45 L). The methanol solution was concentrated to 6 L and then partitioned with an equal volume of *n*-hexane. The remaining layer was evaporated, resuspended in water (6 L), and partitioned with ethyl acetate (6L) and with 1-butanol (6 L), successively.

The *n*-hexane and ethyl acetate fractions were combined (164.8 g), and fractionated by silica gel (1.45 kg) CC with increasing amounts of MeOH in $CHCl_3$ [($CHCl_3$, 6L), $CHCl_3$ -MeOH (30:1, 6 L), (20:1, 6 L), (10:1, 6 L), (7:1, 6 L), (5:1, 6 L), (3:2, 6 L), (2:1, 6 L), and (MeOH, 6 L)], yielding nine fractions (SF-HE 1–SF-HE 9). The fraction SF-HE 1 (46.5 g) was subjected to ODS CC with MeOH- H_2O (30%, 0.5 L), (40%, 0.5 L), (50%, 0.5 L), (60%, 0.5 L), (70%, 0.5 L), (80%, 0.5 L), (90%, 0.5 L), (100%, 0.5 L), and (acetone, 0.5 L), led nine fractions (SF-HE 1-1–SF-HE 1-9). The fraction SF-HE 1-1 (577 mg) was purified by HPLC (25% acetone) to give **3** (vanillin, 6.0 mg), **4** ((3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one, 2.3 mg), and **5** (scopoletin, 2.7 mg). The fraction SF-HE 2 (22.7 g) was

subjected to ODS CC with MeOH-H₂O (20% → 100% of MeOH, 10% step gradient, 0.5 L each), and (acetone, 0.5 L), as mentioned above, led nine fractions (SF-HE 2-1–SF-HE 2-9). The residue of fraction SF-HE 2-1 (1.67 g) was purified by HPLC (25% acetone) to obtain **13** (3-hydroxy-1-(4-hydroxy-3-methoxyphenyl) propan-1-one, 11.4 mg), **14** ((6*S*,9*R*)-vomifoliol, 17.5 mg), **15** (4-hydroxybenzaldehyde, 20.0 mg), and mixture of **16** and **17** ((+)-dehydrovomifoliol and 3-(4-hydroxy-3-methoxyphenyl) propanoic acid, 18.3 mg), respectively. The fraction SF-HE 2-2 (689.1 mg) was purified by HPLC (35% acetone) to give **18** (indole-3-carboxaldehyde, 3.8 mg). The fraction SF-HE 3 (14.0 g) was subjected to ODS CC with MeOH-H₂O (30% → 100% of MeOH, 10% step gradient, 0.5 L each), and (acetone, 0.5 L), as mentioned above, led nine fractions (SF-HE 3-1–SF-HE 3-9). The fraction SF-HE 3-1 (1.15 g) was purified by HPLC (25% acetone) to give **19** (isovanillic acid, 26.2 mg), **20** (grasshopper ketone, 11.9 mg). The fraction SF-HE 3-2 (854 mg) was purified by HPLC (35% acetone) to give **10** (maltol β-D-glucopyranoside 6'-*O*- benzoate, 300 mg). The fraction SF-HE 3-3 (855 mg) was purified by HPLC (40% acetone) to obtain new compound **2** (16.2 mg). The fraction SF-HE 4 (13.4 g) was subjected to ODS CC with MeOH-H₂O (20% → 100% of MeOH, 10% step gradient, 0.5 L each), and (acetone, 0.5 L), as mentioned above, led ten fractions (SF-HE 4-1–SF-HE 4-10). The fraction SF-HE 4-4 (1.29 g) was purified by HPLC (60% acetone) to give new compound **1** (70.0 mg) and **11** (helonioside A, 12 mg). The fraction SF-HE 4-5 (929 mg) was purified by HPLC (50% acetone) to give **6** ((9*S*,12*S*,13*S*)-*E*-9,12,13-trihydroxy-10-octadecaenoic acid, 20.0 mg). The fraction SF-HE 5 (10.3 g) was subjected to ODS CC with MeOH-H₂O (20% → 100% of MeOH, 10% step gradient, 0.5 L each), and (acetone, 0.5 L), as mentioned above, led ten fractions (SF-HE 5-1–SF-HE 5-10). The fraction SF-HE 5-2 (878 mg) was purified by HPLC (30% acetone) to give **7** (20-hydroxy-ecdysone, 15.0 mg). The fraction SF-HE 5-3 (630 mg) was purified by HPLC (35% acetone) to give **12** (22-*O*-acetyl 20-hydroxy-ecdysone, 7.6 mg). The fraction SF-HE 6 (9.23 g) was subjected to ODS CC with MeOH-H₂O (20% → 100% of MeOH, 10% step gradient, 0.5 L each), and (acetone, 0.5 L), as mentioned above, led ten fractions (SF-HE 6-1–SF-HE 6-10). The fraction SF-HE 6-3 (715 mg) was purified by HPLC (25% acetone) to give **8** (luteolin 3'-*O*-methyl-6-*C*-β-D-glucopyranoside, 8.8 mg) and **9** (apigenin-6-*C*-β-D-glucopyranoside, 4.5 mg).

Firmoside A (1)

White amorphous powder; $[\alpha]_D^{26} +54.4$ ($c=0.93$, MeOH); IR (film) ν_{\max} cm⁻¹: 3344, 2923, 2851, 1716, 1697, 1686, 1519, 1508, 1270, 1161, 1028; UV λ max (MeOH) nm (log ϵ): 237 (4.18), 264 (3.69), 300 (sh, 4.17), 327 (4.34); ¹H NMR

(CD₃OD, 600 MHz) and ¹³C NMR (CD₃OD, 150 MHz), as shown in Table 1; negative-ion HR-ESI-MS m/z 869.2501 $[M-H]^-$ (calcd for C₄₂H₄₅O₂₀: 869.2501)

Firmoside B (2)

White amorphous powder; $[\alpha]_D^{26} +46.9$ ($c=1.03$, MeOH); IR (film) ν_{\max} cm⁻¹: 3344, 2921, 2851, 1717, 1699, 1684, 1520, 1507, 1270, 1161, 1030; UV λ max (MeOH) nm (log ϵ): 236 (4.06), 264 (3.62), 300 (sh, 4.12), 327 (4.25); ¹H NMR (CD₃OD, 600 MHz) and ¹³C NMR (CD₃OD, 150 MHz), as shown in Table 2; positive-ion HR-ESI-MS m/z 935.2581 $[M+Na]^+$ (calcd for C₄₄H₄₈O₂₁Na: 935.2580)

Mild alkaline hydrolysis of 1 and 2

Compounds **1** and **2** (2 mg each) were dissolved in MeOH (1.8 mL), and 200 μL of 1 M NaOCH₃ methanol solution was added to start the reaction. After stirring for 30 min at 25 °C, the reaction mixture was neutralized with ion exchange resin (Organo IR120B, H⁺-form) and evaporated. The residue was partitioned with EtOAc and H₂O. The organic and aqueous layers were analyzed to identify methyl ferulate and sugar, respectively. The methyl ferulate was isolated by preparative TLC and identified by HR-ESI-MS and ¹H NMR compared to those reported values. The sugar was analyzed by HPLC by comparing their retention time and optical rotation sign with authentic sucrose; t_R : 10.8 min (positive optical rotation).

DPPH free radical scavenging activity

The samples were dissolved in 100 μL of MeOH in 96-well microtiter plates at various concentrations. The initial absorbance was measured at 515 nm, as A_{S0} . DPPH solution (100 μL, 200 μM) was added to each well and incubated in a dark place at room temperature. After the incubation for 30 min, the absorbance was measured again, as A_{S30} . The % inhibition of free radicals was calculated according to the following equation:

$$\% \text{ Inhibition} = \left[1 - (A_{S30} - A_{S0}) / (A_{D30} - A_{D0}) \right] \times 100$$

where A_D is the absorbance of the control reaction mixture containing DMSO and all reagents without test compounds [33]. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is an analog of vitamin E and widely used for antioxidant reagent.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest associated with this manuscript.

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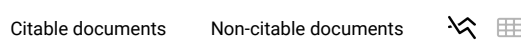
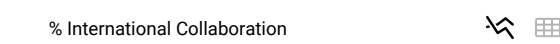
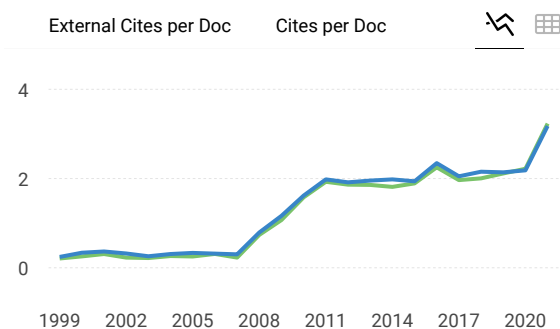
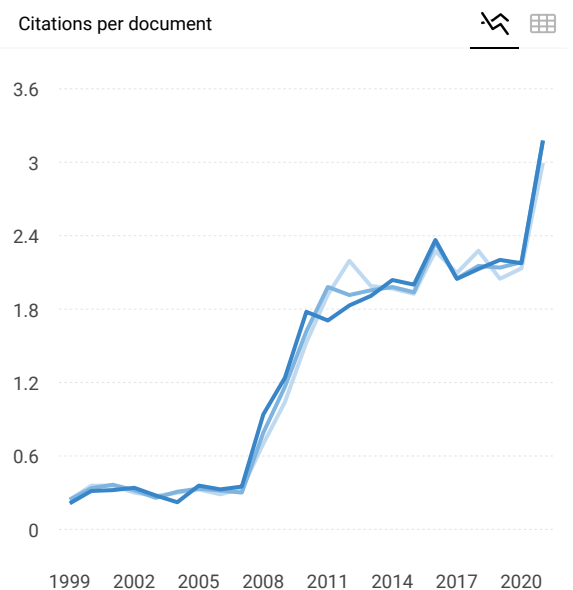
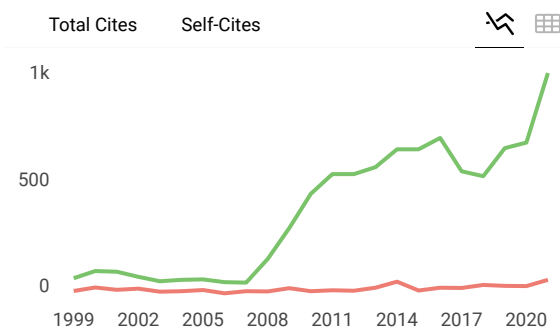
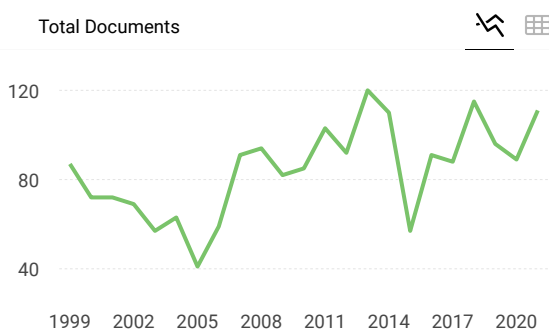
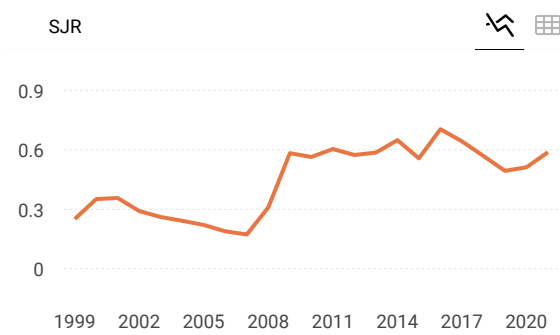
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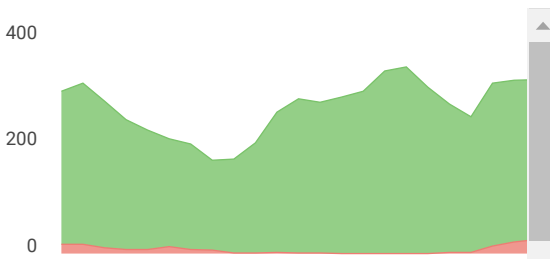
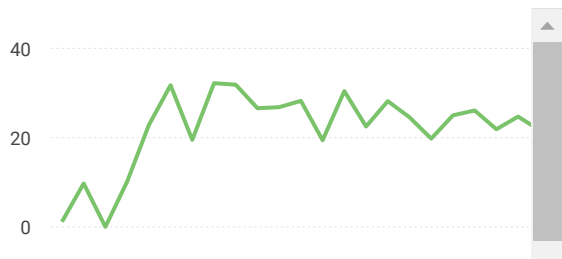
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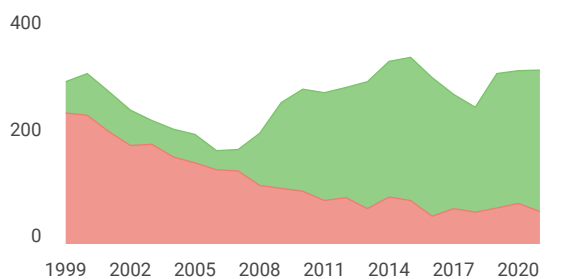
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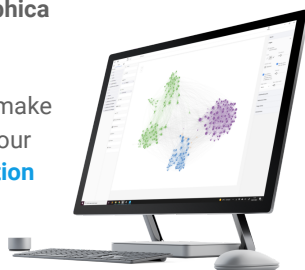
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