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Ethnobotanical survey on medicinal plants (*Carica papaya* L. and *Agelanthus dodoneifolius* (DC.) Polhill & Wiens) used in the treatment of Hepatitis in Burkina Faso, phytochemistry and antioxidant activity

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Abstract

Hepatitis are pathologies of various etiologies affecting millions persons and the management by modern medicines still faces many difficulties. Our study aimed to make a repertory of medicinal plants used in the treatment of hepatitis in the Urbans areas of Bobo-Dioulasso, Dédougou and Fada N'Gourma followed by the phytochemical quantification and antioxidant activity of the most cited ones. We conducted an ethnobotanical survey among traditional Heath practitioners in the three studied locations to achieve this objective. Methanolic extracts of the plant organs were obtained using an extractor apparatus. Polyphenolic compounds contents quantification was done by spectrophotometry using Follin-Ciocalteu reagent and aluminum trichloride. Antioxidant activity was evaluated by three methods ($ABTS^{\bullet +}$, $DPPH^{\bullet}$, FRAP) and the reading of optical densities was performed with the spectrophotometer. A total of, 101 traditional healers were interviewed and 52 species were inventoried as being used in hepatitis care in the three localities. Among the species regularly cited in the different localities were *Carica papaya* (8%) and *Agelanthus dodoneifolius* (13%), and these two species were selected for the further investigation. Among the parts of plants most used there are roots (45%). Phytochemical investigations of *C. papaya* and *A. dodoneifolius* revealed that the best polyphenolic compound content was obtained by the unripe seeds of *C. papaya* with respectively 14.06±0.68 mg EAG and 4.37±0.57 mg EQ for 100 mg of extract. *A. dodoneifolius* extract was given the best antioxidant activity on $ABTS^{\bullet +}$ radical inhibition method with 9279.19±416.37 µmol EAA/g. All these activities could partially justify the use of screened species in the traditional treatment of hepatitis.

Keywords: Ethnobotanical; Hepatitis diseases; Antioxidant; Polyphenolics; Flavonoids

1. Introduction

Hepatitis are diseases with diverse etiologies affecting millions of people and causing a tremendeous number of deaths each year worldwide [1-3]. The number of people with chronic hepatitis B infection worldwide in 2015 was estimated at 257 millions and 71 millions with hepatitis C infection [4; 5]. Hepatitis B is the most prevalent viral hepatitis worldwide [6].

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In Africa, hepatitis is encountered in several countries such as Tunisia, Egypt, Morocco [5]. Sub-Saharan Africa, located in a zone of high endemicity has about sixty-five millions chronic carriers including 1.9 million cases in Burkina Faso [2; 7; 8]. According to Word Heath Organisation, Burkina Faso is classified as an high-prevalence country (≥ 8%) for hepatitis B virus and a low-prevalence country (1-2.5%) for hepatitis C virus [8].

Management of this affection depends on the etiology, the type of hepatitis, the condition of the liver and the patient [2]. The treatment proposed by modern medicine is done by nucleoside analogues and pegylated interferon, a molecule that is not widely available and this type of treatment is long and expensive [6]. Antivirals do not cure the hepatitis B virus, they do not eradicate HBV DNA, and treatment must be continued for life [1]. Although treated by modern medicine, people still reley to traditional medicine for the care of these diseases [9].

Moreover, according to the Center for Economic and Social Policy Analysis [2; 10], 90% of the Burkina Faso population use traditional medicine and pharmacopoeia for their care needs. This is due to inadequate health coverage, the inaccessibility of modern treatments and the exorbitant costs of pharmaceuticals [11]. Ethnobotanical and pharmacognostic research is essential to document and thus perpetuate traditional knowledge on health management using local plants. To this end, various scientific studies on hepatoprotective plants have been undertaken. Some have covered Africa [12], others have focused on smaller areas in the sub-region such as Benin and Mali [13-15]. However, in Burkina Faso, few studies focus on these medicinal plants used in the traditional treatment of liver diseases. As such, we can cite the work of [16] in the Hauts-Bassins, [17] et al. (2018) in the Cascades and [2] in the city of Bobo-Dioulasso. However, there are still several regions and many ethnic groups that have never been the subject of work on medicinal plants used against hepatitis. Also, natural antioxidants of plant origin have the property of trapping free radicals produced in excess due to the attack of liver cells by viruses, thus limiting and/or repairing the damage caused to the liver [2]. Thus, the aim of the present study was to conduct an ethnobotanical survey on medicinal plants used in the treatment of hepatitis in the cities of Bobo-Dioulasso, Dédougou and Fada N'Gourma in order to evaluate the interest of natural substances of some medicinal plants traditionally used against hepatitis and to select the plants with the hight citation rates for their phytochemical study and to evaluate the antioxidant activities.

2. Material and methods

2.1. Materials

2.1.1. Study setting

The ethnobotanical survey was carried in three localities of Burkina Faso. The phytochemical study and the antioxidant activity were carried out at the Laboratory of Research and Teaching in Animal Health and Biotechnology (LARESBA) at the Unit of Training and Research in Sciences and Techniques at the Nazi BONI University of Bobo-Dioulasso (U.N.B). Below the representative map of the survey (Figure 1).

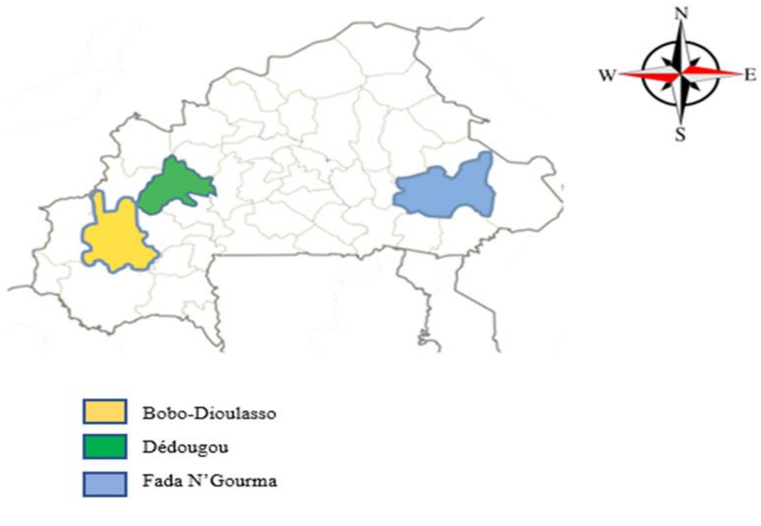

Figure 1 Map of the survey area (made by SAWADOGO Mamadou 12/01/21)

2.1.2. Plant material

Plant material consisted of bark and leaf stems of *Agelanthus dodoneifolius* (DC.) Polhill & Wiens (N 11°51'46.9" ; W 003°38'24.8") and the fruits of *Carica papaya* L. (N 11°51' 22.1" ; W 004°26 '56.7") collected respectively in December 2020 in Ouakara (Dédougou) and in the classified forest of Dindérésso (Bobo-Dioulasso). The two species were previously identified by Dr. Yempabou Herman OUOBA Botanist and Phytoecologist at the University Nazi BONI before the harvest. Then, the samples were dried in the laboratory protect from the sun, at room temperature and pulverized with an aluminum mortar to obtain powder. The powders obtained were packaged and labeled in zip lock bags that were finally used for the different operations in the laboratory.

2.1.3. Solvent and Reagents

All solvents were analytical grade. Agilent Cary 60 UV-Vis Spectrophotometer was used in all spectrophotometric measurements. Ascorbic acid, ferric chloride ($FeCl_3$), aluminum chloride ($AlCl_3$), potassium acetate, quercetin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic (ABTS), Folin-Ciocalteau reagent, gallic acid, sodium carbonate, methanol was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Millipore deionized water was used throughout. Thiazolyl Blue Tetrazolium Bromide (Sigma Aldrich, USA), Dimethyl Sulfoxide (Sigma Aldrich, USA).

2.1.4. Ethnobotanical survey

It was conducted from the month of August to October 2020 among traditional practitioners in three cities. In Bobo-Dioulasso, we surveyed traditional practitioners belonging to the "Jigi Sémè (culture of hope) Association of Houët Traditional Practitioners". In Dédougou, we surveyed traditional practitioners belongning to the "Regional Union of Traditional Practitioners' Association of Mouhoun Loop" and in Fada N'Gourma, we surveyed traditional practitioners belonging to the "Gulmu Traditional Practitioners Association". Information was collected in the local language in both areas (Dioula, Mooré, Gourmacèma) using a pre-established ethnobotanical survey form. It contained specific questions about the informant, the local identity of the plant drug, the part used, and the methods of preparation. This was a semi-structured interview with each traditional practitioner.

2.1.5. Extraction

15 g of plant powder from each sample was loaded into extracted cartridges with 200 mL using the soxhlet for at least 4 hours. After recovery of the solvent, the extract was concentrated, collected in a petri dish and dried under ambient laboratory conditions. The yields (R) of the extractions were calculated by the following formula.

$$\text{R} = \frac{mass\ of\ extract}{\text{mass extracted}} x100$$

2.2. Determination of polyphenolic compounds

2.2.1. Quantification of total phenolics

The estimation of total extractable phenolic compounds was performed by the Follin-Ciocalteu method described by [2]. The sample solution diluted to one hundredth from the stock solution was used. We used three tubes into which a 0.125 mL volume of the diluted extract solution plus a 625 µL volume of the 0.2 N Follin-Ciocalteu reagent incubated for 5 min was introduced. After a volume of 0.5 mL of a solution of sodium carbonate at 75 g/L in distilled water is then added and the mixture incubated for two (02) hours. A fourth tube was used for the preparation of the blank which contained a volume of 125 µL of distilled water plus 125 µL of Follin-Ciocalteu reagent and sodium carbonate. At the end of the incubation, the optical densities are read at 760 nm with a spectrophotometer. The standard calibration curve was plotted using gallic acid (0-200 mg/ L) ($y = 0.004668x + 0.034$; $R^2 = 0.9991$). A total of three (03) readings are taken for each extract and the result given is an average from these analyses. The results are expressed as mg Gallic Acid Equivalent per 100 mg extract or fraction (mg GAE/ 100 mg extract).

2.2.2. Determination of total flavonoids

The method used for the estimation of flavonoid levels in plant extracts and fractions is that described by [18]. The sample solution diluted to the hundredth was used to perform the operation. A total of four (04) tubes were prepared in which a volume of 625 µL of the diluted solution of each sample was introduced then we added to the first three (03) tubes 625µL of $AlCl_3$. The fourth tube considered as the control received 625 µL of methanol and then incubated for 10mn in the dark. Quercetin (0-100 mg/L) was used as a standard for the development of the calibration curve ($y = 0.01259x$; $R^2 = 0.9990$). After incubation three readings are taken per extract sample using a spectrophotometer at 415 nm wavelength the result given is an average of the three. The results are expressed as mg Quercetin Equivalent (QE) per 100mg of extract (mg QE/100mg).

2.3. Antioxidant activities

Reducing power by the FRAP method: 0.5 mL of the solution diluted to the hundredth is introduced into three (03) test tubes and 0.5 mL of distilled water into another tube for the blank. To these different tubes, a volume of 1.25 mL of phosphate buffer (0.2 M; pH = 6.6), then a volume of 1.25 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] is added. The whole is heated in a water bath at 50°C for thirty (30) min. After this operation, 1.25 mL of trichloroacetic acid (10%) is added and the mixture is centrifuged at 3000 rpm for ten (10) min. 625 µL of supernatant is removed from each tube and added to tubes containing 625 µL of distilled water. 125 µL of freshly prepared Trichloroferrate [$FeCl_3$ (0.1%)] is added to the resulting mixture. The resulting solution is stirred and then run on a spectrophotometer for a series of three (03) absorbance and concentration readings at a wavelength of 700 nm against a standard (y = 0.00327x; R^2 = 0.9990) established from ascorbic acid [2].

Anti-radical activity by the DPPH● radical inhibition method: in three (03) test tubes 375 µL of the 1/100th diluted solution and 750 µL of a DPPH solution (20 mg/L) were introduced and then 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 using a spectrophotometer at 517 nm against a standard (y=-0.02224x +0.348; R^2 = 0.9966) obtained from ascorbic acid. The method used is described according to the protocol of [2].

Reducing power by ABTS$^{\bullet+}$ method: For each extract, a methanolic solution (10 mg/mL) is diluted to 100th in distilled water. Ten (10) µL of sample (diluted solution) is taken and then mixed with 990 µL of fresh ABTS$^{\bullet+}$ solution. The whole mixture is incubated in the dark for 15 minutes. Absorbances and concentrations were read three (03) times at a wavelength of 734 nm on a spectrophotometer against a standard curve established from ascorbic acid (y= -0.0007874x +0.709; R^2 =0.9993) [2]. The results of the antioxidant activities are determined by the formula:

C = ((c*D))/ (M*Ci)

C = concentration of anti-free radical compounds in µmol AAE/g extract or fraction.

c = concentration of the sample read on the standard curve

D = dilution factor of the sample (100)

Ci= initial concentration of the solution to be determined (10mg/ml)

M= molar mass of ascorbic acid (176.1 g/mol)

Data analysis and processing: Data entry and analysis were done with Microsoft Word 2010 and Excel 2007. The parameters studied were gender, parts of the plant used, mode of preparation and the frequency of citation (FC) of each plant was calculated by the formula:

$$FC = \frac{Nc}{Nt} * 100$$

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

3. Results and discussion

3.1. Ethnobotanical survey

3.1.1. Characteristics of the subjects surveyed (according to sex)

In the study, both sexes practice traditional medicine, i.e., 85% of men and 15% of women in the Bobo-Dioulasso area; 69% of men and 31% of women in Dédougou and 71% of men and 29% women in Fada N'Gourma (Figure 2). According to the survey data, we have as many women in traditional medicine in Dédougou and Fada N'Gourma (30%) and twice as many in the city of Bobo-Dioulasso (15%). However, we note that the male sex predominates over the female sex. Similar studies in other communities show the same trends [2; 19]. This is the case for Nayala and Sourou (another province of Burkina Faso) conducted by [19] in 2011 and that of [2] in 2020 of Bobo-Dioulasso. These researchers also observed repectively 80% against 20% of female sex and 52% against 48% for the female sex, respectively. This low participation could be related to certain ancestral and cultural practices of traditional healers that most often exclude women. We can therefore say that traditional medicine is a practice reserved for men in Burkina Faso.

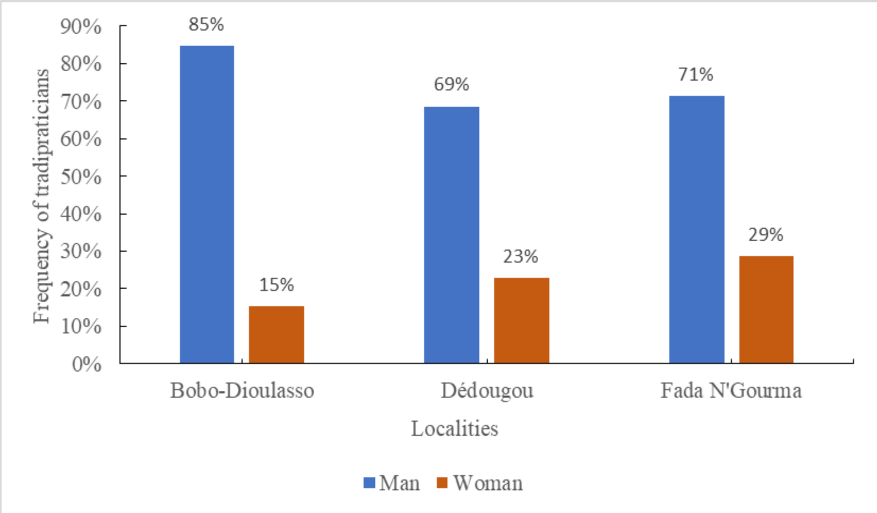

Figure 2 Distribution of traditional healers

3.1.2. Distribution of subjects according to professional experience

The majority of the traditional healers surveyed have 15 to 30 years of experience (Figure3). In addition, these respondents claim to have inherited medicinal knowledge about plants from their parents. This could be endogenous knowledge transmitted from generation to generation. Studies have shown that experience accumulated with age is the main source of information at the local level. In particular, according to [20] it has been recognized that in Africa, the oldest people, who hold the traditional knowledge of treating diseases.

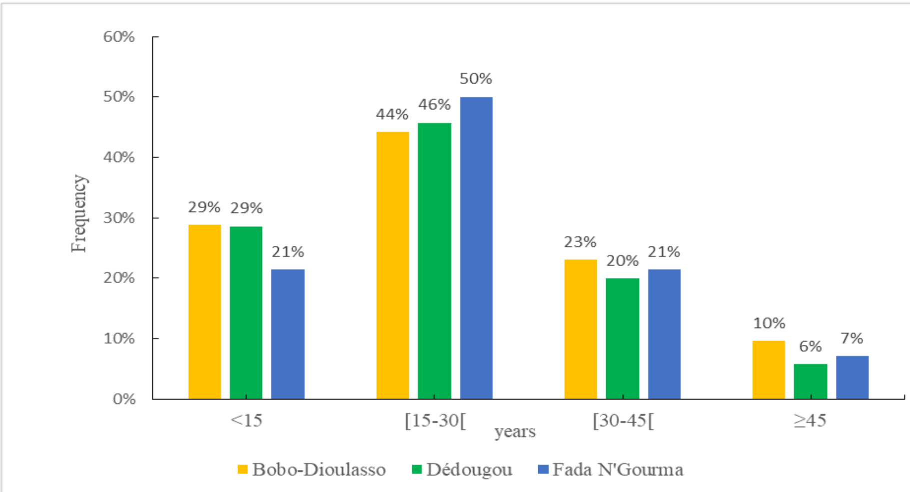

Figure 3 Distribution of herbalists surveyed according to professional experience

3.1.3. Frequency of plant citation in each zone

In Dédougou, thirty-five (35) traditional practitioners were interviewed and 31 species belonging to 22 botanical families were identified. Of all the species, *Agelanthus dodoneifolius* (21%), *Carica papaya* (12%) and *Cassia obstisufolia* (12%) were the most frequently cited (Figure 4a). We surveyed fifty-two (52) traditional healers and identified seventeen (17) species belonging to fourteen (14) botanical families in Bobo-Dioulasso, *Combretum micranthum* had the highest frequency of citation (47%), followed by *Carica papaya* (35%) (Figure 4b). In Fada N'Gourma, fourteen (14) traditional practitioners were interviewed and sixteen (16) species belonging to fourteen (14) botanical families were identified. Of all the species, *Combretum micranthum*, *Parkia biglobosa* and *Chysanthellum americanum* were the most frequently cited with 13% of citation (Figure 4c). The frequency of quotations reveals that the variation of quotations from one zone to another could be related to the number of traditional healers interviewed. However, overall, four species are the most cited because of their higher frequency of citation and their regular citation in the different study areas: *Combretum micranthum* (15%), *Entada africana* (12%), *Agelanthus dodoneifolius* (13%), and *Carica papaya* (8%) (Figure 5). We chose the last two of these four species, namely *Agelanthus dodoneifolius* (13%), *and Carica papaya* (8%) (Figure 5). Indeed, the first two species were the subject of a similar study conducted by other author [2] and collalorators (2020) in Bobo-Dioulasso (same city of Burkina Faso).

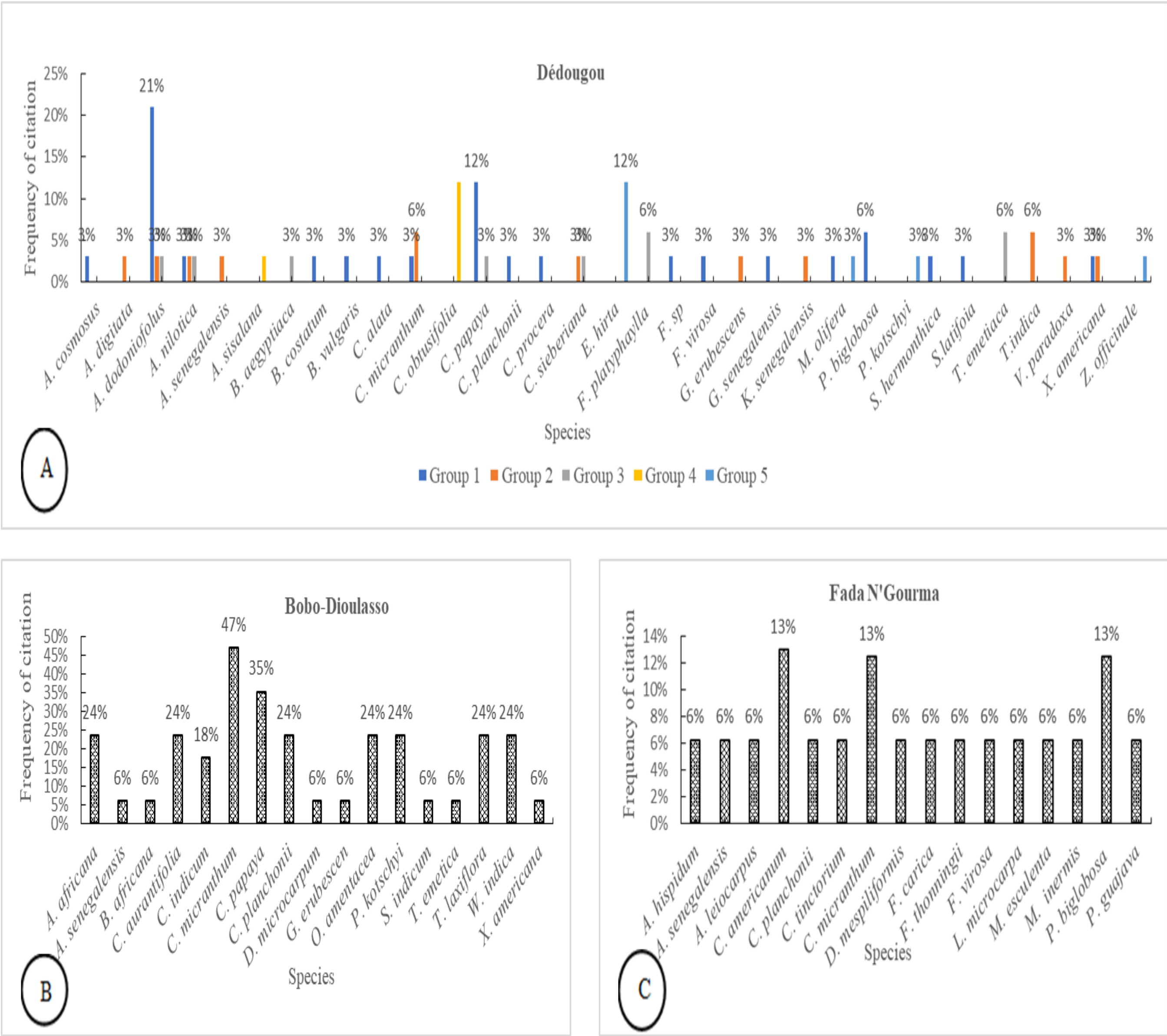

Figure 4 Hepatoprotective medicinal plants identified with citation frequency

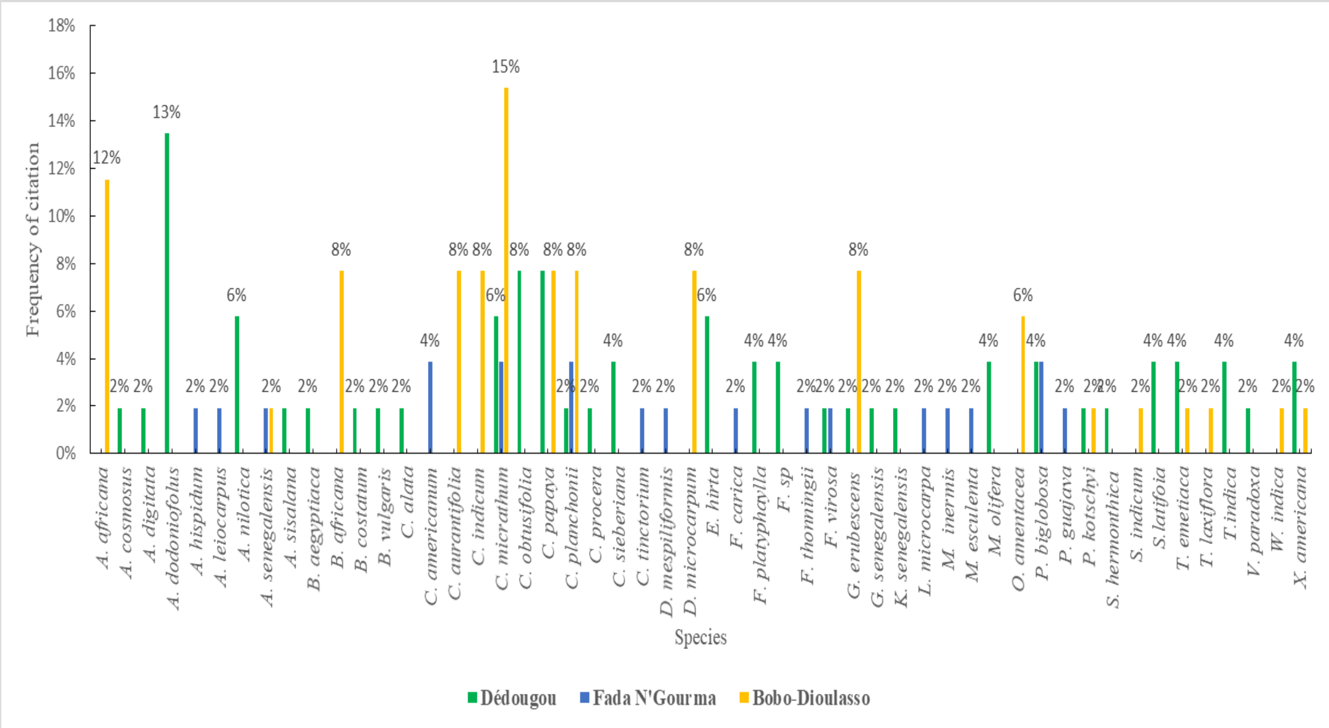

Figure 5 Frequency of citation of medicinal plants used against hepatitis

3.1.4. Distribution according to the parts used

The distribution of the parts used shows that roots and leaves use are predominant in each area (Figure 6). We observe that roots ant leaves are more exploited. These important uses of roots (45%) constitute a threat for biodiversity. The same observation was made by [21] in 8 villages in Soum (another city of Burkina Faso), finding that roots were used at 41%. Comparing the parts used by traditional healers in these four areas, traditional healers use plant resources in almost the same way for health care. According to [21; 22] the preferential use of leaves is to be encouraged because it presents a double advantage. The leaves regenerate more easily than the other organs of the plant (root, bark) and also their only uses make it possible to safeguard biodiversity. Then, the use of these leaves avoids the destruction of the plant and preserves its perenniality [20; 21].

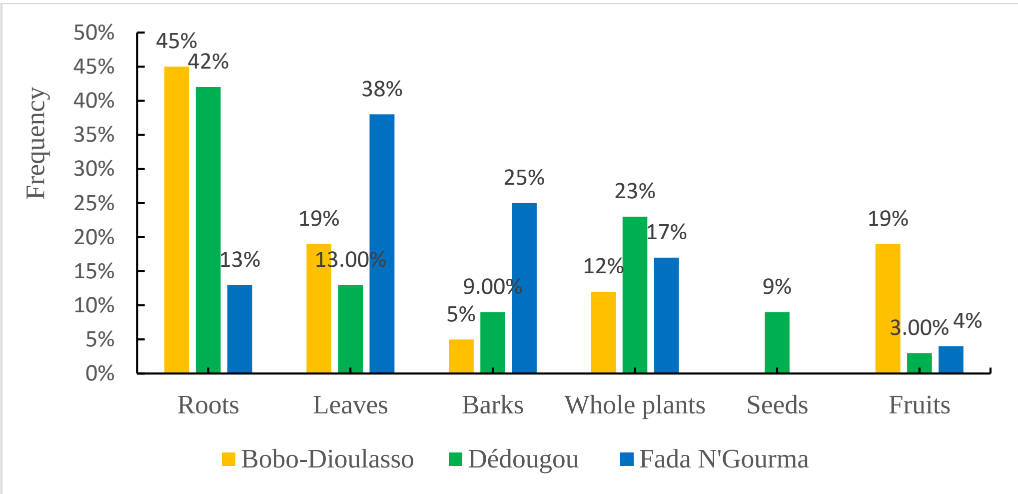

Figure 6 Frequency of the different parts used

3.1.5. Distribution according to the method of preparation of the drug

Various methods are used by traditional practitioners in each area (decoction, powder, maceration and infusion). Analysis of the surveys revealed that decoction was the main mode of use (60%, 89% and 71%) in Bobo-Dioulasso, Dédougou and Fada N'Gourma respectively (Figure 7). This distribution shows that in the Dédougou area, more than half of the traditional healers use decoctions to treat hepatitis. Also, [23] in Senegal showed that decoction was the main mode of use (66,66%). At the end of this study, we can say that African populations tend to use traditional medicine in the decoction form for their care needs.

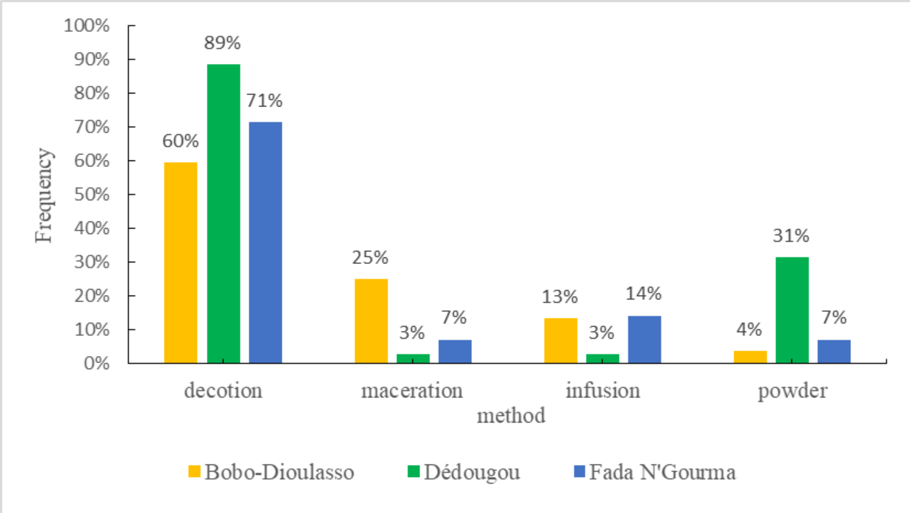

Figure 7 Distribution according to preparation method

3.2. Determination of polyphenolic compounds and antioxidant activities

For the quantification of total phenolics the Follin-Ciocalteu reagent was used and for flavonoids the aluminum trichloride ($AlCl_3$) reagent.

3.2.1. Determination of polyphenolic compounds

The total phenolics content ranged from 5.84 ± 0.32 to 14.06 ± 0.68 mg GAE/100 mg extract (Table 1). The best content was obtained by the extract of unripe seeds of *Carica papaya* (14.06 ± 0.68 mg EAG/100 mg extract). Total flavonoids content is ranged from 0.84 ± 0.04 to 4.37 ± 0.54 mg QE/ 100 mg extract (Table 1). The unripe seeds extract of *Carica papaya* have given the best content with 4.37 ± 0.54 mg QE/100 mg extract.

Table 1 Results of the determination of phenolic compounds

Species	Parts used	TP mg (EAG)/100mg	TF mg (EQ)/100mg
C. papaya	Ripe seed	8,78±0,77^{c}	0,84±0,04^{d}
	Unripe seed	14,06±0,68^{a}	4,37±0,54^{a}
A. dodoneifolius	Barks	5,84±0,32^{d}	2,42±0,60^{b}
	Leaf stem	9,97±0,67^{ab}	1,77±1,37^{c}

TP = total phenolics, TF = total flavonoids. Values are mean ± SD (n = 3).
Different letters in the same column indicate significant difference ($p < 0.05$).

These results allow us to say that polyphenolic compounds contents vary from one species to another, from one organ to another and from one stage of development of the same organ to another. On the other hand, this variation in content may be due to the influence of biotic and abiotic factors on the synthesis of secondary metabolites which are quantified here. This value is 7 times higher than ours which is 14. This discrepancy in our results could be justified by the organ used which is different from ours. Also, this variation in content may also be related to the type of solvent used which is an ethanolic maceration for [24] and collaborators (2020). In Côte d'Ivoire, other researchers who worked on *Carica papaya* leaves found different results than ours [24]. Indeed, they showed that the leaves of *Carica papaya* contains 96.66 mg EAG/g d'extract related to total phenolics and total flavonoids contents (57.67 mg EQ/g d'extract). Flavonoids are known to be endowed with such properties in plants. Its can exert a wide range of biological activities such as anti-inflammatory properties, decrease of oxidative stress, anti-carcinogenic, anti-ulcer [25]. The polyphenolic compounds could probably be responsible for the antiviral and hepatoprotective activities attributed to these species.

3.3. Antioxidant activity

The antioxidant activity of our extracts was evaluated according to the method (ABTS$^{\bullet +}$, DPPH$^{\bullet}$ and FRAP). The DPPH$^{\bullet}$ radical inhibition values ranged from 178.74 ± 46.8 to 828.960±7.80 µmol EAA/g (Table 2). The best radical scavenging activities were obtained from *A. dodoneifolius* (828,960±7,80 µmol EAA/g). It is better than quercetin and trolox (Figure 8). The lowest activities were obtained with *C. papaya* i.e., (178.74 ± 46.8 µmol EAA/g

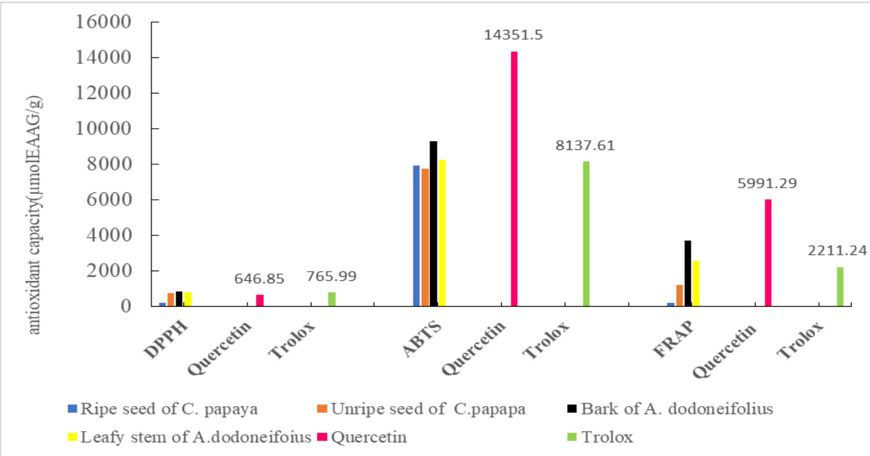

Figure 8 Antioxidant activity by the three methods

As for ABTS$^{\bullet +}$ method, the antioxidant capacity varied from 7019.49 ± 181.49 to 9279.19 ± 416.37 µmol EAA/g (Table 2). The best activities were held with barks of *Agelanthus dodoneifolius,* 9279.19 ± 416.37 µmol EAA/g). It is better than trolox (8137.61 µmol EAA/g) (Figure 8). The lowest was obtained with Ripe seed of *C. papaya* (7019,49 ± 181,49 µmol EAA/g).

For that of reducing power by FRAP method, the best activities were obtained by barks of *Agelanthus dodoneifolius* (3704.67 ± 20.03 µmol EAA/g), followed by *Agelanthus dodoneifolius* steam-leaves. The results of these activities are reported in Table 2.

Table 2 results of antioxidant activities

Extracts	Parts used	DPPH$^{\bullet}$ (µmol EAA)	ABTS$^{\bullet +}$ (µmol EAA)	FRAP (µmol EAA)
C. papaya	Ripe seed	178,74 ± 46,8d	7019,49 ± 181,49^{d}	214,19 ± 20,06^{d}
	Unripe seed	733,65 ± 21,25c	7740,67 ± 110,16^{c}	1186,67 ± 65,75^{c}
A. dodoneifolius	Barks	828,960 ± 7,80^{a}	9279,19 ± 416,37^{a}	3704,67 ± 20,03^{a}
	Leaf stem	801,76 ± 2,55^{ab}	8221,46 ± 124,91^{b}	2546,96 ± 101,76^{b}

Values are mean ± SD (n = 3). Different letters in the same column indicate significant difference ($p < 0.05$).

Taking into account the three antioxidant tests carried out, it is necessary to note on the one hand that *Agelanthus dodoneifolius* gives the best activities and on the other hand that the bark contains the best antioxidant molecules (anti-DPPH$^{\bullet}$: 828,960±7,80 µmol EAA/g < anti-FRAP: 3704.67 ± 20.03 µmol EAA/g < anti-ABTS$^{\bullet +}$: 9279.19 ± 416.37 µmol EAA/g). By content with the parts used the activities are arranged in the following descending order: barks > stem-leaves >unripe seed > ripe seed. These results obtained vary depending on the organ and species used.

If the activities were only influenced by the total phenolic contents, then the extracts of the unripe seed or steam-leaves would have given the best activities on the three antioxidant methods. This is not the case. Indeed, the best anti-ABTS$^{\bullet +}$ activity is obtained by the extracts of the barks of *A. dodoneifolius*. Similarly, the best anti-FRAP activity (3704.67 ± 20.03 µmol EAA/g) is given by the bark of *A. dodoneifolius* (2.42 ± 0.60 mg EQ/100g extract). Which logically should be the unripe seeds (4.37 ± 0.54 mg EQ/100g extract). Then, if we try to link the observed activities to that of the total flavonoid contents we are also confronted with a difficulty because the unripe seeds having given the best total flavonoid contents also possess the lowest activities. Thus, it probable to have a random distribution of the contribution on the observed antioxidant activities. A similar observation was made by several other authors [26 ; 27]. According to the previous results on the ability of the extracts to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). It was difficult to establish a very clear link between the level of flavonoids and reducing power [26].

Particularly at the level of this present study, it emerged that the molecules contained in *A. dodoneifolius* bark have a great antioxidant capacity. And if traditional uses were linked to methanolic extracts, then those uses would be easily justified.

4. Conclusion

The surveys allowed us to interview 101 traditional practitioners and to identify 52 medicinal plants divided into 31 botanical families. The study mainly revealed that the majority of practitioners were men (at least 69%) and majority used decoction (89%). According to the frequency of quotation, *A. dodoneifolius* and *C. papaya* were most species used in the treatment of hepatitis diseases. Interesting results were obtained in terms of polyphenolic compounds contents and antioxidant activity. The activities are functions of the plant organs and *A. dodoneifolius*'s barks possess the best molecules to trap free radicals (828.96 ± 7.80 µmol EAA/g), cation radicals (9279.19 ± 416.17 µmol EAA/g) and reduce iron. (3704.67 ± 20.03 µmol EAA/g). The hepatoprotective properties are thought to be related to the presence of these secondary metabolites in the organs of medicinal plants. Thus, the polyphenolic compounds and antioxidant activity could partially justify the use of these plant species in the treatment of hepatitis. Our next study will attempt to identify the different recipes used, (1) to repeat the different analyses, (2) to test total extracts and fractions obtained *In vitro* and *In vivo* in order (3) to carry out bio-guided techniques for the marketing of traditional improved medicinal products.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

If two or more authors have contributed in the manuscript, the conflict of interest statement must be inserted here.

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