

(RESEARCH ARTICLE)



Ethnobotanical study, phytochemistry and antioxidant activity of medicinal plants used in the treatment of inflammation in two cities of Burkina Faso

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Abstract

Background and Objective: The present study aimed to contribute to the knowledge of medicinal plants used in the treatment of inflammation in Burkina Faso, as well as to quantify the polyphenolic compounds content and to evaluate the antioxidant activity of two most solicited species.

Materials and Methods: An ethnobotanical survey was carried out in the urban areas of Bobo-Dioulasso and Fada N'Gourma in order to collect sociodemographic and ethnobotanical data from traditional practitioners. Methanolic extracts of the plant organs were obtained using an extractor apparatus. The quantification of polyphenolic compounds contents was done by spectrophotometry, respectively with the Folin Ciocalteu reagent and aluminum trichloride. Likewise, antioxidant activity was evaluated by three methods (ABTS^{•+}, DPPH[•] and FRAP) and the reading of optical densities was performed with the spectrophotometer.

Results: One hundred and twelve (112) traditional practitioners were interviewed and seventy-three (73) plant species were identified in both areas. According to the frequency of quotation, *Entada africana* and *Khaya senegalensis* species were retained for further study. Phytochemical study revealed that the total phenolic content was high in the trunk bark of *K. senegalensis* (73.12 ± 1.8 mg GAE/100mg) and the total flavonoid content in the trunk bark of *E. africana* (8.42 ± 0.78 mg QE/100mg). Of the antioxidant activity, the highest value was observed at the level of the ABTS^{•+} radical inhibition method with the best result 13247.2 ± 219.84 µmol AAE/g (leaves of *E. africana*).

Conclusion: The phytochemical analyses of these species indicate their richness in total phenolics and flavonoids contents, and could justify their use in the treatment of inflammatory diseases.

Keywords: Ethnobotany; Inflammation; Antioxidant; Polyphenols; Flavonoids.

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1. Introduction

Management of worldwide health and control of diseases becomes more difficult, due to the persistence, the resurgence of chronic diseases and because of the appearance of many uncontrolled diseases [1, 2]. Among these chronic diseases are inflammatory ones [2]. Inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the body whose goal is to eliminate the pathogen repair tissue damage and promote a return to homeostasis [3, 4]. Sometimes, the inflammatory response can be detrimental due to aggressiveness, persistence of the pathogen, site of inflammation, by abnormalities in the regulation of the inflammatory process, or by quantitative or qualitative abnormality of the cells involved in the inflammation process [5, 4]. During the inflammatory response, Reactive Oxygen Species (ROS) are produced and contribute to amplify the phenomenon of oxidative stress [6]. Regulation of reactive species has been shown to mitigate inflammation [7]. Drugs used to treat different forms of inflammatory diseases are known to cause severe side effects such as digestive damage and renal toxicities regardless of their route of administration [8-11], so there is an urgent need to find a new source of effective drugs with minimal side effects [7].

Herbal medicine proves to be a good alternative as it has been used for centuries to treat ailments [12]. Plants are a valuable source of natural products because they are less toxic. These medicinal plants can therefore be important resources for new substances with therapeutic potential and expensive costs [13]. Thus, [14] and collaborators in 2015 estimated that about 80% of the world population and more than 90% of the population of developing countries resort to traditional medicine for primary health care [14, 15]. Indeed, plants possess secondary metabolites capable of mitigating oxidative damage induced by Reactive Oxygen Species and interacting with different physiological mediators and effectors of inflammation [7]. Previous studies in Africa have shown that *Entada africana* and *Khaya senegalensis* are used extensively in traditional medicine, to treat various pathologies most notably in the treatment of inflammatory pathologies [16-18]. Other researchers have also shown that plant secondary metabolites are functions of climatic and edaphic conditions [19, 20]. The general objective of this work is to contribute to the knowledge of medicinal plants used in the treatment of inflammatory diseases in Burkina Faso in order to develop improved traditional drugs in the future.

2. Material and methods

2.1. Materials

2.1.1. Study setting

We conducted ethnobotanical surveys in the urban areas of Burkina Faso (Figure 1). 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).

2.1.2. Plant material

Plant material consisted of leaves, trunk bark and root barks of *Entada africana* Guill. Perr. Fabaceae-Mimosoideae and *Khaya senegalensis* (Desr.) A. Juss. Meliaceae collected in December 2020 in the classified forest of Dindéréso (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. Solvents 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-Ciocalteu 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).

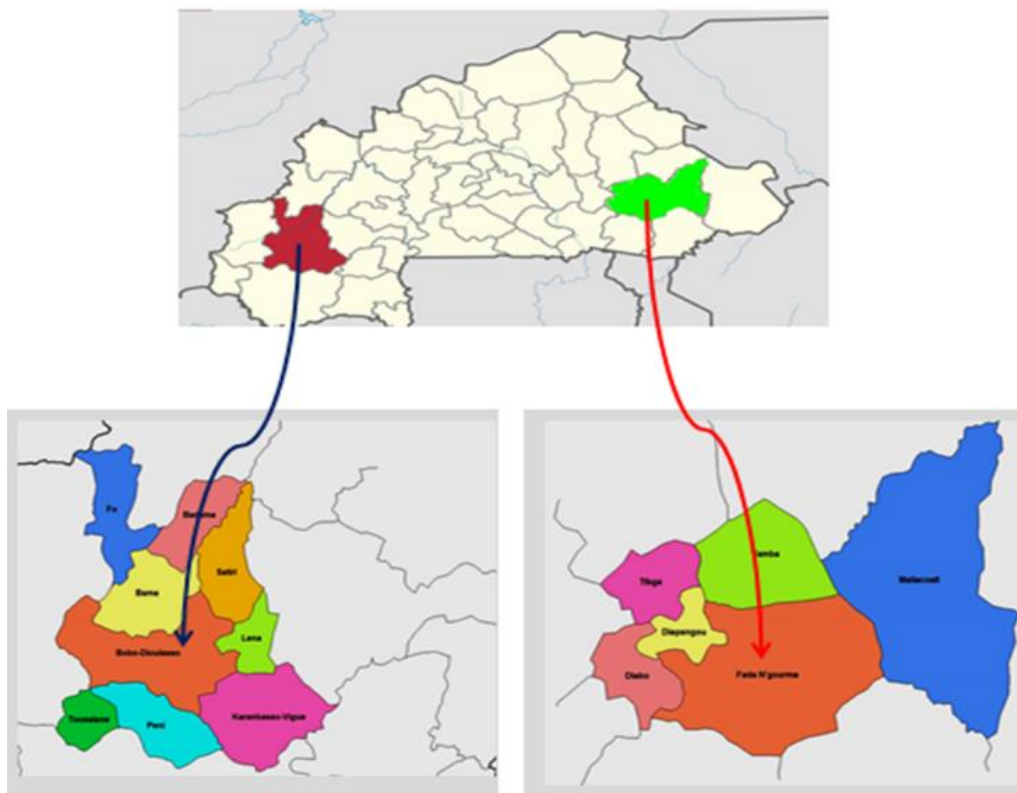


Figure 1 Mapping of study sites (made by LEMA Armandine 03/01/21)

2.1.4. Ethnobotanical survey

It was conducted from the month of August to October 2020 among traditional practitioners in the cities of Bobo-Dioulasso and Fada N'Gourma. In Bobo-Dioulasso, we surveyed traditional practitioners belonging to the " Jigi Sémè (culture of hope) Association of Houët Traditional Practitioners " 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

$$R = \frac{\text{Mass of extract}}{\text{Mass extracted}} \times 100$$

2.2. Determination of polyphenolic compounds

2.2.1. Quantification of total phenolics

The estimation of total extractable phenolic compounds was performed by the Folin-Ciocalteu method described by [21]. 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 μ L volume of the 0.2 N Folin-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 μ L of distilled water plus 125 μ L of Folin-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 100mg extract (mg GAE/ 100 mg extract).

2.2.2. Determination of total flavonoids

The method used for the estimation of flavonoid levels in plant extracts is that described by [22]. 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 625 μL 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

2.3.1. 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 [$\text{K}_3\text{Fe}(\text{CN})_6$] 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 ($y = 3.270 \cdot 10^{-3}x$; $R^2 = 0.9990$) established from ascorbic acid [21].

2.3.2. 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 ($y = -2.224 \cdot 10^{-2}x + 0.348$; $R^2 = 0.9966$) obtained from ascorbic acid. The method used is described according to the protocol of [21].

2.3.3. Reducing power by ABTS^{®+} 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^{•+} solution. The whole mixture is incubated in the dark for 15 minutes. Absorbance and concentrations were read three (03) times at a wavelength of 734 nm on a spectrophotometer against a standard curve established from ascorbic acid ($y = -7.874 \cdot 10^{-4}x + 0.709$; $R^2 = 0.9993$) [21]. The results of the antioxidant activities are determined by the formula:

$$C = \frac{[c \times D]}{M \times Ci}$$

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/mole)

2.3.4. 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:

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

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., 80% of men and 20% of women in the Bobo-Dioulasso area, and 70% of men and 30% of women in Fada N'Gourma (Figure 2a and figure 2b). We note that the male sex predominates over the female sex. Studies conducted by [23] in the city of Banfora also showed that the vast majority of the traditional practitioners interviewed were men (91.11%). Our results are in line with those of [23] and colleagues 2009. We can therefore say that traditional medicine is a practice reserved for men in Burkina Faso.

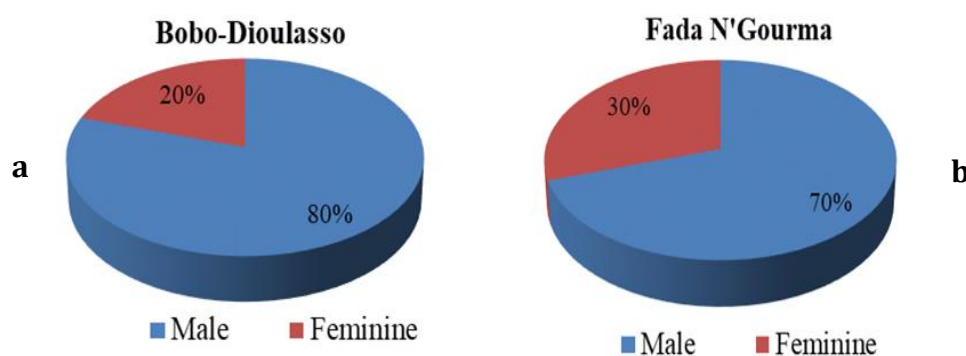


Figure 2 Distribution of respondents by gender

3.1.2. Distribution of subjects according to professional experience

All of the traditional healers surveyed have been practicing this profession for at least three years. The majority have more than 20 years of experience (Figure 3).

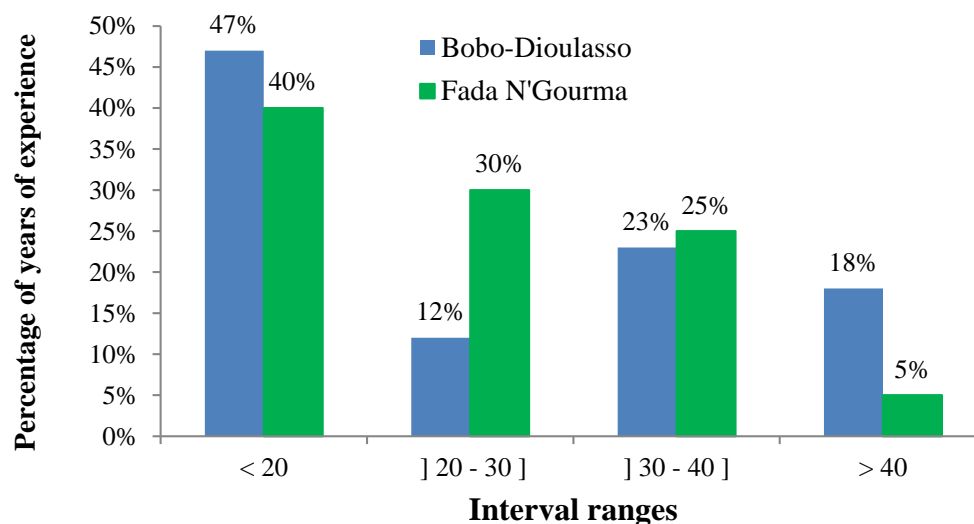


Figure 3 Distribution of herbalists surveyed according to professional experience in Bobo-Dioulasso and Fada N'Gourma

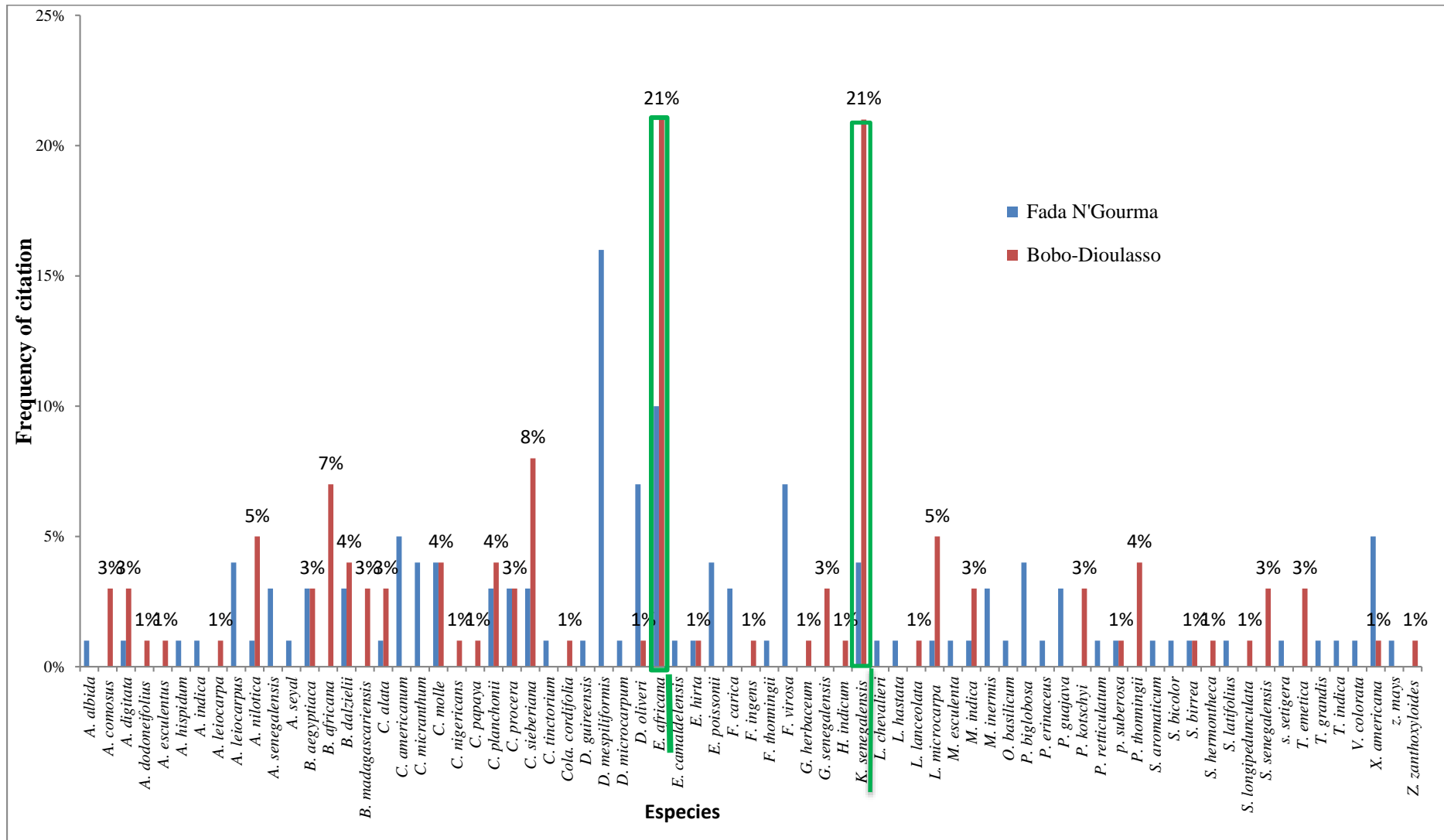


Figure 4 distribution of species according to study areas by frequency of citation

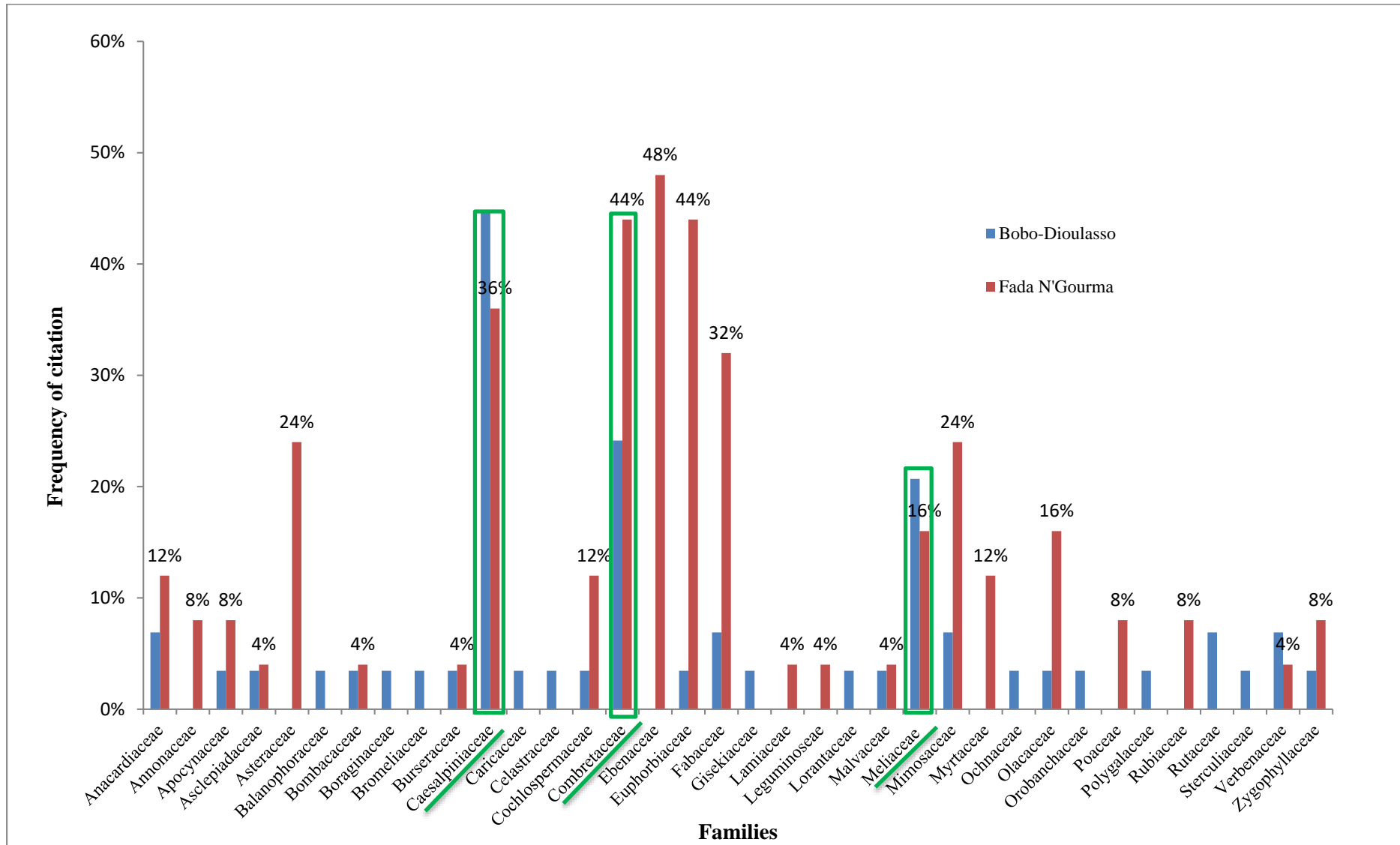


Figure 5 Distribution of families according to the different study area

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, it has been recognized that in Africa, it is the wise, i.e., the oldest people, who hold the traditional knowledge of treating diseases [24]. Other authors have also shown that valorizing the therapeutic experience of local Sahelian populations could contribute to developing improved traditional medicines or isolating new bioactive molecules for the management of infectious diseases that continue to claim more victims each year [25].

3.1.3. Frequency of plant citation in both zones

In Bobo-Dioulasso, 60 traditional practitioners were interviewed and 41 species belonging to 29 botanical families were identified. Of all the species, *Entada africana* (21%) and *Khaya senegalensis* (21%) were the most frequently cited (Figure 4). Similarly, the most represented botanical families were Caesalpiniaceae (13 species with 45%), Combretaceae (7 species with 24%) and Meliaceae (6 species with 21%) (Figure 5). We surveyed 52 traditional healers and identified 51 plant species belonging to twenty-five (25) botanical families in Fada N'Gourma. *Entada africana* and *Khaya senegalensis* had 10% and 4% respectively (Figure 5). Among the most cited families in the area families; we have Caesalpiniaceae (6 species with 36%), Combretaceae (4 species with 24%) and Meliaceae (21%) (Figure 5). Other researchers, such as [21] and collaborators (2020) also found that the best represented families were the Caesalpiniaceae and Combretaceae in their study. Our data is similar to that of [21]. We deduce that plant species belonging to these two families (Caesalpiniaceae and Combretaceae) are better known by traditional healers for their medicinal properties.

3.1.4. Distribution according to the parts used

The distribution of the parts used shows that leaves and barks use are predominant in both areas (Figure 6). We note that roots and bark are more exploited. These important uses of these organs constitute a threat for biodiversity. The same observation was made by [25] and collaborators in 2019 in 8 villages in Soum (another city of Burkina Faso), finding that roots and bark were used at 41%, compared to 43% for leaf uses. Comparing the parts used by traditional healers in these three areas, traditional healers use plant resources in almost the same way for health care. According to [25-27] the preferential use of leaves is to be encouraged because it presents a double advantage, on the one hand leaves are the place of synthesis of secondary metabolites [24], contain many chemical groups, on the other hand the use of these leaves avoids the destruction of the plant and preserves its perenniality [25].

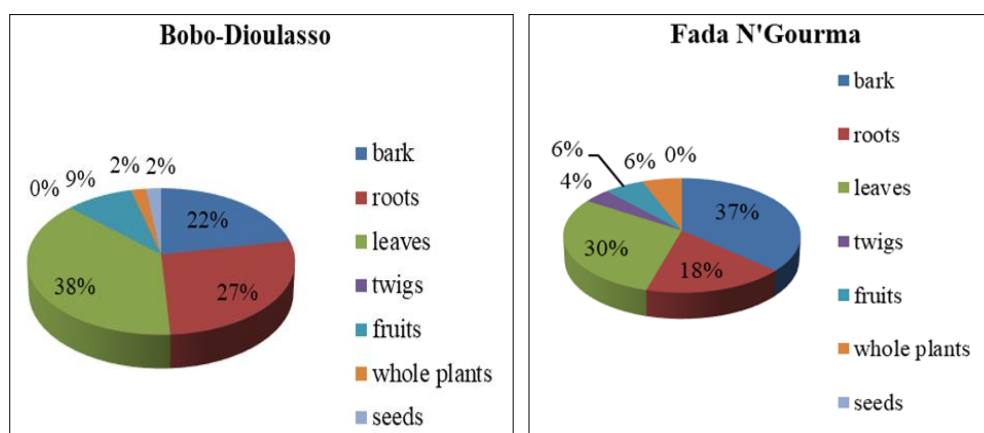


Figure 6 Frequency of the different parts used

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

Various methods are used by traditional practitioners in both areas (decoction, incineration, powder, maceration and infusion). Analysis of the surveys revealed that decoction was the main mode of use (51.28% and 82%) in Bobo-Dioulasso and Fada N'Gourma respectively (Figure 7). This distribution shows that in the Fada N'Gourma area, more than half of the traditional healers use decoctions to treat inflammations. [28] In 2018 in Mali also showed that decoction was the main mode of use (81.3%). By comparing these results with those of Fada N'Gourma, we deduce that they two populations use the similar mode.

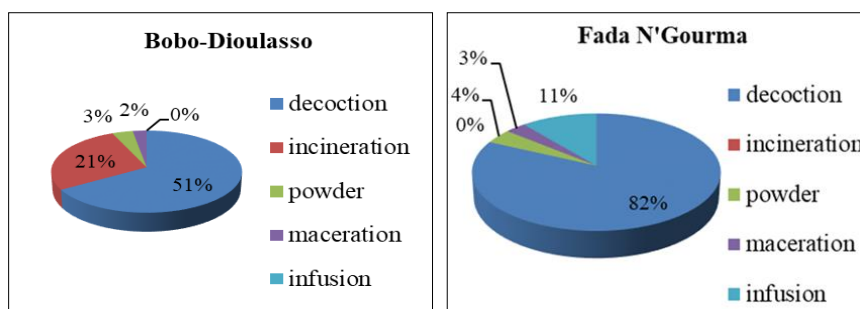


Figure 7 Distribution according to preparation method

3.2. Determination of polyphenolic compounds and antioxidant activities

For the quantification of total phenolics the Folin-Ciocalteu reagent was used and for flavonoids the aluminum trichloride (AlCl_3) reagent (Table 1).

Table 1 results of the determination of phenolic compounds

Species	Extracts	Total phenolics mg (GAE)/100mg	Total flavonoids mg (QE)/100mg
<i>E. africana</i>	Leaves	28.35 ± 2.10 ^e	7.62 ± 1.07 ^b
	Trunk bark	50.34 ± 5.70 ^c	8.42 ± 0.78 ^a
	Root barks	30.35 ± 0.1 ^d	4.47 ± 1.06 ^c
<i>K. senegalensis</i>	Leaves	25.56 ± 0.25 ^f	4.63 ± 1.00 ^c
	Trunk bark	73.12 ± 1.8 ^a	2.6 ± 0.52 ^f
	Root barks	60.48 ± 7.58 ^b	3.1 ± 1.65 ^e

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

3.2.1. Determination of polyphenolic compounds

The total phenolic contents of the methanolic extracts varied from 25.56 ± 0.25 to 73.12 ± 1.8 mg GAE/100mg extract (Table 1). The highest contents were obtained with the trunk (73.12 ± 1.8 mg GAE/ 100mg extract) and root barks (60.48 ± 7.58 mg GAE/ 100mg extract) of *K. senegalensis* and the trunk barks (50.34 ± 5.70 mg GAE/ 100mg extract) of *E. africana*, respectively. On the other hand, the lowest contents of total phenolics were obtained with the leaves of *Khaya senegalensis* (25.56 ± 0.25 mg GAE/ 100mg extract), followed by those of *E. africana* (28.35 ± 2.10 mg GAE/ 100mg extract).

Total flavonoid contents ranged from 2.6 ± 0.52 to 8.42 ± 0.78 mg QE/100mg extract, with high contents of 8.42 ± 0.78 and 7.62 ± 1.07 mg QE/100mg for *E. africana* barks and leaves. The low values were obtained with *K. senegalensis* (trunk bark: 2.6 ± 0.52 mg QE/100mg extract and root bark: 3.1 ± 1.65 mg QE/100mg extract). The results are reported in Table 1.

These results show that polyphenolic compounds vary from one species to another and from one organ to another organ of the same species. Studies conducted by [14] in 2006 on *Combretum micranthum*, *Khaya senegalensis*, *Pterocarpus erinaceus* and *Sida acuta*, four plant species from Burkina Faso, also found that the bark of *K. senegalensis* had the highest content of phenolic compounds content (47.19 μg GAE). This trend shows that phenolic compounds are more concentrated in the bark than in the leaves.

3.3. Antioxidant activity

The antioxidant activity of our extracts was evaluated according to the method (ABTS^{•+}, DPPH[•] and FRAP). Regarding the DPPH[•] radical scavenging assay, the radical scavenging capacity of the extracts ranged from 659.62 ± 22.90 μmol AAE to 825.57 ± 9.64 μmol AAE (Table II). The best radical scavenging activities were obtained with the trunk and root barks of the two species, i.e. 825.57 ± 9.64 μmol AAE and 822.14 ± 0 μmol AAE for *E. africana*, 808.57 ± 3.90 μmol AAE and 813.66 ± 6.40 μmol AAE for *K. senegalensis*. The lowest activities were obtained with the leaves of *E. africana* and *K. senegalensis* i.e. 659.62 ± 22.90 μmol AAE and 799.18 ± 28.77 μmol AAE, respectively.

Table 2 results of antioxidant activities

Species	Parts used	DPPH• (µmol EAA)	ABTS•+ (µmol EAA)	FRAP (µmol EAA)
<i>E. africana</i>	Leaves	799.18±28.77 ^e	13247.2±219.84 ^a	1615.01±90.22 ^e
	Trunk bark	825.57±9.64 ^a	5601.1±41.63 ^e	4243±350.90 ^a
	Root bark	822.14±0 ^b	7163.7±110.16 ^d	1748.11±100.29 ^d
<i>K. senegalensis</i>	Leaves	659.62±22.90 ^f	4879.9±41.6 ^f	1163.48±17.37 ^f
	Trunk bark	808.57±3.90 ^d	9423.4±300.2 ^c	3652.60±130.33 ^b
	Root bark	813.66±6.40 ^c	10770.3±292.4 ^b	3397.82±70.27 ^c

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

For the cation radical ABTS•+, the results ranged from 4879.9±41.6 µmol AAE to 13247.2 ± 219.8 µmol AAE (Table 2). The best activities were held with *E. africana* leaves (13247.2 ± 219.84 µmol AAE /g) and *K. senegalensis* root bark (10770.3 ± 292.4 µmol AAE /g). The lowest was obtained with *K. senegalensis* leaves 4879.9 ± 41.6 µmol AAE /g. For that of reducing power by FRAP method, the best activities were obtained by *E. africana* trunk bark (4243 ± 350.90 µmol AAE /g), followed by *K. senegalensis* trunk bark (3652.60 ± 130.33 µmol AAE /g). The results of these activities are reported in Table 2. Through these results and taking into account the three methods that allow appreciating the total antioxidant activity, there are divergences.

Indeed, the trunk barks of *E. africana* gave the best anti-DPPH• (825.57 ± 9.64µmol AAE) and anti-FRAP (4243 ± 350.90 µmol AAE) activities showed the lowest anti- ABTS•+ activity (5601.1 ± 41.63 µmol AAE). Similarly, *E. africana* leaves that gave the best anti-ABTS•+ activity (13247.2 ± 219.84 µmol AAE), showed low anti-FRAP activity (1615.01 ± 90.22 µmol AAE). What could be the contribution of total phenolics and total flavonoids contents to the overall activity observed?

3.4. Contribution of total phenolics and total flavonoids to the antioxidant activity

The best contents of total phenolics are given by the bark of trunk; that is to say 50.34 ± 5.70 mg GAE/100mg (*E. africana*) and 73.12 ± 1.8 mg GAE//100mg (*K. senegalensis*). Similarly, the best total flavonoid levels were obtained with the leaves of both plant species with 7.62±1.07 mg QE /100mg extract and 4.63 ± 1.00 mg QE/100mg extract respectively. If the activities were only influenced by the total phenolic contents, then the extracts of the trunk barks would have given the best activities on the three antioxidant methods. This is not the case. Indeed, the best anti-ABTS•+ activity is obtained by the extracts of leaves of *E. africana* and this activity seems to be two times higher than that of the trunk bark and roots. If we try to link the observed activities to that of the total flavonoid contents we are also confronted with a problem because the leaves having given the best total flavonoid contents also possess the lowest activities.

Thus, it seems to have a random distribution of the contribution of the contents of the various parts on the observed antioxidant activities. This has been summarized as controversial by several other authors [20, 29]. [20] And collaborators in 2013 demonstrated that often there is a correlation between total phenolics and/or total flavonoids and antioxidant activity. According to these authors three reasons could account for this. Indeed, (i) either the compounds contained in the extracts are macromolecules, (ii) or the majority of flavonoids are not antioxidant or (iii) that these flavonoids are underestimated in the consideration by aluminum chloride [20, 29]. In our case, flavonoids and total phenolics would account for the observed antioxidant activities.

4. Conclusion

Ethnobotanical surveys of medicinal plants used in the treatment of inflammatory diseases in the urban areas of Bobo-Dioulasso and Fada N'Gourma allowed us to interview 112 traditional practitioners and to identify 73 medicinal plants divided into 29 botanical families. The study mainly revealed that the majority of practitioners were men (at least 70%). According to the frequency of quotation, *E. africana* and *K. senegalensis* were much species used in the treatment of inflammatory diseases. The most represented families are Caesalpiniaceae (45%), Combretaceae (24%) and Meliaceae (21%). The method of preparation and the parts used are respectively the decoction (51%: Bobo-Dioulasso and 82%: Fada N'Gourma) and the roots (41% of case). Interesting results were obtained in terms of polyphenolic compounds and antioxidant activity (ABTS•+: 13247.2 ± 219.84 µmol AAE/g (leaves of *E. africana*)). 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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