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Phytochemical screening and antioxidant activity of leaves of eggplant, African spinach and cowpeas grown in Korhogo (North of Côte d'Ivoire)

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Abstract
Leafy Vegetables are more used as culinary ingredients than for their therapeutic properties. The aim of this study is to contribute to study phytochemical constituents and antioxidant activity of leaves of eggplant (*Solanum macrocarpon*), african spinach (*Talinum fruticosum*) and cowpeas (*Vigna unguiculata*) grown in Korhogo. Phytochemical compound of decocted, aqueous and hydroethanolic extracts of these three leaves were revealed by staining method in test tube after chromatographic separation of secondary metabolites. Polyphenolic compounds were determined by colorimetric method. Antioxidant property, free radicals scavenging capacity of extracts were assessed *in vitro* by 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. Phytoconstituants analysis revealed that different extracts of the three leafy vegetables contain secondary metabolites that are polyphenols, flavonoids, alkaloids and saponins. Total polyphenols of leafy vegetables showed levels between 4.91 and 82.98 mg GAE/g. The hydroethanolic extract of *S. macrocarpon* (82.98 mg GAE/g) and decocted extract of *V. unguiculata* (35.51 mg GAE/g) gave the highest values in both extracts. In addition, evaluation of antioxidant power with DPPH revealed IC50 of 45.87 µg/ml with 5extract of *S. macrocarpon*. Also with FRAP method the reducing power of extracts was between 52.90 and 1.06 µmol TE/g. Chemical composition of leafy vegetables studied has proved to be very interesting because of these secondary metabolites which have a beneficial role for health. Then these polyphenol compounds may contribute to fight against diseases related to oxidative stress.

Keywords: Leafy vegetables, *Solanum macrocarpon*, *Talinum fruticosum*, *Vigna unguiculata*, phytochemical screening, antioxidant activity

Introduction
Leafy vegetables were consumed by population to cover their food needs in Côte d'Ivoire. They are commodities that go into making of family meals. Also, ethnobotanical studies revealed that most people in Côte d'Ivoire consume indigenous green leafy such as *Amaranthus hybridus* (borombrou), *Andasonia digitata* (baobab), *Ceiba patendra* (fromager), *Hibiscus sabdariffa* (dah), *Basella alba* (épinard"), *Colocasia esculenta* (taro) and *Corchorus olitorius* (kplala)[1, 2, 3]. These leafy 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. Therefore, this study aims to investigate about phytochemical constituents and antioxidant activity of leaves of eggplant (*Solanum macrocarpon*), african spinach (*Talinum fruticosum*) and cowpeas (*Vigna unguiculata*), three culinary plants grown in Korhogo (Northen of Côte d'Ivoire).

Materials and methods

Collection of plant material

Leaves from *S. macrocarpon* (SM), *T. fruticosum* (TF) and *V. unguiculata* (VU) were collected in february 2019 in Korhogo city (Northern Côte d'Ivoire).

Preparation of leaves extracts
These three leaves were separately washed, cut up and dried shelter from sun light for two (2) 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 three fine powders obtained were used for preparation of different extracts. For powder of each species of leaf, three extracts were prepared (aqueous, hydroethanolic and decocted extracts). The aqueous extract (Aq) of each leaf was obtained by mixing 100 g of powder in 1 liter of distilled

water for 24 hours with constant stirring at 25°C. The mixture was filtrated on hydrophilic cotton and filtrate was dried in oven at 50 °C during 48 hours to give aqueous extract of each leaf. For hydroethanolic extract (Et/W) of each plant, 100 g of powder were stirred in 1 liter of mixture containing ethanol (70%) and water (30%) for 24 hours. The hydroethanolic mixture of the leaf obtained was filtrated on hydrophilic cotton and filtrate was dried in oven at 50 °C during 48 hours to give the hydroethanolic extract. The decocted extract (D) was prepared with 50 g of powder of each leaf dissolved in 1 liter 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 during 48 hours to obtain decocted extract of each leaf.
After 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).

Phytochemical screening of extracts

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 plates with size 7 cm x 5 cm. Starting and front lines were then drawn on each small plate with silica gel. A 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 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 separating spots are surrounded with a pencil and observed in visible light 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 (Rf) according to the formula:

Rf = (Distance of substance) / (Distance of solvent front)

A high frontal ratio of 0.2 – 4, will characterized the apolar compounds, 0.3 -0.5 for average polar compounds and 0.1 – 0.2 for polar compounds.

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 was revealed that the extract test contains alkaloids [4, 5].

Polyphenols: After migration of particles, the chromatographic plates for polyphenols test was treated with 2 ml of iron chloride (2 %). The appearance of blue-blackish or green coloration more or less dark showed the presence of phenolic compounds in tested extract [6].

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 [7].

***Saponins*:** The various extracts was 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 [8].

***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 (hexane). The evaporated extract was taken up with 1 ml of anhydride acetic acid and 1 ml of chloroform before put 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 presence of terpenes in extract [9].

***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. 1ml of concentrated sulfuric acid was then added to the whole. Cardiotonic glycosides in extract was revealed by a brown ring observed in test tube characteristic of a desoxysucre of cardenolides. In addition, a purple ring appears below the brown ring, while a greenish ring is gradually formed in the acetic acid phase [10].

Determination of total polyphenols

Total polyphenols of the methanolic extracts of different leaves were determined by the Folin-Ciocalteu (0.5N) colorimetric method [11]. 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 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).

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-pycrylhydrazyl (DPPH) scavenging activity and reducing power of iron (FRAP method).

DPPH (2,2'-diphenyl-1-pycrylhydrazyl) 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*. [12] with some modifications and testing. 2 ml of methanolic solution of DPPH (100 µM) was mixed with 0.5 ml of different concentrations (0-100 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 (P_I) is calculated according

to the formula below:
$P_I = [(A_0 - A_1) / A_0]x100$
P_I (%): Percentage of inhibition;
A_0: absorbance of DPPH solution without extract (white);
A_1: 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]).

Ferric Reducing Antioxidant Power (FRAP) of extracts
The FRAP of the extracts were carried out according to the method described by Pulido *et al*. [13]. 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 (20mM) and 25 ml of acetate buffer (300 mM sodium acetate, pH led to 3.6 with acetic acid). To 140 µl of tests 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 [14].

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).

Results and discussion
Results
Extraction yields
The extraction yields recorded in Table I have shown that among solvents used, the hydroethanolic mixture gave the high rates of extraction between 6.40 to 7.76 %. The two others extractions (aqueous and decoction) were revealed a low extraction efficiency with water. However, cold extraction (25°C) with water has shown higher yields (3.64 to 6.57 %) than it decocted extraction (50°C) which rates varied from 2.47 to 3.44 %. In addition, the leaves of *T. fruticosum* gave the best yields (3.44 to 7.76 %) whatever extraction method. Hydroethanolic and aqueous extractions of *V. unguiculata* have shown respectively intermediate yields of 6.8 and 6.26%. Aqueous extraction of *S. macrocarpon* (3.64%) and decocted extractions of *S. macrocarpon* (3.15 %) and *V. unguiculata* (2.47 %) have the lowest yields.

Phytochemical screening
Phytochemical screening revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, polyphenols (Table II). Alkaloids are present only in the aqueous extracts the three vegetables leave studied. The polyphenol compounds were present in all extracts analyzed but only the hydroethanolic extract of *S. macrocarpon* contains them in abundance. The presence and abundance of flavonoids were Respectively observed in hydroethanolic extracts of *S. macrocarpon* and *T. fruticosum*. The saponins were reveal in all decocted extracts, in aqueous extract of *T. fruticosum* and hydroethanolic extract of *V. unguiculata*. Cardiotonic glycosides and terpenes were absent in all extracts of leaves studied.

Table 1: Extraction yields of different extracts leafy vegetables

Leafy vegetables	Extracts	Extraction yields %
S. macrocarpon (SM)	Et/W_{SM}	6.40
	Aq_{SM}	3.64
	D_{SM}	3.15
T. fruticosum (TF)	Et/W_{TF}	7.76
	Aq_{TF}	6.57
	D_{TF}	3.44
V. unguiculata (VU)	Et/W_{VU}	6.86
	Aq_{VU}	6.26
	D_{VU}	2.47
Decocted extract (D) Aqueous extract (Aq) Hydroethanolic extract (Et/W).		

Table 2: Phytochemical screening of different extracts of vegetables leaves

Secondary metabolites	Extracts of leafy vegetables								
	S. macrocarpon (SM)			*T. fruticosum* (TF)			*V. unguiculata* (VU)		
	D_{SM}	Aq_{SM}	Et/W_{SM}	D_{TF}	Aq_{TF}	Et/W_{TF}	D_{VU}	Aq_{VU}	Et/W_{VU}
Alkaloids	-	+	-	-	+	-	-	+	-
Polyphenols	+	+	++	+	+	+	+	+	+
Flavonoids	-	-	+	-	-	++	-	-	+
Carditonic glycosides	-	-	-	-	-	-	-	-	-
Terpenes	-	-	-	-	-	-	-	-	-
Saponins	+	-	-	+	+	+	+	-	+
Abundance of compounds (++) Presence of compounds (+) Absence of compounds (-) Decocted extract (D) Aqueous extract (Aq) Hydroethanolic extract (Et/W).									

Total polyphenols compounds of extracts
Concentrations of polyphenolic compounds of different extracts analyzed are shown in Figure 1. These results reveals variable contents of total polyphenols according to the extracts of the leaves. The highest concentrations of polyphenols were observed with hydroethanolic extract of *S. macrocarpon (*82.98 ± 0.52 mg GAE/g) followed by decocted extract of *V. unguiculata* (35.51 ± 7.3 mg GAE/g) and hydroethanolic extract of *T. fruticosum* (27.76 ± 1.64mg GAE/g). The lowest presence of polyphenols was revealed with aqueous extract of *T. fruticosum* (4.91 ± 1.14 mg GAE/g), decocted extract of *S. macrocarpon* (10.5 ± 1.3 mg GAE/g) and hydroethanolic extract of *V. unguiculata* (17.21 ± 1.81 mg GAE/g). The decocted extract of *T. fruticosum* (21.29 ± 1.72 mg GAE/g), aqueous extracts of *S. macrocarpon* and *V. unguiculata* (22.89 ± 0.00 mg GAE/g and 25.29 ± 0.24 mg GAE/g) gave intermediate concentrations of polyphenols. In view of the above, the hydroethanolic extract of *S. macrocarpon* and the decoction of *V. unguiculata* have shown the best levels of total polyphenols. In addition, hydroethanol extraction and decoction gave the best yields extractions of total polyphenols.

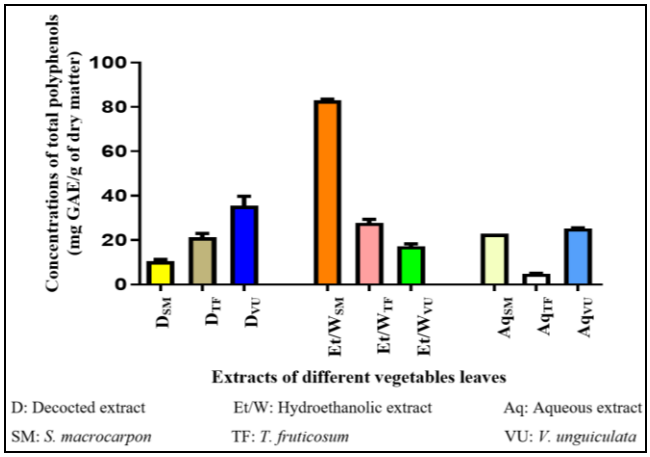

Fig 1: Concentrations of total polyphenols of different vegetables leaves studied

Antioxidant activities of extracts of leaf vegetables

Inhibition power of extracts on DPPH radical

Inhibition powers of hydroethanolic and decocted extracts of *S. macrocarpon* and *V. unguiculata* comparatively to vitamin C on DPPH radical were presented in Figure 2. These antiradical activities against DPPH were increased with concentrations of extracts and vitamin C. The highest antiradical activities were recorded for vitamin C and hydroethanolic extract of *S. macrocarpon* with respectively IC50 of 9.58 and 72.08 µg/ml. The three others extracts, hydroethanolic extract of *V. unguiculata* and decocted extracts of *S. macrocarpon* and *V. unguiculata* were exhibited the low antiradical powers.

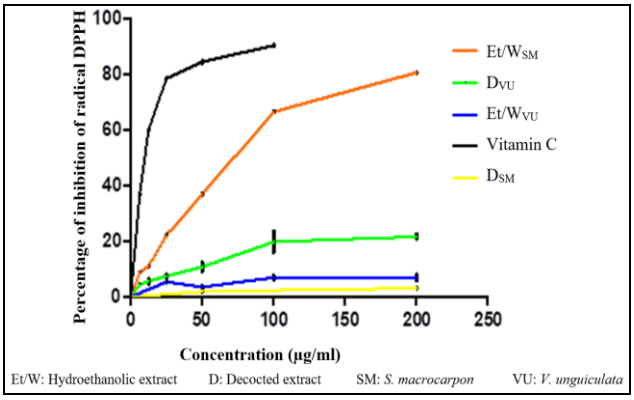

Fig 2: Inhibition of radical DPPH (%) by extracts of vegetables leaves and vitamin C

Ferric Reducing Antioxidant Power (FRAP) of extracts

The results of FRAP of hydroethanolic and decoction extracts of extracts *S. macrocarpon* and *V. unguiculata* were recorded in Figure 3. The hydroethanolic extract of *S. macrocarpon* gave the highest ($p<0.05$) reducing power (52.90 µmol TE/g) followed by decocted extract of *V. unguiculata* (13.06 µmol TE/g). Comparatively to the two above extracts, hydroethanolic extract of *V. unguiculata* and decocted extract of *S. macrocarpon* possessed the lowest antioxidant activities with respectively reducing power of 3.22 ± 1.1 and 1.06 ± 0.01 µmol TE/g.

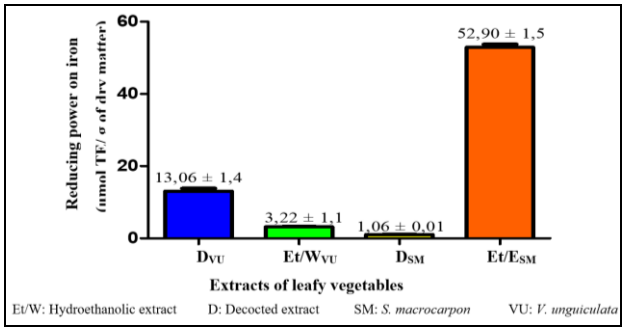

Fig 3: Reducing power of iron by extracts of leafy vegetables

Discussion

Study of the phytochemical compounds of leaves of *S. macrocarpon*, *T. fruticosum* and *V. unguiculata* allowed to determine the extraction yields of different extracts according 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, only the hydroethanolic extraction of each leafy vegetable had the highest yield than extraction with water (aqueous and decocted extractions). These results corroborate those of Quy *et al.* (2014) [15], which showed that the extraction yield of *Limnophila aromatica* consumed in Vietnam depends on several parameters such as solvent, temperature, extraction time and composition of the sample. Indeed, the polarity of hydroethanol solvent, seems to have ability to extract various substances in the two phases of different polarity (alcohol and water). In addition, Rhazi (2015) [16] found that ethanol and water mixture was more effective than water alone in extracting *Acacia mollissima* polyphenols.

Phytochemical screening of extracts of leaves of *S. macrocarpon*, *T. fruticosum* and *V. unguiculata* showed the absence cardiotonic glycosides and terpenes. These results corroborate the work of Kpètèhoto *et al.* (2017) [17], which showed the absence of cardiotonic glycosides and terpenes in decoction and infusion of *Ocimum gratissimum* in Benin. According to these authors, the absence of these two compounds in the extracts was explained by the extraction process used, which would degrade these metabolites. However, Brou *et al.* (2010) [18] found cardiotonic glycosides in the hydromethanolic extracts of six (6) different *Manihot esculenta* cultivars in Côte d'Ivoire. Also, through their investigation, Adjatin *et al.* [19] showed the presence of terpenes and cardiotonic glycosides in aqueous extracts of *T. fruticosum*. This difference could be due to the species of plants, the extraction solvents and the geographical origin of plants. Among phytochemical compounds studied, alkaloids, polyphenols, flavonoids and saponins were presented in aqueous, decocted and hydroethanolic extracts of *S. macrocarpon*, *T. fruticosum* and *V. unguiculata*. The weak presence of alkaloids in the aqueous extracts of leafy vegetables studied agrees with the work of Djeffel (2017) [20] on chalice of *Carlina acaulis*. This author justifies the less concentrations of alkaloids in chalice because of *Carlina acaulis* is edible and the alkaloids would be toxic in large quantities.

The polyphenols are present in variable proportions in the decocted, aqueous and hydroethanolic extracts analyzed. Their presence in *S. macrocarpon*, *T. fruticosum* and *V. unguiculata* was also observed in study conducted by Oulai *et al*. (2014) [21]. In addition, the presence of flavonoids in the various extracts of this study was confirmed by the work of Trichopoulou *et al*. (2001) [22], which indicates the presence of flavonoids such as myricetin, quercetin, kaempferol, isorhamnetin and luteolin in leafy vegetables. However, the presence of saponins in the aqueous, decocted and hydroethanolic extracts of *T. fructicosum* does not agree with the findings of Adjatin *et al*. (2012) [19]. These authors reported the absence of saponins in the aqueous extracts of *Crassocephalum rubens* (Juss ex Jacq.) S. Moore and *Crassocephalum crepidioides* (Benth.) S. Moore in Benin. This would be explained by the treatment of the leaves which were directly dried for two weeks before grinding them.

For antioxidant activities, the hydroethanolic extract of *S. macrocarpon* and decocted extract of *V. unguiculata* leaves gave the high reducing powers. The strong antioxidant activities of these extracts were justified by their high content of polyphenols. Indeed, according to Yildirim *et al*. (2001) [23] 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, condense nucleophilic molecules and make redox reactions [24].

Conclusion

This study investigated on phytochemical contents of antioxidant activities of three leafy vegetables (*S. macrocarpon*, *T. fruticosum* and *V. unguiculata*) consumed by the populations in Korhogo city (Northen of Côte d'Ivoire). Phytochemical screening of aqueous, hydroethanolic and decocted extracts of these leafy vegetables allowed to highlight secondary metabolites compounds such as alkaloids, saponins, flavonoids and polyphenols. The determination of total polyphenols showed its presence and variable concentrations in the various leaf extracts. The evaluation of the antioxidant activity showed a highest reducing power of the hydroethanolic extract of *S. macrocarpon* and the decocted extract of *V. unguiculata*. This study revealed that the consumption of these leaves could effectively 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.

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