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Phytochemistry, metabolites quantification and antioxidant activity of *Calotropis procera* (Ait.) and *Ficus umbellata* (Vahl.), plants traditionally used against hemorrhoids in Benin

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Abstract

Hemorrhoidal disease is a very present ailment that manifests itself in our societies and sometimes leads to cases of surgery. The traditional treatment in Benin is based on the use of medicinal plants. *Calotropis procera* (Ait.) and *Ficus umbellata* (Vahl.) are two plants well known in West Africa, precisely in Benin. They are among the most used plants in traditional medicine. Their different preparations are mainly used against several sickness as tuberculosis, uterine myomas and fibroids, malaria, hemorrhoids... These uses of the two plants were reported in the cities of Porto-Novo and Abomey-Calavi. In order to verify the effects of these plants on hemorrhoids, preliminary studies were carried out. Phytochemical studies of fresh leaves and leaf powders revealed, among other things, the presence of total polyphenols, flavonoids, tannins and traces of essential oil. The different assays showed that *F. umbellata* is richer in total polyphenols (377.97 mg.EqAG/g extract), flavonoids (10.97 mg.EqRUT/g extract) and tannins (33.13 mg.EqPYR/g extract) than *C. procera*. The extract without potash of *C. procera* is richer in phenolic compounds (139.107 mg.EqAG/g extract) than which with potash (63.54 mg.EqAG/g extract). Antioxidant activity was measured for all extracts, and the aqueous extract of *F. umbellata* exhibited the highest activity (14.24 µg / mL).

Keywords: Medicinal plants, *Calotropis procera*, *Ficus umbellata*, Hemorrhoids, Treatment

Introduction

Hemorrhoids are anatomical structures normally present in all healthy individuals. Their function is to contribute to anal continence (Morgado et *al*., 1988). The onset of clinical manifestations transforms this normal anatomical state into « hemorrhoid disease » (Vignon et *al*., 2011). Hemorrhoids are defined not by their appearance, but by their essential manifestations: hemorrhages (Boureima, 2005). Hemorrhoidal crisis motivates the annual hospitalization of 13 subjects per million inhabitants in the United States (Siegfried et *al*., 2015). Although the precise cause is not well understood, the actual frequency is difficult to establish (Drissa, 2008). Hemorrhoids are associated with conditions that increase pressure in the hemorrhoidal venous plexus, such as straining during bowel movements secondary to constipation.

Like modern medicine, traditional medicine has its own value system; its own units of measurement and its own way of protecting the body (Boureima, 2005). Endogenous practices lead African populations, and Beninese populations in particular, to use plants to treat themselves. As these plants are within their reach in their living environment, they obtain them for free. *Calotropis procera* (Asclepiadaceae) and *Ficus umbellata* (Moraceae) are two of the many plants whose leaves are traditionally used to treat hemorrhoids in Benin. These plants are widely used in traditional medicine and pharmacology, for their cytotoxic, antibiotic, antifungal, insecticide activities, etc. For this purpose, we were interested in their phytochemical study and the quantitative determination of their secondary metabolites, to the study of the antioxidant activity of aqueous extracts of plants and to research the family of organic compounds which would be responsible for the antioxidant activity of the leaves of *Calotropis procera* and *Ficus umbellata* used in the traditional treatment of hemorrhoids in Benin.

Materials and Methods

Plant material

The plant material consists of the leaves of *Calotropis procera* (*C. procera*) and *Ficus umbellata* (*F. umbellata*). Both plants are used in traditional medicine to treat several diseases including hemorrhoids. They are certified by the National Herbarium of Benin, and are listed in the document of the "FLORE ANALYTIQUE DU BENIN" [(Akoegninou, 2006)6]. The fresh leaves of *C. procera* are collected between october and november 2019 at the Cathedral of Our Lady of the Immaculate Conception of Porto-Novo at the geographical coordinates: ALTI: 17 m; N 06°28.303'; E002°37.143' with an accuracy of 8 m.

Fresh leaves of *F. umbellata* are also collected between october and november 2019 in Zogbadjè, Calavi (BENIN). The plant is located at the geographical coordinates: ALTI: 19 m; N W6°25.514'; E002°20.357' with the precision on 8 m.

The coordinates are taken with a GARMIN hiking GPS made in America in 2012, under the Etrex software version 3.51. The leaves of both plants are harvested, dried at the Laboratory of Pharmacognosy of Essential Oils (LaPHE) under the shelter of the sun at the temperature of 16°C, ground and then extracted. Aqueous and ethanolic extracts are used for experiments.

Methods

Ethnobotanical and ethnomedicinal inventory and selection of plants studied

This inventory was based on a literature review covering the period from January 2018 to December 2019, following three steps:

- First, surveys conducted among tradithérapeutes, market herbalist tradipraticians, medicinal plant resellers, public health and private specialists to collect information on hemorrhoidal disease and its treatment.
- Then, in order to understand the physiopathology of hemorrhoidal disease and its effects on human organs, documentary research is carried out, and previous works related to our theme are listed.
- Finally, the selection of our plants is based on studies and their accessibility.

Harvesting and preparation of the different extracts

The leaves of *C. procera* and *F. umbellata*, harvested between October and November 2019, were washed with tap water and left dripping before the study operations. A portion of the fresh leaves were used to make the 10% decoction extraction following the traditional method (E1, E2 and E5). E1: 308 g of fresh leaves of *C. procera* + 1.5 L of distilled water; E2: 308 g of fresh leaves of *C. procera* + 10 g of traditional potash + 1.5 L of distilled water and E5: 308 g of fresh leaves of *F. umbellata* + 1.5 L of distilled water.

The rest of the leaves were dried at the laboratory temperature of 16°C, then powdered and stored at -4°C. Extracts E3 and E6 are 10% decoctions of *C. procera* and *F. umbellata* powders. The ethanolic extracts E4 and E7 at 10% are obtained by maceration.

Phytochemical analysis

The phytochemical screening is based on differential staining and precipitation reactions of the main groups of chemical compounds contained in the plants according to the method of Houghton and Raman (1998) revised and adapted to the conditions of the pharmacognosy and essential oils laboratory. Several chemical groups are measured as well as the extraction of essential oils according to the same method.

Qualitative analysis of essential oils by thin layer chromatography (TLC)

The presence of essential oils detectable during the tests is confirmed by hydrodistillation during 4h with a Clevenger type apparatus from harvested plant material. The distillate is collected in a graduated tube, in the presence of xylene. 10 µL of the essential oil-xylene mixture is placed on a TLC plate. Development is done with a 95v/5v toluene-ethyl acetate solution mixture. The plate is developed by spraying with 1% vanillin and then heated to around 105°C. The number of spots present on the plant indicates the number of compounds present in the essential oil according to the method of Houghton and Raman (1998).

Determination of total polyphenols

The determination of total polyphenols was carried out according to the method of Singleton et *al*. (1999), modified. The absorbance, by reference to a standard range obtained with a phenolic acid (gallic acid) allowed to determine the quantity of total phenols present in the extract expressed in mg gallic acid equivalent/g extract. We took 125 µL of the sample which was dissolved in 625 µL of Folin-Ciocalteu reagent. After incubation for 5 min we added 500 µL of sodium carbonate Na_2CO_3 at 75 mg/mL. The vortexed mixture is incubated for 2 hours. The absorbance reading is done with a GENESYS spectrophotometer at 760 nm.

Flavonoid determination

The total flavonoid content of the plant extracts was determined by the aluminum trichloride ($AlCl_3$) method reported by Assogba (Assogba, 2016). Rutin at 1 mg/mL is used as a reference compound to make the calibration curve. We took 500 µL of $AlCl_3$ solution (2%) and added 500 µL of the sample. To this mixture we added 2 mL of water. The blank consists of 500 µL of $AlCl_3$ and 2 mL of water. The absorbance readings are taken with a spectrophotometer at 415 nm after an incubation of 15 min.

Determination of condensed tannins

The method used to determine the content of condensed tannins is the vanillin sulfur method, modified by Heimler et *al* (2006) and adapted to laboratory conditions. The concentrations of condensed tannins are deduced from the calibration ranges established with pyrogallol and are expressed as µg pyrogallol equivalent per milligram of extract. To 50 µL of each sample or standard, we added 2 mL of the sulfuric vanillin solution (2%) in ethanol. The mixture was incubated for 15 min and the absorbance was read at 500 nm.

Determination of the antioxidant activity of the extracts

The antioxidant effect of the three aqueous extracts of Calotropis procera and Ficus umbellata is determined by two methods: the DDPH method using 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and the FRAP method.

Antioxidant activity by DPPH is performed by the method of Lamien-Meda et *al*. (2008). In dry and sterile test tubes, a stock solution of concentration 1 mg/mL of the aqueous solution of the extract is prepared and diluted in geometric series of reason 2 to have different concentrations: 0.048875; 0.09775; 0.1955; 0.391; 0.781; 1.562; 3.125; 6.25; 12.5; 25 µg/mL. Then, 1 mL of each extract (diluted solution) is mixed with 1 mL of a 96° ethanolic solution of DPPH (Cm = 0.04 mg/mL). After vortexing, the mixture is incubated, left in the dark for 15 minutes at 16°C and the absorbance is measured at 517 nm with a spectrophotometer (GENESYS 6, USA). The positive control is represented by ascorbic acid (Vitamin C) which is treated under the same conditions as the tested samples.

The results were expressed as the average of three measurements. The IC_{50} value was determined for each extract. The FRAP (Ferric reducing antioxidant power) method is based on the ability of extracts to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The total antioxidant capacity of each plant extract was determined by the Hinneburg et *al*. (2006) reported by Bakasso (2009) and applied to laboratory conditions. Thus 1 mL of an aqueous solution of each extract (20 mg/mL diluted to 100 to obtain 0.2 mg/mL), of ascorbic acid, was mixed with 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 mL of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After 30 min of incubation at 50°C; 2.5 mL of trichloroacetic acid (10%) was added. The mixture was then centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was then mixed with the same volume of water and 0.5 mL of a freshly prepared aqueous $FeCl_3$ solution (0.1%) was added.

The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-200 mg/L).

The reducing power was expressed as ascorbic acid equivalents (EqAA) (mmol ascorbic acid /g dry extract) considering 1 mM equals FRAP of 1 mL of the dry extract according to the following formula:

$$C = \frac{C_2 \times D}{C_1 \times M}$$

with $\boldsymbol{C}$ = concentration of reducing compounds in mol EAA/mg dry extract; $\boldsymbol{C_2}$ = concentration of the sample read from the calibration curve; $\boldsymbol{D}$ = dilution factor of the stock solution; $\boldsymbol{C_1}$ = concentration of the stock solution; $\boldsymbol{M}$ = molar mass of ascorbic acid (176.1 g/mol). All measurements are repeated 3 times.

Correlation between polyphenol content and antioxidant properties

This study allows us to quantitatively identify the types of phenolic compounds (flavonoids and tannins) contained in the extracts and responsible for their antioxidant properties. Some previous studies have also shown that the reducing power of a compound can be a significant indicator of its potential antioxidant activity (Siddhuraju and Becker, 2007).

It has also been shown that antioxidant chemical groups such as flavonoids and tannins reduce and decolorize DPPH due to their ability to release hydrogen (Ahoton et *al*., 2019). Each type of polyphenol in the extracts is likely responsible for the antioxidant activity of these extracts.

Statistical analysis

Statistical data was processed with GraphPad Prism® software for Windows version 5.00, March 7, 2007 and Microsoft Excel® spreadsheet software version 2016.

Results

Ethnobotanical survey

Among the many medicinal plants used in the treatment of hemorrhoids in the south, in Benin, the most cited and most used are summarized in table 1 below:

Table I : Some medicinal plants used in the treatment of hemorrhoids

Families	Genus and Species	Common names
Anacardiaceae	*Mangifera indica*	Amanga tin (fongbé)
Apocynacea	*Carissa spinarum*	Ahanzo ɖɔ (fongbé)
Arecaceae	*Cocos nucifera*	Agɔnkɛ tin (fongbé)
Arecacees	*Elaeis guineensis*	Dé tin (fongbé)
Asclepiadacees	***Calotropis procera***	**Amɔn man (fɔngbé-gungbé)**
Caricacee	*Carica papaya*	Kpin tin (fongbé)
Euphorbiaceae	*Jatropha gossipifolia*	Gbaguidi pkotin vè (fɔngbé)
Fabacéae	*Pterocarpus erinaceus Poir*	Gbɛ-gbɛ (gungbé)
Lamiaceae	*Ocimum gratissimum*	Tchiáyo (fongbé)
Meliaceae	*Kaya senegalinsis*	Zunza (Caïlcédrat)
Moraceae	***Ficus umbellata***	**Voli man (gungbé)**
Portulacaceae	*Talinum triangulare*	Glace man (fɔngbé)
Rubiaceae	*Morinda lucida*	Huìnsi (fongbé)
Rutaceae	*Zanthoxylum fagara*	Hɛtin (fɔngbé)
indicum	- indicum	Kokloxù adjaxi afɔ vɛ (fɔngbé)
Pteridophyte	*Nephrolepis biserrata*	La fougère (plante parasite du dé tin)

Phytochemical analysis

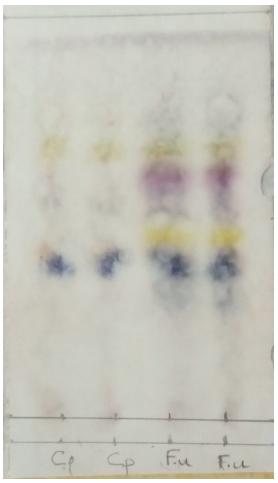
Phytochemical screening revealed the presence of traces of essential oil in the leaves of *C. procera* and *F. umbellata*. Alkaloids, tannins, flavonoids, triterpenoids and O-heterosides are present only in the leaves of *F. umbellata*. Cardenolides and coumarins are found only in the leaves of *C. procera*, and saponosides, mucilages, anthocyanins and leuco-anthocyanins, and reducing compounds are found in all extracts studied (Table II).

Table II: Chemical compounds of extracts obtained by phytochemical screening

	Leaves of Calotropis procera				*Leaves of Ficus umbellata*		
Extracts **Chemical groups**	**E1 Aqueous extract without KOH, fresh leaf**	**E2 Aqueous extract with KOH, fresh leaf**	**E3 Powder, Aqueous extract**	**E4 Powder, EtOH extract**	**E5 Aqueous extract, fresh leaf**	**E6 Powder, Aqueous extract**	**E7 Powder, EtOH extract**
Alkaloids	-	+	++	-	+	-	-
Tannins	+	+	+	+	++	++	+
Catechic tannins	+	+	+	+	++	+	+
Gallic tannins	+	-	+	+	+	+	+
Flavonoids	+	+	+	-	++	++	-
Anthocyanins	-	-	-	+	-	-	+
Leuco-anthocyanins	++	+	+	-	+++	++	+++
Quinone derivatives	-	-	-	-	+	-	-
Saponosides	++	+	+	-	+++	+++	-
Triterpenoids	-	-	+	-	-	-	-
Steroids	-	-	-	-	-	-	-
Cardenolides	+	+	+	-	-	-	-
Cyanogenic derivatives	-	-	-	-	-	-	-
Mucilages	+	++	++	-	+++	+++	-
Coumarins	+	+	-	+	-	-	-
Reducing compounds	++	++	+	++	+++	+	++
Free anthracene compounds	-	-	-	-	-	-	-
	-	-	+	-	-	+	-
	-	-	-	-	-	-	-
Essential oils	**Trace**				**Trace**		

+ : presence; ++ : average presence; +++ : strong presence; - : absence; EtOH = ethanol

Thin layer chromatography (TLC) of essential oils (EO) of *C. procera* (**Cp**) and *F. umbellata* (**Fu**) revealed the presence of four readable spots (picture 1) which will be studied later in detail.



Picture 1: TLC of EO of (Cp) and (Fu)

Total polyphenols

The content of total phenols in the three extracts is evaluated and expressed in mg gallic acid equivalent per gram of extract (mg GAE/g extract). The results show that the aqueous extract of *F. umbellata* is richer in polyphenols (377.976 mg.EAG/g extract), followed by the aqueous extract of *C. procera* without potash (139.107 mg EAG/g extract). The potash aqueous extract of *C. procera* has the lowest content of phenolic compounds (63.54 mg.EAG/g extract) (Figure 1).

Dosage of Flavonoids

The total flavonoid content is evaluated and expressed in mg Rutin equivalent per gram of extract (mg.EqRUT/g extract). The aqueous extract of *F. umbellata* records the highest content (10.972 mg.EqRUT/g extract), followed by the no potash aqueous extract of the leaves of *C. procera* (7.502 mg ERUT/g extract). The aqueous extract with potash of *C. procera* contains the lowest total flavonoid content (2.574 mg.EqRUT/g extract) (Figure 2).

Determination of condensed tannins

The content of condensed tannins is evaluated in mg pyrogallol equivalent per gram of extract (mg.EqPYR/g extract). The results show the highest value with the aqueous extract of *F. umbellata* (33.13 mg.EqPYR/g extract). The lowest content is found with the extract with potash of *C. procera* (5.555 mg.EqPYR/g extract) (Figure 3).

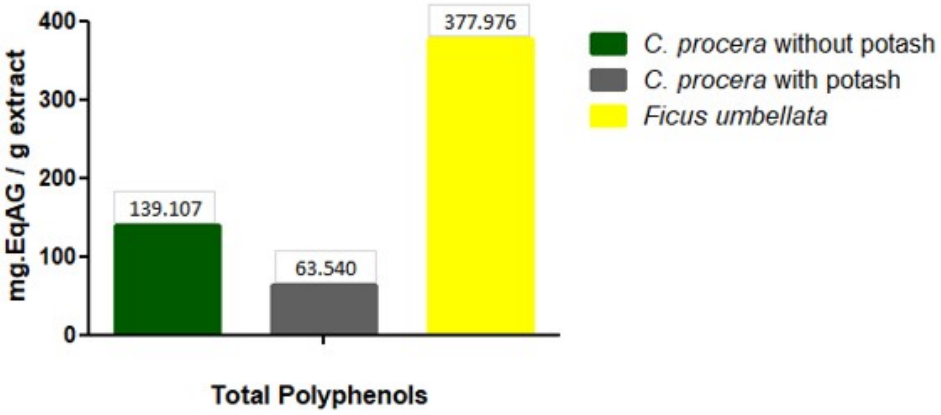


Figure 1 : Total polyphenol content of the three

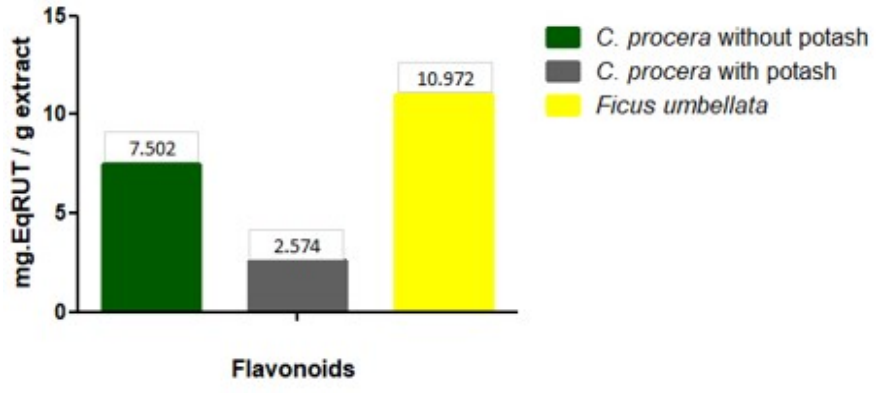


Figure 2: Flavonoid content of the three extracts

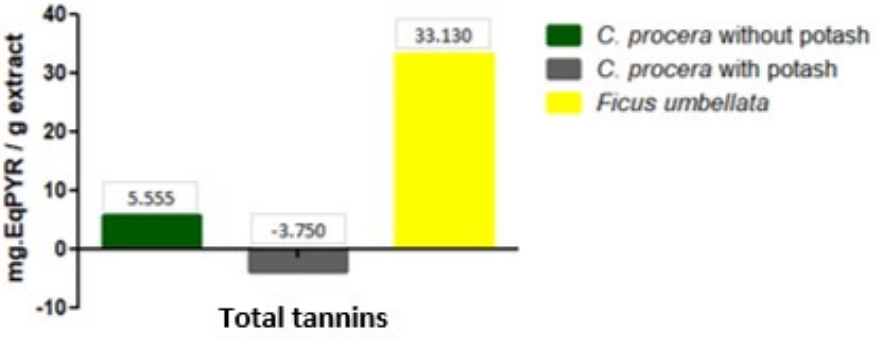


Figure 3: Condensed tannin content of the three extracts

Antioxidant activity

Determination of the IC_{50} of the extracts

The following Figure 4 shows the IC_{50} determined using half the optical density (OD) of DPPH used for each of the three extracts and ascorbic acid.

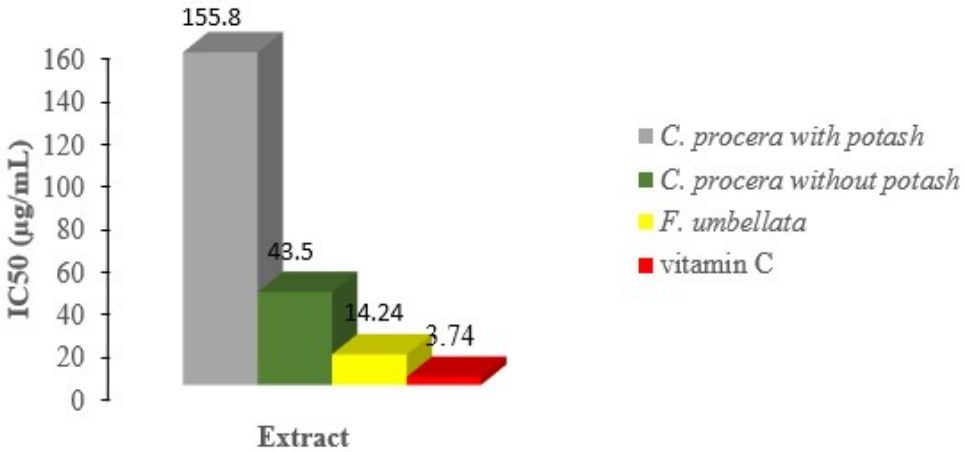


Figure 4: The IC_{50} inhibitory concentrations of the extracts

The antiradical power (APR) of each extract is given in Figure 5.

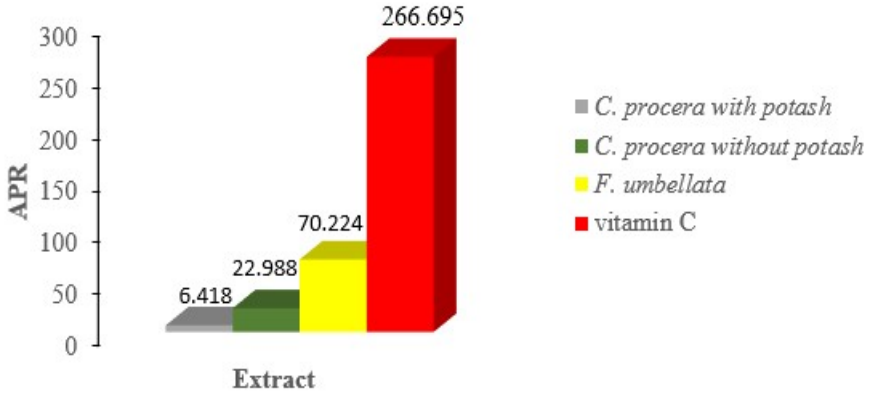


Figure 5: Evaluation of the antiradical powers (APR) of the three extracts and vitamin C

The aqueous extract of *F. umbellata* has the lowest value of IC_{50} (14.24 µg/mL), EC_{50} (356 µg/mg DPPH) and the highest value of APR (70.224) followed by the aqueous extract without potash of *C. procera* with IC_{50} (43.5 µg/mL), EC_{50} (1087.5 µg/mg DPPH) and APR of 22.988. The aqueous extract with potash of *C. procera* has the highest value of IC_{50} (155.8 µg/mL), EC_{50} (3895 µg/mg DPPH) and the lowest value of APR (6.418).

Determination of the iron reducing power (FRAP) of the extracts

The values obtained with the extracts are illustrated through figure 6. The best activity is obtained with the extract of *F. umbellata* (430.211 mmol EqAA/mg extract) followed by the extract of *C. procera* without potash (2.435 mmoL EqAA/mg extract). The extract of *C. procera* with potash studied under the same conditions as the previous ones showed no reducing power because from the calculations performed, a negative value (-146.472 mmol EqAA/mg extract) is obtained for the same concentration of extract used for each extract.

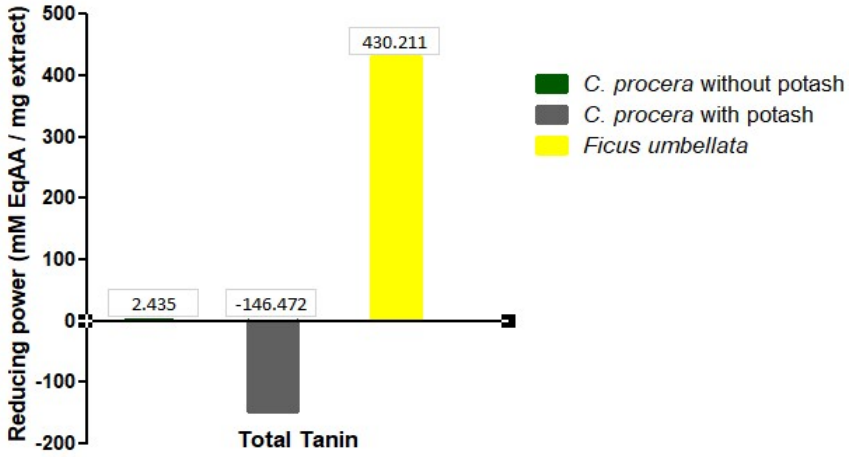


Figure 6 : Reducing power of *C. procera* and *F. umbellata* extracts

Correlation between polyphenols, flavonoids and tannins contents with antioxidant activities of *C. procera* and *F. umbellata* leaves extracts

The different results obtained show very good and significant linear correlations between polyphenol and tannin content (0.9998) and between polyphenols and flavonoids (0.8546).

Regarding the relationship between antioxidant activity and flavonoid and tannin contents (figure 7), the results showed that a very good linear correlation for flavonoids (0.9495) and a modest linear correlation for tannins (0.6764).

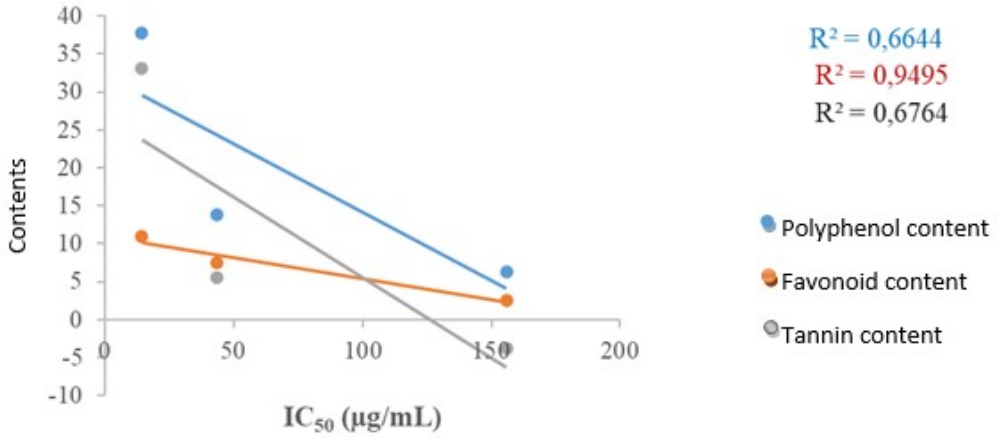


Figure 7: Correlations between IC_{50} and polyphenol, flavonoid and tannin contents of *C. procera* and *F. umbellata* extracts

Discussion

The studies carried out on the extracts of *Calotropis procera* and *Ficus umbellata* for the treatment of hemorrhoids have recorded chemical and pharmacobiological information. The ethnomedicinal survey carried out shows that the use of these plants for therapeutic purposes takes into account all their parts. In general, the most used traditional preparation is the herbal tea by decoction. The most used plant is *Calotropis procera*, according to the information collected during our meetings with traditional therapists and hemorrhoidal patients.

The phytochemical screening carried out on the fresh leaves and powders of the two plants revealed a slight variation of the compounds according to the extracts.

The aqueous extract of *Calotropis procera* powder allowed to highlight the following compounds: alkaloids, tannins, flavonoids, leuco-anthocyanins, saponosides, cardenolides, mucilages, coumarins, and reducing compounds, identified in the extracts of the fresh leaf but also others such as terpenoids and O-heterosides. These results are close to those of Belalem et *al*. (2018) concerning the study of the antioxidant activity of the flavonoid extract and of Nikema (2005) concerning the pharmacochemical properties of *Calotropis procera* (Ait). However, they are different from those found by Koko et al. in 2009 on the main galactogenic and emmenagogue plants used in the territories bordering the Pendjari hunting area (Koko et *al*., 2009). This variation in the chemical groups present in the aqueous extracts of fresh leaves *C. procera* and those of dry leaf powders would be due to the method of extraction (with or without KOH), the state of the leaves (fresh or dry), or the size of the plant material used in the extraction. Several other factors could also explain this difference, as pointed out by Deschepper in 2017 during his work on the variability of the composition of essential oils and interest of the concept of chemotype in aromatherapy (Deschepper, 2017).

Indeed, according to Sofowora (2010), reported by Assogba (2016), the composition of a plant in secondary metabolites varies according to the geographical location, the organ collected, the period or season, the time of collection and storage conditions, which brings up the notion of chemotype. This difference could also be explained by the type of solvent used for the extracts.

The ethanolic extract (96°) contains only tannins, anthocyanins and reducing compounds that were not highlighted by the different aqueous extracts. This could be explained by the fact that water extracts more chemical compounds compared to alcohol as reported again by Akakpo et *al*. (2018). The solubility of chemical compounds in plants varies depending on the type of solvent used. Therefore, 96° alcohol would not be suitable for the extraction of the majority of compounds from *C. procera*. This would explain the choice made on the aqueous solvent in the traditional use by decoction of the plant during the treatments.

The aqueous extracts by decoction of fresh leaves of *F. umbellata* contain alkaloids, polyphenols such as tannins, flavonoids, leuco-anthocyanins, saponosides, mucilages, and reducing compounds and quinone derivatives. The fresh leaves of *F. umbellata* do not contain anthocyanins, triterpenoids, steroids, cardenolides, cyanogenic derivatives, coumarins, and free anthracenics.

Ethanol (96°) was only able to extract tannins, anthocyanins, leuco-anthocyanins and reducing compounds. This solvent would therefore not be suitable to extract many chemical compounds from *F. umbellata*. This would explain the use of water for traditional herbal tea preparations based on this plant, as for *C. procera*.

The results of the phytochemical study on the different aqueous extracts of the leaves of *C. procera* and *F. umbellata* revealed that both leaves are rich in phytochemical compounds, which would justify their use in traditional medicine. These different chemical groups found in the leaves of *C. procera* and *F. umbellata* are endowed with biological activities such as antioxidant and anti-inflammatory activities (Bruneton, 1993, 2009). This result confirms one of our hypothesis (in the works) that the presence of chemical groups, thus pharmacobiological properties, would justify the use of the leaves in the traditional treatment of hemorrhoids. The use of these two plants is confirmed respectively by De Souza (2009) and by Akoegninou (2006).

The results of the general assays have allowed us to determine the content of certain chemical groups present in our different aqueous extracts of *C. procera* and *F. umbellata* leaves. The extracts of our plants are rich in polyphenols. Phenolic compounds are secondary metabolites in medicinal plants and are very important because of their contribution to the maintenance of their health, because thanks to secondary metabolites plants have means of defense or adaptation (Heimler et *al*., 2006) but also to human health because they are endowed with several activities such as antioxidant, anti-inflammatory … (Bruneton, 2009 ; Achat, 2013 ; Lamien-Meda et *al*., 2008). The extract of *F. umbellata* is richer in polyphenols than the extracts of *C. procera*.

The total tannin content is significant in the aqueous extract of the fresh leaf of *C. procera* without potash (5.555 mg.EqPYR/g extract) compared to the extract with potash (-3.75 mg. EqPYR/g extract). The traditional potash used being basic, would have reacted with the hydroxyl functions of the gall tannins of the *C. procera* plant. The highest value of tannin content is recorded in the aqueous extract of *F. umbellata* (33.13 mg. EqPYR/g extract). These results confirm our qualitative analyses of the phytochemical screening performed on the different extracts. The reduction of the functional groups of these metabolites would have an impact on the curative properties of the plant according to our theory.

The use of traditional potash for the preparation of the decoction of the fresh leaf of *C. procera* for the treatment of hemorrhoidal attacks would therefore not be a good practice, since the secondary metabolites are reduced in the extract. It seems important to draw the attention of

traditherapists who use this technique, to the destructive character of potash in the extract. Potash (in the case of potassium hydroxide) is known for its corrosive properties (Leleu and Triolet, 2003) as it reacts violently with many organic or mineral compounds. Their concentrated aqueous solutions are caustic to the skin and mucous membranes (Bonnar et *al*., 2010). Given the use of potash in treatment, it seems important to expand research to further understand its precise role in *C. procera*.

The no potash aqueous extract of leaves of *C. procera* is 3.58 times more active than the aqueous extract of *C. procera* with potash. The aqueous extract of leaves of *F. umbellata* is 3.05 times more active than the aqueous extract without potash of leaves of *C. procera* and 10.94 times more active than the aqueous extract with potash of leaves of *C. procera*. Thus, we noticed that traditional potash (6.66 g/L) decreased the antioxidant activity three times compared to the extract without potash. Comparing with the antioxidant activity of the positive control (Vitamin C), our study showed that ascorbic acid (Vitamin C) was 3.80 times more active than the aqueous extract of leaves of *F. umbellata*, 11.63 times more active than the potash-free extract of leaves of *C. procera*, and, 41.65 times more active than the aqueous extract with potash of *C. procera*.

For Lee et *al*. (1999) according to Assogba (2016), any extract with an IC_{50} less than 400 µg/mL is an extract with good antioxidant activity. In the case of our study, all our tested extracts have an IC_{50} lower than this value, which indicates that our extracts have a good antioxidant activity. These results confirm that the extracts of leaves of *Calotropis procera* and *Ficus umbellata* have antioxidant activities. The antioxidant activity of *C. procera* was proven by several authors including Nekiema (2005) and Belalem et *al*. (2019).

The results on ferric ion reduction (FRAP) showed that the *F. umbellata* extract of the studied species is more reducing than the *C. procera* extracts studied by the same method. Similarly, the extract with potash of *C. procera* lacked ferric iron reducing power compared to the extract with no.

The presence of flavonoids in plants gives them antioxidant activities, detoxification and many health promoting effects (Assogba, 2016; Gbenou et *al*., 2011 ; Prakash et *al*., 2007). Among the activities attributed to flavonoids are anti-inflammatory, hepato-protective and diuretic activities (Bruneton, 2009 ; Andriveau, 2018). Flavonoids also protect against free radicals, microbes, ulcers, viruses, sinusitis, asthma, etc. (Achat, 2013 ; Prakash et *al*., 2007). In addition, they participate in strengthening the elasticity and tightness of blood vessels. They help to improve the irrigation and dilation of the arteries and thus regulate blood pressure, and fight against the deterioration of collagen fibers, which are essential for maintaining cellular health (Achat, 2013). They are reputed to protect against various chronic affections (cardiovascular diseases, cancers) and are used in the treatment of hemorrhoidal crises (Futura Sante, 2018). Their presence in our extracts, and the good linear correlation (0.9495) highlighted with the antioxidant activity for (0.6764) for tannins, would justify their use by phyto-therapists in the treatment of hemorrhoidal attacks. These various properties of flavonoids would be what motivated pharmaceutical companies to develop anti-hemorrhoidal drugs whose active ingredients are mostly flavonoids (Andriveau, 2018). Tannins, belonging to the flavonoid family, have mainly astringent properties, but also antioxidant, antibacterial and sometimes calming properties, and are mainly used externally, especially against wounds, sores or hemorrhoids. This would prove the interest of their use as active principle of some anti-hemorrhoidal drugs (Andriveau, 2018).

Conclusion

Our study allowed us to determine the phytochemical groups of leaves of *Calotropis procera* and *Ficus umbellata* used in the traditional treatment of hemoroids in Benin. The secondary metabolites necessary to fight the disease are listed in the family of polyphenolic

compounds such as flavonoids. The aqueous extracts of the studied medicinal plants contain compounds with phytochemical groups useful for the treatment of hemorrhoids. The quantitative assay allowed us to detect the aqueous extract of *F. umbellata* leaves as the extract richest in phytochemicals and would be the most effective in treating hemorrhoids. The inhibitory effect of traditional potash on the activity of the aqueous extract of *C. procera* traditionally used for the treatment of hemorrhoidal pain is revealed. The DPPH test allowed us to classify the aqueous extract of *Ficus umbellata* as the extract with the highest antioxidant activity, and the correlation study made it possible to identify Flavonoids as precursors of this activity. Ferric ion reduction (FRAP) showed that the *F. umbellata* extract of the species studied is more reducing than the extracts the extracts of *C. procera*.

References

1. Morgado, JP., Suarez, AJ., Gomez, LG., Morgado, JP Jr. 1988. Histoclinical basic for a new classification of hemorrhoidal disease. *Dis Colon Rectum*, 31(6): 474-480.
2. Vignon, RK., Kodjoh, N., Hountondji, A., Eroume, A., Akadiri, R. 2011. Thromboses hemorroïdaires externes asymptomatiques révélées par une boule anale. Le Bénin Médical N° 48.
3. Boureima, D. 2005. Connaissances, Attitudes et Pratiques Comportementales liées aux Hémorroïdes dans le Service de Chirurgie Générale du CHU Gabriel Touré et auprès des Thérapeutes Traditionnels au Mali. Faculté de Médecine de Pharmacie et d'Odonto-Stomatologie, Université de Bamako.
4. Siegfried, DD., Patrice Brice, MO., Deli, V. 2015. Ethnobotanique des plantes médicinales anti hémorroïdaires des marchés et villages du Centre et du Littoral Cameroun. *Journal of Applied Biosciences*, 96: 9072-9093. http://dx.doi.org/10.4314/jab.v96i1.5
5. Drissa, S. 2008. La maladie hémorroïdaire interne dans les centres d'endoscopie digestive du CHU Gabriel Touré et des cabinets médicaux. Faculté de Médecine, de Pharmacie et d'Odonto-Stomatologie, Bamako.
6. Akoegninou, A. 2006. Flore analytique du Benin. Cotonou: éditeur, Backhuys.
7. Houghton, JP., Raman, A. 1998. Laboratory handbook for the fractionation of natural extract. *Pharmacogn Res Lab Dep Pharm King's Coll Lond*.
8. Singleton, VR., Lamuela-Raventos, RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 299:152-79.
9. Assogba, MF. 2016. Phytothérapie et propriétés pharmacobiologiques d'extraits des feuilles de *Eleais guineensis* Jacq (Arecaceae). Thèse de Doctorat Unique, d'Abomey-Calavi.
10. Heimler, D., Vignolini, P., Din, MG., et *al*. 2006. Antiradical activity and polyphenol composition of local Brassiccaceae edible varieties. *Food Chemistry*. 99:464-9.
11. Lamien-Meda, A., Lamien, CE., Compaoré, MMY., Meda, RNT., Kiendrebeogo, M., Zeba, B., et *al*. 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. *Mol Basel Switz*. 13: 581-94.
12. Hinneburg, I., Damien, DHJ., Hiltunen, R. 2006. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.* 97: 122–129.
13. Bakasso, S. 2009. Etudes phytochimiques et potentialités biologiques de cinq espèces *d'indigofera* (fabaceae) utilisées en médecine traditionnelle au Burkina Faso. Docteur Es Sciences Biologiques Appliquees 2009, Université de Ouagadougou. https://www.secheresse.info/spip.php?article70721

14. Siddhuraju, P., Becker, K. 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chem*. 101: 10–19.
15. Ahoton, D., Assogba, MF., Ladekan Yayi, EC., et *al*. 2019. Phenolic compounds' dosage and antioxidant activities of aqueous and ethanolic extracts of *Lophira lanceolata*'s Van Tiegh. ex Keay (Ochnaceae) leaves. *Int J Phytopharm*. 9: e5262–e5262.
16. Belalem, MA., Labed, B., Darraji, H., Bouhrira, A., Reggani, A., Kebbab, RM., et *al*. 2018. Study of the antioxidant activity of flavonoic extract of a medicinal plant (*Calotropis procera*) of *Ahaggar tamanrasset*. *Journal of Fundamental and Applied Sciences*, 11(1): 315-324.
17. Nikiema, WPR. 2005. Propriétés pharmacochimiques de *Calotropis procera* ait. (Asclepiadaceae) recolte au mali : étude préclinique des effets anti-inflammatoires et antimicrobiens des extraits des écorces de racines. Bamako, Faculté de Médecine de Pharmacie et d'Odonto-Stomatologie.
18. Koko, KD., Djego, J., Hounzangbe-Adote, H., Sinsin, B. 2009. Etude ethnobotanique des plantes galactogènes et emménagogues utilisées dans les terroirs riverains à la Zone Cynégétique de la Pendjari. *Int J Biol Chem Sci*., 3(6): 1226-1237.
19. Deschepper, R. 2017. Variabilité de la composition des huiles essentielles et intérêt de la notion de chémotype en aromathérapie. 172. ⟨dumas-01515314⟩.
20. Sofowora, A. 2010. Plantes médicinales et médecine traditionnelle d'Afrique by Abayomi Sofowora*(2010-03-10)*. Karthala.
21. Akakpo, BH., Akpovi, CD., Medehouenou, TCM., Assogba, FM., Dansou, PH., Gbenou, JD. 2016. *Conyza aegyptiaca* (L.) Dryand ex. Aiton extracts exhibite antioxidant activity and prevent hepatic glucose liberation *in vitro*. I*nt J of Biosci*. 431-9.
22. Bruneton, J. 1993. Plantes toxiques végétaux dangereux pour l'Homme et les animaux. 2e ed.
23. Bruneton, J. 2009. Pharmacognosie, phytochimie, plantes médicinales. 3e ed. Paris: Technique et Documentation Lavoisier.
24. De Souza, S. 2008. Flore du Bénin. 2e ed. Bénin: Imprimerie TUNDE.
25. Achat, S. 2013. Polyphénols de l'alimentation: extraction, pouvoir antioxydant et interactions avec des ions métalliques. Thèse de doctorat en Biologie, option Sciences Alimentaires. université d'Avignon, p261.
26. Lamien-Meda, ALCE., Compaoré, MM., Meda, RN., et *al*. 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. *Molecules*. 13:581-94.
27. Leleu, J., Triolet, J. 2003. Réactions chimiques dangereuses. France: INRS. p308. https://www.inrs.fr/dms/inrs/CataloguePapier/ED/TI-ED-697/ed697.pdf
28. Bonnard, N., Brondeau, MT., Jargot, D., et *al*. 2020. Hydroxyde de potassium et solutions aqueuses. 8 April 2020, www.inrs.fr/fichetox (access April 8th, 2020).
29. Lee, K-K., Kim, J-H., Cho, J-J., et *al*. 1999. Inhibitory Effects of 150 Plant Extracts on Elastase Activity, and Their Anti-inflammatory Effects. *Int J Cosmet Sci*, 21: 71–82.
30. Belalem, M., Labed, B., Derradji, H., Bouhrira, A., Reggani, A., Kebbab, R., et *al*. 2019. Study of the antioxidant activity of flavonoic extract of a medicinal plant (*Calotropis procera*) of *Ahaggar* Tamanrasset. *J Fundam Appl Sci*., 11(1): 315-324.
31. Gbenou, JD., Ahounou, JF., Ladouni, P., Agbodjogbé, WKDD., Dansou, P., Moudachirou, M. 2011. Propriétés antiinflammatoires des extraits aqueux de *Sterculia setigera* Delile et du mélange *Aframomum melegueta* K. Schum-*Citrus*

aurantifolia Christm et Panzer. *Int J Biol Chem Sci*. 5: 634-41.
32. Prakash, D., Upadhyay, G., Singh, NB., Singh, HB. 2007. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). *Food Chem.* 104(2): 783-790.
33. Andriveau, M. 2018. Les médicaments hémorroïdes : Quels sont les meilleurs ? les-hemorroides.com (accessed February 7th, 2020).
34. Futura Sante. Les bienfaits des flavonoides, www.futura-sciences.com, (accessed February 7th, 2020).

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