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Research Article

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Chemical Analysis, Total Phenolic and Flavonoid Content, Fractionation of *Croton dibowskii* Hutch Extracts

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ABSTRACT

This article presents a phytochemical study of the roots of Croton dybowskii Hutch. Colorimetric methods, UV-visible spectrophotometry and fractionation on a silica gel column were used for chemical analysis, the determination of total polyphenols and flavonoids. The chemical screening allowed us to highlight six chemical families out of the eight expected. These qualitative analyzes have shown that the Croton dybowskii species is rich in alkaloids, flavonoids, anthracene derivatives, terpenes, coumarins and saponosides. Quantitative analyzes, in particular, the contents of flavonoids and total polyphenols, gave results which vary according to the extraction solvent. The flavonoid contents are less significant for the aqueous extracts and significant for the hydroethanolic and ethanolic extracts, they vary between 0.4 ± 0.02 and 5.8 ± 0.1 mg EC/g DM) whereas those in polyphenolic compounds reveal a more significant concentration for hydro-ethanolic extracts followed by those of ethanolic and aqueous extracts, respectively; 15.2 ± 0.3; 3.6 ± 0.1 and 3.3 ± 0.2 mg EAG/gMS. This study also enables the production of 14 fractions after separation by chromatography on an open column and the isolation of a compound after purification on a Thin Layer Chromatography (TLC). The interest of the Croton dybowskii Hutch root could be justified by its richness in secondary metabolites.

Keywords: *Croton dybowskii* Hutch; Chemical screening; Polyphenols; Flavonoids content; Fractionation; Euphorbiaceae

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INTRODUCTION

Plants, real chemical factories, play a very important role in our daily life [1]. Since ancient times, they have been used as firewood, raw materials in real estate, decoration, food, and the traditional pharmacopoeia [1-3]. Today, they represent a real niche for medicine because, they are fully integrated into African customs and intervene in

traditional pharmacopoeia to fight against many diseases such as malaria, dysentery, yellow fever and many others [4,5]. In Congo, on an area of 342,000 km^2, the floristic resources often used in the pharmacopoeia, represent 60% of the total area [6], but they suffer from under exploitation despite their strong involvement in certain treatments related to traditional medicine. To solve these specific problems, in particular their under exploitation, it is today imperative, to develop the Congolese flora in particular and African in general, in order to make it available to the populations, Improved Traditional Medicines (MTA). In so doing, chemical and pharmacological knowledge of these natural resources is inevitable. Owing to this fact, this work is dedicated to carry out the chemical analysis, the fractionation and the dosage of the polyphenols and flavonoids contained in the total extracts of *Croton dibowskii* Hutch.

MATERIALS AND METHODS

Plant materials

Croton dybowskii Hutch is a medicinal plant of the African pharmacopoeia which belongs to the family of Euphorbiaceae. The plant is found in Central Africa, mainly in Congo, Gabon, and coastal Guinean basin [7,8]. Its roots with a strong aromatic flavour are used as masticatory and in aqueous maceration for the treatment of sexual asthenia, pelvic pain and as a diuretic. Further to its misuse due to its intensive advertisements in the markets and drinking places (bars) in Brazzaville, Pointe Noire, Cabinda and Kinshasa, the plant is disappearing. The roots of *Croton dibowskii* Hutch were harvested in Congo Brazzaville in the department of Pointe-Noire at the level of the Indian point village (Figure 1) based on the following geographical coordinates: S4 40.244 E11 48.861, Alt: -14 m. The different organs of the plant have been compared to those of the National Herbarium of the National Research Institute for Exact and Natural Sciences (IRSEN) under the number 188.

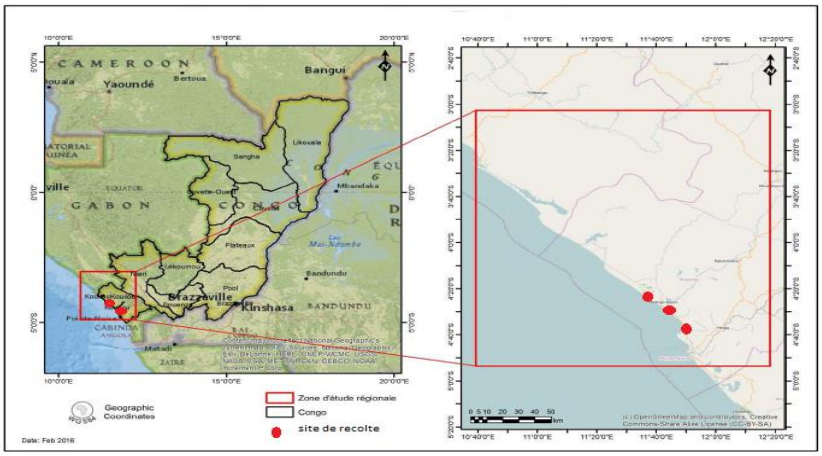

Figure 1. Map of the study area

Preparation of extracts

The roots were dried at room temperature, for about 10 days. The dry vegetable matter is ground with an IKA-WERKE Gmbh-CO-KG, D-79219, Staufen-type device, with a sieve of granulometry 0.25 mm. For measurement, Three extracts: Water (100%), hydro-ethanolic (EtOH-H_2O, 50:50 v/v) and ethanolic (ETOH:100%) were obtained on mixing 2 g of vegetable matter with 2 × 20 ml of each solution. The mixture is shaken up for 72 hours, then

filtered. The filtrate obtained dried concentrated with a rotary evaporator is kept in a cool place (+4°C) waiting to be analyzed.

Chemical screening and qualitative analysis

Three extracts, Water (100%), EtOH-H_2O (50:50 v/v) and ETOH (100%) were screened for their classes of bioactive compounds. The extracts were tested qualitatively for the presence of chemical constituents such as tannins, saponins, flavonoids, anthocyanins, alkaloids, terpenes, Anthracene Derivatives and coumarins. The test for these chemical families was carried out according to the methods described by Oua et Ouabonzi [9,10].

Measurement of total polyphenols

The reagent of Folin-Ciocalteu was used for the evaluation of total phenols of aqueous, hydroethanolic and ethanolic extracts. Folin-Ciocalteu is a mixture of phosphotungstene acid (H3PW12O40) and phosphomolybdene (H3PMo12O40) of yellow color. The method is based on the oxidation of the phenolic compounds by this reagent. This oxidation draws the formation of a new complex molybdenum tungsten of blue color which absorbs to 725 nm. The evaluation of TP is done by comparison of the optic density (D.O) observed to the one got from a stallion of known acid Gallic concentration. The total phenol compounds are measured as follows: 0.1 ml of the extract hydroethanolic is introduced in an Eppendorff tube of 2 ml, the extract is diluted with 0.9 ml of distilled water. 0.9 ml of the reagent of Folin-Ciocalteu (1N) is immediately put after the addition of 0.2 ml of Na_2CO_3 (20%) solution. The resultant mixture is hatched to the ambient temperature for 40 minutes safe from light. The absorbance is measured with the spectrophotometer at 725 nm against a solution of ethanol used like white (control). A right of standardization achieved previously with the Gallic acid in the same conditions as the samples to analyze, permitted to calculate the total phenols contain. The results are expressed in mg equivalent Gallic acid by gram of dry matter (mg E GA/gMs).

Measurement of total flavonoids (FVT)

The colorless solutions of sodium nitrite ($NaNO_2$, 5%) and of aluminum chloride ($AlCl_3$, 10 %) have been used for the evaluation of total flavonoids in aqueous, hydroethanolic and ethanolic extracts. The method is based on the oxidation of the flavonoids by these reagents; oxidation which draws the formation of a brownish complex that absorbed at 510 nm. The comparison of the optic density (D.O) observed to the one issued from a stallion of known concentration Catechin permits to value the total content in flavonoids by colorimetric effect. In a ball of 10 ml are introduced 250 µl of extract and 1 ml of distilled water successively. At the initial time (0 minute) are added 75 µl of a $NaNO_2$ (5%) solution. After 5 min, 75 µl of $AlCl_3$ (10%) are added; 6 minutes later, 500 µl of NaOH (1N) and 2.5 ml of distilled water are added successively to the mixture. A curve of standardization is elaborated with solutions standards to catechin prepared at different concentrations. The results are expressed in mg equivalent Catechin by gram of dry matter (mg EC/gMs).

Fractionation and purification

A fractionation of the crude extract, petroleum ether/chloroform (50%, V/V), of *Croton dybowskii* Hutch (4 g), is carried out by adsorption chromatography on a column of silica gel 50 cm long and 3 cm in diameter. The elution is carried out with petroleum ether gradually enriched with chloroform and ethanol. The fractions obtained are

combined and analyzed by thin layer chromatography on silica gel on an aluminum support and viewed under UV (254 and 366 nm) then revealed by sulfuric Anisaldehyde.

RESULTS AND DISCUSSION

Determination of the main chemical families

The results of the chemical screening carried out on the bark of *C. dybowskii* Hutch shows the presence of six chemical families. We note the presence of Saponosides, Flavonoids, Alkaloids, Anthracene derivatives, terpenoids and coumarins. Terpenes and coumarins are the most representative families. However, there is a total absence of tannins and anthocyanins. Indeed, the abundant presence of secondary metabolites: terpenoids, coumarin and alkaloids, has been reported in certain species of the genus Croton [11-16].

Dosage of total polyphenols (PPT) and total flavonoids (FVT)

The gallic acid calibration curve y=5.0509x+0.0049 was used for the determination of total polyphenols. The results are expressed in mg gallic acid equivalent per gram of dry matter (mgEGa/gMS) with a correlation coefficient R^2=0.996. The reference compound used for the determination of total flavonoids (FVT) is catechin. Its curve y=2.1901x+0.0175 is established with a correlation coefficient R^2=0.986 and the results are expressed in mg catechin equivalent per gram of dry matter (mgEC/gMS). The results of the quantitative analyzes by spectrophotometry of the aqueous (AQ), Hydro Ethanolic (HE) and Ethanolic (ET) extracts are shown in Table 1. They signal out that the contents of flavonoids and total polyphenols vary depending on the nature of the solvent. This quantitative analysis in total Flavonoids (FVT) gives a less significant concentration for the aqueous extracts than for the Hydro-Ethanolic (HE) and Ethanolic (ET) extracts. These values are respectively 0.4 ± 0.02; 2.1 ± 0.1 and 5.8 ± 0.1 mg EC/gMS, whereas that in polyphenolic compounds reveals a more significant concentration for the hydro-ethanolic extracts followed by that of the ethanolic and aqueous extracts, namely: 15.2 ± 0.3; 3.6 ± 0.1 and 3.3 ± 0.2 mgEAG/gMS. It therefore appears that the alcoholic extracts would favor the extraction of flavonoids while the hydro-ethanolic extracts would be favorable for the extraction of polyphenolic compounds. These results confirm those of chemical analysis [11-18].

Table 1. Total Flavonoids (FVT) and Polyphenols (PPT) content in the three extracts of *C. dybowskii* Hutch.

Extracts	Polyphenols (mg EAG/g MS)	Flavonoids (mg EC/g MS)
AQ	3.3 ± 0.2	0.4 ± 0.02
HE	15.2 ± 0.3	2.1 ± 0.1
ET	3.6 ± 0.1	5.8 ± 0.1
AQ: Aqueous; HE: Hydro-ethanolic; ET: Ethanolic		

Fractionation of total extracts

Qualitative analysis of physico-chemical parameters: Separation on an open column gave 14 fractions whose physicochemical properties vary according to the nature of the fraction (Table 2).

Table 2. Nature and Physical Properties of Fractions

Fraction	Color	Physical Nature	Odor	Mass (mg)
Extrait	Orange			4000
F1		liquid		700
F2	Yellow	liquid and crystals		100
F3	Orange		aromatic	1000
F4	Yellow orange	pasty liquid		200
F5	Yellow		semi aromatic	40
F6				370
F7				100
F8				60
F9				40
F10				300
F11				200
F12	Yellow orange	Solid	semi aromatic	20
F13				50
F14				35

Examination of these results shows that, for 4 g of extract deposited on a column filled with silica gel, 14 fractions were obtained whose physical properties (color, physical state flavour and mass) vary with the elution of the fraction. The color varies between yellow and orange yellow. The physical state of the fractions oscillates between the liquid and solid state except for the fraction F2 which is in an intermediate state. The plant being an aromatic plant, the flavour of the fractions keeps the strongly perfuming side, for the fractions F1 to F4 and semi aromatic for the fractions F5 to F14. This can be justified by the presence of terpene compounds reported by some authors in essential oils extracted from this plant [12-16]. The high mass concentrations are obtained in the liquid and highly aromatic fractions because the extract is produced by a mixture of a medium-polar solvent consisting of chloroform and ethanol.

Qualitative qnalysis by Thin Layer Chromatography (TLC)

The chromatographic profile (Figure 2) of the Chloroform/ethanol extract (50% v/ v) of the barks of *Croton dybowskii* shows a series of spots of light yellow, orange-yellow and purple color with different frontal retention (Table 3). These spots materialize the strong presence of terpenes. The yellow colors could highlight the presence of mono terpenes, sesquiterpenes and di-terpenes. The presence of these compounds has been reported by several authors in other species of the genus croton [11-16].

Figure 2. Chromatogram of the choroformic/Ethanol extract (50% v/v)
Migration solvent: Hexane/Ethyl acetate (70/30, v / v).
Developer: Sulfuric Anisaldehyde

Table 3. Chromatographic profiles of the Chloroform/Ethanol extract (50% v/v)

Spots	Frontal Reference (R_f)	Color
1	0.23	yellow
2	0.59	violet
3	0.87	yellow
4	0.93	yellow

The chromatographic profile of fractions F1 to F14 shows a series of spots according to the variation of the polarity of the mobile phase materializing the presence of terpene compounds in all their ranges (mono terpenes, sesquiterpene, di terpenes, tri terpenes and sterols) (Figure 3). In accordance with Wagner and Blatt [19], the light yellow colors will be comparable to mono terpenes and sesquiterpenes while the violets and green to triterpenes and sterols. On the other hand, the brown and blue spots highlighted in the fractions F10 to F14 are indicative of the diterpene compounds. The F10 and F11 fractions were combined and analyzed by preparative thin layer chromatography to check their purity. It appeared that these two fractions are identical and constitute the isolated compound D (Figure 4). Analysis of this compound on a TLC, followed by revelation with sulfuric anisaldehyde, shows a brown compound like diterpene, structures reported in the bark of other species of the genus croton.

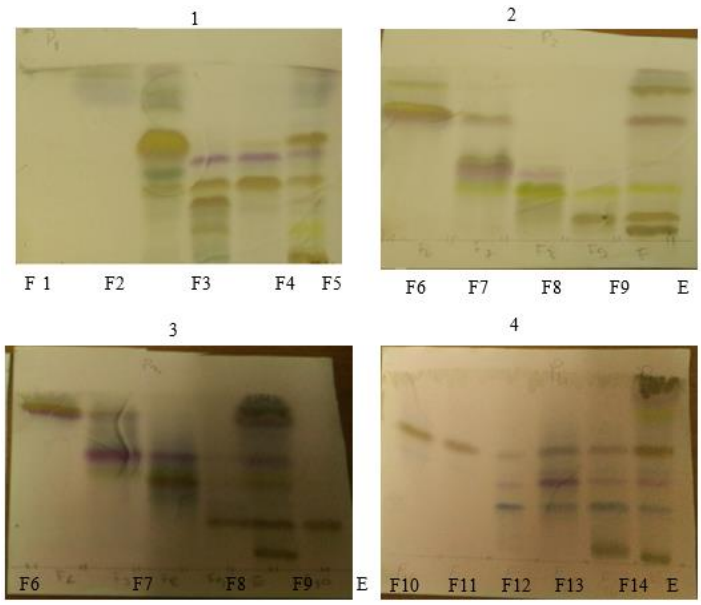

Figure 3. Chromatographic profile of fractions F1 to F14.
Eluent 1: Hexane/chloroform (8/2 v/v); 2: Hexane/chloroform (5/5 v/v); 3: Chloroform /Ethanol (9/1 v/v) and 4: chloroform /Ethanol /Water (8/1/1v/v).
Developer: Sulfuric Anisaldehyde

Figure 4. TLC of compound D isolated.
Eluent: chloroform/Ethanol/Water (8/1/1v/v).
Developer: Sulfuric Anisaldehyde

CONCLUSION

This study examined the phytochemical study of *Croton dybowskii* Hutch. It comes out that, in addition to its aphrodisiac properties which make it very popular to the population, this root contains secondary metabolites such as flavonoids, anthracene derivatives, terpenes, coumarins, alkaloids and saponosides. The quantitative analysis of total flavonoids (FVT) gave a less significant concentration for the aqueous extracts and significant for the hydro-ethanolic and ethanolic extracts, respectively: 0.4 ± 0.02; 2.1 ± 0.1 and 5.8 ± 0.1 mg EC/g MS) whereas that of total polyphenols (PPT) revealed a more significant concentration for hydro-ethanolic extracts followed by that of ethanolic and aqueous extracts, 15.2 ± 0.3; 3.6 ± 0.1 and 3.3 ± 0.2 mg EAG/gMS. The separation, purification, and

isolation of the plant compounds, by TLC, enabled to obtain 14 fractions and the isolation of a compound D whose structure remains to be identified. It should be noted that the analysis of physical parameters suggests compounds of the terpene family. It is suggestive that further studies are necessary to assess the aphrodisiac activity of the *Croton dybowskii* Hutch root.

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