Ethno-botanical Survey and Chemical Study of Medicinal Plants Traditionally used to Treat Anemia in Yakoma Territory (Nord Ubangi), Democratic Republic of the Congo

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims of the Study: To identify plant species traditionally used to treat anemia in Yakoma territory and to evaluate their chemical composition.
Place and Duration: Yakoma Territory (survey) and University of Kinshasa (Phytochemical study), from August and October 2019.
Methods: Ethnobotanical survey according to the "snowball" sampling technique among traditional

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healers (based on the free consent of the respondents), chemical analyses of plant materials (chemical screening, TLC, phytomarkers content, minerals composition) according to standard methods. ED-XRF was used for mineral analysis. Microsoft Excel version 2010, Origin version 8.5 Pro and IBM SPSS statistics version 20 software packages were used for data processing and analysis.

Results and Discussion: The survey showed that 18 plant species are traditionally used by Ngbandi traditional healers to treat anemia in Yakoma territory. They belong to 16 families and 17 genera. The most used organs are the leaves (68.4%) and the roots (10.5%). Decoction and infusion are the most used mode of preparation (33.3% each), followed by cooking (22.2%) and maceration (11.1%). The oral route (77.8%) is the most used mode of administration followed by the enema (16.7%) and the anal route (5.6%). Morphological types consist of herbs and trees (33.3% each) and shrubs and lianas (16.7% each). The biotope types consist of forest plants (44.4%), cultivated plants/Crops (38.9%), marshy ground plants (11.1%) and ruderal plants (5.6%). These plants belonging to five biological types: erected therophytes (44.4%), mesophanerophytes (27.8%), microphanerophytes and climbing phanerophytes (11.1% each) and lianescent phanerophytes (5.56%). These anti-anemic plant species are mostly Pantropical and Afro-tropical (39% of species each) followed by Guineo-Congolese (17% of species) and American (5%). Chemical analysis revealed the presence of alkaloids, anthocyanins, flavonoids, anthraquinones and terpenoids and various minerals including iron, zinc, copper, calcium, magnesium and manganese.

Conclusion: In the current state of knowledge, the survey of anemic plants from this part of the Democratic Republic of the Congo is reported for the first time.

1. INTRODUCTION

Plants have always been a part of man's daily life throughout the ages. Man has been able to rely on nature to provide for his basic needs: food, shelter, clothing, medicine [1]. Nowadays, medicinal plants are still the first reservoir of new medicines, they are considered as an essential source of raw material for the discovery of new molecules necessary to develop new drugs [1]. For centuries and even millennia, our ancestors have used plants to relieve their pains, heal their ailments and heal their wounds. From generation to generation, they passed on their knowledge and simple experiences by word of mouth. Thus, even today, despite the progress of pharmacology, the therapeutic use of medicinal plants is present in some countries of the world and especially in developing countries, in the absence of a modern medical system [2]. The use of plants in phytotherapy is very old and is currently experiencing a revival of interest among the public. The reasons why the population resorts to Traditional Medicine are linked to the difficulties that accompany the treatment of diseases by modern medicine, notably the high cost, the progressive resistance of the pathogen to the active substances and the manifestation of severe side effects or even toxicity in certain cases [3]. The Democratic Republic of Congo (DRC), with its cultural diversity and the richness and diversity of its flora and fauna, constitutes a reservoir of biodiversity as indicated by the results of recent studies, which allows it to occupy a privileged place among the countries of the Congo Basin with traditional know-how based on medicinal plants and/or animals [4]. Anemia (defined as a drop in the level of hemoglobin, Hb, or the number of red blood cells below the value necessary to meet the physiological needs of an individual) is a major public health problem throughout the world and particularly in Africa. It is classified by the WHO (World Health Organization) as one of the ten most serious problems in the world and is caused by micronutrient deficiency. This disease has a prevalence of 24.8% and affects about 2 billion people worldwide, 90% of whom live in developing countries [5]. The people most at risk are children, elderly people, pregnant women and sickle cell disease patients. Apart from sickle cell disease (polymerization of hemoglobin S under hypoxic conditions), iron, folate and vitamin B_{12} deficiencies are the main causes of anemia [6]. Biologically, the mean Hb level is 8 g/dL, with extremes ranging from 3.4 g/dL to 11.4 g/dL. A severe anemia occurs when the Hb level is below 6 g/dL [7].

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Yakoma Territory, North Ubangi Province. This territory has three Sectors and 34 clusters (Fig. 2). These 34 clusters are made up of 169 villages, including the Abumombazi Sector with 16 clusters and 56 villages; the Wapinda Sector: 6 clusters and 35 villages and the Yakoma Sector: 12 clusters and 78 villages. Longitude: 22° 35' and 23° 37' East longitude and 3° 48' and 4° 6' North latitude, Altitude: 470 m above sea level.

Yakoma Territory, with an estimated population of approximately 409,444, is located in the North Ubangi Province, Democratic Republic of the Congo (DRC). Yakoma Territory is bounded by the Uélé and Ubangi Rivers, which in turn border the Central African Republic, to the northeast by the territories of Bondo and Aketi (Bas-Uélé Province), to the northwest by the territories of Mobayi-Mbongo and Businga, and to the south by the territory of Bumba. The Yakoma territory belongs to the AW_4 climate (humid tropical climate). This territory of Yakoma where the surveys were conducted is characterized by the savanna vegetation; the predominant species in this ecosystem is an invasive plant species *Chromolaena odorata* (also called fuga or sida in Ngbandi dialect) [8].

2.2 Ethnobotany and Floristic Study

2.2.1 Ethnobotanical survey

The "snowball" sampling technique (which consists of identifying a knowledgeable informant for the subject of study and then this informant after being surveyed indicates in turn another knowledgeable informant from the same community) was used. This process continued until all the competent expert informants selected in this study were investigated [9]. The interview was conducted in Ngbandi and the survey forms designed in French were used to support the interviews. Respondents were interviewed individually on the basis of a survey form. The main ethnobotanical data (vernacular name, part used, method of preparation of medicinal recipes, etc.) were collected in the form of a questionnaire.
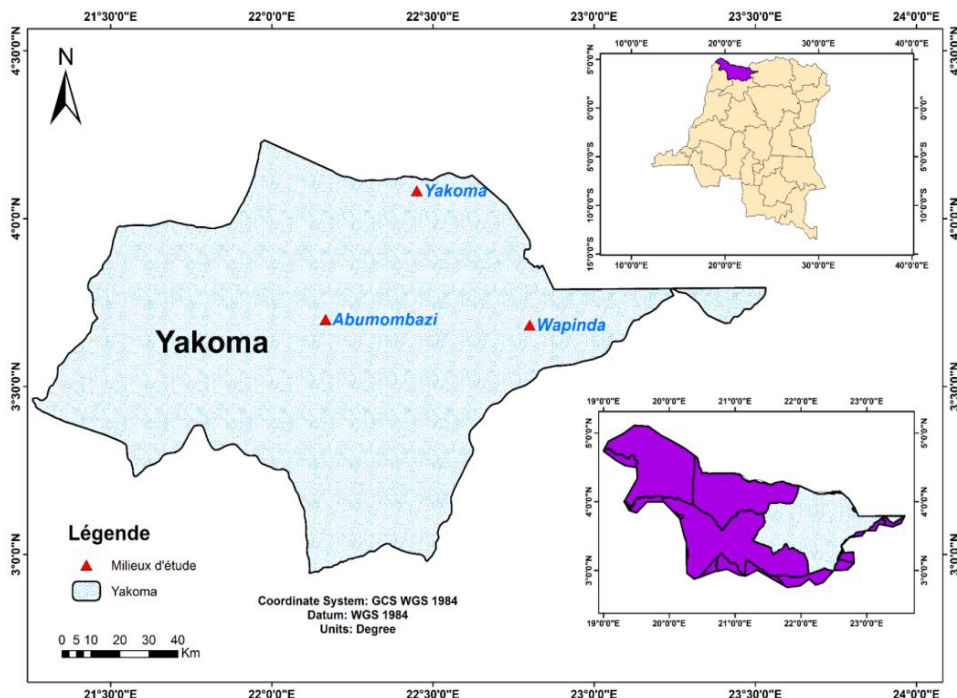

Fig. 1. Localization of Yakoma Territory
(Longitude: 22° 35' and 23° 37' East longitude and 3° 48' and 4° 6' North latitude, Altitude: 470 m above sea level)

2.2.2 Collection of plants and ecological characterization

The collection of samples for the making of herbariums consisted of the collection of the sample; the pressuring of the sample; the drying or conservation in a liquid or in a fixative and finally the mounting of the sample on cardboard sheets. The plant species were identified by Mr. Ngulo (Botanist at the Gbado-Lite botanical garden) and voucher specimens were prepared and deposited in the herbarium of the Department of Biology (Faculty of Science, University of Kinshasa) under Ngunde Samy collection name. The inventoried plants namely *Alchornea cordifolia* (Schumach. & Thonn.) Mull. Arg. (Euphorbiaceae); *Anthocleista chweinfurthii* Gilg. (Gentianaceae) ; *Basella alba* L. (Basellaceae) ; *Bidens pilosa* L. (Compositae*) ; Carica papaya* L. (Caricaceae) ; *Coffea canephora* Pierre ex A. Frochner (Rubiaceae) ; *Cola acuminata* (P. Beauv.) Schott & Endl. (Malvaceae*); Elaeis guineensis* Jacq. (Arecaceae); *Hibiscus acetosella* Welw. Ex Hiern.(Malvaceae) ; *Hibiscus sabdariffa* Rottb. (Malvaceae); *Periploca nigrescens* Afzel.(Apocynaceae) ; *Morinda lucida* Benth. (Rubiaceae) ; *Musa paradisiaca* L. (Musaceae) ; *Phytolacca dodecandra* L'Her. (Phytolaccaceae); *Portulaca triangularis* Jacq. (Portulacaceae) ; *Psidium guajava* L. (Myrtaceace) and *Psophocarpus scandens* (Endl.) Verdc. (Leguminosae), were identified according to the versions of APG II, III and IV and through the comparison of the samples to excicata kept at the herbarium.

Ecological spectra were determined using the Raunkiaier classification [10] adapted for tropical Africa by Schmitz [11]. The phytogeographical distribution types defined in this study were established using the work of Lebrun [12]. Thus, the morphological types consisted of Trees: plant species over 15 m in height; Shrubs: small trees with a total height of less than 7 m; Subshrubs: small trees with a total height of less than more or less 2 m; Lianas: Climbing plants that insert the trunk of a support plant; Annual herbs: non-woody plants whose above-ground parts including the stem die each year; Perennial herbs: non-woody plants whose above-ground and below-ground parts are kept alive for several years. The biological types were Mesophanerophytes (MsPh), trees from 10 to 30 m high; Microphanerophytes (McPh), trees from 2 to 10 m high; Climbing phanerophytes (Phgr), these are climbing plants that can reach 20 to 25 cm in diameter; Lianating phanerophytes (LPh): these are woody lianas with claws. The types of biotopes considered in this work are listed as: Forest; Swampy; Ruderal; Culture (Crops). The phytogeographic distribution retained concerns Pantropical (Pan) species, known in tropical Africa, America and Asia; Afro-tropical (At), distributed in tropical Africa; American (Am) and Guineo-Congolese (GC) distributed in central and western Africa.

2.2.3 Data processing and analysis

Microsoft Excel version 2010, Origin version 8.5 Pro and IBM SPSS statistics version 20 software packages were used for data processing and analysis.

2.2.4 Chemical screening and phytomarkers quantification

Dried and pulverized plant material (10 g) was repeatedly extracted by cold percolation with 95% methanol (MeOH) and water (100 mL X 2) for 48 hours. Chemical screening (identification of total polyphenols, flavonoids, anthocyanins, leuco-anthocyanins, tannins, alkaloids, quinones, saponins, steroids and triterpenoids) and the quantification of total polyphenols, flavonoids, anthocyanins and tannins were performed according to the standard protocol as previously reported [13-15].

2.2.5 Determination of mineral composition

Leaves of *A. cordifolia*, *B. alba*, *P. nigrescens*, *P. dodecandra*, *P. scandens* and *M. lucida* barks used in this study were collected in July and August 2019 in Yakoma Territory, North Ubangi Province (DRC). These samples were prepared (washed, dried, and ground) at the University of Kinshasa Basic Science Laboratory. The detection and quantification of the mineral composition was done by ED-XRF at the Centre Régional d'Etudes Nucléaires de Kinshasa (CRENK). Briefly, the analysis of mineral composition of plant samples was carried out using the X-ray fluorescence spectrometer (EDP-XRF, XEPOS III brand). Molybdenum (39.76 KV of voltage and 0.88 mA of current), Aluminum oxide (49.15 KV of voltage and 0.7 mA of current), Cobalt (35.79KV of voltage and 1mA of current) and last HOPG Bragg Crystal (17.4KV voltage and 1.99 mA current) of the anode in palladium were used as secondary targets. The pellet is placed under a beam of X-rays and under the effect of these rays, the

sample resonates and re-emits X-rays which are its own and they are fluorescent. The energy spectrum of fluorescent X-rays is characterized by peaks that are characteristic of the different elements present in the sample, which makes it possible to identify the chemical elements present and the height of these peaks makes it possible to determine the quantity of these elements. The Kα1 peak (3.313 Kév) of the K was used for the calculation; Bragg's HOPG Crystal target (17.4KV voltage and 1.99 mA of current) gave surfaces that were normalized compared to the peak from coherent and incoherent diffusion.

3. RESULTS AND DISCUSSION

Table 1 provides information on the ethnobotanical and ecological data of the plants listed.

It appears from the Table 1 that 18 medicinal plants belonging to 17 genera and 16 botanical families have been listed in Yakoma Territory (North Unangi, DRC). The Malvaceae alone represent 16.67% or 3 species in terms of citation and the others: Arecaceae, Asteraceae, Basellaceae, Caricaceae, Euphorbiaceae, Fabaceae, Gentianaceae, Lamiaceae, Musaceae, Myrtaceae, Phytolaccaceae, Portulacaceae, Rubiaceae and Rutacea have one species each. These results are similar to those of Gnagne et *al.* [16] who show that the high representation of *Malvaceae* (3 species) could be justified by the grouping of two subfamilies into one botanical family.

Fig. 2 presents in terms of percentage the different parts used of the plants listed or selected.

Fig. 2 shows that six organs are used as drugs. These include the leaves (68.4%), followed by the roots (10.5%). The other parts represent (5.26%) each. These results are consistent with Tahri et *al.* [17] who indicate that leaves are the most used parts in the preparation of recipes and that the use of leaves is a non-invasive (non-destructive) and sustainable practice in phytotherapy. On the other hand, the large-scale use of roots is a practice that contributes to the erosion of these plant genetic resources [18]. These plants are necessary plant resources for the management of anemia for future generations. To this end, they must be integrated into the sustainable management of agroresources for their conservation.

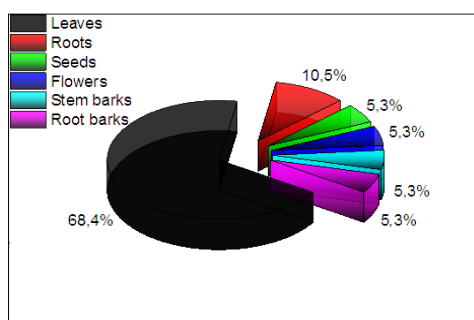Fig. 3 gives the modes of preparation of anti-anemic recipes by traditional healers.
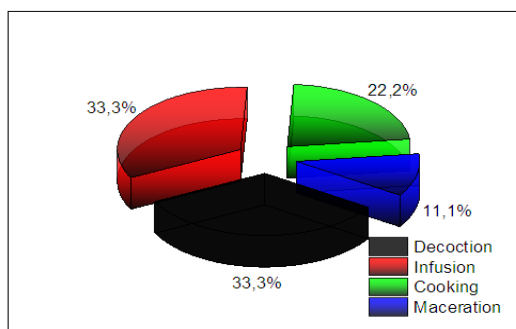

Fig. 2. Plants used parts

Fig. 3. Mode of preparation of plant-based recipes by traditional healers

Table 1. Ethnobotanical and ecological data of the listed anti-anemic plants

Scientific Name (Family)	Vernacular name	Herbarium number	FR(%)	PU	MP	MA	TM	TB	DP	Habitat
Morinda lucida Benth. (*Rutaceae*)	Gere	P. Compère 1824	5	Root bark	Maceration	Enema	arb	Msph	At	For
Bidens pilosa L. (*Asteraceae*)	Ngenge	R. Devred 3326	2	Sheet	Decoction	Oral route	H	Thd	Pan	Rud
Elaeis guineensis (*Arecaceae*)	Mbulu		2	Flower ash	Infusion	Oral route	A	Thd	Pan	Cult
Basella alba L. (*Basellaceae*)	Singo	R. Pierlot 310	6	Leaf	Cooking	Oral route	H	Thd	Pan	Cult
Parquetina nigrescens Afzel.	Kpumanza	H. Breyme 4754	5	Leaf	Maceration	Enema	Lia	Phgr	Pan	For
Coffea caniphora (*Rubiaceae*)	Kafé	P. Compère 391	3	Seed	Infusion	Oral route	A	Thd	Gc	Cult
Carica papaya L.(*Caricaceae*)	Papaye		3	Dry leaf	Infusion	Oral route	arb	Msph	Pan	Cult
Hibiscus acetosella (*Malvaceae*)	Ngaingai	Dumont 4	1	Leaf	Decoction	Oral route	H	Thd	At	Mar
Hibiscus sabdariffa (Malvaceae)	Ngaingai		1	Leaf	Decoction	Oral route	H	Thd	At	Mar
Alchornea cordifolia (Schumach. & Thonn.) Müll.Arg. (*Euphorbiaceae*)	Mbombonzi	C. Evrard 1201	8	Leaf	Decoction	Oral route	arb	Mcph	At	For
Portulaca triangularis (*Portulacaceae*)	Kpelekpele	Kalanda 2	1	Leaf	Cooking	Oral route	Ha	Thd	At	Cult
Psophocarpus scandens (Endl.) Verdc. (*Fabaceae*)	Ngasâ	R. Devred 561	7	Leaf	Cooking	Oral route	Lia	Phgr	At	Cult
Anthocleista schweinfurthii Gilg. (Gentianaceae)	Benzangu	J. Dubois 8	3	Root/Leaf	Infusion	Anal route	A	Msph	Gc	For
Persea americana Mill. (*Lamiaceae*)	Avoka	R. Devred 1366	1	Root	Decoction	Oral route	A	Msph	Pan	For
Musa sp (Musaceae)	Fondo		2	Dry leaf	Infusion	Enema	Ha	Thd	Pan	Cult
Cola acuminata (P.Beauv.) Schott & Endl. (*Malvaceae)*	Liyo, Makasu	R. Germain 8503	2	Stem bark	Decoction	Oral route	A	Msph	GC	For
Psidium guajava L. (*Myrtaceace*)	Kangele	P. Compère 689	3	Leaf	Infusion	Oral route	A	mcph	At	For
Phytolacca dodecandra L'Hér. (*Phytolaccaceae*)	Singo ngbandi	L. Liben 927	5	Leaf	Cooking	Oral route	Lia	Lph	Am	For

FC: Frequency of citation, PU: Part used, MP: Mode of preparation, MA: Mode of administration of recipes, TM: Morphological types, TB: Biotope types, DP:

Fig. 3 shows that four methods of preparation are used to facilitate drug administration, namely decoction and infusion (33.3% each), followed by cooking (22.2%) and maceration (11.1%). Decoction and infusion are widespread in Africa. Indeed, it has been reported by several authors that the local population considers the decoction as an adequate mode to warm the body and disinfect the plant [19, 20]. On the other hand, the decoction allows collecting the most active principles and attenuates or cancels the toxic effect of some recipes [21]. On the other hand, the infusion prevents the thermal denaturation of the active principles when they are exposed for a very long time to the fire.

Fig. 4 gives the modes of administration of recipes during treatment of anemia by traditional healers.

Fig. 4 shows that the oral route (77.8%) is the most used in the administration of phytomedicines in Yakoma territory; followed by the enema (16.7%) and the anal route (5.6%). These results corroborate with Gnagne's research [16] which shows that the oral route is

the most used in the administration of phytodrugs.

Figs. 5 and 6 provide information on the morphological types and biotopes of the plants surveyed respectively.

Fig. 5 shows that the anti-anemic plants listed in Yakoma Territory have four morphological types, namely herbs and trees (33.3% each) as well as shrubs and lianas with 16.7% each.

According to biotope types (Fig. 6), the flora listed classifies these plants in four habitat categories with the predominance of forest plants (For: 44.4%), followed by cultivated plants (Crops: 38.9%), marshy ground plants (11.1%) and ruderal plants (5.6%). The predominance of forest plants is justified by the fact that the study area is located in a forest ecosystem (humid tropical zone). These same results were found by Ngbolua [19] in the city of Gbado-Lite in North Ubangi province.

The results on the biological types and phytogeographical distribution of the anemic plants identified are shown in Figs. 7 and 8.
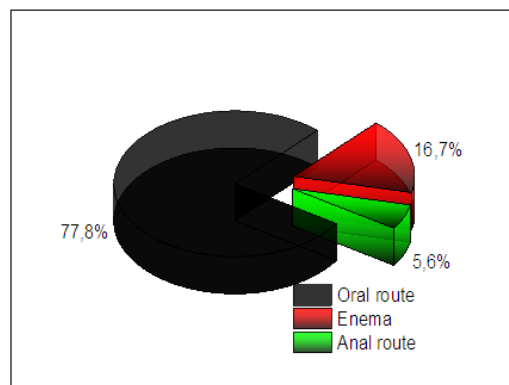

Fig. 4. Mode of administration of recipes during treatment of anemia by traditional healers
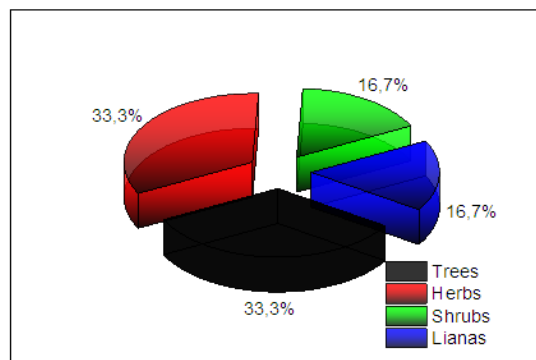

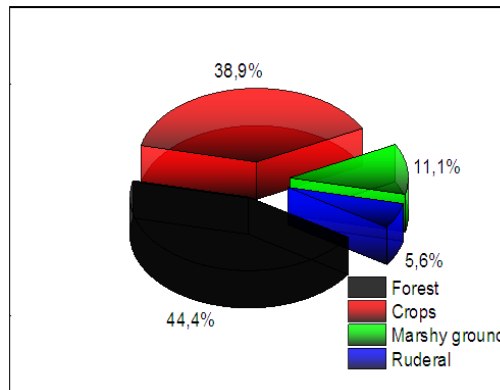**Fig. 5. Morphological types of inventoried plants**

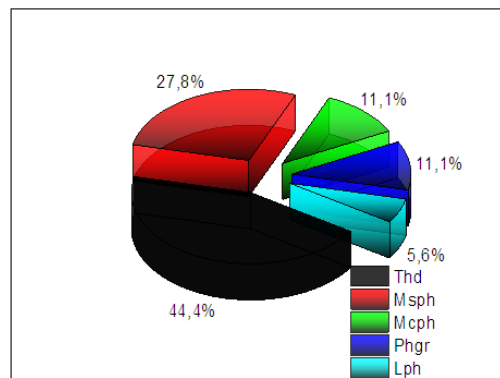**Fig. 6. Habitat types of inventoried plants**

Fig. 7. Biological types of inventoried plants
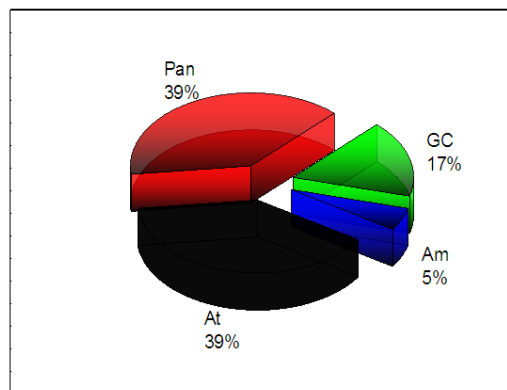

Fig. 8. Phytogeographical distribution of inventoried plants

As shown in Fig. 7, the species recorded are divided into five biological types. In terms of citation, the erected therophytes (thd: 8 species or 44.4%) are in the majority, followed respectively by the mesophanerophytes (Msph: 5 species or 27.8%), the microphanerophytes (Mcph) and the climbing phanerophytes (Phgr): 2 species or 11.1% each, and finally the lianescent phanerophytes (Lph) one species or 5.56%. From the chorological point of view (Fig.

8), these anti-anemic species are mostly Pantropical and Afro-tropical (39% of species each) followed by Guineo-Congolese (17% of species) and finally 5% of American species (one species). The predominance of pantropical and Afro-tropical species has also been reported in several studies including [22-24].

Table 2 gives the results of the phytochemical screening in solution.

Table 2. Results of chemical screening in tubes

Chemical groups	Results						
	Ac/F	Ba/F	Pn/F	Pd/F	Ps/F	Ml/ER	Total
1. Polyphenols	++	+	+	+	+	+	6
o Flavonoids	-	-	+	+	+	+	4
o Anthocyanins	+	+	+	+	-	+	5
o Tannins	+	+	-	+	-	+	4
o Leuco-anthocyanins	-	-	+	+	+	+	4
2. Alkaloids	++	+++	+	+	+++	+++	6
3. Linked Quinones	+	+	-	-	-	++	3
4. Saponins	+	+++	-	+++	+	+++	5
5. Steroids and Triterpenoids	+	+	+	+	+	+	6
6. Free Quinones	++	-	-	-	-	+++	2
Total	8	7	6	8	6	10	

(Legend: +: presence of the desired substance; -: absence of the desired substance, F: leaf, ER: root bark, Ac: Alchornea cordifolia, Ba: Basella alb, Pn: Parquetina nigrescens, Pd: Phytolaca dodecandra, Ps: Psophocarpus scandens and Ml: Morinda lucida)

From the Table 2, it appears that of the six plants analyzed, we found the presence of polyphenols, alkaloids, steroids and terpenoids in all plant species. Anthocyanins are present in five species including *Alchornea cordifolia*, *Basella alba*, *Morinda lucida*, *Phytolaca dodecandra*, and *Parquetina nigrescens* but absent in *Psophocarpus scandens*. Saponins are also present in five species: *A. cordifolia*, *Basella alba*, *P. dodecandra*, *P. scandens* and *M. lucida* but absent in *P. nigrescens*. The presence of flavonoids and leuco-anthocyanins was detected in four species including *M. lucida*, *P. dodecandra*, *P. nigrescens* and *P. scandens* but absent in two species *A. cordifolia* and *B. alba*.

Tannins are also present in four species *A. cordifolia*, *B. alba*, *M. lucida* and *P. dodecandra* absent in *P. nigrescens* and *P. scandens*. Linked quinones are present in three species *A. cordifolia*, *B. alba* and *M. lucida* and absent in three species also including *P. nigrescens*, *P. dodecandra* and *P. scandens* while free quinones are present in two *A. cordifolia* and *M. lucida* but absent in four species *B. alba*, *P. nigrescens*, *P. dodecandra* and *P. scandens*. From the quantitative point of view, the six plants are richer in poly-phenols, alkaloids, steroids and terpenoids followed by anthocyanins and saponins then flavonoids, leuco-anthocyanins and tannins. The chemical screening carried out on the organic phase indicated that all our plants contain steroids and terpenoids while free quinones are present only in two species *A. cordifolia* and *M. lucida*. The presence of the different secondary metabolites in the plants is a very interesting indication because they are endowed with anti-anemic properties; this justifies the use of these plants in traditional medicine in the Yakoma territory according to [18] and [25]. The results of the phytochemical screening by TLC showed the presence of phenolic compounds including (phenolic acids, alkaloids, anthocyanins, coumarins and flavonoids; terpenoids, terpenes and iridoids as well as antraquinones are also present as shown in some chromatograms below (Figs: 9-11).

The anthracene compounds were revealed by the presence of red spots after revelation at 366 nm and 10% ethanolic KOH.

Table 3 below shows the results of the phytomarker content of selected plant species.

The results represented in the Table 3 revealed high total polyphenol content in *P. scandens* (282.96±0.891 mgEqAG/g), followed by *A. cordifolia* (229, 92±8.592 mgEqAG/g), *B. alba* (171.2±0.898 mgEqAG/g), *P. nigrescens* (145.84 mgEqAG/g), *P. dodecandra* (145.68±3.538 mgEqAG/g) and *M. lucida* (104±11.230 mgEqAG/g), respectively. While *P. scandens* is the richest in phenolic compounds followed by *A. cordifolia*, *B. alba*, *P. nigrescens*, *P. dodecandra* and *M. lucida* respectively. However, *P. nigrescens* and *P. scandens* are the richest in flavonoids followed by *B. alba*, *M. lucida*, *P. dodecandra* and *A. cordifolia* while as far as Anthocyanins are concerned, *P. dodecandra* is richer in Anthocyanins than *P. scandens*, *B. alba*, *A. cordifolia*, *M. lucida* and *P. nigrescens*. While in those concerning tannins, hydrolyzable tannins are much more in our plants than condensed tannins see Table 3. Studies have shown that extrinsic factors (such as geographical and climatic factors), genetic factors, but also the degrees of ripening of the plant and the duration of storage have a strong

influence on the content of polyphenols [26] and [27].

The elemental composition of different anti-anemic plants is given in Figs. 12 and 13.
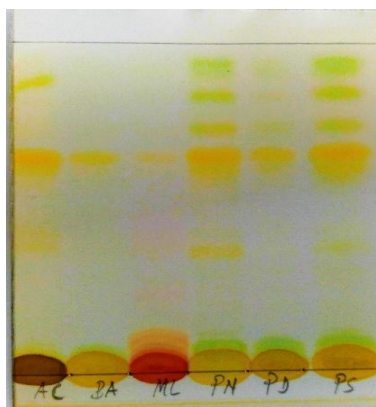

Fig. 9. Chromatogram for alkaloids [Mobile phase: Toluene/ethyl acetate/dietthylamine (35: 10: 5); Detection: NaNO$_2$ 5%]. Alkaloids are characterized by the presence of orange spots
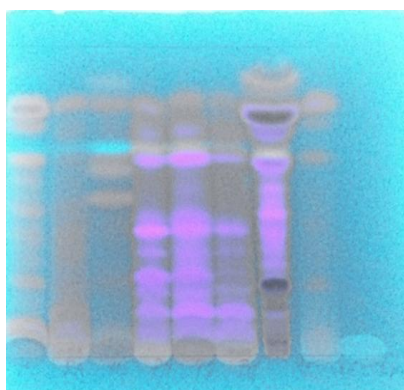

Fig. 10. Chromatogram for terpenes [Mobile phase: Toluene/Ethyl acetate (31: 2.5); Detection: Sulfuric anysaldehyde]. The triterpenes are characterized by blue spots at 366 nm

As can be seen in Fig.14, the six plants studied contain six macroelements, including potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), phosphorus (P), and chlorine (Cl) while sodium (Na) is found only in one plant. The chemical elements K, Ca, S, Mg, P and Cl present in the leaves of five plants and the root bark of one plant: *A. cordifolia*, *B. alba*, *P. nigrescens*, *P. dodecandra*, *P. scandens* and *M. lucida* while Na is absent in Five species but present only in the bark of *M. lucida*. It was also found that K and

Ca content were higher in the leaves of *P. dodecandra*, *P. nigrescens, B. alba* and *P. scandens* than in that of *M. lucida* and *A. cordifolia* while as regards Mg content is higher in the leaves of *B. alba*, *P. scandens* and *P. nigrescens*. According to these results, the six species are much richer in K followed by Ca, P, S, Mg and Cl. Potassium and calcium remain the most abundant elements in the leaves of *P. dodecandra*, *P. nigrescens*, *B. alba* and *P. scandens* followed by K, Cl, Mg, S, P and Na. Potassium and sodium are electrolytes necessary for normal body function and help maintain blood fluidity and volume in the body. Phosphorus in the body is located in the bones and teeth, where it is combined with calcium to provide strength. Calcium makes up a large part of bone, human blood and extracellular fluid, and is necessary for the proper functioning of cardiac muscles, blood clotting and regulation of cell permeability [28]. In humans, Mg is required in plasma and extracellular fluid, where it helps maintain osmotic balance. In addition, magnesium can play an important role as a cofactor of enzymes, reduce the number of abnormal erythrocytes in anemia and improve hydration of red blood cells [29,30]. The presence of these biological electrolytes justifies the use of these plants in traditional medicine for the management of anemia.

From this Fig. 13, it appears that all six plants are rich in Manganese (Mn) and Iron (Fe). With the exception of aluminum (Al), the other microelements are in trace amounts. The presence of iron justifies the use of these plants by Yakoma traditional healers for the treatment of anemia. Indeed, iron is important in the production of hemoglobin. An iron deficiency causes the body to produce fewer and smaller red blood cells, resulting in anemia. In addition, Mn is a co-factor of hydrolases, decarboxylases and transferases, enzymes that are essential for homeostasis [29]. Other important trace elements such as Zn and Cu are present the selected plants. In fact, zinc is a co-factor for many enzymes necessary for the production of red blood cells; therefore, zinc deficiency may be associated with anemia. Impaired iron absorption can be caused by a decrease in trace elements such as zinc, which is found in the structure of enzymes that coordinate or catalyze iron metabolism. Copper (Cu) is also a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an enzyme that oxidizes iron in the blood. The observation of anemia in Cu

deficiency can probably be related to its role in facilitating iron absorption and incorporation of iron into hemoglobin [28,30,31]. The high Al content means that the plants were harvested from a strongly acidic soil [32,33].
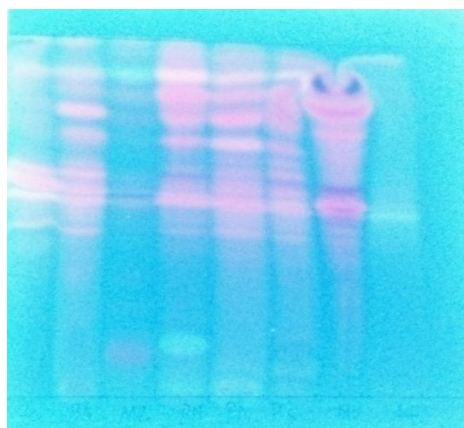

Fig. 11. Chromatogram of the methanolic extract of *A. cordifolia, B. alba, M. lucida, P. nigrescens, P. dodecandra* and *P. scandens* for anthraquinones; Mobile phase: Ethyl acetate/Methanol/Water (50: 8.5: 6.5). Detection: by UV light at 366 nm

Figs 14 (a) and 14 (b) give the graphs of individuals (plants) and variables (minerals) with a large contribution from the design.

The labeled individuals are those with the greatest contribution to the construction of the plan. These individuals are therefore the following anti-anemic plants: *M. lucida, A. cordifolia, P. nigrescens* and *P. dodecandra.*

The labeled variables are those best represented on the plot. The variables are mineral elements measured in the different plants. Dimension 1 distinguishes particularly the individual R. *M. lucida* on the right of the graph, characterized by a strongly positive coordinate on the axis. This individual has a high value for the variables Na, Ti, Al, Zr, Fe and Si (from the most extreme to the least extreme). Note that the variables Na, Ti, Fe and Zr are extremely correlated to this dimension (correlations of 0.92, 0.95, 0.98, 0.94 respectively). They could therefore summarize the dimension 1 by themselves.

Fig. 15 shows the hierarchical ascending classification of plants (individuals).

The classification carried out on the individuals shows 3 classes. Class 1 is composed of individuals such as F.*P. nigrescens*. This group is characterized by high values for the variable Mg. Class 2 is composed of individuals such as F.*A. cordifolia* and F.*P. dodecandra*. This group is characterized by low values for the variable Rb. Class 3 is composed of individuals such as R.M. lucida. This group is characterized by high values for the variables Na, Ti, Al, Zr, Fe and Si (from most extreme to least extreme).

The Principal Component Analysis of Macroelements was performed on six plants (individuals) and seven macroelements (or variables). The analysis of the graphs does not reveal any singular individual. The inertia of the factorial axes indicates whether the variables are structured and suggests the appropriate number of principal components to study. The first two axes of the analysis express 68.64% of the total inertia of the data set; this means that 68.64% of the total variability of the cloud of individuals (or variables) are represented in this plane. The observed inertia on the first factorial plane is lower than the reference value of 82.28%, and therefore low in comparison (this reference inertia is the 0.95-quantile of the distribution of inertia percentages obtained by simulating 747 random datasets of comparable dimensions based on a normal distribution). Moreover, the observed inertia on the first principal component is lower than the reference value 55.7%. The variability expressed by the analysis is therefore not significant.

Table 3. Phytomarkers content of six selected medicinal plants

| Plants	Total polyphenols (mgEAqG/g)	Flavonoids (mg/g)	Anthocyanins (mg/g)	Hydrolyzable tannins (mg/g)	Condensed tannins (mg/g)
A. cordifolia	229,92±8,592	34,356±0,347	0,435±0,0007	1,347±0,013	0,468±0,0003
B. alba	171,2±0,898	57,111±0,281	0,498±0,031	0,314±0,253	0,355±0,003
M. lucida	104±11,230	54,222±0,272	0,246±0,048	0,731±0,008	0,454±0,002
P. nigrescens	145,68±3,538	108,956±0,303	0,354±0,406	0,652±0,005	0,368±0,013
P.dodecandra	133,6±1,12	48,222±0,575	0,997±0,007	0,916±0,009	0,453±0,002
P. scandens	282,96±0,891	88,778±0,141	0,0774±0,001	0,580±0,001	0,418±0,0002
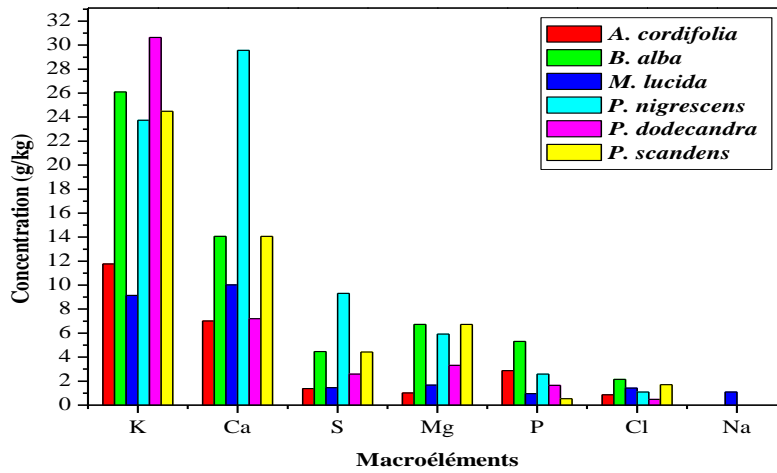
Fig. 12. Macro-element content of the six selected plants
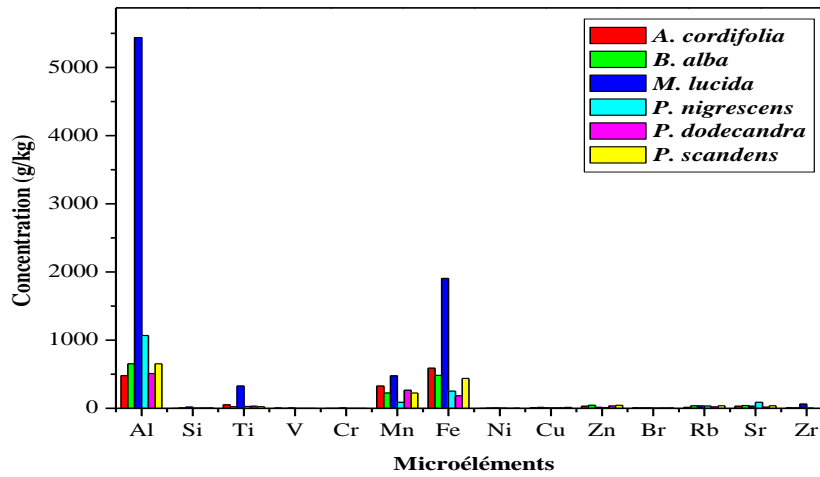

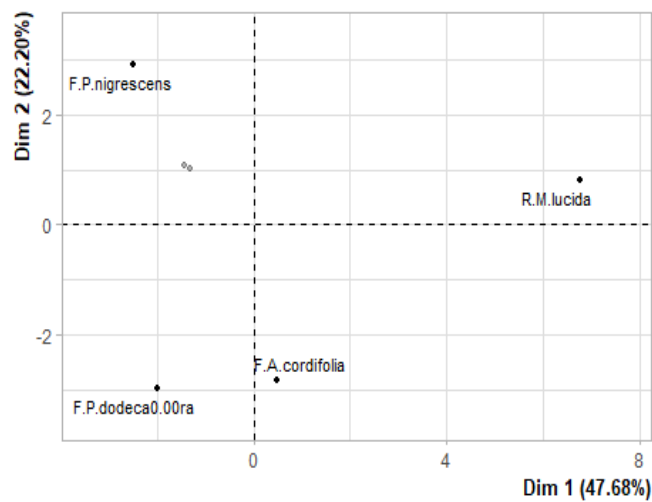**Fig. 13. Concentration of microelements in the six selected plants**

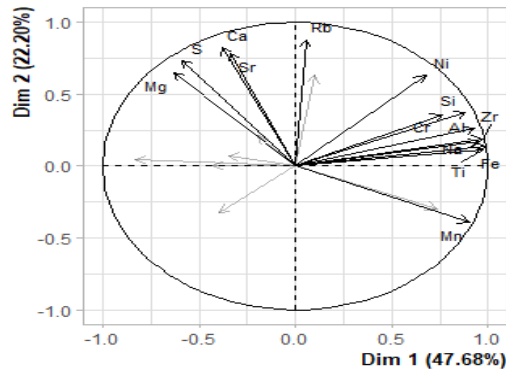**Fig. 14 (a). Plot of individuals (PCA)**
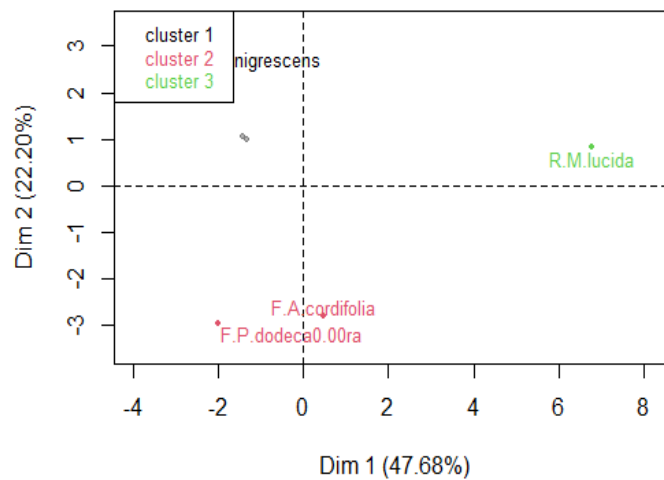
Fig. 14 (b). Variable plot (PCA)

Fig. 15. Hierarchical ascending classification of individuals

Figs. 16 (a) and 16 (b) show the graphs of individuals and variables.

The labeled individuals are those having the greatest contribution to the construction of the plan.

The labeled variables are those best represented on the plot. Dimension 1 opposes individuals such as *P. nigrescens* and *B. alba* on the right of the graph, characterized by a strongly positive coordinate on the axis, to individuals such as *P. dedocandra*, *A. cordifolia* and *M. lucida* (on the left of the graph, characterized by a strongly negative coordinate on the axis). The group to which individuals F.*P. nigrescens* and F.*B. alba* belong (characterized by a positive coordinate on the axis) shares: high values for the variable Mg. The group to which the individual R.*M. lucida* belongs (characterized by a negative coordinate on the axis) shares: high values for the variable Na. Dimension 2 particularly distinguishes individuals such as F.*P.*

dodecandra F.*A. cordifolia* (at the bottom of the graphs, characterized by a strongly negative coordinate on the axis). These individuals form a group sharing variables whose values do not differ significantly from the mean.

Fig. 17 gives the hierarchical ascending classification of individuals (anti-anemic plants).

The classification carried out on the individuals reveals 3 classes. Class 1 is composed of individuals such as R.*M. lucida*. This group is characterized by high values for the variable Na. Class 2 is composed of individuals such as F.*A. cordifolia* and F.*P. dodecandra*. This group is characterized by variables whose values do not differ significantly from the mean. Class 3 is composed of individuals such as F.*B. alba* and F.*P. nigrescens*. This group is characterized by high values for the variable Mg.

The Principal Component Analysis of trace elements analysis was performed with six

13

individuals (plants) and fourteen variables (trace elements). The analysis of the graphs does not reveal any singular individual.

Figs. 18 (a) and 18 (b) show the graphs of individuals (plants) and variables (trace elements) respectively.

The labeled individuals are those having the greatest contribution to the construction of the plan.

The labeled variables are those best represented on the plot. Dimension 1 (inertia

rate 54.34%) particularly distinguishes individuals such as R.M.lucida (on the right of the graph, characterized by a strongly positive coordinate on the axis). These individuals form a group sharing strong values for the variables Ti, Al, Zr, Fe and Si (from the most extreme to the least extreme). Note that the variables Al, Ti, Fe and Zr are extremely correlated to this dimension (correlations of 0.94, 0.96, 0.99, 0.99 respectively). These variables alone could therefore summarize dimension 1.

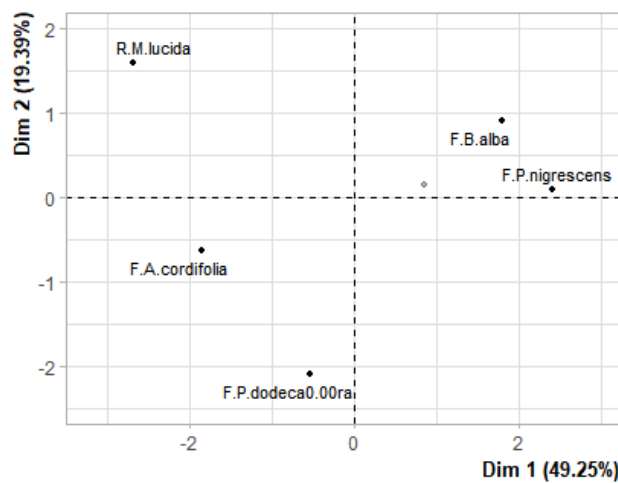Fig. 19 shows the hierarchical ascending classification of anti-anemic plants.

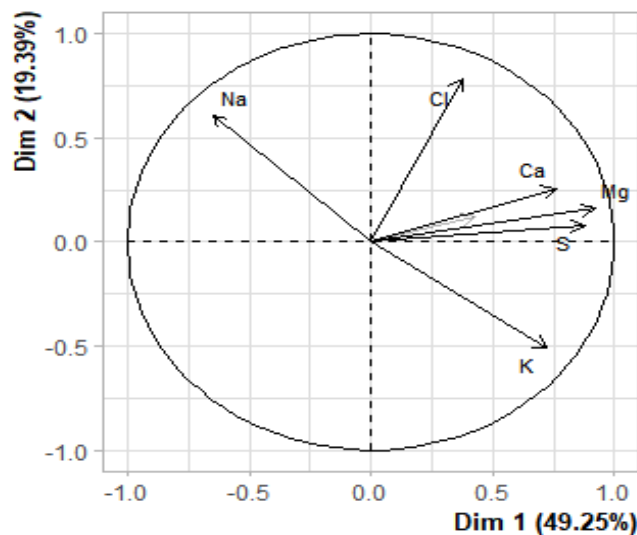**Fig. 16 (a). Graph of individuals (PCA)**
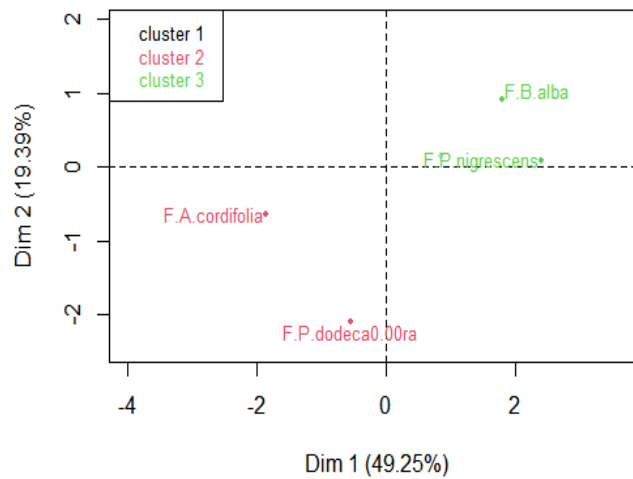

Fig. 16 (b). Graph of variables (PCA)

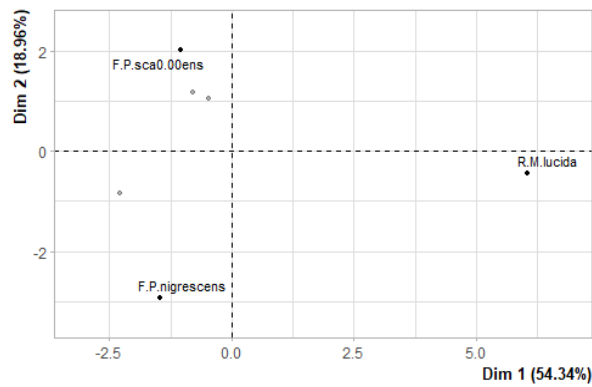**Fig. 17. Hierarchical ascending classification of individuals**

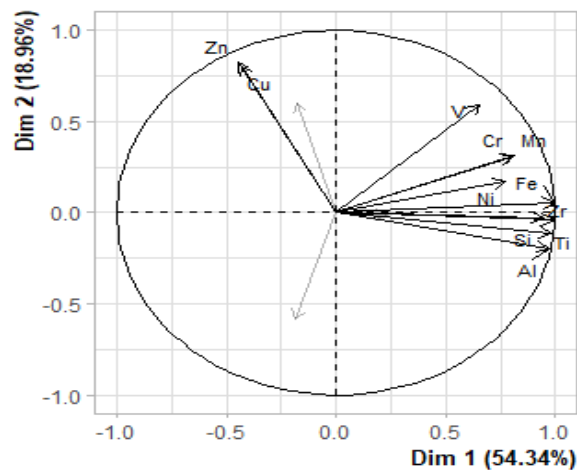**Fig. 18. (a) Graph of individuals (PCA)**

Fig. 18 (b). Graph of variables (PCA)

The classification carried out on the individuals reveals three classes. Class 1 is composed of individuals such as F.*P. nigrescens*. This group is characterized by variables whose values do not differ significantly from the mean. Class 2 is composed of individuals such as F.*P. scadens*. This group is characterized by high values for the variable Cu. Class 3 is composed of
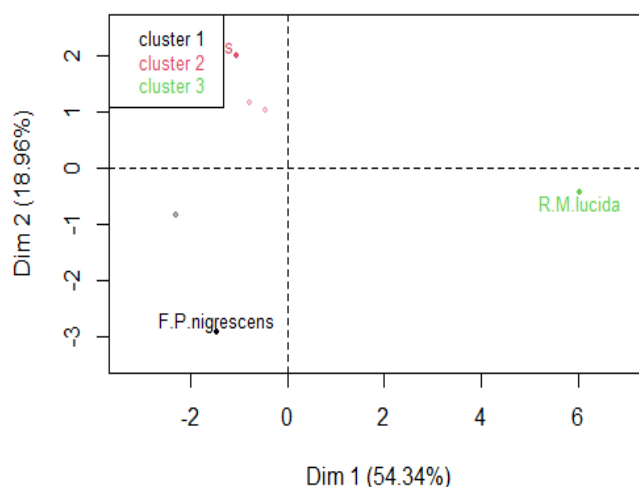
Fig. 19. Hierarchical ascending classification of individuals

individuals such as R.*M. lucida*. This group is characterized by high values for the variables Ti, Al, Zr, Fe and Si (from the most extreme to the least extreme). Thus, by combining *M. lucida* root bark with *P. scandens* leaves, a copper-enriched anti-anemic phytomedicine can be formulated.

The results of this study revealed also that eight of these plants are food (*A. cordifolia*, *B. alba*, *C. canephora*, *H. acetosella*, *H. sabdariffa*, *P. dodecandra*, *P. triangularis* and *P. scandens*) were identified in this study and four species of these 18 plants (*A. cordifolia*, *A. schweinfurthii*, *C. papaya* and *P. scandens*) were previously validated for their anti-sickle cell activity *in vitro* [34-38].

4. CONCLUSION AND SUGGESTIONS

The aim of the present study was to identify the anti-anemic plants used in traditional medicine in Yakoma territory, to evaluate the chemical composition of these plants in view of their scientific validation. The study revealed that 18 plants belonging to 15 families and 17 genera are used. The *Malvaceae* family was the most represented botanical family in terms of species. Leaves were the most used part; decoction and infusion were the most used modes of preparation and the oral route was the most used for drug administration. Chemical analysis of selected plants revealed the presence of alkaloids, anthocyanins, flavonoids, anthraquinones and terpenoids and various minerals including iron, zinc, copper, calcium, magnesium and manganese. However, statistical analysis by PCA revealed that by

combining *M. lucida* root bark with *P. scandens* leaves, a copper-enriched anti-anemic polyherbal can be formulated from Ngbandi indigenous knowledge.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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