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Research Paper

Ethnopharmacological surveys and pharmacological studies of plants used in traditional medicine in the treatment of HIV/AIDS opportunistic diseases in Gabon

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ABSTRACT

Ethnopharmacological relevance: Ethnopharmacological surveys were conducted in two regions of Gabon. This led to highlighting some of the medicinal plants used by local populations in the management of HIV/AIDS opportunistic diseases. Two regions with the highest occurrence of HIV/AIDS cases were visited and ethnopharmarcological data was gathered. These regions were the Estuaire Province (Libreville and its neighborhood) and the Haut-Ogooué Province (Franceville and its neighborhood). The opportunistic diseases and symptomatic conditions considered during this study were: diarrhea, respiratory tract infections, cough, tuberculosis, abscesses, stomach ache, skin rashes, venereal diseases, typhoid fever, anemia, general tiredness, hepatitis and vomiting.

Materials and methods: The reported species were evaluated through three parameters: specificity, reliability and frequency. Plant parts of relevant species were harvested and extracted with an aqueous alcohol solution (ethanol/water: 1/1). The extracts obtained were submitted to phytochemical screening and *in vitro* microbiological assays on some clinical isolates and ATCC strains, involved in HIV/AIDS opportunistic diseases through the Agar well diffusion and Microbroth dilution methods.

Results: Among the 52 species identified during this survey, *Coelocaryon klainei* Pierre ex Heckel (Myristicaceae), *Dacryodes klaineana* (Pierre) H.J. Lam (Bursecaceae), *Phyllanthus diandrus* Pax (Euphorbiaceae), *Saccoglotys gabonensis* (Baill.) Urb. (Humiriaceae) and *Tetrorchidium didymostemon* (Baill.) Pax & K. Hoffm. (Euphorbiaceae) were submitted to *in vitro* microbiological assays. *Phyllanthus diandrus* bark and leaves show best antibacterial activities against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with MIC value of 0.25 respectively. Phytochemical screening revealed the presence in all the plant parts extracts of potentially bioactive molecules, including polyphenols, especially flavonoids and tannins.

Conclusion: It is concluded that some of these plants might be submitted to further scientific studies, including the identification and isolation of bioactive principles, that could be developed to drugs for the treatment of HIV/AIDS opportunistic diseases.

1. Introduction

The tropical forest in general, and the gabonese forest in particular, constitutes not only a key element in the fight against global warming, but, also an unvaluable source of species used in food and in the treatment of several diseases.

The rainforest covers 85% of the Gabon territory, and still today, about 70% of the gabonese population, most of which living below

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the poverty threshold, depend either partially or entirely on traditional medicines for the management of various diseases including HIV/AIDS. HIV/AIDS is one of the most prevalent infections in Gabon, with a national prevalence of 5.3% (approximately 82,466 people among 1,556,000 for the whole country), which constitutes the highest level in the Central African region (WHO, 2009). The high spread combined with the poor management of HIV/AIDS in Gabon can be considered as an obstacle in the fight against poverty and underdevelopment since the majority of infected people are among the economically active population (15–45 years old) (UNAIDS, 2006, 2008). However today, in spite of the decrease of HIV prevalence in Gabon, among an estimated 24,000 people who need antiretroviral therapy, only 67% of them are said to get access to the treatment (UNAIDS, 2013).

The factors involved are certainly limited accessibility for the majority of AIDS patients to antiretroviral therapy and the appearance of opportunistic diseases or infections caused by the acquired immune deficiency (Hodgson and Rachanis, 2002). These infections are the leading cause of the death of AIDS patients in Gabon and in the world (NIMR, 2004; UNAIDS, 2006). It should be important to know that these infections are particularly frequent in patients with HIV/AIDS, because a study conducted at a hospital in Libreville, by Okome-Nkoumou et al. (2006), showed that opportunistic infections due to HIV/AIDS in terms of prevalence were: prurigo (100%), cerebral toxoplasmosis (100%), oral candidiasis (88%), bacteremia (87.8%), shingles (84.6%), minor salmonellosis (72%), tuberculosis (53%), typhoid (29.4%), pneumonia (28%), bacterial meningitis (26.3%), hepatitis B (20%), and malaria (14%).

In fact, the spread of poverty, the expensive cost of medicines, the bacterial resistance to conventional drugs and the side effects of antiretroviral therapy lead most HIV/AIDS patients in Gabon to consult traditional healers whose merits in the treatment of HIV/AIDS opportunistic diseases by herbal medicines is particularly defended by a greater part of them. In Gabon, to date, no study based on the plants used in traditional medicine to treat HIV/AIDS opportunistic diseases has yet been carried out. On the other hand, in Tanzania, a study on the management of HIV/AIDS patients by traditional medicine, demonstrated the relevance of herbal therapies in the treatment of HIV/AIDS opportunistic diseases (Kisangau et al., 2007).

This work was carried out with the aim of providing scientific data with regards to these therapies, and to offer to science and pharmaceutical industries, active molecules against microbial strains responsible of HIV/AIDS opportunistic infections. This preliminary work included an ethnopharmacological survey, the harvest of plant organs, the phytochemical screening of plant extracts and *in vitro* microbiological testing.

2. Materials and methods

2.1. The study area

Estuaire province is one of the nine provinces of the Republic of Gabon (Fig. 1). It is a flat terrain zone that borders Equatorial Guinea Republic to the North, Woleu-Ntem province to the North East, Moyen-Ogooué province to the South East, Ogooué-Maritime province to the South and the Atlantic Ocean to the west. This province is the most popular of Gabon and it includes the capital of the country Libreville, the most popular town of Gabon, with about 493,351 inhabitants in 2012, among 1,556,000 inhabitants of the whole country (MHUESD, 2011; UNDESA, 2012). It is an important industrial and port zone, and a very cosmopolitan area with the highest concentration of AIDS patients in Gabon, who generally consult surrounding traditional healers.

Haut-Ogooué province is located at the South East of Gabon. It borders Ogooué-Ivindo province to the North, Congo-Brazza Republic to the East and the South, and Ogooué-Lolo province to the West. It is a mountainous zone at altitude that varies between 400 and 600 m over the sea level. The capital of the Haut-Ogooué province is Franceville, one of the most populous towns of Gabon, with about 39.096 inhabitants (MHUESD, 2011; UNDESA, 2012). In addition to manganese exploitation, agriculture is one of the economic mainstays of the province and the main crops are coffee, cocoa, bananas, cassava, yams and other food-producing products. The region is occupied by many tribes, among whom the Pygmies, who have the reputation of being very efficient in traditional medicine, and so, they are frequently consulted by citizens, among whom, people living with HIV/AIDS.

2.2. Ethnopharmacological surveys

Ethnopharmacological surveys were carried out in four localities in the Estuaire Province (Northwest Gabon) and four localities in the Haut-Ogooué Province (Southeastern Gabon) from November 2009 to June 2010.

In each province, a pre-established questionnaire was used to collect field information from traditional healers and people with declared knowledge on medicinal plants. The interviewees were identified with the assistance of traditional rulers, and local administrative officers. The questions focused essentially on the local names of the plants, parts used, preparation, harvest modalities, administration, disease condition treated age and the education level of the informant.

During investigations, symptoms of various HIV/AIDS opportunistic diseases/infections were described to practitioners, in order to enable them provide species they used to treat the considered disease (Kisangau et al., 2007; CDC, 2013). In fact, at the beginning of our study, we knew that it would be difficult for traditional healers to distinguish diseases, since many diseases share similar symptoms. In order to avoid the difficulty, we just asked the practitioners to identify symptoms of ailments they were treating, giving us the opportunity to identify and name the possible disease.

The opportunistic diseases considered during this study were hepatitis, respiratory tract infections, tuberculosis and venereal diseases. Other symptomatic but undefined conditions included anemia-general tiredness, abscess, diarrhea, skin rashes, stomach ache, typhoid fever and vomiting.

A sample of each plant species cited in the survey was collected, and identified either on site or sent for identification at the National Herbarium of Gabon at Libreville. A specimen of each species was kept in the Scientific and Technical University of Masuku, Franceville, Gabon. Samples were collected with pruning shears, labeled and transported in plastic bags.

To analyze the ethnobotanical importance of the reported plant species, coherence and convergence method was used including three parameters as described by Ben Haj Jilani et al. (2007) and adjusted to our study. This helped in the classification of species according to their potential importance, and the selection of the most interesting that would undergo more relevant scientific studies.

In the first screening, criteria based on the absolute number of citations was considered. Thus, those mentioned several times, at least by five informants were identified and selected.

In a second screening, the degree of specificity of plants for a disease was considered. Thus, during this second screening, only the species mentioned by at least three different informants for the same disease were selected for the next stage (Ben Haj Jilani et al., 2007).

At the end, the selection of the most relevant species was done in terms of specificity for the disease, reliability and frequency, because the value of a drug is due to its specific efficiency on a

Fig. 1. A map showing the location of the study areas. Legend: ▨ the study areas.

particular disease, and its reliability is associated with the results obtained in use (Abdou Sidi, 1994).

These indices expressed in percentages (%) are defined as follows (Ben Haj Jilani et al., 2007):

$$Specificity = \frac{1}{\text{number of opportunistic diseases treated by a species}} \times 100$$

$$Reliability = \frac{\text{number of citation of a species for the treatment of a disease}}{\text{total number of citation of species for the treatment of the same disease}} \times 100$$

$$Frequency = \frac{\text{number of citation of a species for the treatment of a disease}}{\text{absolute number of citation of that species}} \times 100$$

2.3. Harvest and extraction of the plants organs

Plant material were harvested as indicated by traditional healers. The barks and leaves were harvested with pruning shears, labeled and carried in plastic bags. The identification of the species was done

by Mr. Thomas NZABI, a botanist of the National Herbarium of Gabon. The plant material was then dried for four days at room temperature and finely powdered

The powder obtained was extracted by tederation at room temperature in an aqueous alcohol solution (ethanol/water: 1/1) for two days, and the extract freeze-dried.

2.4. Phytochemical screening

The plants extracts were screened for their qualitative chemical composition, using standard methods (Feuya Tchouya, 2005; Mengome et al., 2009). The identification of the following groups was considered: alkaloids, coumarins, flavonoids, polyphenols, saponosides, sterols and triterpenes, sugars and tannins.

Alkaloids: 0.5 g of each extract was agitated with 5 ml of hydrochloric acid in a steam bath, then 1 ml aliquots of filtrate were treated with a few drops of Mayer's reagent or Dragendorff's reagent. The presence of a precipitate after treatment with either reagent is a preliminary indicator of the presence of alkaloids. To remove non-alkaloid compounds that could lead to false-positive reactions, part of the extract was alkalinized with 40% ammonia solution then treated twice with chloroform. The second chloroform extract was concentrated and then retested with the Mayer and Dragendorff reagents. Coumarins: examined in ultraviolet light, the TLC of drugs with coumarins present spots whose colouring, in presence of ammonia atmosphere, varies from blue to yellow and purple. Flavonoids were detected by using the Shibata reaction or cyanide test. Briefly, 3 ml of extract was evaporated and the residue was dissolved in 2 ml of 50% methanol, then a few magnesium shavings and a few drops of concentrated hydrochloric acid were added. The development of a red–orange or purplish color indicates the presence of flavones aglycones.

Polyphenols and tannins: boiled aqueous extract (1 ml) was mixed with 1% ferric chloride. A black–blue color indicates the presence of gallic tannins and a dark green color tannin catechists. When both were detected in the same extract, they were separated with Styasny's reagent. A drop of the extract was placed on a slab of silica gel and eluted in an atmosphere saturated with chloroform/acetic acid/formic acid (5:4:1). Then the plates were sprayed with 10 ml of methanol solution at 5% nitrous acid and heated in an oven at 80 °C for 10 min. The presence of tannins is revealed by the appearance of blue spots, while polyphenols are revealed by a violet–blue, pink–orange, pink–violet, or red coloration. Saponosides: 1% of each sample decoction was returned gradually in 10 ml test tubes for a final volume of 10 ml. After two vigorous shakes, the tubes were left to stand for 15 min and the height of foam was measured. The tube in which the height of the foam was at least 1 cm, showed the presence of saponosides. However, the height of the foam indicated the value of the foam index. Sterols and triterpenes: these families of compounds were identified by using the Lieberman–Burchard reaction. Briefly, 0.5 g of extract was dissolved in 0.5 ml of chloroform with 0.5 ml of acetic anhydride, and cooled on ice before carefully adding sulfuric acid. A change in color from purple to blue indicates the presence of sterols, while a green or purple–red color indicates the presence of triterpenes. Sugars: a little quantity of extract was dissolved in an ethanol/α-naphthol (99%/1%) solution contained in a test tube, then allowed to run on the tube wall few drops of concentrated sulphuric acid. Sugars presence was detected by the emergence of a red ring at the interface.

For all the families tested, according to the precipitation or color intensity of each tube, following evaluations were given: (+++); (++); (+).

2.5. Microbiological surveys

2.5.1. Microorganisms

Seven bacterial species, Gram-positive (*Staphylococcus aureus* and *Streptococcus B*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Shigella flexneri*) obtained from "Centre Pasteur du Cameroun", and four American Type Culture Collection (ATCC) *Candida albicans* strains (ATCC L26, ATCC 12C, ATCC P37039 and ATCC P37037) were used for the antimicrobial tests. The strains were maintained on agar slant at 4 °C in the laboratories where the antimicrobial tests were performed. The strains were activated at 37 °C for 24 h on Tryptica soy agar or Sabouraud glucose agar supplemented with chloramphenicol respectively for bacteria and fungi prior to any screening. Mueller Hinton agar and Brain Hearth Infusion (BHI) were used for the antimicrobial assays (NCCLS, 1990).

2.5.2. Antimicrobial activity

2.5.2.1. Agar well diffusion method. The antimicrobial activities of the extracts were determined using the Berghe and Vlietnick agar-well diffusion method (Berghe and Vlietinck, 1991). Plates containing 30 ml of sterile nutrient broth were inoculated with standardized inoculate prepared using a cell suspension of about 1.5×10^6 CFU/ml "colony forming units per milliliter" obtained following a 0.5 McFarland turbidity standard. Four wells of 5 mm diameter were made on each plate with sterile Pasteur pipette and 50 μL of the plant extract dissolved in DMSO (10%) at a concentration of 10 mg/ml (Ebi, 2001) was dispensed into each well. The extract was allowed to diffuse into the medium for 1 h at room temperature and then, it was incubated for 24 h at 37 °C. The zones of growth inhibition was measured and recorded in millimeter. The negative control was set up in a similar manner except that the extract was replaced with sterile distilled water. All the testing was done in duplicate.

2.5.2.2. Microbroth dilution method. The Minimum Inhibitory Concentration (MIC) on bacteria and yeasts was determined by the microbroth dilution method (Carbonnelle et al., 1987). This method was applied on extracts that showed some efficacy against microorganisms by the agar well diffusion method (inhibition diameters above or equal to 8 mm). Stock solutions were prepared by dissolving 10 mg of extracts in 1 ml dimethyl sulphoxide (DMSO 10%).

2.5.2.2.1. Antibacterial method. For bacteria, in the well of the first line (line 1), 40 μL of culture medium broth (Mueller Hinton broth) was introduced and 100 μL in the remaining wells of the plates. Later on, 160 μL of stock solution of crude extracts (10 mg/ml) was added to the first well. The medium and sample in the first well were mixed thoroughly before transferring 100 μL of the resultant mixture to the well of the second line. Ten two-fold serial dilutions of the test samples were made from line 1 until line 11 and 100 μL of inoculum standardized at 0.5 McFarland standards was introduced in the entire well containing the test substances except the columns of blank which constitute the sterility control. The concentration range was 4–0.0039 mg/ml for crude extracts and reference antibiotics (Ceftriazon, Gentamicin and Rifampicine) used as positive control. After an incubation period at 37 °C for 24 h, turbidity was observed as indication of growth. Thus the lowest concentration inhibiting the visible growth of bacteria was considered as the Minimum Inhibitory Concentration (MIC). All the testing was done in duplicate.

2.5.2.2.2. Antifungal method. For yeast, in the well of the first line (line 1), 40 μL of culture medium broth (Sabouraud broth) was introduced and 100 μL in the remaining wells of the plates. Later on, 160 μL of stock solution of crude extracts (10 mg/ml) was added to the first well. The medium and sample in the first well were mixed thoroughly before transferring 100 μL of the resultant mixture to the

Table 1
Plant species used in the treatment of various HIV/AIDS opportunistic diseases in Gabon.

No.	Family	Plant name	Local name	Part used	Disease(s) treated	Collection code no.
1	Acanthaceae	*Acanthus montanus* (Nees) T. Anderson	Gèbanganbalè	B, L	Respiratory tract infections; stomach ache	Am 105/UM
		Whitfielda elongata (P. Beauv.) De Wild. & Th. Dur.	Mande–mande	L	Diarrhea	We 072/UM
2	Anacardiaceae	*Lannea welwitschii* (Hiern) Engl.	Gongo	B; fr	Respiratory tract infections	Lw 036/UM
		Trichoscypha abut Engler & V. Brehmer	Ghésè	B	Venereal diseases	Ta 126/UM
3	Apocynaceae	*Alstonia boonei* (De wild)	Nkuka	B	Stomach ache	Ab 005/UM
4	Araceae	*Anchomanes difformis* (Blume) Engl.	Moèngè-a-abongo	Tub	Venereal diseases	Ad 194/UM
5	Burseraceae	*Dacryodes buettneri* (Engl.) H.J. Lam	Ozigo	B, L	Respiratory tract infections	Db 123/UM
		Dacryodes klaineana (Pierre) H.J. Lam	Hombe	B; fr	Skin rashes	Dk 019/UM
		Santiria trimera (Oliv.) Aubrév.	Nkungu	B	Skin rashes	St 063/UM
6	Caesalpinaceae	*Gilbertiodendron deweri* (De Wild.) J. Léonard	Imbembe	B	Respiratory tract infections	Gd 028/UM
7	Compositeae	*Eclipta prostrata* (L.) L	Miténdèmitendè	L	Skin rashes	Ep 196/UM
		Vernonia smithiana Less	Matèngo	B, L	Stomach ache	Vs 149/UM
8	Dilleniaceae	*Tetracera alnifolia* Willd.	Mvughe mambe	L	Stomach ache	Ta 201/UM
9	Euphorbiaceae	*Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg.	Mbonzeni	L, S	Anemia – general fatigue; chronic diarrhea; respiratory tract infections; abcess	Ac 074/UM
		Bridelia ferruginea Benth.	Givala	B	Stomac ache	Bf 208/UM
		Euphorbia hirta L.	Ambèningo	L; S	Skin rashes	Eh 210/UM
		Phyllanthus diandrus Pax	Bumbaha	Wp;	Diarrhea; vomiting	Pd 053/UM
		Plagiostyles africana (Müll. Arg.) Prain	Moukodo	B	Venereal diseases	Pa 084/UM
		Ricinodendron heudelotii (Bail.) Heckel	Ndjoé	B, L	Stomach ache	Rh 108/UM
		Tetrorchidium didymostemon (Baill.) Pax & K. Hoffm.	Niodjè	B, L	Skin rashes; venereal diseases	Td 066/UM
10	Flacourtiaceae	*Oncoba glauca* (P. Beauv.) Planch.	Ghévaghavagha	L	General tiredness	Og 092/UM
11	Gramineae	*Setaria megaphylla* (Steud) T.Durand & Schinz.	Mbongo motoutou	Wp	Venereal diseases	Sm 098/UM
12	Guttiferaceae	*Harungana madagascariensis* Lam. ex Poir.	Tonukwé	B, L	Hepatitis	Hm 030/UM
13	Humiriaceae	*Saccoglotys gabonensis* (Baill.) Urb.	O-suga	B, L	Venereal diseases	Sg 127/UM
14	Irvingiaceae	*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.	Mpetche	B; fr	Respiratory tract infections	Ig 035/UM
15	Lauraceae	*Persea americana* Mill.	Mvoku	B, L	Venereal diseases	Pa 143/UM
16	Lecythidaceae	*Petersianthus macrocarpus* (P. Beauv.) Liben	Mbizo	B	Respiratory tract infections	Pm 051/UM
17	Leguminoseae – Cœsalpinoideae	*Cassia alata* L. (*Senna alata* (L.) Roxb,)	Novonga	L, S	Skin rashes; stomach ache; venereal diseases	Ca 148/UM
18	Liliaceae	*Chlorophytum laxum* R.Br.	Murina-syèsi	B, L	Anemia – general fatigue	Cv 150/UM
		Smilax anceps Willd.	Mokuenguendje	L	Stomach ache	Sa 225/UM
19	Loganiaceae	*Anthocleista schweinfurthii* Gilg	Ororo	R	Venereal diseases	As 226/UM
		Anthocleista vogelii Planch.	Orowo–orowo	L, R	Venereal diseases	Av 131/UM
20	Marattiaceae	*Marattia fraxinea* Sm.	Izendji ya kola	Wp	Skin rashes	Mf 039/UM
21	Melastomataceae	*Dissotis decumbens* (P. Beauv.) Triana	Mioghu mine	L, S	Stomach ache	Dd 139/UM
		Tristemma mauritianum J.F. Gmel.	Koka	L	Respiratory tract infections	Tm 228/UM
22	Meliaceae	*Carapa procera* DC.	Katchimboma	B, L	Respiratory tract infections	Cp 009/UM
23	Moraceae	*Musanga cecropioides* R.Br. ex Tedlie	Moghombo	B, L	Tuberculosis	Mc 076/UM
24	Myristicaceae	*Coelocaryon klainei* Pierre ex Heckel	mabégni	B, L, R	Venereal diseases	Ck 117/UM
		Pycnanthus angolensis (Welw.) Warb.	Ilomba	L	Respiratory tract infections	Pa 081/UM
		Scyphocephalium ochocoa Warb.	Otsoko	B; L	Abscess; anemia – general fatigue; respiratory tract infections; tuberculosis	So 083/UM
25	Olacaceae	*Coula edulis* Baill.	Ngomba	B; fr	Respiratory tract infections	Ce 016/UM
26	Piperaceae	*Piper umbellatum* L.	Yembembe	L	Skin rashes	Pu 054/UM
		Peperomia pellucida L. Kunth	Djokèlo	Wp	Stomach ache	Pp 112/UM
27	Polygalaceae	*Carpolobia alba* G. Don	Kudu	L	Venereal diseases	Ca 179/UM
28	Portulacaceae	*Portulaca oleracea* L.	O-tsamba	L; S	Diarrhea	Po 235/UM
29	Rubiaceae	*Psychotria gilletii* De Wild.	Gè-botabota	B	Venereal diseases	Pg 238/UM
		Sarcocephalus latifolius (Sm.) E.A. Bruce	gébowaï	B; R	Diarrhea; stomach ache	Sl 240/UM
30	Simaroubaceae	*Quassia Africana* Baill. (Baill.)	Issindou ighal	L	Stomach ache; venereal diseases	Qa 242/UM
31	Sterculiaceae	*Cola nitida* (Vent.) Schott & Endl.	Ibendou/Cola	B; fr	Respiratory tract infections; typhoid fever	Cn 165/UM
32	Verbenaceae	*Lippia multiflora* Moldenke	Afi	B, L	Respiratory tract infections	Lm 244/UM
33	Vochysiaceae	*Erismadelphus exsul* Mildbr.	Izendji dja kola	B, L	Skin rashes	Ee 026/UM
34	Zingiberaceae	*Costus afer* Ker Grawl.	Moandu	S	Respiratory tract infections; tuberculosis	Ca 015/UM

B=barks; fr=fruits; L=leaves; R=roots; Tub=tuber; Wp=whole plant.

well of the second line. Ten two-fold serial dilutions of the test samples were made from line 1 until line 11and 100 µL of inoculum standardized at 2.5×10^3cells/ml was introduced in the entire well containing the test substances except the columns of blank which constitute the sterility control. The concentration range was 4 mg/ml to 0.0039 mg/ml for crude extracts and reference antifungals (Fluconazole and Nystatin) used as positive control. After an incubation period at 37 °C for 48 h, turbidity was observed as indication of

growth. Thus the lowest concentration inhibiting the visible growth of yeast was considered as the Minimum Inhibitory Concentration (MIC). All the testing was done in duplicate.

3. Results and discussion

During this study, a total 31 people were interviewed, with an age average of 48.5 years old, among whom, 22 were men and 9 women (percentages of 71% and 29% respectively). Most respondents were farmers whose education levels were essentially that of the primary. Nevertheless, they were able to recognize symptoms of HIV/AIDS opportunistic diseases and related conditions we described to them. In this study, we registered a set of 188 citations for a total of 52 plants belonging to 50 genera and 34 families (Table 1). The Burseraceae, the Euphorbiaceae and the Myristicaceae families were the most represented, constituting about 25% of the listed species, including seven species of the Euphorbiaceae family and 3 of the Burseraceae and the Myristicaceae families each (Table 1). The Euphorbiaceae family is among the most important families recorded in most ethnobotanical studies (Kisangau et al., 2007; Moshi et al., 2010; Asiimwe et al., 2013; Razafindraibe et al., 2013; Lamorde et al., 2014; Mugisha et al. 2014). In Uganda, Lamorde et al. (2014) found that plant species from families Asteraceae, Euphorbiaceae and Mimosaceae were used by traditional healers to manage HIV/AIDS opportunistic infections. The plants frequently used by traditional medicine practitioners were *Aloe sp*. (7), *Erythrina abyssinica* DC (5), *Sarcocephalus latifolius* (Sm) E.A. Bruce (5), *Psorospermum febrifugum* Spach.(5), *Mangifera indica* L. (4) and *Warburgia salutaris* (Bertol.f.) Chiov. (4). *Aloe sp*. was used in the treatment of fever and hepatitis; *Erythrina abyssinica* was used for cough; *Sarcocephalus latifolius* was used for diarrhea and sexually transmitted diseases. In this study, *Sarcocephalus latifolius* (Sm) E.A. Bruce was used for diarrhea and stomach ache. The frequency of mention could be an indication of the therapeutic value of a species (Kamatenesi et al., 2011).

The disease treated by the greatest number of plants were respiratory tract infections with 15 listed species, followed by venereal diseases (14 plants), stomachache (12 plants), skin rashes (9 plants), diarrhea (5 plants), anemia and general tiredness (4 plants), tuberculosis (3 plants), abscess (2 plants), hepatitis (1 plant), typhoid fever (1 plant) and vomiting (1 plant). The predominance of remedies for bronchopulmonary and gastrointestinal troubles agrees with the data from other regions (Kisangau et al., 2007; Kamatenesi et al., 2011; Namukobe et al., 2011; Asiimwe et al., 2013; Razafindraibe et al., 2013; Mugisha et al. 2014). These kinds of ailments along with those linked to daily rural activity, constitute a very high percentage of health problems (Bonet et al., 1999).

41 of the 52 registered plants were each used in the treatment of just one of the 11 diseases identified in our study, 8 were used in the treatment of two different diseases, one was used in the treatment of three diseases and only two plants, namely *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg. (Euphorbiaceae) and *Scyphocephalium ochochoa* Warb. (Myristicaceae) were each used in the treatment of 4 diseases. This unlikelihood could be due to the imperfect transfer of traditional medicine knowledge in the studied areas (suburban areas), and the fact that among the diseases treated by a particular species, only those mentioned in our studies were considered. The ability of some symptomatic conditions in this study to be linked to several diseases is also to be considered, since diarrhea could be associated with Bacterial enteric infections, cytomegalovirus disease, isosporiasis, salmonellosis etc. (CDC, 2013). Most of the remedies were prepared using a single plant or occasionally mixture of plants, for example, *Scyphocephalium ochochoa* with *Petersianthus macrocarpus* (P. Beauv.) Liben (Lecythidaceae) for respiratory tract infections. Several recipes were thus documented.

The methods of drug administration was by oral administration (69%) mainly in cases of respiratory tract infections, tuberculosis, venereal diseases, stomach ache, anemia and typhoid fever, by external application (28%), in cases of skin rashes and in some cases of venereal diseases, and by anal route (03%), in some cases of tuberculosis, vomiting, hepatitis, stomach ache and even venereal diseases (Fig. 2).

The parts of the plants most used for medicinal purposes were, in decreasing order, leaves (36%), barks (32%), fruits (11%), whole plant (07%), stems or trunks (07%) and roots or tuber (07%). Several methods of preparation of herbal medicines were documented, including: chewing (32%), stewed (29%), maceration (25%) and decoction (14%) (Fig. 3). A constancy also appeared in other studies for leaves as the plant part mostly used for medicinal purposes (Kisangau et al., 2007; Moshi et al., 2010; Asiimwe et al., 2013; Razafindraibe et al., 2013; Lamorde et al., 2014; Mugisha et al., 2014).

Some species were notable for the number of people who cited them, for the number of uses which they have or for both aspects. *Alchornea cordifolia* was cited by 10 informants, who attributed to its, four uses involving the leaves and the stem – bark. Other species, like *Dacryodes klaineana* and *Scyphocephalium ochochoa*, were also cited in both areas by a large number of informants for several uses. This finally resulted in high values of the calculated indices for certain species. At the end of our screening, we did a list of the most interesting of them with a reliability degree relatively high for the diseases they were said to treat and frequently cited by populations. These species included: *Acanthus montanus* for respiratory tract infections and stomach ache; *Alchornea cordifolia* for abscess, anemia-general tiredness, diarrhea and respiratory tract infections; *Cassia alata* for skin rashes and stomach ache; *Coelocaryon klainei* for venereal diseases; *Cola nitida* for respiratory tract infections and typhoid fever; *Dacryodes*

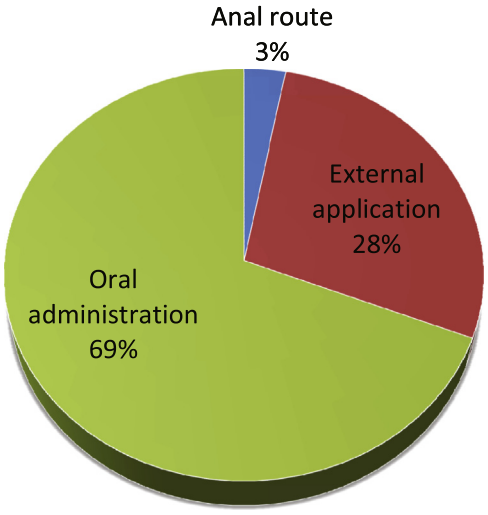

Fig. 2. Percentage forms of drug administration.

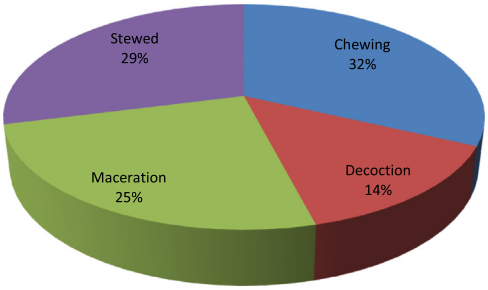

Fig. 3. Percentage forms of herbal preparations.

klaineana for skin rashes; *Phyllanthus diandrus* for diarrhea and vomiting; *Saccoglotys gabonensis* for venereal diseases; *Sarcocephalus latifolius* for diarrhea and stomachache; *Scyphocephalium ochocoa* for abscess, anemia-general tiredness, respiratory tract infections and tuberculosis; and *Tetrorchidium didymostemon* for skin rashes and venereal diseases.

Knowledge on the efficacy and safety of some priority plants derived mainly from a few *in vitro* studies. Results revealed some concordance with their medicinal uses. The seeds aqueous extract of *Alchornea cordifolia* showed high antiviral indice against HIV-2 (Ebi, 2001; Ayisi Nana and Nyadedzor, 2003; Agbor et al., 2004; Igbeneghu et al., 2007). *Acanthus montanus* extracts showed antimicrobial, anti-inflammatory and immunological properties (Okoli et al., 2008); *Cassia alata* aqueous extract exhibited antimicrobial activities against many pathogenic strains, especialy *Dermatophilus congolensis* (Ali-Emmanuel et al., 2002; Idu et al., 2007). *Cola nitida* presented good antimicrobial activities, the seeds extract of this species associated with ciprofloxacin, pefloxacin and levofloxacin, resulting in the decrease of the Minimum Inhibitory Concentration (MIC) of these antibiotics against a clinical isolate of *Escherichia coli* (Ibezim et al., 2006). *Sarcocephalus latifolius* and *Scyphocephalium ochochoa* appeared respectively as antimalarial and antimicrobial plants (Abreu and Pereira, 2001; Hu et al., 2005). Many chemicals isolated from these species exhibited multiple interesting biological activities, including analgesic, anti-inflammatory, antimicrobial and cytotoxic properties on cancer cells (Hu et al., 2005; Mavar-Manga et al., 2006, 2008; Villasenor and Sanchez, 2009).

Tables 2–4 show respectively the phytochemical screening and the antimicrobal results through Inhibition Diameters and Minimum Inhibitory Concentrations (MIC) of *Coelocaryon klainei*, *Dacryodes klaineana*, *Phyllanthus diandrus*, *Saccoglotys gabonensis* and *Tetrorchidium didymostemon*. These species, among the most relevant have not yet undergone, to the best of our knowledge, any pharmacological study. Their barks and leaves aqueous alcohol extracts were evaluated against some clinical isolates and ATCC strains involved in HIV/AIDS opportunistic diseases. MIC values varied from 0.25 to >4 mg/ml. Analysis of the antimicrobial efficacy of the plant extracts was based on the following criteria: strong inhibitors – MIC up to 0.5 mg/ml; moderate inhibitors – MIC between 0.6 and 1.5 mg/ml and weak inhibitors – MIC above 1.6 mg/ml (Aligiannis et al., 2001).

Dacryodes klaineana stem bark and leaves extracts, *Phyllanthus diandrus* stem bark and leaves extracts, *Tetrorchidium didymostemon* stem bark and leaves extracts, and *Saccoglotys gabonensis* stem bark and leaves extracts appeared as strong inhibitors against one or more tested microorganisms (Table 4). The less sensitive strains were *Candida albicans* (*ATCC P37037*), for which only the leaves extracts of *Coelocaryon klainei* showed a very weak inhibition (MIC $>$ 4 mg/ml), and *Candida albicans* (*ATCC L26*), for which none inhibition was observed with any plant part extract. *Pseudomonas aeruginosa* and *Streptococcus B* appeared as the most sensitive microorganisms, respectively being strongly inhibited by the greatest number of extracts (3 extracts) for the first strain, and having showed sensitivity to the greatest number of extracts (8 extracts), for the second strain (Table 4). *Phyllanthus diandrus* bark and leaves show best antibacterial activities against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with MIC value of 0.25 respectively (Table 4). These interesting activities on Gram-negative bacteria confirmed the relevance of this species, since the hydrophilic surface of the outer membrane of this bacterial species, rich in lipopolysaccharide molecules, is presenting a barrier to the penetration of antibacterial substances and is also associated with enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (Rosas-Piñón et al., 2012). The barks of *Tetrorchidium didymostemon* also showed very good activity against *Streptococcus B,* resistant to two tested standard antibiotics (Ceftriazon and Gentamicin). These activities were above those obtained from other plants used in respiratory infections (Madikizela et al. 2014). Along our studies, all the species tested showed some activities (strong, moderate or weak) against strains of which their uses in traditional medicine were not oriented, implied that these plants may possess more therapeutic properties than traditionally recognized. This fact is consistent with the statement of Bonet et al. (1999) that some plants and their active principles can be detected through folk use and then be used much more widely in general medical practice than they are in popular phytotherapy.

Selected plants contained biomolecules potentially active against pathogenic strains, including alkaloids, coumarins, flavonoids, polyphenols, saponins, sugars, sterols, tannins and triterpenes (Table 2). Flavonoids are known to have antimicrobial, anti-inflammatory, anticancer and antiviral activities (Havsteen, 2002). Reports about antibacterial activities of flavonoids through inhibiting enzymes involved in biosynthesis of mycolic and fatty acid have been made (Kuete et al., 2010). Their presence along with that of other potentially active biomolecules justifies antimicrobial activities of the tested species. Nevertheless, the isolation and identification of pure molecules through chromatographic and spectroscopic methods would be necessary in the case of the study of the structure-activity relationships. Good antimicrobial activity is sometimes associated with toxicity (Madikizela et al., 2014). Hence, the real therapeutic interest of the tested species could not be declared before being submitted to other biological evaluation including toxicity analysis.

Moreover, the fact that the age average of respondents was above 48 years and that most of the respondents were farmers whose level of education was essentially that of the primary, implies that the future of the use of traditional medicine in the management of HIV/AIDS and its opportunistic diseases is very hopeless if a considerable effort is not made in the appropriation and standardization of these therapies.

4. Conclusion

It would be very interesting that more enhanced phytochemical and pharmacological studies been conducted on some of these

Table 2
Phytochemical screening of the species that have not yet undergone any scientific study.

Plants	Chemical constituents							
Aqueous extracts	Polyphenols	Tannins	Flavonoids	Sterols/Triterpenes	Coumarins	Saponosides	Alkaloids	Sugars
Coelocaryon klainei (B)	+++	+++	+++ (flavonone)	+++ (Sterol)	+++	++	–	+++
Coelocaryon klainei (L)	++	++	+++ (flavonone)	–	+++	+++	–	–
Dacryodes klaineana (B)	+++	++	+++ (flavone)	–	–	–	+++	–
Dacryodes klaineana (L)	+++	+++	+++ (flavone)	–	–	–	+++	–
Tetrorchidium didymostemon (B)	+++	++	+++ (flavonone)	+++ (Sterol)	–	–	+++	–
Tetrorchidium didymostemon (L)	+++	++	++ (flavonone)	+++ (Triterp)	–	–	+++	–
Saccoglotys gabonensis (B)	+++	+++	++ (flavone)	–	–	+++	+++	+++
Saccoglotys gabonensis (L)	+++	+++	++ (flavonone)	–	–	++	+++	+
Phyllanthus diandrus (B)	+++	+++	+++ (flavonone)	++ (Sterol)	–	+	++	++
Phyllanthus diandrus (L)	+++	+++	+++ (flavonone)	++ (Triterp)	+++	–	++	+++

B: barks; L: leaves; Triterp=triterpenes. +++ very intense, ++ intense, +weak, and – absent.

Table 3
Antimicrobial activities of the selected plants (results obtained as inhibition diameters for extract concentration of 10 mg/ml).

	Inhibition diameters (mm)										
	Microbial strains	***Shigella flexneri***	***Pseudomonas aeruginosa***	***Salmonella typhimurium***	***Streptococcus B***	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***	***Candida albicans* (*ATCC P37037*)**	***Candida albicans* (*ATCC P37039*)**	***Candida albicans* (*ATCC L26*)**	***Candida albicans* (*ATCC 12C*)**
Extracts[a] (10 mg/ml)	***Coelocaryon klainei* (B)**	–	–	12	12	–	–	–	–	–	–	–
	***Coelocaryon klainei* (L)**	–	–	13	–	–	–	–	8	22	–	–
	***Dacryodes klaineana* (B)**	16	16	–	14	15	–	–	–	20	–	–
	***Dacryodes klaineana* (L)**	9	12	–	13	15.5	13	–	–	–	–	–
	***Phyllanthus diandrus* (B)**	–	15	–	14	14	14	–	–	–	–	15
	***Phyllanthus diandrus* (L)**	11	–	–	15	11	–	15	–	–	–	–
	***Tetrorchidium didymostemon* (B)**	–	–	–	11	10	8	12	–	–	–	–
	***Tetrorchidium didymostemon* (L)**	–	9	9	18	12	11	16	–	8	–	–
	***Saccoglotys gabonensis* (B)**	12	15	–	13	14	–	–	–	–	–	–
	***Saccoglotys gabonensis* (L)**	11	14	–	12	14	–	–	–	22	–	9

[a] B: barks; L: leaves.

Table 4
Minimum Inhibitory Concentrations of the selected plants and reference antibiotics.

	Minimum Inhibitory Concentrations (mg/ml)										
	Microbial strains	***Shigella flexneri***	***Pseudomonas aeruginosa***	***Salmonella typhimurium***	***Streptococcus B***	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***	***Candida albicans* (*ATCC P37037*)**	***Candida albicans* (*ATCC P37039*)**	***Candida albicans* (*ATCC L26*)**	***Candida albicans* (*ATCC 12C*)**
ATM	**Ceftriazon**[a]	< 0.125	< 0.125	< 0.125	0.5	< 0.125	< 0.125	< 0.125				
	Gentamicin[a]	< 0.125	< 0.125	< 0.125	0.5	< 0.125	< 0.125	< 0.125				
	Rifampicin[a]	< 0.125	< 0.125	< 0.125	< 0.125	< 0.125	< 0.125	< 0.125				
	Fluconazol[b]								< 0.125	< 0.125	< 0.125	< 0.125
	Nystatin[b]								< 0.125	< 0.125	< 0.125	< 0.125
Extracts[c] **(10mg/ml)**	***Coelocaryon klainei* (B)**	–	–	0.75	2.25	–	–	–	–	–	–	–
	***Coelocaryon klainei* (L)**	–	–	1	–	–	–	–	> 4	> 4	–	–
	***Dacryodes klaineana* (B)**	> 4	1.5	–	0.5	> 4	–	–	–	> 4	–	–
	***Dacryodes klaineana* (L)**	1	0.75	–	1.5	1	0.5	–	–	–	–	–
	***Phyllanthus diandrus* (B)**	–	0.25	–	0.75	1.25	0.75	–	–	–	–	> 4
	***Phyllanthus diandrus* (L)**	2.5	–	–	0.75	3	–	0.25	–	–	–	–
	***Tetrorchidium didymostemon* (B)**	–	–	–	0.25	4	> 4	1.5	–	–	–	–
	***Tetrorchidium didymostemon* (L)**	–	1.25	4	> 4	1.5	0.5	2.25	–	> 4	–	–
	***Saccoglotys gabonensis* (B)**	0.75	0.5	–	1	2.5	–	–	–	–	–	–
	***Saccoglotys gabonensis* (L)**	0.75	0.5	–	4	0.5	–	–	–	> 4	–	> 4

ATM = reference antibiotics.

[a] For bacteria.

[b] For yeast.

[c] B: barks; L: leaves.

plants, since all the extracts tested exhibited antimicrobial activities against many pathogenic strains. It is also interesting to do some microbiological tests involving combinations of extracts from the species tested, for example, those of *Dacryodes klaineana* and *Tetrorchidium didymostemon* which were used together in some medicinal recipes. Further studies will permit, not only to isolate active chemicals from these plants, but also help in the development of improved traditional medicines, inexpensive, more effective and more accessible to the local population for the management of HIV/AIDS and its opportunistic diseases.

Authors' contributions

GRFT was involved in the conception, acquisition, analysis and interpretation of data, drafting and final revision of the manuscript. AS was involved in data analysis and the revision of the manuscript. JFY, MB and RMM were involved in the antibacterial evaluation of the extracts. FFM and EZM were involved in the antifungal evaluation of the extracts. HF and GDNO were involved in the phytochemical screening. DTN, JL and JCT were involved in the literature search of data on selected species and the critical revision of the manuscript for important intellectual content. All authors read and approved the manuscript.

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