The Ethnobotanical investigation, phytochemistry and antioxidant activity of a medicinal plant recipe directed against *Candida albicans* and Enterobacteria stains producing ESBL CTX-M-15 type in Burkina Faso

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ABSTRACT

Aims: The objectives are to carry out an ethnobotanical survey among traditional healers in order to choose the most recipe used in the treatment of vaginal candidiasis in cities of Burkina Faso followed by phytochemical quantification, antioxidant activity and antimicrobial of the best recipe.

Methodology: Ethnobotanical surveys were conducted in Bobo-Dioulasso and Dédougou by semi-structured interview amount traditional healers. The extracts were obtained by ethanolic and hydroethanolic maceration. Characterization of the secondary metabolites was revealed by the tests in tubes. Polyphenolic compounds contents quantification was done by spectrophotometry using Follin-Ciocalteu reagent, aluminum trichloride and vanilic acid. The antioxidant activity was evaluated by two methods: DPPH• and FRAP. The Minimal inhibitory concentration on Enterobacteria strains was determined by the dilution method and the minimal bactericidal concentration was determined by inoculation on agar. The susceptibility test of *Candida albicans* strains was carried out using the disc diffusion assay.

Results: In total, 52 traditional healers were surveyed with a predominance of men (75%) in the both cities. 38 recipes of medicinal plants have been obtained. Decoction is the most frequent method of preparation in Bobo-Dioulasso (87.5%) and in Dédougou (27.27%). The leaves (44%) and the bark of the trunk (31.82%) are respectively the most parts used of the plants. Colorimetric tests revealed the presence of compounds such as flavonoids, alkaloids, tannins and saponosides. The best results of quantification tests of total phenolics, flavonoids and tannins, were obtained with the hydroethanolic extract (80.27±20.27(mgEAG/10 mg); 0.27±0.01(mgEQ/10 mg) and 73.31±4.65 (mgEC/10 mg)). With antioxidant activity, the highest value was obtained with the DPPH radical inhibition method (85.59±0.001%). Minimal inhibitory concentration revelead that the highest value was 0.781 mg/ml and the best minimal bactericidal concentration was 3.123 mg/ml.

Conclusion: Phytochemical and biological analysis realized could be partially justified by the recipes used in the treatment of vaginal candidiasis.

Keywords: Investigation; vaginal candidiasis; polyphenols, Antioxidant.

1. INTRODUCTION

Vaginal candidiasis is one of the most common female infections and affects 138 to 140 million women every year worldwide [1-2]. In tropical Africa, its prevalence varies between 33 and 47% of opportunistic infections [2]. In Burkina Faso, these candidiases affect more than 179,000 women per year [3]. It is a pathology caused by fungal agents that constitute a major public health risk [4]. Furthermore, it is the species *Candida albicans*, an opportunistic fungus, very virulent in immunocompromised people who are responsible for it [5-6-7].

The treatment of this pathology is done through conventional medicine by the use of antifungals such as amphotericin B and azoles (fluconazole, itraconazole, voriconazole) which are often expensive and inaccessible to the population [8-9-10-11-12]. In addition, *Candida albicans* develops resistance and/or recurrences which render the treatment ineffective [13-14-4]. However, according to the WHO [15] more than 80% of the population use traditional medicine for their primary health care. Thus, this medicine could be an alternative care for humanity. Also, several authors have shown that the heritage of plant biodiversity has compounds with antifungal properties such as flavonoids, tannins, phenol acids, coumarins, saponosides [16-17]. It is in this perspective that the present study was initiated with the aim of carrying out an ethnobotanical survey among traditional healers to identify the recipes of medicinal plants used in the treatment of vaginal candidiasis in order to study (i) the phytochemistry, (ii) the antioxidant and antimicrobial activities of the selected recipe.

2. MATERIAL AND METHODS

2.1 Study framework

Ethnobotanical surveys were conducted in two towns in Burkina Faso. All the activities were carried out at the Ecotoxicology Laboratory of the Environment and Agricultural Research Institute (INEAR/NCRST) in Bobo-Dioulasso, at the Food and Nutritional Biotechnology Laboratory and at the Research Center in Biological Food and Nutritional Sciences in Ouagadougou.

2.2. Plant material

The plant material consisted of fruits of *Acacia nilotica* (Linn) willd ex. Del. The collection took place in the month of April 2022 in the classified forest of Dindéresso. The species was previously identified on the site www.plantlist.com before the samples were taken. Then, at the Laboratory, the samples were washed and spread out on the benches for drying for three weeks under ventilation sheltered from the sun. Spraying was carried out with an aluminum mortar to obtain powder. This powder obtained was packaged and labeled in zip bags which were used for the various operations in the laboratory following the method of Nguemo Dongock et al., [43].

2.3. Microbial strains

The microbial material consisted of *Candida albicans* and Gram-negative bacteria consisting of: *Escherichia coli* Castellani and Cahlmers, *Shigella sonnei* (Levine 1920) Weldin 1927, *Salmonella sp* Lignières 1900, *P. aeruginosa* (Schroeter 1872) Migula 1900, *Citrobacter freundii* (Braak 1928) Werkman and Gillen 1932, *Klebsiella pneumonia* (Schroeter, 1886)

Trevisan, 1887, *Acinobacter Baumannie* str. 1656-2, *Salmonella Typhi* (Loeffler 1892) Castellani and Chalmers 1919, *Citrobacter sp* Werkman and Gillen, 1932 . All these strains were isolated from vaginal samples, urine, pus and stool from all kinds of patients received at the Bacteriological Laboratory of SCHIPHRA Hospital.

2.4. Reagents and solvents

The reagents and solvents used during the manipulations were the following: ascorbic acid, ferric chloride ($FeCl_3$), aluminum chloride ($AlCl_3$), quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, sodium carbonate, ethanol, sodium chloride, glycerol, distilled water, bleach, concentrated hydrochloric acid (HCl), magnesium (Mg), vanilic acid, trichloroacetic acid, potassium hexacyanoferrate [$K_3Fe(CN_6)$], catechol.

2.5. Ethnobotanical Survey

The surveys were conducted during a period between November 15, 2021 and February 20, 2022 among traditional healers in the city of Bobo-Dioulasso and Dédougou. Among the traditional healers surveyed, some belonged to an association (the *Jigi Sèmè*: association in Bobo-Dioulasso and the *Soong-Taaba*: association in Dédougou). The approach used was a semi-structured interview in the local language (*mooré* and *bôbô*) using a mini questionnaire sheet. A semi-structured interview was done with the traditional healers who agreed to answer our questions. The survey sheet included questions on the local name of the species, the plant organs used, their methods of preparation and administration.

2.6. Extraction

15 g of vegetable powder were weighed and introduced into an erlenmeyer flask which previously contained 150 ml of pure ethanol for the ethanolic extraction and 150 ml 20% of ethanol for the hydroethanolic extraction under stirring for 48 hours. After filtration, the filtrate was evaporated then concentrated and poured into empty Petri dishes previously weighed and labeled for drying in the oven (Evenamede et al. [44]). The yield (R) of the extractions was calculated by the following formula: R= (Mass of the extract)/ (Sample mass) *100

2.7. Determination of polyphenolic compounds

2.7.1. Characterization of chemical groups

Phytochemical screening consisted of highlighting the presence of certain chemical groups in the extracts obtained using the tube tests described by Nga et al. [18]: alkaloids (Dragendorff's reagent), flavonoids (Shibata test), tannins (2% of $FeCl_3$) and saponins (foam index).

2.7.2. Quantifications of total phenolics

- Assay of total phenolics: Total phenolic compounds were estimated using the Folin-Ciocalteu method described by Singleton et al. [19]. the sample solution was diluted to the nearest $1/10^{th}$ from the stock solution. To a volume of 100 µl of extract, we added a volume of 400 µl of folin-ciocalteu reagent and a volume of 420 µl of sodium carbonate ($NaCO_3$). the solution obtained is then incubated at 37°C for 1 h 30 min. The absorbance was read at the end of the incubation with a UV-visible spectrophotometer at 760 nm against a blank consisting of 100 µl of extract, 400 µl of ethanol and 420 µl of sodium carbonate ($NaCO_3$) prepared at a concentration of 0.9 mg/ml. gallic acid is used as a reference standard for

establishing the calibration curve and for quantifying the total polyphenol content. the results are expressed in mg EAG/10 mg of extract.

- Assay of total flavonoids**:** The method used is that described by Arvouet-Grant et al. [20]. A volume of 500 µl of the extract solution from each sample then 500 µl of $AlCl_3$(2%) were placed in each tube (3). Another tube is considered as the control and which received 500 µl of the extract solution and 500 µl of ethanol then incubated for 15 min in the dark. Quercetin is used as a reference standard and for the quantification of flavonoid contents, the values are expressed as mg EQ/10 mg of extract. After incubation, three readings are taken for each extract sample using a spectrophotometer at a wavelength of 415 nm. The result given is an average of the three. The equation of the curve is: $y = 34.595x + 0.0189$; R^2=0.9981.

- Dosage of total tannins: The reference method of the European Community for the determination of tannins [21] was used. A quantity of 200 µl of each hydroethanolic extract was added to 1000 µl of the vanillin solution (c=0.06 mg/ml) against a blank consisting of 1000 µl of vanilin and 200 µl 80% of ethanol. The mixture obtained is incubated in the dark to react at room temperature for 20 min. Absorbance was measured at a wavelength of 500 nm. Three tests were carried out for each sample. A stock solution of catechol was used as a reference standard for establishing the calibration curve and for quantifying the tannin content, then the results are expressed as mg EC/10 mg of dry matter. The calibration curve is: $y = 0.1239x + 0.0005$ with R^2=0.9986.

2.8. Antioxidant activities

2.8.1. Reducing power of iron by the FRAP method

The reducing power of the extracts is determined by the Fluorescence Recovery After Photobleaching (FRAP) method [22]. 0.5 ml of extract is mixed with 1.25 ml of phosphate buffer (0.2 M; pH=6.6) and 1.25 ml of potassium hexacyanoferrate [$K_3Fe(CN)_6$] at 1%. The mixture is placed in a water bath at 50°C for 30 minutes and then 1.25 ml of 0.1% trichloroacetic acid is added thereto. The tubes are centrifuged at 3000 rpm for 10 minutes. Then, 125 µl of the supernatant of each tube is mixed with 25 µl of $FeCl_3$ (0.1%) solution and 125 µl of distilled water then left to stand in the dark for 15 minutes before measuring the absorbances at 700 nm against a blank composed of 250 µl of distilled water and 25 µl of $FeCl_3$ (0.1%). Ascorbic acid is used to draw the reference standard curve expressed in mmolEAA/10 mg.

2.8.2. Anti-radical activity by the DPPH radical inhibition method

The determination of the antiradical activity by the 2.2-Diphenyl-1-Picrylhydrazyl (DPPH) test was carried out using the method described by Dieng et al. [23]. In test tubes we introduced 400 µl of the diluted solution (extract) and 800 µl of a DPPH solution (10 mg/50 ml of ethanol) then incubated for 15 min in the dark. A blank was prepared with 400 µl of ethanol and 800 µl of the DPPH solution. Absorbances and concentrations were read using a spectrophotometer at 517 nm.

2.9. Antimicrobial tests

- Preparation of the standard inoculum: For each germ previously inoculated into petri dishes containing Sabouraud agar, an isolated colony is removed using a loop 2 mm in diameter and then homogenized in 10 ml of sterilized 0.9% NaCl solution.

- Preparation of culture media: All culture media, namely: Muller Hinton (MH) agar; Muller Hinton broth; Sabouraud agar; Luria-Bertani (LB) were prepared according to the manufacturers' instructions.

2.9.1. Susceptibility test of *Acacia nilotica* on strains of *Candida albicans*

This test is carried out according to the method of Arias et al. [24] with some modifications. After the solidification of the Sabouraud agar in the petri dishes, a certain quantity of inoculum is poured into each petri dish so as to flood the boxes with the germs and then leave them to dry near the flame for 30 minutes. After this step, the sterile Wathman n°5 paper discs, 6 mm in diameter, are placed in the petri dishes using a sterile brush on which 10 µl of each extract diluted at different concentrations have been deposited, then an antifungal such as nystatin was used as positive control and 80% ethanol considered as negative control were added, the whole is incubated for 72 h. After incubation, sensitivity was measured using a ruler.

2.9.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined according to the method of Arias et al., [24] after an incubation time of 18 to 24 hours. This test was carried out by determining the turbidity with respect to the 11^{th} well which is the control induced by the growth of the germs studied. The stock solutions of the extracts were prepared by dissolving 500 mg of dry extract in 100 ml of distilled water. Thus, the MIC corresponds to wells where there is no turbidity. Using a micro pipette, each well (the 96-well plates) received 100 µl of MH broth and 100 µl of plant extract is put in the very first wells of the plate with which a cascade dilution was performed up to the 12^{th} well skipping every 11^{th} well. Thus, 100 µl of inoculum of each bacterium was added. After incubation of the plates at 37°C for 18 to 24 hours, the reading of the turbidity was done with the naked eye.

2.9.3. Determination of the minimum bactericidal concentration (MBC)

The MBC is defined as the smallest concentration at which the extract prevents growth of bacteria after subculturing [25]. This technique was performed by taking 100 µl from each clear well and then introducing it into the Petri dish containing Muller Hinton (MH) agar. For inoculation, sterile Petri dishes previously poured with MH agar are inoculated by plating using a sterile rake; the inoculation is carried out in such a way as to ensure a homogeneous distribution of the bacteria. After 18 to 24 h of incubation at 37°C, the MBC was determined by counting colonies.

2.10. Statistical analyses

All the data obtained were processed and analyzed with Microsoft Excel 2016 software. The data collected on the survey sheets were sociodemographic and ethnobotanical characteristics. The citation frequency (Fc) of each plant was determined by the following formula: Fc=Nc/Nt; With Nc: number of citations of the most cited plant and Nt: total number of people surveyed.

3. RESULTS AND DISCUSSION

3.1. Ethnobotanical survey

3.1.1. Distribution of traditional healers by gender

The ethnobotanical surveys made it possible to interview 52 traditional healers, including 36 in Bobo-Dioulasso against 16 in Dédougou with a predominance of the male gender, i.e., 75% in the two cities (figure 1). Although our sample size is small, we find that both genders practice traditional medicine in these cities. Other researchers from Burkina Faso such as Traoré et al. [26] who worked on the medicinal plants used in the treatment of malaria in Banfora found on a sample of 45 traditional healers 91.11% of men. In terms of participation in relation to gender, we find that the male gender predominates. This is found in the locality of Benin where Guinnin et al. [27], by conducting an ethnobotanical study on medicinal plants used in the traditional treatment of viral hepatitis B and C obtained a predominance of 60.97% of men. After analyzing her results, we note that the low proportion observed among women could be due to the fact that in Africa, certain ritual practices are often associated [28]. In addition, women are generally taken up with household chores, which considerably limit their integration into the practice of traditional medicine.

3.1.2. Distribution of medicinal plants according to species

The ethnobotanical surveys carried out made it possible to identify 38 species used in the composition of 38 medicinal plant recipes (figure 2). From these results, we note that in terms of contribution of species in the recipes, the most cited were respectively *Acacia nilotica* (11.5%), followed by *Balanites aegyptiaca* (L.) Delile, *Calotropice procera* (Ait.) Ait. F., *Crescentia cujete* L. and *Zanthoxilum Zanthoxiloide* (Lam.) Watermann with a frequency of 11% each. These frequencies could be explained by the fact that traditional healers are unanimous on the use of certain species and in addition they have regular and effective knowledge of the therapeutic virtues of these plants. Other authors such as Lema et al. [29]; Sawadogo et al. [30] and Thiombiano et al. [31] have also found the involvement of these species in the composition of recipes against ulcers, hepatitis and breast cancer.

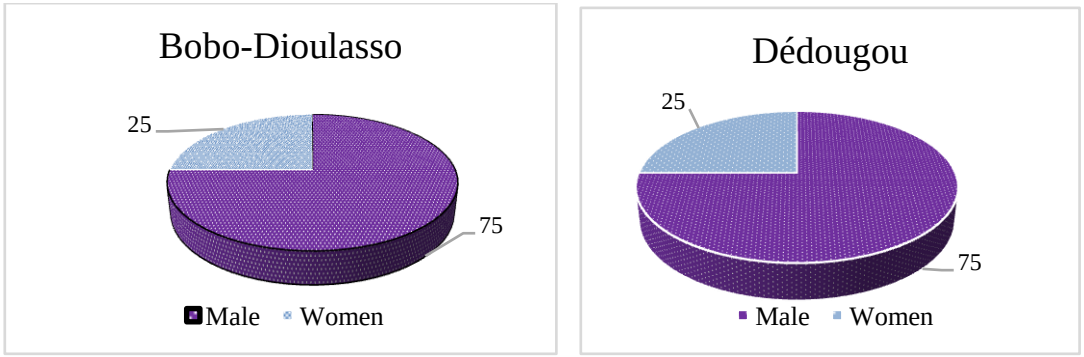

Figure 1: Distribution of traditional healers by gender

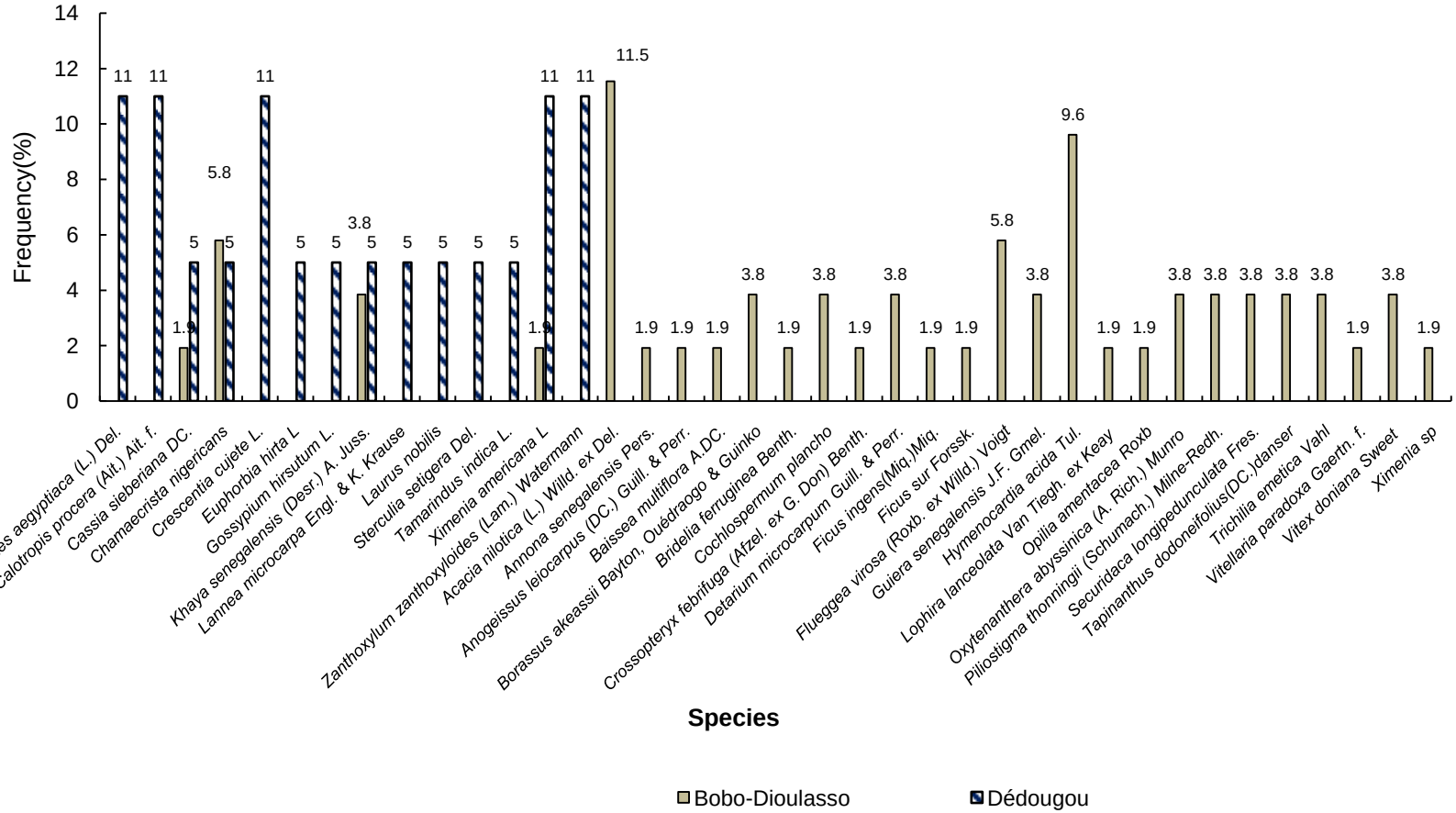

Figure 2: The distribution of the species of the two cities

3.1.3. Distribution of medicinal plants according to family

The medicinal species identified belong to 25 botanical families (Figure 3) whose analysis reveals that the Fabaceae (18) and Phylanthaceae (18) families are the most represented in the city of Bobo- Dioulasso. The studies carried out by Kpodji et al. [32] in southern Benin on medicinal plants used in the treatment of inflammatory diseases, through a series of ethnobotanical surveys of 42 herbalists had shown that out of 28 different botanical families, the most represented were respectively the families of Euphorbiaceae and Fabaceae. This approximation of families would indicate that the species of these families are the most sought after by populations in the treatment of various pathologies including microbial infections.

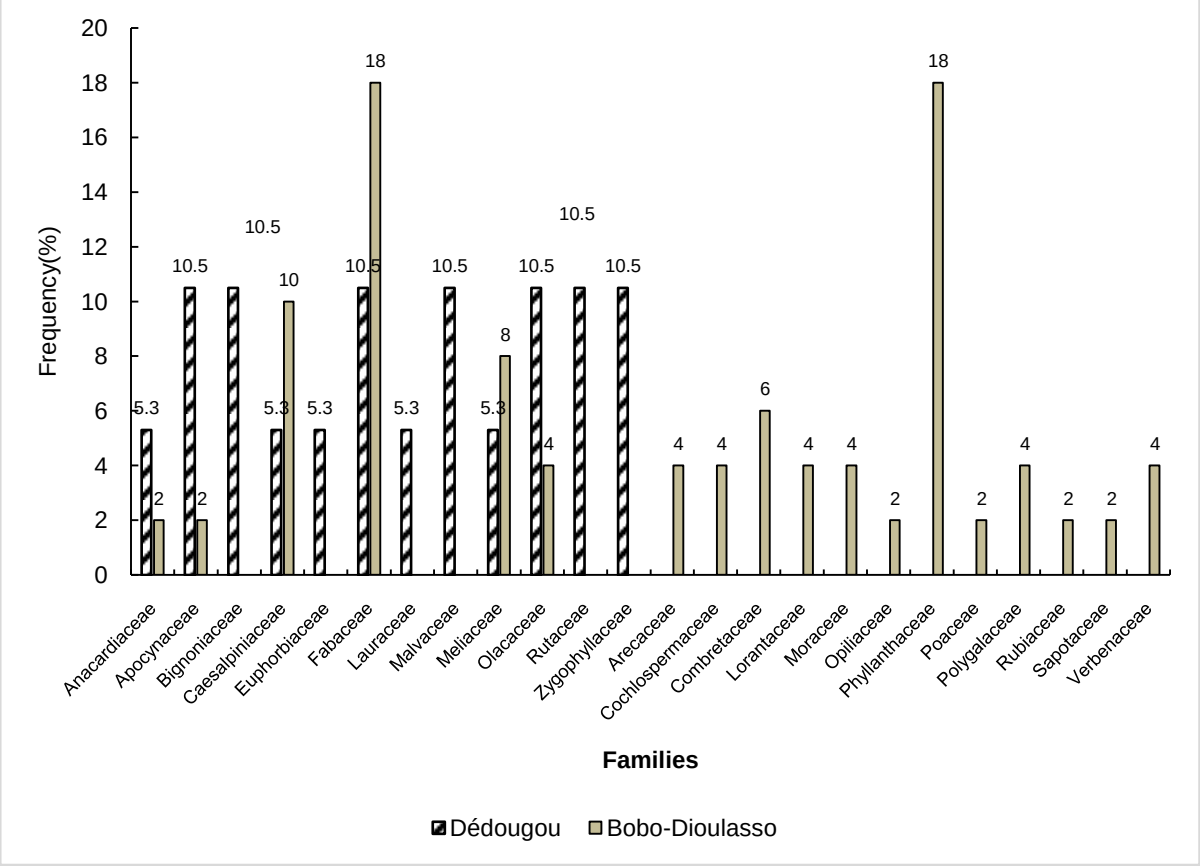

Figure 3: Distribution according to families

3.1.4. Distribution of medicinal plants according to the parts used

Various plant organs are used in traditional medicine for the treatment of vaginal candidiasis. In our case, all parts of the plant are involved in the composition of the recipes (figure 4). It appears that in Bobo-Dioulasso, the leaves (44%) and roots (22%) constitute the most used parts of the plant, while in Dédougou, the bark of the trunk (31.82%) and the des roots (18.18%) are the most used. These results are close to those of Dakio et al. [33] which showed that a diversity of plant organs is used with a dominance of leaves (36%). Therefore, we can say that in Bobo-Dioulasso, traditional healers are concerned about the preservation of nature, which would

justify the strong use of leaves in the composition of recipes against candidiasis.

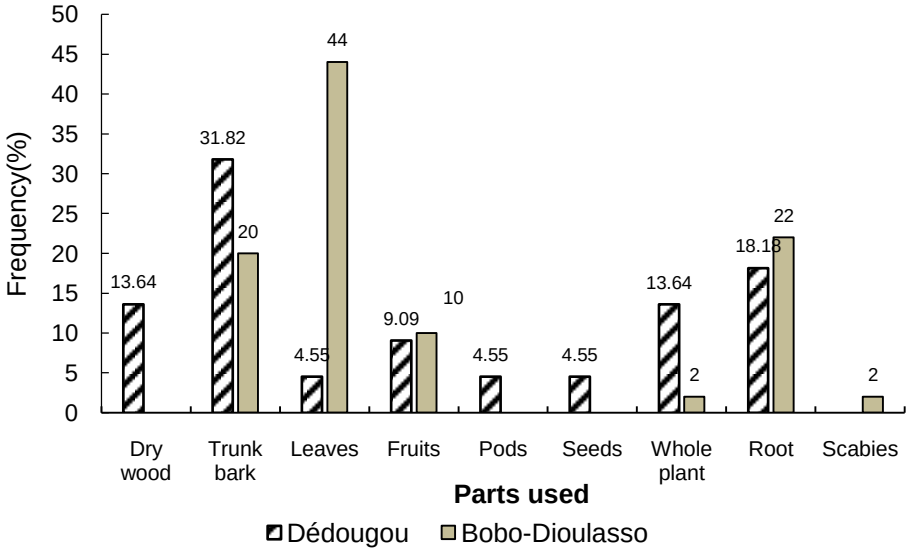

Figure 4: Parts used in traditional medicine

3.1.5. Distribution of medicinal plants according to the method of preparation

During the survey, three methods of preparation were the most cited, of which decoction is the most common method in the two cities (figure 5). As a result, it appears that the most common method of preparation in Bobo-Dioulasso is the decoction with a citation rate of 87.5% followed by the method of powder added to *Vitellaria paradoxa* C.F.Gaertn butter 5%. In addition, in Dédougou, the most used methods are decoction and calcination (27.27%) followed by calcination with added *Vitellaria paradoxa* C.F.Gaertn butter (22.73%). These results are similar to those of the work of Lema et al. [29] who showed that out of 290 traditional healers surveyed (Bobo-Dioulasso, Boromo, Dédougou and Fada N'Gourma) that the decoction (70%) was the main mode of preparation of these recipes. Also, Zerbo et al. [34] (in the North-West of Burkina Faso) showed that the decoction (58%) was the method most frequently used. Following these findings, these methods seem to be the easiest to perform and can reduce the toxicity of the extracts.

3.1.6. Distribution of medicinal plants according to the mode of administration

Several modes of administration are used by traditional healers in the treatment of candidiasis. Thus, the most cited mode of administration in Bobo-Dioulasso is the bath associated with intimate toilet with a frequency of 80% and in Dédougou, the method of massage and that of the beverage added to intimate toilet occupy the first place. with a frequency of 35% each (Figure 6). Studies by Lema et al. [29] on the recipes of medicinal plants used in the management of ulcers in Burkina Faso on an entity of 290 traditional healers showed that administration by the method of drink (47%) and bath (28%) were the modes the most used. This could be due to the simplicity of these different methods.

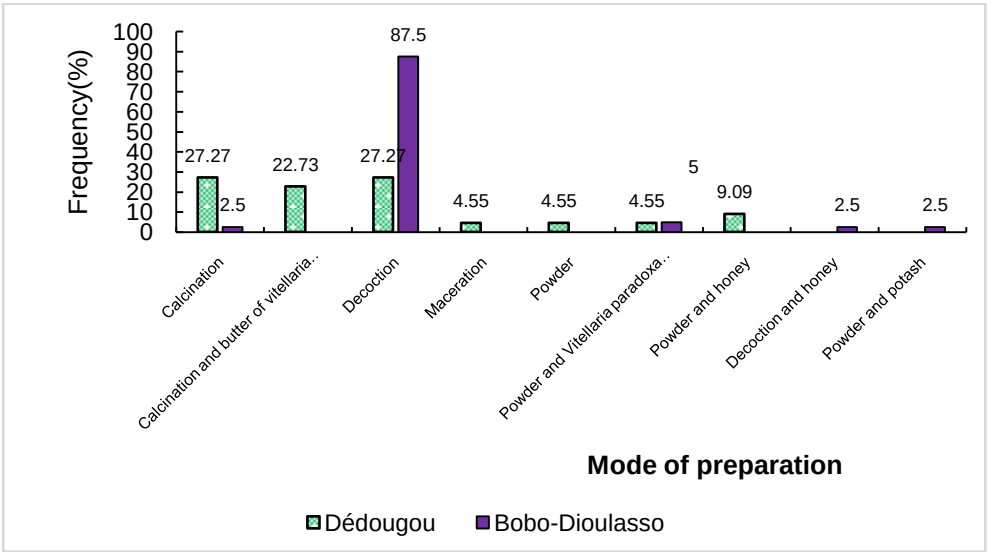

Figure 5: Breakdown by method of preparation

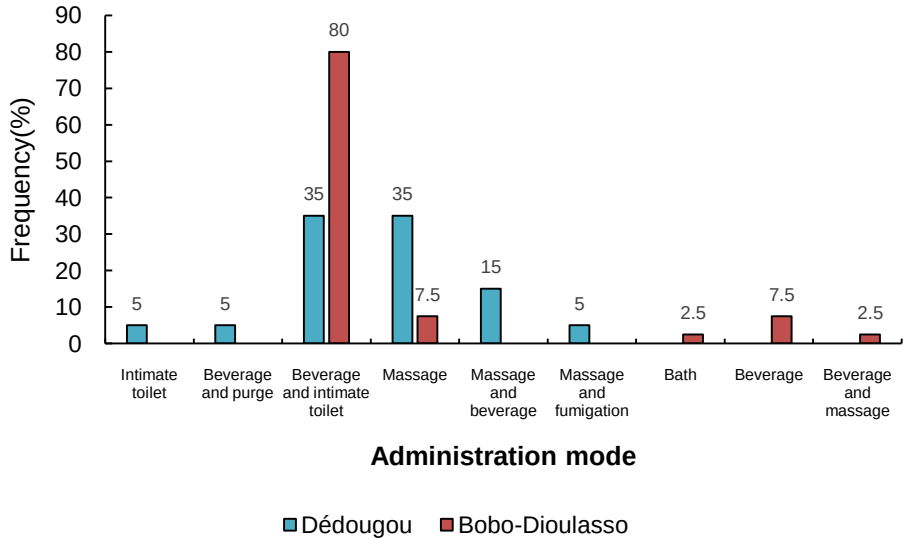

Figure 6: Distribution according to mode of administration

3.2. Phytochemical study

3.2.1. Extraction yield

The highest yield was obtained with the hydroethanolic extract of *A. nilotica* fruits (60.33%) and the lowest yield with the ethanolic extract (48%). Our results are different from those of Doumbia et al. [35] in Mali who used the same organs but an infusion and obtained a yield of 37.15%. This difference could be explained by the extraction technique used.

3.2.2. Characterization of chemical groups

Characterization tests in tubes revealed the presence of tannins, alkaloids, flavonoids and saponosides in both types of *A nilotica* fruit extracts (table 1). These results corroborate the work of Ali et al. [17] who had also reported the presence of polyphenols, tannins and alkaloids in the extracts of the study plant.

Table 1: Characterization tests for chemical groups

	Chemical groups			
Extracts	Tannins	Flavonoids	Alkaloids	Saponosides
Ethanolic	+	+	+	+
Hydroethanolic	+	+	+	+

Legend: + presence.

3.2.3. Quantification of polyphenolic compounds

The total phenolic, flavonoids and tannin of ethanolic and the hydroethanolic extract are summary in table (table 2).

Dosage of total phenolics: The total phenolic contents were 76.62 ± 7.23 and 80.26 ± 20.271 mg EAG/10 mg of dry extract respectively for the ethanolic and the hydroethanolic extract. We find that the total phenolic contents of the two extracts are equivalent. Our results are different from those of Doumbia et al. [35] who obtained 182.59 ± 1.41 (mg EAG/g) of total phenolics with infused fruits of *Acacia nilotica*. This difference in results could be explained by the nature of the extracts extraction method.

Assay of total flavonoids: The dosage of total flavonoids revealed that the content of hydroethanolic extracts was 0.23 ± 0.012 mg EQ/10 mg of extract and ethanolic was 0.274 ± 0.09 mg EQ/10 mg of extract. Our results are different from those of Doumbia et al. [35] who obtained 9.49 ± 0.66 (mg EQ/g) in total flavonoids with *Acacia nilotica* fruit infused.

Tannin content: The tannin contents of the two extracts were 4.43± 2.13 and 73.31± 4.65 mg EC/10 mg of extract respectively for the ethanolic and the hydroethanolic extract. Studies by Metowogo et al. [36] in Togo on hydro ethanolic extracts obtained 12.8 ± 1.3 mg EC/g of extract. What is different from ours, the difference could be due to the extraction technique.

Table 2: Total polyphenols assay results

species	**Extracts**	**Phenolics (mg EAG/10 mg)**	**Flavonoids (mg EQ/10 mg)**	**Tannins (mg EC/10 mg)**
Acacia nilotica L.	Ethanolic	76.63±7.23[b]	0.23±0.018[b]	4.44±2.13[b]
	Hydroethanolic	80.27±20.27[a]	0.274±0.01[a]	73.31±4.65[a]

Mean values(n=3) ±SD, letters in columns are significantly different(P<0.05)

3.3. Biological activities

3.3.1. Evaluation of antioxidant activity

The antioxidant activities were evaluated by two methods (DPPH and FRAP). With regard to these methods, the percentages of inhibition of DPPH were 76.33±0.099% and 85.59±0.001% respectively for the ethanolic and hydroethanolic extract and the reducing power (FRAP) of the extracts was of 3.97±0.23 and 6.22±0.09 mmolEAA/10 mg of extract respectively for the ethanolic and hydroethanolic extract (Table 3). Furthermore, studies conducted by Mansouri et al. [37] on the antioxidant activities (DPPH and FRAP) on the species in Morocco against inflammations with the aqueous extract of the gum and found respectively 0.04 ± 0.00 and 0.013 ± 0.006 Mm Equivalent Trolox/g of plant. Rather et al. [16], on methanolic extracts of the flowers and pods of *Acacia nilotica* showed that in terms of antioxidant activity by the DPPH method, the plant has an inhibition rate of 65.86% for the methanolic extract of flowers and 63.86% for the extract of the pods for a concentration of 100 mg/ml. These methods led to the conclusion that the species have an interesting antioxidant activity which could be due to the contribution of either phenolics and/or flavonoids in the recipe.

Table 3: Table representing the results of the antioxidant activities

Specie	Extracts	DPPH (%)	FRAP (mmolEAA/10mg)
Acacia nilotica L.	ethanolic	76.33±0.099[b]	3.97±0.23[b]
	hydroethanolic	85.59±0.001[a]	6.22±0.09[a]

Mean values(n=3) ±SD, letters in columns are significantly different(P<0.05)

3.3.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

3.3.2.1. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) represents the smallest concentration that can inhibit the growth of microorganisms by 99.99%. The MIC was observed from the first well to the 7th well depending on the types of microbial strains. Thus, these MICs carried out on the different strains with the ethanolic and hydroethanolic extracts all presented interesting results. In general, the MICs varied from 0.781 mg/ml to 6.25 mg/ml (Table 4). The 100% AnF_1 extract was very active on strains of *Acinobacter baumannii*, *Shigella sonnei*, *P. aeruginosa*, certain *Escherichia coli* with a concentration of 0.781 mg/ml and the 20% AnF_1 extract was very active on *Acinobacter baumannii* and a single strain of *E. coli* at a concentration of 0.781 mg/ml. According to the work of Bashige-Chiribagula et al. [38], the antibacterial activity of plant extracts can be strong, moderate or weak. Several previous works such as Ali et al. [17] and Banso, [39] had already established the antibacterial properties of *Acacia nilotica* by linking them to the presence of certain bioactive groups including tannins, flavonoids, alkaloids. The same is true for Guta et al in [40] who obtained a powerful antibacterial activity with the ethyl acetate fraction of *Acacia nilotica* fruits with a concentration of 0.25 mg/ml. We can admit that the inhibition of microbial growth could be linked to the presence of flavonoids and tannins in our fruit extracts. This antibacterial activity can be explained by the fact that these metabolites disrupt the stability of the cell membrane, as well as by increasing its permeability [41].

3.3.2.2. Determination of the minimum bactericidal concentration (MBC)

On all bacterial strains, the extracts showed bactericidal activity, except on *P.aeruginosa* harboring the SHV type bla gene which showed countless colonies on agar medium. The best minimum bactericidal concentration (MBC) was 3.123 mg/ml (Table 4). Gouffi and Mabrouk. [41] performed MBC on strains of *Escherichia coli* ATCC 25922 En. cloacae, *R. ornithinolytica*, *Klbesiella pneumoniae*, ESBL-producing *Escherichia coli* and obtained positive results from a concentration of *Laurus nobilis* essential oil of 05 µl/ml; 2.5 µl/ml and 1.25 µl/ml. The MBC/MIC ratio makes it possible to determine whether an extract is bactericidal or bacteriostatic. According to Okou et al. [42], if the MBC /MIC ratio ≤ 4, the tested substance is bactericidal and if the MBC/MIC ratio > 4, the tested substance is bacteriostatic. The 100% AnF_1 extract is bactericidal on 5 strains of Enterobacteriaceae and bacteriostatic on the other 13 strains of Enterobacteriaceae. Also, the AnF_1 20% extract is bactericidal on 5 strains of Enterobacteriaceae and bacteriostatic on the other 13 strains of Enterobacteriaceae. The bactericidal and bacteriostatic activity of our extracts could also be due to the presence of secondary metabolites, in particular phenolics and/or flavonoids.

425 **Table 4 : Results of the antibacterial activity.**

Type of extracts	Strains	Strain names	MIC (mg/ml)	MBC (mg/ml)	Report MBC /MIC	Number of colonies	Genotype
AnF_1 100%	Uc 2704	*Escherichia coli*	3.125^{b}	12.5^{c}	4^{h}	2	
	Sc 1148	*Salmonella sp*	6.25^{a}	50^{a}	8^{f}	1	
	Uc 982	*P. aeruginosa*	0.781^{d}	50^{a}	64.02^{a}	ND	SHV
	Uc 2525	*Citrobacter freundii*	3.125^{b}	50^{a}	16^{d}	5	
	Uc 2532	*Acinobacter Baumannii*	0.781^{d}	50^{a}	64.02^{a}	2	
	273H	*Klbesiella pneumoniae*	1.563^{c}	25^{b}	15.99^{e}	3	CTM-X15
	Uc 176	*Salmonella Typhi*	1.563^{c}	12.5^{c}	7.99^{g}	4	
	Sc 1264	*shigella.sonnei*	0.781^{d}	25^{b}	32.01^{b}	3	
	Uc 2457	*Escherichia coli*	0.781^{d}	50^{a}	64.02^{a}	5	CTM-X15
	Uc 2455	*Escherichia coli*	0.781^{d}	50^{a}	64.02^{a}	2	
	565P	*Escherichia coli*	1.563^{c}	25^{b}	15.99^{e}	1	CTM-X15
	Uc 241	*Escherichia coli*	1.563^{c}	3.125^{d}	2^{i}	1	CTM-X15
	Uc 763	*P. aeruginosa*	3.125^{b}	25^{b}	8^{f}	3	CTM-X15
	Uc 714	*Escherichia coli*	3.125^{b}	3.125^{d}	1^{j}	2	CTM-X15
	Uc 681	*Klbesiella pneumonia*	1.563^{c}	6.25^{b}	4^{h}	3	CTM-X15
	Uc 1028	*Citrobacter sp*	3.125^{b}	25^{b}	8^{f}	1	CTM-X15
	Uc 2582	*klebsiella pneumoniae*	3.125^{b}	25^{b}	8^{f}	3	
	Uc 2527	*Klbesiella pneumoniae*	3.125^{b}	12.5^{c}	4^{h}	2	
AnF_1 20%	Uc 2704	*Escherichia coli*	0.781^{d}	25^{b}	32.01^{b}	3	
	Sc 1148	*Salmonella sp*	3.125^{b}	50^{a}	16^{d}	2	
	Uc 982	*P. aeruginosa*	1.563^{c}	50^{a}	31.99^{c}	ND	SHV
	Uc 2525	*Citrobacter freundii*	6.25^{a}	12.5^{c}	2^{i}	3	

	Uc 2532	*Acinobacter Baumannii*	0.781^{d}	6.25^{b}	8^{f}	5	
	273H	*Klbesiella pneumoniae*	1.563^{c}	50^{a}	31.99^{c}	1	CTM-X15
	Uc 176	*Salmonella Typhi*	3.125^{b}	12.5^{c}	4^{h}	2	
	Sc 1264	*shigella.sonnei*	1.563^{c}	12.5^{c}	7.99^{g}	4	
	Uc 2457	*Escherichia coli*	1.563^{c}	12.5^{c}	7.99^{g}	3	
	Uc 2455	*Escherichia coli*	1.563^{c}	12.5^{c}	7.99^{g}	3	
	565P	*Escherichia coli*	3.125^{b}	3.125^{d}	1^{j}	5	CTM-X15
	Uc 241	*Escherichia coli*	3.125^{b}	50^{a}	16^{d}	2	CTM-X15
	Uc 763	*P. aeruginosa*	3.125^{b}	12.5^{c}	4^{h}	4	CTM-X15
	Uc 714	*Escherichia coli*	3.125^{b}	25^{b}	8^{f}	1	CTM-X15
	Uc 681	*Klbesiella pneumonia*	1.563^{c}	25^{b}	15.99^{e}	1	CTM-X15
	Uc 1028	*Citrobacter sp*	3.125^{b}	12.5^{c}	4^{h}	2	CTM-X15
	Uc 2582	*klebsiella pneumoniae*	3.125^{b}	50^{a}	16^{d}	1	
	Uc 2527	*Klbesiella pneumoniae*	3.125^{b}	50^{a}	16^{d}	1	CTM-X15

Mean values (n=3) ±SD, different letters in columns are significantly different (P<0.05). ***Sc****:Stool culture;* ***Uc****: Urine culture*
AnF$_1$100%*: Ethanolic extract;* ***AnF$_1$ 20%:*** *Hydroethanolic extract.*

3.3.2.3. Antifungal susceptibility test of Acacia nilotica extracts

All the strains of *Candida albicans* isolated (13 strains in total) were the subject of a sensitivity study to extracts of *Acacia nilotica* in vitro. We note that some strains showed resistance to nystatin. In addition, the control considered negative (80% ethanol) showed resistance and/or sensitivity depending on the strain considered. Also, all ethanolic extracts showed antifungal activity on most of our *Candida albicans* strains tested. It appears that the diameters of inhibition varied from 6 to 14 and 6 to 30 mm respectively for the negative control and the positive control. With regard to the hydro ethanolic extract, the diameters varied from 12 to 54; 12 to 27 and 8 to 16 mm respectively for disc 1, 2 and 3. Similarly, the inhibition diameters of the ethanolic extract varied from 17 to 54; 13 to 28 and 12 to 28 mm respectively for disk 1, 2 and 3 (table 5). By comparing the discs of our extracts to the references, we note that our extracts have the best inhibition diameters. These results could explain the use of *Acacia nilotica* in the treatment of candidiasis.

Table 5: Results of antifungal susceptibility of strains

Type of extracts	N° of *Candida albicans* strains	Sensitivity: Diameter (mm) in superscript
AnF_1 20%	Sc 1228	T^{9}, NY^{12}, D_1^{14}, D_2^{13}, D_3^{13}
	Sc 1170	T^{6}, NY^{6}, D_1^{34}, D_2^{14}, D_3^{12}
	Vs 301	T^{13}, NY^{6}, D_1^{17}, D_2^{16}, D_3^{13}
	Vs 324	T^{6}, NY^{6}, D_1^{44}, D_2^{27}, D_3^{10}
	Vs 339	T^{8}, NY^{12}, D_1^{12}, D_2^{12}, D_3^{11}
	Sc 1164	T^{10}, NY^{6}, D_1^{23}, D_2^{17}, D_3^{13}
	Vs 302	T^{14}, NY^{26}, D_1^{52}, D_2^{14}, D_3^{12}
	Sc 1229	T^{11}, NY^{24}, D_1^{13}, D_2^{12}, D_3^{8}
	Vs 319	T^{12}, NY^{23}, D_1^{48}, D_2^{22}, D_3^{16}
	P 235	T^{12}, NY^{22}, D_1^{32}, D_2^{25}, D_3^{21}
	Vs 310	T^{8}, NY^{30}, D_1^{52}, D_2^{14}, D_3^{10}
	Vs 297	T^{12}, NY^{21}, D_1^{30}, D_2^{17}, D_3^{13}
	Vs 338	T^{12}, NY^{22}, D_1^{54}, D_2^{28}, D_3^{16}
AnF_1 100%	Sc 1228	T^{6}, NY^{12}, D_1^{28}, D_2^{25}, D_3^{12}
	Sc 1170	T^{6}, NY^{6}, D_1^{28}, D_2^{25}, D_3^{18}
	Vs 301	T^{13}, NY^{6}, D_1^{22}, D_2^{19}, D_3^{17}
	Vs 324	T^{6}, NY^{6}, D_1^{26}, D_2^{18}, D_3^{12}
	Vs 339	T^{8}, NY^{12}, D_1^{17}, D_2^{15}, D_3^{13}
	Sc 1164	T^{10}, NY^{6}, D_1^{20}, D_2^{17}, D_3^{13}
	Vs 302	T^{14}, NY^{26}, D_1^{26}, D_2^{15}, D_3^{10}
	Sc 1229	T^{11}, NY^{24}, D_1^{18}, D_2^{13}, D_3^{10}
	Vs 319	T^{12}, NY^{23}, D_1^{38}, D_2^{20}, D_3^{16}
	P 235	T^{12}, NY^{22}, D_1^{48}, D_2^{25}, D_3^{14}
	Vs 310	T^{8}, NY^{30}, D_1^{32}, D_2^{24}, D_3^{16}
	Vs 297	T^{12}, NY^{21}, D_1^{50}, D_2^{15}, D_3^{13}
	Vs 338	T^{12}, NY^{22}, D_1^{54}, D_2^{28}, D_3^{16}

*Negative control **T**=80% ethanol; **D_1**= disc 1 (50mg/ml(extracts); **D_2**=disc 2 (25mg/ml(extracts); **D_3**= disc 3(12.5mg/ml(extracts); **NY**=nystatin (conventional antibiotic).*
***Sc**:Stool culture; **Uc**: Urine culture; **Vs**: Vaginal samples; **P**: Pus.*
***$ANF_1$100%**: Ethanolic extract; **$ANF_1$20%**: Hydroethanolic extract.*

4. CONCLUSION

The investigations made it possible to question 52 traditional healers and to identify 38 medicinal plants distributed in 25 botanical families. The study revealed that the majority of respondents are men (75%) in each city. The best contents of total phenolic compounds were given by the hydroethanolic extract; i.e., 80.27 ± 20.27 mg GAE/10 mg. Similarly, the best values for total flavonoids were obtained from 0.274 ± 0.01 mg QE / 10 mg extract and that of tannins were 73.31 ± 4.65 mg EC / 10 mg extract. Regarding the anti-DPPH• and anti-FRAP activities, the best were 85.59±0.001% and 6.22±0.09 mmolEAA/10 mg. According to the frequency of citation, *Acacia nilotica* was the species most used in the treatment of vaginal candidiasis. the most represented families were the Fabaceae (18%) and the Phyllantaceae (18%). The mode of preparation and the parts used were the decoction respectively (87.5%: Bobo-Dioulasso and 27.27%: Dédougou), the leaves (44%

Bobo-Dioulasso) and the bark of the trunk (31.82% Dédougou). Interesting results were obtained in terms of antimicrobial activity, with the best values of MIC and MBC respectively. of 0.781 and 3.125 mg/ml. In addition, the extracts possess strong inhibitory activity on *Candida albicans* strains with large diameters up to 54 mm. It would be more interesting to deepen the investigations on this recipe of medicinal plants which has a strong activity on the microbial stocks in order to propose the most effective treatment without risk of toxicity for the man.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

The authors declare that they have no competing interests regarding this article.

AUTHORS' CONTRIBUTIONS

Mindiédiba Jean Bangou A' led the ethnobotanical survey, analyses and supervision; Jacob Koudbi Zongo B' was in charge of the phytochemical and antimicrobial activities; Pierre Alexandre Eric Djifaby Sombié C' who directed the extractions; Bibata Traore C was in charge of collections, tests in the Laboratory and documentary research. All authors have read and approve the final manuscript.

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APPENDIX