Ethnobotanical survey and phytochemical screening of anti-snakebite plants used in Bissok District of Gabon

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Abstract. Mengome, Mewono L, Mboma R, Engohang-Ndng J, Angone SA. 2021. Ethnobotanical survey and phytochemical screening of anti-snakebite plants used in Bissok District of Gabon. Biodiversitas 22: 3264-3275. Snakebites remain a major health issue in tropical and subtropical regions in the world. The limited access of antivenom sera in remote areas of many countries forces populations to rely on plant-based remedies to manage snakebite-induced injury. In this study, we conducted an ethnobotanical survey of medicinal plants used for the management of snakebites in the district of Bissok (Northern Gabon). After collecting and verifying the authenticity of the plants, we further performed their phytochemical analysis. The procedure used in this study involved a structured questionnaire and direct interviews of local populations. Overall, data collected on-site included local names, the part of the plant used, the preparation, and the route of administration. The presence of some phytochemical compounds was determined according to standard methods. Overall, a total of 29 species of plants belonging to 20 different families were reported to be used to treat snakebites. The plants used were herbs (44.80%), trees (24.10%), shrubs (20.70%), liana (10.30%), and rhizomes (6.90%). They were mainly used as poultice or crush, and to a lesser extent as decoction and maceration. Regarding the route of administration, interviewees reported mainly external use on the site of the bite. Concerning the chemical composition, we found that the antivenom plants were rich in chemical compounds known to have antivenom, antipyretic and antimicrobial properties, e.g. alkaloids, terpenoids, flavonoids, steroids, coumarins, phenols, tannins gallic Our results open avenues to develop venom enzyme inhibitory assays.

Keywords: Medicinal plants, snake envenomation, phytochemicals, ethnobotany, phytochemical, Bissok District, Gabon

INTRODUCTION

According to the World Health Organization (WHO), snakebites are a neglected public health issue in many countries including those located in tropical and subtropical regions (de Moura et al. 2015; Dhananjaya et al. 2016). Snakebites are responsible for more deaths and disabilities than some tropical infectious diseases such as dengue fever, cholera, Japanese encephalitis, Chagas disease, and leishmaniasis (WHO 2017). Each year, about a million snakebites occur in Africa. However, the highest-burden is recorded in sub-Saharan Africa, causing between 100,000 and 500,000 envenoming cases and around 10,000 to 30,000 deaths (Chippaux 2011; Dhananjaya et al. 2016). Due to the importance of the threat, the World Health Organization (WHO) has re-added snakebite envenoming to the list of Neglected Tropical Disease (NTD) by the year 2017 (WHO 2017; Gómez-Betancur et al. 2019).

It is generally admitted that most snakebite cases are not reported. The above-mentioned data are likely underestimated (Chippaux 2011). In that respect, Chippaux (2011) and de Moura et al. (2015) claimed that the underreporting of snakebite-associated mortalities and disabilities may be explained by: insufficient access to antivenoms (healthcare centers), poorly trained healthcare personnel, and lack of appropriate epidemiological surveillance. In addition, it appears that the lack of antivenoms in most healthcare centers, mainly in rural areas, is a major drawback for the management of snakebites.

In sub-Saharan Africa and Gabon in particular, the use of medicinal plants is a reality and they are a common resource for dealing with snakebites (Omara 2020; Omara et al. 2020). Gabonese communities use traditional medicine for several reasons including economic, familiarity and knowledge of plants, attachment to their traditions, treatment of physical ailments, and in some instances belief in spiritual healing (Makita et al. 2011; Ngoua et al. 2019). Although the treatment for snakebites is effective, it is not readily available due to geographical areas, and anti-venom is usually administrated after local effects have already developed to some extent (Otero-Patiño et al. 2012). Research of alternative treatment from snake envenomation can be applied in the field to reduce the local and systemic actions induced by snake venoms.
Medicinal plants are a common source of active drugs for treating a wide range of diseases all over the world. Most of these medicinal plants are consumed as food or used for their rich phytochemical compounds that are known to have both preventive and curative effects on consumers for the treatment of various diseases (Prolhp and Onoagbe 2012; Shabbir et al. 2014). According to the World Health Organization, Traditional Medical Program has provided evidence that ethnomedical information can lead to valuable drug discovery (Susana et al. 2019). Since the dawn of time, several active compounds have been discovered from plants on the basis of ethnobotanical information. Presently, the World Health Organization estimated that 80% of the population in some Asian and African countries use medicinal plants as an alternative form of primary healthcare. Regarding the management of snakebite in rural areas of the world, several plant species and plant part are commonly used (WHO 2019). In Nigeria for instance, Mucuna pruriens (L.) DC. was used for prophylactic for snakebite (Gómez-Betancur et al. 2019), while Akindle et al. (2020) reported that methanolic extracts of Annona senegalensis Pers. were able to reduce Naja nigricollis’ venom-induced both mortality and hyperthermia in rats. This study aims to report an ethnobotanical survey and phytochemical compounds obtained in aqueous extracts of medicinal plants used to treat snakebites in Bissok, a District located in northern Gabon.

MATERIALS AND METHODS

Materials and reagents

Botanical materials were obtained by informants when the species were not very far from the house. Field guides were used to help in locating the district of Bissok and offering translation services. Chemical solvents and all other reagents were obtained from Sigma-Aldrich (Sigma-Aldrich GmbH, Stemhein, Germany) or Merck (France).

Study area

The survey was carried out in Bissok District, Woleu-Ntem Province, the northern province of Gabon, a country located in central Africa. This province covers an area of 38,465 km², meaning 14.4% of the national territory. It is bordered on the western side by Equatorial Guinea, on the north by Cameroon, on the east by the Republic of Congo Brazzaville, on the south by the provinces of Ogooué-Ivindo, Moyen-Ogooué, and Estuaire (Figure 1). Access to health centers is extremely limited for populations of Woleu-Ntem because on one hand roads are overall in very bad conditions in almost all seasons of the year which makes them impassable and on the other hand when existing, health centers are very poorly equipped and lack anti-serum to treat snakebites, such as Gabon viper (Bitis gabonica); Mamba de Jameson (Dendroaspis jamesoni); mamba vert (Dendroaspis viridis); Cobra aquatique (Naja annulate).

Figure 1. A map showing the location of the study area in Bissok District, Woleu-Ntem Province, Gabon. Adapted from an original version published by Geology.com (https://geology.com/world/gabon-satellite-imageshtml)
Field interviews
We conducted a survey of plants used to treat snakebites by connoisseurs and by traditional healers in the district of Bissok (N:1°16’0”; E:11°35’0”) which is an area predominantly occupied by the Fang ethnic group. The survey was conducted between June 2014 and August 2016. In this district, populations relied extensively on subsistence agriculture and hunting. Interviews were based on a direct questionnaire on the plants used by the populations to treat snakebites. Interviewees belong to the following ethnic groups: Fang, Pygmy, Kota, and Punu. While Fang and Pygmies are native to the area, the Kota and Punu seem to have arrived through marriage to a Fang or Pygmy (Wily 2012). Practically, people were asked information about the use of plants including the local name, the parts of the plant used, methods of preparation, and routes of administration.

Plants collections
Plants were identified by botanists of the National Herbarium of Gabon (NHG). We completed identification with other support such as Checklist of Gabonese Vascular plants (Sosef et al. 2006) and web sources such as the Plant List (http://www.thelplantlist.org/) and the Plants of the World Online (http://www.plantsoftheworldonline.org/). Both the scientific names and corresponding voucher numbers were attributed.

Preparation of the antivenom plant extracts
After botanical identification of plants, samples were dried using a dehumidifier (Bioblock Scientific LMS Cooled Incubator) at 35°C for one week. Further, they were cut into small pieces and reduced to fine powders using an electric grinder.

Ten (10) g of powdered samples were mixed with 100 ml of distilled water. The mixture was then heated in a water bath at 100°C for 1 hour. Samples were then allowed to cool at room temperature, after which decoctions were filtered using a 33 mm diameter sterile syringe Millipore filter with 0.45 μm pore size (Millipore, Biebford, MA), dried, and used for further tests.

The phytochemical screening of the anti-snake venom plant extracts
The prepared aqueous extracts were screened for their qualitative chemical composition using standard methods described elsewhere (Mengome et al. 2010; Badjin et al. 2016b). The identification of the following groups was considered: alkaloids, sterols, triterpenes, coumarins, flavonoids, tannins, polyphenols, carotenoids, reducing sugars, carbohydrates, and saponosides. According to the precipitate or color intensity in each sample and test, the following evaluations were given: (i) +++: represents very high, (ii) ++: indicates moderate, (iii) +: indicates little/traces, and (iv) –: indicates absent (Gul et al. 2017; Suleiman and Ateeq 2020).

Data analysis
Raw data were entered in Microsoft Excel and analyzed using simple descriptive statistics. This was followed by the computation of frequency of use, family abundance, and the determination of preparation procedure and route of administration.

For each medicinal plant a Use Index (UI%) was calculated to give a ranking of the importance of the use of medicinal species. The Use Index is based on information collected and the UI% is calculated from the following formula:

\[ UI = \left( \frac{na}{Na} \right) \times 100 \]

Where: “na” is the number of interviewees who cited a given species of plant as useful and “NA” is the total number of people interviewed (Randriamiharisoa et al. 2015; Bajin et al. 2016a).

RESULTS AND DISCUSSION

Ethnobotanical survey
Information on the plants used to treat snakebites in the Bissok District in northern Gabon is summarized in Table 1. In 18 villages of the district, 51 people belonging to two social groups were interviewed, i.e. healers and professionals of the traditional use of plants. Four different ethnic groups were interviewed among whom were the Fang people constituting 58.8% followed by Pygmies (17.6%), Kota (13.7%), and Punu (9.8%).

A total of 29 plants from 20 families and 29 genera were reported to be used in the treatment of snakebites and were further classified in alphabetical order according to their families. Local names or common names are provided in Fang language because it is the most common language spoken throughout the surveyed region. Further, in Table 1, these plants are shown to be used alone or in combination. Among the 20 families, the most commonly mentioned were Asteraceae with six species. The Apocynaceae, Piperaceae, Rubiaceae, and Zingiberaceae families had each two species while all other families listed in Table 1 were represented by only one species. We further sought to determine which plants were mostly cited by interviewees. The highest index was found for Emilia coccinea (Sims) G.Don (62.7%), followed by Adenia reticulata (De Wild. & T.Durand) Engl., Cyanthillium cinereum (L.) H. Rob., and Mangifera indica L (all having 52.9% of respondents), and Drymaria cordata (L.) Willd. ex Schult. (50.9%). Twelve plants had between 35% and 50% respondents such as Alstonia congensis Engl.; Eclipta prostrata (L.) L. (35.3%), Carica papaya L.; Plectranthus bojeri (Benth.) Hedge (37.2%), Piper umbellatum L., Jateorhiza macrantha (Hook.f.) Exell & Mendonça; Manihot esculenta Crantz (39.2%), Dacyrcodes edulis (G.Don) H.J.Lam and Oldenlandia herbacea DC. (43.1%), Zanthoxylum gilletii (De Wild.) P.G.Waterman (45.1%), Anthochoista vogelii Planch. (47.1%), and Costus ligularis Baker (49%).

Analysis of ethnobotanical data
It appears that 44.8% of plants reported to be used for treating snakebites were herbaceous plants. Trees and shrubs accounted for 24.1% and 20.7% of plants used while lianas...
and rhizomes represented respectively 10.3% and 6.9% of all mentioned plants (Figure 2).

Furthermore, the survey revealed that the use of leaves and that of the whole plant were the most commonly used parts of the plant, with 51.7% and 24.1% respectively reported by respondents. The use of stems, roots, and rhizomes was only marginal (Figure 3). Gabon Poultice and crush were the most occurring mode of preparation of plants (25% and 21% respectively) (Figure 4).

**Phytochemical profile of the aqueous extracts**

The phytochemical screening of aqueous extract of medicinal plants reported in this study is summarized in Table 2. Fifteen types of chemical compounds were screened including flavonoids (Fla), tannins gallic (Tang) or tannins catechic (Tanc), quinones (Qu), phenols (Phe), sterols (Ste), triterpenes (Trit), carotenoids (Car), total sugars (Sug), reducing sugar (Res), deoxysugar (Deso), Cardiotonic sugar (Cars), mucilage (Muc), saponosides (Sap), and organs (Org). In addition, alkaloids were screened using the Draggendorff test for AlkD and the Mayer test for AlkM. Overall, 208 assays were performed to screen the targeted chemical compounds. Out of the 208 tests carried, 143 tests were positive while 65 were negative.

A close look at the chemical analysis of samples shows that plants reported by respondents could be divided into five categories according to chemical diversity and chemical abundance found in the extract as shown in Figure 5. The first category of plants includes *Mangifera indica* and *Emilia coccinea* which both contained 13 bioactive chemical groups with a relative abundance of 81.3% of chemicals found in the extract. The second category includes *Anthocleista vogelii* and *Cissus quadrangularis* containing 12 bioactive chemical groups with a relative abundance of 75%. The third category includes five species (*Dacryodes edulis*, *Drymaria cordata*, *Manihot esculenta*, *Piper umbellatum*, and *Lantana camara*) and contained 11 bioactive chemical groups with a relative abundance of 68.8%. *Alstonia congensis* and *Peperomia pellucida* made the fourth category of plants with 10 types of bioactive chemicals and 62.5% of relative abundance. Finally, the fifth category included *Ageratum conyzoides* and *Zanthoxylum gilletii* which both contained nine bioactive chemical groups with 56.3% of relative abundance.

Furthermore, the relative abundance of phytochemicals was analyzed in plant organs used to treat snakebites. Thus, Figure 6 indicates barks which use was mentioned for 55 positive profiles, containing about 38.5% of bioactive chemicals while whole plants (43 positive profiles), and leaves (33 positive profiles) contained respectively 31.5% and 23.1% of bioactive compounds. Finally, liana in 12 plants mentioned and assayed contained only 8.4% of bioactive chemicals.

When looking into the relative abundance of each type of chemical compound (Figure 7), it appears that nine compound types are particularly dominant, including alkaloids (88.5%), flavonoids (92.3%), phenols (100%), triterpenes (76.9%), total sugars (100%), reducing sugars (84.6%), deoxy sugars (84.6%), cardiac glycoside (100%) and mucilage (76.9%).
Table 1. Plant species used in the management of snakebites in Bissok District (Northern of Gabon). Plants are listed according to family/scientific name/voucher, local name, parts used, methods of preparation, manners of use, and routes of administration.

| Family/scientific name/voucher | Local or common name (Fang) | Part used | Method of preparation | Routes of administration | Incidence | Use Index (UI) |
|--------------------------------|-----------------------------|-----------|-----------------------|--------------------------|-----------|---------------|
| Acanthaceae | Brillantaisia owariensis P. Beauv. | Nyar-élc | Whole plant | Macerate the plant and mix with Emilia coccinea | Oral | 12 | 23.5% |
| Anacardiaceae | | Andok ntangha | Barks | Crush all fresh herbal and place them in poultice on the snakebite | External | 27 | 52.9% |
| Apocynaceae | Aistonia congensis Engl. | Oyomtsë, oyomtë | Roots | Crush fresh roots and place them in poultice on the snakebite | External | 12 | 23.5% |
| Asteraeae | Agaratum conyzoides L.1017 | Oyomtsë, oyomtë | Roots | Crush fresh roots and place them in poultice on the snakebite | External | 12 | 23.5% |
| Bidens pilosa L. 2934 | | | Leaves | Crush fresh herbs and applied on the snakebite | Oral | 5 | 9.8% |
| Eclipta prostrata L. 2299 | | | Whole plant | Crush fresh herbs and applied on the snakebite | Oral | 18 | 35.3% |
| Emilia coccinea (Sims) G.Don | Alô-mvu | Whole plant | Crush fresh whole plants and place them in poultice on the snakebite | Oral | 18 | 35.3% |
| Cyanthillium cinereum (L.) H.Rob. 3134 | Ayapana sauvage | Leaves | Crush fresh whole plants and place them in poultice on the snakebite | Oral | 32 | 62.7% |
| Baccharoides guineensis (Benth.) H.Rob. 2952 | | | Crush fresh whole plants and place them in poultice on the snakebite | External | 27 | 52.9% |
| Burseraceae | | | Crush fresh whole plants and place them in poultice on the snakebite | External | 13 | 25.5% |
| Caryophyllaceae | Drymaria cordata Wild. ex Schult 5629 | | Leaves | Crush fresh leaves, mix it with the sap, and place the mixture in poultice on the snakebite | External | 19 | 37.2% |
| Clusiaceae | Carica papaya L. 1365 | | Sap | Crush fresh leaves, mix it with the sap, and place the mixture in poultice on the snakebite | External | 26 | 50.9% |
| Clausiaceae / Guttiferae | Garcinia manii Oliv. 212 | | Sap | Apply the sap to the wound caused by a snakebite | External | 15 | 29.4% |
| Euphorbiaceae | Manihot esculenta Grantz 11412 | | Leaves | Crush fresh leaves or stem to release the sap and apply on the snakebite | External | 20 | 39.2% |
| Lamiales | Plectranthus bojeri (Benth.) Hedge 978 | | Leaves | Crush fresh leaves or stem to release the sap and apply on the snakebite | External | 19 | 37.2% |
| Loganiaceae | Anthocleista vogelii Planch. 811 | | Leaves | Clean fresh young leaves and place them in poultice on the snakebite | External | 24 | 47.1% |
| Family               | Species                                      | Part       | Preparation and Usage                                                                 | Method   | Effect |
|----------------------|----------------------------------------------|------------|----------------------------------------------------------------------------------------|----------|--------|
| Melastomaceae        | *Dissotis multiflora* Triana 69              | Leaves    | Crush fresh leaves and mix with the *Costus ligularis* juice. The mixture is used as a poultice on the snakebite | External | 10     |
| Menispermaceae       | *Jateorhiza macrantha* (Hook. f.) Exell & Mendonça-4422 | Liana     | Place the crushed liana in poultice on the snakebite                                    | External | 20     |
| Passifloraceae       | *Adenia reticulata* (De Wild. et Th. Durand) Engl. 920 | Whole plant | The whole plant is hand-rubbed, and its juice is mixed with crushed leaves of *Plectranthus bojeri* and *Cyanthillium cinereal*. Drink the macerate of the whole plant. The mixture is then spread on the snakebite | Oral     | 27     |
| Piperaceae           | *Piper umbellatum* L. 2339                   | Leaves    | Boil leaves in water then drink the infusion warm                                       | External | 20     |
| Menispermaceae       | *Piperomiar pellucida* 927                  | Whole plant | Chew the whole plant and swallow                                                        | Oral     | 15     |
| Rubiaceae            | *Oldenlandia herbacea* (L.) Roxb 1571        | Mille grains | Chew the whole plant, put it in water then drink and chew the plant                     | Oral     | 22     |
| Rutaceae             | *Otomeria volubilis* (K.Schum.) Verdc 608    | Leaves    | Crush leaves and apply the juice on the snakebite                                       | Oral     | 16     |
| Verbenaceae          | *Zanthoxylum gilletii* (De Wild.) P.G. Waterman 2551 | Leaves    | Triturated leaves are macerated in the juice of *Costus ligularis* and the mixture is applied to the snakebite | External | 23     |
| Vitaceae             | *Cissus quadangularis* L. 125                | Liana     | The raping of the liana is used as a poultice on the snakebite                           | External | 17     |
| Zingiberaceae        | *Costus ligularis* Bak. 264                  | Leaves, stem | The juice of the whole plant is said to be a snake repellent                            | External | 25     |
| Zingiberaceae        | *Zingiber officinale* Roscoe 159             | Rhizome   | Chew and crush rhizomes and apply them as a poultice on the snakebite                   | Oral     | 15     |
Table 2. Phytochemical screening of some species of plants used to treat snakebites in Gabon

| Family/scientific name/voucher | AlkD | AlkM | Fla | Tang | Tanc | Qun | Phe | Ste | Trit | Car | Sug | Res | Deso | Cars | Muc | Sap | Org | R+ |
|--------------------------------|------|------|-----|------|------|-----|-----|-----|------|-----|-----|-----|------|------|-----|-----|-----|-----|
| Anacardiaceae/Mangifera indica L./1588 | +++ | + | +++ | - | +++ | +++ | - | +++ | + | + | +++ | +++ | +++ | ++ | - | B | 13 (81.3%) |
| Apocynaceae/Alstonia congensis Engl./1093 | +++ | +++ | +++ | + | - | + | + | + | +++ | + | +++ | - | +++ | - | + | B | 10 (62.5%) |
| Asteraceae/Ageratum conyzoides L./1017 | +++ | ++ | +++ | - | - | +++ | ++ | - | - | +++ | +++ | + | +++ | - | +++ | Wp | 9 (56.3%) |
| Asteraceae/Emilia coccinea (Sims) G.Don/1065 | +++ | +++ | +++ | + | - | +++ | - | +++ | +++ | +++ | +++ | +++ | - | +++ | - | Wp | 13 (81.3%) |
| Burseraceae/Dacryodes edulis (G.Don) H. J.Lam./1114 | +++ | +++ | + | + | - | - | +++ | - | +++ | +++ | +++ | +++ | - | B | 11 (68.8%) |
| Caryophyllaceae/Drymaria cordata Wild. ex Schult/5629 | +++ | +++ | - | + | - | - | ++ | - | +++ | +++ | +++ | ++ | - | +++ | - | Wp | 11 (68.8%) |
| Euphorbiaceae/Manihot esculenta Grantz/11412 | +++ | +++ | +++ | - | - | +++ | - | +++ | +++ | +++ | +++ | ++ | - | +++ | - | L | 11 (68.8%) |
| Loganiaceae/Anthocleista vogelii Planch./811 | +++ | +++ | +++ | - | - | +++ | - | +++ | ++ | +++ | +++ | + | ++ | - | B | 12 (75%) |
| Loganiaceae/Anthocleista vogelii Planch./811 | +++ | +++ | +++ | - | - | +++ | - | +++ | ++ | +++ | +++ | + | ++ | - | L | 11 (68.8%) |
| Rutaceae/Zanthoxylum gilletii (De Wild.) P.G.Waterman/2551 | +++ | +++ | +++ | - | - | +++ | - | +++ | +++ | +++ | +++ | ++ | - | B | 9 (56.3%) |
| Vitaceae/Cissus quadrangularis L./125 | +++ | +++ | +++ | - | - | +++ | - | +++ | +++ | +++ | +++ | ++ | - | L | 11 (68.8%) |

Note: AlkD: Alkaloids according to Dragendorff test, AlkM: Alkaloids according to Mayer test, Fla: Flavonoids, Tang: Tannins gallic, Tanc: Tannins catechic, Qun: Quinones, Phe: Phenols, Ste: Steroids, Trit: Triterpenes, Car: Carotenoids, Sug: Total sugars, Res: Reducing sugars, Deso: Desoxysugars, Cars: Cardiotonic sugars, Muc: Mucilages, Sap: Saponosids, B: Barks, Wp: Whole plant, L: Leaves, Li: Liana, Org: organs, (+): positive result, (-): negative result, R+: Percentage of positive results. +++ represents very high, ++ indicates moderate, + indicates little/traces, and – no presence.
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Figure 5. Plants were grouped into five categories according to the diversity and relative abundance of chemical compounds screened.

Figure 6. Plant organs used were screened for the relative abundance of bioactive chemical compounds.

Figure 7. The affluence of different types of chemical compounds.

Discussion
Snake envenomation is a neglected tropical disease that accounts for between 10,000 and 30,000 deaths per year (Chippaux 2011; Dhananjaya et al. 2016). The threat is particularly important in remote tropical and subtropical areas where health facilities are hardly available. When present, they are often poorly supplied with medical consumables and equipment. Inevitably, in these areas, traditional medicine becomes the medical approach of the first intention. The main signs developed after snakebite are characterized by inflammation, edema, necrosis, hemorrhage, and collapsing of the patient. Moreover, kidney and neuronal failure, respiratory dysfunction, and cardiotoxicity can all be experienced by the patient (Nalbantsoy et al. 2013; Gómez-Betancur et al. 2014; Pompermaier et al. 2018; Elgorashi and McGaw 2019). Hemorrhage is one of the most relevant signs of local and systemic envenoming by viper snakes (Gómez-Betancur et al. 2014).

In this study, we are reporting the results of an ethnobotanical survey we conducted in the district of Bissok (Northern Gabon). Of the 29 species of plants collected, 24 of them were reported in a number of studies to be used against snakebites (Ezuruike and Prieto 2014; Molander et al. 2014). More specifically, *Manihot esculentus* Crantz (Lebbie and Turay 2017) in Sierra Leone, *Lantana camara* L. and *Carica papaya* L. (Kamatenesi et al. 2011; Kodi et al. 2017) in Uganda, *Zanthoxylum gilletii* (De Wild Waterman) in Kenya (Okeyo et al. 2011), *Cissus quadrangularis* L. in South Sudan (Doka and Yagi 2009), have been reported for treating snakebites.

Interestingly, only three plants, i.e. *M. indica*, *E. prostrata*, and *P. umbrellatum* have been shown through experimental data to have anti-venom activities against snake venom enzymes and/or toxins (Nanjara et al. 2014; Singh et al. 2017). Indeed, aqueous extract of *E. prostrata* known in Brazil and China as an antidote against snakebite...
was successfully tested against South American rattlesnake venom (Félix-Silva et al. 2017). Furthermore, antinflammatory, antihemorrhagic, antiprotozoal and anti- phospholipase properties of those plant extracts were revealed (Hasan et al. 2016; Strauch et al. 2019). Aqueous stem bark extracts of *M. indica* have been shown to inhibit the toxic and lethal effect of the *Indian Russell’s viper* venom (Dhananjaya et al. 2011). Moreover, many secreted phospholipase A2 (sPLA2) inhibitors have been isolated from various medicinal plants (Kim et al. 2020; Fatahiya et al. 2021). Other studies have shown antivenom activity of aqueous stem bark extract of *M. indica* by inhibiting NN-XIIb-PLA2 of *India cobra* venom with IC50 = 7.6 µg/ml (Dhananjaya et al. 2016). Extracts and pure compounds derived from various parts of *P. umbellatum* have shown several pharmacological activities, including anti-inflammatory, analgesic, anti-atherogenic, cytotoxic, anti-platelet, anti-rheumatism, antimarialar, antifungal, antibacterial, anti-leishmanial, pain, oedema, and anti-trypansosomal activities (Agbor et al. 2012; da Silva et al. 2014; 2016; Didjour et al. 2019). Several studies from *P. umbellatum* have shown the presence of alkaloids, flavonoids, sterols, and terpenes. Many of them are found in essential oil and other classes of secondary metabolites (Didjour et al. 2019). These results suggest that knowledge on virtues of traditional medicine is shared across countries, continents and support our findings.

As previously reported elsewhere, medicinal plants were used either alone or in combination. During our interviews, we were unable to clearly decipher why particular plants were required to be used in combination with others. However, we hypothesize that these plants act synergistically in order to prevent snakebite-induced tissue damages. We were further interested in knowing which type of plants and plant parts were commonly used by local populations in the District of Bisok to manage cases of snakebite. Overall, our results showed that herbaceous plants were mostly used, whilst leaves and stem barks were the most used part of the plant. These results are in accordance with our previous report (Mengome et al. 2010) and those from other authors (Bajin et al. 2016a) showing that herbaceous plants and leaves are the prominent plants and parts used. Nevertheless, it is important to note that the use of either plant type or plant part depends on the anatomical place where the injury occurs and, on the availability and accessibility of the material.

It is obvious that plants’ secondary metabolites drive their biological activities (Khameneh et al. 2019). Regarding plant-based management of snakebite, Singh et al. (2017) investigated the effect of plant secondary metabolites on either snake products or venom-induced damages. Their report highlighted the role of alkaloids, terpenoids, flavonoids, steroids, and coumestans in the inhibition of the snake enzyme PLA2 on a snake bite-related injury. For instance, alkaloids extracted from the *Azadirachta indica* (neem) have been shown to inhibit PLA2 activity from various snakes’ venoms (Yirgu and Chippaux 2019); other coumarin-type polyphenols isolated from leaves of *Ageratum conyzoides* act against visceral hemorrhages (Jasvidianto et al. 2020). In addition, *Emilia coccinea* has shown to be effective for treating infectious diseases (Prinsloo et al. 2018).

In this study, our preliminary qualitative analysis of chemical compounds in our batches of plants revealed the presence of phenols, total sugars, cardiotonic glycosides, flavonoids, tannins gallic, alkaloids, reducing sugar, deoxy sugars, terpenes, and mucilages. To a lesser extent, our plant extracts contained sterols, quinones, carotenoids, and saponosides. The relative abundance of chemical compounds in our plant extracts shows the presence of compounds with biological activity against snake envenomation. That observation is supported by results published by many other research teams. Indeed, over a decade ago, a team of researchers isolated alkaloids from the leaves of *Rauwolfia vomitoria* to treat edema (Fidele et al. 2014; Jasvidianto et al. 2020). More recently, reserpine alkaloids were isolated from *R. vomitoria* and showed to exhibit sedative properties on patients. Furthermore, it is documented that triterpenoids (lupeol acetate) neutralize the venom activity of *Naja kaouthia* by canceling the lethality, cardiotoxicity, neurotoxicity, and respiratory injury effects of the venom (Shabir et al. 2014).

Flavanoids are recognized for their numerous biological properties including, but not limited to anti-inflammatory, anticaner, cardioprotective, antioxidiant, and vein-tonic activities (Mpondo et al. 2012; Borah et al. 2019). Moreover, flavonoids isolated from root aqueous extracts obtained from the plant *Ophirrhiza mungos* showed to inhibit PLA2 contained in the venom of *Daboia russelii* (Krishnan et al. 2014; Vanessa et al. 2020). Also, rutin, a flavonoid glycoside is effective in increasing the survival time of rats injected with cobra venom. Rutin, quercitin, hesperidin, and bioflavonoids produce significant antinociceptive and/or anti-inflammatory activities (Gómez-Betancur et al. 2014). Phenolic compounds also present in our plant extracts are frequently mentioned in the literature as being responsible for inhibiting damaging activities induced by snake venoms (Gomes et al. 2010; Ambikabathy et al. 2011; da Silva et al. 2016; de Moura et al. 2018). *In vitro* assays of the polyphenol compounds obtained from aqueous extracts of seeds of *Pithecocobium dulce* and *Pentace burmanica* and nutgalls of *Quercus infectoria* indicated that these chemicals were able to neutralize cobra venom (Pereañez et al. 2011; Shabir et al. 2014). Similarly, several sesquiterpene types and flavonoids isolated from *Artemisia* species have shown to exhibit anti-inflammatory properties (Emami et al. 2010; Ruikar et al. 2011). In addition, many studies on polyphenolic compounds, including flavonoids and phenolic acids, have reported various activities for these secondary metabolites, including anti-ulcer, anti-inflammatory, and antioxidant (Kumar and Pandey 2013; da Silva et al. 2016; Borah et al. 2019). Tannins have an astringent activity that promotes vasoconstriction, an important parameter in hemostasis. These hemostatic and vasoconstrictor effects on small vessels, justify the use of tannins against varicose veins, wounds, and hemorrhoids (Ghedadba et al. 2014; Dibong et al. 2015). Tannins are typically used orally to assure vasoconstrictive function and limit the loss of body fluid (Pereañez et al. 2011; Ghedadba et al. 2014; Dibong et al. 2015). Tannins have also an inhibitory effect on snake venom enzymes (Gomes et al. 2010; Ambikabathy et al. 2011; Khameneh et al. 2019).
2011; Pereañez et al. 2011; Silva et al. 2016; de Moura et al. 2018). Not only tannins interfere with enzymatic activities of the snake venoms, but they also chelate metal ions such as zinc, copper, and iron found for instance in the venom of Bothrops (Patilito et al. 2012). The chelating effect of the extracts of Aniba fragrans was confirmed by SDS-PAGE when mixing the Bothrops atrox venom with the plant extracts (de Moura et al. 2018). Tannins isolated from aqueous extract of Mimoso pudica roots displayed inhibitory effects on the lethality, myotoxicity, and enzyme activities of Naja kaouthia venom (Sia et al. 2011; Pereañez et al. 2011). Detoxification, neutralization of neurotoxic and hemorrhagic effects of tannins were revealed from extracts of young persimmon fruits of Diospyros kaki (Ying and Chun 2016). Although sterols are not abundant in species we analyzed, it appears however that β-sitosterol and stigmasterol isolated in root extracts of Pluchea indica using methanol as solvent carry protective effects against the lethality of Naja kaouthia. Moreover, they showed to have neutralizing effects on respiratory dysfunction, neurotoxicity, cardiototoxicity, and on phospholipase A2 activity. Interestingly, sterols increased anti-serum activity by up to 35.5% (Shabir et al. 2014). Taken altogether, these results confirm the interest in medicinal plants as potential remedies against snakebites. In addition, this study opens new avenues for the use of natural compounds in clinical settings.

In conclusion, several plants have been mentioned to be traditionally used for the treatment of snakebite envenomation. However, only a few of them have been experimentally and scientifically validated. This report on medicinal plants used for treating snakebites in Gabon opens new avenues for the use of natural compounds in clinical settings. Further investigation at a national scale would bring new insight into ancestral knowledge and practices regarding the management of snakebites. Moreover, experimental studies would be of interest to scientifically valid or not, the use of plants that require such validation in treating snakebites in rural areas.

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