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Medicinal Plants used in the Treatment of Hepatitis in Bobo-Dioulasso: Studying the Availability and Analyzing the Phytochemical Properties of *Combretum micranthum* G. Don and *Entada africana* Guill. et Perr.

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Abstract

This paper focuses on studying the inventory of the medicinal plants used in hepatitis care in Bobo-Dioulasso and evaluating their availability in local vegetation and their phytochemical properties. To achieve this objective, several approaches were developed which include: (1) an ethnobotanical survey among 111 traditional health practitioners (THP); (2) a dendrometric study to evaluate the abundance and spatial distribution of these species in Dindérésso Classified Forest; and (3) an evaluation of polyphenolic compounds and antioxidant activity of the two most quoted species roots using three methods such as anti-DPPH*, anti-FRAP, and anti-ABTS. The results show that *Entada africana* and *Combretum micranthum* were the most quoted species among 40 species used in the treatment of liver disease. The availability study revealed that juvenile and adult individuals of E. africana are frequent in woody savannah, shrubby savannah, and grassy savannah. As for juvenile and adult *C. micranthum* individuals, they are only frequent in wooded savannah (RI<60%). These populations are therefore declining due to anthropogenic pressure. Phytochemical analysis reveal polyphenols contents of 37.91 and 20.71 mg EAG/100 mg respectively for *C. micranthum* and *E. africana* and flavonoids contents of 0.85 ± 0.09 and 0.66 ± 0.05 mg EQ/100 mg respectively for *C. micranthum* and *E. africana*. Finally, the results show that there is an anti-oxidant activity for the two species. There were about 198 and 13 µmol EAA/g for the two species i.e.,

ABTS and DPPH, respectively. The results show that the antioxidant activity could partially justify the traditional use of this plant.

Keywords: Hepatitis, Ethnopharmacology, Traditional medicine, Phytochemistry

Introduction

Liver disease or hepatitis is a major global public health problem (WHO, 2016). The most crucial are viral and alcoholic hepatitis according to the World Gastroenterology Organization (WGO, 2012). Viral hepatitis is the most common and widespread hepatitis in the world (Guinnin *et al.*, 2015). According to the Francophone alliance of health actors in the fight against HIV and chronic viral infections, they are more dreadful than the three major pandemics (HIV, tuberculosis and malaria) in terms of the number of infections and deaths they cause (AFRAVIH, 2018). Worldwide, about 328 million people are infected annually and 1.4 million people die annually from these viral hepatitis infections (WHO, 2016). In Africa, about 19 million people are infected by hepatitis C virus and 75 million people suffer from hepatitis B, including 1.9 million people in Burkina Faso (WHO, 2016; Meda *et al.*, 2018). Although these diseases are treated by modern medicine, people still resort to traditional medicine for their cure (Sourabié *et al*., 2012).

According to the Centre for Economic and Social Policy Analysis (CAPES, 2004), 90% of the Burkinabè population uses traditional medicine and pharmacopoeia for treatment. This is because of the country's inadequate health and pharmaceutical coverage and the inaccessibility to modern treatments by financially deprived populations (Zerbo *et al*., 2007). Ethnobotanical and pharmacognosic research is needed to document and thus perpetuate this traditional knowledge of health management using local plants. In this context, various scientific works on hepatoprotective plants have been undertaken. Some have covered Africa (Bitsindou *et al*., 1993), while others have focused on smaller areas in the sub-region such as Benin and Mali (Sangaré, 2005; Sangaré *et al*., 2012; Guinnin *et al*., 2015). However, in Burkina Faso, few studies have been conducted on these medicinal plants used in the traditional treatment of liver diseases. Also, plants natural antioxidants have the property to trap free radicals produced in excess due to the attack of liver cells by viruses, thus limiting and/or repairing liver damage (Twedt, 2006). Hence, the interest in evaluating the natural substances of medicinal plants traditionally used against hepatitis has grown significantly.

Moreover, the craze for plants, combined with the lack of official texts regulating the collection of medicinal plants, increase the pressure on

vegetation. In addition, the fragility of the ecological balance requires that particular attention should be paid to the rational management of renewable natural resources, in general, and medicinal species in particular (Yelkouni, 2004). Therefore, in addition to ethnobotanical and pharmacognosic work, investigations must be carried out on the diagnosis of the state of the populations of these medicinal species for their sustainable management. The general objective of this study is to contribute to the knowledge of plants used in hepatitis treatment by evaluating photochemistry properties and their availability. Specifically, it is about:

- inventorying the medicinal species used in the hepatitis treatment by the traditional health practitioners of Bobo-Dioulasso,
- determining the availability of these species in the Dindérésso classified forest,
- measuring the total polyphenols and flavonoids of the two species and their antioxidant power.

Material and Methods

Plant Material: The roots of *Entada africana* and those of *Combretum micranthum* were harvested in October 2019 in the classified forest of Dindérésso (Bobo Dioulasso). Also, it was identified in the Laboratory of Biology and Plant Ecology of the Nazi BONI University (NBU). They were firstly washed and dried in the chemistry laboratory (room temperature), and made into powder. This activity was carried out in the Laboratory of Research and Teaching in Animal Health and Biotechnology (LARESBA).

Solvent and Reagents: The solvents used are analytical grade methanol and distilled water. The reagents are: DPPH (1,1-diphenyl-2-picrylhydrazyl), phosphate buffer, potassium hexacyanoferrate [$K_3Fe(CN)_6$], trichloroacetic acid, trichloroferrate [$FeCl_3$], potassium persulfate, ABTS (2,2'-azinobis-[3- ethylenzothiazoline-6-sulfonic acid]) for evaluation of antioxidant activity, and Follin-ciocalteu and sodium carbonate $AlCl_3$ (aluminum chloride) for the determination of total phenolics and flavonoids.

Extraction: A mass of 15 g of vegetable powder from each sample was weighed and loaded into extraction cartridges and placed in a Soxhlet. A volume of 200 mL of methanol was placed in the extraction flask and the temperature was maintained at 70°C. This operation lasted at least 4 hours for each sample. After then, the extract was concentrated and placed in empty petri dishes previously labelled and weighed. The crude extracts thus obtained were then placed in the open air to evaporate the solvent and produce a dry residue.

Medicinal Plants Inventory: The ethnobotanical survey was used to select the two most quoted species on which availability and phytochemistry

studies were conducted. For the ethnobotanical survey, we used ethnobotanical survey sheets to collect the different information from the interviewees. This survey was carried out on four (4) groups of traditional health practitioners in the city of Bobo-Dioulasso, namely: the Provincial Association of Traditional Practitioners of "Houet" (Group 1), the Association of Traditional Practitioners and Herbalists (Group 2), the Association of Dozos of "Houet" (Group 3), and the National Federation of Traditional Practitioners of Bobo Dioulasso (FENATRAB) (Group 4). We interviewed the Traditional Health Practitioners, having agreed to answer our questions, and having at least ten (10) years of experience in the use of plants in traditional medicine and pharmacopoeia. The approach used was the semi-structured interview in the local language Dioula. The data collected covered their knowledge of hepatitis, the names and parts of the plants they use to treat hepatitis, and their years of experience as a Traditional Health Practitioners. For species identification, several flora were used (Maydell, 1992 ; Arbonnier, 2000 ; Guinko, 2009).

Availability: At the end of the ethnobotanical surveys, the two most quoted species were selected and their abundance was evaluated in the Dindérésso Classified Forest (Figure 1). Dindérésso Classified Forest is a natural reserve near Bobo-Dioulasso with a significant floristic potential. The stratified random sampling inventory method was adopted. Indeed, this method has several advantages, including the possibility of all vegetation units to be sampled. The dendrometric inventories of the selected species were carried out during the month of December 2019. Data collection plots were installed in the different plant formations. The size of the plots we used is 900 m^2 (30 m x 30 m). It was used by Ouédraogo *et al*. (2009), Bognounou *et al.* (2009), and Sop *et al*. (2010). Diameter measurements of trees were made at chest height (DBH), i.e., at 1.30 m above ground level for trees using the forest compass.

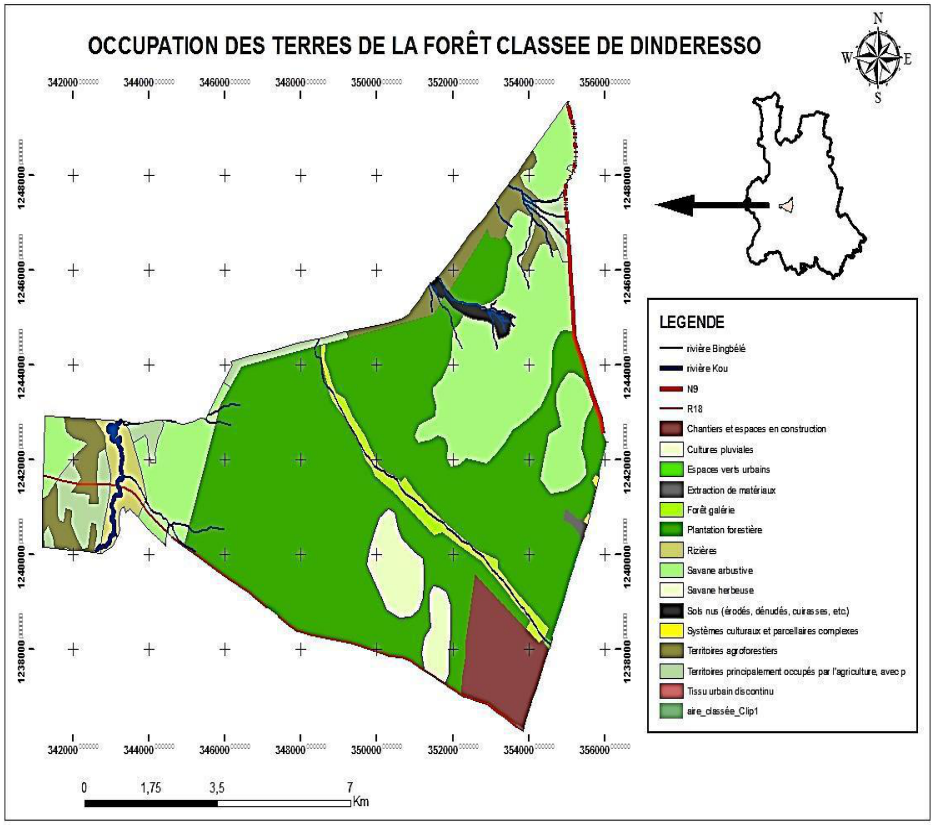

Figure 1: Land occupation of Dindérésso's classified forest (DCF). DCF land use map (Bahiré, 2016)

Antioxidant Activity Determination

Stock solution of 10 mg/mL concentration was prepared for various determinations and tests.

DPPH Method: The different stock solutions of the extracts (10 mg/mL) were diluted to the hundredth in methanol to have a test concentration of 100 µg/mL. Thus, in three (3) test tubes, 375 µL of the diluted solution and 750 µL of a DPPH solution (20 mg/L) were introduced and incubated for 15 min in the dark. A blank was prepared with 375 µL of the sample and 750 µL of methanol. Absorbances and concentrations were read with a spectrophotometer (Thermo Fisher, GENESYS 30, Serial number: 9A1W198106) at 517 nm against a standard ($y=-2.224\cdot10^{-2}x + 0.348$; $R^2=0.9966$) obtained from ascorbic acid. The method used is described according to the protocol of Meda *et al*. (2010).

Iron (III) to Iron (II) Activity (FRAP)

The method used is described according to the protocol of Meda *et al.* (2010). Stock solutions (10 mg/mL) were diluted to the hundredth in distilled water to give a final test concentration of 100 µg/mL. In 3 test tubes, 0.5 mL of the diluted solution and 0.5 mL of distilled water were added to another tube for the blank. A volume of 1.25 mL phosphate buffer (0.2M; pH 6.6) and 1.25 mL potassium hexacyanoferrate [$K_3Fe(CN)_6$] were added to these tubes. This was heated in a water bath at 50°C for 30 min. After this operation, a volume of 1.25 mL trichloroacetic acid (10%) was added and the mixture was centrifuged at 3000 revolutions per minute for 10 min. 0.625 mL of the supernatant were removed from each tube and added to tubes containing 0.625 mL of distilled water. Freshly prepared trichloroferrate [$FeCl_3$ (0.1%)] 125 µL was added to the resulting mixture. The solution obtained was stirred and then run through a spectrophotometer for a series of three (3) absorbance and concentration readings at a wavelength of 700 nm against a standard ($y = 3.270.10^{-3}x$; $R^2 = 0.9990$) established from ascorbic acid.

ABTS Radical Cation Decolorization Assay

ABTS Method: The method used is described according to the protocol of Coulidiati *et al.* (2010). For each extract, a methanolic solution (10 mg/ mL) was diluted 100-fold in distilled water. 10 µL of sample (diluted solution) were taken and then mixed with 990 µL of the fresh solution from $ABTS^{\bullet+}$. The whole was incubated in the dark for 15 minutes. Absorbances and concentrations were read 3 times at a wavelength of 734 nm with the spectrophotometer against a standard curve established from ascorbic acid ($y = -7.874.10^{-4}x+0.709$; $R^2=0.9993$).

Polyphenols Quantification

Total Phenolics (Bangou *et al*., 2019): Stock solutions were diluted 1/100 with distilled water to a concentration of 100µg/mL. 0.125mL of this solution was taken and 0.625mL of follicle reagent were added. After 5 min, 0.5mL of sodium carbonate were added and incubated for 2h in the dark. The blank was prepared with 0.125mL of distilled water. Measurement was made with the spectrometer.

Total Flavonoids (Bangou *et al*., 2019): We used the same dilution principle to obtain a final concentration of 100µg/mL. A total of 4 tubes were prepared into which a volume of 375µL of the diluted solution of each sample was introduced. We added to the first three tubes 750µL of $AlCl_3$, and it was incubated for 10min in the dark. The 4th tube considered as a control received 750µL of methanol.

Data Analysis

All of the data collected was entered in the Microsoft Excel spreadsheet version 2013, which was also used to calculate the different parameter values.

Ethnobotanical Study: The frequency of citation (Fc) of each plant was determined by the following formula:

$$\boldsymbol{Fc = \frac{Nc}{Nt} \times 100}$$

Nc: number of citations of the plant considered and Nt: total number of people surveyed.

Availability Study: In addition to the Excel software, The XLSAT software version 2007 made it possible to make the various analysis to be specific to this activity. The following parameters, used to analyze the structure of the species, were calculated via Excel:

- The density (D), in individuals/ha, of each species for each plant formation:

$$D = n/s$$

- n: number of individuals of the species considered; s: surface of the plot in ha.The mean diameter (DM) of each species for each plant formation:

$$DM = \frac{\sum_{t=1}^{t} di}{T}$$

With di: the diameter of the tree measured at 1.30 m from the ground and T: the total number of individuals of the species.

- The basal area (G) of each species for each plant formation

G=($\boldsymbol{\pi di^2/4}$).

With di: the diameter of the tree measured at 1.30 m from the ground.

To establish the distribution in class intervals, all individuals of the inventoried species were collected in five (5) class intervals with a diameter of 5 cm ([5-10]; [10.1-15]; [15.1-20]; [20.1-25] and >25 cm).

The subdivision of the juvenile population into height classes highlights the problem of the development of woody species (Steven, 1984). To this end, the demographic structure of the juvenile population in the regeneration stratum was analyzed on the basis of height classes: [0-0.5]; [0.51-1]; [1.1-1.5]; [1.51-2]; and >2 m.

The availability of the species used was assessed on the basis of surveys carried out on 40 plots. For this purpose, a Rarity-weighted Richness Index was calculated according to the equation:

$$RI = \left[1 - \left(\frac{ni}{N}\right)\right] \times 100$$

With RI: the rarity index, ni: number of records in which species *i* is present, and N: total number of records.

Rarity classes are constituted using the scale proposed by Traoré et al. (2011).

RI < 60%: Very frequent species in the plant formation.

60 ≤ RI < 80%: Medium-frequency species in plant formation.

RI ≥ 80%: Rare species in plant formation.

Results and Discussion

Ethnobotanical Study: A total of 111 people from 11 ethnic groups (bobo, dioula, mossi, San, wolfo, tiéfo, dafi, senoufo, bissa, dogosè, and toussian) were interviewed. The youngest in the field had 10 years of experience and the oldest had 60 years of experience (Table I). Both sexes were represented with approximately the same degree, i.e., 52% against 48% for the female sex. The similarity of the proportions of the two genders is explained by the fact that the majority of herbalists were women, who most often settle in the market place. Thus, this is a group we have taken into account in this study. They belonged to different ethnic groups, of which the most represented were the bobos (26%), the dioulas (23.5%), and the mossis (17%). The ethnobotanical survey also identified 40 species that were used in the treatment of hepatitis in Bobo-Dioulasso. These species were distributed among 23 botanical families (Table II). Table II presents these species, their parts used, and how they are used. The use of these species for this care would mean that these species possess biological activities, specifically antioxidant, antiviral, and hepatoprotective activities. The most represented botanical families are Combretaceae (15%), Mimosaceae (13%), and Caesalpiniaceae (10%). Depending on the group surveyed, different species have been identified with citation frequencies that differ from one group to another (Figure 2). From this figure, it appears that the species *Combretum micranthum* and *Entada africana* are regularly cited. Indeed, out of the four groups surveyed, all three cited these two species, compared to the other species that are cited by one or two groups. This could mean that these two species are commonly the most used in the treatment of hepatitis in Bobo-Dioulasso. Furthermore, their use in traditional medicine in the treatment of hepatitis is reported in various studies in Burkina Faso, Mali, Benin, and Africa in general (Bitsindou *et al.*, 1993; Sangaré, 2005; Bangou *et al.*, 2011; Sangaré *et al.*, 2012; Guinnin *et al.*, 2015). This guided our choice of these species for the two other activities of the study, namely: the availability study and phytochemical analysis.

Table I: Overall socio-demographic characteristics of respondents (N=111)

Variable	Categories	Staff	percentage (%)
Genre	Man	58	52
	Woman	53	48
number of years' experience	[10-20]	80	72
	]20-30]	16	14
	]30-40]	11	10
	]40-50]	2	2
	]50-60]	2	2
ethnic groups	Bissa	4	4
	Bobo	29	26
	Dafi	7	6
	Dioula	26	23,5
	Dogosè	5	4,5
	Mossi	19	17
	Samoa	5	4,5
	Senoufo	7	6
	Tiéfo	5	4,5
	Toussian	2	2
	Wolfo	2	2

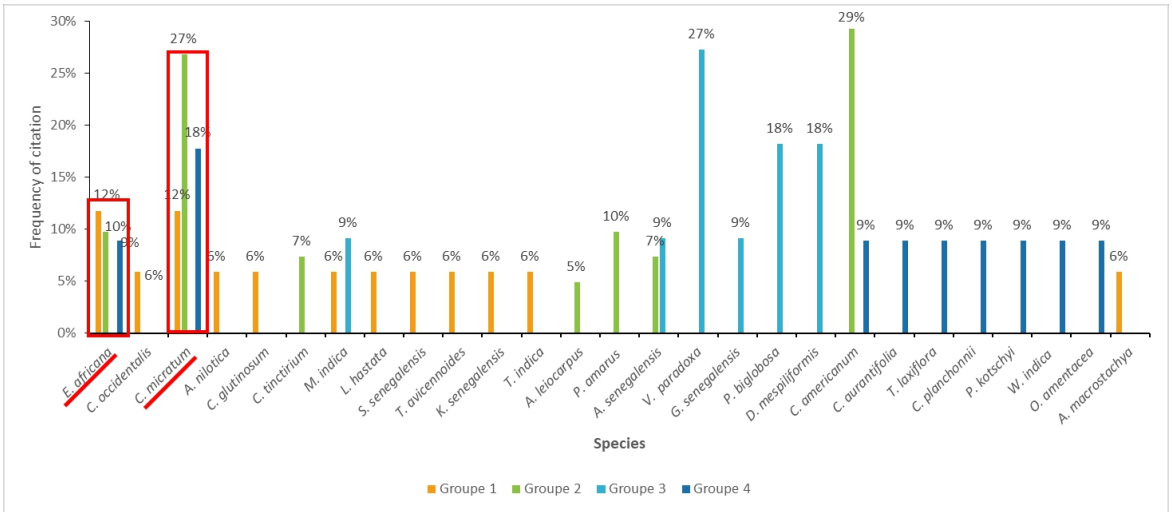

Figure 2 : Frequency of citation of species used in the treatment of hepatitis in Bobo-Dioulasso

As for the used parts of the plants, the leaves were the most commonly used (36%), followed by the bark of the trunk (23%), the roots

(23%), and fruits (10%) (Figure 3). The used parts of *C. micranthum* and *E. africana* were mainly the roots. This could mean that it is their roots that possess the hepatoprotective power sought in these plants. However, the removal of these organs represents a danger to the survival of these species. Indeed, the woody plants that intervene in health care through organs such as bark and roots are doomed to disappear because of bad harvesting practices (Bognounou *et al*., 2009). It is therefore imperative to find alternatives to limit the exploitation of these organs, with a view to sustainable management of these species.

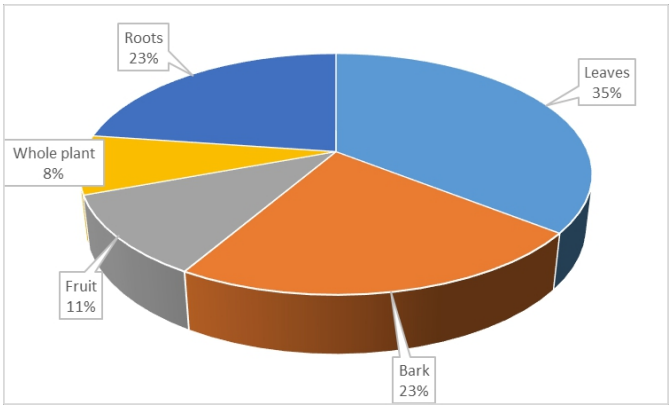

Figure 3 : Proportion of plant parts used

Availability Study

Species Abundance: Table III shows the densities obtained in adult and juvenile stands of *E. africana* and *C. micranthum* in the different plant formations. In general, the adult stand of *E. africana* was relatively denser in the grassy savannah with 107 individuals/ha and that of *C. micranthum* was densely represented in the wooded savannah with 56 individuals/ha. As for the density of the juvenile stand, the juvenile feet of *E. africana* were densely present in wooded and grassy savannah with 373 and 429 individuals/ha respectively for each plant formation. For that of *C. micranthum*, juvenile individuals were densely present in wooded savannah with 1941 individuals/ha. Hence, these two species are not present in gallery forests.

Table II : List of medicinal species identified in the ethnobotanical survey for the treatment of hepatitis

Family	Species	Used parts	Mode of preparation	Mode of administration
Anacardiaceae	*Mangifera indica* L.	Leaves	Decoction	Oral use
	Sclerocarya birrea A. Rich) Hochst	Bark		Drink ; Bath
	Lannea microcarpa Engl. et K. Krause			Oral use
Annonaceae)	*Annona senegalensis* Pers.	Leaves	Decoction	Oral use

Apocynaceae	*Saba senegalensis* (A. DC.) Pichon	Fruit	Powder	
Asclepiadaceae	*Leptadenia hastata* (Pers), Decne.	Leaf root	Decoction	
Asteraceae	*Chrysanthellum indicum* DC.	Whole Plant	Infusion	
Bombacaceae	*Adansonia digitata* L.	Bark	Powder	
Burseraceae	*Commiphora africana* (A. Rich.) Engl.	Leaves	Decoction	
Caesalpiniaceae	*Detarium microcarpum* Guill et Perr.	Bark	Decoction, Powder	Oral use
	Swartzia madagascariancis Desv.	Roots	Powder	
	Cassia occidentalis L.	Leaves		
	Tamarindus indica L.	Roots	Decoction	
Caricaceae	*Carica papaya* L.	Fruit	Decoction	Oral use
Cochlospermaceae	*Conchlospermum tinctirium* Perr. Ex A. Rich.	Roots	Decoction, Powder	
	Cochlospermum planchonnii			
Combretaceae	*Combretum micrantum* G. Don	Roots	Decoction Powder	Oral use
	Combretum glutinosum Perr. ex DC.	Whole Plant	Powder	
	Anogeissus leiocarpus (DC.) Guill. & Perr.	Leaves	Decoction	Oral bath ; Seat bath
	Guiera senegalensis J. F. Gmel.	Roots	Infusion of Powder	Oral use
	Terminalia laxiflora Engl.		Decoction	
	Terminalia avicennoides Guill. et Perr.	Bark	Decoction Infusion	
Euphorbiaceae	*Philantus amarus* Shumach. et Thonn.	Whole Plant	Decoction, Powder	Oral use
Ebenaceae	*Diospyros mespiliformis* Hochst. ex A. DC	Bark	Powder Infusion	
Fabaceae	*Pterocarpus lucens* Gull et Perr	Roots	Decoction	
Meliaceae	Khaya *senegalensis* (Desr.) A. Juss.	Bark		
Meliaceae	*Pseudocedrela kotschyi* (Schweinf.) Harms			
Mimosaceae	*Entada africana* Guill. et Perr.	Roots	Decoction, Powder	
	Acasia nilotica (L) Willd. ex Del.	Seeds	Decoction	
	Parkia biglobosa (Jacq.) R. ex G. Don	Bark	Infusion	
	Prosopis africana Guill. Perr. et Rich.		Decoction	
	Accacia macrostachya Reichenb. ex Oc.	Leaves		
Lamiaceae	*Hyptis spicigera* Lam.	Leaves	Decoction	Oral use
Opiliaceae	*Opilia amentacea* (Guili. et Perr) Endl. ex Walp.			
Palmae	*Hyphaena thebaica* (L.) Mart.			
Poaceae	*Oxytenanthera abyssina* (A. Rich.)			

	Munro		
Rutaceae	*Citrus aurantifolia* (Chnstm.) SWingle	Fruit	Infusion
Sapotaceae	*Vitellaria paradoxa* C. F. Gaertn.	Leaves	Powder Infusion
Sterculiaceae	*Sterculia setigera* Del.		Decoction
	Walteria indica L.	Whole Plant	

Table III: Density of adult and juvenile individuals of both species

plant formation type	*Entada africana*		*Combretum micranthum*	
	Number of adult plants / ha	Number of juvenile plants / ha	Number of adult plants / ha	Number of juvenile plants / ha
Forest gallery	00.00	00.00	00.00	00.00
Wooded savannah	66.70	373.33	56.30	1941.33
Shrubby savannah	25.93	186.67	4.94	355.56
Grassy savannah	107.07	429.09	5.05	123.64

Rarity Index of Adult Species: Rarity indices (Table IV) revealed that adult individuals of *E. africana* were very common in wooded (RI=27%), shrubby (RI=44%), and grassy (RI=9%) savannahs. As for those of *C. micranthum*, they were very frequent only in wooded savannah (RI=47%). As for the juvenile stratum, Table V shows that juvenile individuals of *E. africana* were very frequent in grassy savannahs (RI=55%) and moderately frequent in wooded (RI=60%) and shrubby (RI=70%) savannahs, while those of *C. micranthum* were only very frequent in wooded savannahs (RI=50%) (Table IV).

Table IV: Frequencies and Rarity indices of adult individuals of the two species

Formation	*Entada africana*		*Combretum micranthum*	
	Frequency (%)	Rarity Index (%)	Frequency (%)	Rarity Index (%)
Forest gallery	0	100	0	100
Wooded savannah	73	27	53	47
Shrubby savannah	56	44	11	89

Table V: Frequencies and Rarity indices of juvenal individuals of the two species

Formation	*Entada africana*		*Combretum micranthum*	
	Frequency (%)	Rarity Index (%)	Frequency (%)	Rarity Index (%)
Forest gallery	0	100	0	100
Wooded savannah	40	60	50	50
Shrubby savannah	30	70	10	90
Grassy savannah	45	55	10	90
Grassy savannah	91	9	9	91

Analysis of these different parameters show that adult and juvenile individuals of *E. africana* were denser in the grassy savannah, with 107 and 429 individuals/ha respectively, when compared to the other plant formations of the Dindérésso Classified Forest. It is therefore easier to find those species in the said formation. Its absence in gallery forests could be explained by its ecology. Concerning *C. micranthum*, adult and juvenile individuals were abundant in the wooded savannah. Considering the average density of juvenile individuals higher than that of adult individuals, both species had a good regeneration capacity in the two plant formations indicated (Taïta, 1997). This good regeneration of these species should ensure the recruitment of juveniles in the different successive height classes. This good regeneration capacity could be explained by the fact that these species find favorable conditions for their development, such as soil types.

Structural Characterization of Populations

The adult stand structure of the species shows a predominance of small individuals for both species. Indeed, whatever the type of formation concerned, the individuals are included in the classes [5;10] [10.1;15] (Figure 4 and Figure 5).

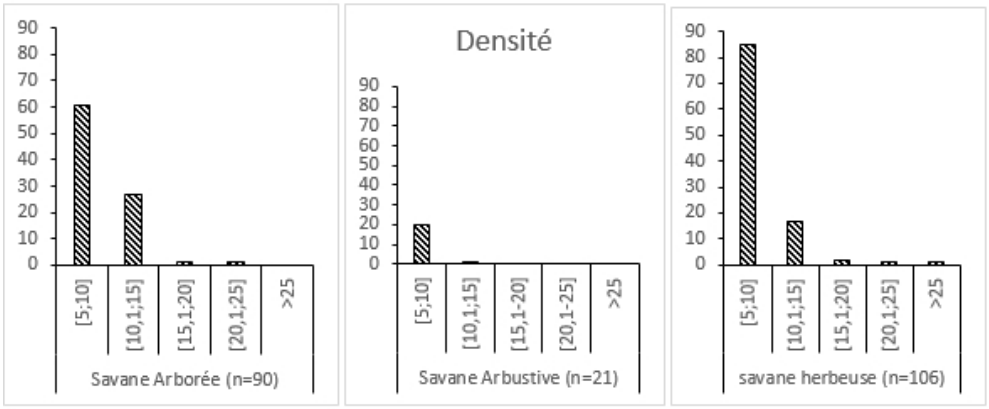

Figure 4: Diameter class structure of adult individuals of *E. africana* in (A) woody savannah, (B) shrubby savannah and (C) grassy savannah.

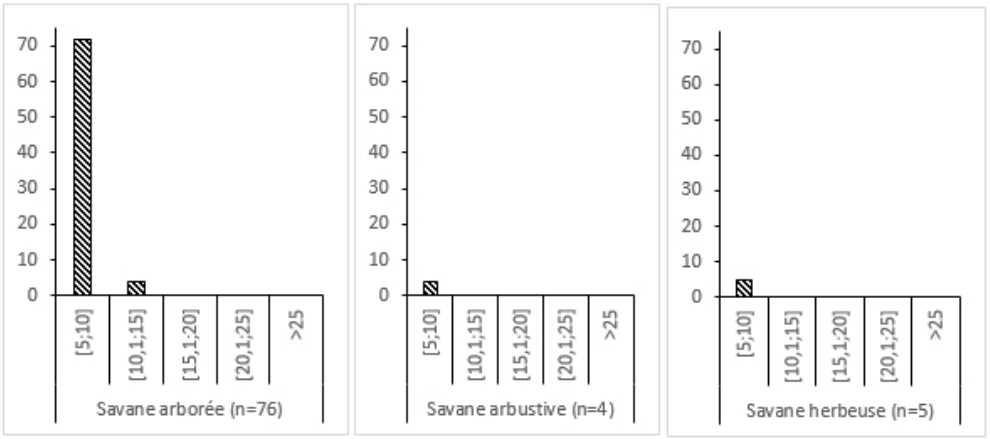

Figure 5: Diameter class structure of adult's individuals of *C. micranthum* in (A) woody savannah, (B) shrubby savannah and (C) grassy savannah.

The height class structure of juvenile individuals of *E. africana* reveals the presence of individuals in all height classes in the woody and grassy savannah (Figure 6). Also, *C. micranthum* reveals the presence of individuals in all height classes in the woody savannah (Figure 7).

Species diameter class structures showed a predominance of small size individuals for both species. Indeed, the number of individuals is higher in the smaller diameter classes. This predominance of small-diameter individuals in the stands of both species could be explained by the fact that these are shrubs generally dominated by small-diameter individuals. Also, this predominance would characterize a stable population of these species if regular recruitment of the juvenile population in the various successive diameter classes could be ensured. Unfortunately, the structure of the juvenile populations of both species appears unstable. In fact, a low presence or absence of individuals in some height classes has been observed, indicating a low or irregular recruitment from the juvenile to the adult stratum. This could be due to anthropogenic pressures such as bush fires and methods of collection for their use in traditional medicine. The irregular recruitment of juveniles to the adult stratum suggests that populations of both species are declining, and their continued exploitation would be a threat to their survival. Specifically, the exploitation of their roots in the treatment of the hepatitis concerned in the present study may even lead to the disappearance of these species (Bognounou *et al.*, 2009). Alternatives should therefore be considered to reconcile the preservation of medicinal species and traditional medicinal needs. One approach could be the planting and assisted regeneration of medicinal species. However, this technique presents weaknesses when the pressure is high, beyond the development capacity of

these species. Also, knowledge of the phytochemistry of these species could be exploited to limit root harvesting.

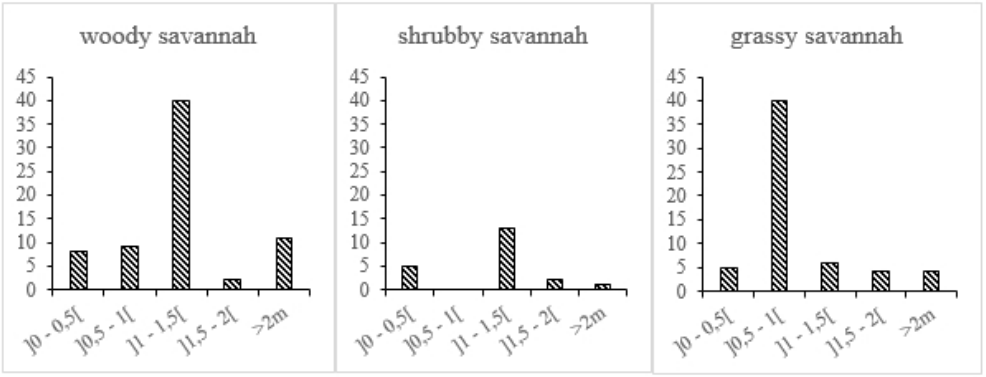

Figure 6: Height class structure of juvenile individuals of *E. africana* in woody savannah, shrubby savannah and grassy savannah.

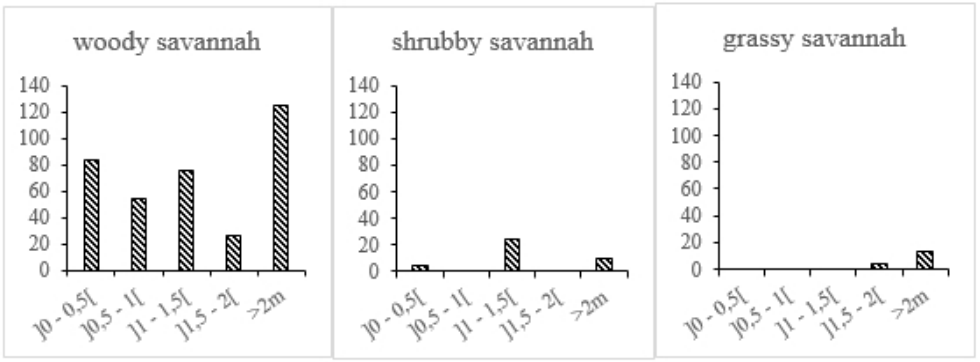

Figure 7: Height class structure of juvenile individuals of *C. micranthum* in woody savannah, shrubby savannah and grassy savannah.

In addition, other investigations could be carried out on the architecture of the root system of these species because, according to Kaboré (2015), the knowledge of such architecture of the species whose roots are harvested could result to good harvesting practices that would minimize the destruction of the latter.

Phytochemical Analysis

Determination of Total Polyphenols: The results of the determinations are shown in Table VI. Total phenolics contents were 20.70 ±0.24 mg EAG/100mg extract for *E. africana* and 37.91 ±1.30 mg EAG/100mg extract for *C. micranthum*. The best content was obtained by *C. micranthum* which was 37.91 ±1.30. For total flavonoids, the contents were 0.66 ±0.04 and 0.87 ±0.09 mg EQ/100 mg extract for *E. africana* and *C. micranthum* respectively.

Table VI: Content of total polyphenols and flavonoids

Species	Total Polyphenols (mg EAG/100mg extract)	Total Flavonoids (mg EQ/100mg extract)
Combretum micranthum	37.91 ± 1,30	0,85 ± 0.09
Entada africana	20.71 ± 0,25	0.66 ± 0.05

Assessment of Antioxidant Activity: These results are recorded in Table VII. Indeed, we obtained a value of 198.54± 3.66, 13.58 ± 0.36 and 14.88 ± 0.17 µmol EAA respectively with the ABTS, DPPH and FRAP methods for *C. micranthum* species. For the species *E. africana*, the values were 197.27± 1.47, 13.70 ± 0.07 and 18.84 ± 1.23 µmol EAA by using the ABTS, DPPH and FRAP methods, respectively.

Table VII: Results of ABTS, DPPH and FRAP methods

Species	ABTS (µmol EAA)	DPPH (µmol EAA)	FRAP (µmol EAA)
Combretum micranthum	198.54± 3.66	13.58 ± 0.36	14.88 ± 0.17
Entada africana	197.27± 1.47	13.70 ± 0.07	18.84 ± 1.23

The Soxhlet methanolic extraction yield of *C. micranthum* was 6.90% and that of *E. africana* 8.87%. The contents of total phenolics and flavonoids were respectively 37.91 ±1.30 mg EAG/100mg extract and 0.85 ± 0.09mg EQ/100 mg for *C. micranthum*. For *E. africana* species, we obtained 20.70 ±0.24 mg EAG/100mg total phenolics extract and 0.66 ± 0.05 mg EQ/100 mg total flavonoids extract. The latter values were slightly lower than those obtained from *C. micranthum* extracts. Total polyphenols were therefore present in the root extracts of the two species in the study. These compounds could probably be responsible for the antiviral and hepatoprotective activities attributed to these species. Indeed, it is these compounds that possess such properties in plants.

Evaluation of antioxidant activities by three complementary methods, ABTS, DPPH and FRAP, yielded significant values for each method. These values were approximately the same for both species. This could mean that *C. micranthum* and *E. africana* do possess antioxidant activities. The antioxidant activity of a plant extract is of particular interest. It corresponds to the capacity of the extract to protect the organism against oxidative stress damage, which is involved in the induction and amplification of various pathologies, such as liver diseases. The presence of these secondary metabolites and their associated pharmacological properties in these species would be a reason for their use in the care of hepatitis. Their roots are therefore collected in order to research the pharmacological properties of these metabolites. Thus, the knowledge of the phytochemistry of these species could be used to limit root harvesting. Indeed, previous investigations (Bangou *et al*., 2011; Kwaji, 2017; Kwaji *et al*., 2018) have

revealed that the leafy twigs of *C. micranthum* and the trunk bark of *E. africana* would have the same properties as those obtained in the root extracts of these two species in the present study. Such comparative analysis could provide strong arguments for orienting the population towards the use of other parts of these species whose harvesting will compromise less the sustainability of these species.

Conclusion

This study showed that the ethnobotanical survey conducted among traditional practitioners in Bobo-Dioulasso revealed that a diversity of medicinal plants was used in the treatment of hepatitis in this region of Burkina Faso. The survey identified 40 species belonging to 23 botanical families used in the treatment of these dreaded diseases. Citation frequencies also revealed that *Combretum micranthum* and *Entada africana* were regularly cited and were thus the subject of a phytochemical study. This phytochemical study showed that these two species possess antioxidant properties. The structure of the adult populations showed a predominance of small individuals for both species. An overall analysis of the adult and juvenile strata leads to the conclusion that the populations of these two species are declining due to anthropogenic pressure. Furthermore, their exploitation must therefore be appropriate for their effective conservation and preservation.

Our next studies on these plants' species will aim at making a bio-guided evaluation to (1) identify the polyphenolic compounds implied in the hepatoprotective activity in vivo, (2) to check literatures informations such as anti-inflammatory and cyto-toxicity properties for the extend purpose of this type of inventory to other regions of Burkina Faso.

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