Extension of the EU *"Traditional Herbal Medicine"* concept to an oral transmission context: the traditional uses of the five anti-infectious medicinal plants most widely used in Burundi

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Research

Abstract

Background: In Burundi, five plants, namely *Urtica massaica* Mildbr., *Mikania natalensis* DC., *Senecio maranguensis* O. Hoffm., *Justicia nyassana* Lindau and *Helichrysum congolanum* Schltr. & O. Hoffm., are widely cited for the treatment of infectious diseases. The present work aims to compare the local knowledge and uses of these five popular anti-infectious plants.

Methods: A survey was carried out among 43 traditional healers from different regions of Burundi to fully repertory the uses of these five medicinal plants and define consensus in their uses through their fidelity levels and use values for each cited disease. Phytochemical analyses of these plants allowed to identify their main classes of secondary metabolites.

Results: For the five investigated plants, the leaves and aerial parts are the organs mainly used. Preparation methods include maceration, decoction and squeezing of juice, powdering and calcination. From their fidelity levels and use values, the studied plants appear extensively reported for infectious diseases, except for *U. massaica*, that is mainly used in inflammatory conditions. The phytochemical classes we detected in these plants [terpenoids, (poly)phenolic compounds including flavonoids and tannins, saponins] may explain their uses and warrant further studies.

Conclusions: The survey indicates that the five plants studied are widely used in the treatment of infectious diseases. The convergence of some usages indicates a plausibility of efficacy and safety, coherent with the EU concept of "*Traditional Herbal Medicine*", which points to possible rational recommendations of treatments. A study of the antimicrobial activities of these plants would complete this work.

Keywords: Medicinal plants, traditional medicine, infectious diseases, phytochemistry, Burundi.

Background

Traditional medicines (TMs) are notably widespread in sub-Saharan Africa (Kahumba *et al.* 2015) where they coexist with conventional ("modern") medicine (Falisse *et al.* 2018) and are sometimes considered as an attractive alternative or complementary source of care. However, for many patients, geographical and/or economic reasons often make TMs the only medicines accessible (Mutombo *et al.* 2022) and plants are then a primary source of healthcare (Dias, Urban, and Roessner 2012; Hussain *et al.* 2024; Manoharachary and Nagaraju 2017).

In Burundi, as in many African countries, the knowledge associated with medicinal plants is mostly held by traditional practitioners (phytotherapists, herbalists, spiritualists, bonesetters, …), who are often reluctant to share information outside of their family, thus commonly perpetuating their know-how through intergenerational transmission, most often father-to-son (OBPE 2016). The transmission of traditional knowledge is then mainly vertical (i.e. parental), although it can sometimes be horizontal (peer learning) (Castiñeira Latorre, Canavero, and Arim 2020; Santoro *et al.* 2018; Soldati *et al.* 2015). Nowadays however, young people are often unaware of or unconcerned about natural resources, which represents a definite risk of knowledge loss (Rahman *et al.* 2016; Tamang *et al.* 2023).

Given the major importance of TMs for the Burundese population, and in line with WHO recommendations (WHO 2013), an obvious need appears to improve the regulation of practices, practitioners, and products, and to develop an integrative medicine which would combine both conventional and alternative therapies. This ideally would require knowing more about the herbal products used, and objectively evaluating the effectiveness of treatments through clinical case studies; but, due to financial and ethic constraints, this is often not possible. And so, to initiate and progress in the regulation of TMs, surrogate methods are needed to develop a portfolio of products reasonably safe and plausibly effective. In the European Union (EU), the concept of "*Traditional Herbal Medicine*" has been developed to allow a simplified marketing authorization for traditional herbal medicinal products ; this registration under a drug status is based on a defined medical purpose, on the quality and non-genotoxicity of herbs, and on their market seniority, i.e. the proven presence, non-problematic, of the considered herbal drug on both a EU and non-EU market, for at least 15 years and 30 years, respectively (Peschel 2007). Such a concept of "*Traditional Herbal Medicine*" is a pragmatic solution for the regulation of phytomedicines which, in the context of a future integrative medicine, deserves to be considered for developing countries. But, obviously, the extension of this regulatory concept to an oral transmission context requires formal documentation of what can be considered as "*traditional use*", which should be achieved by reconciliating and condensing information obtained from chemical studies and ethnopharmacological enquiries.

Although quite limited information is available on the Burundian ethnomedical knowledge, previous studies inventoried a series of medicinal plants and the ailments they are believed to treat: *(i)* an ethnobotanical survey, conducted between 1979 and 1984 among 129 human and veterinary healers, resulted in the collection of 5696 plants from 499 species (107 families), used in 2729 recipes to treat, prevent or exorcise 136 symptoms (Baerts & Lehmann 1993); *(ii)* a 1995 survey identified 750 medicinal species, from 109 families and 364 genera, treating some 250 diseases or symptoms, including intestinal parasitosis, diarrhea, childhood diseases, gynecological problems, and dermatoses; the most represented families were the Asteraceae (85 species), the Fabaceae (65 species), the Euphorbiaceae (46 species) and the Lamiaceae (40 species) (Bigendako & Bukuru 1995); and *(iii)* another survey, conducted in 2012-2013, inventoried in the city of Bujumbura the most frequent infectious diseases treated by traditional medicine; these included diarrheal, skin, and respiratory diseases that were treated by 155 species grouped in 139 genera and 51 families, dominated by the Asteraceae, Fabaceae, Lamiaceae, Rubiaceae, Solanaceae and Euphorbiaceae (Ngezahayo *et al.* 2015). Through this last survey, five plants, the Urticaceae *Urtica massaica* Mildbr., the Asteraceae *Mikania natalensis* DC., *Senecio maranguensis* O. Hoffm. and *Helichrysum congolanum* Schltr. & O. Hoffm., and the Acanthaceae *Justicia nyassana* Lindau, were widely cited for the treatment of "diseases compatible with a microbial infection" i.e. probably infectious diseases (Ngezahayo *et al.* 2015).

These 5 plants are present in other African countries, including Rwanda, the Central African Republic, Sudan, Ethiopia, the Democratic Republic of Congo, Uganda, Kenya and Tanzania. Regarding their traditional uses, *(i) M. natalensis* is reported to be used in South African Zulu, Sotho and Xhosas traditional medicines (Hutchings and van Staden 1994; Mhlongo and Van Wyk 2019); *(ii) U. massaica* in Rwanda (Nahayo *et al.* 2008) and Kenya (Kamau *et al.* 2016; Keter and Mutiso 2012; Njoroge and Bussmann 2006); and *(iii) H. congolanum* in the Democratic Republic of Congo (Latham and Ku Mbuta 2014). Apart from a few papers on *U. massaica* (Nahayo *et al.* 2008) and limited data on *M. natalensis* (Koorbanally *et al.* 2004), these plants have not so far been the subject of biological or phytochemical studies.

As the clinical efficacy and safety of these highly used anti-infective medicinal plants have not been assessed so far, the present work aims to investigate a method to evaluate the plausibility of their safe use, through a survey comparing the recommendations and eventual interdicts of tradipraticians from different regions of Burundi, including the city of Bujumbura.

Materials and Methods

Survey on the indications claimed by Burundian tradipraticians for the selected antimicrobial plants

To repertory the likely antimicrobial indications of the five plants in Burundian traditional medicine, a survey was carried out among traditional healers from different regions of Burundi. The traditional healers met in the markets were selected because they are grouped into associations approved by the "Ministry of Public Health and the Fight against AIDS", the other ones were indicated to us either as being esteemed locally (case of the Bugenyuzi commune) or by a responsible member of a traditional healers' association (case of the Buta region). Each traditional healer was met at his place of work and answered our pre-established questionnaire with the freedom to give their name or not. The survey questionnaire was subdivided into three main parts, centered on the identification of the traditional healer (sex, marital status, age, level of study, places of work and residence, etc.), on their profession (seniority and education in the profession, generalist or specialist, types of diseases treated, etc.) and on the use of each of the investigated five plants (parts used, diseases treated, method of preparation and administration, prescribed dose, whether it is used in combination or not, duration of treatment, possible prohibitions during treatment, side effects, treatment failures, etc.). In this study, the principles of the declaration of Helsinki (World Medical Association 2014) were followed.

Collection and identification of plant samples

Samples of the 5 plants were collected with the support of a traditional healer who assisted in identification and collection. For each plant, a reference specimen was collected in duplicate, and was prepared following standard ethnobotanical procedures. One specimen per plant was deposited in the Herbarium of the Department of Biology of the Faculty of Sciences at the University of Burundi (UB) and registered under the numbers NJA001, NJA002, NJA003, NJA004 and NJA005 for *J. nyassana*, *U. massaica*, *M. natalensis*, *S. maranguensis* and *H. congolanum*, respectively. The family, genus and species of each of the five plants were determined by *(i)* comparing with existing herbarium specimens; and *(ii)* referencing to specialized databases such as the African Plant Database "https://africanplantdatabase.ch/" and WFO Plant List "https://wfoplantlist.org/plant-list". The plants that are combined with the five plants to form different recipes were cited in the national language (Kirundi), and translated to scientific names with the help of the botanists of the Department of Biology of the Faculty of Sciences at UB.

Graphical representation and statistical analysis of data

The relationship between recipes and plants was graphed as an interaction network using Cytoscape 3.9.1 (https://cytoscape.org/), with the layout organic (Mukazayire *et al.* 2011, Ngezahayo *et al.* 2015, Shannon *et al.* 2003). To determine the significance of differences between groups, a Chi-square analysis was performed using the IBM SPSS Statistics 20 software.

Informant consensus

Based on the work of (Hoffman & Gallaher 2007, Phillips & Gentry 1993), we assessed the degree of consensus of the informants (interviewed traditional practitioners) regarding the uses of the five plants in traditional Burundian medicine by calculating two indices of importance in ethnobotany, namely the level of fidelity and the use values for each plant.

Fidelity level (FL)

The fidelity level or fidelity index, which is expressed as a percentage, calculates a ratio between the number of interlocutors who cite the use of a plant for the same major purpose and the total number of interlocutors who mentioned any use of the plant (Asiimwe *et al.* 2021, Friedman *et al.* 1986, Hoffman & Gallaher 2007, Phillips & Gentry 1993). A high FL value indicates

the frequency with which the plant is used to treat a particular disease in the study area (Asiimwe *et al.* 2021). It is calculated according to Equation 1.

Equation 1: $FL = I_p/I_u \times 100$

where I_p is the number of interlocutors who reported using a given plant for a given use and I_u is the total number of interlocutors who cited the plant for any use.

Use values

The use value for each plant and disease category was calculated in two stages, the use value of a plant for an interlocutor (Equation 2) and then the use value of the same plant for all interlocutors (Equation 3) (Hoffman & Gallaher 2007, Phillips & Gentry 1993).

Equation 2: $UV_{is} = \Sigma U_{is}/N_{is}$

where UV_{is} = use value of a plant s for informants i; U_{is} = number of uses mentioned for a plant s by interlocutor i and N_{is} = number of "events" in which interlocutor i cites a use for plant s. In our case, N_{is} (number of events) = 1.

Equation 3: $UV_s = \Sigma_i UV_{is}/N_i$

where UV_s = use value of a plant s for all interlocutors; N_i = total number of interlocutors interviewed for plant s. The use values of the interlocutors for a species s are added together and divided by the total number of interlocutors.

Phytochemical screening

Plant material

Two of the five plants, namely *J. nyassana* and *H. congolanum*, were harvested on February 7, 2019 on the Biyorwa hill of Butaganzwa Commune in Ruyigi Province, at 3°27’29.40’’ S, 30°05’30.42’’ E (altitude 1508 m), and 3°27’29.46’’ S, 30°05’30.30’’ E (altitude 1508 m), respectively. A third plant, *M. natalensis*, was harvested on February 7, 2019 on the Kizigama hill of Butaganzwa Commune in Ruyigi Province at 3°28’05.22’’ S, 30°09’16.08’’ E (altitude 1569 m). *U. massaica* and *S. maranguensis* were harvested on February 23, 2019 on the Zingi-Nyaruyaga hill of the Bugarama Commune in Rumonge Province, at 3°42’55.86’’ S, 29°27’55.86’’ E (altitude 2033 m) and 3°42’58.38’’ S, 29°27’36.78’’ E (altitude 1941 m), respectively (Figure 1). After harvesting, the samples were dried in open air, protected from direct sunlight, in the "Centre de Recherche Universitaire en Pharmacopée et Médecine Traditionnelle (CRUPHAMET)" of the Faculty of Sciences at UB, crushed in a mortar and sieved through a 1 mm sieves to obtain a fine powder.

Extraction

The solvents used for extraction were n-heptane (99+%, for analysis, Acros organics), dichloromethane (stabilized by 0.2% ethanol, for analysis, VWR), ethyl acetate (for analysis, VWR), methanol (for analysis; Merck) and water. In a separating funnel lined with a wadding buffer, 150 g of each powder were macerated for 48 h in 1.5 L of the extraction solvent and percolated out until exhaustion, as shown by thin-layer chromatography (TLC) derivatized with a vanillin-sulfuric acid reagent. Based on previous works (Bekro *et al.* 2007, Ngezahayo *et al.* 2017), successive extractions were performed with five solvents of increasing polarities (*n*-heptane, dichloromethane, ethyl acetate, methanol and water). The 20 organic extracts were obtained after evaporation of the solvents using a rotary evaporator set at 40°C while freeze-drying was applied to obtain the five dry aqueous extracts.

Procedure for phytochemical screening

Based on published procedures, a series of analytical reactions (Adou *et al.* 2016, Bekro *et al.* 2007, N’guessan *et al.* 2011, N’Guessan *et al.* 2009) and high performance thin-layer chromatographies (Merck 1975, Wagner & Bladt 1996) specific to the class of compounds of pharmacological interest were systematically carried out on the different extracts for the identification of alkaloids (Dragendorff’s reagent) (N’Guessan *et al.* 2009), flavonoids (magnesium-HCl reduction) (Adou *et al.* 2016; Bekro *et al.* 2007; N’Guessan *et al.* 2009), terpenoids (Liebermann-Burchard reagent) (Bekro *et al.* 2007 ; N’Guessan *et al.* 2009), tannins and (poly)phenolic compounds ($FeCl_3$ complexation) (Adou *et al.* 2016; Bekro *et al.* 2007), saponins (foaming test) (N’Guessan *et al.* 2009).

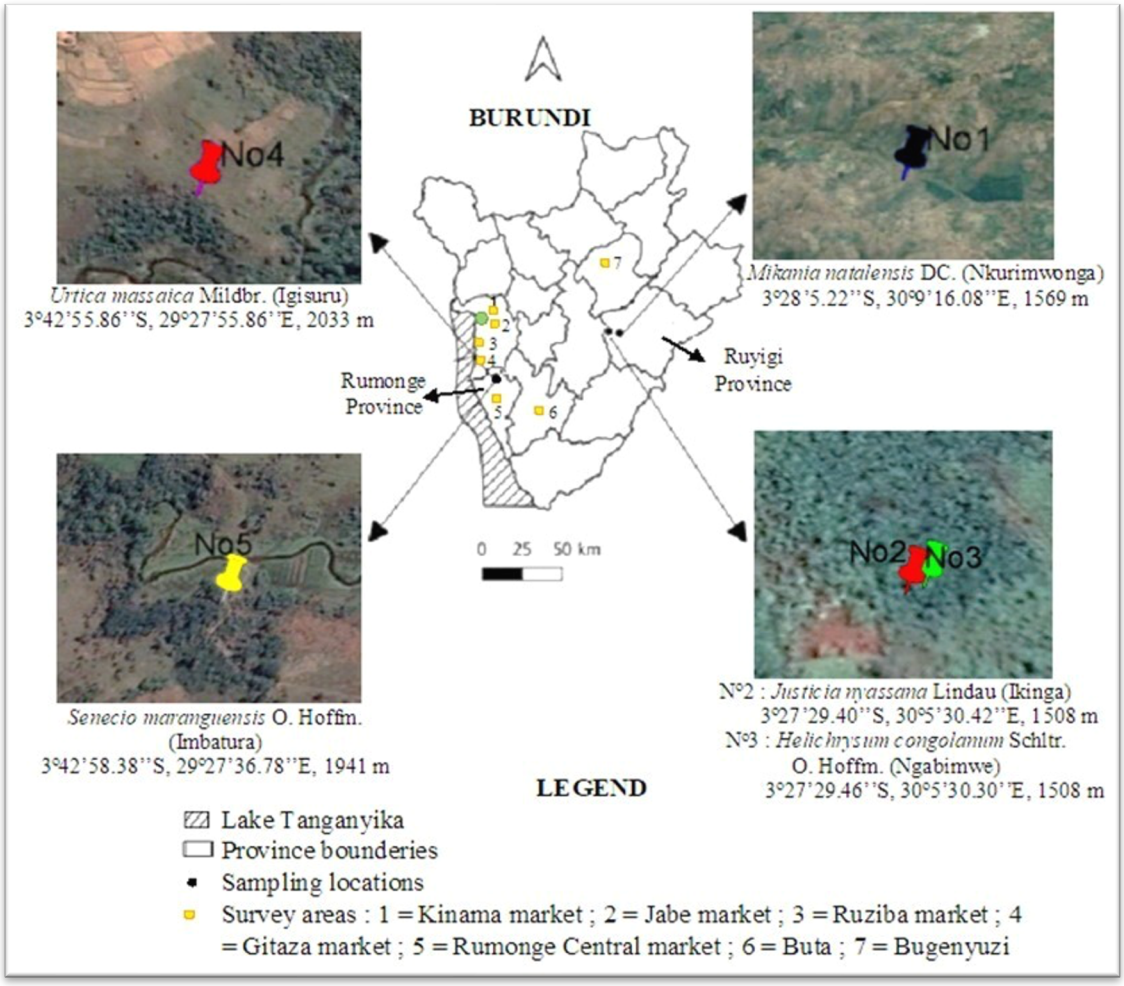

Figure 1. Locations of sample collection and ethnopharmacological survey areas.

Results and discussion

Profile of interviewed tradipraticians

In total, 43 traditional healers, 26 men and 17 women, were interviewed (Table 1). This gender imbalance may be due to a tradition of father-to-son transmission, as observed in many Burundian professions (Manirakiza 2020). This is confirmed by the results of our survey according to which 91% of the traditional healers interviewed inherited this profession from their parents. Twenty-eight of them work in the markets of the city of Bujumbura (Jabe, Kinama and Ruziba Markets), nine in the markets of the Rumonge Province (Rumonge Central and Gitaza Markets), four were met in the Bugenyuzi Commune of the Karusi Province (Centre of the country) and two in the Buta region of the Bururi Province in the South of Burundi (Figure 1 & Table 1). Thirty-eight of these tradipraticians are grouped into associations recognized by the Ministry of Public Health and AIDS Control and 5 are independent. The age of the traditional practitioners interviewed ranged from 20 to 79 years, with a peak between 40 and 49 years. This aligns with prior research indicating that the traditional knowledge and cultural practices related to the utilization of medicinal plants for healthcare predominantly reside among older community members, while younger individuals frequently exhibit disinterest (Hussain *et al.*, 2024). Most of traditional practitioners interviewed either have not pursued education or have attended only primary school; traditional knowledge, being mostly inherited from ancestors (Table 1), does not require higher formal education, which may pose problem in communication with modern practitioners when pursuing the development of an integrative medicine.

Table 1. Categories of interviewed tradipraticians

Distribution of respondents		Men	Women	Total
Distribution of respondents by age group	20-29	0	2	2
(n = 43)	30-39	6	3	9
	40-49	5	7	12
	50-59	5	3	8

	60-69	5	0	5
	70-79	5	2	7
Distribution of respondents according to whether or not they are grouped into associations (n = 43)	Atraprabu [(a)]	13	7	20
	Aguebu[(a)]	7	7	14
	Aguetrabu[(a)]	3	1	4
	Independant	3	2	5
Distribution of respondents by survey area (n = 43)	Jabe Market	12	6	18
	Kinama Market	3	6	9
	Rumonge Central Market	4	2	6
	Gitaza Market	2	1	3
	Ruziba Market	1	0	1
	Bugenyuzi-Karusi	3	1	4
	Buta	1	1	2
Distribution of respondents by level of education (n = 43)	University	1	1	2
	Secondary	0	2	2
	Primary	13	4	17
	Yaga Mukama[(b)]	1	1	2
	Didn't study	11	9	20

Legend:

(a) Atraprabu, Aguebu and Aguetrabu are the 3 major local associations of tradipraticians: ATRAPRABU = Association des Tradi-praticiens du Burundi; AGUEBU = Association des Guérisseurs du Burundi; AGUETRABU = Association des Guériseurs Traditionels du Burundi.

(b) Yaga Mukama corresponds to a parish school where children learned to read and write and where they learned catechism lessons to prepare them for the various sacraments.

Indications for the selected five plants

Of the 43 traditional healers interviewed, 40 claim to use *J. nyassana*, 34 *U. massaica*, 38 *M. natalensis*, 36 *S. maranguensis* and 34 *H. congolanum*. As shown in Table 2, the leaves and aerial parts are the main parts used and the preparation methods include maceration, decoction, squeezing of juice, drying and powder reduction or calcination. Modes of administration include the oral route for macerations, decoctions and juice, the enema for macerations and decoctions, the oral or dermal routes (coating with powder/ash mixed with butter or scarification) for powder or ash. Except for *J. nyassana*, these plants are used in combination with other medicinal plants; such combinations may suggest a probable synergy of action or an eventual need to counter side effects. In total, 35 different diseases are treated by the 5 plants: (i) dermatoses by 4 plants; (ii) skin rashes by 3 plants; (iii) diarrhea, anemia, pustules and varicella by 2 plants; and (iv) all other cited diseases by one plant. *U. massaica* is the plant that treats the most diseases (12 diseases including dermatoses, diarrhea, diabetes, anemia) followed by *H. congolanum* (11 diseases including ringworm, skin rashes, pustules, dermatoses), *M. natalensis* (10 diseases including skin rashes, diarrhea, pustules, varicella, hemorrhoids), *J. nyassana* (9 diseases including dysentery, measles, diarrhea, cholera) and *S. maranguensis* (7 diseases including dermatoses, scabies, skin rashes, pustules). Our survey indicates that *M. natalensis* is used in 15 recipes including 2 monorecipes, *S. maranguensis* in 14 recipes including 3 monorecipes, *H. congolanum* in 18 recipes including 5 monorecipes, *U. massaica* in 17 recipes including 4 monorecipes. For *J. nyassana*, it is not combined and was cited in 9 monorecipes (Figure 2). Chi-square analyses (https://www.spss-tutorials.com/) indicate no significant difference between men and women in the use of all these plants ($p > 0.05$): *U. massaica* [χ^2 (1, N=43)=3.505, p=0.061]; *J. nyassana* [χ^2 (2, N=43)=1.596, p=0.450]; *M. natalensis* [χ^2 (1, N=43)=0.112, p=0.738]; *S. maranguensis* [χ^2 (1, N=43)=1.084, p=0.2985] and *H. congolanum* [χ^2 (2, N=43)=1.052, p=0.591]. Similarly, for the 5 plants, the Chi-square analyses show no significant difference between traditional practitioners in Bujumbura city and those in other parts of Burundi in the use of *U. massaica* [χ^2 (1, N=43) =3.318, p=0.069], *J. nyassana* [χ^2 (2, N=43) =1.736, p=0.420], *M. natalensis* [χ^2 (1, N=43) =0.045, p=0.832], *S. maranguensis* [χ^2 (1, N=43) =1.881, p=0.170] and *H. congolanum* [χ^2 (2, N=43) =1.786, p=0.409].

This Chi-square analysis shows that the five plants studied are used by women and men, whether in the city of Bujumbura or in other regions of Burundi, in the treatment of infectious diseases such as rashes, pustules, dermatoses, diarrhea, dysentery, etc. but also other conditions such as diabetes, rheumatism, high blood pressure, etc. The results of our survey are consistent with previous work. These include treatment of diarrhea, measles and dysentery by *J. nyassana* (Ngezahayo *et al.* 2015), treatment of ringworm (Ngezahayo *et al.* 2015, Polygenis-Bigendako 1990), skin rashes (Ngezahayo *et al.* 2015) and dermatoses (Polygenis-Bigendako 1990) by *S. maranguensis*, treatment of dermatoses (Polygenis-Bigendako 1990), ringworm (Ngezahayo *et al.* 2015) and skin infections (Latham & ku Mbuta 2014) by *H. congolanum*, treatment of cough with *M. natalensis* (Hutchings & van Staden 1994, Mhlongo & Van Wyk 2019), treatment of wounds with *S. maranguensis* (El-

Kamali 2009) as well as treatment of diabetes (Kamau *et al.* 2016, Keter & Mutiso 2012) and diarrhea (Gahamanyi *et al.* 2021; Munyaneza & Bigendako 2008) with *U. massaica*.

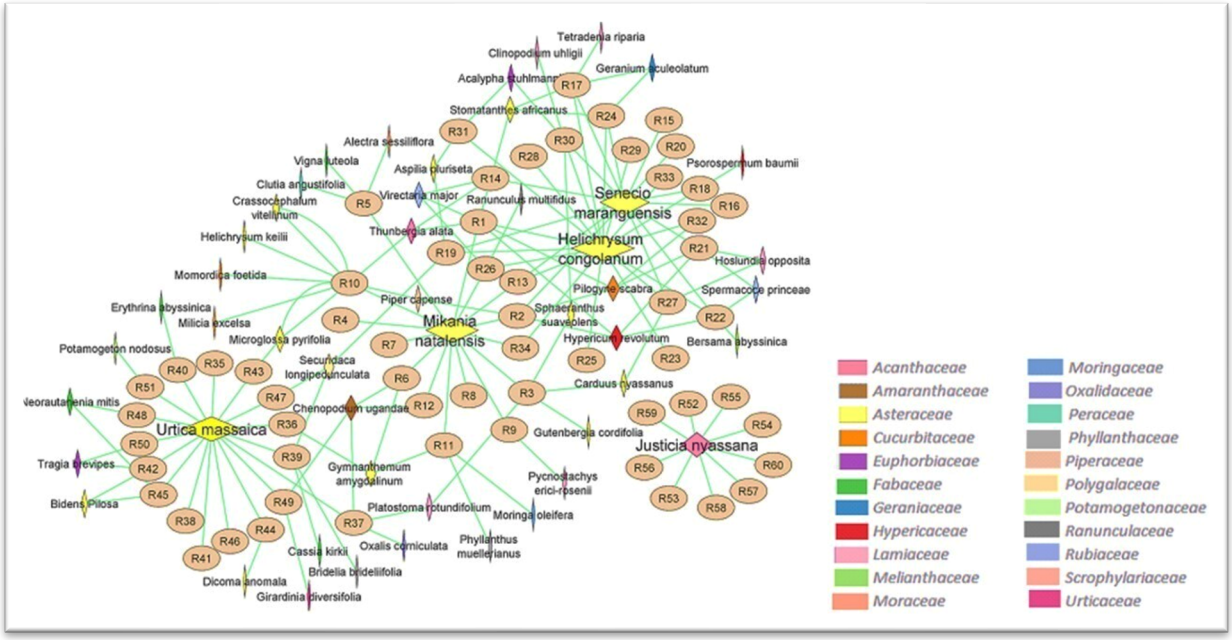

Figure 2. Relationship between recipes and medicinal plants. Recipes are represented by circles, medicinal plants by diamonds. The size of the diamond is proportional to the frequency of citation of a medicinal plant. The colors of the diamonds correspond to the botanical family of the plant (Mukazayire *et al.* 2011, Ngezahayo *et al.* 2015, Shannon *et al.* 2003).

Fidelity level and use values

In total, 35 diseases were cited as being treated by the 5 plants studied. The Fidelity level varies from 2.50% to 97.22% (Table 3). The highest FL value (97.22%) is observed for *S. maranguensis* for the treatment of pustules. Based on the values found for FL, *M. natalensis* is most plausible for the treatment of hernia (FL = 83.78%), skin rashes (FL = 81.08%) and pustules (FL = 62.16%); *S. maranguensis* is much more plausible for the treatment of pustules (FL = 97.22%), dermatoses (FL = 94.44%) and skin rashes (FL = 91.67%); *H. congolanum* for the treatment of skin rashes (FL = 88.24%), ringworm (FL = 76.47%) and dermatoses (FL = 73.53%); *U. massaica* is more plausible for the treatment of rheumatism (FL = 73.53%), colopathy (FL = 64.71%) and diabetes (FL = 52.94%) and *J. nyassana* is much more plausible for the treatment of dysentery (FL = 90.00%) and cholera (FL = 72.50%). Previous work confirms the use of *S. maranguensis* in the treatment of dermatoses (Polygenis-Bigendako 1990), and rashes (Ngezahayo *et al.* 2015), the treatment of dermatoses with *H. congolanum* (Polygenis-Bigendako 1990) as well as the treatment of dysentery with *J. nyassana* (Ngezahayo *et al.* 2015).

The 35 diseases reported by the traditional healers were divided into 13 categories. The category of skin diseases came out on top with 45.78% of mentions, followed by the category of gastrointestinal infections (15.77%), diseases of the digestive tract (8.99%), inflammations (7.47%) and diseases of the circulatory system (7.05%). The other categories have less than 5% of mentions. All 5 plants are involved in the first category while in other categories only 1, 2, 3 or 4 plants out of 5 are involved. These high citations for the first two categories of diseases indicate that, in Burundian traditional medicine, these plants are much more used in the treatment of infectious diseases (Table 4).

Table 2. Indications of the investigated plants in Burundian traditional medicine

Plant name (Familly, voucher specimen number)	Parts used	Diseases treated	Combinations with	Mode of preparation	Mode of administration
***Mikania natalensis* (Asteraceae, NJA003)**	Aerial parts (leaves and stems)	Skin rashes	*Helichrysum congolanum* Schltr. & O. Hoffm, *Hypericum revolutum* Vahl, *Pilogyne scabra* (L. f.) W.J. de Wilde Duyfjes, *Stomatanthes africanus* (Oliv. & Hiern) R.M. King & H. Rob., *Thunbergia alata* Bojer ex Sims, *Virectaria major* (K.Schum.) Verdc.	Maceration	Dermal route (washing the body)
			Pilogyne scabra (L. f.) W.J. de Wilde Duyfjes	Decoction	Enema
			Piper capense L. f., *Senecio maranguensis* O.Hoffm.		Oral route
		Pustules	*Carduus nyassanus* (S. Moore) R.E. Fr., *Gutenbergia cordifolia* Benth. Ex Oliv., *Platostoma rotundifolium* (Briq.) A.J. Paton, *Sphaeranthus suaveolens* (Forssk.) DC.	Decoction/maceration	Oral route
		Hemorrhoids	Single plant (no combination)	Decoction	Enema
		Varicella	*Alectra sessiliflora* (Vahl) Kuntze, *Clutia angustifolia* Knauf, *Vigna luteola* (Jacq.) Benth.	Maceration	Oral route
		Diarrhea	*Chenopodium ugandae* (Aellen) Aellen, *Gymnanthemum amygdalinum* (Delile) Sch. Bip. ex Walp.	Maceration	Oral route
		Colopathy	Single plant (no combination)	Decoction	Enema
		Constipation	Single plant (no combination)	Decoction	Enema
		Cough	*Pycnostachys erici-rosenii* R.E.Fr.	Maceration	Oral route
		Hernia	*Milicia excelsa* (Welw.) C.C.Berg	Decoction	Enema
			Momordica foetida Schumach.	Calcination	Oral route
			Crassocephalum vitellinum (Benth.) S. Moore, *Microglossa pyrifolia* (Lam.) Kuntze, *Piper capense* L. f.	Decoction	Enema
			Microglossa pyrifolia (Lam.) Kuntze	Decoction	Oral route
			Crassocephalum vitellinum (Benth.) S. Moore, *Helichrysum keilii* Moeser, *Thunbergia alata* Bojer ex Sims	Decoction	Enema

Plant name (Familly, voucher specimen number)	Parts used	Diseases treated	Combinations with	Mode of preparation	Mode of administration
		Back pain	*Gymnanthemum amygdalinum* (Delile) Sch. Bip. ex Walp., *Moringa oleifera* Lam.	Squeeze the juice	Oral route
			Chenopodium ugandae (Aellen) Aellen, *Phyllanthus muellerianus* (Kuntze) Exell	Decoction	Enema
***Senecio maranguensis* (Asteraceae, NJA004)**	Leaves	Dermatoses	Single plant (no combination)	Powder	Dermal route (coating the body)
			Helichrysum congolanum Schltr. & O. Hoffm, *Mikania natalensis* DC., *Thunbergia alata* Bojer ex Sims, *Virectaria major* (K. Schum.) Verdc.	Decoction/maceration	Oral route
		Skin rashes	Single plant (no combination)	Powder	Dermal route (coating the body)
		Pustules	*Geranium aculeolatum* Oliv., *Helichrysum congolanum* Schltr. & O. Hoffm., *Stomatanthes africanus* (Oliv. & Hiern) R.M. King & H.Rob., *Tetradenia riparia* (Hochst.) Codd	Calcination	Oral route
		Scabies	Single plant (no combination)	Powder	Dermal route (coating the body)
			Aspilia pluriseta Schweinf. Ex Engl., *Helichrysum congolanum* Schltr. & O. Hoffm., *Psorospermum baumii* Engl., *Securidaca longepedunculata* Fresen.	Maceration/decoction	Oral route
		Wounds	Single plant (no combination)	Squeeze the juice	Dermal route
		Hair loss	*Hoslundia opposita* Vahl, *Hypericum revolutum* Vahl, *Pilogyne scabra* (L. f.) W.J. de Wilde Duyfjes, *Spermacoce princeae* (K. Schum.) Verdc.	Maceration/decoction	Oral route
		Anorexia	*Bersama abyssinica* Fresen., *Helichrysum congolanum* Schltr. & O. Hoffm	Calcination	Oral route
			Hypericum revolutum Vahl, *Spermacoce princeae* (K. Schum.) Verdc.	Maceration	Oral route
***Helichrysum congolanum* (Asteraceae, NJA005)**	Leaves	Ringworm	Single plant (no combination)	Calcination	Oral route
			Geranium aculeolatum Oliv., *Senecio maranguensis O. Hoffm.*, *Stomatanthes africanus* (Oliv. & Hiern) R.M. King & H.Rob.	Decoction	Enema
		Fever	Single plant (no combination)	Maceration	Oral route

Plant name (Familly, voucher specimen number)	Parts used	Diseases treated	Combinations with	Mode of preparation	Mode of administration
		Skin rashes	*Ranunculus multifidus* Forssk.	Powder/ash	Dermal route (coating the body)
			Mikania natalensis DC., *Virectaria major* (K. Schum.) Verdc.	Decoction	Enema
		Pustules	*Carduus nyassanus* (S. Moore) R.E.Fr., *Hoslundia opposita* Vahl, *Senecio maranguensis O. Hoffm.*	Calcination	Oral route
		Varicella	Single plant (no combination)	Decoction	Oral route
		Lack of growth in children	Single plant (no combination)	Calcination	Oral route
		Dermatoses	*Acalypha polymorpha* Müll. Arg., *Clinopodium uhligii* (Gürke) Ryding, *Senecio maranguensis O. Hoffm.*, *Sphaeranthus suaveolens* (Forssk.) DC.	Decoction	Enema
		Urinary tract infection	*Acalypha polymorpha* Müll. Arg., *Aspilia pluriseta* Schweinf. Ex Engl.	Maceration	Oral route
		Dietary supplement	*Pilogyne scabra* (L. f.) W.J. de Wilde Duyfjes	Powder	Oral route
		Kwashiorkor	Single plant (no combination)	Powder	Oral route
		Psychosomatic disorders	*Mikania natalensis* DC.	Decoction	Steam bath
***Urtica massaica* (Urticaceae, NJA002)**	Aerial parts (leaves and stems)	Diabetes	Single plant (no combination)	Powder	Oral route
			Gymnanthemum amygdalinum (Delile) Sch. Bip. ex Walp.	Decoction	Oral route
			Oxalis corniculata L.	Maceration	Oral route
			Chenopodium ugandae (Aellen) Aellen, *Platostoma rotundifolium* (Briq.) A.J. Paton	Squeeze the juice	Oral route
		Hernia	Single plant (no combination)	Decoction/maceration	Oral route
			Bridelia brideliifolia (Pax) Fedde, *Cassia kirkii* Oliv., *Securidaca longepedunculata* Fresen.	Decoction	Oral route
		Dermatoses	*Erythrina abyssinica* Lam. Ex DC.	Powder	Oral route

Plant name (Familly, voucher specimen number)	Parts used	Diseases treated	Combinations with	Mode of preparation	Mode of administration
		Rheumatism	Clay	Maceration	Oral route
			Bidens Pilosa L., *Tragia brevipes* Pax	Decoction	Dermal route (the remedy is pressed to the joints)
		Anemia	*Microglossa pyrifolia* (Lam.) Kuntze	Squeeze the juice	Oral route
		Neuropathy	*Dicoma anomala* Sond.	Crushing	Dermal route (the remedy is pressed where the pain is felt)
		Diarrhea	*Bidens Pilosa* L., *Neorautanenia mitis* (A.Rich.) Verdc.	Decoction/maceration	Oral route
		Amoeba	Single plant (no combination)	Maceration	Oral route
		Constipation	*Securidaca longepedunculata* Fresen.	Decoction	Oral route
		Colopathy	*Neorautanenia mitis* (A.Rich.) Verdc.	Decoction	Oral route
		Venous thrombosis	*Chenopodium ugandae* (Aellen) Aellen, *Girardinia diversifolia* (Link) Friis	Decoction	Dermal route (the remedy is pressed on the body)
		Male impotence	*Tragia brevipes* Pax	Calcination	Dermal route
			Potamogeton nodosus Poir.	Decoction	Oral route
***Justicia nyassana* (Acanthaceae, JNA001)**	Leaves	Measles	Single plant (no combination)	As vegetable	Oral route
				Squeeze the juice	Oral route
				Decoction	Enema
		Diarrhea	Single plant (no combination)	Maceration	Oral route
				As vegetable	Oral route
				Squeeze the juice	Oral route
		Dysentery	Single plant (no combination)	Decoction	Enema
				Maceration	Oral route

Plant name (Familly, voucher specimen number)	Parts used	Diseases treated	Combinations with	Mode of preparation	Mode of administration
				As vegetable	Oral route
		Hepatitis	Single plant (no combination)	Maceration	Oral route
		Dermatoses	Single plant (no combination)	As vegetable	Oral route
				Squeeze the juice	Oral route
				Decoction	Oral route
					Steam bath
					Enema
		High blood pressure	Single plant (no combination)	As vegetable	Oral route
		Meningitis	Single plant (no combination)	Decoction	Steam bath
		Cholera	Single plant (no combination)	Decoction	Enema
				Maceration	Oral route
				As vegetable	Oral route
		Anemia	Single plant (no combination)	As vegetable	Oral route
				Decoction	Oral route
				Squeeze the juice	Oral route

Table 3. Fidelity levels (FL) for each plant/disease combination

Treated diseases	Number of citations (I_p)	FL (Ip/Iu x 100)
M. natalensis **(I_u = 37)**		
Skin rashes	30	81.1
Pustules	23	62.2
Hemorrhoids	5	13.5
Varicella	18	48.7
Diarrhea	21	56.8
Colopathy	2	5.4
Constipation	15	40.5
Cough	2	5.4
Hernia	31	83. 8
Back pain	5	13.5
S. maranguensis **(I_U = 36)**		
Dermatoses	34	94.4
Skin rashes	33	91.7
Pustules	35	97.2
Scabies	10	27.8
Wounds	17	47.2
Hair loss	3	8.3
Anorexia	3	8.3
H. congolanum **(I_u = 34)**		
Ringworm	26	76.5
Fever	4	11.8
Skin rashes	30	88.2
Pustules	18	52.9
Varicella	8	23.5
Lack of growth in children	4	11.8
Dermatoses	25	73.5
Urinary tract infection	6	17.7
Dietary supplement	21	61.8
Kwashiorkor	2	5.9
Psychosomatic disorders	3	8.8
U. massaica **(I_u = 34)**		
Diabetes	18	52.9
Hernia	17	50.0
Dermatoses	6	17.7
Rheumatism	25	73.5
Anemia	18	52.9
Neuropathy	13	38.2
Diarrhea	4	11.8
Amoebiasis	3	8.8
Constipation	2	5.9
Colopathy	22	64.7
Venous thrombosis	11	32.4
Male impotence	6	17.7
J. nyassana **(I_u = 40)**		
Measles	20	50.0
Diarrhea	14	35.0
Dysentery	36	90.0
Hepatitis	7	17.5
Dermatoses	15	37.5
High blood pressure	9	22.5
Meningitis	1	2.5
Cholera	29	72.5
Anemia	13	32.5

Table 4. Diseases belonging to each category with the number of plant citations

Categories of diseases	Pathologies	Citations
Skin diseases [331 [(a)], 5 [(b)]]	Dermatoses	80
	Pustules	76
	Ringworm	26
	Skin rashes	93
	Varicella	26
	Scabies	10
	Measles	20
Gastro-intestinal infections [114[(a)], 2[(b)]]	Amoebiasis	3
	Cholera	29
	Diarrhea	39
	Hepatitis	7
	Dysentery	36
Digestive tract disorders [65[(a)], 2[(b)]]	Constipation	17
	Hernia	48
Vascular problems [5[(a)], 1[(b)]]	Hemorrhoids	5
Circulatory system disorders [51[(a)], 2[(b)]]	Anemia	31
	Venous thrombosis	11
	High blood pressure	9
Inflammations [54[(a)], 2[(b)]]	Back pain	5
	Colopathy	24
	Rheumatism	25
Metabolic disorders [18[(a)], 1[(b)]]	Diabetes	18
Genito-urinary tract disorders [12[(a)], 2[(b)]]	Male impotence	6
	Urinary tract infection	6
Nutritional disorders [33[(a)], 2[(b)]]	Dietary supplement	21
	Kwashiorkor	2
	Anorexia	3
	Lack of growth in children	4
	Hair loss	3
Respiratory tract disorders [2[(a)], 1[(b)]]	Cough	2
Wounds and injuries [17[(a)], 1[(b)]]	Wounds	17
Central nervous system disorders [17[(a)], 3[(b)]]	Psychosomatic disorders	3
	Neuropathy	13
	Meningitis	1
Body's reaction to an infection [4[(a)], 1[(b)]]	Fever	4

Legend: (a): Sum of citations for the category of disease; **(b)**: Number of plants among the five studied that are used in the treatment of diseases in the category.

Overall, the use values were 3.56, 3.35, 3.35, 3.14 and 3.42 for *M. natalensis*, *U. massaica*, *J. nyassana*, *S. maranguensis* and *H. congolanum* respectively. If we look at the disease categories, which can be called usage categories, we note that *M. natalensis* has the highest use values in the categories of skin diseases (1.65), digestive tract disorders (1.07) and gastrointestinal infections (0.51). For *U. massaica*, the highest use values are recorded in the categories of inflammations (1.07), digestive tract disorders (0.51) and metabolic disorders (0.42). *J. nyassana* shows high use values for gastrointestinal infections (2.00), skin diseases (0.81) and circulatory system disorders (0.51). *S. maranguensis* shows the highest use value (2.60) in only one disease category, that of skin diseases, while *H. congolanum* has a high use value for the skin diseases category (2.49) followed by that of nutritional disorders (0.63). In general, the plants studied, with the exception of *U. massaica* which has a high use value in the inflammations category, have the highest use values in the skin diseases category, 2 plants (*J. nyassana* and *M. natalensis*) have the highest use values in the gastrointestinal infections category and 2 plants (*M. natalensis* and *U. massaica*) have the highest use values in digestive tract disorders (Table 5). These results, combined with the results on the fidelity level, show that the five plants studied are used much more extensively against infectious diseases, with less emphasis on *U. massaica*, which is used much more extensively against inflammatory diseases. As fidelity levels measure the frequency of use of a given plant to treat a particular disease in a study area (Asiimwe *et al.* 2021), the high frequencies of use we recorded for these 5 plants indicates their importance in the treatment of infectious diseases in Burundi.

Table 5. Use values by disease category for all 5 plants

Plant	Use values (UVs)													
	Total	Skin diseases	Gastro-intestinal infections	Digestive tract disorders	Vascular problems	Circulatory system disorders	Inflammation	Metabolic disorders	Genito-urinary tract disorders	Nutritional disorders	Respiratory tract disorders	Wounds and injuries	Central nervous system disorders	Body's reaction to an infection
Mikania natalensis	3.56	1.65	0.51	1.07	0.12	0.00	0.16	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Urtica massaica	3.34	0.14	0.16	0.51	0.00	0.60	1.07	0.42	0.14	0.00	0.00	0.00	0.30	0.00
Justicia nyassana	3.34	0.81	2.00	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Senecio maranguensis	3.14	2.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.40	0.00	0.00
Helichrysum congolanum	3.42	2.49	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.63	0.00	0.00	0.07	0.09

Phytochemical screening of extracts from the selected five plants

As indicated in Table 6, all the hexane, dichloromethane and ethyl acetate extracts contain sterols and terpenes, except for the ethyl acetate extract of *J. nyassana* (JN/Ac). Tannins and/or (poly)phenolic compounds are detected in aqueous extracts of *U. massaica* (UM/Aq), *J. nyassana* (JN/Aq) and *M. natalensis* (MN/Aq), in all five methanolic extracts and in all five ethyl acetate extracts. The methanolic extracts JN/Me, SM/Me, MN/Me and HC/Me as well as the ethyl acetate extracts JN/Ac, MN/Ac and HC/Ac contain flavonoids that are absent in *U. massaica*. Saponins are found in all aqueous extracts except SM/Aq and in two methanolic extracts JN/Me and HC/Me. Alkaloids are absent in all extracts of all five plants. It is important to note that HPTLC is a more efficient method than analytical reactions (in tubes) in phytochemical screening. In fact, (poly)phenolic compounds were not detected by tube reactions or analytical reactions, but they were detected using HPTLC (Figures 3 and 4).

Table 6: Major phytoconstituents detected in the 5 plants studied.

Plant	Extract	Abbreviation	Chemical group				
			Sterols and terpenes	Tannins and/or (poly)phenolic compounds	Flavonoids	Saponins	Alkaloids
Urtica massaica	Hexane	UM/He	+	-	-	-	-
	Dichloromethane	UM/Di	+	-	-	-	-
	Ethylacetate	UM/Ac	+	+	-	-	-
	Methanol	UM/Me	-	+	-	-	-
	Aqueous	UM/Aq	-	+	-	+	-
Justicia nyassana	Hexane	JN/He	+	-	-	-	-
	Dichloromethane	JN/Di	+	-	-	-	-
	Ethylacetate	JN/Ac	-	+	+	-	-
	Methanol	JN/Me	-	+	+	+	-
	Aqueous	JN/Aq	-	+	-	+	-
Senecio	Hexane	SM/He	+	-	-	-	-
maranguensis	Dichloromethane	SM/Di	+	-	-	-	-
	Ethylacetate	SM/Ac	+	+	-	-	-
	Methanol	SM/Me	-	+	+	-	-
	Aqueous	SM/Aq	-	-	-	-	-
Mikania natalensis	Hexane	MN/He	+	-	-	-	-
	Dichloromethane	MN/Di	+	-	-	-	-
	Ethylacetate	MN/Ac	+	+	+	-	-
	Methanol	MN/Me	-	+	+	-	-
	Aqueous	MN/Aq	-	+	-	+	-
Helichrysum	Hexane	HC/He	+	-	-	-	-
congolanum	Dichloromethane	HC/Di	+	-	-	-	-
	Ethylacetate	HC/Ac	+	+	+	-	-
	Methanol	HC/Me	-	+	+	+	-
	Aqueous	HC/Aq	-	-	-	+	-

Legend: HC/Ac, JN/Ac, **MN/Ac**, **SM/Ac**, **UM/Ac**: ethyl acetate extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*. **HC/Aq**, **JN/Aq**, **MN/Aq**, **SM/Aq**, **UM/Aq**: aquous extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*. **HC/Di**, **JN/Di**, **MN/Di**, **SM/Di**, **UM/Di**: dichloromethane extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*. **HC/He**, **JN/He**, **MN/He**, **SM/He**, **UM/He**: hexanic extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*.

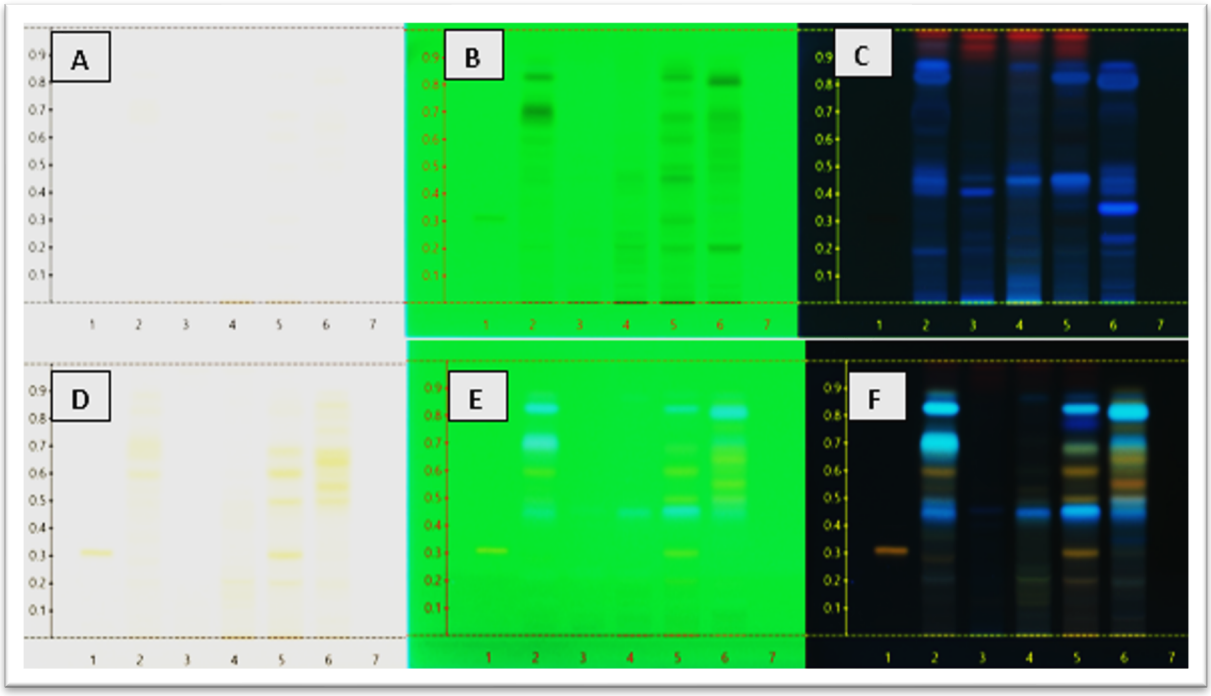

Figure 3. HPTLC chromatograms of the methanolic extracts of *M. natalensis* (track 2), *U. massaica* (track 3), *J. nyassana* (track 4), *H. congolanum* (track 5) and *S. maranguensis* (track 6). Tracks 1 and 7 correspond to rutin and quercetin respectively. Amount applied on the plate: 10 µg (10 µL, 1 mg/mL). Mobile phase: THF-Toluene-Formic Acid-Water (59 :30 :7 :4 v/v/v/v). Visualization of the spots under visible light (A), UV 254 nm (B), UV 366 nm (C) before derivatization. Derivatized with NP/PEG reagent and visualized under visible light (D), UV 254 nm (E), UV 366 nm (F).

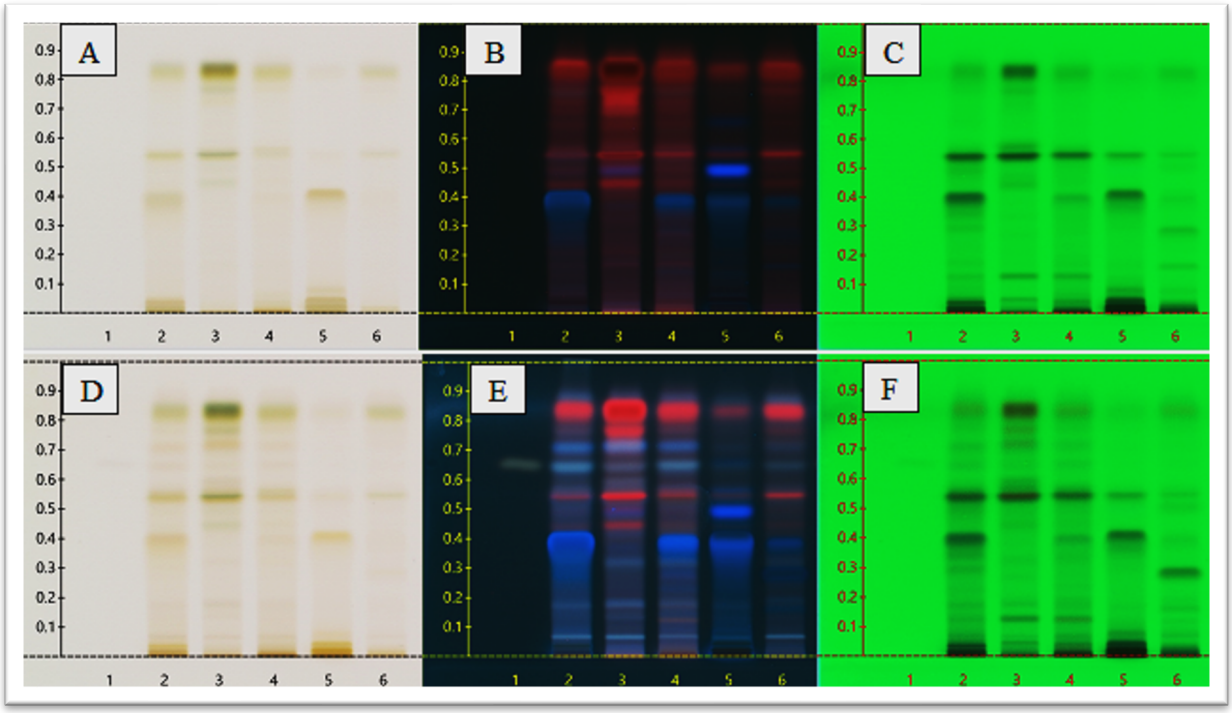

Figure 4. HPTLC chromatograms of the ethyl acetate extracts of *M. natalensis* (track 2), *U. massaica* (track 3), *J. nyassana* (track 4), *H. congolanum* (track 5) and *S. maranguensis* (track 6). Track 1 corresponds to ursolic acid. Amount applied on the plate: 10 µg (10 µL, 1 mg/mL). Mobile phase: Chloroform-Ethyl acetate-Acetic acid (12:10:1 v/v/v). Visualization of the spots under visible light (A), UV 366 nm (B), UV 254 nm (C) before derivatization. Derivatized with Liebermann-Burchard reagent and visualized under visible light (D), UV 366 nm (E), UV 254 nm (F).

The presence of sterols, terpenoids and flavonoids in *J. nyassana* is coherent with previous studies on other *Justicia* species (Corrêa & de Alcântara 2012). The presence of tannins and the absence of alkaloids in *U. massaica* agrees with the results of a previous work (Nahayo *et al.* 2008) although other authors indicate the presence of alkaloids in this plant (Oloro *et al.* 2016). Our results also agree with those of the latter author regarding the non-detection of flavonoids in *U. massaica*. Alkaloids were not detected in any of the investigated plants; nevertheless, as pyrrolizidine alkaloids, common in *Senecio genus*, are genotoxic, a toxicity that is manifest at very low levels (Zhou *et al.* 2013), their presence and levels should be evaluated for this *Senecio maranguensis* species that appears widely used in Burundi.

Conclusion

The five plants studied are used, whether in the Bujumbura city or in other parts of Burundi, in the treatment of infectious diseases such as skin rashes, pustules, dermatoses, diarrhea, dysentery, infections, etc., but also in other conditions such as diabetes, rheumatism, high blood pressure, etc. Mostly, these plants are used in combination with other plants, which suggests a possible synergy of action in the treatment of various infectious diseases. All the traditional practitioners interviewed confirmed that treated patients were "cured". Nevertheless, six of them stated that treatment failures can be observed, but rarely and especially when the patient has not respected the prescriptions. These plants contain various secondary metabolites that may justify their therapeutic effects, which remains to be confirmed. A study of their biological activities, in particular the antimicrobial activities, and of their combination with other plants would complete this work. Interviews are a quite interesting survey method to apprehend usages of drugs, but the information on efficacy, side effects and interdicts appears particularly difficult to obtain; and so, the efficacy and safety of remedies are difficult to assess from such data. Although the marked convergence of some usages indicates a plausibility of efficacy, coherent with the EU concept of "*Traditional Herbal Medicine*", there is a need to develop a rational strategy to legally define whether a given use could be considered as "*traditional*".

Declarations

List of abrevations: ADC2: automatic developing chamber 2; ATS: Automatic TLC Sampler; CRUPHAMET: centre de recherche universitaire en pharmacopée et médecine traditionnelle; FL: fidelity level; HPTLC: high performance thin-layer chromatography; HC/Ac, JN/Ac, MN/Ac, SM/Ac, UM/Ac: ethyl acetate extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*. HC/Aq, JN/Aq, MN/Aq, SM/Aq, UM/Aq : aquous extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*. HC/Di, JN/Di, MN/Di, SM/Di, UM/Di: dichloromethane extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*. HC/He, JN/He, MN/He, SM/He, UM/He: hexanic extracts of *H. congolanum*, *J. nyassana*, *M. natalensis*, *S. maranguensis* and *U. massaica*; NP/PEG: natural product/polyethylene glycol; TMs: traditional medicines; TLC, thin-layer chromatography; UB: University of Burundi; UVs: use values; WFO: world flora online.

Ethics approval: All participants provided oral prior informed consent.

Consent for publication: not applicable

Availability of data and materials: All the data are presented in the manuscript.

Competing interests: The authors declare that they have no competing interests

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Autors' contributions: Japhet Nzoyisubiziki: prepared the study, conducted field surveys, obtained and analyzed the survey data, wrote the article ; Jérémie Ngezahayo : supervised the study and revised the article ; Alexis Ngendahimana: conducted field surveys ; Amandine Nachtergael : supervised the study and revised the article ; Vestine Ntakarutimana : supervised the study and revised the article ; Mohamed Tabyaoui: supervised the study and revised the article ; Anicet Sindayihebura: catography of survey and sampling sites ; Anatole Bukuru : helped manipulate cytoscape software ; Pierre Duez : supervised the study, revised the analysis of survey and botanical data, corrected and approved the article.

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