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Isolation of (+)-catechin and (-)-epicatechin from the leaves of *Amaranthus cruentus* L. (Amaranthaceae)

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Abstract
The methanolic and ethyl acetate extracts from the leaves of *A. cruentus* L. were investigated for highlight its bioactive components. This study led to the isolation and identification of two polyphenols namely (+)-catechin and (-)-epicatechin which recognized biologically important. Their structures were elucidated thanks to their spectral data (NMR, MS, IR and UV). The presence of these two compounds in extracts of *A. cruentus* L. showed that the consumption of its leaves have a biological interest on human health.

Keywords: *Amaranthus cruentus,* Amaranthaceae, (+)-catechin and (-)-epicatechin, biological interest.

1. Introduction
The genus *Amaranthus* is an ancient crop belonging to the Amaranthaceae family with origin in the Americas. Current interests in *Amaranthus* reside in the great amount of genetic diversity, phenotypic plasticity and its extreme adaptability to adverse growing conditions (Rastogi & Shuckla, 2013) [18]. It is known as a high-protein crop that is being broadly introduced into America, Europe and Southeast Asia. *Amaranthus* comprises over 70 recognized species (Espitia-Rangel *et al*., 2012; Kena *et al*., 2014) [6, 10]. Three of these species (*Amaranthus cruentus*, *A. caudatus* and *A. hypochondriacus*) are predominantly used for grain production (Huerta-Ocampo & Barba de la Rosa, 2011) [8]. *Amaranthus cruentus* L. is an annual pseudo-cereal with broad leaves and is known for its nutritional value. It is used as a leafy vegetable and a forage crop. The promotion and consumption of indigenous vegetables is very important when considering the increase in the human population, food insecurity and alleviation of malnutrition in developing countries. Concerning its chemical composition, pectinic polysaccharides were isolated and characterized from the aerial part of *A. cruentus* L. (Minzanova *et al*., 2014) [14]. The grain of this species has high protein, as well as a high fat content. There is a potential to use it as an energy food. The balance of carbohydrates, fats and protein, allow amaranth the opportunity to achieve a balanced nutrient uptake with lower amounts of consumption than with other cereals (Morales *et al*., 1988) [15].
They are currently being studied as a source of bioactive compounds, including fibers and proteins that present hypocholesterolemic, anti-hypertensive and antioxidant functions, amongst others (Mendonça *et al*., 2009; Tiengo *et al*., 2009) [12, 22].

2. Material and methods
2.1 General
The NMR spectra were recorded on a Brüker Advance-300 operating at 300 MHz, using TMS as internal standard. Chemical shifts were quoted in δ ppm and coupling constant *J* was measured in Hertz (Hz). One-dimensional ^{1}H and ^{13}C spectra were acquired under standard conditions. Currently, ^{1}H–^{1}H homonuclear (COSY, NOESY) and ^{1}H–^{13}C heteronuclear (HSQC, HMBC) correlation techniques were routinely applied in field of constitutional analysis. These techniques were recorded on a Brücker Avance-400 operating at 400 MHz. Column chromatography was performed on silica gel (Kieselgel 60, particle size 0.040–0.063 mm) and Sephadex® LH-20. TLC was run on silica gel precoated glass plates (Merck silica gel 60 F_{254}). Spots were detected by spraying with phosphomolibdic acid. This operation was

followed by a heating. ESIMS were obtained with ITQ 900 spectrometer using an Agilent DB-5HT (30 x 0.32 x 0.1) column. IR spectra were recorded with a PerkinElmer type 257 spectrometer.

2.2. Plant material

The leaves of *A. cruentus* L. (Amaranthaceae) were collected in February 2017 in Korhogo (north of Côte d'Ivoire). They were identified by Pr. Ipou Ipou Joseph (Centre National de Floristique, Université Félix Houphouët-Boigny).

Isolation

Air-dried pulverized leaves of *Amaranthus cruentus* L. (300 g) were repeatedly defatted with petroleum ether, and then successively extracted with CH_2Cl_2 and MeOH. After evaporation to dryness under reduced pressure, three crud extracts were obtained: the petroleum ether (9.7 g), CH_2Cl_2 (6.4 g), AcOEt (3.2 g) and MeOH (2.8 g). The methanol extract (2.8 g) was chromatographed over silica gel column chromatography. Elution was performed with the system cyclohexane-dichloromethane and dichloromethane-methanol, according to an increasing gradient of polarity to give eight fractions (F-1 to F-8). Fraction F-6 was purified using repeated Sephadex®LH-20 [MeOH/CH_2Cl_2 (1/2)] then column chromatography on silica gel using dichloromethane-methanol gradient, to yield 7.9 mg of compound 1 (Fig. 1).

The ethyl acetate extract (3.2g) was chromatographed over silica gel column chromatography, eluting with cyclohexane-dichloromethane gradient and dichloromethane-methanol systems to give ten fractions (F-1 to F-10). Fraction F-4 was purified using successive Sephadex®LH-20 [MeOH/CH_2Cl_2 (1/1)] and column chromatography on silica gel using dichloromethane-methanol gradient to yield 6.4 mg of compound 2 (Fig. 1).

The structures of these compounds (Fig. 1) were established according to their spectral data (NMR, IR and MS).

2.3 Identification of compounds 1 and 2

(+)-catechin (**1**): yellow amorphous solid; mp: 175-176°C; $[\alpha]^{15}{}_{D}$ +11.8 (c 1.00 g/ml, MeOH, 589 nm); ^1H and ^{13}C NMR (400 MHz) data in table 1; IR ($CHCl_3$): υ_{max} (cm^{-1}) = 3255, 2926, 1626, 1521, 1240, 1080, 820; UV (MeOH): λ_{max} (nm) = 277; 222; ESI-MS (*m/z*): 291 $[M+H]^+$
(Molecular formula $C_{15}H_{14}O_6$)

(-)-epicatechin (**2**): milky white amorphous powder; mp: 238-240°C; $[\alpha]^{15}{}_{D}$ -56.8 (c 0.33 g/ml, MeOH, 589 nm); ^1H and ^{13}C NMR (400 MHz) data in table 1; IR ($CHCl_3$): υ_{max} (cm^{-1}) = 3293, 2910, 1607; UV (MeOH): λ_{max} (nm) = 278; 230; ESI-MS (*m/z*): 291 $[M+H]^+$ (molecular formula $C_{15}H_{14}O_6$).

3. Results and discussion

Compound 1 (Fig. 1) was obtained as yellow amorphous solid. The ESI-MS showed a peak at *m/z* 291 $[M+H]^+$ corresponding to the molecular formula $C_{15}H_{14}O_6$. The IR absorption band at 3255 cm^{-1} belongs to OH stretching of phenol, those at 2926 and 1626 cm^{-1} were assigned to saturated C-H stretching and aromatic C=C stretching, respectively. The calculated 9 degrees of unsaturation were attributed to six C=C aromatics bonds and three rings with two aromatic rings. The ^1H and ^{13}C NMR spectra of compound 1 (Table 1) closely resembled those of flavanols (Rayar *et al*., 2016) [19]. The ^{13}C NMR spectrum (Table 1) revealed the presence of 15 carbons signals, among which: one methylene, seven methine and seven quaternary carbons. Assignement were done as follows: δ = 82.9 (C-2), 68.1 (C-3), 29.3 (C-4), 157.1 (C-5), 96.5 (C-6), 158.1 (C-7), 95.4 (C-8), 158.5 (C-9), 100.8 (C-10), 132.0 (C-1'), 115.8 (C-2'), 146.9 (C-3'), 146.8 (C-4'), 116.2 (C-5') and 119.5 (C-6'). The ^1H NMR spectrum (Table 1) showed one methylene group (δ_H 3.31, H-4a; δ_H 3.67, H-4b) and seven methine groups [δ_H 5.18 (H-2), 4.59 (H-3), 6.64 (H-6), 6.67 (H-8), 7.64 (H-2'), 7.24 (H-5') and 7.20 (H-6')].

The proton H-3 (δ_H 4.59 ppm) was split by H-2 (δ_H 5.18 ppm) and H-4a (δ_H 3.31 ppm). The protons at H-4, were split by H-3, giving a doublet of doublet at δ_H 3.31 and δ_H 3.67. The position of the H-2 (δ_H 5.18) suggests that the flavan structure possesses the correct cis-2,3 stereochemistry.

The distinctive signals of the B-ring aromatic protons are of the ABX-type. The basic structure of this compound was therefore deduced as 3, 3', 4', 5, 7-pentahydroxyflavan.

The ^1H-^1H COSY correlations showed coupling between H-2 and H-3, and also between H-3 and H-4. These positions were further confirmed by long-range coupling observed in the HMBC. In HMBC spectrum, correlations between the proton at δ_H 4.59 (H-3) to the carbon at δ_C 132.0 (C-1') and 100.8 (C-10) corroborated the attachment of the hydroxyl group to C-3. The methine and methylene groups were assigned on the basis on HSQC and COSY analysis. Stereochemistry at position C-2 and C-3 was determined by the positive value of optical rotation data. It showed that the protons H-2 and H-3 were respectively in β and α-orientations. The NMR data (Table 1) thus showed signals typical of and it is comparable to the ^1H and ^{13}C NMR data reported in the literature (Michael *et al*., 2007) [13].

To the best of our knowledge, compound 1 was (+)-catechin. This compound was isolated for the first time in the leaves of *A. cruentus* L. (Amaranthaceae). (+)-catechin was already isolated from *Annona reticulata Linn* fruit (Rayar & Manivannan, 2016) [19]. It is a substance, which diminish or arrest the action of some hormones; thus "tyronorman" injected with thyroxin suppresses the rise in basal metabolism. The tanning property of catechin in human skin may be supposed to be the active ingredient for the treatment of leucoderma (shiti). Catechin has antihormone activity. Further its activity has also been correlated with those of vitamin P (Hye *et al*., 2009) [9].

Compound 2 was obtained as milky white amorphous powder. The ESI-MS of 2 showed a peak at *m/z* 291 $[M+H]^+$ corresponding to the formula $C_{15}H_{14}O_6$.

The stereochemistry of this compound was proposed on the basis of optical rotation measurements, in comparison with the literature. The ^1H and ^{13}C NMR spectra of 2 (Table 1) were almost similar to those of compound 1 (Rayar *et al*., 2016) [19]. The difference between compound 1 and compound 2 were the value of their optical rotation measurements. Optical rotation data obtained in MeOH being negative for 2 and positive for 1

Compound 2 was thus identified as (-)-epicatechin (Vogel *et al*., 1967; Nikkon *et al*., 1995; Aher *et al*., 2010) [23, 16]. Its physical and spectral data are consistent to those reported by literature (Byeng Wha Son *et al*., 1989; Rayar A. *et al*., 2016) [4, 19]. The (-)-epicatechin is isolated here for the first time in the leave of *A. cruentus* L. (Amaranthaceae). But, it was previously isolated from the leaves and wood of *Acacia catechu*, apple peels, mango kernels and pear skin (Abdullahi U., *et al*., 2016) [2].

The flavanol (−)-epicatechin enhances exercise capacity in mice and Epi-rich cocoa improves skeletal muscle structure in

heart failure patients. It may thus, hold promise as treatment for sarcopenia (Gabriela *et al*., 2017). It has been isolated from the leaves and wood of *Acacia catechu*, apple peels, mango kernels and pear skin (Marek *et al*., 2010; Shen *et al*., 2006; Sunday, 2000) [11, 20, 21]. It has also been reported to showed antioxidant activity, good antilisterial activity at IC_{50} greater than 200 µg/mg and antimicrobial activity at MIC value greater than 500 µg/ml (Abdullahi *et al*., 2016) [2]. Epicatechin is one of the many abundant flavonoids in nature. The high safety margin of epicatechin contributed to its therapeutic successes in the management of diabetes and cancer. However, its exact mechanism of action in these two conditions is still being explored (Abdulkhaleq *et al*., 2017) [1].

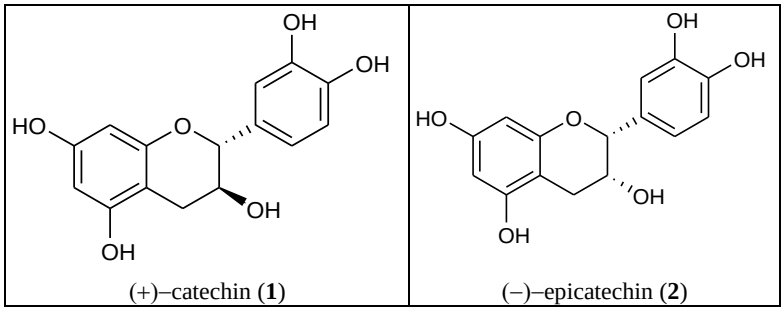

Fig 1: Structures of compounds 1 and 2.

Table 1: ^{1}H and ^{13}C NMR spectral data of (+)−catechin (1) in DMSO and (−)−epicatechin (2) in CD_3OD

Position	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
2	82.9	5.18 (1H,*d*, J = 7.4 Hz)	79.9	4.83 (br,s)
3	68.1	4.59 (1H, *ddd*, J = 5.5, 7.5; 8.5 Hz)	67.5	4.19 (1H, m)
4a	29.3	3.31 (1H, *dd*, J = 8.5, 16.0 Hz)	29.3	2.86 (1H, *dd*, J = 4.8, 16.80 Hz)
4b	29.3	3.67 (1H, *dd*, J = 5.5, 16.0 Hz)	29.3	2.73 (1H, *dd*, J = 2.7, 16.80 Hz)
5	157.1		157.4	
6	96.5	6.64 (1H,*d*, J = 2.0 Hz)	96.4	5.93 (1H,*d*, J = 2.3 Hz)
7	158.1		157.9	
8	95.4	6.67 (1H,*d*, J = 2.0 Hz)	95.9	5.96 (1H,*d*, J = 2.3 Hz)
9	158.5		157.7	
10	100.8		100.1	
1’	132.0		132.3	
2’	115.8	7.64 (1H,*d*, J = 1.8Hz)	115.3	6.99 (1H,*d*, J = 1.7 Hz)
3’	146.9		145.9	
4’	146.8		145.8	
5’	116.2	7.24 (1H,*d*, J = 8.0 Hz)	115.9	6.77 (1H,*d*, J = 8.2 Hz)
6’	119.5	7.20 (1H, *dd*, J = 1.8, 8.0 Hz)	119.4	6.81 (1H, *dd*, J = 1.7, 8.2 Hz)

Conclusion

The phytochemical examination of the leaves from *Amaranthus cruentus* L. (Amaranthaceae) was effectively carried out. From their chemical evidences and spectral data, (+)-catechin (1) and (-)-epicatechin (2) had been isolated and identified for the first time in this species. Their structures are in agreement with those reported by literature.

The presence of these two compounds in extracts of *A. cruentus* L. showed that the consumption of its leaves have a biological interest on human health.

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