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Antioxidant and antibacterial activities of constituents and semi-synthetic derivatives from *Senna siamea* (Lam.) H. S. Irwin & Barneby (Caesalpiniaceae)

Isaac Nde Chedjou[a], Roland TchuenteuTchuenguem[b], Billy Toussie Tchegnitegni[a], Francis Tatong Ngouafong[a], Jean Paul Dzoyem[b], Beaudelaire Kemvoufo Ponou[a*], Rémy Bertrand Teponno[a], Luciano Barboni[c], Léon Azefack Tapondjou[a*]

[a] *Research Unit of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon*
[b] *Department of Biochemistry, faculty of Science, University of Dschang, Box 67, Dschang, Cameroon*
[c] *School of Science and Technology, Chemistry Division, University of Camerino, via Madonna delle Carceri 62032 Camerino, Italy*

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Abstract

Eleven compounds (**1–11**) were isolated from the methanolic extract of leaves and stem bark of *Senna siamea*. Benzylation of compound **2** gave two new hemi-synthetic derivatives: 4'-*O*-benzylvitexin **(12)** and 7,4'-*O*-dibenzylvitexin **(13).** The structures of these compounds were established on the basis of their spectroscopic (1D and 2D NMR) and mass spectrometric (FAB-TOF-MS) data. The extracts, fractions, some isolated compounds as well as the hemi-synthetic derivatives were evaluated for their antibacterial activity using the broth microdilution method and for their antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging potential and ferric reducing antioxidant power (FRAP). The ethyl acetate fraction of the stem bark showed moderate activity against *E. faecalis* and *P. mirabilis* with MIC value of 256 µg/mL. Compound **6** showed moderate activity towards *P. mirabilis* (MIC = 64 µg/mL). The methanol, ethyl acetate and *n*-BuOH soluble fractions of stem bark as well as the *n*-BuOH fraction of the leaves were more active against DPPH compared to vitamin C with EC_{50} values of 1.58 ± 0.25 µg/mL, 1.12 ± 0.67 µg/mL, and 1.02 ± 0.87 µg/mL, respectively. Additionally, compound **4** was the most active against DPPH (EC_{50} = 1.05 ± 0.38) µg/mL.

1. Introduction

Senna siamea is a tree whose irregular crown can reach 25 m high with compound leaves [1]. It belongs to the genus *Senna* (Synonym: *Cassia*) which has about 600 species belonging to the family Ceasalpiniaceae [1]. *Senna* species are widely spread in tropical and subtropical areas [1]. It is used in traditional medicine in the treatment of diseases caused by reactive oxygen species (ROS) such as hypertension, asthma and diabetes [2]; and infectious diseases such as constipation and dysentery [3]. Previous pharmacological works showed that the alcoholic extract of *S. siamea* leaves is endowed with antibacterial properties [4]. In addition, the methanolic extract of the leaves has antioxidant activities [5]. Previous phytochemical works on the seeds, leaves and stem bark of *S. siamea* led to the isolation of flavonoids, alkaloids, triterpenes and anthraquinones [3]. In continuation of our ongoing search for bioactive compounds from Cameroonian medicinal plants, we have previously reported the isolation of flavonoids and their glycosylated derivatives (**2-6**, **10** and **11**) from the major fractions of methanolic extracts of *S. siamea* [6]. These results incited us to investigate the minor fractions. Herein we describe the isolation of four additional compounds (**1**, **7**, **8** and **9**).

*Corresponding authors : *Research Unit of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon*, tapondjou2001@yahoo.fr (TAL) Tel.: +237 675004826; beaudelaireponou@yahoo.fr (PKB) Tel.: +237 699094250

Several research works reported the enhancement effect of prenyl groups and alkyl chains on the antibacterial activity of flavonoids [7]. To the best of our knowledge, no result reported the effect of benzyl group. In order to study the effect of benzyl groups, a benzylation reaction of the major isolated compound (Vitexin, **2**) was carried out and afforded two new hemi-synthetic derivatives. Given the traditional uses of this plant in the treatment of microbial diseases, extracts, fractions, some of the isolated compounds as well as hemi-synthetic derivatives were evaluated for their antibacterial and anti-oxidant activities. The results of the biological activities are also described in this paper.

2. Materials and Methods

2.1. General experimental procedures

High-resolution mass spectra were obtained with a Spectrometer (JEOL-600-H2 and JOEL HX 110) equipped with a FABMS and HRFABMS source. The 1 D (^{1}H and ^{13}C NMR) and 2 D NMR spectra (HSQC, ^{1}H-^{1}H COSY, HMBC, TOCSY, NOESY) were performed in DMSO-d_6 using a Varian INOVA-600 NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C spectra) and BRUKER DRX-500 NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C spectra). All chemical shift (δ) values are given in ppm units. Spectra were calibrated using deuterated solvent peaks. The coupling constants (*J*) are in Hz. Silica gel (40-63 *µm*, 63-200 *µm*, Merck 64271) and Sephadex LH-20 (Sigma 9041-37-6) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) plates developed with Hex-EtOAc, EtOAc-

MeOH, and EtOAc-MeOH-H_2O mixtures. Spots on TLC plates were visualized by spraying with 10% H_2SO_4 and heating for 5 min at 70 °C. The absorbance of each sample was measured with a microplate spectrophotometer reader FLUOstar Omega at 517 nm.

2.2. Collection and identification of *Senna siamea*

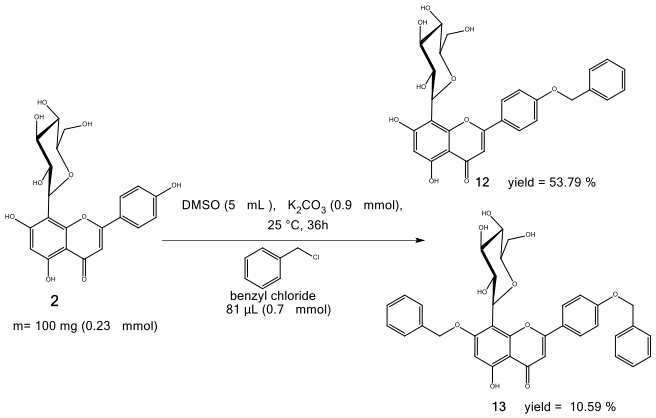
The leaves and stem bark of *S. siamea* were collected in Dschang (West region of Cameroon) in November 2017 and identified by Mr Victor Nana. Specimens documenting the collection were deposited (Nº 25661/HNC) at the National Herbarium of Cameroon (HNC) in Yaounde.

2.3. Extraction and isolation

The air-dried and ground leaves (4.5 kg) of *S. siamea* were extracted by maceration with MeOH (13 L) followed by filtration. The filtrate obtained was concentrated under reduced pressure to give 439 g of a crude extract. Part of this extract (429 g) was suspended in distilled water (1 L) and successively extracted with EtOAc and *n*-BuOH to afford the EtOAc (146 g) and the *n*-BuOH (64 g) fractions, respectively. Part of the EtOAc-soluble fraction (133 g) was subjected to silica gel (63-200 µm, Merck) column chromatography, eluted with *n*-hexane-EtOAc (from 100:0 to 0:100) and EtOAc-MeOH (from 100:0 to 50:40) to afford five main fractions [A (36 g), B (25.5 g), C (16 g), D (16.5 g) and E (25.5 g)]. Part of *n*-BuOH soluble fraction (60 g) was chromatographed under silica gel column chromatography (63-200 µm) eluted with EtOAc-MeOH (from 100:0 to 40:60) leading to six sub-fractions [I (12 g), II (3.2 g), III (6 g) IV (6.2 g), V (13 g) and VI (14 g)]. The purification of sub-fraction III (6 g) on a silica gel column chromatography with EtOAc as eluent led to the isolation compound **7** (10 mg). Dried and pulverized stem bark (6 Kg) was extracted with methanol 95% (16 L) for 72 h at room temperature to yield the corresponding crude extract (345 g) after evaporation under reduced pressure. Part of this extract (340 g) was suspended in distilled water (1 L) and successively extracted with EtOAc and *n*-BuOH to afford EtOAc (42 g) and *n*-BuOH (70 g) fractions, respectively. The EtOAc fraction (40 g) was subjected to column chromatography using silica gel 60 (63 -200 µm) eluted with the mixture *n*-hexane-EtOAc (from 100:0 to 0:100) and EtOAc-MeOH (from 100:0 to 50:50) to yield eight main fractions [F (6 g), G (2 g), H (1 g) I (1.8), J (10.8 g), K (1.8 g), L (1.5) and M (12.5 g)]. Column chromatography of fraction G (2 g) on silica gel (40 - 60 µm) eluted with *n*-hexane-EtOAc of increasing polarity (95:5, 90:10, then 85:15) yielded four sub-fractions (G_1—G_4). The sub-fraction G_3 (36 mg) was chromatographed using a Sephadex LH-20 column, methanol being used as the elution solvent to afford compound **1** (8 mg). Sephadex LH-20 column chromatography of I (1.8 g) gave sub-fractions (I_1—I_4). Sub-fraction I_3 (94 mg) was purified on Sephadex LH-20 to give compound **8** (20 mg). The fractionation of 70 g of the *n*-butanol extract from the stem bark by means of silica gel column chromatography using AcOEt, then AcOEt-MeOH by increasing polarity led to five (05) main fractions A (7 g), B (6 g), C (21 g), D (14 g) and E (18 g). Further fractionation of B (6 g) under silica column chromatography eluted with AcOEt gave sub-fractions B_1 (1.6 g) to B_2 (1 g) and B_3 (3 g). The purification of sub-fraction B_1 (1.6 g) using a Sephadex column LH-20 led to the isolation of **9** (30 mg). Compounds **2**—**6**, **10** and **11** were isolated as previously described by Chedjou et al. [6].

2.4. Benzylation of vitexin (2)

Vitexin (**2**) (100 mg, 0.23 mmol) was dissolved in 5 mL of DMSO and to the resulting solution was added successively K_2CO_3 (96 mg, 0.69 mmol) and benzyl chloride (81 µL) (Scheme 1). The obtained mixture was magnetically stirred at room temperature (25—26°C) for thirty-six hours. The evolution of the reaction was controlled via a TLC plate until the total consumption of the substrate. The reaction medium subsequently underwent differential solubilization between 50 mL of distilled water and 200 mL of ethyl acetate. After the evaporation of the ethyl acetate soluble fraction, the residue obtained was purified on silica gel column chromatography using an *n*-hexane-ethyl acetate (60:40) mixture as eluent to afford compounds **12** (65 mg; 53.79%) and **13** (15 mg; 10,59%).



Scheme 1: Semi-synthesis of 4'-*O*-benzylvitexin (**12**), and 4',7-*O*-dibenzylvitexin (**13**) from Vitexin (**2**)

The antibacterial activity of the methanol extracts, fractions and compounds was assessed by determining the minimum inhibitory concentration (MIC) using the broth microdilution method as previously described by Dzoyem *et al.* [8]. Five bacterial strains were used: *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 6539), *Staphylococcus aureus* (ATCC 1026), *Enterococcus faecalis* (ATCC 29212) *and Proteus mirabilis (Isolate)*. Briefly, the test sample and the selected antibiotic were dissolved in dimethylsulfoxide-Mueller Hinton broth (DMSO-MHB) and dimethylsulfoxide-Sabouraud Dextrose broth (DMSO-SDB). The solution obtained was then added to MHB and SDB and serially diluted twofold in 96-well microplates to give a final concentration range of 2 to 1024 µg/mL for extracts and from 0.5 - 128 µg/mL for pure compounds and references. One hundred microliters of inoculums prepared in MHB at a concentration of 1.5 × 10 CFU/mL were then added, even for SDB. The plates were covered with a sterile plate sealer and then agitated with a shaker to mix the contents of the wells and incubated at 37°C. The final concentration of DMSO was less than 2.5%, and DMSO did not affect the microbial growth. Wells containing only MHB or SDB, 100 µL of any inoculum and DMSO at a final concentration of 2.5% served as the negative control. The MICs of samples were detected after 18 h, following addition of 40 µL of INT 0.2 mg/mL and

incubation at 37°C for 30 min. The strains were obtained from the American Type Culture Collection (ATCC). The isolate was obtained and identified from clinical samples at the Research Unit of Microbiology and Antimicrobial Substances of the University of Dschang. Ciprofloxacin (Sigma, Germany) was used as a reference drug.

2.6. Antioxidant assay

MeOH extracts, fractions, some of the isolated compounds and semi-synthetic derivatives were evaluated for their antioxidant activities using two methods: DPPH▪ scavenging and ferric reducing antioxidant power (FRAP) as previously described by Mensor *et al.* [9] and Benzie and Strain [10], respectively.

DPPH assay

In each well of a 96-well plate, 20 µL of methanol was added to the last seven rows. This was followed by the introduction of 20 µL of the methanolic solutions of the samples to be tested (2 mg/mL) into the first two wells of each column (4 columns were used for one sample) and successive serial dilutions of factor 2 were made in the other wells, maintaining the volume at 20 µL. A volume of 180 µL of methanolic solution of DPPH (0.08 mg/mL) was again introduced into each well of the first three columns, while 180 µL of methanol was introduced into each well of the fourth column. Plates containing 200 µL of final solution per well were incubated for 30 min in the dark and at room temperature. At the end of the incubation, the optical densities were read on a spectrophotometer (FLUOstar Omega microplate reader) at 517 nm and converted into percentages of antioxidant activity as shown below.

$$\% \text{ of antioxidant activity} = \frac{[A_{DPPH} - (A1-A2)]}{A_{DPPH}} \times 100$$

A_{DPPH} = Absorbance of DPPH, A1 = Absorbance of sample + methanolic solution of DPPH
A2 = Absorbance of the methanolic solution of sample

FRAP assay

The FRAP (Ferric reducting Antioxidant Power) reagent was prepared by mixing a buffer solution of sodium acetate (300 mM, pH 3.6), a solution of 2,4,6-tris (2-pyridyl)-1,3,5-s-triazine TPTZ (10 mM) and a solution of $FeCl_3$ in the proportions 10:1:1. A volume of 5 µL of sample (2 mg/mL) was mixed with 95 µL of FRAP reagent. The mixture was incubated for 30 min at 37°C in the dark. After incubation, the optical density was read on a spectrophotometer (FLUOstar Omega microplate reader) at 593 nm. Vitamin C was used as a positive control. The antioxidant power of the sample was calculated from the calibration curve of the $FeSO_4$ solution (The number of moles of the FeSO4 solution varying from 156.25 µmol to 10,000 µmol) and expressed in $FeSO_4$ micromole equivalent per sample gram.

3. Results and discussion

3.1. Phytochemical Investigation

The structures of the isolated compounds **1**, **7–9** (Figure 1) were determined by careful examination of their 1D and 2D NMR data followed by the comparison with those reported in the literature and those of compounds **2–6**, **10** and **11** were determined as described in [6]. They were identified as apigenin (**1**) [11], vitexin (**2**) [12], isovitexin (**3**) [13], quercetin (**4**) [14], quercetin-3-*O*-α-L-rhamnopyranoside (**5**) [15], quercetin-3-*O*-β-D-arabinopyranoside (**6**) [16], quercetin 3-*O*-β-D-galactopyranoside (**7**) [17], luteolin (**8**) [18], apigenin 6-*C*-(2″-*O*-α-rhamnopyranosyl-β-D-glucopyranoside) (**9**) [19], aurantiamide acetate (**10**) [20] and betulinic acid (**11**) [21].

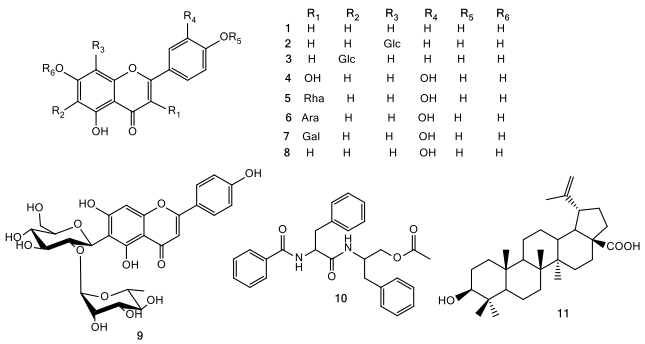


Figure 1: Structure of isolated compounds **1–11**

Various research works reported the enhancement effect of prenyl groups and alkyl chains on the antibacterial activity of flavonoids [7]. To the best of our knowledge, no result reported the effect of benzyl group. In order to study the effect of benzyl groups, a benzylation reaction of the major isolated compound (Vitexin, **2**) was carried out and afforded two new hemi-synthetic derivatives. The yields of compounds **12** (53.79 %) and **13** (10.59 %) could be justified by the difference in acidity of the hydrogen atoms of 4'-OH and 7-OH, and the steric hindrance due to the presence of the glucopyranosyl unit. 5-OH did not undergo substitution because of the chelation with the carbonyl group. The use of a stronger base might improve the yield of this reaction and the substitution at 5-OH could be possible by increasing the temperature.

3.2. Antioxidant activity

From the results of these tests (Table 1), the methanolic extract from leaves and its *n*-BuOH fraction were highly active against DPPH radical with EC_{50} values of 7.43 µg/mL and 1.24 µg/mL, respectively. The methanolic extract from the stem bark, its ethyl acetate and *n*-BuOH fractions also exhibited high antiradical activities with respective EC_{50} values of 1.58 µg/mL, 1.12 µg/mL, and 1.02 µg/mL. The ethyl acetate fraction from the leaves was moderately active against DPPH. This result is in agreement with those previously reported by Kaur and Arora [5]. Among the tested compounds, quercetin (**4**) showed the highest free radical scavenging activity (EC_{50} = 1.05 µg/mL), which was higher than that of vitamin C (EC_{50} = 2.29 µg/mL) used as a reference drug. These results corroborate with those in the FRAP test, where compound **4** showed the best reducing power. Quercetin-3-*O*-α-L-rhamnopyranoside (**5**) (EC_{50} = 1.21 µg/mL) and quercetin-3-*O*-β-D-galactopyranoside (**7**) (EC_{50} = 6.48 µg/mL) were significantly active toward DPPH free radical.

Table 1: Antioxidant potential of the crude extracts, fractions, some isolated and semi-synthetic derivatives.

Samples	DPPH essay [EC_{50} (µg/mL)]	FRAP essay (mmol $FeSO_4$/g)
ME (leaves)	7.43 ± 0.19	94.41 ± 0.15
EAF (leaves)	25.64 ± 0.29	93.45 ± 0.52
n-BF (leaves)	1.24 ± 0.33	95.77 ± 0.23
ME (stem bark)	1.58 ± 0.25	84.71± 0.85
EAF (stem bark)	1.12 ± 0.67	86.45 ± 0.77
n-BF (stem bark))	1.02 ± 0.87	88.3 ± 0.24
2	11.45 ± 0.77	100.09 ± 0.65
3	10.86 ± 0.29	94.75 ± 0.88
4	1.05 ± 0.38	87.14 ± 0.23
5	1.21 ± 0.19	94.85 ± 0.54
7	6.48 ± 0.11	100.27 ± 0.65
9	42.14 ± 0.53	98.48 ± 0.16
12	75335.55 ± 0.73	103.54 ± 0.68
Vitamin C	2.29 ± 0.13	60.52 ± 0.55

ME: Methanol extract; EAF: Ethyl acetate fraction; *n*-BF: *n*-butanol fraction

The antioxidant activities of compounds **4**, **5** and **7** could be justified by the presence of the catechol function on the B ring which is the best hydrogen donor while the activities of compounds **2**, **3** and **9** which lack the catechol group could be explained by the presence of hydroxyl groups at C-5 and C-7 as previously reported [22]. The semi-synthetic 4'-*O*-benzylvitexin (**12**) presented neither antiradical nor reducing power compared to vitexin which showed significant antiradical activity. These results proved that the benzyl group reduced the antiradical activity. In both DPPH and FRAP tests, flavonoid glycosides (**5** and **7**) were less active than their corresponding aglycone (**4**) and it is in perfect agreement the result reported by Rice-Evans *et al.* [23].

3.3 Antibacterial activity

The ethyl acetate fraction of stem bark showed moderate activity (MIC = 256 µg/mL) against *E. faecalis* and *P. mirabilis strains*.

Table 2: Antibacterial activity (MIC) of the extract, fractions, isolated compounds from the leaves and stem bark of *S. siamea* and the semi-synthetic derivatives.

Samples	Microorganisms									
	Ec		*ST*		*Sa*		*Ef*		*Pm*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
EAFL	512	-	512	-	1024	-	512	-	512	-
n-BFL	-	-	512	-	-	-	1024	-	1024	-
EAFS	512	-	512	-	512	-	256	-	256	-
n-BFS	-	-	1024	-		-	1024	-	1024	-
2	-	-	128	-	-	-	-	-	-	-
3	-	-	128	-	-	-	-	-	-	-
4	128	-	-	-	128	-	128	-	128	-
5	128	-	256	-	128	-	256	-	128	-
6	128	-	128	-	256	-	128	256	64	512
7	128	-	-	-	256	-		-		-
8	128	-	-	-	128	-	128	-	128	-
9	-	-	-	-	-	-	-	-	256	-
12	-	-	128	-	-	-	-	-	-	-
13	-	-	128	-	-	-	-	-	-	-
Cipro	2	512	1	8	2	8	0,5	4	1	4

Ec: *Escherichia coli* (ATCC 25922), *ST*: *Salmonella typhi* (ATCC 6539), *Sa*: *Staphylococcus aureus*(ATCC 1026), *Ef*: *Enterococcus faecalis* (ATCC 29212), *Pm* : *Proteus mirabilis* (Isolate), *Ciprofloxac*in: reference antibiotics; ME: Methanol extract; EAFL: Leaf Ethyl acetate fraction; EAFS: Stem bark Ethyl acetate fraction; *n*-BFL: :Leaf *n*-butanol fraction; *n*-BFS: :Stem bark *n*-butanol fraction.. For compounds: - = >256 µg/ml; For extracts and fractions: - = >1024 µg/mL; Cipro = Ciprofloxacin.

The Ethyl acetate fractions of leaves and stem bark exhibited moderate activity against *E. coli* and *S. typhi* with MIC value of 512 µg/mL. Quercetin-3-*O*-*β*-D-arabinopyranoside (**6**) showed moderate activity with *P. mirabilis* (MIC= 64 µg/mL). It was bacteriostatic against *P. mirabilis* (MBC/MIC = 8) and bactericide towards *E. faecalis* (MBC/MIC = 2). Vitexin (**2**), 4'-*O*-benzylvitexin (**12**), 4',7-*O*-dibenzylvitexin (**13**) showed low activity with an MIC value of 128 µg/mL toward *S. typhi*. Base on this result, a benzyl group has no effect on the antibacterial activity. Quercetin (**5**) and quercetin-3-*O*-*α*-L-rhamnopyranoside showed low antibacterial activities (MIC = 128 µg/mL) for *E. coli* and *S. aureus* (Table 2).

3.4. Physical and spectroscopic data for compounds

Apigenin (**1**)**:** yellow from methanol, ^{1}H NMR (CD_3OD ; 500 MHz) : 6.61 (1H, *s*, H-3), 6.24 (1H, *d*, *J* = 2.1 Hz, H-6), 6.48 (1H, *d*, *J* =2.1 Hz, H-8), 7.86 (1H, *d*, *J* = 8.8 Hz, H-2'/H-6'), 6.95 (1H, *d*, *J* = 8.8 Hz, H-3'/H-5') ; ^{13}C NMR (CD_3OD ; 125 MHz) : 164.4 (C-2), 102.3 (C-3), 182.7 (C-4) 161.8 (C-5), 98.8 (C-6), 164.7 (C-7), 93.5 (C-8), 157.9 (C-9), 103.8 (C-10), 121.7 (C-1'), 161.4 (C-4'), 128.0 (C-2'/C-6'), 115.4 (C-3'/C-5').

Vitexin (**2**)**:** yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD; 500 MHz) aglycone**:** 6.79 (1H, *s*, H-3), 6.27 (1H, *s*, H-6), 8.03 (1H, *d*, *J* = 8.3 Hz, H-2'/H-6'), 6.89 (1H, *d*, *J* = 8.3 Hz, H-3'/H-5') ; glucose**:** 4.69 (1H, *d*, *J* =10 Hz, H-1''), 3.84 (1H, *t*, *J* = 9.4 Hz, H-2''), 3.26 (1H, *m*, H-3''), 3.39 (1H, *m*, H-4''), 3.24 (1H, *m*, H-5''), 3.53 (1H, *m*, H-6''), 3.78 (1H, *m*, H-6''); ^{13}C NMR (CD_3OD ; 125 MHz) aglycone**:** 164.3 (C-2), 102.8 (C-3), 182.5 (C-4) 161.5 (C-5), 98.5 (C-6), 163.0 (C-7), 105.0 (C-8), 156.0 (C-9), 104.2 (C-10), 122.0 (C-1'), 160.8 (C-4'), 129.4 (C-2'/C-6'), 116.2 (C-3'/C-5'); glucose**:** 73.8 (C-1''), 71.2 (C-2''), 79.0 (C-3''), 70.9 (C-4''), 82.2 (C-5''), 61.6 (C-6'').

Isovitexin (**3**)**:** yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD ; 500 MHz) aglycone: 6.60 (1H, *s*, H-3), 6.50 (1H, *s*, H-8), 7.84 (1H, *d*, *J* = 8.9 Hz, H-2'/H-6'), 6.95 (1H, *d*, *J* = 8.9 Hz, H-3'/H-5'); glucose: 4.92 (1H, *d*, *J* = 9.9 Hz, H-1''), 4.16 (1H, *t*, *J* = 9.4 Hz, H-2''), 3.50 (1H, *m*, H-3''), 3.51 (1H, *m*, H-4''), 3.44 (1H, *m*, H-5''), 3.77 (1H, *dd*, 12.1 ; 5.4 Hz, H-6''), 3.90 (1H, *m*, *J* = 12.1 ; 2.3, H-6''); ^{13}C NMR (CD_3OD ; 125 MHz) : 164.3 (C-2), 102.6 (C-3), 182.5 (C-4) 160.5 (C-5), 107.7 (C-6), 163.6 (C-7), 93.8 (C-8), 157.3 (C-9), 103.7 (C-10), 121.6 (C-1'), 161.3 (C-4'), 127.9 (C-2'/C-6'), 115.2 (C-3'/C-5'); glucose: 73.8 (C-1''), 71.2 (C-2''), 78.7 (C-3''), 70.4 (C-4''), 81.2 (C-5''), 61.3 (C-6'').

Quercetin (**4**)**:** yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD ; 600 MHz) : 6.43 (1H, *d*, *J* = 2.0 Hz, H-6), 6.20 (1H, *d*, *J* = 2.1 Hz, H-8), 7,75 (1H, *d*, *J*= 2.1 Hz, H-2'), 6,90 (1H, *d*, *J* = 8.5 Hz, H-5'), 7,64 (1H, *dd*, *J* = 8.5 ; 2.1 Hz, H-6'). ^{13}C NMR (CD_3OD ; 150 MHz) : 147.4 (C-2), 135.8 (C-3), 175.8 (C-4) 161.1 (C-5), 97.8 (C-6), 164.1 (C-7), 92.8 (C-8), 156.7 (C-9), 103.1 (C-10), 122.6 (C-1'), 114.4 (C-2'), 144.8 (C-3'), 146.5 (C-4'), 114.8 (C-5'), 120.4 (C-6').

Quercetin-3-*O*-*α*-*L*-rhamnopyranoside (**5**)**:** yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD ; 500 MHz) aglycone: 6.22 (1H, *d*, *J* = 2.1 Hz, H-6), 6.39 (1H, *d*, *J* = 2.1 Hz, H-8), 7.36 (1H, *d*, *J* = 2.1 Hz, H-2'), 6.93 (1H, *d*, *J* = 8.3 Hz, H-5'), 7.33 (1H, *dd*, *J* = 8.3; 2.1 Hz, H-6') ; rhamnose: 5.37 (1H, *d*, *J* = 1.4 Hz, H-1''), 3.76 (1H, *m*, H-2''), 3.52 (1H, *m*, H-3''), 3.37 (1H, *d*, *J* = 2.6 Hz, H-4''), 3.44 (1H, *m*, H-5''),

0.96 (3H, *d*, 2.5 Hz, H-6''). ^{13}C NMR (CD_3OD ; 125 MHz) : 157.9 (C-2), 134.8 (C-3), 178.3 (C-4) 161.8 (C-5), 98.4 (C-6), 164.5 (C-7), 93.4 (C-8), 157.1 (C-9), 104.5 (C-10), 121.6 (C-1'), 115.1 (C-2'), 145.1 (C-3'), 148.5 (C-4'), 115.1 (C-5'), 121.4 (C-6'); rhamnose: 102.1 (C-1''), 70.6 (C-2''), 70.7 (C-3''), 71.8 (C-4''), 70.4 (C-5''), 16.2 (C-6'').

Quercetin-3-*O*-*β*-D-arabinopyranoside (**6**)**:** yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD ; 600 MHz) aglycone: 6.22 (1H, *t*, *J* = 2.1 Hz, H-6), 6.41 (1H, *t*, *J* = 2.1 Hz, H-8), 7.76 (1H, *d*, *J* = 2.2 Hz, H-2'), 6.88 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.59 (1H, *dd*, *J* = 8.4; 2.2 Hz, H-6') ; Arabinose: 5.19 (1H, *d*, *J* = 6.6 Hz, H-1''), 3.91 (1H, *d*, *J* = 1.9 Hz, H-2''), 3.82 (1H, *m*, H-3''), 3.58 (1H, *dd*, *J* = 6.3 ; 3.3 Hz, H-4''), 3.47 (1H, *d*, *J* = 10.6 Hz, Ha-5''), 3.84 (1H, *d*, *J* = 2.5 Hz, Hb-6''). ^{13}C NMR (CD_3OD ; 150 MHz) : 157.4 (C-2), 134.1 (C-3), 178.0 (C-4), 161.6 (C-5), 98.3 (C-6), 164.6 (C-7), 93.1 (C-8), 157.2 (C-9), 104.1 (C-10), 121.5 (C-1'), 115.9 (C-2'), 144.5 (C-3'), 151.3 (C-4'), 114.9 (C-5'), 121.4 (C-6'); Arabinose: 103.0 (C-1''), 71.4 (C-2''), 72.6 (C-3''), 67.7 (C-4''), 65.6 (C-5'').

Quercetin 3-*O*-*β*-D-galactoyranoside (**7**)**:** yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD ; 500 MHz) aglycone: 6.23 (1H, *d*, *J* = 2.1 Hz, H-6), 6.42 (1H, *d*, *J* = 2.1 Hz, H-8), 6.88 (1H, *d*, *J* = 8.5 Hz, H-2'), 7.86 (1H, *d*, *J* = 2.2 Hz, H-5'), 7.60 (1H, *dd*, *J* = 8.5; 2.2 Hz, H-6') galactose: 5.19 (1H, *d*, *J* = 7.8 Hz, H-1''), 3.83 (1H, *d*, *J* = 1.9 Hz, H-2''), 3.58 (1H, *dd*, *J* = 6.3 ; 3.3 Hz, H-3''), 3.87 (1H, *dd*, *J* = 3.4 ; 1.0 Hz, H-4''), 3.50 (1H, *dd*, *J* = 6.1 ; 1.1 Hz, H-5''), 3.57 (1H, *dd*, *J* = 6.3 ; 3.3 Hz, Ha-6''), 3.66 (1H, *dd*, *J* = 6.0 ; 1.1 Hz, Hb-6''). ^{13}C NMR (CD_3OD ; 125 MHz) : 157.4 (C-2), 134.4 (C-3), 178.2 (C-4) 161.6 (C-5), 98.5 (C-6), 164.8 (C-7), 93.3 (C-8), 157.1 (C-9), 104.2 (C-10), 121.6 (C-1'), 114.7 (C-2'), 144.8 (C-3'), 148.5 (C-4'), 116.3 (C-5'), 121.5 (C-6'); galactose: 104.0 (C-1''), 71.6 (C-2''), 73.7 (C-3''), 68.5 (C-4''), 75.7 (C-5''), 60.4 (C-6'').

Luteolin (**8**)**:** yellow powder, soluble in methanol; ^{1}H NMR (CD_3OD; 500 MHz): 6.52 (1H, *s*, H-3), 6.20 (1H, *d*, *J* = 2.2 Hz, H-6), 6.43 (1H, *d*, *J* = 2.2 Hz, H-8), 7.36 (1H, *d*, *J* = 2.0 Hz, H-2'), 6.89 (1H, *d*, *J* = 8.9; 2.1 Hz, H-5'), 7.37 (1H, *m*, H-6'). ^{13}C NMR (CD_3OD ; 125 MHz) : 165.9 (C-2), 100.1 (C-3), 181.7 (C-4) 160.3 (C-5), 97.7 (C-6), 163.5 (C-7), 93.0 (C-8), 156.6 (C-9), 102.7 (C-10), 121.6 (C-1'), 111.4 (C-2'), 144.0 (C-3'), 147.8 (C-4'), 117.5 (C-5'), 119.9 (C-6').

Apigenin 6-*C*-(2''-*O*-*α*-rhamnopyranosyl-*β*-D-glucopyranoside) (**9**)**:** amorphous yellow powder, soluble in methanol, ^{1}H NMR (CD_3OD ; 500 MHz) aglycone: 6.60 (1H, *s*, H-3), 6.43 (1H, *d*, *J* = 2.2 Hz, H-8), 7.84 (1H, *d*, *J* = 8.8 Hz, H-2'/H-6'), 6.92 (1H, *d*, *J* = 8.8 Hz, H-3'/H-5'); glucose: 4.90 (1H, *sl*, H-1''), 4.25 (1H, *m*, H-2''), 3.56 (1H, *m*, H-3''), 3.65 (1H, *m*, H-4''), 3.35 (1H, *m*, H-5''), 3.71 (1H, *m*, Hz, H-6''), 3.86 (1H, *m*, H-6''); rhamnose: 5.22 (1H, *sl*, H-1''), 3.87 (1H, *d*, *J* = 2.3 Hz, H-2''), 3.40 (1H, *m*, H-3''), 3.11 (1H, *m*, H-4''), 2.52 (1H, *t*, *J* = 9.5 Hz, H-5''), 0.71 (3H, *m*, H-6''). ^{13}C NMR (CD_3OD ; 125 MHz) : 165.8 (C-2), 103.6 (C-3), 184.0 (C-4) 161.5 (C-5), 109.6 (C-6), 164.8 (C-7), 95.5 (C-8), 158.7 (C-9), 105.3 (C-10), 122.9 (C-1'), 162.7 (C-4'), 129.1 (C-2'/C-6'), 116.1 (C-3'/C-5'); glucose: 73.6 (C-1''), 77.6 (C-2''), 81.5 (C-3''), 72.7 (C-4''), 82.5 (C-5''), 62.9 (C-6''). rhamnose: 102.2 (C-1''), 72.4 (C-2''), 72.0 (C-3''), 73.6 (C-4''), 70.0 (C-5''), 18.1 (C-6'').

Aurantiamide acetate (**10**)**:** Beige powder, soluble in methanol, ^{1}H NMR (CD_3OD; 600 MHz): 4.79 (1H, *s*, H-2), 4.31 (1H, *m*, H-4), 3.91 (1H, *dd*, *J* = 11.2 ; 6.1 Hz, Ha-5), 3.98 (1H, *dd*, *J* = 11.2 ; 4.4 Hz, Hb-5), 2.01 (3H, *s*, H-7), 3.01 (1H, *dd*, *J* = 13.4; 8.4 Hz, H-8a), 3.14 (1H, *dd*, *J* = 13.4, 6.8 Hz, H-8b), 2.80 (1H, *dd*, *J* = 13.8, 7.9 Hz, H-9a), 2.85 (1H, *dd*, *J* = 13.8, 6.7 Hz, H-9b), 7.72 (1H, *m*, H-2'/6'), 7.44 (2H, *t*, *J* = 7.7 Hz, H-3'/5'), 7.52 (1H, *d*, *J* = 7.4 Hz, H-4'), 7.19 (1H, *d*, *J* = 6.6 Hz, H-2''/6''), 7.10 (1H, *m*, H-3''/5''), 7.26 (2H, *m*, H-4''), 7.26 (2H, *m*, H-2'''/6'''), 7.10 (1H, *d*, *J* = 6.6 Hz, H-3'''/5'''), 7.19 (1H, *m*, H-4''') ; ^{13}C NMR (CD_3OD ; 150 MHz) : 170.0 (C-1), 56.8 (C-2), 173.3 (C-3) 51.3 (C-4), 66.3 (C-5), 172.7 (C-6), 20.1 (C-7), 39.1 (C-8), 32.8 (C-9), 135.4 (C-1'), 128.6 (C-2'/ C-6'), 127.7 (C-3'/ C-5'), 132.9 (C-4'), 139.0(C-1''), 127.9 (C-2''/ C-6''), 127.7 (C-5'''/ C-5'''), 130.4 (C-4''), 138.8 (C-1'''), 129.6 (C-2'''/ C-6'''), 130.4 (C-3'''/ C-5'''), 129.7 (C-4''').

Betulinic acid (**11**)**:** white powder, soluble in methanol,^{1}H NMR (CD_3OD; 600 MHz): 3.14 (1H, *m*, H-3), 3.05 (1H, *m*, H-18), 1.57 (1H, *s*, H-23), 0.97 (1H, *s*, H-24), 10.87 (1H, *s*, H-25), 0.77 (1H, *s*, H-26), 1.03 (1H, *s*, H-27), 4.60 (1H, *sl*, Ha-29), 4.73 (1H, *sl*, H-29b), 1.72 (1H, *s*, H-30) ; ^{13}C NMR (CD_3OD ; 150 MHz) : 38.6 (C-1), 27.6 (C-2), 77.6 (C-3), 38.7 (C-4), 55.4 (C-5), 18.1 (C-6), 34.3 (C-7), 40.6 (C-8), 50.5 (C-9), 36.6 (C-10), 20.7 (C-11), 25.5 (C-12), 38.1 (C-13),42.3 (C-14), 29.6 (C-15), 31.8 (C-16), 55.8 (C-17), 47.4 (C-18), 49.0 (C-19), 150.4 (C-20), 30.4 (C-21), 37.0 (C-22), 27.4 (C-23), 15.6 (C-24), 15.7 (C-25), 15.1 (C-26), 14.1 (C-27), 176.6 (C-28), 108.9 (C-29), 18.6 (C-30).

4'-*O*-benzylvitexin (**12**)**:** yellow powder, soluble in DMSO, FABMS: *m/z* 523.1581 $[M+H]^+$ (cal. 523.1526) (for $C_{28}H_{26}O_{10}$). ^{1}H NMR (DMSO-d_6; 500 MHz) aglycone: 6.92 (1H, *s*, H-3), 6.57 (1H, *s*, H-6), 8.17 (1H, *d*, *J* = 9.0 Hz, H-2'/ H-6'), 7.19 (1H, *d*, *J* =9.0 Hz, H-3'/ H-5'); glucose: 4.94 (1H, *d*, *J* = 5.7 Hz, H-1''), 3.86 (1H, *ddd*, *J* = 9.9 Hz; 8.7 Hz; 5.2 Hz, H-2''), 3.26 (1H, *m*, H-3''), 3.45 (1H, *m*, H-4''), 3.24 (1H, *m*, H-5''), 3.58 (1H, *m*, Hz, H-6a''), 3.76 (1H, *m*, H-6b''). Benzyl : 5.22 (1H, *d*, *J* = 11.2 Hz ; H-1'''a), 5.30 (1H, *sl* ; H-1'''b), 7.48 (1H, *m*, H-4'''/ H-6'''), 7.40 (1H, *d*, *J* = 7.5 Hz, H-3'''/ H-5'''), 7.58 (1H, *d*, *J* =7.5 Hz, H-5'''). ^{13}C NMR (CD_3OD ; 125 MHz) : 163.9 (C-2), 103.2 (C-3), 182.6 (C-4), 161.4 (C-5), 96.5 (C-6), 164.3 (C-7), 105.0 (C-8), 155.6 (C-9), 104.4 (C-10), 123.9 (C-1'), 129.0 (C-2'/6'), 115.2 (C-3'/5'), 161.4 (C-4'); glucose: 73.6 (C-1''), 71.2 (C-2''), 79,6 (C-3''), 70.4 (C-4''), 82.3 (C-5'') 61.4 (C-6''), Benzyl : 70.0 (C-1'''), 136.7 (C-2'''), 127.2 (C-4'''/ C-6'''), 128.5 (C-3'''/C-7'''), 127.0 (C-5''').

4',7-*O*-dibenzylvitexin (**13**)**:** yellow powder, soluble in methanol, FABMS: *m/z* 613.0 $[M+H]^+$ (for $C_{35}H_{32}O_{10}$). ^{1}H NMR (CD_3OD ; 500 MHz) aglycone: 6.77 (1H, *s*, H-3), 6.53 (1H, *s*), H-6), 8.10 (1H, *d*, *J* = 8.9 Hz, H-2'/ H-6'), 7.18 (1H, *m*, H-3'/ H-5'); glucose: 4.86 (1H, *d*, *J* = 5.7 Hz, H-1''), 3.68 (1H, *d*, *J* = 9.3 Hz, H-2''), 3.44 (1H, *m*, H-3''), 4,23 (1H, *dd*, *J* = 9.9 Hz ; 8.9 Hz, H-4''), 3.32 (1H, *m*, H-5''), 3.77 (1H, *dd*, *J* = 12.3 Hz ; 5.3 Hz, H-6a''), 3,90 (1H, *dd*, *J* = 12,1 Hz ; 2,3 Hz, H-6b'' ; Benzyl : 5.20 (1H, *m*, H-1'''), 4,82 (1H, *m*, H-1a''''), 5,05 (1H, *d*, *J* = 11,3 Hz, H-1b''''), 7,45 (1H, *d*, *J* = 7,6 Hz, H-4'''/ H-6'''), 7.45 (1H, *d*, *J* = 7.6 Hz, H-4'''/ H-6'''/ H-4''''/ H-6''''), 7.38 (1H, *m*, H-3'''/ H-7'''/ H-3''''/ H-7''''). ^{13}C NMR (CD_3OD ; 125 MHz) : 166.7 (C-2), 104.9 (C-3), 184.9 (C-4), nd (C-5), nd (C-6), 164.8 (C-7), nd (C-8), 156.2 (C-9), 108.6 (C-10), 125.2 (C-1'), 129.9 (C-2'/6'), 117.0 (C-3'/5'), 163.8 (C-4'); glucose: 77.0 (C-1''), 71.9 (C-2''), 80.0 (C-3''), 72.4 (C-4''), 82.9 (C-5'') 63.0 (C-6''), Benzyl : 71.1 (C-1'''), 78,9 (C-1''''), 138,0

(2'''/2''''), 128,8 (C-4'''/ C-6'''/ C-4''''/ C-6''''), 129.7 (C-3'''/ C-7'''/ C-3''''/ C-7''''), 129.1 (C-5'''/C-5'''').

Conclusion

This work was undertaken in order to investigate the chemical constituents of *S. siamea*, evaluate their antibacterial and antioxidant activities and study the effect of the benzyl group. The chemical investigation of the leaves and stem bark of *S. siamea* led to the isolation of eleven known compounds. This is the first isolation of compounds **7**, **8** and **9** from *S. siamea*. Benzylation of vitexin gave two new semi-synthetic derivatives: 4'-*O*-benzylvitexin (**12**) and 7,4'-*O*-benzylvitexin (**13**). The MeOH extract, fractions, isolated compounds and semi-synthetic derivatives were tested for their antibacterial properties against five bacterial strains, and they were also evaluated for their antioxidant activity. The methanolic extract, the *n*-BuOH and EtOAc fractions from the stem bark showed the highest antioxidant activities as judged by the DPPH scavenging assay and Ferric reducing antioxidant power. The semi-synthetic derivatives did not exhibit antioxidant activity. Compound **6** showed moderate activity with respect to *P. mirabilis*. It was bacteriostatic against *P. mirabilis* and bactericidal toward *E. faecalis*. The benzyl group showed a decreasing antioxidant effect but it has no effect on the antibacterial activity. The above results could justify the use of *S. siamea* in the traditional medicine for the treatment of infectious diseases.

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