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RESEARCH ARTICLE

Ethnobotanical survey on Medicinal Plants used in the treatment of tumors in two cities of Burkina Faso: Phytochemistry and Antioxidant activities

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Abstract

In Burkina Faso, phytotherapy is the main medical alternative used by the population to manage various diseases, including tumors. The objective of the present study was to assess the medicinal plants with antitumor properties in the cities of Bobo Dioulasso and Fada N'Gourma and to evaluate the phytochemical and antioxidant activity of two most solicited species. In this perspective, an ethnobotanical survey using a semi-structured interviews were conducted. Then, methanolic extract was performed to quantify total phenolic and flavonoid contents using Folin-Ciocalteu and aluminum chloride reagents, respectively. The results of the ethnobotanical survey allowed us to identify 40 species belonging 32 families of medicinal plants. A total of 104 tradipraticians were surveyed in both cities. *Diospyros mespiliformis* hocht and *Daniellia oliveri* Hutch. & Dalz were retained at the end of the analysis with (7.4%) and (6.8%) as frequency of citations respectively. *D. mespiliformis* leaf extract had the highest total phenolic content (56.34±6.49 mg EAG/100mg) and the highest total flavonoid content (2.35±0.58 EQ/100mg) was obtained by the *D. oliveri* extract. The best antioxidant activity was observed in the $ABTS^{\bullet+}$

radical inhibition method especially with the total methanolic extract of *D. mespiliformis* leaves (12308.15±333.09µmol EAA/g). The contents of polyphenolic compounds associate of antioxidant activities could justify the various uses of these plant species in traditional medicine.

Keywords: Ethnobotany, Traditional Medicine, Polyphenols, Antioxidant, Antitumor

Introduction

Phytotherapy according to the World Health Organization[1] remains the most widely used medical treatment in the world. It has been used for centuries to treat ailments for historical, cultural, and social reasons.[2] Several researches show that traditional medicine and pharmacopoeia remain the main sources of primary health care for 80% of the population.[3,4] In Burkina Faso, nearly 30,000 traditional health practitioners perform this function, or one traditional practitioner per 500 inhabitants.[5] Although there are 20,000 plants used in the world for their medicinal properties without counting those that have not yet been listed or discovered only about 3000 have been studied at the scientific level.[1] According to the authors, the active ingredients of medicinal plants are related to the secondary metabolites of plants. [6,3,7] Numerous epidemiological and experimental studies on humans and animals suggest that compounds of the flavonoid group, which are very abundant in plants, could play a role in the prevention of cardiovascular diseases and cancers because of their antioxidant powers and their abilities to activate natural antitumor defense mechanisms.[8,9,10] The overproduction of reactive oxygen species beyond the capacities of biological systems to eliminate them gives rise to oxidative stress that is implicated in the development of several diseases ranging from arteriosclerosis to cancer.[11,12] In addition, breast and cervical cancers are the most frequent cancers in the female population.[10] In 1971, cervical cancer accounted for 12.56% of malignancies treated at the Yalgado Ouédraogo Hospital in Ouagadougou.[13,10] Synthetic products such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are certainly effective but are likely to have side effects and even be toxic.[14]According to Dakio et al,[4] the richness of biodiversity and the knowledge of our traditional therapists could help improve the management of certain patients. Their exploitation could lead to new areas of scientific research. In this particular context, it is important to evaluate the potential of plants used in the treatment of tumors. It is in this context that the present study was conducted. Its general objective was to Evaluate the antioxidant activity and phytochemical study of *Diospyros mespiliformis* hocht (Ebenaceae) and *Daniellia oliveri* Hutch. & Dalz (Caesalpiniaceae), two most solicited species.

Material And Methods

Ethnobotanical Survey Area

Ethnobotanical study was realized in both cities of Burkina Faso, specifically in Bobo-Dioulasso and Fada N'Gourma, Bobo-Dioulasso is the second capital city which is approximatively at360 km away from Ouagadougou, the capitalcity. The investigation site covers about 136.8 km and is located in the South-Western part of Burkina Faso, at11°15'04,9'North latitude, 004°26'08,6' West longitude, and an altitude of 445 m. The region is mainly inhabited by Bobos, Mossi, Dafing, and other ethnic groups such as Samogo, Fulani and Lobi/Dagara. The local language is Dioula.Fada N'Gourma is located in the eastern region of Burkina Faso with coordinates of 12°03'00" North and 0°22'01" East. This city is populated with 180356 inhabitants[15] covering an area of 36 Km^2. The climate is of the South Sudanese type with an average temperature of 28.3 C° and an average rainfall of 565 mm (Köppen-Geiger). Fada N'Gourma has a vegetation characterized by a shrubby savanna [16]. The population is very homogeneous in these study areas (Figure 1) and the local languages commonly spoken are Mooré, Fulfuldé, and Dioula. The local language is Gourmanchtèma.

The antioxidant activities and phytochemical study were carried out at the Laboratory of Research and Teaching in Animal Health and Biotechnology (LARESBA) at Nazi BONI University of Bobo-Dioulasso.

Ethnobotanical survey

The ethnobotanical survey was conducted among traditional healers in the eastern region in the city of Fada N'Gourma and in the Hauts-Bassins region in the city of Bobo Dioulasso during the months of August and October2020. The data were collected using the structured interview method among traditional Heath practitioners. The information collected concerns the profile of the respondents, their knowledge of tumors and the plants used for their treatment.

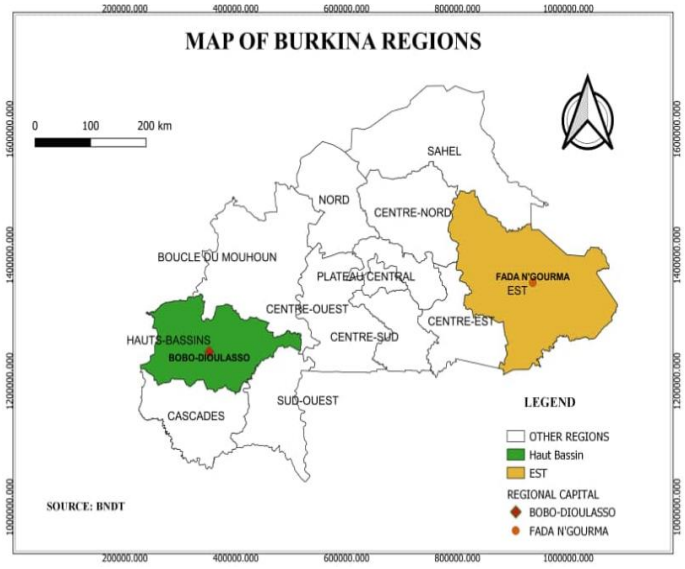

Figure 1. Regional map of the study area.

Plant material

The plant material consists of leaf powder, root bark, and plant trunks of *Diospyros mespiliformis* hocht and *Daniellia oliveri* Hutch. & Dalz. These different organs were identified and collected in December 2020. The two species were previously identified by Dr. Yempabou Hermann OUOBA Botanist and Phytoecologist at the Nazi BONI University before the harvestin compliance with the sustainable management policy according to the organs concerned, then rinsed and cut in order to dry and pulverize.

Solvents And Reagents

All solvents were analytical grade. Agilent Cary 60 UV-Vis Spectrophotometer was used in all spectrophotometric measurements. Ascorbic acid, ferric chloride (FeCl3), aluminum chloride (AlCl3), 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).

Extraction

We made a methanolic extraction with soxhlet. For that 15g of powder of each sample are put in a cartridge and extracted with 200ml of methanol. The duration of each extraction was at least 4 hours. Then, the extract was concentrated, and collected in a petri dish. The yields (R) of the extractions were calculated by the following formula:

$$R = \frac{\text{extracted mass}}{\text{mass of the extract}} * 100$$

Determination of polyphenolic compounds

Determination of total polyphenols

The extracts are dissolved in pure methanol to have a concentration of 10 mg / mL, then diluted to 1/100 in distilled water. A volume of 0.125mL of the diluted solution was then mixed with 0.625mL of Folin-Ciocalteu reagent at 0.2 N and incubated for 5 min. 0.5Ml of a solution of sodium carbonate at 75 g / l in distilled water is

then added and the mixture incubated for 2 hours. At the end of the incubation, the optical densities were read at 760 nm using a spectrophotometer. The standard calibration curve was plotted using gallic acid (0-200 mg / L) ($y = 0.004668x + 0.034$; $R^2 = 0.9991$).[8] A total of 3 readings are taken for each extract and fraction and the result given is an average from these analyses. Results are expressed as mg Gallic Acid Equivalent per 100mg extract or fraction (mg GAE/ 100 mg extract).

Determination of total flavonoids

The method used for estimation of flavonoid levels in plant extracts is that described by Meda et al[8]. A volume of 625µl of 2% $AlCl_3$ in pure methanol is mixed with an equal volume of extract or fraction at 1 mg / ml in methanol. Optical densities are read after 10mins of incubation in the dark at 415 nm using a spectrophotometer. Quercetin (0- 100 mg /l) was used as a standard for the development of the calibration curve ($y = 0.01259x$; $R^2 = 0.9990$). A mixture of 625 µL of extract or fraction and 625µl of methanol without $AlCl_3$ was used as a blank. Three readings are taken per extract and fraction sample and results are expressed as mg Quercetin Equivalent (QE) per 100mg extract (mg QE/100mg).

Antioxidant activities

Reducing power by the FRAP method

The Ferric Reducing Antioxidant Power (FRAP) method is based on the ability of extracts to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The total antioxidant capacity of each plant extract was determined by the method described by Meda et al[8]. The sample solution was prepared with distilled water. To 0.5mL of the sample solution, we add 1.25mL of phosphate buffer (0.2 M) and 1.25mL of potassium hexacyanoferrate. We incubate this mixture for 30 minutes in a water bath at 50°C. After that we add 1.25mL of trichloroacetic acid (10%) to it and centrifuge it for 10 minutes at 300 rpm. 0.625mL of the supernatant is added to 0.625mL of distilled water and 0.125mL of iron chloride ($FeCl_3$) freshly prepared with distilled water (0.1%). The absorbance of the latter mixture is read at 700nm by a spectrophotometer. Ascorbic acid was used to produce the calibration curve ($y = 0.003270x$; $R^2 = 0.9990$). A blank was also prepared under the same conditions with distilled water. Determination of iron (III) reducing activity was performed in triplicate and expressed as µmol of ascorbic acid equivalent (AAE)/g of extract.

Anti-free radical activity by the DPPH• radical inhibition method.

The antiradical activity of the extracts by the DPPH method, is their ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The method used is the one described by Meda et al[17]. 375µl of methanolic sample solution is mixed with 750µl of DPPH solution (20mg/L). The mixture is incubated for 15 min in the dark. A blank is also prepared with methanol instead of DPPH solution (20mg/L). Measurements are performed by a spectrophotometer at 700nm. Ascorbic acid was used as the standard ($-0.02224x+0.348$; $R^2=0.9966$). The average of three readings was used and the results expressed as µmol ascorbic acid equivalent (AAE)/g extract.

Reducing power by the $ABTS^{\bullet+}$ method.

The method used was as described by Meda et al[8]. The sample solution is prepared with distilled water. 990µL of $ABTS^{\bullet+}$ (2,2'-azinobis (3- ethylbenzothiazoline)-6-sulfonic) solution (0.1mg/mL) is added to 10µL of sample solution. The blank here is ethanol. Measurements are performed by a spectrophotometer at 734 nm. Ascorbic acid was used as the standard ($y = -0.0007874x + 0.709$; $R^2 = 0.9993$). The average of three readings was used and the results expressed as µmol ascorbic acid equivalent (AAE)/g extract. The results of the antioxidant activities are determined by the formula:

$$C = \frac{c * D}{M * ci}$$

C = concentration of free radical scavenging compounds in µmol EAA/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)

Statistical analysis

The statistical data analyzed was the tabulation of the survey forms and the statistical analysis of the results. Thus, the responses to the questionnaires were coded, entered and processed using the MICROSOFT Excel 2019 software.

Results And Discussion

Data from the ethnobotanical survey

In total, the ethnobotanical study involved 104 traditional practitioners spread over the both cities, including Fada N'Gourma, with 54 traditional practitioners divided into two (2) groups. These were full-time traditional healers who were generally part of a large association called

"Association des Tradipraticiens du "*Gulmu*" or "*Laafia mani*" representing group 1. Their professional experience ranged from 3 to 45 years; part-time practitioners rarely belonged to an association and considered that they had entered traditional medicine by inheritance or association through training. The survey in Bobo Dioulasso took place within a single association called "*Jigisémé*"(culture of hope), of which there were 50. The experience of these traditional practitioners ranged from 3 to 40 years. This could be justified by passing on knowledge from generation to generation.

Plants cited

In the town of Fada N'Gourma, 48 species were cited by the traditional healers. Some of these plants are used in combination for the treatment of tumors. The best cited in the treatment of tumors in this city by the two groups are *Diospyros mespiliformis*, *Chrysanthellum americanum*, *Daniellia oliveri*, *Fluggea virosa*, *Khaya senegalensis*, and *Ximenia americana*. We evaluated the citation rate of species in Bobo Dioulasso according to the number of plants recorded. These plants are very diversified (Figure 3). The most cited are: *Khaya senegalensis*followed by *Detarium microcarpa*,*Euphorbia hirta*, *Zanthoxylium zanthoxyloides*, *Daniellia oliveri*and *Diospyros mespiliformis*.The plants in the study were found in both groups surveyed with fairly high citation frequencies of (11.76%-12.31%) for *D. mespiliformis* and *D. oliveri* (2.94%-6.15%). Other authors, in an ethnobotanical survey in the dry savannah of North Togo, *D. mespiliformis* was found to be one of the plants most used for personal hygiene, with a use value of 2.48 and a frequency of 13.6%. The species are is used in almost all cities in North Togo.[18] According to the surveys of Fachola et al[19] on ethnobotanical knowledge *D. oliveri* in Benin show that this plant is of important use in traditional medicine with a frequency of about 80% used in different diseases such as cough, pain, ulcers...

The frequencies of quotations according to families showed that almost the same families are quoted in both cities (Figure 2). The most cited families in BoboDioulasso are Caesalpiniaceae, Meliaceae, Mimonaceae, Rubiaceae, Oleaceae and Euphorbiaceae. As for Fada N'Gourma, the most cited families are the Caesalpiniaceae (4 genera, 8 species), the Mimonaceae, the Euphorbiaceae, the Combretaceae, the Asteraceae and the Ebenaceae. These results are similar to those of a study conducted in a location in northwestern Burkina Faso by Zerbo et al[20] where they found that with 76 genera and 39 families, that the best represented families were Caesalpiniaceae, Mimosaceae, Combretaceae and Anacardiaceae. Another ethnobotanical study in the Niangologo forest by Ouoba et al[21] showed that of the 98 genera and 46 families of species surveyed, the most represented families were Fabaceae, Caesalpiniaceae, Combretaceae and Rubiaceae. We can say from these analyses that the knowledge of medicinal plants could be linked to the presence of the species in the region and to the accessibility of the latter. The frequent use of these families could be explained by the richness and diversity of their species in the different areas.

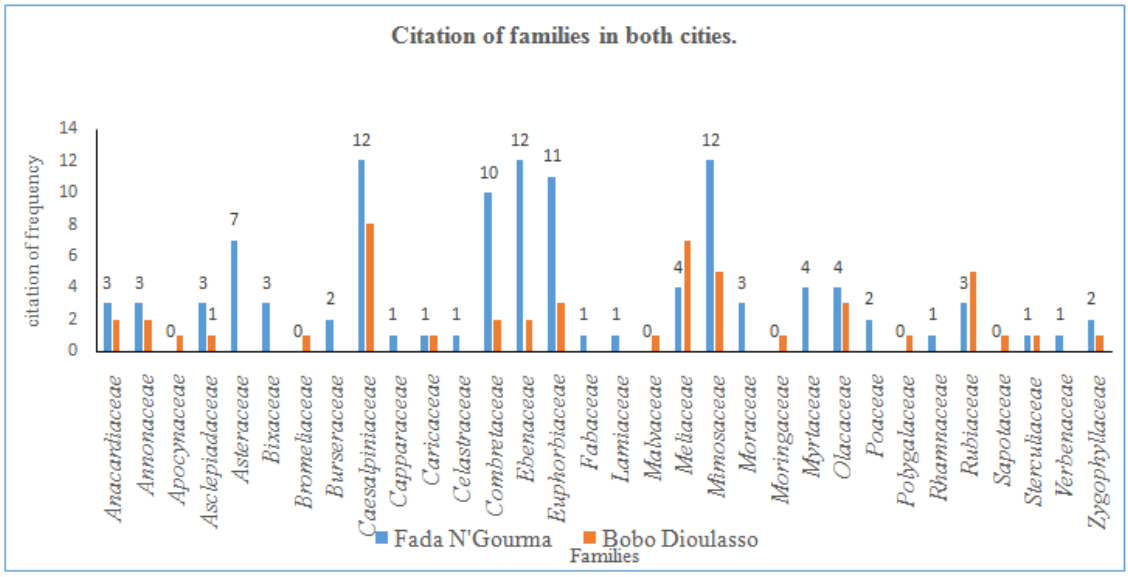

Figure 2. Citation of families in both cities.

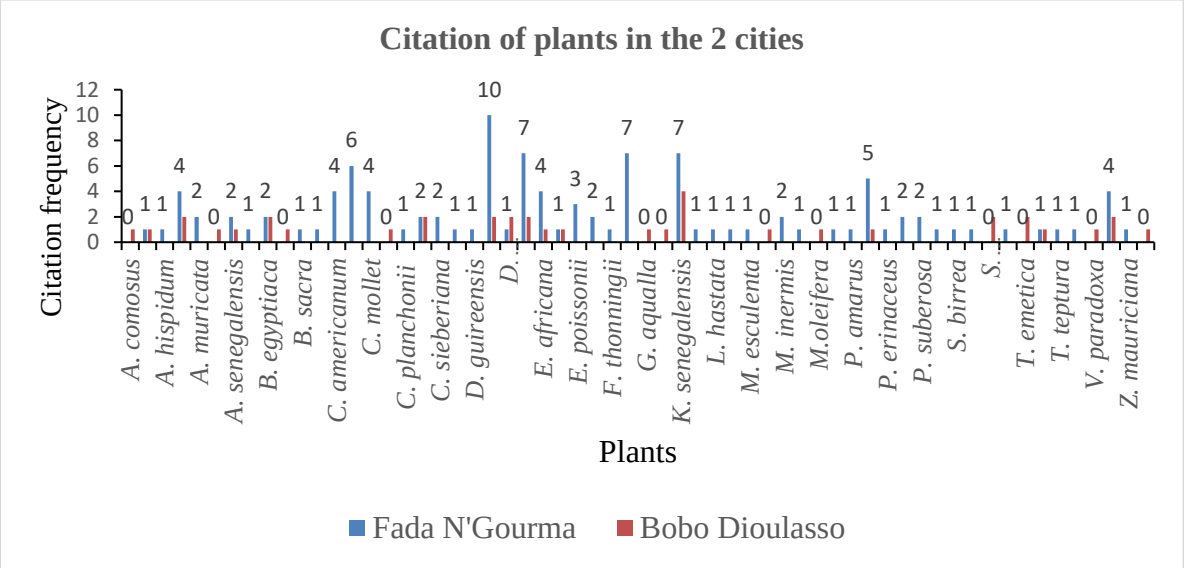

Figure 3. Citation of plants in the 2 cities.

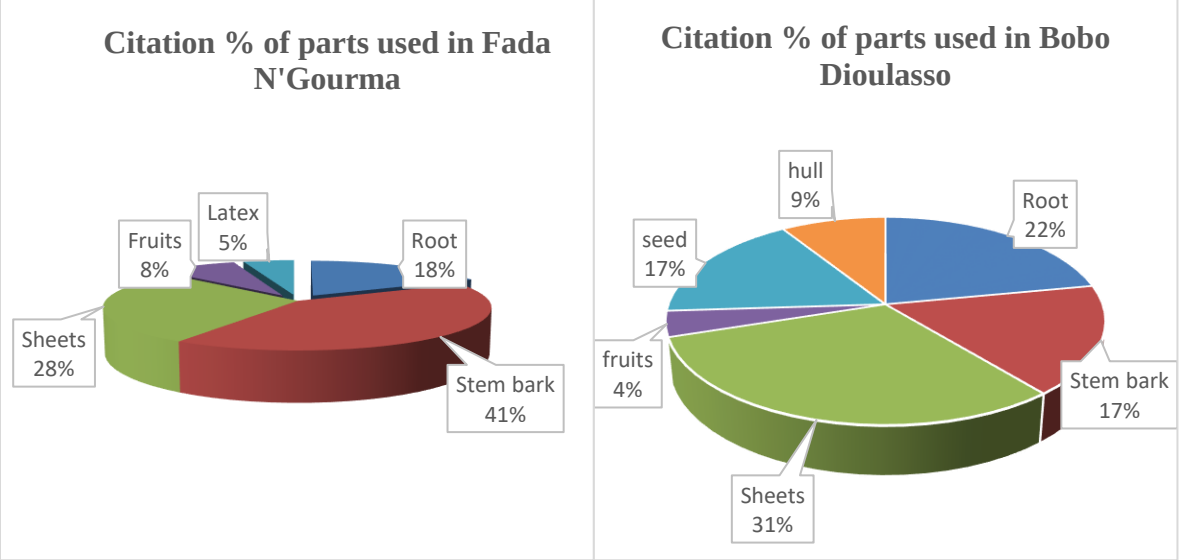

Figure 4. Citations of plant parts used in the study cities.

Parts of the plants used

The organs or parts used by traditional practitioners in Bobo Dioulasso are respectively leaves, roots and stem bark. In contrast, in Fada N'Gourma, stem bark is the most frequently cited with a rate of 41%, followed by leaves (Figure 4). The least cited in both cities are latex and the shells of.

The high use of stem bark (17%-41%), leaves (28%-31%) compared to roots (18%-22%) in the pharmacopoeia by the surveyed traditional practitioners is similar to the results obtained by other authors such as Assogbadjo et al[22]; Ambe et al[23]; Thiombianoet al[24]who showed that in Côte d'Ivoire, Burkina Faso, Senegal, Democratic Republic of Congo and Togo, leaves and trunk bark are the plant organs most used in traditional medicine for the treatment of various ailments. Leaves being the seat of photosynthesis, they would have good biological properties and are more accessible[25], which would motivate the population to use them. This form of use would contribute to the preservation of biodiversity.

Mode of administration

The most common method of administration in Bobo Dioulasso and Fada N'Gourma, with a rate of 39% and 64% respectively, was by mouth, followed by massage (12%-32%). The least used method is bathing, but for 20% of the recipes in Fada N'Gourma, drinking and bathing are combined. The most common method of preparing these recipes is decoction in both cities (Figure 5).

Extraction yields

Extraction yields varied between 5.62% and 17.44%. With 17.24% the trunk bark of *D. oliveri* recorded the highest yield of the plant. In *D. mespiliformis* the leaves recorded the best extraction yield of the plant (17.44%).

Determination of polyphenolic compounds and antioxidant activities

Determination of polyphenolic compounds

Polyphenol contents varied between 10.67 ±1.06 and 56.34± 6.49 mg (EAG)/100mg in both species (Table 1). The best content is obtained in the methanolic extract of *D. mespiliformis* leaves. Other authors have reported polyphenol contents of 2.06 to 7.6 mg (EAG)/100mg in *D. oliveri* extracts[26].Our values are higher than those of Muanda et al.[26] Sombié et al[27] showed that *D. mespiliformis* leaves have a total polyphenol content of 16.41± 0.30 mg (EAT)/100mg.This content is still much lower than the content obtained in our study. Concerning total flavonoids, they vary between 0.68 ±0.04 mg (EQ)/100mg and 2.35± 0.58 mg (EQ)/100mg (Table 1). The highest value was recorded in the extracts of *D. oliveri* root barks. In the different organs of *D. oliveri* the contents of total flavonoids remained almost similar. In the methanolic extracts of *D. mespiliformis*, the trunk barks and the leaves have total flavonoid contents that were close. We therefore observe an accumulation of flavonoids in the root and trunk barks of *D. oliveri*.

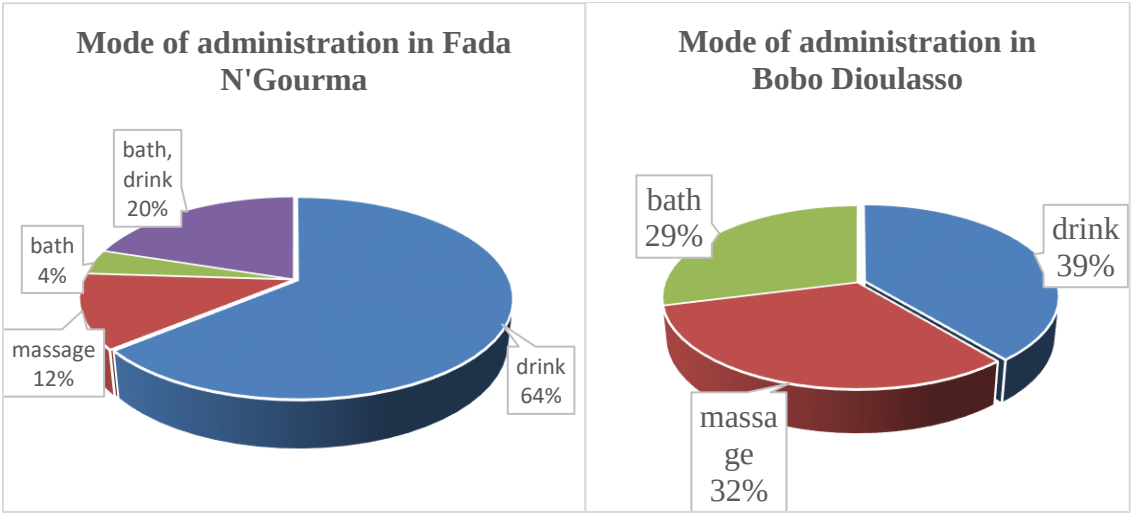

Figure 5. Mode of administration in the study cities.

Table 1. Results of polyphenolic compounds assays

Plants	Parts used	Total polyphenols Mg (EAG)/100mg	Total flavonoids Mg (EQ)/100mg
Daniellia oliveri	Root bark	26,78± 2,97^{d}	2,35± 0,58^{a}
	Stem bark	10,67 ±1,06^{f}	2,20 ±0,18^{c}
	Leaves	29,63± 3,12^{c}	1,17 ±0,36^{e}
Diospyros mespiliformis	Root bark	22,56± 1,07^{e}	0,68 ±0,04^{f}
	Stem bark	44,42 ±0,32^{b}	1,61 ±0,2^{d}
	Leaves	56,34± 6,49^{a}	2,33 ±0,16^{b}

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

Table 2. Antioxidant activities of methanolic extracts and different methods

	Tests	DPPH$^{\bullet}$ (µmolAAE/g)	FRAP (µmolAAE/g)	ABTS$^{\bullet+}$ (µmolAAE/g)
Daniellia oliveri	Root bark	741,11±33,85^{d}	985,24±37,37^{f}	6250,23±220,32^{e}
	Stem bark	822,16±33,77^{b}	3299,49±137,82^{a}	12308,15±333,09^{a}
	Leaves	600,04±48,52^{f}	1192,43±50,12^{d}	6033,88±166,54^{f}
Diospyros mespiliformis	Root bark	750,67±34,34^{c}	1510,81±90,22^{c}	6514,66±166,54^{d}
	Stem bark	726,86±73,40^{e}	1151,83±19,99^{e}	9062,84±41,63^{c}
	Leaves	858,77±10,63^{a}	2800,74 ±142,02^{b}	9591,70±33,77^{b}

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

This content is strongly influenced by factors such as variation in growthtype, season, climate and degree of maturity.[28] In *D. mespiliformis* flavonoids are more accumulated in the leaves. Few studies have focused on extracts from the organs of *D. mespiliformis* but most studies have focused on fruit extracts of the plant. These studies report a flavonoid content of 22.40 ± 0.28 mg EQ/100 g fruit.[29] Our results show that the flavonoid content of leaf extracts and bark are higher than the content in fruits.

Antioxidant activities

In both plants the reducing power of the total methanolic extracts by FRAP test, varied from 985.24±37.37 µmol EAA/g to 3299.49±137.82 µmol EAA/g. The best antioxidant activity was obtained with the stem bark extract of *D. oliveri*. As for *D. mespiliformis* the best activity was obtained with the root bark extract. The antiradical power of the extracts was evaluated using the DPPH$^{\bullet}$ radical. The results obtained with the methanolic extracts ranged from 600.04±48.52 µmol EAA/g to 858.77±10.63 µmol EAA/g. The extract from the stem bark of *D. oliveri* obtained the best activity. In *D. mespiliformis* it was the stem bark extract that showed the best free radical activity. The ABTS$^{\bullet+}$ cation radical scavenging capacity of the total methanolic extracts, ranged from 6033.88±166.54 µmol EAA/g to 12308.15±333.09 µmol EAA/g. The extracts from the stem barks of *D. oliveri* gave a better scavenging capacity of ABTS$^{\bullet+}$ cation radical of 12308.15±333.09µmol EAA/g. The best free radical scavenging activity at *D. mespiliformis* was recorded by the leaf extracts. All these data are recorded in Table 2.

The results of our antioxidant activities reveal that our six methanolic extracts namely leaves and bark of stem and roots of *D. oliveri* and *D. mespiliformis* have antioxidant potential. We find that there is a significant contribution of flavonoids in total phenolic activity. Indeed, extracts from the leaves of *Diospyros mespliformis* which showed the best total phenolic content also gave the best flavonoid content. This correlation is mainly perceived in terms of anti-DPPH$^{\bullet}$ (858,77±10,63 µmol AAE/g) and anti-FRAP (2800.74±142.02 µmol AAE/g) activities. More specifically, at the level of *Daniela olliveri* stem bark extracts on the three antioxidant activities. At this level, the total phenolic content is 10.67 mgEAG/g of extract, six times lower than the case of *Diospyros mespliformis* leaves. However, has the best anti ABTS activity (12308µmolEAA/g extract), anti-FRAP 3299µmolEAA/g extract and anti DPPH (822.16 µmolEAA/g extract).,

The contribution and/or non-contribution of total phenolics and flavonoids is a well-known phenomenon in the scientific community. Indeed, several researchers from various horizons have gone up and noted this.[11,30] Meda et al.[11] showed that this controversy would be due to several possibilities: (i) either the compounds existing in the extracts have large molecular weights or are heterosidic, (ii) or the majority of the flavonoids are not antioxidants (iii) or there is under estimate of the flavonoids by the method of $AlCl_3$. In our case the correlation seems to exist.

Conclusion

The ethnobotanical survey carried out among traditional practitioners allowed us to identify *Daniellia oliveri* and *Diospyros mespiliformis* as plants used in the treatment of tumors. These species were thus the object of a phytochemical study. This study showed that these two species, contain polyphenols and flavonoids in large quantities. The extract of the stem bark of *Diospyros mespiliformis* gave above all the best activities anti-ABTS$^{\bullet+}$ (12308,15±333,09 µmol AAE/g), anti-FRAP (3299,49±137,82 µmol AAE/g) and anti-DPPH$^{\bullet}$ (822,16±33,77 µmol AAE/g), thus showing the contribution of flavonoids in the activities. In order to follow up this study, we envisage through bio-guided methods,

(i) researched the molecules responsible for these activities,
(ii) characterize them and isolate them in a first time.
(iii) then oriented against the stem cells of this tumor.

Acknowledgments

The authors are grateful to the associations of practitioners of the cities of Bobo-Dioulasso and Fada N'Gourma whose practitioners agreed to share their know-how.

Conflict of Interest

We have no conflict of interests to disclose and the manuscript has been read and approved by all named authors.

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