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Phytochemical screening and antioxidant activity of leaves of *Amaranthus hybridus* L., *Corchorus olitorius* L and *Hibiscus sabdariffa* L. grown in northern of Côte d'Ivoire

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Abstract

Our work was based on the study of aqueous and hydroethanol extracts from the leaves of *Amaranthus hybridus* L. (Amaranthaceae), *Corchorus olitorius* L. (Tiliaceae) and *Hibiscus sabdariffa* L. (Malvaceae), and decoction. This work presents for the first time the phytochemical screening and the antioxidant activity of extracts of leaves of these three plants coming from the Côte d'Ivoire. Phytochemical analysis of these extracts revealed the presence of polyphenols and alkaloids in the three leaves. The total phenolic content of the extracts showed variations, between 13.22 and 90.89 mg GAE/g. The hydroethanol extract (90.89 mg GAE/g) and decoction of *C. olitorius* (57.89 mg GAE/g) had the most elevated contents. The evaluation of the reducing power with DPPH method revealed that the hydroethanol extract of *C. olitorius* was the most active (IC₅₀ = 45.58 µg/mL). This result was in agreement with that obtained by FRAP method.

Keywords: *Amaranthus hybridus*; *Corchorus olitorius*; *Hibiscus sabdariffa*; Antioxidant activity; Phytochemical

1. Introduction

Green leafy vegetables are rich sources of proteins, vitamins, dietary fiber and minerals. The consumption of vegetables has increased considerably in recent years mainly due to their positive association with the prevention of varied chronic diseases. Nonetheless, the diets of poor people in most developing countries are predominantly cereal-based with minimal vegetable content. Hence, increased consumption of affordable and available indigenous leafy vegetable is a practical and sustainable approach for solving malnutrition problems in poor communities. Studies showed that vegetables display a wide range of bioactivities including antidiabetic, antihypertensive, antitumor, anti-inflammatory and hypoglycemic properties. Most of these pharmaceutical, health and nutraceuticals benefits have been attributed to the presence of bioactive compounds which include ascorbic acid, carotenoids and phenolics [1-5].

Ethnobotanical studies revealed that most people in Côte d'Ivoire consume indigenous green leafy such as *Amaranthus hybridus* L. (Amaranthaceae), *Corchorus olitorius* L. (Tiliaceae), *Hibiscus sabdariffa* L. (Malvaceae) [6-8]. These leafy-

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green vegetables are more used as culinary ingredients than for their therapeutic properties because of lack of sufficient scientific data about them with regard to chemical groups and their biological activities.

The aim of this study was to determine phytochemical constituents of leaves of *Amaranthus hybridus* L. (Amaranthaceae), *Corchorus olitorius* L. (Tiliaceae) and *Hibiscus sabdariffa* L. (Malvaceae), three culinary plants grown in Korhogo (North of Côte d'Ivoire). Total phenolic content (TPC) was determined by the Folin–Ciocalteu method. The antioxidant activities of the vegetables were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and the Ferric reducing ability of plasma (FRAP) assays.

2. Material and methods

2.1. Plant material

Leaves from *Amaranthus hybridus* L. (Amaranthaceae), *Corchorus olitorius* L. (Tiliaceae) and *Hibiscus sabdariffa* L. (Malvaceae) were collected in February 2019 in Korhogo (North of Côte d'Ivoire) and authenticated by Pr Ipou Ipou Joseph. Voucher specimens (n°10 KABLAN AH-2019, n°11 KABLAN CO-2019 and n°11 KABLAN HS-2019) were deposited in the herbarium of Centre National de Floristique (CNF), Université Félix Houphouët-Boigny, Abidjan (Côte d'Ivoire).

2.2. Preparation of leaves extracts

The extraction method used is the same as that reported by Touré *et al.*, 2020 [8]. The leaves were separately washed, cut up and dried shelter from sun light for two weeks. Each species of dried leaf was reduced to powder using a mechanical grinder. The powder was sieved with 2 mm of particle size before stored in an air tight container. The fine powders were used for preparation of different extracts. For powder of each species of leaf, three extracts were prepared (aqueous, hydroethanol and decoction). The aqueous extract (Aq) of each leaf was obtained by mixing 100 g of powder in 1 L of distilled water for 24 hours with constant stirring at 25°C; the mixture was filtrated on hydrophilic cotton and the filtrate was dried in oven at 50 °C during 48 hours. For hydroethanol extract (Et/W) of each plant, 100 g of powder were stirred in 1 L of mixture containing ethanol (70%) and water (30%) for 24 hours. The hydroethanol mixture was filtrated on hydrophilic cotton and the filtrate was dried in oven at 50 °C during 48 hours. The decocted extract (D) was prepared with 50 g of powder of each leaf dissolved in 1 L of distilled water before boiling the mixture for 1 hour. The boiling mixture was then filtrated, and the filtrate dried in oven at 50 °C for 48 hours. After the different extractions, the yields (R) were calculated according to the following formula:

$$R (\%) = (m / M) \times 100$$

M: mass of the vegetable powder (g); m: mass of the crude extract (g).

2.3. Phytochemical screening of extracts

2.3.1. Thin Layer Chromatography TLC

A TLC aluminum plate, containing the silica gel (stationary phase) of size 20 cm x 20 cm, was cut in small TLC plates with size 7 cm x 5 cm. Starting and front lines were then drawn on each small plate. The TLC plate was spotted with a few drops of each leaf extract on starting line using capillary tubes before air drying these drops. The mobile phase (eluent) composed by three solvents was a mixture with same proportions of methanol, ethyl acetate and dichloromethane. The TLC plate with different extracts was put in a chromatographic tank containing the eluent. Once the eluent reaches the front line, the TLC plate is removed from the tank, air dried and exposed. The separated spots are surrounded with a pencil and observed in visible light first, then to a revealing reagent "vanillin" composed by vanillin (1 g), methanol (100 mL) and sulfuric acid (2 mL). The chromatograms are then examined under UV/Visible and the bands observed were surrounded. The colors of bands on chromatographic plate were observed with an ultraviolet lamp and the front was used to calculate the frontal ratio (R_f) according to the formula:

$$R_f = (\text{Distance of substance}) / (\text{Distance of solvent front}).$$

A high frontal ratio of 0.3–0.5, will characterize the apolar compounds, 0.2–0.4 for average polar compounds and 0.1–0.2 for polar compounds.

2.3.2. Secondary metabolites tests

Alkaloids

After migration and air dried of particles of different extracts, chromatographic plates were impregnated with Dragendorff reagent. The presence of orange stain in the plate revealed that the extract contains alkaloids [9-11].

Polyphenols

After migration of particles, the chromatographic plates for polyphenols test were treated with 2 mL of ferric chloride (2 %). The appearance of blue-blackish or green coloration more or less dark showed the presence of phenolic compounds in tested extract [11].

Flavonoids

Flavonoids were revealed with Godin's reagent on chromatographic plates. The appearance of yellow, pink and orange staining at 365 nm indicates the presence of flavonoids [11].

Saponins

The various extracts were taken up in 5 mL of distilled water, then introduced into a test tube and stirred vigorously. The formation of a foam (height 1 cm) stable for 15 minutes, revealed the presence of saponins [11].

Terpenes

2 g of powder of leaf were mixed in 20 mL of hexane for 24 hours. The mixture was filtered, and 10 mL of filtrate were put in crucible to evaporate solvent. The evaporated extract was taken up with 1 mL of anhydride acetic acid and 1 mL of chloroform before putting this mixture in test tube. A volume of 1 to 2 mL of sulfuric acid was added to the contents of tube. The appearance of a brown-red or violet ring in the test tube reveals the presence of terpenes in extract [11].

Cardiotonic glycosides

To 5 g of each extract in test tube was added 2 mL of glacial acetic acid containing a drop of a ferric chloride solution. 1 mL of concentrated sulfuric acid was then added to the whole. Cardiotonic glycosides in extract were revealed by a brown ring observed in test tube characteristic of a desoxy sugar of cardenolides. In addition, a purple ring appears below the brown ring, while a greenish ring is gradually formed in the acetic acid phase [11].

2.4. Determination of total polyphenols

Total polyphenols of the methanol extracts of the different leaves were determined by the Folin-Ciocalteu (0.5N) colorimetric method [12]. To 0.5 mL of each extract diluted (1/100) were added, 2 mL of a solution of sodium carbonate and 2.5 mL of Folin-Ciocalteu reagent. The whole was incubated at 50 ° C for 15 minutes in a water bath before reading the absorbance at 517 nm against a reference tube (0.5 mL of 100% methanol). The total phenolic compounds were quantified with a linear calibration line ($y = ax + b$) of gallic acid (standard) at different concentrations (0.05 to 0.15 µg/mL) under the same conditions as the sample. The following formula was used to calculate contents of total phenolic compounds (Q) expressed with gallic acid equivalent (GAE) per dry matter of extract used.

$$Q = (V \times C \times d) / m$$

V: volume of crude extract (mL),

C: average concentration (µg/mL), d: dilution factor,

m: mass of sprayed dry matter (g).

2.5. Evaluation of antioxidant activity of extracts

Extracts that showed high polyphenol contents were used to evaluate their antioxidant potencies with methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and reducing power of iron (FRAP).

2.5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

In vitro antiradical activity of extracts was performed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test according to the method of Parejo *et al.* (2002) [13] with some modifications. 2 mL of methanol solution of DPPH (100 µM) was mixed with 0.5 mL of different concentrations (0-200 µg/mL) of the extracts and the standard (vitamin C). The mixture was

then kept in the dark at 25°C for 15 minutes before measuring the absorbance with spectrophotometer at 517 nm. The percentage of inhibition (PI) is calculated according to the formula below:

$$PI = [(A_0 - A_1) / A_0] \times 100$$

PI (%): Percentage of inhibition;

A₀: absorbance of DPPH solution without extract (white); A₁: absorbance of DPPH solution with extract (test). The concentrations of extracts or vitamin C for 50 % of inhibition of the DPPH radicals were determined by graph representing (% Inhibition DPPH = f [extracts]).

2.5.2. Ferric Reducing Antioxidant Power (FRAP) of extracts

The FRAP of the extracts were carried out according to the method described by Pulido *et al.* (2000) [14]. A fresh solution of FRAP reagent (10 mM) was prepared by mixing 2.5 mL of the ferric tripyridyltriazine solution (10 mM in 40 mM HCl) with 2.5 mL of hydrated iron chloride (20 mM) and 25 mL of acetate buffer (300 mM sodium acetate, pH 1 to 3.6 with acetic acid). To 140 µL of test compounds dissolved in methanol, 3500 µL of the FRAP reagent were added. After 30 min of incubation of these mixtures in dark, the absorbance was read at 593 nm with the Trolox as control. A calibration graphic was made with the following concentrations of Trolox: 1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL [8].

2.6. Statistical analysis

The statistical analysis of the results was performed using the Graph Pad Prism 7.0 software for multiple variances analysis (ANOVA). The differences between the means were determined according to the Newman-Keuls test at the 5% threshold (P <0.05 is considered significant). The results were expressed as averages with the standard error on the mean (mean ± SEM).

3. Results

3.1. Extraction yields

The extraction yields recorded in Table 1 showed that among solvents used, the hydroethanol mixture gave the highest rates of extraction between 10.40 to 14.80 %. The two other extractions (aqueous and decoction) revealed a low extraction efficiency. However, cold extraction (25°C) with water showed higher yields (6.81 to 7.87 %) than decocted extraction (50°C) which rates varied from 3.42 to 6.22 %. In addition, the leaves of *H. sabdariffa* gave the best yields (6.22 to 14.80 %) whatever extraction method. Hydroethanol and aqueous extractions of *A. hybridus* showed respectively intermediate yields of 6.81 and 10.40%. Decocted extraction of *A. hybridus* (3.42%), *C. olerius* (4.36 %) and *H. sabdariffa* (6.22 %) furnished the lowest yields.

Table 1 Extraction yields of different extracts from leafy vegetables

Leafy vegetables	Extracts	Extraction yields (%)
<i>A. hybridus</i> (AH)	Et/WAH	10.40
	AqAH	6.81
	DAH	3.42
<i>C. olerius</i> (CO)	Et/WCO	11.75
	AqCO	6.13
	DCO	4.36
<i>H. sabdariffa</i> (HS)	Et/WHS	14.80
	AqHS	7.87
	DHS	6.22

Decocted extract (D) Aqueous extract (Aq) Hydroethanol extract (Et/W)

3.1.1. Phytochemical screening

Phytochemical screening revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins and polyphenols (Table 2).

Alkaloids were present in all extracts of the three vegetables leaves studied. The polyphenol compounds were present in the decocted extracts analyzed but only the hydroethanol and aqueous extracts of the three vegetables contained them in abundance. The presence and abundance of flavonoids were respectively observed in hydroethanol extracts of *A. hybridus*, *C. olerius* and *H. sabdariffa*. The saponins were revealed only in decocted and aqueous extracts of *A. hybridus*. Cardiotonic glycosides and terpenes were absent in all extracts of leaves studied.

Table 2 Phytochemical screening of different extracts of vegetables leaves

Secondary metabolites	Extracts of leafy vegetables								
	<i>A. hybridus</i> (AH)			<i>C. olerius</i> (CO)			<i>H. sabdariffa</i> (HS)		
	DAH	AqAH	Et/WAH	DCO	AqCO	Et/WCO	DHS	AqHS	Et/WHS
Alkaloids	+++	+	+	±	±	±	±	±	±
Polyphenols	+	++	++	+	++	++	+	++	++
Flavonoids	-	-	++	-	-	+	-	-	+
Cardiotonic glycosides	-	-	-	-	-	-	-	-	-
Terpenes	-	-	-	-	-	-	-	-	-
Saponins	++	++	-	-	-	-	-	-	-

Abundance of compounds (++), Presence of compounds (+), Presence of compounds in small quantities (±), Absence of compounds (-), Decocted extract (D) Aqueous extract (Aq) Hydroethanol extract (Et/W)

3.1.2. Total polyphenols compounds of extracts

Concentrations of polyphenolic compounds of the different extracts analyzed are shown in Figure 1. These results reveal variable contents of total polyphenols according to the extracts of the leaves. The highest concentrations of polyphenols were observed with the hydroethanol extract of *C. olerius* (90.89 ± 4.6 mg GAE/g) followed by the decocted extract of *C. olerius* (57.89 ± 2.6 mg GAE/g) and the hydroethanolic extract of *A. hybridus* (21.37 ± 0.1 mg GAE/g). The lowest presence of polyphenols was revealed with decocted extract of *A. hybridus* (13.32 ± 3.8 mg GAE/g). In view of the above, the hydroethanol extract and the decoction of *C. olerius* showed the best levels of total polyphenols. In addition, hydroethanol extraction and decoction gave the best yields extractions of total polyphenols.

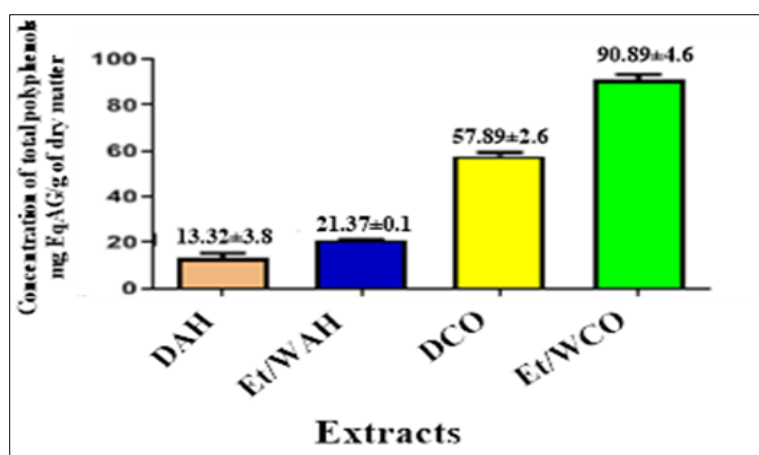


Figure 1 Concentrations of total polyphenols of different vegetables leaves studied

3.1.3. Antioxidant activities of extracts of leaf vegetables

Inhibition power of extracts on DPPH radical

Inhibition powers of hydroethanol and decocted extracts of *A. hybridus* and *C. olerius* comparatively to vitamin C on DPPH radical are presented in Figure 2. These antiradical activities were increased with concentrations of extracts and vitamin C. The highest antiradical activities were recorded for vitamin C and hydroethanol extract of *C. olerius* with respectively IC_{50} of 8.09 and 45.58 $\mu\text{g/mL}$. The other extracts, hydroethanol extract of *A. hybridus* and decocted extracts of *C. olerius* and *A. hybridus* exhibited the lowest antiradical powers.

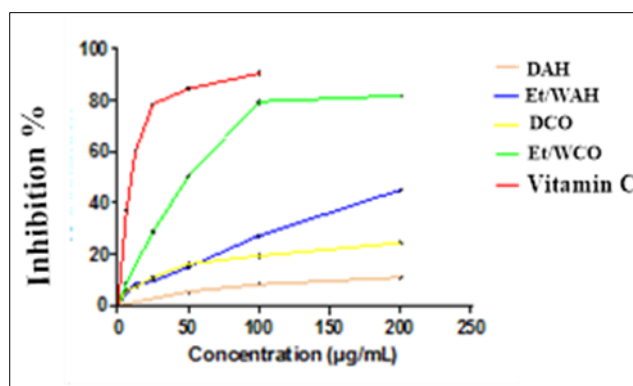


Figure 2 Inhibition of radical DPPH (%) by extracts of vegetables leaves and vitamin C

Ferric Reducing Antioxidant Power (FRAP) of extracts

The results of FRAP of hydroethanol and decoction extracts of *A. hybridus* and *C. olerius* are recorded in Figure 3. The hydroethanol extract of *C. olerius* gave the highest ($p < 0.05$) reducing power ($73.73 \pm 3.08 \mu\text{mol TE/g}$) followed by the decocted extract of the same plant ($27.88 \pm 1.2 \mu\text{mol TE/g}$). Comparatively to the two above extracts, hydroethanol and decocted extracts of *A. hybridus* possessed the lowest antioxidant activities with respectively reducing power of 11.16 ± 1.9 and $3.73 \pm 1.1 \mu\text{mol TE/g}$.

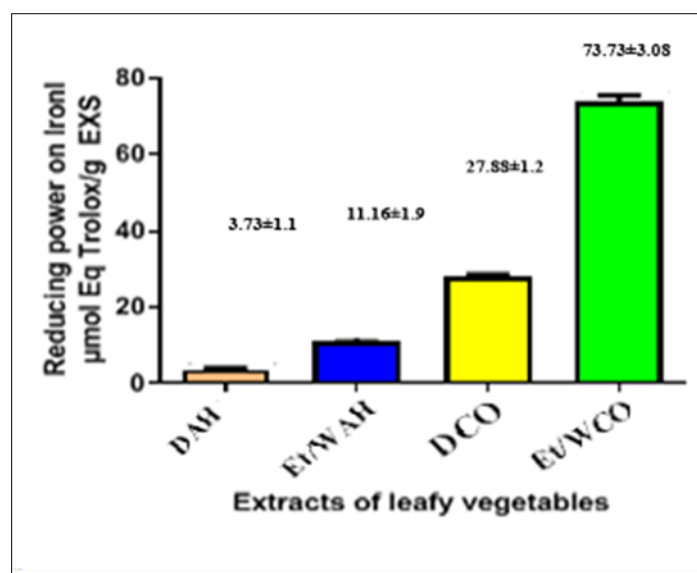


Figure 3 Ferric Reducing power of extracts of leafy vegetables

4. Discussion

Study of the phytochemical compounds of leaves of *A. hybridus*, *C. olerius* and *H. sabdariffa* allowed to determine the extraction yields of different extracts according to solvents and methods used. Analysis of the results shows that the extraction solvent and the process have an influence on the extraction yield and the content in secondary metabolites

of the extracts studied. Also, the yield of the hydroethanol extraction of each leafy vegetable was higher than that of extraction with water (aqueous and decocted extractions). *H. sabdariffa* provided more dry matter than all the three extracts (6.22%; 7.87%; 14.80%). This work is in accordance with that of Grabsi *et al.* (2016) [15] which showed an interesting yield for the species *H. sabdariffa* with alcoholic solvent. However, the species *A. hybridus* gave lower yields compared to those of *H. sabdariffa* (3.42%; 6.81%; 10.40%). According to Akubugwo *et al.* (2007) [1], *A. hybridus* provides a considerable amount of dry matter. The hydroethanolic extracts of all species have the best yields. The hydroethanolic extract of *C. olerius* is better than the same extracts of the two other plants, namely *Amaranthus hybridus* L. and *Hibiscus sabdariffa* L.

The absence of dry matter for the aqueous extract should be noted. This is due to the fact that the different extractions hardly provided any dry matter due to the sticky nature of the plant when in contact with a liquid (water). For the other two solvents used, we were able to obtain a yield of 4.36% for the decocted and 11.75% for the ethanol/ water mixture. These results are in agreement with those obtained by Benalbid *et al.* (2017) [16] which indicated a low yield for leaves of *C. olerius*.

Phytochemical screening of extracts from leaves of *A. hybridus*, *C. olerius* and *H. sabdariffa* showed the absence of cardiotoxic glycosides and terpenes. These results corroborate the works of Mibei *et al.* (2012) and Tangara (2013) [17, 18], which showed the absence of cardiotoxic glycosides and terpenes in decoction and infusion of *C. olerius*. The polyphenols are present in variable proportions in the decocted, aqueous and hydroethanolic extracts analyzed. Their presence in *A. hybridus*, *C. olerius* and *H. sabdariffa* was also observed in study conducted by Akubugwo *et al.* (2007) [1]. In addition, the presence of flavonoids in the various extracts of this study was confirmed by the work of Gardner *et al.* (2000) [19], which indicated the presence of flavonoids such as myricetin, quercetin, kaempferol, isorhamnetin and luteolin in leafy vegetables. However, the presence and abundance of saponins in the aqueous and decocted extracts of *A. hybridus* was confirmed with the findings of Akubugwo *et al.* (2007) [1]. For antioxidant activities, the hydroethanol and decocted extracts of *C. olerius* leaves gave the highest reducing powers. The strong antioxidant activities of these extracts were justified by their high content of polyphenols. Indeed, according to Yildirim *et al.* (2001) [20], there is a link between the content of phenolic compounds and the reducing power. The antioxidant activity of polyphenols is due to the acidic nature of the phenol function (hydrogen donor) and its ability to establish hydrogen bonds. This gives them the ability to complex metals, to condense nucleophilic molecules and to make redox reactions.

5. Conclusion

This study investigated on phytochemical contents and antioxidant activities of three leafy vegetables (*A. hybridus*, *C. olerius* and *H. sabdariffa*) consumed by the populations in Korhogo (Northern of Côte d'Ivoire). Phytochemical screening of aqueous, hydroethanol and decocted extracts of these leafy vegetables allowed to highlight secondary metabolites such as alkaloids, saponins, flavonoids and polyphenols. The determination of total polyphenols showed variable concentrations in the various leaf extracts. The evaluation of the antioxidant activity indicated a highest ferric reducing power of the hydroethanol and the decocted extracts of *C. olerius*. This study revealed that the consumption of these leaves could effectively contribute to fight against oxidative stress. Given to above results, it would be interesting to assay and characterize the polyphenols of the various extracts studied in order to know their therapeutic power during dietary follow-up in patients.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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