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Full Length Research Paper

Erythrophleum africanum Afzel. (Caesalpiniaceae), an African toothpick: Phytochemical screening, total flavonoid content and antioxidant activity

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Erythrophleum africanum **is an endangered West African medicinal plant because of the large-scale use of its stems as toothpicks. Phytochemical screening (preliminary test and thin layer chromatography (TLC) method), a quantitative analysis of total flavonoids, anthocyanidins and radical scavenging activity (2,2'-diphenyl-1-picrylhydrazyl (DPPH) method) of stems of *E. africanum* were undertaken. The study shows that the quantification of the phenolic compounds (flavonic aglycones and anthocyanidins) and the assessment of the antioxidant activity put in evidence that in spite of the weak percentage (0.05%) of total flavonoids in the stem of *E. africanum*, the flavonoids are responsible to 94.46% of its antioxidant propriety.**

Key words: *Erythrophleum africanum*, total flavonoids, toothpick, antioxidant activity, 2,2'-diphenyl-1-picrylhydrazyl (DPPH).

INTRODUCTION

Plants have been used as medicine or drugs all over the world throughout the ages (Apak and Olila, 2006). Our work will consist in showing that their choice, sometimes guided by chance or instinct, is scientifically confirmed by their biological and chemical proprieties .The ethno botanic inquiries carried out in the markets of the district of Abidjan, targeted herbalists led us to list many plants such as "*Erythrophleum africanum".* Our choice is justified by the fact that no photochemical study of its stem has been made to our knowledge. However, research made on the leaves show an important toxicity (Hassan et al., 2007). It is a pan African plant of dry forests, dense or light, and wooded savannah. It is a tree of 10 to 15 m of high that we can mistake for *Burkea africana* (Aubréville, 1950; Michel, 2003) and for *Albizzia cariaria*. It possesses smooth and scaled leaves, hard and breakable interiorly into red orange slice .That tree has alternate leaves of 10 to 15 cm long (Priya, 2008). Its vernacular name differs from one region to another. So in Malinké, it is known as "tali", "gouélé téli", or "kabala". In Bobo-Dioulasso it is called "kiri" or "kiri gbèsè" (gbèsè stands for toothpick). Sénoufo people call it "djéguéljé" (Michel, 2003). It is used to cure many diseases .In fact the stem bark of *E. africanum* is used as diuretic and emetic. It is also used in the treatment of toothache and in the magic – religious practices.

To evaluate the stem bark of *E. africanum* as alternative means in the dental and mouth hygiene, we had to know its phytochemical composition and to test its antioxidant power *in vitro.*

MATERIALS AND METHODS

Plant material

Stems of E. africanum were collected in Longorola (Sikasso/Mali) in July 2009. They were kindly identified by Pr. AKE-Assi Laurent, botanist in the National Center of Floristique (Centre National de Floristique CNF) of the University of Cocody (Abidjan/Côte d'Ivoire). The stems of plant were cut and dried under ventilation at room temperature then finely ground with an electrical grinder (RETSCH type SM 100).

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Solvent, reagents and technical material

All the solvents, reagents and chemical products were purchased from Carlo Erba. Reagents for the phytochemical screening were prepared to the Laboratoire de Chimie Bio Organique et des Substances Naturelles (LCBOSN).The solvents and reagents used for thin layer chromatography (TLC) were analytically graded. TLC was performed over silica plates (20 × 20 cm, $60F_{254}$, Merck). The total phenolic content was determined using a spectrophotometer (MILTON ROY, Spectronic 601).

Preparation of crude extract

For identification of secondary metabolites, 1 g of the plant powder was macerated in 50 ml, 80% (v/v) for 1 h at room temperature. The methanolic crude extract was obtained after filtration under vacuum.

Isolation of total flavonoides by chelating technique

We used the new method of isolation isoflavones by complexation technique described by Priya et al. (2008). The methanolic crude extract of stems (10 ml) was treated with 10 ml of a solution of $AlCl_3$ (0.05 g/ ml) for 5 days at room temperature. The residue was filtered out and washed with MeOH and dried under a vacuum to give a solid residue. The residue was hydrolyzed using a dilute solution of HCl (2 N), to release free total flavonoids which were extracted using AcOEt. The ethyl acetate fraction was analyzed by TLC using Neu's reagent to confirm the presence of flavonoids.

Phytochemical screening and TLC

Tests for quinones, anthracenics, saponins, flavonoids, coumarins, alkaloids, reducing compounds and tannins were performed according to Dohou et al. (2003) and Bekro et al. (2007). TLC for the presence of flavonoids was carried out using TLC plates with eluting system ($CHCl_3$-MeOH-H_2O-AcOH, 10:1.5: 0.3:0.3; v/v/v/v). To develop the spots of chromatograms, reagent of Neu was used (Dohou et al., 2003; Mamyrbekova et al., 2008; N'gaman et al., 2009).

Total flavonoid content

One gram of dry and ground plant material was put in presence of 100 mL of MeOH (80% v/v). After agitation during 30 min, 1 ml of crude extract appropriated was mixed in 50 µl of Neu's reagent and pure MeOH (12 ml). The absorbance was determined at 404 nm and was compared to 1 mL of quercetin solution (0.05 mg/mL) used as positive control. The percentage of total flavonoids was calculated in equivalent quercetin according to the following formula (Lebreton et al., 1967):

$$\text{Percentage of total flavonoids (\%F)} = (0.05 \times A_{ext}/ A_q) \times 100 / C_{ext} \text{ (\%)}$$

Where: A_{ext} is absorbance of crude extract, A_q is absorbance of quercetin and C_{ext} is the concentration of crude extract in plant material, either 10 mg/ml.

Quantitative analysis of anthocyanidins and flavonic aglycones

The anthocyanidins, flavonic aglycones (flavonols and flavones) contents were assessed using the method described by Lebreton et al. (1967) and Dohou et al. (2003). Two gram of plant material was put in cooling at room temperature; the aglycones were extracted treating the aqueous phase by ethylic ether (2 × 50 ml). The ether was evaporated and the extract was taken in a few milliliters of EtOH 95% (v, v). To isolate the anthocyanes, the aqueous phase was treated by n-BuOH (2 × 50 ml). The dosage of anthocyanidins swept the specter from 480 to 600 nm and while keeping the maximal absorbance. The content was calculated according to the following formula (Dohou et al., 2003):

$$\text{Anthocyanidins (T) (mg/g)} = [(\gamma A/\varepsilon) \times M \times V \times d] / P$$

Where: γ is the factor of correction equals to 6 of the yield of transformation of leucoanthocyanidins (of the order of 17%), A is the absorbance to the maximal absorption wave length, ε is the coefficient of molar absorption of cyanidol (= 34700), M is the molar mass of leucocyanidol (= 306), V is the volume of n-butanolic solution, D is the factor of dilution, and p is mass of dry matter of hydrolyzed plant material.

The differential dosage of flavonols and flavones aglycones was realized using chelating properties of ethanolic solution of $AlCl_3$, 1% (1 g of $AlCl_3$ dissolves in 99 mL of EtOH, 95%). An aliquot of crude extract obtained after evaporation of the solvent was dissolved in 60 mL of ethanol 95% and 6 mL of solution of $AlCl_3$ (1%) was added. The all is hatched during 10 min. The specter is swept between 380 to 460 nm and the maximal absorbance was determined. The absorbance of the differential peak against a blank without $AlCl_3$ is proportional to the concentration of the sample flavonic aglycones. The aglycones content calculated was expressed in equivalent of quercetin according the following formula (Dohou et al., 2003):

$$\text{Aglycones (T) (mg/g)} = [(A/\varepsilon) \times M \times V \times d] / P$$

Where: A is the absorbance of the differential peak, ε is the coefficient of molar absorption of quercetin (= 23000), M is the molar mass of quercetin (= 302), V is the volume of ethanolic solution of aglycones, D is the factor of dilution, and P is the mass of the dry matter of hydrolyzed plant material.

Free radical scavenging activity assessment (2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay)

The antioxidant activity of the methanolic crude extract and ethyl acetate extract (total flavonoids) was assessed by the mean of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method (Blois, 1958).

The vitamin C was used like antioxidant control. The percentage of inhibition of the DPPH by the methanolic crude extract and ethyl acetate extract was calculated according the following formula (Wu et al., 2008):

$$\text{Percentage of inhibition (\%I)} = [(A_b - A_e) / A_b] \times 100$$

Where: A_b = absorbance of blanck and A_e = absorbance of sample

RESULTS

Phytochemical screening

Table 1 shows the results of the tests by means of the

Table 1. Preliminary phytochemical analysis of methanolic crude extract of plant material.

Secondary metabolites	Qui	Anth	Tan	Sapo	Cou	Alk	Flavo	Re com
Détection	-	-	+	+ (Im = 250)	+	Tr	+	+

+ : Presence; - : Absence; Tr : Trace ; Im : Index of moss; Tan : tannins; Cou : coumarins; Anth : anthracenics; Qui: quinones; Alk: alkaloids; Sapo: saponins; Re Comp : reducing compounds; Flavo : flavonoids.

Table 2. TLC analysis of the methanolic crude extract of stems of *E. africanum.*

Rf	Color UV/366 nm	Color with reagent of Neu UV/366 nm	Possible flavonoids
0.15	Orange	Blue	Methylated flavones
0.29	Invisible	Orange	Anthocyanidins
0.34	Yellow	Fluorescent yellow	Chalcones/aurones
0.73	Blue	fluorescent blue	Methylated flavones

Table 3. Results of quantitative analysis of stems of *E. africanum.*

Quantified compounds	Total flavonoids	Anthocyanidins	Aglycones
Content	0.05%	1.803 mg/g	0.123 mg/g

Table 4. TLC analysis of ethyl acetate fraction.

R_f	Color with reagent of Neu UV/366 nm	Possible compounds
0.14	Blue	Methylated flavones
0.46	Orange	Anthocyanidins
0.59	Blue	Methylated flavones
0.87	Yellow-green	Flavonols/ aurones

colorful reactions. We were looking for the following groups of phytochemical compounds: quinones, anthracenics, tannins, saponins, coumarins, alkaloids and reducing compounds.

Detection of flavonoids in the methanolic crude extract

The reagent of Neu has been used to reveal flavonoids. This reagent, indeed, reveals them as colorful stains in blue, orange, green, red and yellow fluorescent (Wagner et al., 1996).

So, four spotlights (Rf = 0.15; 0.29; 0.34 and 0.73) blue, orange, fluorescent yellow and fluorescent blue would correspond respectively to the methylated flavones, anthocyanidins, chalcones and to aurones (Wagner et al., 1996) (Table 2).

The results of quantitative analysis of stems and the TLC qualitative analysis of the total flavonoides isolated are presented in Tables 3 and 4.

Isolation of total flavonoids by chelating technique

In order to highlight the intrinsic antioxidant activity of the flavonoids, we extracted them with ethyl acetate after isolation by complexation and acidic hydrolysis. The qualitative analysis on TLC by means of the reagent of Neu of the ethylacetate fraction makes the blue, orange and yellow-green spotlights to appear under UV/366 nm on the chromatogram. While referring us to the works of Wagner et al. (1996), we deduced that these colorful stains correspond respectively to methylated flavones, anthocyanidins, flavonols or aurones (Table 4). The results of the TLC analysis confirm the method of isolation of total flavonoids by complexation. Figure 1 present antioxidant activity of flavonoide contained in stems of *E. africanum.*

The natural antioxydants seem to contribute meaningful manner to the prevention of illnesses such as cancers and cardiovascular illnesses (Krinsky, 2001). Systems of defenses antioxydantes include enzymes and the specific vitamins (essentially A, E and C). They also include

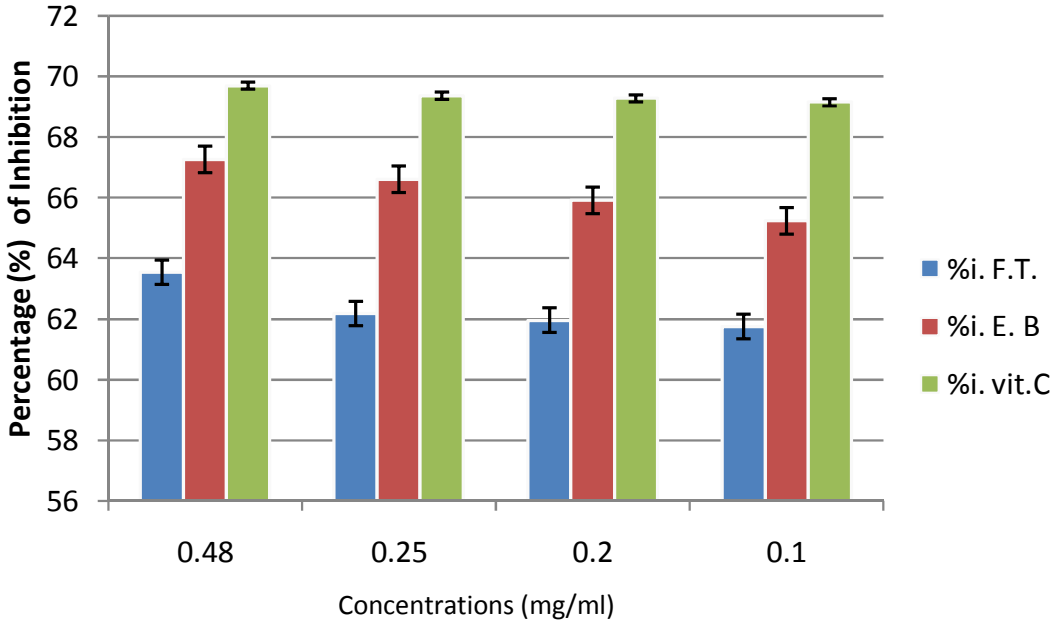

Figure 1. Results of the percentage of inhibition of DPPH. F.T: Total Flavonoids; E.B: Crude Extract; Vit C: vitamine C.

proteins that act indirectly, as the proteins vector of iron and copper. The antioxidant activity of crude extract (E.B) and of the total flavonoids (F.T) has been valued by spectrophotometry in comparison with the one of vitamin C taken as reference antioxidant. For included concentrations between 0.1 and 0.48 mg/ml, the percentage of inhibition of the induced DPPH by vitamin C (69.14 to 69.69%) is distinctly superior to those of E.B and F.T. As for E.B, for included concentrations between 0.1 and 0.48 mg/ml, the percentage of inhibition of DPPH (65.23 to 67.26%) becomes more pronounced as the concentration increases. The raw excerpt E.B is therefore gifted of antioxidant activity. The middle results gotten after 3 experiences permitted to draw the histogram expressing the variation of the percentage of inhibition according to the concentration of E.B (Figure); the maximum rate (67.26 ± 2%) is observed at 0.48 mg/ml. Concerning the total flavonoids, for the same concentrations (0.1 to 0.48 mg/ml) (Figure), we also note an increase of the antioxidant activity to reach its maximum in either 63.54 ± 1.5% or 94.46% of the antioxidant activity of E.B. It is evident from this analysis that in spite of the weak rate of flavonoids (0.05%) in the toothpick, flavonoids are responsible up to 94.46% of its antioxidant activity.

DISCUSSION

Various chemicals such as tannins, saponins, coumarins, alkaloids and flavonoids which are obviously present in stems of *E. africanum,* encourage defense against the microbial fermentation (Diby, 2004; Bitty, 1982).

The quantitative analyses give us the rates of 0.05% of total flavonoids, 1.803 mg/g of anthocyanidins and 0.123 mg/g of flavonic aglycones. These results justify that the stem of *E. africanum* is poor in flavonoids. Nevertheless, the quantity of the anthocyanidins is 14 times superior to the one of the flavonic aglycones (Table 3). Nevertheless, their power antioxydante is important and will be able to justify the daily use of the stems of *E. africanum* in part as toothpick. Indeed, by their antioxidant activities, flavonoïds (Chevalley, 2000) are recognized for their veinotonic properties and are capable of decreasing the permeability of the blood capillaries and to increasing their resistance. It is for this reason that they are used like vasculoprotectors and veinotonics in the treatment of the symptoms of veinolymphatic insufficiency and in the unrests of capillary fragility to the level of skin and the gingival mucous (Chevalley, 2000; Cavin, 1999). This toothpick could probably play a role of struggle against the oxidant stress, supposed to be at the origin of numerous illnesses (Favier, 2003), for the populations who would use it.

Conclusion

The present research achieved on *E. africanum* allowed us to identify some secondary metabolisms: coumarins, flavonoids, alkaloids, saponins, reducing compounds and tannins. Dosages done at the end of the phytochemical

screening, permitted to quantify the flavonoids containing in the stems. In spite of the weak rate (0.05%) of flavonoids in this toothpick, they are responsible to 94.46% of its antioxidant activity, which justifies the large use of the stems of *E. africanum* in part as toothpick.

REFERENCES

Aubréville A (1950). Sudano-Guinean forest flora. Paris, editingGeographic Society maritime and colonial, pp. 207-243.

Bekro YA, Bekro MJA, Boua BB, Tra BFH, Ehile EE (2007). Ethnobotanical studies and phytochemicalscreening of *Caesalpinia benthamiana* (Baill.)Herend. And Zarucchi (Caesalpiniac eae). Sci. Nat., 4: 217-225.

Bitty MJ (1982). Study of some plants used as stick friction toothivory coast, Thesis for the state diploma of doctor of dental surgery in Ivory Coast, p. 99.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical, Nature, 181: 1199-1200.

Cavin A (1999). Phytochemical investigations of the three plantsin Indonesia and radical-scavenging antioxidantproperties. *Tinospora crisp* (Menispernaceae) *Merremia emarginata* (Convovulaceae) and *Orophea enneandra* (Annonaceae), Ph.D. Thesis, Lausanne, p. 243.

Chevalley I (2000). Contribution to the phytochemical study ofSaxifragaceae, isolation of antioxidants fromSaxifraga stellaris L. and Saxifraga cuneifolia I.and an antifungal compound in Ribes rubrum, Ph.D. thesis Lausanne, P. 175.

Diby E (2004). Identification of medicinal plants used intraditional odontostomatology in Ivory Coast, Thesis for the state diploma of doctor of dental surgery, p. 251.

Dohou N, Yamni K, Tahrouch S, Idrissi HLM, Badoc A, Gmira N (2003). *Screening phytochimique d'une endémique ibéro-marocain*e, *Thymelaea lythroides*, Bull. Soc. Pharm. Bordeaux, 142: 61-78.

Favier A (2003). Chemical News-November-December. Pp. 108–115.

Hassan SW, Ladan MJ, Dogondaji RA, Umar RA (2007). Phytochemical and Toxicological Studies of Aqueous Leaves Extracts of *Erythrophleum africanum*. Pak. J. Biol. Sci., 10(21): 3815-3821.

Krinsky NI (2001). Carotenoids as antioxidants, Nutrition, 17: 815–817.

Lebreton P, Jay M, Voirin B (1967). On the qualitative and quantitative analysis offlavonoids. Chim. Anal*.,* 49(7): 375-383.

Mamyrbékova-Békro JA, Koffi MK, Békro YA, Marc G, Tra J, Véronique M, Boua BB (2008). *Phytocompounds of the extracts of four medicinal plants os Côte d'Ivoire and assessment of their* potential antioxidant by thin Layer chromatography. Eur. J. Sci. Res., 24(2): 219-228.

Michel A (2003). Trees, shrubs and vines in dry areas of West Africa, p. 237.

N'Gaman KCC, Békro YA, Mamyrbékova-Békro JA, Bénié A, Goore-Bi S (2009). Composition of the secondary metabolites andthe antioxidant activity of crude extracts of *Gmelina arborea* Roxb. (Verbanaceae) of Côte d'Ivoire, West Africa: Analysis by Thin Layer Chromatography. Eur. J. Sci. Res., 36(2): 161-171.

Priya RR (2008), A new methods of isoflavones from Glycine max (Soya Beans) by complexation technique. Asian J. Sci. Res., 1(2): 176-179.

Wagner H, Bladt S, Zgainski E (1996). Plant drug analysis, a thin-layer, chromatography atlas, Springer Verlag, Berlin Heidlberg, 2nd éd. New York, p. 320.

Wu JH, Hang CY, Jung YT, Chang ST (2008): RP-HPLC-DPPH screening Method for Detection of Radical-Scavenging Phytochemicals from Flowers of Acacia confusa. J. Agric. Chem. Food, 56: 328-332.