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# Antibacterial activities of 13 medicinal plants used against infectious and parasitic diseases in Kinshasa and its surroundings, D.R. Congo

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## Abstract

The present research aims to test the antibacterial activities of a sample of 13 plant species used in traditional medicine against infectious and parasitic diseases in Kinshasa and its surroundings. Ten herbal drugs were tested in the laboratory on *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*, and *Staphylococcus aureus*. With the exception of *Pseudomonas aeruginosa*, three of the four bacterial strains tested are sensitive to the herbal recipes in this study: *Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus*. These results give credibility to the traditional knowledge of local ethnic groups for the antibacterial properties of traditional prescriptions used in traditional medicine.

**Keywords:** Phytotherapy; Endogenous Knowledge; Infectious Disease; Antibacterial Activity; Kinshasa

## 1. Introduction

Medicinal plants play a significant role in health care programs in developing countries. Ancient Indian literature considers "all" plant parts to be potential sources of medicinal substances [1]. Relying on traditional medicine as an alternative in most cases could be explained by several factors including the low cost of treatment compared to conventional medicine, the financial capacity of the patient or his family, the difficulties of access to modern health care, the culture, the nature of the illness, etc.[2]. In the Democratic Republic of the Congo (DRC), urban and rural populations increasingly rely on medicinal plants to solve their health problems[3]–[5]. Several medicinal plants, mainly collected from natural habitats are available on the markets in public places[6] and among traditional healers. Ethnobotanical studies have great potential for understanding the oldest form of human knowledge, which is also the most widespread [7] . To promote endogenous knowledge of medicinal plants, ethnobotanical surveys combined with biochemical and phytochemical analyses are increasingly providing data on their therapeutic activity against various infectious diseases. For example, the anti-sickle cell activity of several medicinal plants used against malaria in Kinshasa supports the correlation between the chemical activity of plants and their use in traditional medicine[8]–[10]. Several pharmaceutical products contain principles derived from natural molecules[11]. Therefore, many plants have been

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used as a basis to develop several synthetic and semi-synthetic drugs[12], [13]. Other authors reported that 62% of drugs introduced between 1981 and 2002 include natural products or their derivatives [14]. They distribute this percentage as follows: 7% natural products; 27% derived from natural products; 5% synthetic derivatives of natural products, and 23% synthetic compounds designed from a natural product. Natural products are an innovative source of therapeutic agents for the treatment of infectious diseases and other ailments [14]. A decreasing susceptibility of infectious agents to antimicrobials has led to the need to increase the therapeutic arsenal of anti-infectious agents, with an emphasis on antibacterial, antiparasitic, and antifungal agents[15]–[17]. Recently, the scientific community and pharmaceutical companies have paid particular attention to medicinal plants because of their potential to develop innovative anti-infectious agents of natural origin[13], [16], [18]. The present study aims to enhance endogenous plant knowledge by linking two semantic systems: the ethnognosic system focusing on the effects of plants and the scientific system searching for the causes of diseases. The purpose of this study is to identify some herbal drugs and to test the antibacterial activities of 13 herbal drugs utilized against infectious diseases in Kinshasa.

## 2. Material and methods

### 2.1. Study area

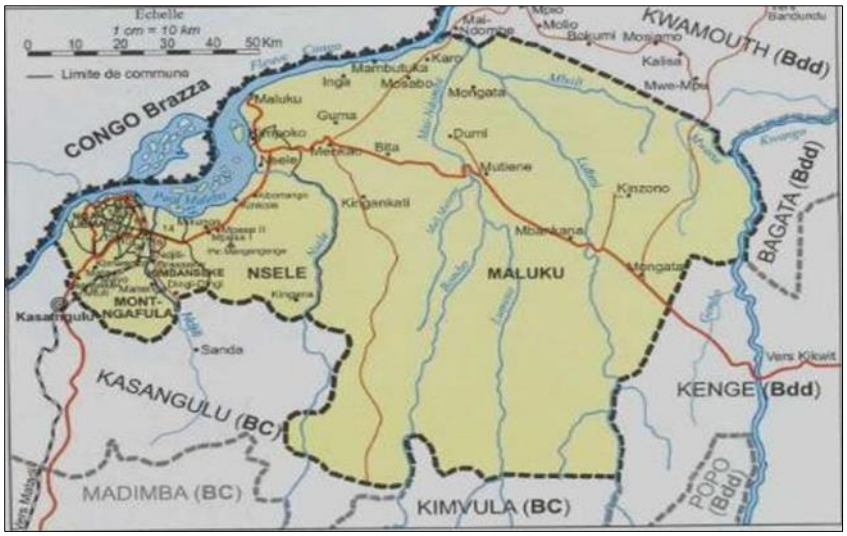


**Figure 1** Map of the city of Kinshasa [21]

**Table 1** Districts of Kinshasa and their respective communes

| Districts | Funa | Lukunga | Mont Amba | Tshangu |
|---|---|---|---|---|
| Communes | Bandalungwa | Barumbu | Limete | Kimbanseke |
| | Bumbu | Gombe | Lemba | Maluku |
| | Kalamu | Kinshasa | Ngaba | Masina |
| | Makala | Kintambo | Kinsenso | N'djili |
| | Kasa-Vubu | Lingwala | Matete | |
| | Ngiri-Ngiri | Ngaliema | | |
| | Selembao | Mont-Ngafula | | N'sele |

The City of Kinshasa is located between 4 ° 18 'and 4 ° 25' South latitude and between 15 ° 18 'and 4 ° 22' East longitude. Its average altitude is 360 m[19]. It is bordered to the north by the left bank of the Congo River, to the east by the Bateke plateau in Bagata, to the south by the Lukaya River and to the west by the Mfuti River. It covers an area of 9,965.2 km $^2$. The city is built on the left bank of the Congo River called Pool Malebo. It is crossed by many rivers of which the three most important (N'djili, N'sele, and Mai-Ndombe) are considered allochthonous. The climate of the city of Kinshasa is

Type AW4, which is a tropical climate. It is characterized by a long rainy season lasting 8 months (often interrupted with a small dry period between January and February), the rainy season lasts from mid-September to mid-May, and a dry season the rest of the year [19], [20]. The vegetation of Kinshasa consists of degraded primitive forests, savannahs, aquatic and semi-aquatic habitats in the valleys and the basin of Malebo. It belongs to the Guinea-Congolese region, to the domain of the Congo basin and to the Congolese-Zambezian transition sector.

Kinshasa is a decentralized entity made of 4 districts and 24 communes (municipalities). Table 1below shows the distribution of municipalities within each district.

*2.1.1. Plant material*

The plant material consists of 13 plant species collected in Kinshasa and its surroundings or purchased at the Central Market of Kinshasa. Other plant material was obtained from the Laboratory of the Phytotherapy Center in Congo (CERPHYTOC).

*2.1.2. Bacterial strains*

The pathogenic bacteria used in the present study were isolated from samples of patients who visited the Bacteriology Laboratory at the National Institute for Biomedical Research of Kinshasa (INRB). The bacteria used for the antibacterial test are ranged into 2 categories: the Gram-negative Enterobacteriaceae: *Escherichia coli, Salmonella typhi*, and *Pseudomonas aeruginosa*, and the Gram-positive cocci: *Staphylococcus aureus* [22].

## 2.2. Methods

*2.2.1. Population and sampling strategy*

The population of our study includes market herbalists, traditional healers, and users of medicinal plants in Kinshasa and surroundings. The selection of participants was based on the inclusion criteria such as being part of the following three groups: market herbalists, traditional healers, and users of medicinal plants. Furthermore, the participant extends his reputation beyond the district of practice; and agrees to participate in the survey on the basis of free consent.

*2.2.2. Data collection*

Data on medicinal plants were collected from ethnobotanical surveys by means of semi structured interviews. The survey was carried out during the period from June 2019 to June 2021(preliminary survey from June 2019 to October 2019. and the final survey from November 2019 to June 2021). The plant material collected was identified in the field by the taxonomist of the Laboratory of Systematic Botany and Plant Ecology of the Department of Biology at the National Pedagogical University of Kinshasa, Prof. Idrissa Assumani. Unidentified material was compared with the specimens from the Herbarium of the National Institute of Agronomic Research (INERA) of the University of Kinshasa or using published works by[23]–[25]. The taxonomy of scientific names is based on the Angiosperm Phylogeny Group standards [26] for the phylogenetic classification of flowering plants. The vernacular names of plants have been facilitated as much as possible by traditional healers and villagers in the field, and by means of ethnobotanical publications [27]–[30].

The selection of a few plants for the antibacterial tests on pathogenic gram-negative Enterobacteriaceae (*Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*) and the gram-positive Cocci (*Staphylococcus aureus*) was made based on plant drugs used by traditional healers. The drugs were administrated for the following diseases or infections: food poisoning, tuberculosis, plague, leprosy, bacillary dysentery, whooping cough, tetanus, gonorrhea, smallpox, measles, shingles, hepatitis, rabies, ringworm, skin mycosis, amebiasis, malaria and filariasis. These pathologies were the main concern for consultation in traditional medicine at the CERPHYTOC Laboratories and other traditional healers.

*2.2.3. Preparation of plant extracts*

A total of 10 plant extracts were prepared from 13 medicinal plants used to treat one or more infectious and parasitic diseases mentioned (Table 2). For decoctions, different concentrations (0.04; 0.06; 0.10; 0, 50 g / mL) were prepared from the powder of plant organs. After boiling for 15 minutes, the extracts were then cooled and filtered. For *Tetradenia riparia*, 250 g of ground fresh leaves were macerated in ethanol (80%) for 5 minutes. After filtrating, the extracts were stabilized with Nipasol M Sodium and then transferred into sterile 150 mL transparent glass vials numbered from 1 to 10. Plant extracts used for the present study were not previously subjected to chemical screening. Both the decoctions and the ethanolic macerate tested *in vitro* are natural products as they are administered to patients by traditional healers. Plant species with the parts used, the concentration, and the preparation mode are listed in table 2.

**Table 2** Preparation of plant extracts

| Recipes | Plant species | Part used | Concentration | Preparation |
|---|---|---|---|---|
| 1 | *Sarcocephalus latifolius* (Sm.) E.A. Bruce | Root | 0.04 g/mL | Decoction |
| | *Aloe buettneri* A.Berger | Fresh Leaves | 0.04 g/mL | |
| | *Heinsia crinita* (Afzel.) G. Taylor | Root | 0.04 g/mL | |
| 2 | *Sarcocephalus latifolius* (Sm.) E.A. Bruce | Root | 0.04 g/mL | Decoction |
| | *Millettia laurentii* De Wild. | Stem bark | 0.04 g/mL | |
| | *Hyptis suaveolens* (L.) Poit. | Stem bark | 0.04 g/mL | |
| | *Morinda morindoides* (Baker) Milne-Redh. | Powder of root | 0.04 g/mL | |
| 3 | *Ageratum conyzoides* (L.) L. | Entire plant | 0.10 g/mL | Decoction |
| 4 | *Desmodium velutinum* (Willd.) DC. | Leaves | 0.06 g/mL | Decoction |
| 5 | *Syzygium guineense* (Willd.) DC. | Stem bark | 0.10 g/mL | Decoction |
| 6 | *Tetradenia riparia* (Hochst.) Codd | Fresh Leaves | 0.50 g/mL | Ethanolic Macerate |
| 7 | *Securidaca longipedunculata* Fresen. | Root | 0.04 g/mL | Decoction |
| | *Pentadiplandra brazzeana* Baill. | Root | 0.04 g/mL | |
| 8 | *Millettia laurentii* Wild. | Stem bark | 0.04 g/mL | Decoction |
| 9 | *Fleroya stipulosa* (DC.) Y.F.Deng | Stem bark | 0.04 g/mL | Decoction |
| 10 | *Sarcocephalus latifolius* (Sm.) E.A. Bruce | Root | 0.04 g/mL | Decoction |

*2.2.4. Preparation of the cultivation medium*

To test the antibacterial activity of 10 recipes, two cultivation mediums were used depending on the category of pathogen and the destination. Agar was used for counting pathogens, the Mueller-Hinton broth was used to store isolated and previously selected strains for 24 hours. The Mueller-Hinton broth is prepared as follows: an infusion of 300 g of dehydrated beef, 17.5 g.L-1 of acid hydrolysate of casein, and 1.5 g.L-1 of corn starch. The pH was maintained at approximately 7.4 ± 0.2 after autoclaving with buffer solution.

*2.2.5. Preparation of the inoculum and evaluation of the antibacterial activity*

Bacterial suspension was prepared by combining 2 mL of physiological water (0.9%) with two colonies isolated from strains growing on Mueller-Hinton broth for 18 to 24 hours stored at $37^0$C. We adjusted the suspension's turbidity by a 1/100 dilution to obtain a density of 106 cells per mL close to the 0.5 Mac Farland standard (1.5x108 CFU /mL-1) or 108 cells per mL with an OD600 between 0.08 and 10.2. The antibacterial activity of the plant extracts was tested according to the West technique [27]. The technique consists of mixing 0.5 mL of the plant extract with 10 mL of liquid agar kept at $45^0$C. The mixture is poured into Petri dishes, cooled to room temperature, then streaked with germs and incubated at $37^0$C for 24 hours. The lecture of the result was made against a sterile sample (a sample in which there was not antibacterial drugs).

## 3. Results

### 3.1. Sociodemographic profile of participants

A total of 60 people have participated in this study. They are males (56%) and females (44%), between the ages of 15 and 75. The majority of the informants are Kongo natives. Figure 2 summarizes the sociodemographic characteristics of the informants.

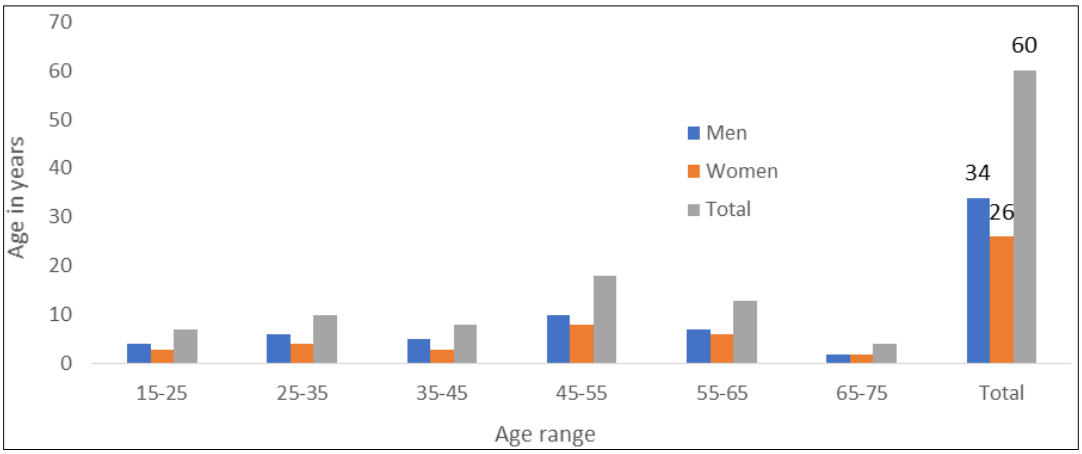


**Figure 2** Distribution of informants according to the age

### 3.2. Importance of pathologies according to the drugs used.

**Table 3** List of 13 medicinal plants tested and their ethnobotanical characteristics

| Family | Scientific name | Vernacular name+language | Disease treated | Part used | Preparation mode | Mode of administration |
|---|---|---|---|---|---|---|
| Asteraceae | *Ageratum conyzoides* (L.) L. | Mpata kasakula (Kiyaka) | Malaria | Whole Plant | Maceration | External Use |
| Lamiaceae | *Hyptis suaveolens* (L.) Poit. | | Malaria | Stem | Decoction | Oral |
| | *Tetradenia riparia* (Hochst.) Codd | Mutuzo (Mashi), Muravumba (Kinyarwanda) | Helminthiasis | Leaves | Maceration | Oral |
| Leguminosae | *Desmodium velutinum* (Willd.) DC. | Kalamata (Tshiluba ) | Helminthiasis | Leaves | Decoction | Oral |
| | *Millettia laurentii* De Wild. | Wenge (Lingala) | Gonorrhea | Stem bark | Maceration | Rectal |
| Myrtaceae | *Syzygium guineense* (Willd) D.C. subsp. Guineense | Kikulu, Nkulu (Kikongo) | Amebiasis | Stem bark | Decoction | Oral |
| Pentadiplandraceae | *Pentadiplandra brazzeana* Baill. | Nkenge kiasa (Kikongo) | Gonorrhea | Root | Decoction | Oral |
| Polygalaceae | *Securidaca longipedunculata* Fresen. | | Gonorrhea | Root | Decoction | Oral |
| Rubiaceae | *Fleroya stipulosa* (DC.) Y.F.Deng | Nlongwa/Nlongo (Kikongo) | Filariasis | Stem bark | Decoction | Oral |
| | *Heinsia crinita* (Afzel.) G. Taylor | Kinkete, Kibwa (Kikongo) | Gonorrhea | Root | Decoction | Oral |
| | *Morinda morindoides* (Baker) Milne-Redh. | Kongo bololo (Lingala) | Helminthiasis | Root | Decoction | Oral |
| | *Sarcocephalus latifolius* (Sm.) E.A.Bruce | Kienga, Mutumbi (Kikongo) | Gonorrhea | Root | Decoction | Oral |
| Xanthorrhoeaceae | *Aloe buettneri* A. Berger | Cigembegembe (Mashi) | Poisoning | Leaves | Decoction | Oral |

Figure 3 shows the importance of the diseases according to the recipes used. An inventory of 20 pathologies in the category of infectious and parasitic diseases was compiled among the traditional healers surveyed.

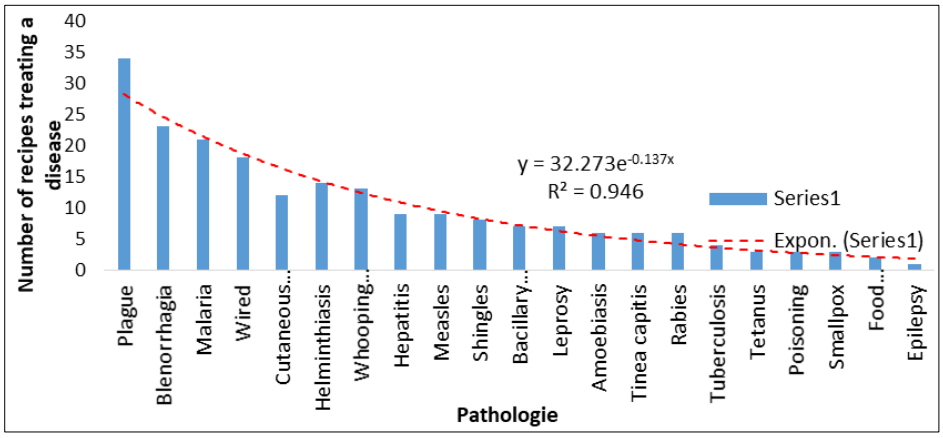


**Figure 3** Importance of pathologies as a function of recipes used

In the present study, plague, gonorrhea (blenorrhagia), malaria, filariasis, helminthiasis, cutaneous mycosis, and pertussis are among the top 10 diseases treated with the most drugs. The number of recipes for the different pathologies is clearly significant ($R^2$=0.946). A total of 104 medicinal plant species were used by the herbalists to treat the above-mentioned pathologies, (data not included in this study), only a sample of 13 plant species was selected for further antibacterial analysis. Table 3 summarizes the floristic list with ethnobotanical description. The majority of the drugs are prepared as decoctions from roots, stem bark, and the leaves of the plant species selected. The drugs are generally orally administrated to the patients. The external use and rectal pathway are less applied.

### 3.3. Antibacterial activities of the tested recipes

The antibacterial activity results of 13 plants tested are summarized in table 4.

**Table 4** Antibacterial activities of 13 plant species used against infectious and parasitic diseases in Kinshasa and surroundings

| **Recipes** | **Composition** | **Preparation** | **Bacterial strains** | | | |
|---|---|---|---|---|---|---|
| | | | *(A)* | *(B)* | *(C)* | *(D)* |
| 1. | *Sarcocephalus latifolius* (Sm.) E. A. Bruce, *Aloe buettneri* A. Berger, *Heinsia crinita* (Afzel.) G. Taylor | Decoction | - | + | - | - |
| 2. | *Sarcocephalus latifolius* (Sm.) E.A.Bruce *Millettia laurentii* De Wild., *Hyptis suaveolens* (L.) Poit, *Morinda morindoides* (Baker) Milne-Redh, | Decoction | - | + | + | - |
| 3. | *Ageratum conyzoides* (L.) L. | Decoction | + | + | - | - |
| 4. | *Desmodium velutinum* (Willd.) DC. | Decoction | + | - | - | - |
| 5. | *Syzygium guineense* (Willd.) DC | Decoction | + | + | - | - |
| 6. | *Tetradenia riparia* (Hochst.) Codd | Ethanolic macerate | + | + | + | - |
| 7. | *Securidaca longipedunculata*Fresen *Pentadiplandra brazzeana* Baill. | Decoction | - | - | - | - |
| 8. | *Millettia laurentii* De Wild., | Decoction | + | - | + | - |
| 9. | *Fleroya stipulosa* (DC.) Y.F.Deng | Decoction | + | - | - | - |
| 10. | *Sarcocephalus latifolius* (Sm.) E. A. Bruce | Decoction | + | - | + | - |

(A)= *Staphylococcus aureus; (B) = Salmonella typhi; (C) Escherichia coli; (D) Pseudomonas aeruginosa.*

The results in table 4 show that recipe 6 was effective against 3 of the 4 strains tested. In addition, these preliminary data suggest that *Staphylococcus aureus* is susceptible to 6 out of 10 recipes. On the other hand, *Salmonella typhi* and *Escherichia coli* are respectively sensitive to 5 and 3 of the recipes tested. While *Pseudomonas aeruginosa* was found to be resistant to all trials, *Tetradenia riparia* ethanolic extract was proven successful against *Staphylococcus aureus, Salmonella typhi*, and *Escherichia coli*.

## 4. Discussion

Based on the socio-demographic characteristics, most participants were between the ages of 15 and 75, with older people knowing more about medicinal plants than younger people. Knowledge of the uses and/or properties of medicinal plants is generally accumulated after a long experience and passed from one generation to the next. Today, this knowledge is in danger of being lost because its transfer is not always guaranteed[31]. Comparing the present study with previous works, 3 plants of the 13 species tested have been scientifically validated to show antibacterial properties *in vitro* in the DR Congo. These include *Tetradenia riparia*, *Morinda morindoides*, and *Sarcocephalus latifolius* [8]. In a study on the evaluation of the effectiveness of anti-diarrheal plants in Ivory Coast, Bouboutou *et al*.(1995) [32] demonstrated the bactericidal activity of dried leaves of *Paulinia pinnata* on *Salmonella typhi* and *Pseudomonas aeruginosa*. On the other hand, hydro alcoholic extracts of *Alchornea cordifolia* and *Spondias mombin* show different zones of inhibition on *Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* [33].. However, none of our recipes inhibited the development of *Pseudomonas aeruginosa.* In another study, authors [34], observed *in vitro* a sensitivity of *Escherichia coli* and *Staphylococcus aureus* in the presence of the total extract of a foliar mixture of *Alchornea cordifolia, Cassia alata,* and *Staurospermum verticillatum.* In the present study, these strains are simultaneously susceptible to *Millettia laurentii, Sarcocephalus latifolius,* and *Tetradenia riparia.* Furthermore, the alcoholic extracts from the leaves of *Sarcocephalus latifolius* act against *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Shigella flexineri* [35]. Laboratory experiments using the aqueous decoction confirmed these results on *Escherichia coli, Salmonella typhi,* and *Staphylococcus aureus.* Ezzedine *et al(1990)* [36] reported the antibacterial activity of etherpetrolic extracts of *Hyptis suaveolens* on various strains including *Escherichia coli, Staphylococcus aureus*, *and Pseudomonas aeruginosa*. In the present study, the efficacy of recipe 2 against *Escherichia coli* could be attributed to *Hyptis suaveolens*. However, the resistance of *Pseudomonas aeruginosa* may result from the insolubility of the active principle in water. Other authors demonstrated the antiplasmodial activity *in vitro* and *in vivo* of some plants including *Morinda morindoides, Rauvolfia vomitoria*., *Sarcocephalus latifolius*, *Senna occidentalis*, and *Vernonia amygdalina* [37]. These species are widespread in the Phytotherapy of several infectious and parasitic diseases in the study area. Although they have not been tested, their frequent use by traditional healers in inventoried recipes is validly justified. For example, Kabena (2020) [38] demonstrated the antibacterial activity of the extracts of *Oncoba welwitschii* on strains *of Escherichia coli* and *Staphylococcus aureus* at a minimum inhibitory concentration.

## 5. Conclusion

The present study made it possible to identify 13 medicinal plants used against various infectious and parasitic diseases in Kinshasa and its surroundings. The medicinal uses of the plants listed mainly involve plague, gonorrhea, and malaria. Drugs are mostly prepared in the form of decoctions orally administered. Microbiological analysis revealed that endogenous plant knowledge has a scientific basis for certain bacteria: *Staphylococcus aureus* is susceptible to 6 of 10 recipes tested; *Salmonella typhi* and *Escherichia coli* are respectively sensitive to 5 and 3 of the drugs tested. The ethanolic extract of *Tetradenia riparia* has been shown to be an effective drug against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Phytochemical screening of the 13 plant species tested in the present study could contribute to extend the application of the present flora to more bacterial strains, and to better understand why *Pseudomonas aeruginosa* resisted most of the samples tested.

## 6. Compliance with ethical standards

### *Acknowledgments*

The Authors thank the CERPHYTOC Laboratories to reveal some drugs used against infectious and parasitic diseases. The Authors are further thankful to the INRB Kinshasa for the collaboration and the facilities provided to allow antibacterial tests.

*Disclosure of conflict of interest*

The authors declare that they have no competing interests.

*Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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