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Traditional knowledge and phytochemical screening of plants used in snakebite prevention in Benin

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Abstract

Background: Snake envenomation is a public health problem that has caused many deaths, disabilities and significant physical, psychological and socio-economic losses worldwide. In many rural communities, some plants have been utilized for preventing snake envenomation. The present study aims to document the knowledge of traditional medicine specialists on the plants used to prevent snakebites or repel snakes in Benin. A survey was conducted between January 2020 and September 2020 to this end. A total of 238 traditional healers and 56 hunters of different socio-cultural groups were interviewed using snowball sampling technique. Survey data were analyzed using Relative Citation Frequency. The diversity of antsnake bite plants was assessed using the Shannon diversity index, Pielou equitability, the Bray–Curtis similarity index and a Factorial Correspondence Analysis. Finally, qualitative phytochemical screening was performed on aqueous extracts following standard methods based on staining and/or precipitation reactions.

Results: A total of 74 plant species belonging to 36 families and 70 genera were identified. The most frequently encountered families were Leguminosae (27.3%), Euphorbiaceae (8.1%), Arecaceae, Asteraceae and Cucurbitaceae (4.5% each). *Annona senegalensis*, *Securidaca longipedunculata*, *Piliostigma thonningii*, *Chenopodium ambrosioides* and *Cymbopogon citratus* were the most cited species. The analysis of the intra-community diversity of antsnake bite plants from socio-cultural and sociolinguistic groups showed a high diversity and strong equi-representativeness of the plants used in the prevention of snakebites in Benin. Preliminary qualitative analysis of the bioactive compounds in extracts of the most cited antsnake bite plants revealed the presence of alkaloids, flavonoids, tannins, saponosides, mucilages, reducing compounds and triterpenoids.

Conclusions: In-depth pharmacological and phytochemical studies would make it possible to rationalize on the effectiveness of the identified medicinal plants as well as provide insights of the compounds responsible for their antsnake bite activity.

Keywords: Snake bites, Phytorepellents, Traditional medicine, Ethnobiology, Ethnobotany, West Africa

Background

Snakebite envenomation (SBE) is one of the 24 top killer neglected tropical diseases that predominantly affect rural communities in tropical and subtropical countries (Bawaskar et al. 2021; Cristino et al. 2021; Goldstein et al. 2021). It mostly affects poor communities in Africa, Asia, Oceania, Central and South America (Benjamin et al. 2020; Gómez-Betancur et al. 2019) especially those who are engaged in farming, gathering and hunting for

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their livelihood (Nduwayezu et al. 2020; Schneider et al. 2021; WHO 2021). Globally, snakebite envenomation affects more than five million people resulting in 25,000 to 125,000 deaths and approximately 400,000 people with permanent disabilities (Bawaskar et al. 2021). In West Africa, the annual cases of SBE mortality and amputations vary from 24 to 28 in Guinea-Bissau to 1,927 to 2368 in Nigeria (Habib et al. 2015). In a recent analysis (Halilu et al. 2019), the total annual burden of SBE in forty-one (41) countries of Sub-Saharan Africa was 1.03 million Disability Adjusted Life Years (DALYs) resulting from 268,471 SBE cases, 12,290 deaths, 14,766 amputations and 55,332 post-traumatic stress disorder (PTSD) cases. In Benin, health facilities record an average of 4,500 envenomations each year resulting in more than 650 deaths (Chippaux 2002a). The incidence and severity of snakebites is, however, unevenly distributed. They decrease along the humidity gradient. Majority of snakebite envenomation occurs in the rainy season with a greater incidence in the northern region of the country, and this is due to the abundance of *Echis ocellatus* in this part of the country (Chippaux 2002b; Fayomi et al. 1997). Treatment delays, like in other parts of the developing world, worsen prognosis and result in high medical care expenses to victims and health systems (Fourn et al. 2005; Massougbody et al. 2002).

Despite the high morbidities and mortalities from ophidic accidents, the only medically recognized therapeutic treatment for this neglected tropical disease is prompt administration of snake antivenom immunoglobulins (Gómez-Betancur et al. 2019; WHO 2021). Unfortunately, antivenom serum is usually not available when needed, is associated with anaphylaxis or serum sickness and does not prevent or reverse the local tissue damage induced by poisonous snake venoms (Devi et al. 2002). Some studies have also indicated that the antivenin sera may not be effective against the venoms of some medically important snakes (Alirol et al. 2015; Harrison et al. 2017; Potet et al. 2019; Visser et al. 2008). This failure of the health system coupled with fragmented records, limited access and prohibitive cost of antivenin sera makes it difficult to manage snakebites (Massougbody et al. 2002). Thus, more than 80% of patients resort to traditional medicine as a first-line treatment (Chippaux 2002b). Herbal remedies used to manage snakebite envenomation have been reported and documented through various ethnobotanical studies in many indigenous communities around the world (Mansoor and Sanmugarajah 2018; Modak et al. 2020; Okot et al. 2020; Omara 2020; Omara et al. 2020; Yirgu and Chippaux 2019). Ethnobotany and phytomedicine have been indicated to have partly failed to deliver novel treatments for poisonous snake envenomation (Trim et al. 2020; Vieira et al. 2021), but the use

of plants as prophylactics for snakebites has been historically practiced (Chekole 2017; Clark and Shivik 2002; Ilondu and Lemy 2018; Mekuanent et al. 2015; Omara et al. 2021b; Renapurkar et al. 1991). However, very few studies have surveyed plants with repellent or anti-snakebite activities. These plants when smoked, planted or sprinkled around farms and homesteads feature as potential irritants capable of repelling or dissuading snakes. In order to contribute to the reduction in mortality and consequences linked to snakebites, these plants should therefore be correctly identified, investigated and publicized which could improve the quality of life of rural communities. Moreover, the inventory of plants used in the treatment or prevention of a disease through ethnobotanical surveys does not necessarily attest to their effectiveness but constitutes a step in the research and development of new herbal remedies (Dossou and Fandohan 2021; Kouchadé et al. 2017). Plant resources are an important source of bioactive compounds responsible for their biological activities (Al-Snafi, 2021; Mengome et al. 2021). However, these bioactive substances as well as their harmfulness or not are unknown by the communities that use them. Ignorance of these aspects could have enormous consequences on the lives of their consumers (Mangambu et al. 2015). The use and promotion of medicinal plants will now have to be based on conclusive pharmacological and phytochemical studies (Kouchadé et al. 2017). This is the justification of the current study, which aimed to assess traditional knowledge of plant species used by rural population of Benin to prevent snakebite envenomation.

Methods

Description of the study area

This study was conducted in rural communities of Benin (Sub-Saharan Africa), one of the regions of the world with high burden of snake envenomation. Located in the “Dahomey Gap”, the Republic of Benin share common frontiers with Niger and Burkina Faso in the north, the Atlantic Ocean in the south, the Republic of Togo in the west and Nigeria in the east. It covers an estimated area of 114,763 km² (Ayihouenou et al. 2016). Depending on climatic and edaphic conditions, Benin is characterized by 10 phytodistricts contracted into 3 biogeographical zones, in particular Sudanian zone, Sudano-Guinean transition zone and Guineo-Congolese zone (White 1983).

According to Judex et al. (2009), 55 local languages are spoken by Beninese. Depending on their ethnicity, the Mahi, Adja, Fon, Aizo, Sahoue and allies (Kwa sociolinguistic group) are the socio-cultural groups encountered mainly in the Guinean-Congolese region. The sociolinguistic group Gur comprising mainly the socio-cultural

groups Berba, Waama, Ditammari, Dendi, Natimba and allies is found much more in the north of the country (Sudanian region). In the center of the country, particularly in the Sudano-Guinean transition zone, Idaasha, Nagot, Yoruba (Yoruboid sociolinguistic group) are the main socio-cultural groups encountered (Lewis 2009). The official language is, however, French. The literacy rate is about 38.4% of the total population (52.2% male and 23.6% females) (Wikipedia 2021). Benin is purely an agrarian country that relies on rain-fed agriculture and fishing activities which contributes to about 70% of employment for 1.2 million households and 30% of the gross domestic product (FindScope Benin 2019; Maboudou and Niehof 2020). At least 550,000 smallholders farming on 1.7 hectares dominate the agricultural sector of Benin (IFAD 2021).

Sampling and data collection

The present study was undertaken between January 2020 and September 2020. It was carried out in the 10 phytodistricts of Benin (Coast, Pobè, Plateau, Ouémé-Valley, Zou, Borgou-Sud, Bassila, Borgou-Nord, Atacora Chain, Mékrou-Pendjari) in two major socio-cultural groups per phytodistrict (Fig. 1). Based on non-probability snowball non-probability technique (N'Danikou et al. 2015), a survey questionnaire was used to collect ethnobotanical data using a semi-structured interview with traditional healers (238) and hunters (56) (major holders of endogenous knowledge) with proven knowledge of plants used in preventing snake envenomation. To identify the informants, the oldest residents in the community were contacted who identified the main informants recognized by the community as experts in the use of medicinal plants in snakebites management. The criteria used to select the respondents was that he/she should have a good knowledge of medicinal plants and must have been living in the area for at least five years (Ribeiro et al. 2017). Herbalists who had no knowledge of used in preventing snakebites or avoiding snakes were excluded from the study. Then, the identified consenting informants were asked to indicate any other experts with similar competence. The number of interviewees was deemed representative when a saturation limit was attained. This saturation limit was defined as the point at which there are no new names of key informants being mentioned by the last interviewee. At this point, the plot of the number of key informants becomes asymptotic with the addition of any new interviewee (N'Danikou et al. 2015; Ribeiro et al. 2017). The plant samples were collected from their sources along with each interviewee and the botanists who performed the botanical identification and authentication. The inventoried species were collected, identified and voucher samples deposited in the herbarium at

Laboratory of Crops, Horticultural and Forest Sciences of “*Université Nationale d’Agriculture*” of Benin. Botanists and documents of Benin flora, in particular “*Flore du Bénin*” (de Souza 2008) and “*Flore Analytique du Bénin*,” were used (Akoègninou et al. 2006) while confirmation of the identified species was also done at the National Herbarium of Benin. The verification of scientific names and families of species was done at the international index of plant names (IPNI: <http://www.ipni.org>) and the website of the phylogeny of species (<http://www.mobot.org/MOBOT/research/APweb>), respectively.

Data analysis

The ethnobotanical data obtained were analyzed using simple descriptive statistics in R software (cran.r-project.org).

Intra-community diversity of antisnake bite plants

Shannon’s Diversity Index and Pielou Equitability Index were used to assess the intra-community diversity of plants used by local communities in the prevention of snakebites. The Shannon–Wiener Diversity Index was calculated using Eq. 1 (Mulya et al. 2021).

$$\left\{ \begin{array}{l} H = - \sum_{i=1}^{U_s} p_i \log_2 p_i \\ p_i = n_i / N \\ N = \sum_{i=1}^{U_s} n_i \end{array} \right. \quad (1)$$

where H = the Shannon diversity index for a given socio-cultural or sociolinguistic group; n_i = number of citations for each species, and N = total number of citations for a given socio-cultural or sociolinguistic group. The Shannon diversity index generally varies between 1 and 6 bits and is distributed as if after:

- If $H \in [1; 2.5 [$, then the antisnake bite flora is not very diversified;
- If $H \in [2.6; 3 [$, then the antisnake bite flora is moderately diversified;
- If $H \in [4; 6 [$, then the antisnake bite flora is very diverse.

Pielou Equitability Index or Pielou Evenness (E) was calculated using Eq. 2 (Heath, 2021).

$$E = \frac{H}{\log_2 S} \quad (2)$$

From which H = Shannon diversity index, S = Number of plant species cited by the group considered.

Pielou Equitability Index makes it possible to measure the degree of equitable representation of each species or

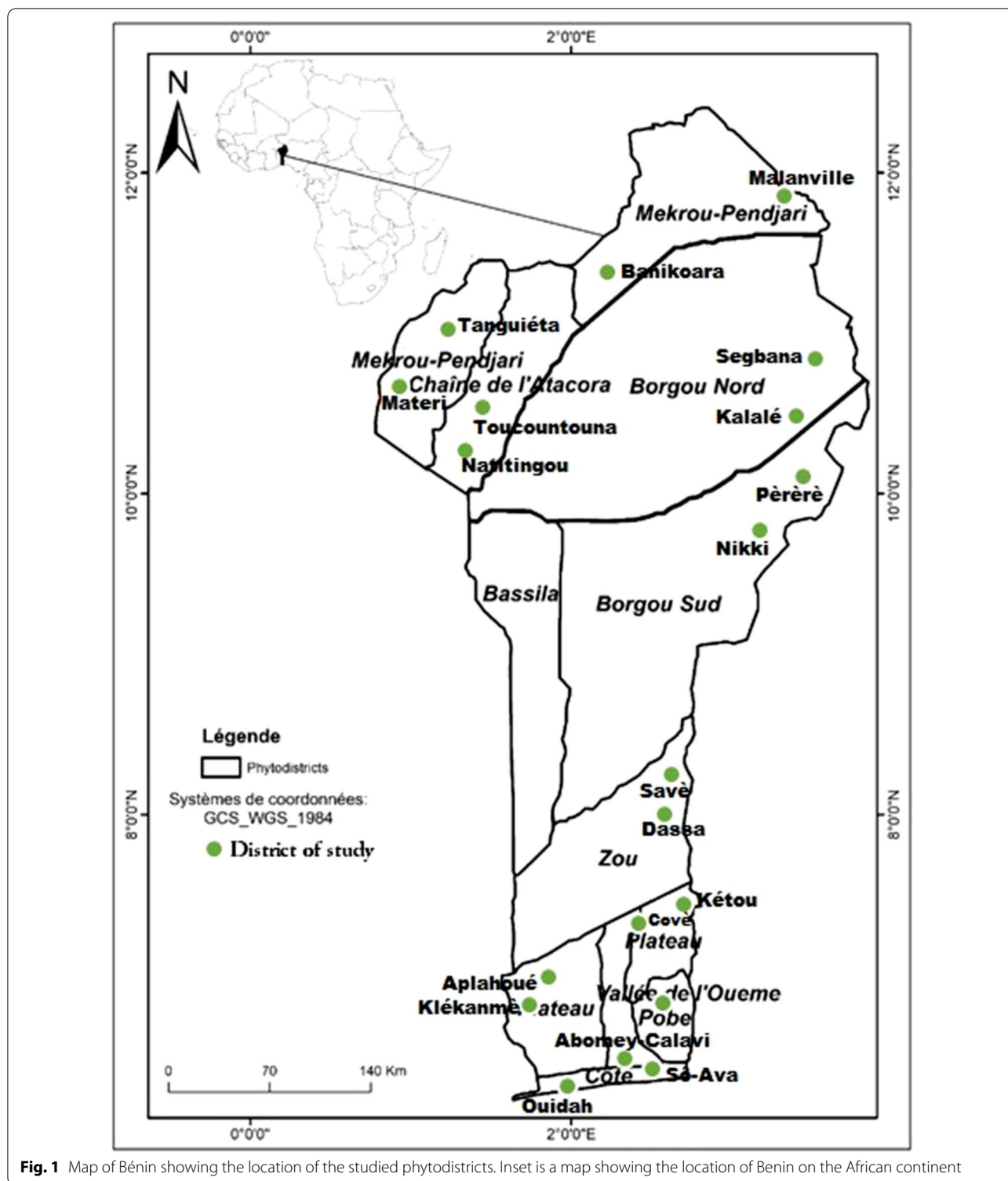


Fig. 1 Map of Bénin showing the location of the studied phytodistricts. Inset is a map showing the location of Benin on the African continent

the relative weight of each species within the antsnake bite flora of a given community. It is between 0 and 1. When;

$E=0-0.6$, a small group of plants are relatively predominant in the antsnake bite flora of the people considered.

$E > 0.6$, then most species have relatively the same importance in the antsnake bite flora of the people considered.

Floristic composition of antsnake bite plants

Assessment of knowledge on plants used to prevent or avoid snakebites in Benin was done using the Relative Frequency of Citation (RFC) utilizing Eq. (3) (Vitalini et al. 2013).

$$\text{RFC} = \frac{n}{N} \times 100 \quad (3)$$

where n = number of respondents who cited the species, and N = total number of respondents who participated in the study.

Variation of knowledge on antsnake bite plants between communities

In order to assess the variation in knowledge within the communities taken in pairs, the Bray–Curtis index (dissimilarity) was determined for two (sociolinguistic or sociocultural) groups A and B using Eq. 4 (Callisto et al. 2021).

$$C_n = 1 - \left[\frac{2JN}{(N_a + N_b)} \right] \quad (4)$$

where in C_n = Bray–Curtis index; JN = sum of the smallest numbers of citations of species common to A and B; N_a = Total number of citation of species by the sociocultural or sociolinguistic group A and N_b = Total number of citation of species by the sociocultural or sociolinguistic group B. Two communities A and B are similar if C_n is equal or close to 0 and dissimilar if C_n equal or close to 1.

Use of significant species by sociocultural and sociolinguistic groups

To appreciate the relationship between sociocultural groups, sociolinguistic groups and endogenous knowledge about plants used to prevent snakebites, a Factorial Correspondence Analysis (FCA) was performed on the frequency of citation of the ten (10) plant species the most cited.

Preliminary phytochemical qualitative analysis of the main plants used in the prevention of snakebites

This study was conducted at Laboratoire de pharmacologie et des huiles essentielles of the Faculty of Sciences and Techniques (FAST) of the University of Abomey-Calavi, Bénin.

Ten (10) plants species most used in the prevention of snakebites in Benin were used for preparation of aqueous extracts. The reported organs of the most cited species

were shade-dried for one month. Each dried organ was then reduced to a fine powder using an electric grinder and stored in a small dry jar, hermetically sealed in order to avoid any contamination. Measured 25 g of dried powder of each organ was separately macerated in 250 ml of distilled water and then boiled for 15 min as per the procedures used by the local people of Benin. The decoctions were filtered separately to obtain the aqueous extracts.

Phytochemical screening

Qualitative phytochemical screening was performed on aqueous extracts using the standard methods based on staining and/or precipitation reactions as described by Houghton and Raman (1998) and Houmènou et al. (2017). The different chemical groups screened and the principle of the reactions are presented in Table 1.

Results

Socio-demographic characteristics of the informants

A total of 294 experienced respondents, including 238 traditional healers (traditional medicine practitioners) and 56 hunters, were interviewed in this study. The population under study is made up of people from 17 socio-cultural groups. The Nagots (12.59%), Mahis (8.84%), Adjias (8.50%), Baribas (7.82%) and Yoruba (7.14%) were the most represented. More than half of the interviewed traditional medicine practitioners were over 50 years old, with a mean age of 51 years. The age of the respondents ranged from 10 to 105 years (Table 2). Two respondents were less than 20 years, and these were included because they were known to have proven knowledge of the use of plants in snakebite management. Majority of respondents in this study were men (98.30%). Majority of the respondents were illiterate (51.02%) followed by those who had the chance to go to primary school (37.07%) while the remaining percentage had gone beyond primary education. About 82% of the people surveyed drew their knowledge from experiences of parents or relatives to prescribe medicinal plants followed by learning which represents only 14%. On the other hand, research, dreams and intuition are very little used modes of transmission and acquisition of endogenous knowledge.

Intra-community diversity of antsnake bite plants

Globally, there is an average diversity of antsnake bite plants both within sociolinguistic and sociocultural groups ($H \in [2.6; 3]$). This diversity is high in Yoruboid and Bariba ($H > 4$) (Table 3). On the other hand, only within the socio-cultural group Toffin there is a low diversity of species. Moreover, this diversity is evenly distributed within the respondents of most of the groups considered ($E > 7$).

Table 1 Test for the detection of phytochemicals. Source: Houghton and Raman (1998)

Phytochemical compounds	Test	Observations (positive results)
Alkaloids	Mayer's test	Yellowish-white precipitate
	Dragendorff's test	Orange-red precipitate
Tannins	Ferric chloride test	Blue-black coloration
Catechic tannins	Stiasny's test	Precipitated pink
Gallic tannins	Ferric chloride and saturation with sodium acetate	blue tint
Flavones	Shinoda test with magnesium powder	Orange coloration
Anthocyanins	Hydrochloric acid and 50% ammonia	Purplish red coloration
leuco-anthocyanins	Shinoda's test	Brown red coloration
Quinonic derivatives	Borntrager's test	pink or purplish red coloration
Saponosides	Foam index test	Significant presence of foam of at least 1 cm
Triterpenoids	Liebermann–Burchard test	Blue, green or purple Coloration
Steroids	Kedde's test	Red wine coloration
Mucilages	Absolute alcohol test	Flaky precipitate
Reductive compounds	Fehling liquor test	Brick red precipitate
Free anthracenes	Test with chloroform and ammonia	No red coloring

Floristic composition of anti-snakebite plants

This ethnobotanical survey identified 74 species of plants used by rural communities in Bénin to prevent or avoid snakebites (Additional file 1: Table S1). These species belong to 70 genera and 36 families, of which the most represented families were Leguminosae (with 20 species), Euphorbiaceae (with 6 species), Arecaceae, Asteraceae and Cucurbitaceae with 3 species each. The genera with the most used plant species were *Acacia*, *Allium*, *Jatropha* and *Piliostigma* which were each represented by two species each. Quantitative assessment of the medicinal plant species identified on the basis of RFC showed that *Annona senegalensis* Pers. (RFC = 35.14%), *Securidaca longipedunculata* Fresen. (RFC = 32.77%), *Piliostigma thonningii* (Schumach.) Milne-Redh. (RFC = 29.39%), *Chenopodium ambrosioides* L. (RFC = 8.45%) and *Cymbopogon citratus* (DC.) Stapf (RFC = 7.09%) were the most mentioned by the respondents.

With regard to the growth habit of the mentioned species, most remedies were made from herbs (36.49%) and trees (35.14%) (Fig. 2) mainly harvested by rural communities from savannas (47.30%), home gardens or around houses (40.54%) and in fallows (39.19%) (Fig. 3).

Parts used, preparation and administration of the antisnake bite plants

Several parts of the identified plants such as roots, leaves, bark, fruits, flowers, resin, stem, bulb, sap and whole plant singly or sometimes in combination are used in the preparation of anti-snakebite recipes by rural communities of Benin. Leaves (36.81%), roots (33.41%), whole plant (11.37%) and bark (9.26%) were the most cited plant

organs used (Fig. 4). On the other hand, tubers, resin and sap are less frequently used.

The methods of preparation and administration of anti-snakebite recipes vary from one plant or individual to another. Five (5) methods have been used for preparing anti-snakebite recipes in Benin. Pounding (43%) and incineration (36%) of plant materials are the most used preparation methods. Other methods of preparation include maceration, trituration and decoction but are used to a lesser extent by rural communities (Fig. 5). The high frequency of pounding (dry or fresh) and incineration of plant organs is due to the need to keep such recipes for a long-time to ensure their availability for the prevention of snakebites. Preparation of these recipes is accompanied by special provisions which are not scientifically explainable but are used by the traditional medicine practitioners surveyed to guarantee effectiveness of recipe.

As for forms of administration, oral intake (48%) and scarification (32%) are the most used. Some plants are also cultivated around houses, or their parts are stored in houses to repel snakes (15%). Herbal baths utilizing the medicinal plants on the other hand is rarely used in the administration of the antisnake bite recipes (Fig. 5). The main solvents used in the preparation and administration of anti-snakebite recipes are water and *sodabi* (a local liquor).

Myths around the use of anti-snakebite plants in Bénin

The interviewees affirmed mostly that success of preparation of anti-snakebite recipes and their effectiveness require some special arrangements. It is necessary, for example, to make special arrangements for harvesting

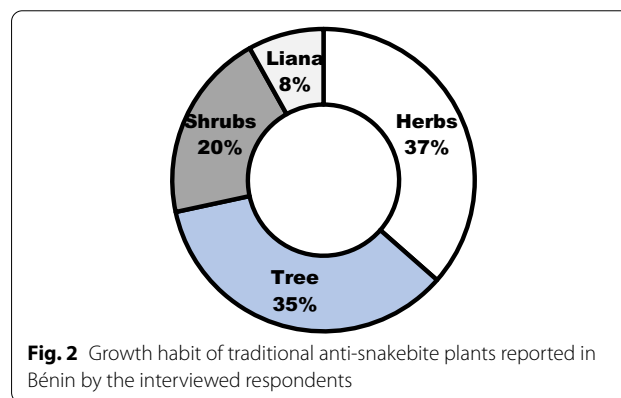
Table 2 Socio-demographic characteristics of respondents

Variables		Number of Respondents	Proportion (%)
Sex	Male	289	98.30
	Female	5	1.70
	Total	294	100
Age (years)	< 20	2	0.68
	20–30	12	4.08
	30–40	43	14.63
	40–50	83	28.23
	50–60	69	23.47
	> 60	83	28.23
Education levels	None	159	51.02
	Primary	109	37.07
	Secondary	31	10.54
	Superior	4	1.36
Occupation	Traditional healers	238	80.95
	Hunters	56	19.05
Socio-cultural groups	Adja	25	8.50
	Aïzo	16	5.44
	Bariba	23	7.82
	Berba	13	4.42
	Dendi	18	6.12
	Fon	20	6.80
	Holli	17	5.78
	Idaatcha	15	5.10
	Lokpa	11	3.74
	Mahi	26	8.84
	Nagot	37	12.59
	Nateni	10	3.40
	Peulh	13	4.42
	Toffin	7	2.38
Waama	12	4.08	
Yom	10	3.40	
Yoruba	21	7.14	

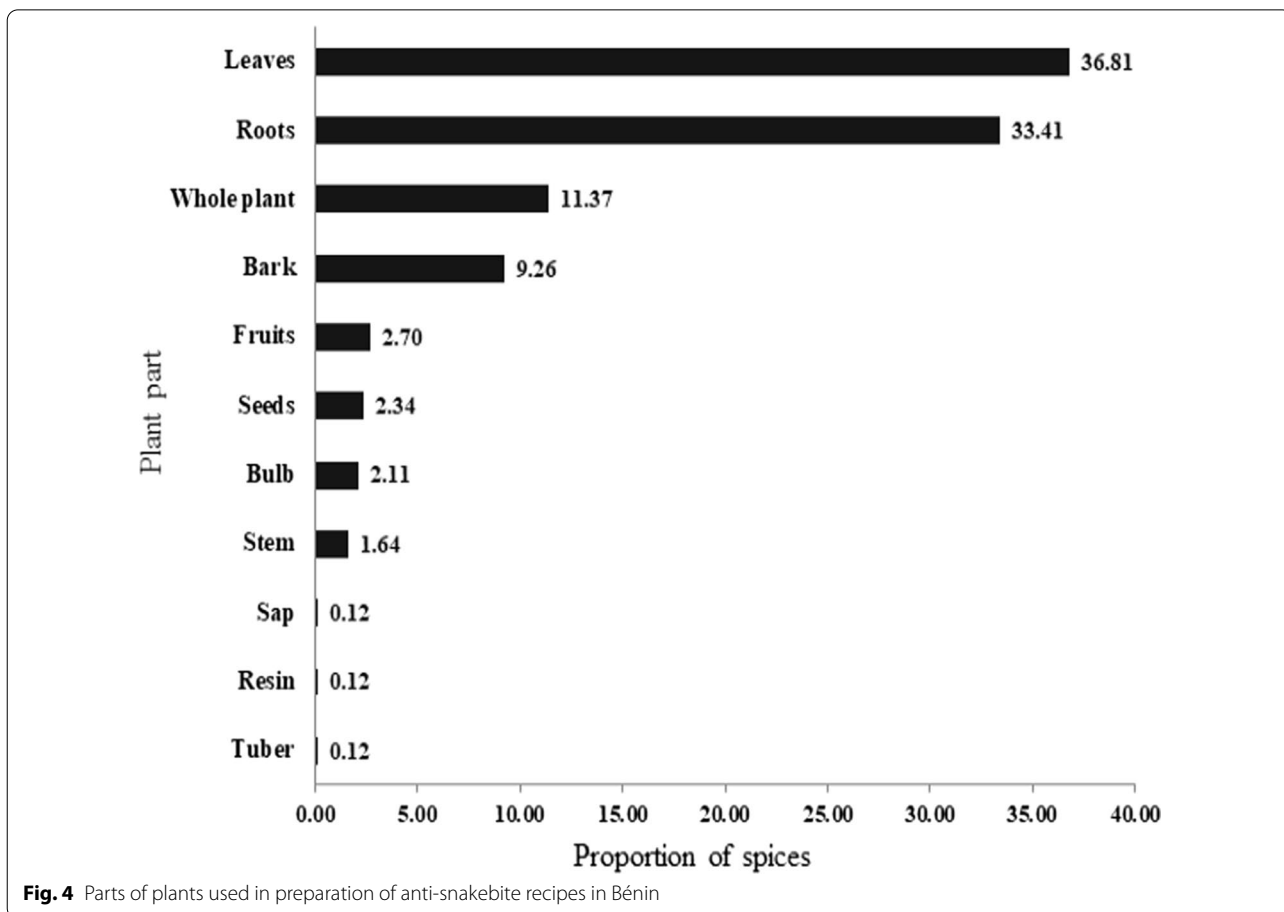
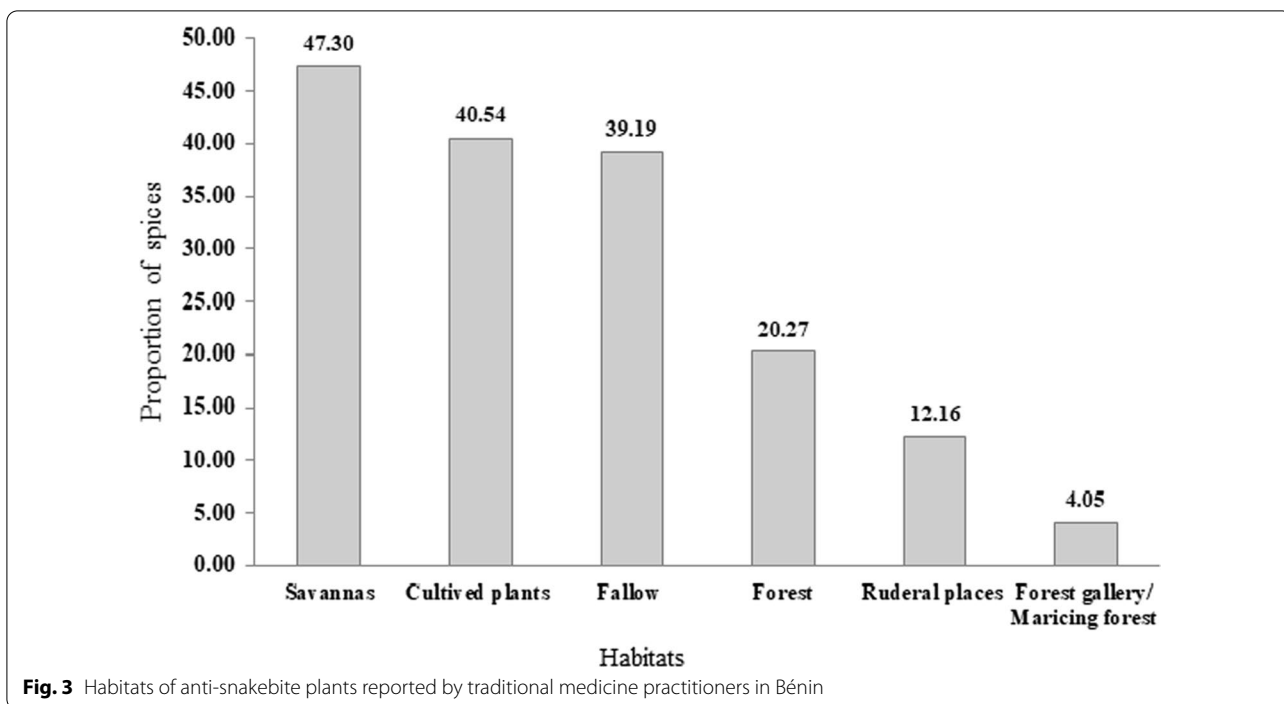
Table 3 Indices of diversity of plant species used to prevent snakebites in Benin

		H	E
Sociolinguistic groups	Kwa	2.82	0.79
	Gur	3.88	0.75
	Yoruboid	4.52	0.85
Socio-cultural groups	Adja	2.93	0.79
	Aïzo	2.81	0.85
	Bariba	4.05	0.92
	Berba	2.37	0.84
	Dendi	2.75	0.74
	Fon	3.41	0.85
	Holli	3.15	0.85
	Idaatcha	3.08	0.93
	Lokpa	2.72	0.91
	Mahi	2.79	0.84
	Nagot	3.34	0.77
	Nateni	2.59	0.92
	Peulh	3.73	0.91
	Toffin	1.99	0.77
Waama	2.85	0.82	
Yom	3.30	0.95	
Yoruba	3.54	0.91	

plant materials. According to the practitioners, plant material collection very early in the morning is recommended because it is believed that wishes formulated early in the morning and before talking to anyone are often granted. Once the plant material has been collected, it is strongly recommended to testify to its gratitude to the plant and above all to leave without looking back. Recipes involving *Piliostigma thonningii* (Schumacher.) and *Schrankia leptocarpa* DC. call for the use of young (unopened) leaves. It is also necessary to be in a state of spiritual purity (not having had a sexual act 24 h before) in order to ensure the effectiveness of the remedies. To all these are added incantations by traditional healers who ensure, and guarantee quality of rituals



linked to the various preparations. Harvesting of some plant parts, for example, *Milicia excelsa* (Welw.) C.C. Berg requires permission from local dignitaries and a ritual requiring a sheep to be offered as a sacrifice to the tree. Preparation of almost all anti-snakebite recipes calls for systematic addition of *Aframomum melegueta* (Roscoe) and parts of snakes including snake sloughing and viper head. Preparation of sacred rings and amulets worn to avoid being bitten or approached by a snake are also done. Scarification cuts are made according to sex of the individual; these are supposed to be seven (7) or nine (9), respectively, in the case of a woman or a man.



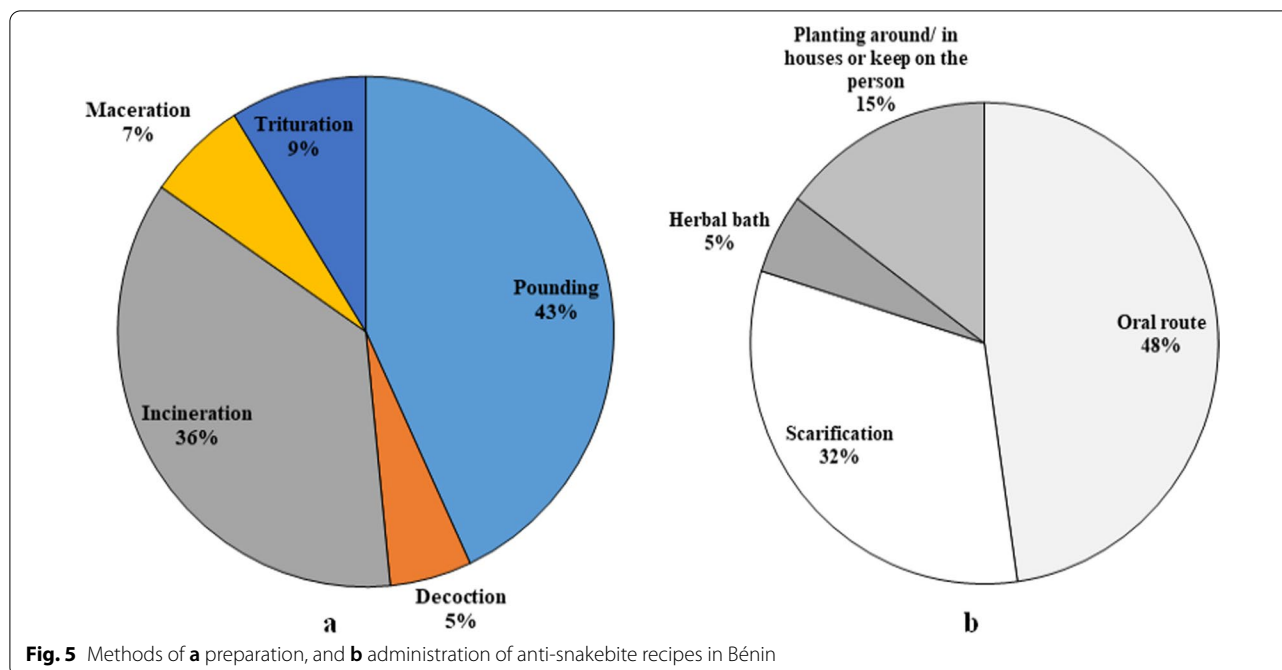


Table 4 Bray–Curtis index (Cn) according to sociolinguistic groups

	Kwa	Gur	Yoruboïd
Kwa	0		
Gur	0,74	0	
Yoruboïd	0,55	0,62	0

Variation of knowledge on antisnake bite plants between communities

The results of the Bray–Curtis index showed that knowledge of antisnake bite plants suggests a certain similarity between the Yoruboïd group and the Gur group on the one hand and the Yoruboïd group and the Kwa group on the other hand (Cn < 0.7). However, the Kwa group and the Gur group show a relatively large dissimilarity (Cn > 0.7) (Table 4). Likewise, the comparison of socio-cultural groups indicates dissimilarity between them in terms of knowledge about the plants used in the prevention of snake bite. A similarity of knowledge and uses of plants is observed at the level of the Adja-Mahi, Aïzo-Fon, Berba-Dendi, Berba-Nateni, Berba-Waama pairs (Additional file 2: Table S2).

Use of significant species by sociocultural and sociolinguistic groups

The results from the FCA showed that the correlation between the variables is explained by the first two axes at

100% and 57.95%, respectively, for sociolinguistic groups and sociocultural groups. These results show that the use of *Parkia biglobosa*, *Cymbopogon citratus* (DC.) Stapf, *Ocimum gratissimum* L. in the prevention of snakebites is more noticeable in the Yoruboïd sociolinguistic group, whereas in the Gur it is rather *Tamarindus indica*, *Momordica charantia* L., *Annona senegalensis*. Moreover, *Chenopodium ambrosioides* L. and *Allium cepa* L. are more used by Kwa. *Annona senegalensis*, *Securidaca longipedunculata* and *Piliostigma thonningii* are more used by the Yoruboïd and Gur.

Considering socio-cultural groups, *Momordica charantia* and *Ocimum gratissimum* are used more by the Holli, Yoruba and Yom. *Tamarindus indica*, *Securidaca longipedunculata*, *Annona senegalensis* are the main plant species used by the Dendi, Nateni, Waama, Berba, Bariba, Peulh, Lokpa to repel or prevent snake bites while the Mahi, Toffin, Adja, Aïzo, Idaatcha prefer use *Chenopodium ambrosioides* L., *Cymbopogon citratus*, *Allium cepa*, *Parkia biglobosa* and *Piliostigma thonningii* for the same purpose (Fig. 6).

Preliminary phytochemical qualitative analysis of the main plants used in the prevention of snakebites

Table 5 presents the 10 plant species most used to prevent snakebites in Benin, selected for qualitative phytochemical analysis based on the value of the RFC.

Several phytochemical compounds have been highlighted through the phytochemical screening of the organs of the main plant species used in the prevention

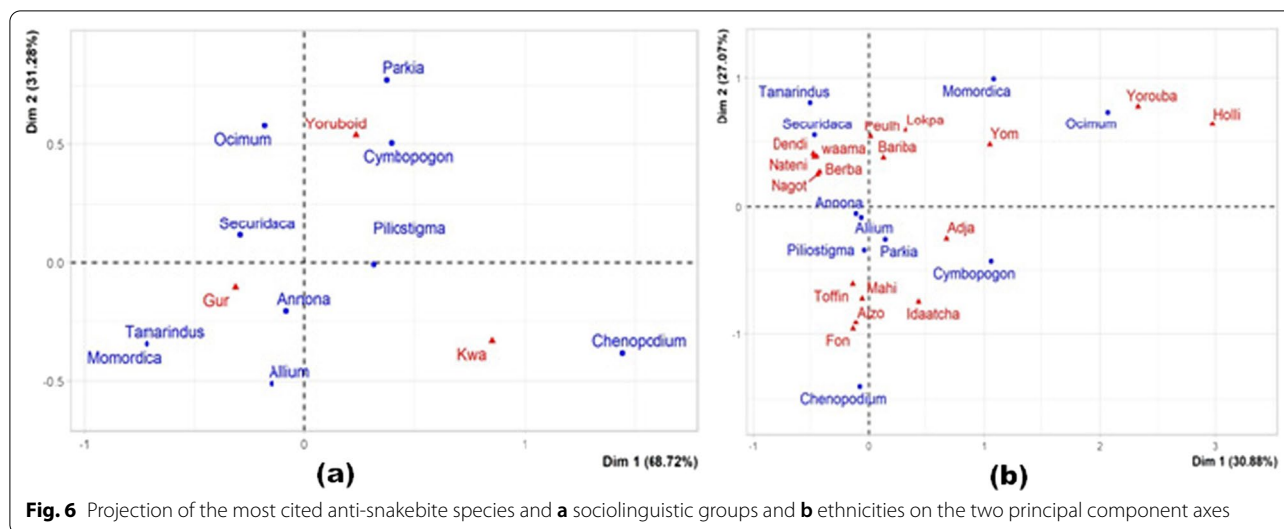


Fig. 6 Projection of the most cited anti-snakebite species and **a** sociolinguistic groups and **b** ethnicities on the two principal component axes

of snakebites in Benin. The chemical analysis of the organ samples of the main plants used shows that all the extracts have a diverse and varied composition of bioactive compounds (Table 6). None of the samples alone has all the chemical compounds. The aqueous extracts of *Ocimum gratissimum* and *Annona senegalensis* are richer in the chemical elements sought (68%) followed by *Cymbopogon citratus* (DC.) Stapf., (56%) and *Piliostigma thonningii* (Schumach.) Milne-Redh., *Securidaca longipedunculata* Fresen, *Parkia biglobosa* (Jacq.) R.Br. ex Benth., which, respectively, contain 50% of the desired chemical compounds. *Allium cepa* L. is the species with the least richness in the chemical elements sought. Tannins (90%), catechic tannins (90%), reducing compounds (80%) and alkaloids (80%) are the most present bioactive compounds in all plants. On the other hand, anthocyanins are the only compounds absent in all the samples.

Table 5 List of plant species and parts used for preparing aqueous extracts for phytochemical screening

S/N	Botanical name	Part used	RFC
1	<i>Annona senegalensis</i> Pers	Leaves	35.14
2	<i>Securidaca longipedunculata</i> Fresen	Leaves	32.77
3	<i>Piliostigma thonningii</i> (Schumach.) Milne-Redh	Leaves	29.39
4	<i>Chenopodium ambrosioides</i> L	Whole plant	8.45
5	<i>Cymbopogon citratus</i> (DC.) Stapf	Leaves	7.09
6	<i>Ocimum gratissimum</i> L	Leaves	6.08
7	<i>Allium cepa</i> L	Bulb	5.74
8	<i>Tamarindus indica</i> L	Leaves	4.73
9	<i>Parkia biglobosa</i> (Jacq.) R.Br. ex Benth	Leaves	4.73
10	<i>Momordica charantia</i> L	Whole plant	4.39

Discussion

Snake envenomation is a medical emergency prevalent in many tropical and subtropical areas of the world (Steinhorst et al., 2021). Because they mostly occur in rural settings, traditional therapists end up being consulted as the first-line defense for treatment. Such traditional healers are rich with knowledge of snakebites, and how they can be prevented or treated. An in-depth understanding of these aspects is critical to improve and extend snakebite-related healthcare (Steinhorst et al. 2021).

This study identified medicinal plant species used to avoid or prevent snakebites in Benin. The respondents were predominantly adult men, which corroborates a previous ethnobotanical survey by Fanou et al. (2020) in Benin where all the traditional healers were men, but women dominated as market herbal dealers. The elderly (greater than 60 years old) and young people (40–50 years old) had more diversified and rich knowledge about plants used to prevent or avoid snakebites. This could be explained by the fact that knowledge of traditional medicine is transmitted mainly from ascendants to descendants so that these two categories of people have almost the same level of traditional knowledge (Agbankpé et al. 2014; Dougnon et al. 2017; Fanou et al. 2020). This is even more justifiable given the fact that most of the interviewed practitioners in this study reiterated that they inherited ethnobotanical knowledge from their families. It should be noted that knowledge on the use of plants and their properties for prevention and treatment of especially complex ailments such as snakebites is generally acquired through experience accumulated over years and is transmitted from one generation to another (Dougnon et al. 2017). This is because such knowledge is usually a guarded family secret. This

Table 6 Secondary metabolites identified in the organs of the most cited plant species used to prevent snakebites in Benin, West Africa

Phytochemicals	A*	B	C	D	E	F	G	H	I	J
Alkaloids	+	-	+	+	+	+	+	-	-	+
Tannins	+	+	+	+	+	+	+	+	+	-
Catechic tannins	+	+	+	+	+	+	+	+	+	-
Gallic tannins	-	-	-	-	+	-	-	-	+	-
Flavonoids	-	+	-	-	-	-	-	+	+	-
Anthocyanins	-	-	-	-	-	-	-	-	-	-
Leuco-anthocyanins	+	-	+	+	-	-	-	+	-	-
Quinonic derivatives	+	-	-	-	+	-	-	-	-	-
Steroids	+	-	-	+	+	-	+	-	-	-
Triterpenoids	+	+	-	-	+	-	-	-	-	+
Saponosides	-	+	+	+	+	+	-	-	+	-
Mucilages	+	-	+	-	+	+	+	+	+	-
Reductive Compounds	+	+	-	+	+	-	+	+	+	+
Free anthracenes	-	-	+	+	-	-	+	-	+	-
Proportion of presence	68.75	43.75	50	50	68.75	37.5	43.75	50	56.25	18.75

*Legend: + means present; - means absent (Omara et al. 2021a). Plant species: A = *Annona senegalensis*; B = *Tamarindus indica*; C = *Securidaca longipedunculata* Fresen.; D = *Parkia biglobosa*; E = *Ocimum gratissimum*; F = *Chenopodium ambrosioides*; G = *Momordica charantia*; H = *Piliostigma thonningii*; I = *Cymbopogon citratus*; J = *Allium cepa*

observation is concordant with reports from other countries, in which knowledge of medicinal plants and antisnake bite plants are acquired and passed on orally from the elders to the young, including apprenticeships from relatives (Okot et al. 2020; Owuor and Kisangau 2006).

This study identified 74 plants used by traditional medicine professionals to prevent or avoid snakebites in Benin. The species belong to 36 families, with the most mentioned being Leguminosae, Euphorbiaceae, Arecaceae, Asteraceae and Cucurbitaceae. *Annona senegalensis*, *Securidaca longipedunculata*, *Piliostigma thonningii*, *Chenopodium ambrosioides* and *Cymbopogon citratus* were the most frequently cited species. These families, genera as well as species have been cited in previous ethnobotanical studies carried out in different geographical areas of the world as having snake repelling and antiophidic potential, particularly in Nigeria, Kenya, Ethiopia, Uganda, Pakistan, Sri Lanka and India (Adzu et al. 2005; Amlabu et al. 2014; Dharmadasa et al. 2016; Ilondu and Lemy 2018; Okot et al. 2020; Omara 2020; Yirgu and Chippaux 2019). For example, *Annona senegalensis* (stem bark), *Allium cepa* and *Allium sativum* (bulbs), *Cymbopogon citratus* (leaves) and *Nicotiana tabacum* (leaves and whole plant) have been reported to be used for wading off snakes from human settlements in Nigeria, East Africa and Ethiopia (Ilondu and Lemy 2018; Omara et al. 2021b). In most cases, the plants are grown in the home vicinity, burnt to produce smoke or plant decoctions are made and sprinkled around the house. As explained by Ilondu and Lemy (2018) and Omara

et al. (2021b), snakes are dissuaded by the bad smell of such plants or the plants causes discomfort and disorientation to the snakes. Further, species such as *Allium cepa* (bulb), *Allium sativum* (bulb), *Annona senegalensis* (leaves and roots) and *Securidaca longipedunculata* (root bark, leaves and roots) have been reported to antagonize the activities of *Naja naja karachiensis* (Asad et al. 2014a, 2013, 2014b), *Echis ocellatus*, *Naja nigricollis nigricollis*, *Bitis arietans* (Adzu et al. 2005; Amlabu et al. 2014; Asad et al. 2014b) and *Naja nigricollis* Hallowel venoms (Sanusi et al. 2014; Wannang et al. 2005). However, it is not direct to relate such antivenom activities to the antisnake bite activity of the plants identified in this study. By making a comparison with the plants used to treat snakebites in Benin (Dossou et al. 2021), we find that the antisnake bite and antivenom plants are more than 50% identical. Indeed, 55 out of 74 plant species (74.32%) identified by local communities in Benin as used to prevent or repel snakebites are also snake antivenom species. Thus, only 19 and 54 species are strictly antisnake bite and antivenom among the plant species identified. Species identified are mainly herbs and trees harvested around houses, in savannas and fallow land. The remarkable presence of herbaceous plants could be explained by proximity and perennial nature of these species, which makes it possible to have at least one organ in each season (Mangambu et al. 2015).

Various myths were identified in the use of medicinal plants to prevent snakebites in Benin. Similar practices as those witnessed in this study have been reported in other

countries. For example, *Cyphostemma adenocaula* roots are tied around the body in Ethiopia as a snake repellent (Giday et al. 2013). Burning of clothes in Kenya and burning of bicycle, motorcycle and vehicle tyres to discourage snakes were reported among the Lango people of Northern Uganda (Omara 2020; Omara et al. 2020). In the same region, maintaining a container of water some distance away from the house has been indicated as an effective strategy of keeping snakes away from homes and thus avoiding snakebites (Omara 2020).

The analysis of the intra-community diversity of anti-snake bite plants from socio-cultural and sociolinguistic groups has shown that there is a strong diversity and a strong equi-representativeness of the plants used in to prevent envenomations by snakebites in Benin. This remarkable and varied diversity of plants used to prevent envenomation by snakebites in Benin could be linked not only to the degree of use and knowledge of plants by local communities (Etame-Loe et al. 2018) but also and especially to the environmental conditions responsible for the availability and distribution of the species listed (Lawin et al. 2019).

Knowledge of the plants used to prevent snakebites has shown the existence of a degree of similarity between the antiophidic flora of the Yoruboid and Kwa groups, on the one hand, and those of Yoruboid and Gur on the other hand, while the similarity is weak between Gur and Kwa. This could on the one hand be due to climatic and environmental conditions and to the availability of the resources (Avocèvou-Ayisso et al. 2012; Fandohan et al. 2017) and on the other hand to the existence of an interaction or an inter-ethnic transfer of endogenous knowledge and knowledge linked to intercultural mixing created either by marriage, cohabitation over a long period of time or even belonging to the same religious denomination (Avocèvou-Ayisso et al. 2012; Etame-Loe et al. 2018; Fandohan et al. 2017). The Yoruboid group is indeed by its geographical position and ancestral links a buffer group between the Kwa and Gur. Various people of southern Benin (in particular the Aïzo, the Fon, the Mahi) originally came from the interbreeding between the Yoruba and Adja people between the thirteenth and fifteenth centuries and from the migrations caused by the creation of the kingdom of Ahomey (Anignikin 2001). In addition, the Yoruba people have a certain historical affinity with the Bariba who share their geographical space with the Peulh, Dendi, Gourmanche and others (Lombard 1960).

The biological activities of plant species are determined by the secondary metabolites they contain (Khameneh et al. 2019). The secondary metabolites responsible for the antiophidic activity have been highlighted by several studies which have shown the presence and the role of

alkaloids, flavonoids, triterpenoids, steroids in the inhibition of certain enzymes contained in the venoms of snakes in particular phospholipases A2 (Kotta et al. 2020; Kulatunga and Arawwawala 2019; Singh et al. 2017; Yirgu and Chippaux 2019). Alkaloids are bioactive substances very well known for their wide range of biological properties including analgesics, antibiotics, antiparasitics and analgesics. This fairly broad therapeutic potential of alkaloids could justify their presence in antiophidic medicinal plants. Previous studies have also shown the neutralizing ability of snake venoms from alkaloids (Kadir et al. 2015). Known to have anti-inflammatory, antioxidant, cardio-protective, anticancer properties (Borah et al. 2019; Gomes et al. 2010), flavonoids neutralize phospholipases A2 known as an important component of snake venoms (Mengome et al. 2021; Moreira et al. 2020). Flavonoids prevent blood clotting and help maintain good blood circulation (Gbenou et al. 2013). Tannins with their astringency are used in the treatment of varicose veins and wounds because of their vasoconstrictor and homeostatic effects (Dibong et al. 2015). They promote vasoconstriction and reduce body fluid loss (Dibong et al. 2015; Pereañez et al. 2011). Several previous studies have revealed the ability of tannins to neutralize enzymes and toxins contained in snake venoms (da Silva et al. 2016; de Moura et al. 2018; Gomes et al. 2010). Zhang and Li (2017) showed the neutralization of hemorrhagic and neurotoxic effects by tannins contained in medicinal plant extracts. Furthermore, the ability of tannins to act on the enzymatic activities of snake venoms has been demonstrated (Patiño et al. 2012). Phenolic compounds also have various properties including antimicrobial, antiparasitic, anti-inflammatory and analgesic (Koné 2009; Liu 2005). The phenolic compounds also present in the plant extracts tested are frequently mentioned in the literature as being responsible for the inhibition of harmful activities induced by snake venoms (da Silva et al. 2016; de Moura et al. 2018; Gomes et al. 2010). The diversity of therapeutic properties of listed medicinal plants attests to their ability to repel or neutralize snake venom. However, the use of these plants must be done with great caution given the major safety issues surrounding their use. It would therefore be important to evaluate the toxicity of the extracts of these plant species and to deepen the investigations to lead to the development of remedies respecting the medical standards of marketing and popularization of use.

Conclusions

This study has shown that rural population of Benin have rich and diversified knowledge on the use of plant resources to prevent snakebites. This knowledge could be put to service for the well-being of rural

communities and disseminated through establishment of a program for enhancement and promotion of plant species used in traditional medicine in snakebite prevention. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, mucilages, reducing compounds and triterpenoids which are potential sources of snake repellents and could explain the antisnake bite activity of these plants. In-depth pharmacological and phytochemical studies are required so as to rationalize on the effectiveness of the medicinal plants as well as provide insights of the compounds responsible for their antisnake bite activity.

Abbreviations

Cn: Bray–Curtis index; FCA: Factorial correspondence analysis; RFC: Relative frequency of citation; S/N: Species number; SBE: Snakebite envenomation.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42269-022-00851-8>.

Additional file 1. Table S1 Medicinal plants used in traditional prevention of snakebites in Benin.

Additional file 2. Table S2 Bray-Curtis index (Cn) according to socio-cultural groups for knowledge on anti-snakebite plants.

Acknowledgements

Our sincere appreciation goes to Fidèle Assogba, Abel Akpovo, Gabin Ganka and Yéyinou Victoire Djotchou for their support and comments on this manuscript. We also thank the local communities, in particular the traditional hunters and healers of Benin, for their warm welcome and for sharing their knowledge of the antisnake bite plants.

Author contributions

AJD, ABF and JG designed the study. AJD collected and analyzed data, and wrote the first draft of the manuscript. ABF analyzed the data, edited the manuscript and supervised the research work. TO and JG reviewed and corrected the manuscript. All authors have read and approved the final version of the manuscript.

Funding

No funding was received for this study.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Declarations

Ethics approval and consent to participate

This study is part of a doctoral study conducted at the Université Nationale d'Agriculture (UNA) of Benin. The ethics committee of the "Ecole Doctorale des Sciences Agronomiques et de l'Eau" of the UNA approved the study (Approval No. 830036710418UNA). The objective of study was clearly explained to the respondents, and each gave their verbal consent before the administration of questionnaire. The choice of verbal consent is justified by the fact that the studied population is composed mainly of illiterates.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 29 April 2022 Accepted: 25 May 2022

Published online: 04 June 2022

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