

Ethnopharmacological plants used to treat hepatitis and their anti-oxidant activity of district of Bobo-Dioulasso (*Burkina Faso*)

Traoré Tata Kadiatou^{*1}; Tibiri André^{2,3}; Ouédraogo Noufou^{1,2}; Sombie, Nogma Ernest³; N'do Jotham Yhi-pênê.³; Ouedraogo Salfo^{1,2}; Guissou, Innocent Pierre^{1,2}

¹Laboratoire du Développement des médicaments (LADME), Ecole doctorale de la santé, Université Ouaga I Pr Joseph Ki-Zerbo 03 BP 7021 Ouaga 03, Burkina Faso

²Département de médecine et pharmacopée traditionnelles-pharmacie (MEPHATRA-PH), Institut de recherche en science de la santé (IRSS/CNRST) 03 BP 7192 Ouaga 03, Burkina Faso

³Laboratoire de Biochimie et de Chimie Appliquée (LABIOCA), Université Ouaga I Pr Joseph Ki-Zerbo, Burkina Faso.

QR Code



*Correspondence Info:

Traore Tata Kadiatou
Laboratoire du Développement des médicaments,
Ecole doctorale de la santé,
Université de Ouaga I Pr Joseph Ki-Zerbo,
03 BP 7021 Ouaga 03, Burkina Faso

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Abstract

Background: Plants are frequently used by traditional healer for the care of the hepatic pathologies.

Objective: This study is part of the valorization of the hepatoprotective potential of some plants used in traditional medicine for the care of hepatitis in the Hauts-Bassins region of Burkina Faso.

Materials and methods: A survey realized in this region allowed retaining 5 species, after the classification according to the frequency of quotation and the bibliographic review, among the 44 species recorded: *Anogeissus leiocarpus* (DC.) Guill. & Perr (Combretaceae), *Balanites aegyptiaca* Delie. (Balanitaceae), *Cassia alata* L. (Cesalpiniaceae), *Opilia celtidifolia* (Guill. & Perr.) Endl. (Opiliaceae) and *Ziziphus mauritiana* L. (Rhamnaceae). The content in phenolic compounds of the aqueous extracts of these plants was determined. Their antioxidant activity was evaluated by two methods: discoloration of radical cation ABTS and iron reduction (FRAP).

Results: *Anogeissus leiocarpus* and *Ziziphu smauritiana* gave the strongest antioxidant activities and high phenolic compounds (total phenolics, total flavonoids, total tannins and flavonols). The antioxidant activity (FRAP, ABTS) was associated with the total phenolic content of the extracts expressed in tannic acid equivalent per 100 grams of dry matter with R² of 0.8148 and 0.7966.

Conclusion: The antioxidant activity and the content phenolic compounds observed during this study with *Anogeissus leiocarpus* and *Ziziphus mauritiana* indicates that these two plants have a high hépato protective potential which can justify their use by the Traditional healer in the care of hepatitis.

Keywords: Hepatitis; Ethnopharmacology; Medicinal plants; Polyphenols; Antioxidant.

1. Introduction

Hepatitis is defined by inflammation of the hepatic parenchyma, associated with a more or less extensive necrosis of hepatocytes [1]. The causes of hepatitis can be of various origins namely viral, drug, toxic or autoimmune. Hepatitis establish a health problem public worldwide, affecting hundreds of millions of people every year in the world[2]. The prevalence of chronic carriers of the hepatitis B virus was 240 million and that of the hepatitis C virus

(HCV) 170 million [3, 4]. Burkina Faso is a country where the prevalence of hepatitis B virus is between 12-14% and HCV of 2.8% [5]. In 2004, acute hepatitis B caused, according to the WHO, 1100 deaths in the Burkina population. Chronic hepatitis is an important risk factor for cirrhosis and primary liver cancer, causing 900 and 1 300 deaths each year in the country[6].

Hepatitis induces an immune reaction of hepatocyte from which inflammation leads to the

production of oxygenated free radicals. These free radicals are neutralized by the antioxidants, allowing the hepatocytes to regenerate. In case of chronicity, the inflammation persists and the production of the free radicals becomes permanent. The intake of antioxidants reduces the progression of the inflammatory process by protecting the liver. These natural or synthetic antioxidants have a protective action. Numerous studies showed that the hepatoprotective properties of the plants are due to the phenolic compounds which they contain [7]. Phenolic compounds are natural antioxidant. Indeed, these compounds are involved in the reduction and trapping of free radicals[7].

The treatment of hepatitis with medicinal plants would be an opportunity for the population given the inaccessibility of modern medicines. In addition, the very high cost of treating hepatitis removes any hope of curing people with hepatitis. Faced with this difficult situation, the resort to medicinal plants appears as the least expensive solution and the easiest to access if only their therapeutic efficiencies are scientifically proven.

They therefore use medicinal plants for the care of their hepatitis. There is an interest growing for the study of healing plants used in the care of liver diseases in various regions of the world these last decades[8]. The frequent use of medicinal plants of the population aroused an interest for the phytochemical and pharmacological investigations of some plants in the care of hepatitis in the region of the Hauts-Bassins.

The objective of this study was to inventory medicinal plants for the care of hepatitis in the Hauts-Bassins region, then to determine the phenolic compounds contents of the extracts of six plants more cited and to evaluate the antioxidant capacity of these extracts.

2. Materials and methods

2.1. Ethnomedical survey

The survey, of qualitative type, was based on the knowledge and the practices of traditional healers in the care of hepatitis. It took place in the town of Bobo-Dioulasso, administrative center of the Hauts-Bassins region. Located 365 km from the capital Ouagadougou with an area of 11540 km² representing 4.21% of the country, it has a climate of the Southern Sudanese type. The population was 537.728 inhabitants in the 2012 census and is made up of a diversity of ethnic group whose bobos, dioulas, senoufos and language commonly spoken is the dioula.

It was to carry out interviews in dioula and/or French language with a semi-structured interview guide conceived by MEPHATRA / PH with the traditional healer.

The investigators received a training of a hepatogastro-enterologist to standardize the clinical signs of hepatitis. The study was conducted on August 2012 and the interview guide focused on issues such as social-demographic characteristics of traditional healers and their knowledge of hepatitis and the plants used. The data collected consisted mainly of including age, sex, and place of residence of traditional healers. The others variables study was clinical signs of hepatitis, target population, knowledge of the plants used (parts of the plant, the mode and harvest period of the parties). The method of preparation and administration, dosage and approximate dose, duration of treatment and adverse effects of plants were also reported.

In the current study medicinal plants reported by the traditional healers were identified by local names and collected in the field. Specimens harvested in the survey area, were identified in the Ecology Laboratory of University of Ouagadougou. A data analysis on the basis of the frequency of quotation, the research articles, books and relevant web pages were studied with the aim to accumulate data of phytochemical compounds present in the plants for the selection of species in experimental studies.

2.2. Plant material

The plant material was harvested in November 2012 in Bobo-Dioulasso. Drying was carried out under dust-free ventilation in the building housing the chemistry and pharmacology laboratories of the MEPHATRA / PH department. After drying, the plant material was crushed (crusher with type Gladiator Est's blades) and stored in vacuum plastic bags in desiccators.

2.3. Extraction methods

Two types of extraction including decoction and maceration were used in accordance with the traditional preparation form described by traditional healers.

Decoction:

30 g of dried plant material were mixed with 500 ml of distilled water and then homogenized. The obtained mixture was brought to a boil under hanging ebb 30 min.

Aqueous maceration:

30 g of vegetable material dried in 500 ml of distilled water were left for 24 hours at room temperature with stirring.

Extracts obtained from decoction and maceration were first filtered with cotton and then centrifuged at 2000 RPM for 10 min. The supernatant of each extract was lyophilized and then stored in the freezer for experimental studies

2.4. Evaluation of antioxidant activities of plant extracts

2.4.1. ABTS (2, 2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic acid]) test

The antioxidant power in Trolox equivalent (Trolox Equivalent Antioxidant Capacity = TEAC) was determined by the ABTS (2, 2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic acid] test [9, 10]. The ABTS cationic radical (ABTS^{•+}) is generated by mixing a solution of 2.45 mm potassium persulfate (K₂S₂O₈) and a stock solution of ABTS at 7 mm, the whole is kept away from light and at room temperature for 12-16 hours before use. The reaction mixture was established (constituted) by 1.0 ml of solution diluted with ABTS^{•+} and of 10 µl of extract (of 1 mg / ml in 31.25 µg / ml) or of Trolox as standard (0-15 µM) in ethanol or the appropriate solvent. The mixture was allowed to incubate for 30 min at room temperature. The absorbance was read at 734 nm on the spectrophotometer (Agilent 8453) and the blank was the diluents solvent of the extract or standard. The curve of inhibition of the absorbance as a function of the concentration of the extract or Trolox was established for the determination of the 50% inhibitory concentration (IC₅₀). This IC₅₀ allowed calculating the anti-radical power (ARP) (ratio 1 / IC₅₀). Finally, TEAC was calculated by making the ratio of the ARP of the extracts to that of the reference compound (Trolox).

2.4.2. The Ferric Reducing Antioxidant Power (FRAP) Test

The Ferric Reducing Antioxidant Power (FRAP) method is based on the reduction of ferric ion (Fe³⁺) to ferrous ion (Fe²⁺). The Hinneburg variant was used [11]. Into a test tube containing 0.5 ml of sample solution (0.1 mg / ml) was added 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and then 1.25 ml of potassium Hexacyanoferrate [K₃Fe(CN)₆] 1% in water.

The whole was heated at 50 °C in a water bath for 30 minutes. The volume 1.25 ml of trichloroacetic acid (10%) was then added and the mixture was centrifuged at 2000 RPM for 10 minutes. A quantity of 0.625 ml of the mixture was taken. To this quantity were added 0.625 ml of distilled water and then 0.125 ml of freshly prepared 1% FeCl₃ in water. A blank without a sample was prepared under the same conditions and the reading was made at 700 nm against a standard curve. The concentration of reducing compounds (antioxidants) in the plant extract was calculated and expressed in mmol Equivalent Trolox (ET) / g of dry extract.

2.5. Determination of phenolic compounds in plant extracts

2.5.1. Total phenolics content determination

The total phenolic compounds were measured according to the Singleton method [12]. These compounds

react with the Folin Ciocalteu reagent (FCR) in an alkaline medium. The reaction mixture consisted of 1 ml of extract (1 mg / ml), 1 ml of 2N FCR and 3 ml of a 20% sodium carbonate solution in a test tube. After incubation for 40 min at room temperature, the absorbance of the mixture was measured at 760 nm on the spectrophotometer (Agilent 8453) against a tannic acid standard curve (R² = 0.999). In the blank control tube, the extract was replaced with distilled water. The tests were realized and the total phenol concentration of the extract, expressed in gram-equivalent tannic acid per 100 g dry matter (MS) (g EAT / 100 g MS), was calculated.

2.5.2. Tannins content determination

Tannins were measured according to the method of Singleton [12] adapted by Tibiri [13]. The polyvinyl polypyrrolidone (PVPP) precipitates tannins by formation of a complex. Seventy-five (75) mg of PVPP was added to 2 ml of extract (1 mg / ml). The mixture was vortexed, kept at 4°C for 15 min and then centrifuged at 3000 g for 10 min. The supernatant contained phenolic compounds other than tannins, which had been precipitated by PVPP. The other compounds are measured according to the total phenol dosage method. The total phenol content of the supernatant was determined and the tannin content (expressed as g EAT / 100 g MS) was calculated by making the difference between the first value of the total phenolic compounds (which contained the tannins) and the second value of total phenolic compounds (in the absence of tannins).

2.5.3. Total flavonoids content determination

The flavonoid dosage was realized according to the Kumaran method [14] adapted by Abdel-Hameed [15]. Two (2) ml of extract (1 mg / ml in methanol) were mixed with 2 ml of aluminium trichloride (2%). After 40 min of incubation at ambient temperature, the absorbance was measured at 415 nm using a spectrophotometer (Agilent 8453) against a quercetin standard curve of R² = 0.999. The blank control tube consisted of 2 ml of methanol. The quantity of flavonoids in the plant extract was determined in gram-equivalent quercetin (EQ) per 100 g dry matter (MS) (g EQ / 100 g MS).

2.5.4. Flavonols content determination

The flavonols were realized according to the Kumaran method [14] adapted by Abdel-Ameed [15]. The dosage is based on the formation of a complex with aluminium whose maximum absorption is at 440 nm. One (01) ml of extract (1 mg / ml in methanol) was mixed with 1 ml of aluminium trichloride (20 mg / ml) and 3 ml of sodium acetate solution (50 mg / ml). After 2 h 30 min of incubation at ambient temperature, the absorbance was measured. The absorbance of quercetin (0.025 mg / ml in methanol) was measured in the same conditions with R² =

0.999. The flavonol content in the extract was calculated in gram-equivalent quercetin (EQ) per 100 g dry matter (g EQ / 100 g MS).

2.6. Analysis of results

A seize mask was created using the Sphinx software (v 5.1.0.8) to seize the data collected during the survey. The results of the determination of the antiradical power and the polyphenol dosage were expressed on average \pm SEM (standard deviation to the average) for three independent tests. The observed differences between the variables were assessed using the analysis of variance (ANOVA) with the GraphPad Prism v. 5 software. Statistical differences were considered statistically significant at the significance level of 5% (p 0.05).

3. Results

3.1. Results of the ethnomedical survey

Twenty (20) traditional healers, of which 16 men and 4 women were, interviewed (20% women and 80% men). The traditional healer's age range was between 30 and 80 with an average age of 54.25 years.

Symptoms of hepatitis according traditional healers were headache, fever, asthenia, anorexia, nausea, abdominal pain, jaundice (palms, foot, and eyes), urine and discoloured stools.

Forty-four (44) species of medicinal plants belonging to 41 genera and 24 families were identified (Table 1).

Table 1: List of listed species used for the treatment of hepatitis during the survey

Species and family	Local Name	Language of denomination	Pathology (s)	Used part	Method of preparation	Administration
<i>Acacia sieberiana</i> DC. (Fabaceae – Mimosoideae)	Wonigue	Dioula	Jaundice	Whole Foot	Water maceration	Oral use
<i>Acacia erythrocalyx</i> Brenan (Fabaceae – Mimosoideae)	Goga	Moore	Hepatitis B	Trunk bark	Decoction	Oral use
<i>Annona senegalensis</i> Pers. (Annonaceae)	Mandesounsoun	Dioula	Jaundice	Whole root	Decoction	Oral bath
<i>Anogeissus leiocarpa</i> DC. (Combretaceae)	Galama	Dioula	Liver disease; Jaundice	Leaves, entire root; Sheets	Decoction	Oral bath; seat bath
<i>Argemone mexicana</i> L. (Papaveraceae)	Argemone	Latin	Liver cancer	Whole Foot	Decoction	Oral use
<i>Balanite saegytiaca</i> (L.) Delile (Balanitaceae)	Zeguene	Dioula	Chronic hepatitis	Bark of trunk, whole root	Decoction powder	Oral use
<i>Bauhinia rufescens</i> Lam. (Fabaceae – Mimosoideae)	Sifletyiri	Dioula	Abscess of liver	Leaves, whole root	Decoction	Oral use
<i>Carica papaya</i> L. (Caricaceae)	Papayer	French	Hépatite	Leaves, fruit	Decoction	Oral bath
<i>Cassia alata</i> L. (Fabaceae – Caesalpinioideae)	Kotama	Dioula	Jaundice	Whole root	Water maceration	Oral bath
<i>Cassia fistula</i> L. (Fabaceae – Caesalpinioideae)	Cassia	Dioula	Jaundice	Sheets	Decoction	Oral, bath, inhalation
<i>Cassia occidentalis</i> L. (Fabaceae – Caesalpinioideae)	Kenkeliba	Dioula	Liver disease; Hepatitis	Whole root; Sheets	Decoction	Oral bath ; Oral use
<i>Cassia sieberiana</i> DC. (Fabaceae – Caesalpinioideae)	Sindjanfing	Dioula	Hepatitis	Whole root	Decoction	Oral use
<i>Cassia tora</i> L. (Fabaceae – Caesalpinioideae)	Kirikiri	Dioula	Severe liver disease	Seeds	Powder	Oral use
<i>Chrysanthellum indicum</i> DC. (Asteraceae)	Toritegue	Dioula	Hepatitis	Sheets	Decoction	Oral bath
<i>Citrus limon</i> L. (Rutaceae)	Citronnier	French	Hepatitis	Fruit	Decoction	Oral, bath, inhalation
<i>Cochlospermum planchonii</i> MS. (Cochlospermaceae)	Dribala	Dioula	Hepatitis A	Whole root	Infusion powder	Oral use
			Hepatitis	Sheets, Whole root	Decoction	Oral bath, seat bath
<i>Combretum micranthum</i> G. don (Combretaceae)	Golobe	Dioula	Hepatitis	Leaves, whole root, seeds; Sheets	Decoction powder	bath, seat bath, bathing, purgation
<i>Cymbopogon citratus</i> (DC.) Stapf (Poaceae)	Citronnelle	French	Hepatitis	Sheets	Decoction	Oral use
<i>Entada africana</i> Guill. & Perr. (Fabaceae – Mimosoideae)	Samanere	Dioula	Hepatitis	Bark of trunk, whole root	Decoction	Oral bath
<i>Erythrina senegalensis</i> A.DC. (Fabaceae – Faboideae)	Fadougalen	Dioula	Viral Hepatitis B	Bark of trunk, whole root	Decoction	Oral use
<i>Flueggea virosa</i> (Roxb. ex Willd.) Voigt (Euphorbiaceae)	Baran-baran	Dioula	Jaundice	Whole root	Decoction	Oral, bath, inhalation
<i>Gardenia aqualla</i> Stapf & Hutch. (Rubiaceae)	Gounleche	Dafing	Jaundice	Whole root	Decoction	Oral use, seat bath
<i>Lanneave lutina</i> A. Rich. (Anacardiaceae)	Souroukoupekoun	Dioula	Hepatitis	Leaves, trunk bark, whole root	Decoction powder	Oral use
<i>Mangifera indica</i> L. (Anacardiaceae)	Manguier	French	Jaundice	Trunk bark	Decoction	Oral use, application

Table 1 continue.....

<i>Maytenuss enegalensis</i> Lam, Boiss (Celastraceae)	Ninkele	Dioula	Hepatitis	Leaves, whole root	Decoction powder	Oral, bathing, purgation
<i>Müragynain ermis</i> (Willd.) Kuntze (Rubiaceae)	Djoun	Dioula	Jaundice	Sheets	Decoction	Oral bath
<i>Opilia celtidifolia</i> (Guill. & Perr.) Endl. (Opiliaceae)	Nimbossi	Dioula	Hepatitis	Sheets	Decoction	Oral bath
<i>Oxytenanthera abyssinica</i> (A.Rich.) Munro (Poaceae)	Bambou	French	Hepatitis	Root whole leaves	Decoction	Oral bath
<i>Pavetta crassipes</i> K. Schum. (Rubiaceae)	Bôbôkougou	Dioula	Liver cancer	Sheets	Decoction	Oral use
<i>Phyllanthus amarus</i> Schumach. & Thonn. (Euphorbiaceae)	Phyllantus	Latin	Hepatitis, viral	Whole Foot	Decoction powder	Oral use
<i>Piliostigmahonningii</i> (Schum.) Milne-Redh. (Fabaceae – Caesalpinioideae)	Gnama	Dioula	Jaundice (hepatitis A)	Seeds	Infusion powder	Oral use
<i>Pseudocedrela kotschyi</i> (Schweinf.) Harms (Meliaceae)	Korogue	Dioula	Hepatitis	Leaves, trunk bark	Infusion	Oral use
<i>Psidium guajava</i> L. (Myrtaceae)	Goyavier	French	Jaundice	Sheets	Decoction	Oral use, application
<i>Pteleopsis suberosa</i> Engl. & Diels (Combretaceae)	Gnodefou	Dioula	Hepatitis B	Trunk bark	Decoction	Oral use
<i>Sarcocephaluslati folius</i> (Sm.) E.A. Bruce (Rubiaceae)	Bati	Dioula	Jaundice	Leaves, whole root	Decoction, Infusion powder	Oral use, Oral bath
<i>Sterculia setigera</i> Delile (Sterculiaceae)	Koungosira	Dioula	Hepatitis	Leaves, trunk bark, whole root	Decoction powder	Oral use
<i>Swartzia madagascariensis</i> Desv. (Fabaceae)	Sindjangue	Dioula	Hepatitis C	Whole root ; Root bark	Powder ; Water maceration	Oral use, Oral bath
<i>Tamarindus indica</i> L. (Fabaceae – Caesalpinioideae)	Tomi	Dioula	Hepatitis	Leaves, trunk bark, whole root	Decoction powder	Oral use
<i>Terminalia macroptera</i> Guill. & Perr. (Combretaceae)	Wolon	Dioula	Jaundice	Whole root	Decoction	Oral, bath, seat bath
<i>Trichiliaemetica</i> Vahl (Meliaceae)	Soulafinsan	Dioula	Hepatitis	Sheets	Decoction	Oral bath
<i>Vitellaria paradoxa</i> C.F. Gaertn. (Sapotaceae)	Siyiri	Dioula	Hepatitis B	Trunk bark	Decoction	Oral use
<i>Ximenia americana</i> L. (Olacaceae)	Minigoli	Dioula	Hepatitis	Leaves, trunk bark, whole root	Decoction	Oral use
<i>Ziziphus mauritiana</i> Lam. (Rhamnaceae)	Tomonon	Dioula	Liver disease	Whole root	Decoction	Oral use

The largest number of species belong to Fabaceae-Caesalpinaceae and Fabaceae-Mimosadeae families followed by Combretaceae and Rubiaceae, Meliaceae,

Poaceae, Euphorbiaceae and Anacardiaceae, and the remaining 15 families were represented by one plant species each (Figure 1).

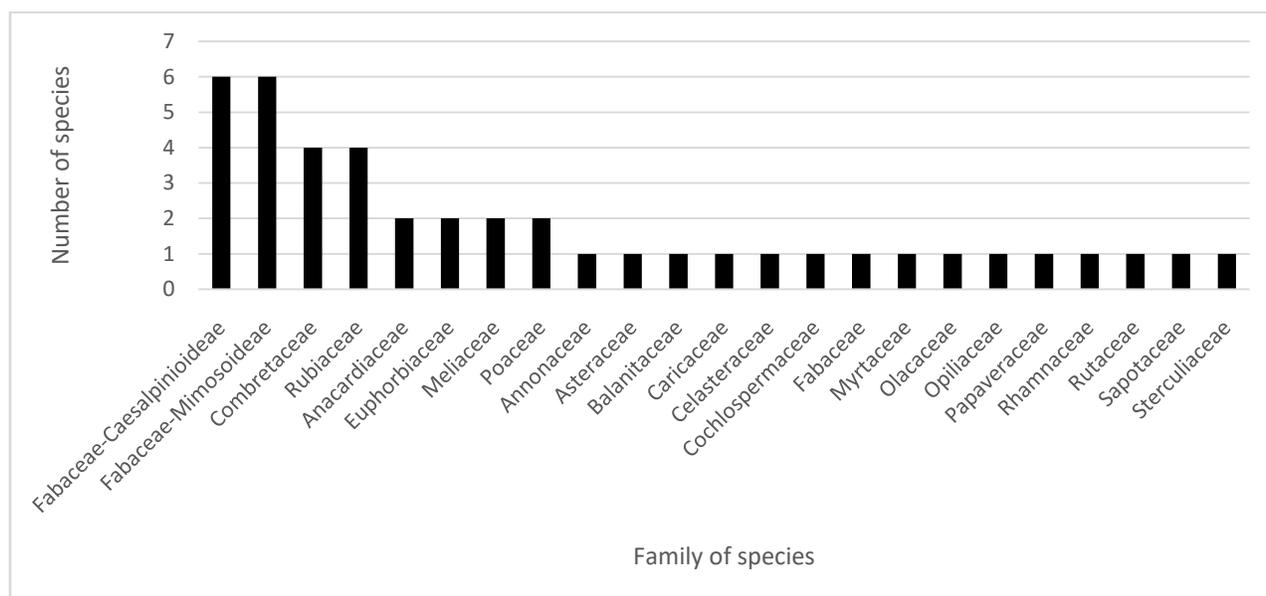


Figure 1 : Distribution of species by family

Among the medicinal plants cited, 55.9% were trees, 25.4% of the herbaceous plants and 18.6% of the shrubs. The pre-selected species were then compared with data from the literature review in order to select the species with antioxidant and hepatoprotective properties were the

least studied. The species selected for the experimental study consisted of *Aleiocarpus*, *B aegyptiaca*, *C alata*, *O celtidifolia* and *Z mauritiana*.

The plant parts most commonly used by traditional healer were roots (37.35%) and leaves (36.14%). (Figure 2)

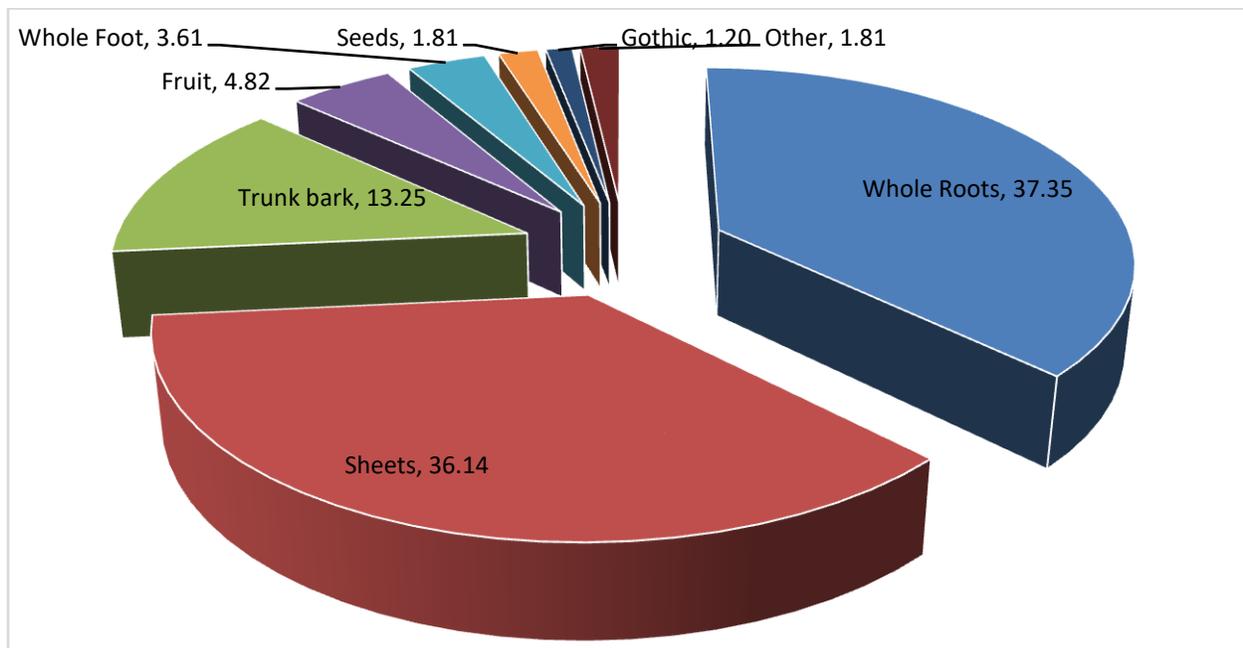


Figure 2: Frequency of quotation of the different parts of plants used

The most common method of preparation was decoction and powder (Figure 3).

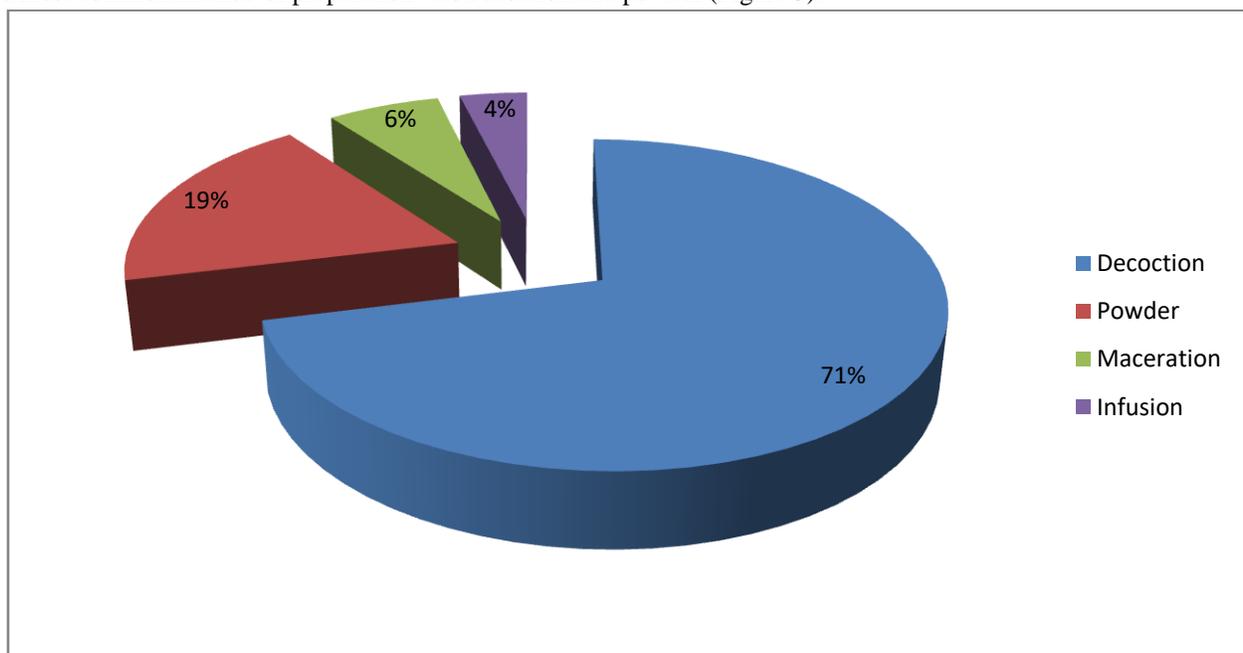


Figure 3: Percentage of species preparation methods

The different modes of administration were the drink, the bath of the body, the bath of the siege and the inhalation (Figure 4).

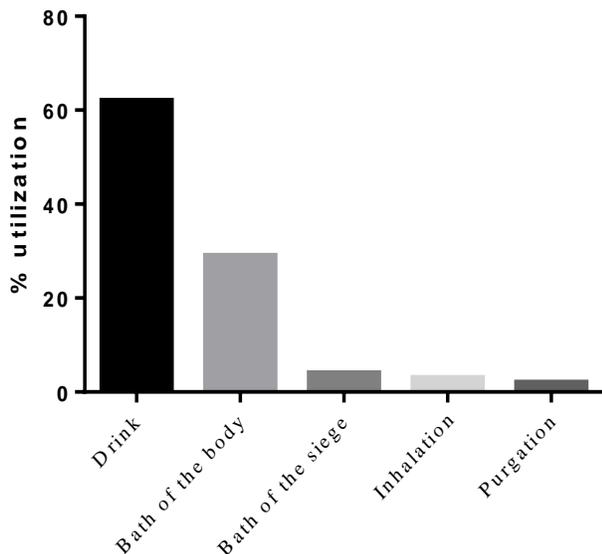


Figure 4 : Percentage of mode of utilization

3.2. Antioxidant activities

The results of the antioxidant activity determined by the ABTS and FRAP methods are shown in Table 2.

Table 2: Antioxidant activity (ABTS, FRAP) of plant extracts

Plants/extracts	ABTS (TEAC)	FRAP (mmol ET/100 g MS)
<i>A. leiocarpus/decoction</i>	0.53±0.12 ^a	82.39±0.34 ^a
	0.29±0.04 ^a	98.53±1.75 ^b
<i>B. aegyptiaca/decoction</i>	0.04±0.00 ^b	27.38±2.24 ^c
	0.02±0.00 ^b	22.80±0.55 ^c
<i>C. alata/maceration</i>	0.07±0.01 ^b	61.11±2.32 ^d
<i>O. celtidifolia/decoction</i>	0.08±0.02 ^b	48.37±3.65 ^e
<i>Z. mauritiana/decoction</i>	0.26±0.17 ^a	71.53±0.75 ^f
Trolox	1.00±0.00	

For the evaluation of the antioxidant activity of the ABTS method, the TEAC data varied from 0.02 to 0.53 ± 0.12. *Anogeissus leiocarpus* and *Zizuphus mauritiana* showed the highest antioxidant capacity in Trolox equivalent (TEAC). The values of the antioxidant activity by the FRAP method varied from 22.8 ± 0.55 to 98.53 ± 1.75 mmol and / 100 g of dry matter. The extracts of *Aleiocarpus* and *Z mauritiana* gave the greatest capacities of reduction of the ferric ion compared to those of the other plants.

3.3. Phenolic compounds Content

The figure 5 shows the total phenolics and tannin contents expressed in g-tannic acid equivalents per 100 g

dry matter (EAT / 100 g MS), and the contents of the flavonoids and flavonols expressed in g equivalent quercetin per 100 g of dry matter (EQ / 100 g MS) (Figure 5).

The total phenol contents vary from 0.83 ± 0.05 g EAT / 100 g MS for *B. aegyptiaca* to 27.77 ± 0.72 g EAT / 100 g MS for *A. leiocarpus*. Values of tannin contents were between 0.68±0.01 g EAT/100 g MS for *B. aegyptiaca* and 19.42±0.34g EAT/100 g MS for *A. leiocarpus*. As for flavonoids, their contents varied 0.17 ± 0.08 for *B. aegyptiaca* to 6.23 ± 0.92 g QT / 100 g MS for *A. leiocarpus*. Finally, the flavonol contents of the plant extracts varied between 0.14 ± 0.12 for *B. aegyptiaca* to 2.12 ± 0.47 g EQ / 100 games for *A. lookups*.

The plant extracts richest in phenolic compounds, after the dosages, were those of *A. leiocarpus* and *Z. mauritiana* compared to the three other plants.

3.4. Correlation between antioxidant activity and phenolic content

Tests of the correlation between the phenolic content and the antioxidant activity (FRAP and ABTS) of the different extracts of the plants studied gave more or less correlated levels according to the antioxidant tests used and the phenolic compound contents. Indeed, a correlation was obtained between the phenolic compounds (total phenolics, tannins) of the various plant extracts studied and the antioxidant activity by the FRAP method (R² = 0.815 and R² = 0.770) as well as with Of ABTS (R² = 0.797, R² = 0.977).

5. Discussion

The survey was based on knowledge related to the care of hepatitis by the traditional healers. On the whole, they gave clinical signs of hepatitis similar to those described by some authors namely headache, fever, asthenia, anorexia, nausea, abdominal pain, jaundice (Palms of hands, foot plant, yellow eyes,...), urine and discoloured stool^[16]. These results give some credibility to traditional healer's knowledge. The majority of traditional healers interviewed were male (Sex ratio = 4). It could explain by the fact that in African societies, men are privileged in the transmission of knowledge, and these women are more interested in paediatric diseases [17].

Among the species counted in near these traditional healers, the most common families were Fabaceae - Caesalpinioideae, Combretaceae and Rubiaceae with 55.9% of trees, 25.4% of herbaceous plants and 18.6% of shrubs. That indicates that the zone consists of a considerable diversity (timbered savannas, raised and shrubby savannas) of plant species. Studies have shown that more than a dozen diseases are cared for by the (most cited) species of the Combretaceae family[17]. Some studies have

also shown that leaves, roots and bark of the trunk are the main parts used in traditional medicine[18]. This could be justified by the fact that these parts of plants are most accessible throughout the year. The strong use of the decoction could be explained by the fact that this form makes it possible to extract faster the active ingredients[19]. In addition the decoction attenuates or cancels the toxic effect of certain recipes [20]. The drink was also the main mode of administration [19]. The administration orally may be explained by the fact that this route allows a good absorption of the active ingredients than the other routes of administration used.

Evaluation of the antioxidant activity of the extracts by the various methods was carried out according to the type of oxidant. Thus, the ABTS test was used to quantify the anti-radical potential of the H⁺ donor compounds contained in the extracts and the FRAP method to evaluate the reducing power of the ferric ions of the extracts. There is some complementarity between these different methods of assessing the anti-free radical activity of plants (ABTS and FRAP), without one of which can be established as a reference[21]. The extracts of *A. leiocarpus* and those of *Z. mauritiana* were distinguished from the other plants with their antioxidant capacities. The high antioxidant capacities of the extracts of these plants would explain their ability to trap reactive oxygen species (ROS) against hepatocellular attacks. These same extracts exhibited good antioxidant activity by the FRAP method, which could confirm their desired antioxidant properties in the treatment of hepatitis.

The antioxidant activity of these plants prevents the reactions of ferric ions with hydrogen peroxide thus blocking the formation of hydroxyl ions that are involved in the cytotoxicity of the liver[22]. Phenolic compounds are known to have antioxidant activities and it is likely that the activity of the extracts is due to these compounds [23].

Quantification of the phenolic compounds of the 5 selected plant extracts showed that *A. leiocarpus* and *Z. mauritiana* had the highest levels compared to the levels of the other plants (*B. aegyptiaca*, *C. alata* and *O. celtidifolia*) (Figure 1). These results are in agreement with the work of the isolated phenolic compounds of *A. leiocarpus* [24, 25]; some studies on the different parts of *Z. mauritiana* have shown high levels of phenolic compounds such as tannins and flavonoids. The tannins, which were the majority compounds of total phenols, are known for their hepatoprotective effect, anti-inflammatory, antibacterial and antiviral properties[26]. Also, flavonoids play a very important role in the treatment of diabetes, gout, inflammations, tumours, hypertension, thrombosis, allergies and bacterial and viral diseases [27]. This anti-inflammatory activity of tannins and flavonoids contained

in different plant extracts could explain the use of these plants by traditional healers in the care of hepatitis.

Several studies on medicinal plants have correlated total phenolics with antioxidant power[14]. A good linear correlation between total phenolics and antioxidant activity would show that the bulk of the antioxidant activity is due in part to the presence of a large proportion of the total phenolics[14]. These compounds are considered to be the main factors contributing to the antioxidant activity of medicinal plants. Indeed, their redox properties would enable them to act as reducing agents, hydrogen donors, singlet oxygen distributors and also chelating agents for metals [28]. Besides, because of their low redox potentials, flavonoids would be able to reduce oxidative free radicals such as superoxide, peroxy, alkoxy and hydroxyl by hydrogen transfer and the resulting flavonoxyl radical could react with another radical to form a stable structure [29]. They would also be able to trap metal ions because they have chelating properties[22]. This antioxidant activity of the two plants (*A. leiocarpus* and *Z. mauritiana*) could be linked to the presence of high phenolic compounds in the different parts of these plants; which may explain their use in traditional medicine by traditional healers.

6. Conclusion

The ethno-medical survey showed a great diversity of species used for the care of hepatitis. Five (5) of these species, in particular *A. leiocarpus*, *B. aegyptiaca*, *C. alata*, *O. celtidifolia* and *Z. mauritiana*, were the subject of the experimental study. Phytochemical analysis showed that *A. leiocarpus* and *Z. mauritiana* are richer in phenolic compounds and are responsible for the antioxidant activities observed in their different extracts. *A. leiocarpus* and *Z. mauritiana* appear to have real potential by their anti-free radical activities and their content of phenolic compounds that can justify their use by traditional healers in the care of hepatitis.

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