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RELATIONSHIP BETWEEN SECONDARY METABOLITES, ANTIRADICAL ACTIVITIES, AND COLOUR CHARACTERISTICS OF *COCHLOSPERMUM TINCTORIUM A. RICH.* (BIXACEAE) ROOT

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Abstract

Cochlospermum tinctorium A. Rich. (Bixaceae) is a widespread herbaceous plant in the African sahelian and soudanian zones. Its root is used as food and medicinal plant. Studies of secondary metabolites, functional activities and trichromatic parameters were realized according to standard methods of laboratory. At a threshold of $\alpha = 0.05$, significant correlations ($p < 0.05$) were found between secondary metabolites contents and functional activities such as radical scavenging activity of antioxidant component or hydroxyl radical scavenging activity. The value of DPPHH was 95.56 ± 0.94% for CT inner Matrix with variation of 0.12% for CT Soaked inner Matrix and 1.63% for CT Peeler. But HSRA was 37.14 ± 5.71 for CT inner Matrix with variation of -38.46% for CT Soaked inner Matrix and -71.79% for CT Peeler. *Cochlospermum tinctorium* root has exhibited efficiency to heal many diseases thanks to multiple bioactive compounds. In addition, the colour of powders depended upon chemical compounds. Finally, *Cochlospermum tinctorium* root was found to neutralize organic radicals and reactive oxygen species.

Introduction

Bixaceae, usually represented by *Bixa orellana L.*, *Cochlospermum planchonii* Hook. F. and *Cochlospermum tinctorium* A. Rich., is a well-known plant family in herbal medicine. The species *Cochlospermum tinctorium* A. Rich., the plant of interest in this study is widely distributed in the savannah area of Western and Central Africa. A recent study has shown that the plant has hepatoprotective effect against carbon tetrachloride induced toxicity in rats (Etuk *et al.*, 2009b). However, a number of medicinal plants have been recommended for the treatment of liver disorders (Sanmugapriya & Venkataraman, 2006). Apart from the preventive actions, drugs are also needed for the treatment of existing pathological conditions (Tiné *et al*,. 1993; Zhang *et al.*, 2013). According to many authors, the root of *Cochlospermum tinctorium* (also called rhizome) is used in the prevention or treatment of malaria (Benoit *et al.*, 1995; Guiguemde *et al.*, 2000; Zederkopff-Ballin *et al.*, 2002; Ahmadu *et al.*, 2014), bacterial diseases (Ouattara *et al.*, 2007;

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Tidjani *et al*., 2009; Magaji *et al*., 2010), hepatic affections such as jaundice, chronic hepatitis B (Etuk *et al*., 2009a; Akinloye *et al*., 2011; Akinloye *et al*., 2012; Musa *et al*., 2012). It is also used in controlling physiological disorders such as hyperglycemia (Ndouyang *et al.*, 2018), convulsion (Maiha *et al*., 2009), inflammation and pain (Nergard *et al*., 2005; Ahmed *et al*., 2011). In addition, the root of plants of the genus Cochlospermum used against viruses was also reported (Solon *et al*., 2012; Musa *et al.*, 2012). In accordance with this view, rhizomes of Cochlospermum tinctorium are used against fever, hepatitis, abdominal pain, helminthes and bilharzias infestations (Ekanem, 1994). The root of C. tinctorium contains chemicals that are efficient against many diseases and its extracts are used as antidote when intoxication by other medicinal plants occur (Musa *et al*., 2012). The factors of hepatic diseases are multiple and developing a single agent capable of preventing hepatic diseases at all times appears elusive. Finding a potent drug that can regenerate hepatic functions irrespective of the initial cause of the damage appears more feasible by using C. tinctorium (Etuk *et al*., 2009a, 2009b). There are no reliable curative drugs for the treatment of hepatic diseases in modern medicine. The efficacies of most of these medicinal plants have to be validated. So, investigations of medicinal plants with potential hepatic regenerative activity are important. The present study examined the ability of the functional activities of C. tinctorium root extract against free radicals.

Experimental

Plant material and drying

The plant materials, the fresh roots of Cochlospermum tinctorium were collected in the village of Mindaore (local name of the plant in Tupuri language: 'Belyewn'), at the Eastern of the region of Mayo-Kebbi in Chad. The sample was taken to the Laboratory of Biophysics, Food Biochemistry and Nutrition of ENSAI/ University of Ngaoundere Cameroon for unit operations by using separately two different processing as reported by Ndouyang *et al.* (2018). The cuticle was discarded, and the first operation being a traditional household process consisted of reducing the inner matrix of the root in slices and of dividing the skin of the same root in small pieces; the second involved soaking processing of a part of the root inner matrix. Here, root slices of known weights (100 - 150 g) were subject to soaking twice in 5 L of water for 3 h before replacing tap water. After all the pretreatments, the samples were dried at 50°C for 18 h, and ground into powder, before storage at 4°C for different analysis.

Determination of secondary metabolites

The secondary metabolites in the *C. tinctorium* root considered in this respect are the following ones: total phenolic compounds; flavonoids; total, condensed and hydrolysable tannins; total and soluble oxalates; phytates, and total cyanides. Their contents were determined as using methods reported by Ndouyang *et al.* (2015).

Alkaloids determination

The alkaloid content was determined gravimetrically (Haborne, 1973), which was recently used by Adeniyi *et al.* (2009). 5 g of each sample was weighed using a weighing balance and dispersed into 50 mL of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 h before it was filtered. The filtrate was then evaporated to one quarter of its original volume on hot a plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to

filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60°C for 30 min, transferred into desiccators to cool and then reweighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed. The experiment was repeated three times for each food stuff sample and recorded as the average of three replicates.

Determination of flavonoids

Flavonoids content of the different samples was determined following the method of Eom *et al*. (2007). Essentially, 1 g of each ground sample was homogenized with 20 mL of extracting solvent (methanol/water/acetic acid, 140/50/10, v/v) and filtered into volumetric flasks and its volume was adjusted to 100 mL by addition of extracting solvent. Aliquots of 2.5 mL were transferred into 50 mL volumetric flasks and their volumes made up of water (analyzed solutions). To each 10 mL of analyzed solution, 2 mL of water and 5 mL of $AlCl_3$ reagent (133 mg crystalline aluminum chloride and 400 mg crystalline sodium acetate were dissolved in 100 mL of extracting solvent) were added. The absorbance was recorded at 430 nm against a blank made of 10 mL of analyzed solution and 5 mL of water. The amount of flavonoids was calculated from the calibration curve of quercetin standard solutions and expressed as mg quercetin/100 g of plant material.

Determination of carotenoids content

In addition, determination of total carotenoids and chlorophylls a and b methanolic solutions of sample extracts of the appropriate concentration (1.0 to 4.0 mg/mL) were analyzed in a visible spectrophotometer (UV/Vis SP8001 Spectrophotometer, Axiom, Germany) at 470, 653 and 666 nm. The concentrations of carotenoids and chlorophylls α and β were determined according to the equations of Lichtentha and Wellburn reported recently by Rainha *et al.* (2011) as follows:

Total carotenoids (mg/L) = 1000 Abs470 - 2.860 Ca - 129.2 Cb/245,

Chlorophyll a Ca (mg/L) = 15.65 Abs666 - 7.340 Abs653

Chlorophyll b Cb (mg/L) = 27.05 Abs653 - 11.21 Abs666.

DPPH radical scavenging activity

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form, DPPH-H. The free radical scavenging activity of all the extracts was evaluated by DPPH according to the previously reported method (Sakthidevi & Mohan, 2013). Briefly, an 0.1 mm solution of DPPH in methanol was prepared, and 1 mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (125, 250, 500 &1000 μg/mL).The the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (UV/Vis SP8001 Spectrophtometer, Axiom, Germany). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. Trolox was used as positive control. All the tests were performed in triplicates and the results were averaged. The capability to scavenging the DPPH radical or DPPH scavenging effect was calculated by using the following formula:

$$DPPH.H(\%) = \frac{A_{control} - A_{essay}}{A_{control}} * 100$$

Hydroxyl Radical Scavenging Activity (HRSA): The antioxidant activity of extracts was also measured as their ability to inhibit non site-specific hydroxyl radical-mediated peroxidation following the method reported by Abdou Bouba *et al.* (2010). The reaction mixture used, contained 100 µL of extract dissolved in distilled water, 500 µL of 5.6 mM 2-deoxy-D-ribose in KH2PO4–NaOH buffer (50 mM, pH 7.4), 200 µL of premixed 100 µM FeCl3 and 104 mM EDTA (1:1 v/v) solution, 100 µL of 1.0 mM H2O2 and 100 µL of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 50°C for 30 min. After this, 1 mL of 2.8% TCA and 1 mL of 1.0% TBA was added to each tube and the samples vortexed and heated in a water bath at 50°C for 30 min. The extent of oxidation was estimated from the absorbance of the solution at 532 nm. The percentage inhibition values were calculated from the absorbance of the control (Acontrol) and of the sample (Asample) using equation where the controls contained all the reaction reagents except the extract or positive control substance. The antioxidant activities of the extracts were expressed as mannitol equivalents (mg mannitol/g extract) as followed:

$$HRSA(\%) = \frac{A_{control} - A_{sample}}{A_{control}}$$

Ferric Iron Reducing Activity (FIRA) The antioxidant potential of the different sample extracts was also evaluated by their ability to reduce iron-(III) to iron-(II) following the method of Oyaizu (1986). In this respect, 1 mL aliquot of each extract, dissolved in distilled water, was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% aqueous K3Fe(CN)6 solution and incubated for 30 min at 50 °C. After this, 2.5 mL of 10% TCA was added, and the mixture centrifuged for 10 min. 2.5 mL aliquot of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous FeCl3, and the absorbance at 700 nm was recorded. Ferric iron reducing activity was determined as ascorbic acid equivalents (mg ascorbic acid/g extract).

Measurement of colour characteristics L, a* and b**

Colour measurements of three samples were carried out using a portable tintometer (Lovibond RT Colour Measurement Kit V2.28) with a 10˚ observer window and a D-65 light source as recently purchased by Abdou Bouba *et al.* (2012). The colour values generated by the equipment were expressed as L* (whiteness or darkness), a* (redness/greenness) and b* (blueness/yellowness). The whiteness index (WI) was calculated by the following formula (Tsai, 1994):

$$\boldsymbol{WI = 100 - \sqrt{(100 - L)^2 + a^2}}$$

Calculation of variation

The variations (Δ) of chemical compounds, colour and functional activities parameters of CTSM (C. tinctorium) and CTP (C. tinctorium) were evaluated as follows (Sonnergaard, 1999):

$$\Delta_{1,2}\ (\%) = \frac{\boldsymbol{Value\ 2 - Value\ 1}}{\boldsymbol{Value\ 1}} * \mathbf{100}$$

Value 2: analyzed value from CTSM or CTP; value 1: analyzed value from CTM only.

Statistical Analysis
Analyses were performed in triplicate for chemicals, and colour measurement was repeated up to five times. The software Statgraphics Plus 5.0 was used for the statistical analysis at a threshold, α=0.05. Principal component analysis and Pearson correlation was done using the Statbox 6.4 statistical software (Paris, France).

Results

Secondary metabolites
The compared study of secondary metabolites (Fig. 1) contained in the three powders of C. tinctorium (CT), namely CT Matrix (CTM), CT Soaked Matrix (CTSM) and CT Peeler or skin (CTP), revealed variable secondary metabolites contents. The total phenolic compounds or phenols are present in all the samples. However, CTM has a higher phenolic content than CTSM and CTP. Tannins and hydrolysable tannins, in particular, constitute important phenolic compounds in the root of C. tinctorium. Followed by phytates and oxalates that are mainly accumulated in the peeler or skin of C. tinctorium (CTP). Compared to CTM, the variation (Δ) of oxalate content was 30.46 % for CTSM and 70.71 % for CTP in the C. tinctorium root (Table 1). But oxalates were reduced from CTM and had oxalate content very low in CTSM (Δ = -47.01%). The phenolic compounds (Δ = -49.28%), including tannins (Δ = -29.06%), were washable by soaking C. tinctorium root slices. Contrary, phytates were not washable (Δ=-74.20%) in the conditions of the present study.

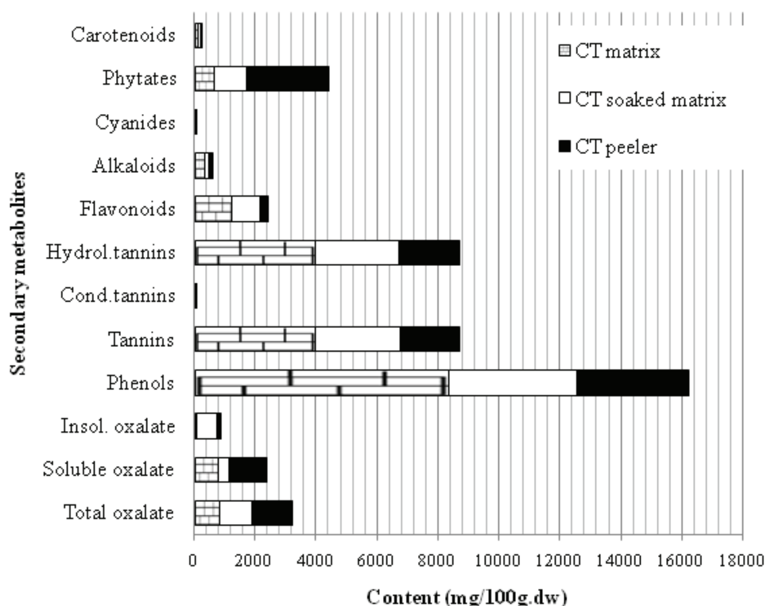

Fig. 1: Comparison of secondary metabolites content in different part of C. tinctorium root Insol = insoluble; cond = condensed; hydrol = hydrolysable

Functional activities

The three functional activities of the *C. tinctorium* root extracts were more or less based on the samples (Fig. 2). The antioxidant power revealed by DPPH•, was important in each sample. At 5% of threshold, no statistical significant difference was detected ($p > 0.05$). However, appreciable values of anti-hydroxyl radical activities (HRSA) were detected at a high level for CTM, an intermediate level for CTSM and a low level for CTP. That can be explained by the presence of high activity compounds against OH• in the root of *C. tinctorium* (CTM). In these conditions, *C. tinctorium* root presented no significant activity nor power of reducing ferric iron (Fe^{3+}) in ferrous iron (Fe^{2+}). The three values (for CTM, CTSM and CTP) stayed at less than 15%. Thus, Table 1 shows, for DPPH test, a low variation at the same time for CTSM ($\Delta = 0.12\%$) and CTP ($\Delta = 1.63\%$). These results revealed that the three samples have the same antioxidant power with no statistical significant difference ($p > 0.05$). In view of this evidence, the whole root of *C. tinctorium* can be considered as more efficient against organic radicals like DPPH•. Meanwhile, variation for HRSA were important for CTSM ($\Delta = -38.46\%$) and for CTP ($\Delta = -71.79\%$). There was a significant difference ($p < 0.05$) in terms of activity against hydroxyl OH•.

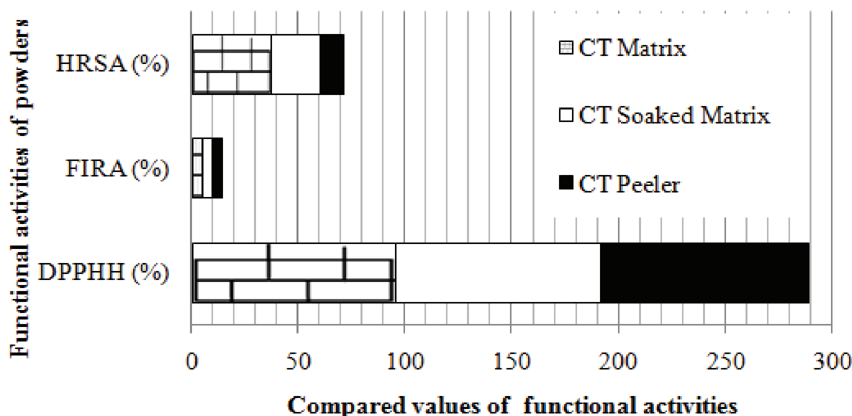

Fig. 2: Comparison of functional activities values of different part of C. tinctorium root DPPH = 1,1-diphenyl-2-picrylhydrazyl; FIRA = Ferric Iron Reducing Activity; HRSA = Hydroxyl Radical Scavenging Activity.

Colours

The three parameters that are in the trichromatic system are designed by the balance L* (Luminance/darkness), a* (redness/greenness), b* (blueness/yellowness), and their combination or whiteness index (WI) are presented in Fig. 3. All the values for the balance L* (luminance/darkness) were almost unique, but have remained low than the reference value. Also, no statistical difference was observed between samples for the balance a* (redness/greenness). Their respective WI were lower than reference value. So, Table 1 shows, for values reported to the CTM ones, variation of -1.32% L* for

CTSM attesting of effect of soaking on this balance, and variation of -0.20% b* for CTP. In the two cases, no statistical significant variation was observed ($p > 0.05$), but the others cases revealed significant difference ($p < 0.05$).

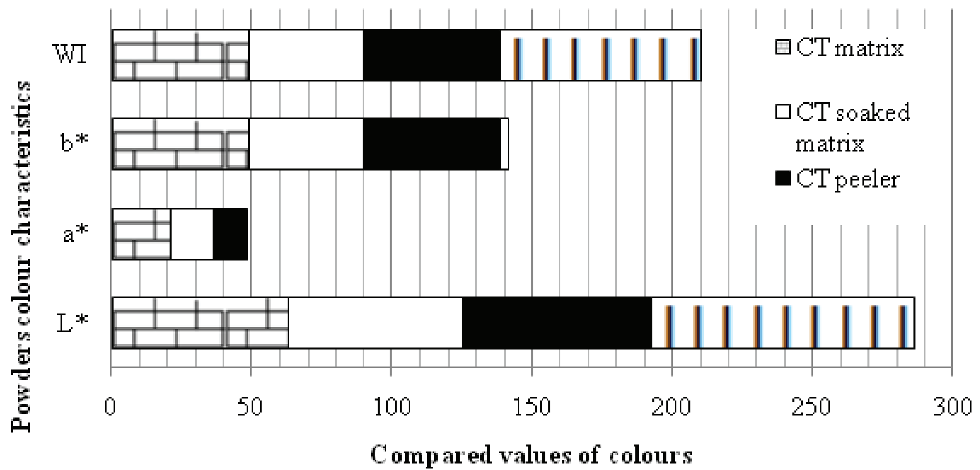

Fig. 3: Comparison of colour characteristics of different part of C. tinctorium root L* = balance luminance/darkness; a* = balance redness/greenness; b* = blueness/yellowness; WI = Whiteness index.

At the end of the presentation of the results, it appears necessary to look for correlation between variables in order to lead a structured discussion.

TABLE 1
Variation of mean values of parameters of CTSM and CTP linked to CTM ones

Variables	CTM	Variation of mean values linked to CTM ones (Δ,%)	
		CTSM	CTP
Total oxalate (mg/100g.dw)	799.81 ± 31.99	30.46	70.71
Soluble oxalate (mg/100g.dw)	739.38 ± 30.81	-47.01	65.92
Insoluble oxalate (mg/100g.dw)	60.43 ± 1.18	978.26	129.24
Phenols (mg/100g.dw)	8327.76 ± 389.96	-49.28	-56.11
Tannins (mg/100g.dw)	3929.48 ± 177.72	-29.04	-50.11
Condensed tannins (mg/100g.dw)	1.50 ± 0.40	12.66	-89.86
Hydrolysable tannins (mg/100g.dw)	3927.98 ± 177.53	-29.06	-50.09
Flavonoids (mg/100g.dw)	1193.30 ± 32.07	-21.75	-79.10
Alkaloids (mg/100g.dw)	305.97 ± 15.26	-59.38	-56.45
Cyanides (mg/100g.dw)	0.33 ± 0.05	112.76	135.67
Phytates (mg/100g.dw)	622.84 ± 132.80	74.20	328.21
Carotenoids (mg/100g.dw)	113.11 ± 12.86	-26.86	-84.45
DPPH (%)	95.56 ± 0.94	0.12	1.63
FIRA (%)	4.57 ± 0.10	1.26	-0.09
HRSA (%)	37.14 ± 5.71	-38.46	-71.79
L*	62.91 ± 0.29	-1.32	6.80
a*	20.81 ± 0.22	-26.80	-43.87
b*	48.88 ± 0.31	-16.33	-0.20
WI	35.20 ± 0.44	19.81	13.78

CTM = CT inner Matrix; CTSM = CT Soaked inner Matrix; CTP = CT Peeler.

Discussion

The antioxidant activity, the anti-hydroxyl radical activity or the ferric iron reduction activity were linked to specific secondary metabolites of C. tinctorium root. Besides, the trichromatic system (L*,a*, b*) was the fact of specific compounds.

The correlation study (Table 2), shows an association between metabolites and functional activities or trichromatic balance. At $p < 0.05$, the antioxidant activity or scavenging capacity of radical 1,1-diphenyl-2-picrylhydrazyl (DPPH•) was significantly correlated to soluble oxalates and phytates contents (r = 0.709 to 0.735), and in a non-significant correlation, to cyanides content (r = 0,581). However, these activities appeared not associated to phenolic compounds (hydrolysable tannins, flavonoids) and carotenoids (r = -0.816 to -0.719).

Meanwhile, HRSA appeared highly correlated to phenolic compounds (including hydrolysable tannins, flavonoids), alkaloids and carotenoids (r = 0.840 to 0.957). Contrarily, the secondary metabolites without anti-hydroxyl activity were hydolysable oxalates, cyanides and phytates (r = -0.972 to -0.857).

Phenolics are secondary metabolites that play a role in the maintenance of the human body. The presence of phytoconstituents, such as phenols, flavonoids and tannin in plants, indicates the possibility of antioxidant activity and this activity helps in preventing a number of diseases through free radical scavenging activity. Therefore, phenolic compounds are antioxidant agents that scavenge free radicals responsible of oxidative damage. Recently, Sakthidevi and Mohan (2013) reported that phenolic compounds have attracted much interest recently because in vitro studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, antitumor and antimicrobial activities. Studies have found that antioxidant properties are due to the presence of phenols and flavonoids (Jose & Radhamany, 2012). Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties. Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids is correlating with their antioxidant activities (Mbaebie *et al*., 2012). Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems.

Many authors have reported activities of C. tinctorium to protect liver against intoxication or affectation (Sanmugapriya & Venkataraman, 2006; Etuk *et al.*, 2009a, 2009b), even infection by viruses (Solon *et al*., 2012; Musa *et al.*, 2012). Nowadays, it is well known that phenolic compounds are highly responsible for the health effects derived from consumption of plant origin food (Villaño *et al*., 2007). According to these authors, phenolic compounds play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals. They attested these reactions:

$$ArOH + DPPH^{\circ} \rightarrow ArO^{\circ} + DPPH\text{-}H \quad [i]$$

$$ArO^{\circ} + DPPH^{\circ} \rightarrow \text{products} \quad [ii]$$

The reaction [i] indicates that

$$-\frac{d[DPPH^{\bullet}]}{dt} = nk_1[DPPH^{\bullet}][\text{ArOH}]$$

where n is the stoichiometric factor pf ArOH. Vegetal compound react with radical DPPH• by giving proton H to reduce DPPH• into DPPHH (Zhang & Yasumori, 2004). The significant positive correlation between HRSA and metabolites such as aromatic compounds and the phytoenes (phenolic compound including tannins and flavonoids, alkaloids, carotenoids) show effective antioxidant reactions. Meanwhile, they are efficient against reactive oxygen species (ROS) such as OH• by establishing single or multiple chemical bonds to produce water in some cases. However, as asserted by Bouba *et al.*, 2010), the C. tinctorium root extracts have shown ability to inhibit non site-specific hydroxyl radical-mediated peroxidation. Hydroxyl radicals OH• react with oxalates, cyanides, and phytates, compounds that are in their neutral forms in acid medium and anions when the medium is basic. These chemicals are involved in antioxidant activities but do not neutralize organic radicals comparable to DPPH•. Thus, C. tinctorium root extracts are

potent and reduce both organic and non-specific radicals chemically similar to DPPH• and OH•. Its uses as medicinal root for treatment of many diseases, is based on its multiple capacities in healing or correcting organic affections (Etuk *et al.*, (2009a, 2009b)). Cellular damages in rats were healed by consumption of C. tinctorium root extracts. The cellular regeneration are enhanced by the C. tinctorium root extracts. According to Auldridge *et al*. (2006), apocarotenoids are responsible of cellular regeneration. Nowadays, cochloxanthine and dihydrocochloxanthine are two principles apocarotenoids recognize in the C. tinctorium root (Akinloye *et al*., 2012; Musa *et al.*, 2012).

If FIRA has no significant correlation with a secondary metabolite of C. tinctorium root extract, it would be linked to the lower reductor content such as vitamin C or citric acid. Lastly, the values of trichromatic system parameters (L*, a*, b*) were also correlated to phytochemical compounds. The balance L* (luminance/darkness) is significantly associated to oxalates (r = 0.816 to 0.949) and to phytates (0.913); they are anions that give white powders. The balance a* (redness/greenness) is correlated with aromatic compounds and/or phytoenes phenols including tannins and flavonoids, alkaloids, carotenoids (r = 0.897 to 0.989). Finally, the balance b* (blueness/yellowness) is correlated to just hydrolysable tannins (r = 0.806).

TABLE 2

Pearson matrix of correlation between colours and secondary metabolites (mg/100 g.dw) in C. tinctorium root.

Variables	DPPHH (%)	FIRA (%)	HRSA (%)	L*	a*	b*	WI
Total oxalate	**0.735**	-0.076	**-0.972**	**0.816**	**-0.971**	0.074	0.609
Soluble oxalate	**0.709**	-0.164	-0.540	**0.949**	-0.471	**0.806**	-0.204
Insol. oxalate	-0.275	0.147	-0.157	-0.518	-0.246	**-0.990**	**0.808**
Phenols	-0.581	-0.114	**0.875**	-0.461	**0.957**	0.408	**-0.912**
Tannins	**-0.717**	-0.079	**0.932**	**-0.703**	**0.989**	0.106	**-0.737**
Cond.tannins	-0.630	0.077	0.656	**-0.839**	0.620	-0.515	-0.075
Hydrol.tannins	**-0.717**	-0.079	**0.932**	**-0.702**	**0.989**	0.106	**-0.738**
Flavonoids	**-0.816**	-0.008	**0.920**	**-0.893**	**0.925**	-0.240	-0.467
Alkaloids	-0.422	-0.075	**0.840**	-0.314	**0.897**	0.537	**-0.952**
Cyanides	0.581	0.298	**-0.857**	0.463	**-0.919**	-0.339	**0.831**
Phytates	**0.782**	-0.069	**-0.916**	**0.913**	**-0.902**	0.290	0.420
Carotenoids	**-0.729**	0.074	**0.957**	**-0.860**	**0.928**	-0.197	-0.495

The correlations in bold character statistically are significant at $\alpha = 0.05$. Insol = insoluble ; cond = condensed; hydrol = hydrolysable; DPPHH = 1,1-diphenyl-2-picrylhydrazyl (reduced); FIRA = Ferric Iron Reducing Activity; HRSA = Hydroxyl Radical Scavenging Activity.

In accordance with the previous view, the graph of different variables on the principal component axis and the biplot of different powders and variables on the principal component axis are illustrative. The axis PC1 and PC2 support the most of information cumulated at 90.62%. Therefore, the eigen values of principle components are significant for PC1 and PC2 (Table 3).

All parameters are well distributed on or in the correlation circle, except the non-significant ones such as FIRA (Fig. 4). The biplot of different powders and variables on the principal component (Fig. 5) show the relationship between secondary metabolites and their functional activities.

TABLE 3
Eigen values of principle components (PC).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigen value	12.417	4.801	1.158	0.355	0.180	0.057	0.024	0.009
% variance	65.350	25.270	6.096	1.869	0.946	0.299	0.125	0.045
% cumulated	65.350	90.620	96.716	98.585	99.531	99.830	99.955	100.000

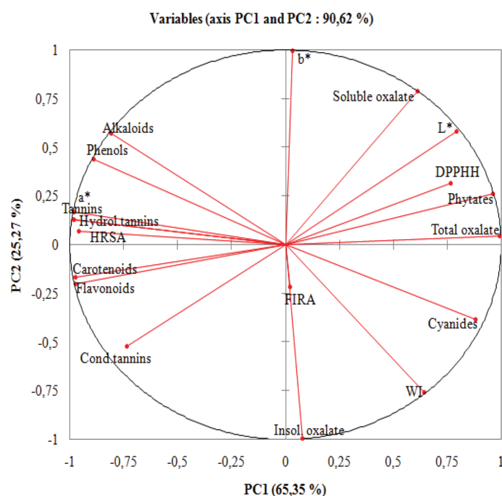

Fig. 4: Graph of different variables on the principal component axis.

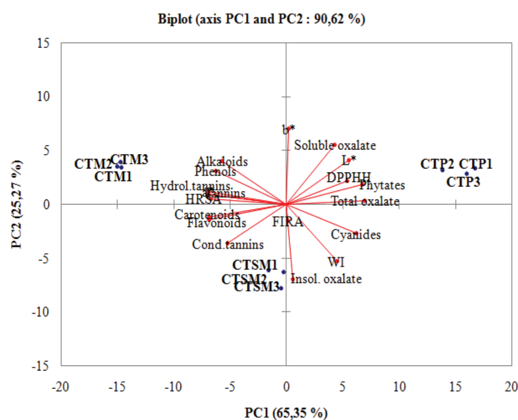

Fig. 5: Graph of different powders and variables on the principal component axis (the different powders are represented by bold points).

Conclusion

The Cochlospermum tinctorium root contains many secondary metabolites that play important role as antioxidant and anti-reactive oxygen species. Its compounds are potent to heal or correct many disorders caused in cellular compartments such as mitochondria. The ability of C. tinctorium root extract in giving hydrogen to neutralize free radicals enhances its application in multiple ways. Meanwhile, further investigations are necessary to facilitate a usage of the C. tinctorium.

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