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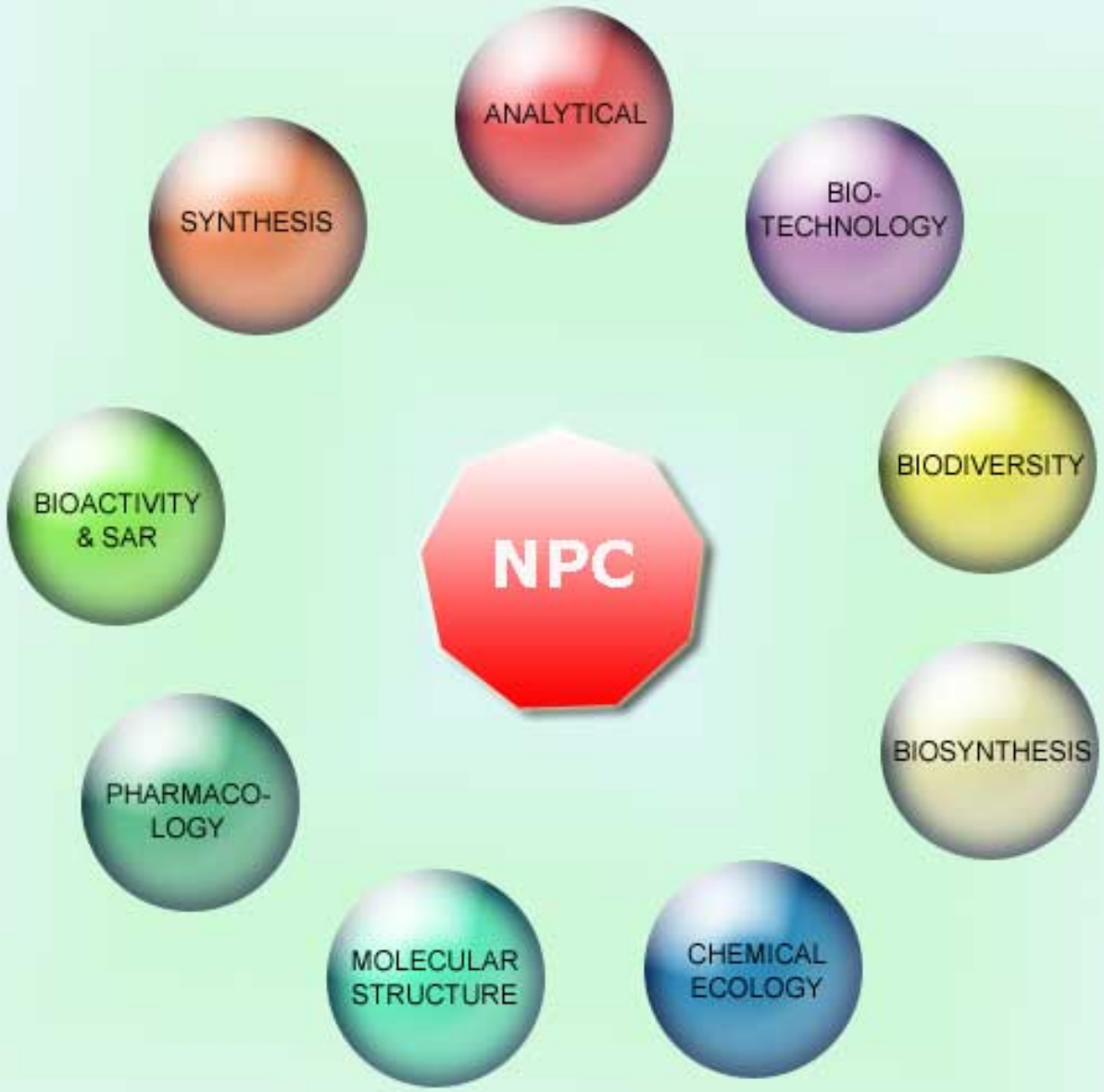

This Issue is Dedicated to
Professor Francesco De Simone
on the Occasion of his 72nd Birthday

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New Phenolic Glycosides from *Securinega virosa* and Their Antioxidant Activity

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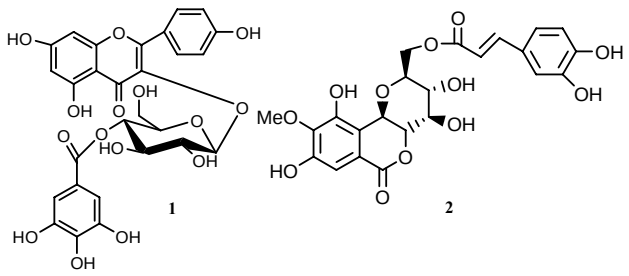
One new flavonoid glycoside, 3-*O*-kaempferol 4-*O*-(galloyl)-β-D-glucoside, one new bergenin derivative, 11-*0*-caffeoylbergenin, along with other known flavonoids and phenolic derivatives, were isolated from the leaves of *Securinega virosa*. Their structures were established on the basis of detailed spectral analysis. *In vitro* biological analysis of the isolated compounds showed that they were able to quench DPPH radicals and had a direct scavenging activity on superoxide anion. Kaempferol 3-*O*-(4-galloyl)-β-D-glucopyranoside (**1**), 11-*0*-caffeoylbergenin (**2**), and glucogallin (**6**) exhibited the highest antioxidant capacity, being also able to modulate hydroxyl radical formation more efficiently than the other compounds, acting as direct hydroxyl radical scavengers and chelating iron.

Keywords: *Securinega virosa*, Euphorbiaceae, flavonoids, phenolic compounds, antioxidant activity.

Securinega virosa (Roxb. ex Willd) A Juss., syn. *Flueggea virosa* (Roxb. ex Willd) Voigt, is a small tree widely distributed in Mali [1,2]. It is used in traditional medicine for many diseases, including diarrhea, rheumatism, malaria, liver disease, inflammation and pain. Extracts of the plant are used for the expulsion of worms and in the treatment of bilharziasis, and for other urinary and genital tract disorders [2]. Some biological activities have already been confirmed. Extracts of the aerial parts were shown to have antispasmodic activity against both histamine and acetylcholine induced spasms, and increased phenobarbitone sleeping time [3]; the extracts also demonstrated antitumor activity against KB, L1210 and P388 cell lines [4]. Alkaloids isolated from the plant caused hypotension in cats and relaxation of rabbit gut smooth muscle [5].

The present paper deals with the antioxidant bioassay-guided fractionation of *S. virosa* methanol extract, leading to the isolation and structural characterization of one new flavonoid (**1**), one new



1 **2**

bergenin derivative (**2**), together with other known compounds, including quercetin 3-*O*-β-D-glucopyranoside (**3**), corilagin (**4**), acalyphidin M (**5**), glucogallin (**6**), geraniin (**7**), and bergenin (**8**).

Compound **1** was isolated as a yellow amorphous powder. Its molecular formula was established as $C_{28}H_{24}O_{15}$ by means of the ESI-MS ([M-H]$^-$ peak at *m/z* 599), and by ^{13}C, ^{13}C-DEPT NMR, and elemental analysis. Analysis of the 600 MHz NMR spectra suggested a flavonoid skeleton for compound **1**. The ^{1}H NMR spectrum (Table 1) indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled

Table 1: ^{1}H- and ^{13}C- NMR spectroscopic data of compound **1** (CD_3OD, 600 MHz)[a].

position	δ_H	δ_C
2	-	159.0
3	-	134.2
4	-	180.0
5	-	163.0
6	6.3 d (1.7)	101.8
7		165.0
8	6.46 d (1.7)	95.6
9		158.7
10		104.8
1'		123.0
2'	7.88 d (8.5)	129.4
3'	6.93 d (8.5)	117.5
4'	-	160.0
5'	6.93 d (8.5)	117.5
6'	7.88 d (8.5)	129.4
3-*O*-Glc'''	5.40 d (7.5)	102.0
2"	3.47 dd (7.5, 9.0)	73.6
3"	3.55 t (9.0)	77.0
4"	4.05 t (9.0)	73.6
5"	3.39 m	76.2
6"a	3.83 dd (5.0, 12.0)	62.7
6"b	3.61 dd (3.5,12.0)	
galloy*l*1'''	-	122.0
2'''	7.07 s	110.0
3'''	-	147.8
4'''	-	139.6
5'''	7.07 s	147.8
6'''	-	110.0
COO	-	169.7

[a] Coupling pattern and coupling constants (*J* in Hertz) are in parentheses.

doublets at δ 6.46 and 6.33, *J* = 1.5 Hz) and a 4'-hydroxylation pattern for ring B (ABX system signals at δ 6.93, d, *J* = 8.0 Hz; 7.88, d, *J* = 8.0 Hz), allowing the aglycon to be recognized as kaempferol [6]. The ^{1}H NMR spectrum of **1** also showed signals ascribable to sugar moieties and a galloyl residue (Table 1). One anomeric proton arising from the sugar moiety appeared at δ 5.40 (1H, d, *J* = 7.5 Hz), which correlated with the signal at δ 102.0 in the HSQC spectrum. All the ^{1}H and ^{13}C NMR signals of **1** were assigned using 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. Complete assignments of proton and carbon chemical shifts of the sugar portion were accomplished by DQF-COSY and 1D-TOCSY experiments and allowed identifying the sugar as a β-glucopyranosyl unit.

The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilylated, and the GC retention times of each sugar were compared with those of authentic sugar samples prepared in the same manner. The lower field shifts of H-4" (δ 4.05) suggested the substitution pattern of a galloyl moiety. Unequivocal information could be obtained by the 2D-NMR spectra; the HMBC experiment indicated connections between δ 5.40 (H-1") and 134.2 (C-3); and δ 4.05 (H-4") and 169.7 (COO). Thus, the structure of **1** was determined as kaempferol 3-*O*-(4-galloyl)-β-D-glucopyranoside.

Table 2: ^{1}H- and ^{13}C- NMR spectroscopic data of compound **2** (CD_3OD, 600 MHz)[a].

position	δ_H	δ_C
2		81.8
3	3.47 t (9.0)	71.9
4	3.84 t (9.0)	75.5
4a	4.05	81.2
6	-	166.0
6a	-	119.7
7	7.11 s	111.0
8	-	149.5
9	-	152.3
10	-	142.3
10a	-	117.3
10b	4.99 d (10.0)	74.2
11a	4.08 dd (5.0, 12.0)	64.5
11b	4.24 dd (3.0, 12.0)	
OMe	3.94 s	60.9
caffeoyl		
1	-	125.0
2	6.70 d (1.8)	114.3
3	-	149.7
4	.	146.0
5	6.45 d (8.0)	116.5
6	6.68 dd (8.0, 1.8)	123.5
α	6.37 d (16.0)	115.0
β	7.42 d (16.0)	146.8
COO		169.0

[a] Coupling pattern and coupling constants (*J* in Hertz) are in parentheses.

The molecular formula $C_{23}H_{22}O_{12}$ for compound **2** was determined from the ESI-MS ion $[M-H]^-$ at *m/z* 489, and from the ^{13}C, ^{13}C-DEPT NMR and elemental analyses. The ^{1}H- and ^{13}C-NMR spectra (see Table 2) indicated that the compound was a bergenin derivative [3]. The ^{1}H NMR spectrum showed a low-field signal at δ 7.11 (1H, s) due to the aromatic proton of the bergenin nucleus [7]. All the ^{1}H and ^{13}C NMR signals of **2** were assigned using COSY, 1D-TOCSY, HSQC, and HMBC experiments. In particular, the COSY spectrum, together with the 1D-TOCSY experiments, allowed the identification of the spin system of a glucosyl unit.

The presence of one caffeoyl moiety was shown in the ^{1}H NMR spectrum by the signals at δ 6.70 (1H, d, *J* = 1.8 Hz), δ 6.68 (1H, dd, *J* = 8.0, 1.8 Hz), and δ 6.45 (1H, d, *J* = 8.0 Hz). The HSQC spectrum showed esterification shifts for C-11 (δ 64.5). An unambiguous determination of the sequence and linkage sites was obtained from HMBC experiment, which showed cross peak correlations between δ 4.08 (H-11) and 169.0 (C-1'), δ 4.99 (H-10b) and 142.3 (C-10), 119.7 (C-6a), 81.8 (C-2), and δ 4.05 (H-4a) and 71.9 (C-3), 117.3 (C-10a), and 166.0 (C-6).

Therefore, the structure 11-*O*-caffeoylbergenin was assigned to compound **2**.

Compounds **3-8** were identified by means of 1D- and 2D-NMR spectroscopy and ESI-MS analysis; a comparison of their data with those reported in the literature led to their identification as quercetin 3-*O*-β-D-glucopyranoside (**3**) [6], corilagin (**4**) [7], acalyphidin M (**5**) [8], glucogallin (**6**) [9], geraniin (**7**) [10], and bergenin (**8**) [11].

Preliminary *in vitro* biological analysis indicated that compounds **1**-**7** were able to quench DPPH radicals and exhibited a direct scavenging activity on the superoxide anion; this radical was in fact produced by the reduction of β-mercaptoethanol, excluding the Fenton-type reaction and the xanthine/xanthine oxidase system (Table 3).

Kaempferol 3-*O*-(4-galloyl)-β-D-glucopyranoside (**1**), 11-*0*-caffeoylbergenin (**2**), corilagin (**4**), acalyphidin M (**5**), and glucogallin (**6**) exhibited the highest antioxidant capacity. The biological activity of the kaempferol derivatives, corilagin, glucogallin and corilagin is reported in the literature [12-14].

Table 3: Scavenger effect on DPPH stable radical and superoxide anion of methanol fractions and compounds **1-8** isolated from *C. senegalensis*

Compound	DPPH test	Effect on $O_2^{-}\cdot$
	[a]IC_{50} (μg/μL) ± [b]SD	
A	253 ± 14	80.0 ± 4.3
B	209 ± 48	57.0 ± 1.3
C	110 ± 17	8.7 ± 0.5
D	53 ± 5	2.3 ± 0.04
E	88 ± 14	1.8 ± 0.5
F	61 ± 23	1.6 ± 0.05
G	6.4 ± 0.5	0.4 ± 0.03
1	3.7 ± 0.9	0.2 ± 0.04
2	2.6 ± 0.1	0.65 ± 0.03
3	12.9 ± 0.5	0.07 ± 0.01
4	4.8 ± 1.1	0.4 ± 0.01
5	1.15 ± 0.08	0.5 ± 0.01
6	2.15 ± 0.04	0.2 ± 0.01
7	6.8 ± 0.9	0.1 ± 0.03
8	168 ± 34	67.0 ± 0.03
[c]Trolox	96 ± 2	
[d]SOD		89 ± 15

[a]concentration that inhibited radicals by 50%. [b]n = 6.
[c]Trolox (50 μM) and [d]superoxide dismutase (SOD) (80 mU/mL) were used as standards; the results are expressed as % of inhibition.

Although both $O_2^{-}\cdot$ and H_2O_2 are potentially cytotoxic, most of the oxidative damage in biological systems is caused by the OH· radical, which is generated by the reaction between $O_2^{-}\cdot$ and H_2O_2 in the presence of transition metal ions [15]. In fact, the OH· radical can react with a number of target molecules, including proteins, membrane lipids, and DNA.

Table 4: Effect of methanol fractions and compounds **1-8** isolated from *S. virosa* (100 μg/mL) on DNA cleavage induced by the photolysis of H_2O_2 and metal chelating activity.

Compound	Supecoiled DNA (% of native DNA)	Ferrozine assay [a]IC_{50} (μg/μL) ± [b]SD
scDNA	100	–
A	10.0 ± 0.6*	–
B	14.0 ± 0.9*	–
C	20.0 ± 0.7*	–
D	46.7 ± 1.4*	–
E	46.7 ± 1.4*	–
F	55.0 ± 4.7*	114 ± 20
G	63.3 ± 1.6*	13 ± 3
1	63.3 ± 1.6*	7 ± 2
2	82.6 ± 7.5	7 ± 1
3	–	232 ± 20
4	52.5 ± 2.3*	85 ± 4
5	94.0 ± 3.6	13 ± 3
6	53.2 ± 5.2*	27 ± 3
7	97.1 ± 3.6*	32 ± 5
8	79.0 ± 2.6*	333 ± 4
[c]DMSO	73.2 ± 3.4*	
[d]DTPA		75.1 ± 2.1*

The hydroxyl radicals generated by the photolysis of H_2O_2 inhibited the supercoiled DNA (SCDNA). Each value represents the mean ± SD of 3 experiments. *Significant vs. supercoiled DNA ($p<0.001$).
[a]concentration that inhibited the ferrozine-Fe^{2+} formation by 50%.
[b]ν = 6. [c]DTPA (5 μM) and [d]DMSO (1mM) were used as standardS;
[c]the result is expressed as % inhibition.

Based on the data obtained from this study, compounds **1**, **2** and **6** might also be able to modulate hydroxyl radical formation more efficiently than other compounds acting as direct scavengers and chelating iron. These natural compounds exhibited a more efficient protection against DNA strand scission induced by OH· radicals generated by UV-photolysis of H_2O_2 (Table 4), and showed metal chelating activity capturing ferrous ions before ferrozine, with IC_{50} values (concentration that inhibited the ferrozine-Fe_{2+} by 50%) of 7.2, 7.3 and 27 μg/mL, respectively (Table 4). These data also suggest that the biological effect of *S. virosa* observed from ethnopharmacological studies is due in part to the antioxidant action of its active components.

Experimental

General experimental procedures: Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. Elemental analysis was obtained from a Carlo Erba 1106 elemental analyzer. UV spectra were recorded on a Perkin-Elmer-Lambda 12 spectrophotometer. A Bruker DRX-600 NMR spectrometer using the UXNMR software package was used for NMR experiments. ESI-MS (negative mode) were obtained using a Finnigan LC-Q Advantage Termoquest spectrometer, equipped with Xcalibur software. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany); compounds were detected by

spraying with $Ce(SO_4)_2/H_2SO_4$ (Sigma-Aldrich, St. Louis, Mo, USA) and NTS (Naturstoffe reagent)-PEG (Poliethylene glycol 4000) solutions. Column chromatography was performed over Sephadex LH-20 (Pharmacia); reversed-phase (RP) HPLC separations were conducted on a Waters 515 pumping system equipped with a Waters R401 refractive index detector and Waters U6K injector, using a C_{18} μ-Bondapak column (30 cm x 7.8 mm) and a mobile phase consisting of MeOH-H_2O mixtures at a flow rate of 2 mL/min. GC analyses were performed using a Dani GC 1000 instrument.

Plant material and chemicals: The leaves of *Securinega virosa* were collected in Bandiagara, Mali, in 2005 and identified by Prof. N'Golo Diarra of the Departement Medicine Traditionelle (DMT), Bamako, Mali where a voucher specimen is deposited.

pBR322 plasmid DNA, 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), and 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma Aldrich Co (St. Louis, USA); β-nicotinamide-adenine dinucleotide (NADH) was obtained from Boehringer Mannheim GmbH (Germany). All other chemicals were purchased from GIBCO BRL Life Technologies (Grand Island, NY, USA).

Extraction and isolation: The air-dried powdered leaves of *Securinega virosa* (300 g) were defatted with *n*-hexane and extracted successively by exhaustive maceration (3 x 1 L, for 48 h) with $CHCl_3$, $CHCl_3$-MeOH 9:1, and MeOH. The extracts were concentrated under reduced pressure to afford 7, 4, 6, and 10 g of dried residues, respectively. A portion of the MeOH extract (6 g) was partitioned between *n*-BuOH and H_2O to give a *n*-BuOH soluble portion (2.5 g); this residue was chromatographed over a Sephadex LH-20 column (100 cm x 5 cm) with MeOH as the eluent. A total of 95 fractions were collected (10 mL each). These were combined according to TLC analysis [silica 60 F_{254} gel-coated glass sheets with *n*-BuOH-AcOH-H_2O (60:15:25) and $CHCl_3$-MeOH-H_2O (40:9:1)] to give 8 pooled fractions (A-H). Fraction D (90 mg) was purified by RP-HPLC using MeOH-H_2O (1:1) to give compounds **1** (6 mg, t_R= 11 min) and **3** (5 mg, t_R= 13 min). Fraction E (58 mg) was purified by RP-HPLC using MeOH-H_2O (3:7) to give compounds **2** (7 mg, t_R= 12 min) and **8** (22 mg, t_R= 10 min). Fraction F (70.5 mg) was purified by RP-HPLC using MeOH-H_2O (25:75) to give compounds **6** (12.5 mg, t_R= 8 min) and **4** (10.8 mg, t_R= 11 min). Fraction H yielded compound **7** (29.2 mg). Fraction G (100 mg) was chromatographed over a Sephadex LH-20 column (25 cm x 2 cm) with MeOH as the eluent, yielding compound **5** (14.0 mg).

Kaempferol 3-*O*-(4-galloyl)-β-D-glucopyranoside (1)

Yellow amorphous powder.
$[\alpha]_D$: -27 (*c* 0.1, MeOH).
^{1}H NMR (600 MHz, CD_3OD): Table 1.
^{13}C NMR (600 MHz, CD_3OD): Table 1.
ESIMS: *m/z* 599 $[M - H]^-$.
Anal. Calcd for $C_{28}H_{24}O_{15}$: C, 56.01; H, 4.03. Found C, 54.49; H 4.05.

11-*O*-Caffeoylbergenin (2)

Yellow amorphous powder.
$[\alpha]_D$: -42.5(*c* 0.1, MeOH).
MP: 210-212ºC
^{1}H NMR (600 MHz, CD_3OD): Table 2.
^{13}C NMR (600 MHz, CD_3OD): Table 2.
ESIMS: *m/z* 489 $[M - H]^-$.
Anal. Calcd for $C_{23}H_{22}O_{12}$: C, 56.33; H, 4.52. Found C, 56.38; H 4.57.

Acid hydrolysis of compound 1: A solution of compound **1** (2.0 mg) in 1 N HCl (1 mL) was stirred at 80°C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60°C for 5 min. After drying the solution, the residue was partitioned between water and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a L-CP-Chirasil-Val column (0.32 mm x 25 m). Temperatures of the injector and detector were 200°C for both. A temperature gradient system was used for the oven, starting at 100°C for 1 min and increasing up to 180°C at a rate of 5°C/min. Peaks of the hydrolysate were detected by comparison with retention times of an authentic sample of D-glucose (Sigma Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine

Antioxidant activity in cell-free systems

Quenching of DPPH: The free radical-scavenging capacity of extracts, fractions and pure compounds was tested by their ability to bleach the stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) [16]. The reaction mixture contained 86 μM DPPH and

different concentrations of the natural compounds in 1 mL of ethanol. After 10 min at room temperature the absorbance at λ = 517 nm was recorded. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

Scavenger effect on superoxide anion: Superoxide anion was generated *in vitro* as described by Paoletti *et al*. [17]. The assay mixture contained, in a total volume of 1 mL, 100 mM triethanolamine-diethanolamine buffer, pH 7.4, 3 mM NADH, 25 mM/12.5 mM EDTA/$MnCl_2$, and 10 mM β-mercapto-ethanol; some samples contained the natural compounds at different concentrations. After 20 min incubation at 25°C, the decrease in absorbance was measured at λ = 340 nm. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

DNA cleavage induced by hydrogen peroxide UV-photolysis: The experiments were performed, as previously reported [18], in a volume of 20 μL containing 33 μM in bp of pBR322 plasmid DNA in 5 mM phosphate saline buffer (pH 7.4), and the natural compounds at different concentrations. Immediately prior to irradiating the samples with UV light, H_2O_2 was added to a final concentration of 2.5 mM. The reaction volumes were held in caps of polyethylene microcentrifuge tubes, placed directly on the surface of a transilluminator (8000 μW cm^{-1}) at 300 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 μL of a mixture, containing 0.25% bromophenol blue, 0.25% xylen cyanol FF, and 30% glycerol, were added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer (45 mM Tris-borate, 1 mM EDTA). Untreated pBR322 plasmid was included as a control in each run of gel electrophoresis, conducted at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 μg/mL; 30 min) and photographed on Polaroid-Type 667 positive land film. The intensity of each scDNA band was quantified by means of densitometry.

Metal chelating activity: The chelating of ferrous ions by fractions and pure compounds was estimated by the ferrozine assay [19]. Briefly, natural compounds were added to a solution of 0.15 mM $FeSO_4$. The reaction was initiated by the addition of 0.5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 mins. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The results were expressed as percentage inhibition of the ferrozine-Fe^{2+} complex formation. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

References

[1] Adjanohoun EJ, Aké Assi L, Floret JJ, Guindo S, Koumaré M, Ahyi AMR, Raynal J. (**1985**) Contribution aux études ethnobotaniques et floristiques au Mali. Médecine traditionnelle et pharmacopée. ACCT Ed, Paris, 250 p.

[2] Kerharo J, Adam JC. (**1974**) *Pharmacopée sénégalaise traditionnelle*: *Plantes médicinales et toxicologiques*. Paris Vigot et Frères Ed, 1011p.

[3] Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. (**1969**) Screening of Indian plants for biological activity. Part II. *Indian Journal of Experimental Biology*, ***7***, 250-262.

[4] Tatematsu H, Mori M, Yang TH, Chang JJ, Lee TTY, Lee KH. (**1991**) Cytotoxic principles of *Securinega virosa*: virosecurinine and viroallosecurinine and related derivatives. *Journal of Pharmaceutical Science*, ***80***, 325-327.

[5] Paris RR, Moyse H, Le Men J. (**1955**) Alkaloids from new Euphorbiaceae: *Flueggea virosa*. *Annales Pharmaceutiques Francaises*, ***13***, 245-250.

[6] Fico G, Braca A, De Tommasi N, Tomè F, Morelli I. (**2001**) Flavonoids from *Acontuym napellus* subsp. n*eomontanum. Phytochemistry*, ***57***, 543-546.

[7] Nawwar MAM, Hussein SAM, Merfort I. (**1994**) NMR spectral analysis of polyphenolics from *Punica granatum. Phytochemistry*, ***36***, 793-798.

[8] Liu Y, Cui C, Zhao M, Wang J, Luo W, Yang B, Jiang Y. (**2008**) Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. *Food Chemistry*, ***109***, 909-915.

[9] Kashiwada Y, Nonaka G, Nishioka I. (**1984**) Isolation and characterization of new dimeric and trimeric procyanidins. *Chemical & Pharmaceutical Bulletin*, ***32***, 3461-3470.

[10] Adesina SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO, Pias M. (**2000**) Antimicrobial constituents of the leavs of *Acalypha wilkesiana* and *Acalypha hispida*. *Phytotherapy Research*, ***14***, 371-374.

[11] Lee YY, Jang DS, Jin JL, Yun-Choi HS. (**2005**) Anti-platelet aggregating and anti-oxidative activities of 11-*O*-(4'-*O*-methylgalloyl)-bergenin a new compound isolated from *Crassula cv*. 'Himaturi'. *Planta Medica*, ***71***, 776-777.

[12] Bors W, Michel C. (**1999**) Antioxidant capacity of flavanols and gallate esters: pulse radiolysis studies. *Free Radical Biology & Medicine*, ***27***, 1413-1426.

[13] Kinoshita S, Inoue Y, Nakama S, Ichiba T, Aniya Y. (**2007**) Antioxidant and hepatoprotective actions of medicinal herb, *Terminalia catappa* L. from Okinawa Island and its tannin corilagin. *Phytomedicine*, ***14***, 755-762.

[14] Maldonado PD, Chánez-Cárdenas ME, Pedraza-Chaverrí J. (**2005**) Aged garlic extract, garlic powder extract, S-allylcysteine, diallyl sulfide and diallyl disulfide do not interfere with the antibiotic activity of gentamicin. *Phytotherapy Research*, ***19***, 252-254.

[15] Halliwell B, Gutteridge JM. (**1995**) The definition and measurement of antioxidants in biological systems. *Free Radical Biology & Medicine*, ***18***, 125-126.

[16] Latté KP, Kolodziej H. (**2004**) Antioxidant properties of phenolic compounds from *Pelargonium reniforme*. *Journal of Agricultural and Food Chemistry*, ***52***, 4899-4902.

[17] Paoletti F, Aldinucci D, Mocalli A, Caparrini A. (**1986**) A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Analytical Biochemistry*, ***154***, 536-541.

[18] Russo A, Acquaviva R, Campisi A, Sorrenti V, Di Giacomo C, Virgata G. (**2000**) Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell Biology and Toxicology*, ***16***, 91-98.

[19] McGown EL, Rusnak MG, Lewis CM, Tillotson JA. (**1982**) Tissue ascorbic acid analysis using ferrozine compared with the dinitrophenylhydrazine method. *Analytical Biochemistry*, ***119***, 55-61.

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