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Journal of Medicinal Plant Research

Full Length Research Paper

In vivo antimalarial activity of the crude extract and solvent fractions of the leaves of *Zehenria scabra* (Cucurbitaceae) against *Plasmodium berghei* in mice

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Zehenria scabra **is among the Ethiopian folk medicine for malaria like fever and other infectious diseases. But it lacks apposite pharmacological investigation. This study aimed at evaluating the antimalarial activity and safety profile of *Z. scabra*. *Plasmodium berghei* was used for induction of malaria, kept in a refrigerator and maintained by serial passage of blood from mouse to mouse. The crude extract, chloroform and ethylacetate fractions were obtained from air dried aerial part of *Z. scabra*. Activity was tested *in vivo* against chloroquine-sensitive strain of *P. berghei* by measuring the parasite load using light microscope. 80% methanolic extract yield was 18%. The 2000 mg/kg body weight of the crude extract was devoid of any signs of toxicity. Crude extract of the plant provided 62.5, 72.85 and 76.01% suppression with increasing doses of 100, 200 and 400 mg/kg, respectively. Two solvent fractions of the plant have been also assessed for the same parameter. The ethyl acetate fraction was found to be the most active of all with suppression of 71.88% for 25 mg/kg, 62.47% for 50 mg/kg and 77.62% for the 400 mg/kg dose. Similarly, the 25, 50 and 100 mg/kg of chloroform fractions had also shown 53.57, 73.95 and 61.31%, respectively. In the case of survival of the animals, after 7 days of treatment groups, the ethyl acetate groups had shown better outcome which was 100% for the medium (50 mg/kg) and maximum doses (100 mg/kg). Activities as well as safety studies of this plant confirm the ethnopharmacological usefulness as antimalarial, thus its usage by the folkloric medicine. It can also be used as a base for characterization of some active compounds that could be used as markers for standardization of the extracts for use as traditional antimalarial.**

Key words: *Antimalarial acitivy*, *Plasmodium berghei, Zehneria scabra,* parasitemia.

INTRODUCTION

Malaria infection and associated complications continue to be a major health problem in many parts of the world including the America, Asia and Africa. It is one of the leading causes of morbidity and mortality in some of the poorest tropical and subtropical regions. Particularly it remains to be one of the most important illnesses in sub-Saharan Africa where 20% of children under the age of 5 die as a result of this infection. The World Health

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Organization (WHO) estimates that every year 250 million people become infected and nearly one million die (Agusto et al., 2013, Yetein et al., 2013).

In Ethiopia, malaria constitutes a major public health preoccupation particularly for children and pregnant women. In 2005, the Ethiopian government launched a massive expansion of the malaria prevention and control program aimed mainly at the reduction of malaria in populations living below 2,000 meters above sea level. However, global warming has been implicated in the increase in prevalence of malaria in some highlands of the country (Woyessa et al., 2012). In the past decades, the situation has been aggravated by increasing spread of drug-resistant *Plasmodium falciparum* strains. In Ethiopia, *P. falciparum* and *Plasmodium vivax* account for 60 to 70% and 30 to 40% of malaria cases, respectively (Kinfu et al., 2012). *P. falciparum* has been the major cause of epidemics, and of most malaria deaths and drug resistance are common for this particular protozoa species (Kiszewski and Teklehaimanot, 2004).

Despite challenges of drug resistance, modern chemotherapeutic agents are the main stay of treatment for patients having access to health services, but majority of the population in the rural areas use herbal remedies to treat malaria like fever. New antimalarial drug leads are therefore urgently needed. Traditional healers have long used plants to prevent or cure infections. Herbal medicine or phytomedicine refers to the use of any plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes (Barrett et al., 1999). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which are based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people (Kirby, 1996; Yetein et al., 2013).

Countries in Africa, Asia and Latin America use herbal and traditional medicine to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care (Kaur and Jaggi, 2010). In industrialized countries, adoption of traditional medicine termed ‘‘Alternative medicine’’ is getting much higher attention in recent years. Current research shows medicinal plants continue to play an important role in health aid (Edwards, 2012).

Zehneria scabra (L.F.) Sond is an important climber which belongs to the family Cucurbitaceae, which has been used in many traditional medicinal systems. It has many medicinal values such as to cure fever, diarrhea, skin diseases, stomach pain and treat livestock. Extracts of *Z. scabra* is mentioned in Ethiopian folklore for the treatment of a variety of diseases. The 80% methanolic extract of *Z. scabra* exhibits antimicrobial activity against common bacterial pathogens such as *Staphylococcus aureus* and *Escherichia coli*. The plant is also used traditionally for the treatment of inflammation and pain management (Bruck, 2004; Berhanemeskel, 2007). Medicinal uses of this plant have also been mentioned in other African regions such as Tanzania and Cameroon. Although there are no experimental studies conducted on the antimalarial activity of *Z. scabra,* yet studies on plant species of Cucurbitaceae family demonstrated significant (30%) reduction in parasitemia levels (Amorim et al., 1988; Bickii, 2007; Moshi et al., 2012).

In vivo antimalarial tests generally assess the development of rodent specific parasites *Plasmodium berghei* Vincke and Lips, *Plasmodium yoelii* Landau, Michel and Adam or *Plasmodium chabaudi* Landau in mice, after subcutaneous and/or oral administration. Activity is expressed as a decrease of parasitemia after a certain time which is examined in smears or as survival time. These parasite models are indispensable for the development of antimalarial even if they do not always perfectly mirror *Plasmodium falciparum* infection in human (Fidock et al., 2004; Sullivan et al., 2011). Despite widespread development of resistance, currently used and potent antimalarial drugs such as artemether, chloroquine and quinine are obtained from plant sources. Hence, it is imperative to focus on traditionally used medicinal plants for the discovery of possible new innovative antimalarial sources for the future. In line with this, the antimalarial activity of different solvent fractions of leaves of *Z. scabra*, a traditionally used plant for malaria like fever in Northwest Ethiopia has been assessed in mice models.

METHODS

Chemicals and materials used

Normal isotonic saline, 80% methanol, hexane, ethyl acetate, chloroform, tween 2%, citrate dextrose, giemsa, distilled water, 1 ml syringes, needles, feeding tube, vials, electronic balance, stopwatch, gloves, laboratory glass wares, microscope were used.

Collection of plant material

Large amounts of the leaves of *Z. scabra* were collected from the suburb of Gondar, Azezo district, 10 km away from the city, on the way to Addis Ababa. It was identified by Mr. Abyot Endale in Pharmacognosy Department, University of Gondar. The dried voucher specimen (No. SoP UoG21001) was deposited in the herbarium of the School of Pharmacy. The plant material has been collected from communal grazing land, for which specific permission was not required. The plant is not considered as endangered species, since it grows in many parts of the country, both in private and communal lands. Moreover, the amount of used in this study is small in quantity, and it does not affect the environment.

Extraction and fractionation

The leaves of the plant were air-dried at room temperature under the shade and pulverized. Subsequently, the pulverized leaves were extracted using 80% methanol. A total of 200 g dried leaves

were extracted by maceration (100 g of dried leave in 600 ml of 80% methanol) for 72 h. The extraction process was facilitated by using a shaker at 120 rpm. The mixture was first filtered with Whatman filter paper No. 1 (Whatman®, England). The residue was re-macerated twice for the same duration of hours and then filtered. The combined filtrates were then dried by Rota evaporator (Buchi Rota vapor, Switzerland) at a temperature of 40°C. After drying, a total of 36 g of dry extract was harvested (18% yield) and the dried extract was kept at -20°C until use. The crude 80% methanol extract was then successively partitioned using hexane, ethyl-acetate, chloroform and water. The fractions collected were dried and kept in the same condition as the crude extract at the same temperature.

Acute toxicity study

Acute toxicity study was conducted for the active extracts (Single dose 2 g/kg) using Organization for Economic Cooperation and Development (OECD, 2008) guidelines. The first animal was given with a limit dose of 2000 mg/kg, and then four other mice were sequentially treated based on the outcome of the first animal. The animals were observed for toxicities like diarrhea, weight loss, absence of tremor, lethargy and paralysis periodically for the first four hours during the 24 h period and later followed for 14 days for any lethality (OECD, 2008).

Animals

Antimalarial activity was assessed on mice of either sex. Swiss albino mice (18 to 25 g) were maintained at a temperature of 22 ± 2°C with 12 h light/12 h dark cycle and given food and water. Animal handling and care in the experimental procedure were conducted according to local ethical guidelines. This study was approved by the Bioethics Committee of School of Pharmacy, University of Gondar. All animals were also acclimatized to the working environment one week prior to the experiments.

In vivo antimalarial activity testing

Plasmodium berghei was used for induction of malaria in experimental mice. The parasites kept in a fridge before they were administered to the mice and maintained by serial passage of blood. The infected blood was then collected in test tubes by the tail bleeding and diluted with isotonic saline before it was given to the different groups of mice. The *in vivo* antimalarial activity was evaluated using the method described by Mohanty et al. (2013). Methanol, ethyl acetate and chloroform fractions were obtained from air dried aerial part of *Z. scabra,* while the hexane fraction could not result in a yield which can be administered to the animals. The fractions were tested *in vivo* against *P. berghei* infected mice by measuring the parasite load and mean survival time. In this test, five mice in each group were infected by intraperitoneal (i.p.) injection of *P. berghei* infected erythrocytes, diluted in 0.5 ml of sterile acid citrate dextrose. The blood was then diluted with physiological saline (0.9%) based on parasitemia level of the donor mice and the red blood cell (RBC) count of normal mice in such a way that 1 ml blood contains 5×10^7 infected RBCs. Each mouse was then given 0.2 ml of this diluted blood intraperitoneally which contained 1×10^7 *P. berghei* infected RBCs (Kiszewski and Teklehaimanot, 2004).

Mice were orally treated following Peters 4-Day suppression test (Peters, 1993). Animals were infected on day 0, (D0) while treatment started 24 h after infection (D1). Malaria infection was established in mice by i.p. inoculation of 200 µl of 1×10^6 parasitized cells/ml on the first day (D0) of the experiment. The animals in the test groups have received different doses of the preparations based on their group 24 h post infection. The dose was maintained for 4 consecutive days. Drugs were administered orally. To ascertain the parasitemia, on the third day of experiment, thin blood smears were made and stained with 10% giemsa in phosphate buffer, pH 7.2 for 20 min. A blood sample for the slide preparation was taken using tail bleeding method. The slide was examined under a microscope at 100×. Percentage parasitemia was determined by counting the parasitized red blood cells on at least four random fields of the giemsa stained slide. The mean parasitemia of each group was determined and the standard deviation values were also included in the result. Each experiment had a positive control group and a negative control group. The positive control group received chloroquine (reference drug) at a dose of 25 mg/kg while the negative control group received tween 2%. *In vivo* antimalarial efficacy was examined by evaluating percent suppression, percent survival and mean survival time. Percent suppression was calculated as:

$$\text{Percent suppression} = 100 \times [(A-B) / A]$$

Where A is the mean percent parasitemia of the mice taken as negative control and B is the mean parasitemia in the test group. In addition, mortality in the mice was followed up to 28 day post-infection to evaluate the percent survival and mean survival time. According to ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guideline (Kilkenny et al., 2012), we used the humane endpoints for the survival study. The animals were humanely euthanized and the criteria used for their killing includes weight loss up to 25%, weakness or inability to obtain feed or water and when they are at their critical stage of their lives. Cervical dislocation method was used for killing the animals after the experiment. Any pain or distressed which occurred after the ends of the experiment were thoroughly followed and appropriate measures like humanely killing of the animals were used. Animals were checked for these symptoms twice a day until the end of the experiment. Nonetheless, throughout the experiments, we did not notice a distress on the animals with respect to the guideline. No anesthetic or analgesic was used as the procedure and method did not require the use of analgesics or anesthetics for such a study.

Phytochemical screening

The crude extract and solvent fractions were screened for the presence of different secondary metabolites following standard procedures (Gavamukulya et al., 2014).

Statistical analysis

Results were expressed as means ± SEM and analyzed using SPSS version 19. Comparisons were made between negative control (vehicle), positive control (chloroquine) and treatment groups of various doses using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Body weight differences were also analyzed using paired t-test. P-values of 0.05 were considered statistically significant.

RESULTS

Extraction

The dried pulverized leaves of 200 g of *Z. scabra* were extracted by maceration with 80% methanol and the yield

Table 1. The average percentages of parasitemia, chemosuppression and survival effect of the different extracts, standard drug and negative control in mice (N = 5).

Plant ZS extract	Doses (mg/kg)	Parasitemia% (Mean ± SEM)	Chemosuppression% (Mean ± SEM)	Survival (%) on day 10
80% methanolic ZS	100	29.05 ± 7.7	62.54 ± 9.9	40
	200	21.05 ± 2.5	72.85 ± 3.17 [b]***	40
	400	18.6 ± 2.9	76.01 ± 3.75[b]***	60
TWEEN 2%		77.55 ± 2.4	0 ± 0[a]***	0
Chloroquine	25	0.0000	100 ± 0 [b]***	100
Chloroform	25	36 ± 6.4	53.57 ± 8.26	40
	50	20.2 ± 6.3	73.95 ± 8.17 [b]***	40
	100	30 ± 8.2	61.31 ± 10.58	60
Ethyl acetate	25	21.8 ± 5.6	71.88 ± 7.25 [b]***	60
	50	29.1 ± 4.1	62.47 ± 5.23	60
	100	17.35 ± 3.9	77.62 ± 5.13 [b]***	60

a - against Chloroquine, b - against Tween 2 %, * - p<0.05, 2 - **<0.01, 3 - ***<0.001.

was 36 g (18%), slightly higher than the findings of Akele (2012). There was no yield for n-hexane while the yields for chloroform and ethyl acetate were approximated to 10% each.

Acute toxicity

The 2000 mg/kg body weight of the crude extracts was devoid of any signs of toxicity in the first mouse. The other four animals also showed a similar outcome. These were evidenced by the lack of signs of toxicities like diarrhea, weight loss and absence of tremor, lethargy and paralysis during the first four hours, within the 24 h period and throughout the 14 days. Although there are no studies which supports the safety of the plant studies elsewhere (Moshi, 2007; Daniel, 2008) showed the relative safety of the plant. This study was also in line with the previous reports and supports the usage of the plant by the society without great concerns of safety.

Parasitemia and chemo-suppression

Malaria mortality was slightly but not significantly reduced by extracts of *Z. scabra* leaves in relation to the mortality in non-treated mice (P > 0.05). However, the mice survival time was prolonged due to the different extracts of the plant (Table 1). The 80% methanolic extract of the plant provided 62.5, 72.85 and 76.01% suppression with increasing doses of 100, 200 and 400 mg/kg, respectively. Two of the most active fractions (choloroform and ethyl acetate) of the plant have been also assessed for the same parameters. The ethyl acetate fraction was found to be the most active extract with suppression of 71.88% for (25 mg/kg), 62.47% for (50 mg/kg) and 77.62% for the maximum dose (100 mg). Similarly, the 25, 50 and 100 mg/kg of chloroform fractions also showed 53.57, 73.95 and 61.31% suppression, respectively. In the case of survival time for the animals, after ten days of treatment, the ethyl acetate group had shown a better result in maintaining the life of the mice, i.e. 100% for the 50 and 100 mg/kg.

Weight variation of animals

As shown in Table 2, the body weight of the animals in different groups was measured before the experimentation and afterwards. The chloroquine group together with the ethyl acetate fractions had kept the initial body weight, while the chloroform fractions, i.e. 25 mg and 100 mg/kg doses, and the negative control (Tween 2%) did not show any tendency of prevention of weight loss.

Phytochemical screening

Table 3 shows preliminary phytochemical tests and it has been revealed that a variety of secondary and primary metabolites were detected in the crude extract.

DISCUSSION

Nature has been the source of medicine for thousands of

Table 2. The variation in body weight of the animals in different groups before and after the experiment (N=5).

Group	Body wt. before (Mean ± SEM)	Body wt. after (Mean ± SEM)	*P*-value
ZS100	26.3 ± 0.72	24.4 ± 0.51	0.035
ZS200	25.9 ± 0.57	22 ± 1.04	0.071
ZS400	26.2 ± 1.16	25 ± 1.22	0.076
Chloroform 25	24.08 ± 0.30	18.8 ± 0.37	0.000
Choloroform 50	26.38 ± 0.87	26.6 ± 0.81	0.514
Chloroform 100	20 ± 1.21	17.8 ± 1.2	0.009
EA 25	22.5 ± 0.51	19.2 ± 0.49	0.014
EA 50	22.4 ± 1.20	20.2 ± 0.37	0.054
EA 100	22.56 ± 1.22	25.04 ± 1.08	0.742
Tween	23.68 ± 1.72	21.94 ± 1.68	0.252
Chloroquine	23 ± 1.08	22.94 ± 1.66	0.939

years, and an impressive number of new drugs have been isolated from natural sources, many which are based on their use in traditional medicine. Various therapeutic plants have been used for years in daily life, especially in those areas are short of modern medicine to treat a variety of diseases. Natural products cover a diversity space not yet available from synthetic libraries, with an unrivalled success rate as drug leads. Bioactive plant compounds have served as templates for several synthetic drugs and as precursors for the production of semi synthetic drugs (Wessjohann, 2000; Newman et al., 2003).

Z. scabra Sond is a perennial herb, climber or trailing to 6 m, widespread in tropical and southern Africa. It is mentioned in Ethiopian folk medicine for treatment of a variety of infectious diseases. In an ethnomedicinal study conducted in Ankober district of Ethiopia, highest fidelity level values were recorded for *Z. scabra* (95%) and *Hagenia abyssinica* (93.75 %) showing conformity of knowledge on the species of best healing potential (Lulekal et al., 2013). Some prominent uses of the plant include treatment of skin diseases, gonorrhea, syphilis, cleansing uterus before a child is delivered and malaria. It has also been reported that 80% methanol extracts of *Z. scabra* exhibits antimicrobial activity against most common bacterial pathogens like *S. aureus* and *E. coli* (Bruck, 2004). Studies on the plant use have also shown that the leaves are prepared by boiling, and then the decoction taken in the form of a drink. Sometimes, the plant is mixed with several other plants for treatment of malaria. The ethanol extract of the plant has exhibited antibacterial activity against some gram negative bacteria (Desta, 1993; Giday et al., 2007).

Parasitemia and chemo-suppression

The plant was extracted using various solvents; 80% methanol was used for the preparation of the crude extract followed by successive extraction using chloroform, ethyl acetate and hexane. The chloroform and ethyl acetate fractions were checked for antimalarial activity, while the hexane fraction was devoid of enough yields for evaluation of its activity. Thus, it is plausible to assume the active constituents for this plant would lie in either of the two extracts. The crude as well as the fractions of *Z. scabra* resulted in chemo suppression of various percentages. The 80% methanolic extracts provided 62.5, 72.85 and 76.01% suppression with increasing doses of 100, 200 and 400 mg/kg, respectively. The ethyl acetate fraction was found to be the most active with suppression of 71.88% for 25 mg/kg, 62.47% for 50 mg/kg and 77.62% for the 100 mg/kg. Similarly, the 25, 50 and 100 mg/kg of chloroform fractions also showed 53.57, 73.95 and 61.31% suppression, respectively.

In the case of survival of the animals, after seven days of treatment, the ethyl acetate groups had shown better outcome, i.e. 100% for both 50 and 100 mg/kg doses. In another study about the *in vitro* antibacterial activity of the different solvent fractions of the plant, it has been revealed that ethanolic and methanolic extracts showed the highest growth inhibition while ethyl acetate and chloroform extracts showed moderate inhibition. It has been shown in the study that ethanolic extracts of the plant effectively inhibit growth of both gram-positive and gram-negative bacteria while the aqueous extract showed no activity (Anad, 2012). This contrasts with our findings where the ethyl acetate fraction was found to be more potent. The difference might be attributed to differences in sensitivity between the protozoa and bacteria, and study models applied. Assuming the results obtained, it is suggested that the most potent antimalarial principles present in *Z. scabra* could be concentrated in the ethyl acetate fraction and organic solvent extraction method could be better for the isolation of antimalarial compounds from this particular medicinal plant.

Drugs like chloroquine lead to decreased parasitemia and resultant recovery of severe malaria. They also reduce parasitemia through various ways like reducing parasite nutrient intake, interfering with parasite metabolic

Table 3. Phytochemical screening test of crude extracts of *Zehneria scabra*.

Phytochemical	Test	Test result
Alkaloid	Wanger's test	+
Carbohydrates	Molisch's test	-
Amino acids	Ninhydrin test	-
Glycosides	Borntarger's test	+
Tannins	Ferric chloride test	+
Saponins	Foam test	-
Flavonoids	Lead acetate test	+
Phenols	Ferric chloride test	+
Diterpenes	Copper acetate test	+

pathways like heme metabolic pathway which is involved in the metabolism of iron. Drugs also negatively affect parasite reproduction and growth (Ziegler et al., 2002; de Villiers and Egan, 2009). The plant extracts reduced the level of parasitemia and prolonged survival up to the 10th day. Chloroquine had a good chemo-suppression of 100% as determined on the fourth day post-infection and a 100% survival rate by 10th day post-infection.

The activities as well as the safety studies of this plant confirm the ethnopharmacological usefulness as antimalarials, thus its claim by the folkloric medicine. The plant extracts with chemo-uppression like the ethyl acetate fraction offer potential for isolation of lead antimalarial compounds. It can also be used as a base for the characterization of some active compounds that could be used as markers for standardization of the extracts for use as traditional antimalarial and thus contribute to the development of potential antimalarial medicine from the biodiversity of Ethiopia. The extracts prolonged the mean survival time of the mice indicating that the extracts suppressed *P. berghei* and reduced the overall pathologic effect of the parasite on the mice. However, neither the extracts nor the standard drug cured the infection. This could be due to recrudescence of *P. berghei* parasites after apparent cure (Bhat and Surolia, 2001).

As shown in Table 3, preliminary phytochemical tests reveal the presence of a variety of secondary and primary metabolites in the crude extract. At the same time, the ethyl acetate fractions were found to be more active. This fraction also minimized body weight reduction. The survival time percentage of mice treated with this fraction was also longer than the mice treated with the other extracts. This can also suggest the potential for isolating pure compounds with much higher antimalarial activity. However, this is not always the case as the anti-plasmodial activity of plants could also emerge as a result of the synergistic interactions of the different bioactive components. Some examples include alkaloids, flavonoids, triterpenoids, phenols and other compounds (Mengiste, 2012).

Conclusion

The activities as well as the safety studies of this plant confirm the ethno-pharmacological usefulness as antimalarial medicine, thus its highest utilization by the folkloric medicine. The ethyl acetate fraction with chemo suppression offer potential for isolation of lead anti-malarial compounds. It can also be used as a base for the characterization of some active compounds that could be used as markers for standardization of the extracts for use as traditional antimalarial and thus contribute to the development of potential antimalarial medicine from the biodiversity of Ethiopia.

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Conflict of Interest

Authors have not declared any conflict of interest.

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Anticonceptive, estrogenic and antiestrogenic potentials of methanol extract of *Garcinia kola* seed in rodents

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The anticonceptive, estrogenic and antiestrogenic potential of methanolic extract of *Garcinia kola* seed in rodents was investigated. The anti-conceptive effect of extract showed that the extract dose-dependently protected female mice and rats from conception for two to three gestational periods. Changes observed in the length and weights of pups were not statistically significant relative to control. There were no abnormalities observed in the pups over thirty days. In ovariectomized immature rats treated with extract (100 to 300 mg/kg), there was a significant increase in uterine wet weight. The extract also induced uterotrophic effects, namely, immature vaginal opening and cornification, when comparedwith control. These findings agree with the traditional use of *G. kola* seed in control of fertility. The contraceptive property of the extract may be associated with the direct effects of its chemical constituents.

Key words: *Garcinia kola*, anti-conceptive potential, estrogenic.

INTRODUCTION

In the last two decades, the scientific world has recorded an increased pharmacological evaluation of medicinal plants that could be of benefits as fertility regulatory agents (Farnsworth et al., 1980; Gupta and Rakhi, 2006). The search for these agents became very intense due to some adverse effects of the synthetic drugs. Besides, the high cost and non-affordability of these drugs, especially in developing countries, made it more imperative for an alternative search of drugs that could be accessible and affordable.

A large number of plants which have been screened for contraceptive activity, include among others, Gossypol seeds (Udoh et al., 1992), *Azadirachta indica* (Joshi et al., 1996), *Asparagus pubescens* (Nwafor et al., 1998), *Cassia nigricans* (Nwafor and Okwuasaba, 2001), *Similax krausinia* (Idiong, 2010) and *Carpolobia lutea* (Ettebong et al., 2011). Garcinia kola seed (Guttiferae), also known as bitter kola is one of the medicinal plants used by some indigenes in Nigeria, to control fertility in females. Despite its bitter taste, *G. kola* seed is widely used in African

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Traditional Medical practice. Studies carried out by Iwu and Igboko (1982) showed that the phytochemical principles, in G kola seed stimulated an increase in gastric acid secretion, exhibited anti-hepatotoxic biochemical effects (Iwu, 1985, Akintowa and Essien, 1990). Garcinia kola is also used in the treatment of such conditions as common cold, catarrh, cough, hoarseness of voice (Okunji and Iwu 1991), dysentery, diarrhoea. Other studies using methanolic extracts showed that the phytochemical principles, exhibited antidiabetic effect (Iwu et al, 1990); antipyretic, anti- inflammatory effects (Braide, 1993, Iwu 1993). It has also been shown that ingestion of G. kola seed caused mild bronchodilatation in man (Orie and Ekon, 1993). Udoh (1998) reported that G. kola seed diets fed for durations lasting 6 weeks or longer caused testicular atrophy and degeneration of spermatozoa in male rats. A similar work was carried out by Braide et al. (2003) on female rats to determine the effects of the seed on female reproductive system. It was observed that the seed caused a decrease in serum concentration of the gonadotropins (FSH and LH) and prolactin, while coincidentally causing marked increase in serum level of estradiol and progesterone in female rats. The seed also caused marked proliferation of the uterine endothelial cells and dilation of the lumen. In another study, Akpanta et al. (2005) reported that ethanolic extract of *G. kola* seed blocked ovulation in female rats. The present study, designed to investigate the anticonceptive potential of the plant was instigated by the findings from the works mentioned earlier.

MATERIALS AND METHODS

Plant

Fresh seeds of *G. kola* were purchased from the local markets in Uyo, Akwa Ibom State, Nigeria. The plant was authenticated by Dr (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo and a voucher specimen with number UUH220 was assigned to it and was deposited at the herbarium of Department of Botany and Ecological Studies, University of Uyo.

Extraction procedure

The seeds were peeled to remove testa, washed and air-dried for 8 h, then subsequently dried in an electric oven (Astell Hearson, England) thermostatically controlled at 40°C for 12 h. The dry seeds were pulverized to a fine powder with the aid of a mortar and pestle. The pulverized powder was exhaustively de-fatted and was further extracted by cold maceration in methanol for 72 h. The filtrate was concentrated and evaporated to dryness using the yield calculated and then stored in a refrigerator at -4°C until when needed.

Phytochemical screening

Phytochemical screening of the extract was done to determine the presence of chemical constituents such as flavonoids, simple sugar, alkaloids, tannins, saponins, phlobatannins, cardiac glycosides and anthraquinones and the methods of Odebiyi and Sofowora (1978) and Trease and Evans (1989) were adopted.

Animals

Adults and immature albino rats (weighing 180 to 220 g and 60 to 90 g, respectively) and mice (18 to 25 g) were obtained from the University of Calabar, Calabar, Cross River State, Nigeria. They were not quarantined for 2 weeks and subsequently quarantined for two weeks, and then were maintained strictly under favorable environmental conditions of 12 h light/12 h dark cycle, temperature 22 ± 2.5°C and fed with growers pellets feed (Bendel Feeds and flour Mills Ltd, Edo State) with water *ad libitum*. All animal experiments were conducted in accordance with internationally accepted laboratory animal use and care (Based on Helsinki Convention) and guidelines and rules of Faculty of Pharmacy, University of Uyo, Ethical Committee Report on Animal Experimentation.

Determination of median lethal dose (LD_{50})

The method of Miller and Tainter (1944) was used to determine the median lethal dose of the extract. Thirty-six healthy albino mice weighing 18 to 25 g were divided into six groups of six mice per group. Different doses (100 to 2000 mg/kg) of the extract were administered intraperitoneally (i.p). Physical signs of toxicity were observed for 24 h and recorded. The mortality values obtained were used to plot a graph of log probit versus concentration.

Anticonceptive activity

The anticonceptive activity was determined using the method of Nwafor et al. (1998). Adult albino female rats and mice showing regular estrus cycle through daily vaginal smear analysis and those having at least two successive 4-day estrus cycles were selected. The animals were randomized and separated into six groups consisting of six animals per group. Group I received 5 ml/kg of Tween 80, intraperitoneally in divided doses for 4 days. Groups II, III, and IV received different doses (100 to 300 mg/kg) of the extract intraperitoneally in divided doses for 4 days. Group V received 0.1 µg/rat of 17β-estradiol, while group VI received 17β-estradiol concurrently with 200 mg/kg of extract. On the 5th day, fertile males were introduced in the ratio of one male to three females and allowed to remain until experiment was terminated. The number, weight and length of pups were recorded (Telleria et al., 1997; Nwafor et al., 1998).

Estrogenic and anti-estrogenic potentials

Estrogenic and antiestrogenic activities of the extract were assessed in bilaterally ovariectomized immature albino rats (weighing 70 to 90 g) using the methods of Edgren and Calhoun (1957) and Nwafor et al. (1998). The end point used to determine the estrogenic effects included: uterine wet weight, degree of vaginal cornification and quantal vaginal opening. Exactly one week after bilateral ovariectomy, the rats were randomized and divided into six groups of six animals per group. Group I received 5 ml/kg Tween 80 (s.c) in divided doses for four consecutive days and served as control. Groups II to IV received 100 to 300 mg/kg, respectively by the same route for four consecutive days. Group V received 0.1 µg/rat of 17-β-estradiol dissolved in corn oil by the same route for four consecutive days. Group VI received 200 mg/kg of extract concurrently with 17-β-estradiol for four consecutive days, to evaluate the antiestrogenic activity. The animals were observed for degree of vaginal opening and cornification. All animals were sacrificed 24 h after the last treatment and the uterine wet weight

Table 1. Phytochemical screening of extract.

	Test	Observation	Inference
	Alkaloid test		
a	Dragendorff's reagent	Brick red precipitate formed	++
b	Mayer's reagent	Yellow Precipitate formed	++
c	Wagner's reagent	Brownish Precipitate formed	++
	Saponin test		
a	Frothing test	Formed frothing, that lasted for a while	+++
b	Fehling's test	Brown precipitate formed	+++
c	Haemolysis test	Haemolysis in tubes with extract	+++
	Tannins		
a	Ferric Chloride test	Turned blue black	+++
b	Bromine test	Decolourized bromine water	+++
	Anthraquinones		
a	Borntrager's test	No violet colour observed in the ammonia phase	-
b	Combined Anthraquinones test	No violet colour observed in ammonia phase	-
	Cardiac glycoside		
a	Salkowski test	Steroidal ring present	+++
b	Keller Killiani test	Brown ring formed at interface	+++
c	Lieberman's test	Colour change from violet to blue to green	+++
	Flavoniod test	Crimson colour precipitate	+++
	Terpenes	No pink colour in the interface	-

+: Trace; ++: Positive; +++: Strongly positive; -: Absent.

Table 2. Anti-conceptive effect of methanol extract in adult female rats.

Dose (mg/kg)	Mean No. of pups	Protection over n-gestational period	Percentage of animals protected
Control (5 ml/kg Tween 80)	5.60 ± 0.45	(0/6)	0
100	5.17 ± 0.62	2 (3/6)	50
200	4.80 ± 0.00	2 (4/6)	67
300	4.50 ± 0.75	3 (4/6)	67
17-β	4.00 ± 0.52	3 (5/6)	93.33
17- β+ 200	3.85 ± 0.32	3 (5/6)	93.33

Numerator indicates the number of rats protected

for degree of vaginal opening and cornification. All animals were sacrificed 24 h after the last treatment and the uterine wet weight recorded (Rubin et al., 1951).

Statistical analysis

Results were expressed as multiple comparison of mean ± standard error of mean (SEM). Significance was determined using one way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5% was considered significant.

RESULTS

Acute toxicity test

The mean lethal dose (LD_{50}) was calculated to be 1000 ± 66.40 mg/kg. The physical signs of toxicity included excitation, paw-licking, and decreased motor activity. Others were increased respiratory rate, convulsion and death

Table 3. Anti-conceptive effective of extract in adult mice.

Dose (mg/kg)	Mean No. of pups	Protection over n-gestational period	Percentage of animals protected
Control (5 ml/kg Tween 80)	4.8 ± 1.06	(0/6)	0.00
100	3.00 ± 1.35	2 (3/6)	33.33
200	2.23 ± 1.24	2 (3/6)	50.00
300	2.00 ± 1.48	3 (4/6)	67.00
17-β	2.15 ± 1.06	3 (3/6)	50.00
17- β + 200	2.10 ± 1.15	3 (3/6)	50.00

Numerator indicates the number of mice protected.

Table 4. Estrogenic and anti-estrogenic effect of extract.

Dose (mg/kg)	Weight of Animals		Uterine wet weight (mg/100 g body weight)	Vaginal opening	Cornification
	Initial (g)	Final (g)			
Control (5 ml/kg Tween 80)	101.30	112.70	0.05 ± 0.01	-	-
100	121.30	123.00	0.07 ± 0.02	+	+
200	102.80	104.80	0.29 ± 0.15[c]	+	+
300	113.70	116.00	0.31 ± 0.01[c]	2+	2+
Standard (17-β estradiol)	111.70	115.50	0.42 ± 0.02[c]	4+	4+
200 + 17-β estradiol	94.00	108.30	0.56 ± 0.01[b]	4+	4+

Values represent Mean± SEM. Significance relative to control; [b]p<0.05, [c]p<0.001 (n=6).

(Figure 1).

Phytochemical screening

The phytochemical screening of the extract revealed the presence of the following secondary metabolites: tannins, saponins, flavonoids, alkaloids and cardiac glycosides. Phlobatanins and anthraquinones were however absent (Table 1).

Anticonceptive effect of the extract

The extract (100 to 300 mg/kg), protected the rats from conception. The protection lasted for 2 to 3 gestational periods, equivalent to 50 to 67% degrees of protection relative to control (Table 2). Similar effects were observed in mice (33.33 to 67%) (Table 3). Similarly, the effects of the extract on weight and length of pups in both rats and mice, showed no significant discriminatory changes (Figures 2 to 5).

Effect of extract on lengths and weights of pups

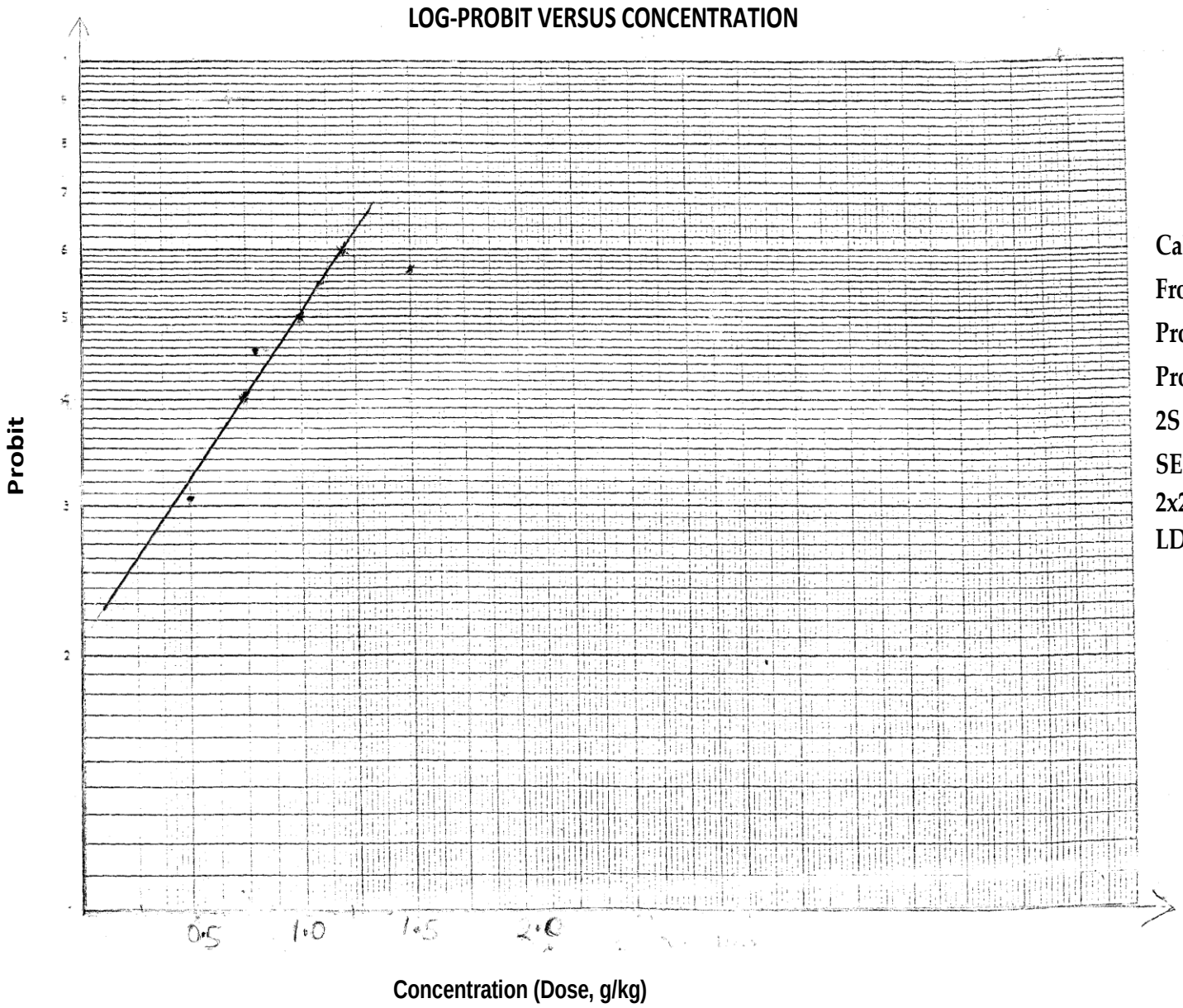
The extract caused changes in lengths and weights of pups, which were statistically not significant relative to control as shown in Figures 2 to 5.

Estrogenic and anti-estrogenic effects of extract

The methanolic extract of *G. kola* showed a dose dependent increase in uterine wet weight. This increase was statistically significant (p<0.05 to p<0.01). There were also vaginal opening and cornification in a dose-dependent manner. However, the extract showed a weak estrogenic effect relative to the standard (Table 4).

DISCUSSION

These results show that the methanolic extract of *G. kola* seed, possesses anti-conceptive activity in rats and mice. The extract also possesses weak estrogenic potential in rodents. This is predicated upon the fact that animals previously treated with extract and kept with sexually active males were protected over varied gestational periods. There was an increase in uterine weight which was dose dependent. The premature vaginal opening and cornification were observed in young overiectomized rats. These effects are associated with endometrial growth and proliferation (Jacobs et al., 1996). From the phytochemical screening, the extract contains flavonoids, which are known to have anti-inflammatory effects



Calculation of LD_{50}

From probit graph LD_{50} = 1000 mg/kg

Probit 4 = 740

Probit -6 = 1200

2S = Probit 6. – Probit 4 = 1200-740 = 460

SEM = $\sqrt{2}S/2 = \sqrt{460}$

2x24 =66.395

LD50 = 1000 ± 66.40 mg/kg

Figure 1. Graph for acute toxicity.

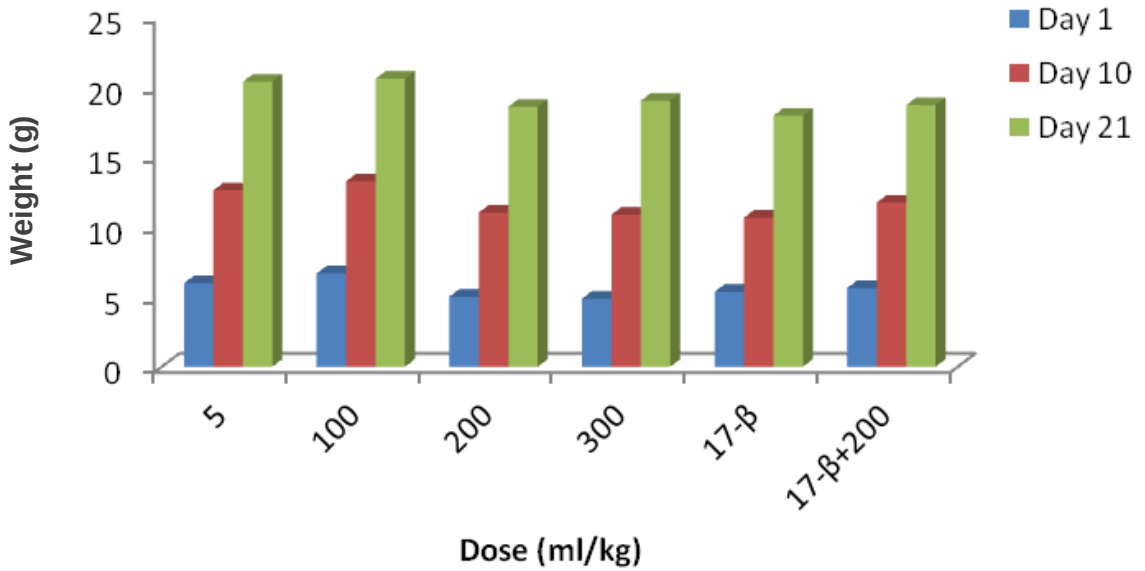


Figure 2. Effect of extract on the weight of rat pups.

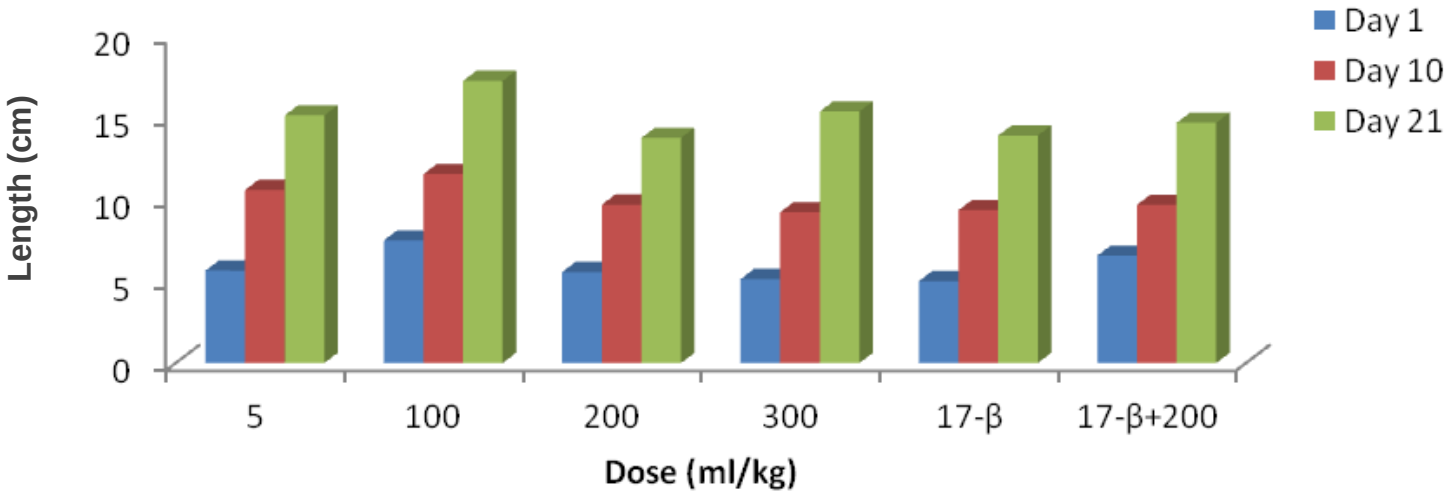


Figure 3. Effect of extract on the length of rat pups.

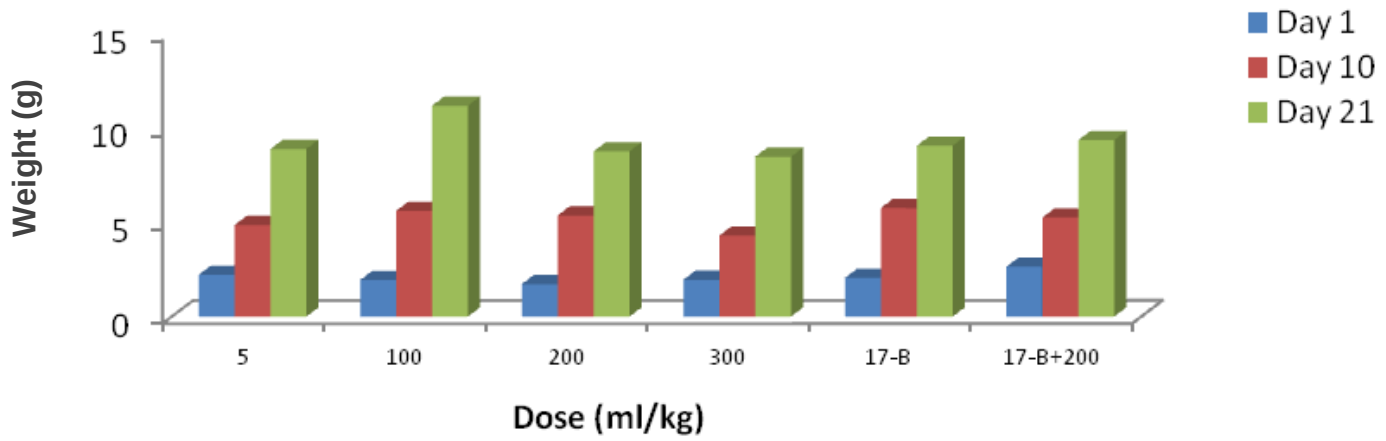


Figure 4. Effect of extract on the weight of mice pups.

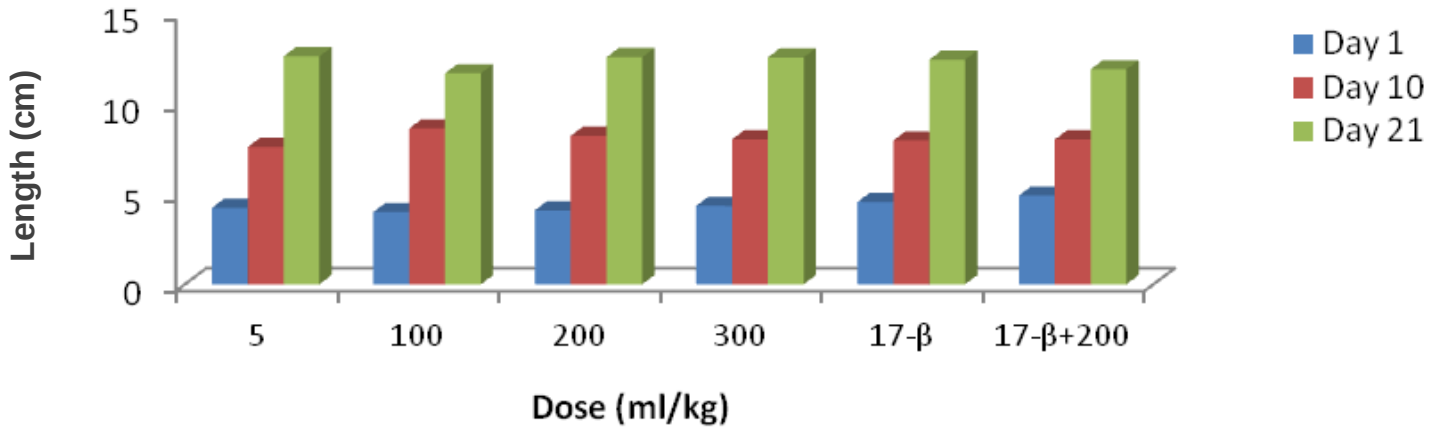


Figure 5. Effect of extract on the length of mice pups.

(Braide, 1993 and Liang et al., 1999). Ovulation is a type of inflammatory reactions which were blocked by anti-inflammatory drugs (Gaytan et al., 2002).

This may explain the anti-conceptive effect observed in the study. The weak estrogenic activity may also be responsible for the anti-conceptive effect of the extract. The study carried out by Braide et al. (2003), showed that the alkaloid fraction of *G. kola* seed, caused changes in gonadal hormones in female rats. It was observed that the seed caused a decrease in serum concentration of the gonadotropins (FSH and LH) and prolactin, while the alkaloid fraction of *G. kola* seed, caused changes in gonadal hormones in

female rats. It was observed that the seed caused a decrease in serum concentration of the gonadotropins (FSH and LH) and prolactin, while coincidentally causing marked increase in serum level of estradiol and progesterone in female rats. In another study, Akpanta et al. (2005) reported that ethanolic extract of *G. kola* seed blocked ovulation in female rats. The findings in the present study, coupled with other works which had been reported earlier, corroborate the rationale behind the traditional use of *G. kola* seed as contraceptive among women in some parts of Nigeria.

Conclusion

The findings in this study reveal that *G. kola* seed possesses anti-conceptive and weak estrogenic properties. Therefore, this work corroborates the rationale behind the traditional use of *G. kola* seed as contraceptive for women.

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Conflict of Interest

Authors have not declared any conflict of interest.

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Full Length Research Paper

Contribution to the knowledge of affinities of traditional medicine of Bantu of high and low lands in the territories of Beni and Lubero

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The investigations were conducted during 2012 on the Nande Bantu ethnic majority in the villages of Beni and Lubero territories which are located in North Kivu province in the eastern part of Democratic Republic of the Congo (DRC). Thus 6 villages in the zone of high altitude and 6 others in the zone of low altitude have been chosen randomly, by taking the farthest ones among them. Two-hundred and forty (240) persons were interviewed in 12 villages; 120 persons in each agro-climatic zone. In spite of the investigation, 309 species have been recorded, among these, 15 species treat at least 10 different diseases, such as: *Allium sativum, Allium cepa, Aloe vera, Bidens pilosa, Carica papaya, Citrus limon, Conyza sumatrensis, Crassocephalum montuosum, Dichrocephala integrifolia, Elaeis guineensis, Erythrina abyssinica, Persea americana, Plantago palmata, Ricinus communis* and *Solanum aculeastrum.* Plants in the family of Asteraceae are the most represented or (26%), followed by Alliaceae (13%). Two species were cited in more than 5 villages in low and high altitude, namely: *C. limon* and *R. communis*. In terms of the diseases treated by the species, 4 species are the most interesting such as: *A. sativum* and *C. limon* (26 diseases for each), *R. communis* (24) and *A. vera* (20).

Key words: Plants, popular traditional medicine, Bantus, high and low altitude, Beni and Lubero.

INTRODUCTION

The local socio-economic situation in the villages surveyed in the territory of Beni and Lubero is susceptible to strong degradation. Exchanges from one village to another are very limited because of the destruction of communication infrastructures and armed conflicts with their attendant problems. Commercial exchanges and access to farming resources are among the sub-conditions seen earlier. Thus, people mainly refer to

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traditional medicine based on the use of medicinal plants. This agrees well with observing World Health Organization (WHO) (1993) cited by Balagizi (2007), Sofowora (1986) and Augero (2008) cited by Ilumbe (2014), which estimated 75 to 80% of the populations of developing countries use traditional herbal medicine on the basis of plants for first aid. According to the secretary of the convention on Biological diversity, global sales of drugs base totaled 60 million dollars USD in 2000 (OMS, 2003). Some authors perceive this kind of situation as a profitable element that is real (Anstey, 1993; Kalpers, 2005).

The average rate of the use of health services, according to the report of the Use of Health Services and State Health and poverty in Democratic Republic of Congo, is about 0.5 consultations per inhabitant yearly (DR Congo, 2006). WHO estimates the utilization of traditional medicine for curing diseases as 60%. The same organization has given guidelines on good agricultural and collection practices for medicinal plants in order to ensure quality control (OMS, 2003).

On the demographic level, our study is on two territories diametrically opposed in terms of special occupation. The two territories, Beni and Lubero are located at the extreme north of North Kivu Province. The two areas cover 25.580 km^2, or 42.9% of area of the province of North Kivu, from which 18,096 km^2 for the territory of Lubero and of 7484 km^2 for Beni (Kujirakwija, 2006). On one hand, we have the mountain region of North Kivu, the densest of Democratic Republic of Congo, with a high density of 105 inhabitants on a squared kilometer (Nicolai, 1988, Mafikiri, 1994); on the other hand, the oriental valley of the territory of Beni was characterized by a weak density.

The vegetation varies greatly in the two territories according to the stages defined by the topography of the area. It gives knowledge on the use of plants in a very diversified traditional medicine. The transition forest occupies the foothills of Western ridge gardens up to 1600 m, for secondary forest. It is formed as a result of human activities which include cropping, clearing and burning where the soil is protected from sun and rain, and appears to be the most fertile. The periodic abandonment of this field follows the appearance of some softwood trees and the forest is gradually formed (Tailfer, 1989). The high stage which comes before warm lands presents the physiognomy of arbustive savannas, and the forest of natural mountains is transformed by the concentrated action of people in fallow and artificial tree-planting, for example *Eucalyptus* and *Acacia*. The sector of Beni is, on the contrary, the domain of semi-caducifolized forest. This latter formation has even undergone a profound degradation because of itinerant agriculture, which is intense in this territory.

This work builds on the idea that the Nande Bantus living in two areas of different altitudes may have different knowledge about the use of medicinal plants. We would like to name the diseases treated and the recipes used in popular traditional medicine of Nande Bantus of high and low altitude in order to decelerate the convergence and divergence among them.

Importance of the study

The use of plants in traditional medicine has attracted our attention to the extent that we now make reference to the phytotherapy of a good proportion of the population of Beni and Lubero territories, especially the ones who stay in the rural area, which is still land-locked. This use of plants without a preamble study exposes the users to several cases of intoxications, which can be confusion in identifying the plants or not preparing for exigencies. This work constitutes a tool which is used for the criblage of plants having real medicinal virtue, for visualizing the convergence in their use throughout the villages of these two territories and thus determining the most efficient means.

Area of study

The investigations focus on the Nande Bantu ethnic majority in the villages of Beni and Lubero. Thus 6 villages in the zone of high altitude and 6 others in the zone of low altitude have been chosen randomly, by taking the farthest ones among them. The territory of Lubero is situated in the East of the DRC and West of Edward Lake between longitude 28° 30' E and latitude 0° 30' N and 0° 30' S. The territory of Beni is situated between longitude 29° and 30° E and latitude 0° 30' S and 1° N. It is limited in the North by Ituri District, in the South by the territory of Lubero and the Republic of Uganda (Kasay, 1988). The high concentration of people and fast growth of the population in the high lands have resulted in land conflicts. This has caused a shift of part of this great population towards the zones of the less populated low altitude (Vyakuno, 2006). Figure 1 show the axes followed by this population during their settlement in the two territories.

MATERIALS AND METHODS

The ethno-botanic surveys were done in 12 villages where Nande Bantus live: 6 villages of high altitude and 6 other villages of low altitude. We have used a semi-structured questionnaire administered to 240 subjects; 120 persons per topographic area. The data collected from the different households throughout the villages were completed with the information collected during the group discussions. The administered questions focused on the treated diseases, use of plants for the preparation of medicines, the recipes and their preparatory mode, the administration mode and pharmaceutical form of medicines. The same questions were given to the participants during the group discussions; there, they enumerated all the species used for the preparation of medicines

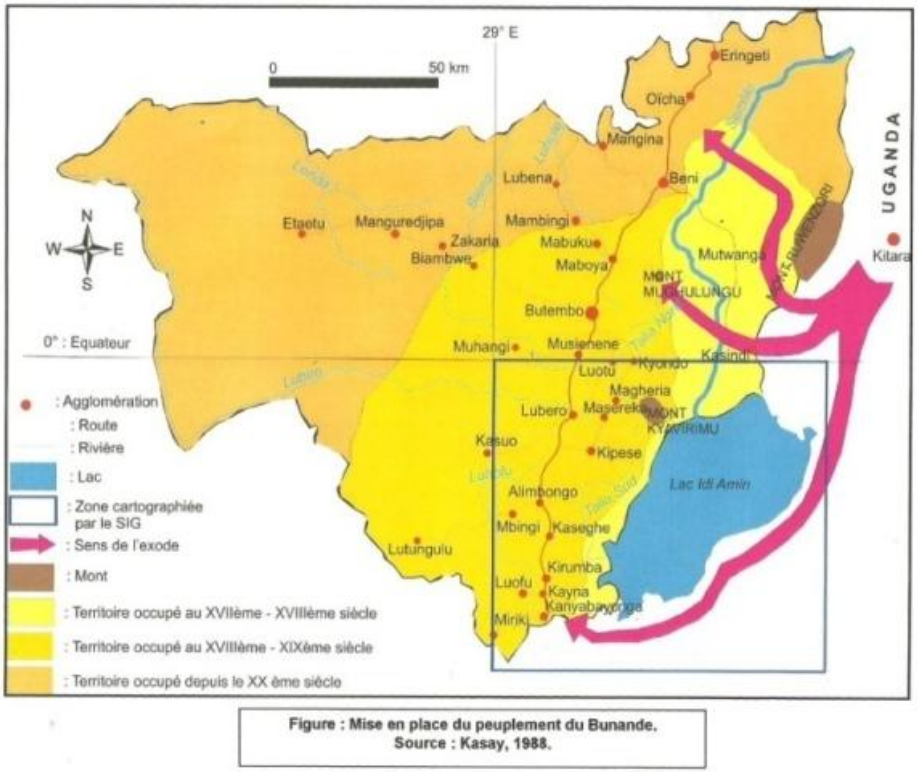

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Figure 1. Map of Bantu settlement in Beni and Lubero territories.

against a well determined infection. We based our approach on the guidelines of the database of traditional medicine and pharmacopoeia (Adjanohoun et al., 1970). The species were identified on site, with many guide documents on the flora of woody plants of Rwanda (Troupin, 1982), Catalogue of Vascular Plants of sub regions of Kisangani and Tsopo (High Zaire), 3rd edition (Lejoly et al., 1988) and the Central African Rainforest Tome 1 and 2 (Tailfer, 1989). Those that were difficult to identify on site were identified in the herbarium of the Faculty of Sciences, University of Kisangani. The results of the data collected from the Bantus living in the two agro-climatic zones have been analyzed and compared to the results of similar therapeutic use of plants done by other researchers in African countries.

RESULTS

Treated diseases

Many diseases are treated with the popular traditional medicine of the Bantus living in the villages of low and high altitude in Beni and Lubero territories. In total, 175 diseases are treated: 60 are treated by the Bantus living in the villages of high altitude and 46 by those who live in the villages of low altitude. The two groups (Coefficient of Jaccard) have 39.4%. Because this value is inferior to 50%, we conclude that the Bantus living in the low lands treat, in a great deal, diseases differently from the Bantus living in the villages of high altitude. Five diseases were cited by more or less 20% of the surveyed population and were considered as recurrent diseases. These diseases are: malaria, diarrhea, poisoning and cough.

Characteristics of recipes

The recipes used in traditional medicine require three characteristics which depend on the form of medicine they desire to obtain for the target disease and of the organ infected by the disease. These characteristics vary in the following manner between the two entities under investigation.

Preparatory mode of recipes

The preparation methods of recipes estimated as the simplest and mostly used by all without much supervision during their execution are the ones mostly used by the Bantus of Beni and Lubero territories. The Figure 2 visualizes the evolution of means obtained from expressed frequencies. It can be noticed that, decoction and distillation are the two modes of preparation mostly used by the Bantus of the two zones. The test of Wallis applied on the crossed table of the preparation modes of recipes by the Bantus of high and low altitude shows a great dispersion of frequencies in the same entity. The Chi-square calculated is $\chi^2 = 127$, dl = 14, and the P-value is $2.2e^{-16} < 0.05$. The Bantus of the two blocks use

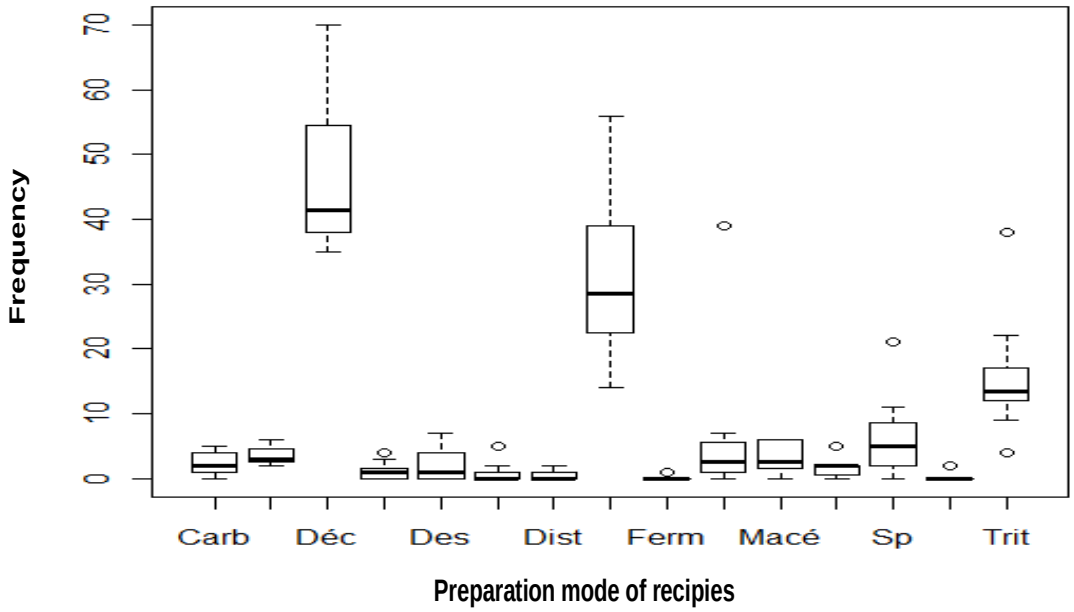

Figure 2. Variation of preparation mode of recipes in the villages of low and high altitude taken separately (Carb= carbonization, Dec= decoction, Des= desiccation, Dist= distillation, ferm= fermentation, Mac= maceration, Sp= without preparation, Trit= trituration).

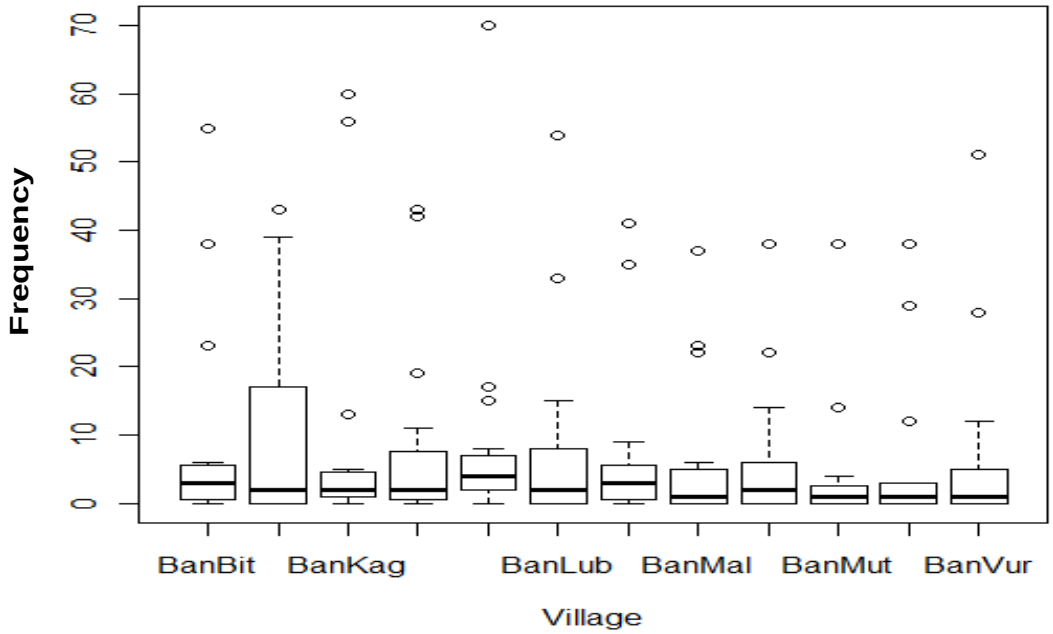

Figure 3. Variation of preparation modes of recipes between the villages of low and of high altitude (BanBit= Bantus of Bitungwe, BanKag= Bantus of Kagheri, BanLub=Bantus of Lubero, BanMal=Bantus of maleki, BanMut= Bantus of Mutwanga, BanVur= Bantus of Vurusi).

the same modes for preparing recipes. Figure 3 show the different variations of frequencies. The analysis of distribution of frequencies between the two villages using the test of Chi square of Wallis or χ^2 (6, 8), dl (11), and P-value (0, 81) > 0.05 makes us to conclude that there is no difference in the preparation of recipes between the villages.

Mode of administration of medicines

The analysis of the table of frequencies related to the administration of recipes showed the preponderant application of the oral ingestion followed by the administration through rectal way and local application (Figure 4). The modes of administration of medicines

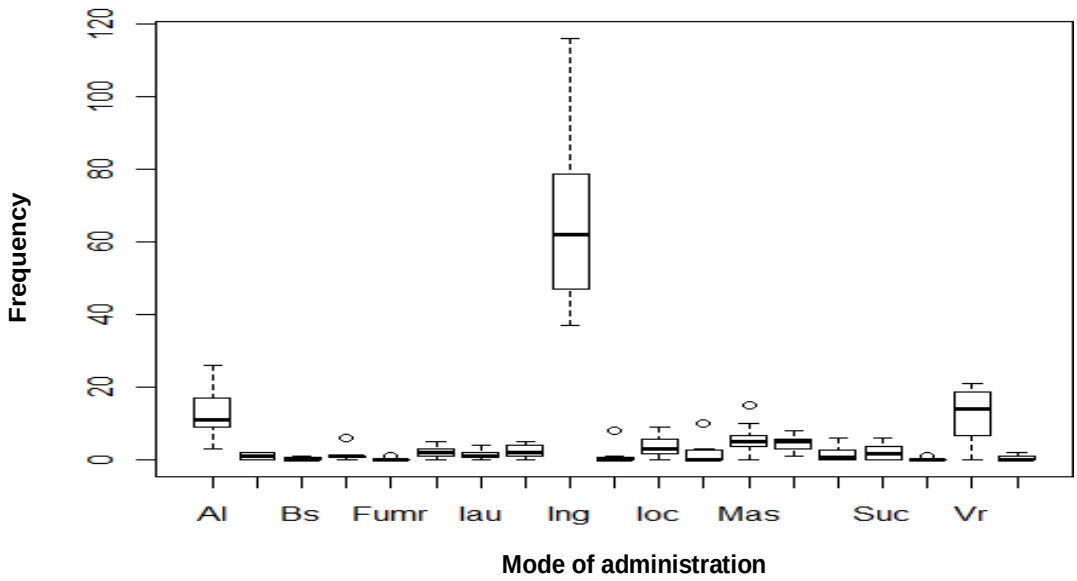

Figure 4. Variation of modes of administration of recipes through the villages (Al= local application, Bs= Bath of seat, Fumr= smoke, Iau= Auricular instillation, Ing= Ingestion,Ioc= Ocular instillation, Mas= Mastication, Suc= Succion, Vr= renal way).

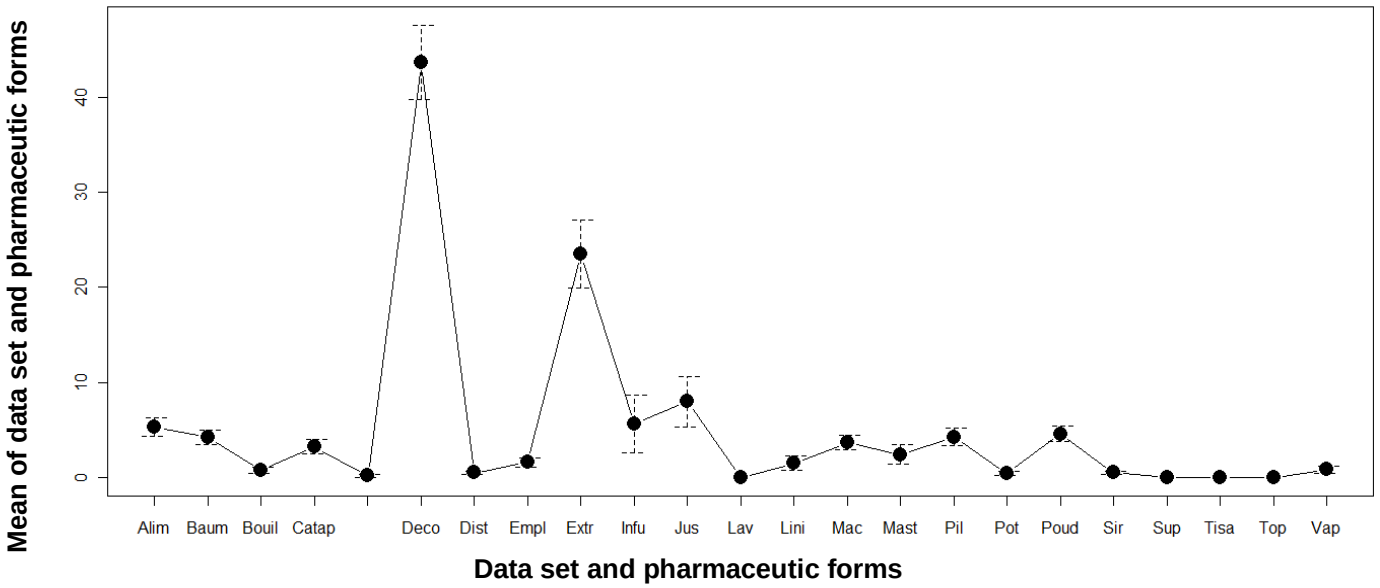

Figure 5. Variation of middle frequencies of the pharmaceutic forms used in the villages of high and low altitude of Beni and Lubero.

vary considerably in the villages of Beni and Lubero territories. The analysis of χ^2 gives 141, dl = 18 and the p-value = 2.2^{e-16}, very inferior value to the critical value of 0. 05. Whereas, the situation is the same in the two villages (P-value = 0.63 > 0.05).

Pharmaceutical forms of recipes

The distribution of forms of recipes used in the traditional medicine of the villages is represented in Figure 5. There are notable differences between the frequencies of the two forms: decocted, extra and other pharmaceutic forms. This shows the predominance of the forms of Bantus traditional medicine used for treating diseases. The test of Wallis gives a Chi-squared equivalent to 186, dl (22) and p-value (2.2^{e-16} < 0.05). This makes us to conclude that there exists a significant difference in the pharmaceutics forms used in Bantus traditional medicine of different villages.

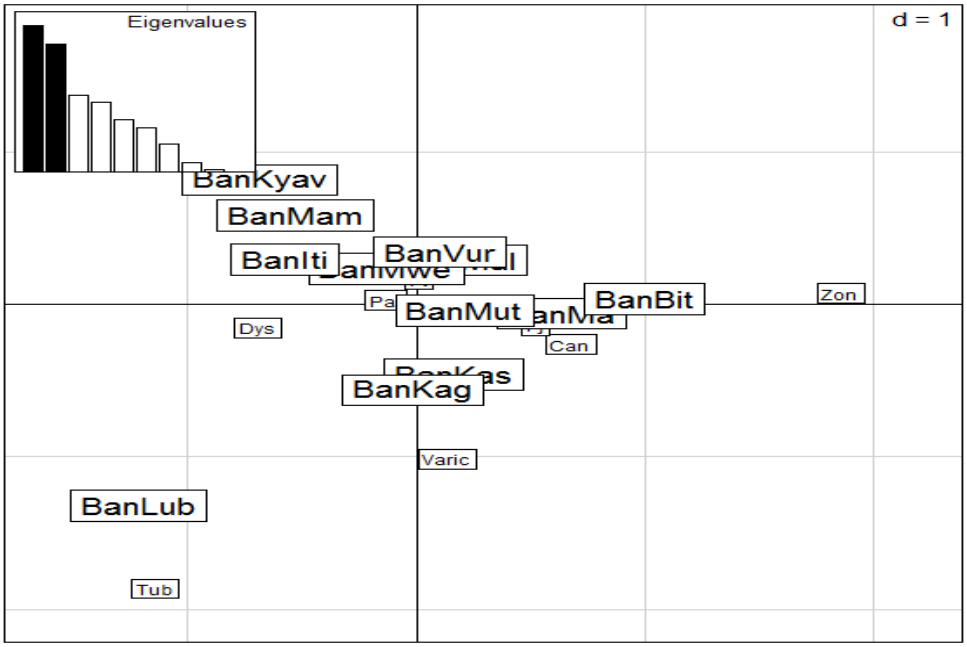

Figure 6. Representation of the AFC of the crossed table of infections and parasitory diseases-villages of high and low altitude in Beni and Lubero territories.

Floristic composition

The vegetal species which were recorded in popular traditional medicine of Bantus living in the villages of high and low lands are 309. Among these, 14 species used for the preparation of recipes are used to treat 10 different diseases. Families of plant species and the number of villages where they have been cited are listed in Table 1. This table shows that the plants of the family of Asteraceae are the most represented (26.6%), followed by the family of Alliaceae (13.3%). Most of the plants used by the Bantus are generally collected from hut garden. The two species have been cited in more than 5 villages in low and high altitude, namely: *Citrus limon* and *Ricinus communis*. In terms of diseases treated by species, 4 species intervene in more than 20 different diseases. These species are respectively, *Allium sativum* (26 diseases) *C. limon* (26 diseases), *R. communis* (23 diseases) and then *Aloe vera* (20 diseases).

Convergence in the treatment of diseases and the use of plants and recipes in Bantu villages

Figure 6 presents the regrouping of the villages of two eco-regional blocks (high and low altitude) in charge of infectious diseases. The test of Freidman shows that there do not exist, differences in treating infectious and parasitory diseases between the Bantus living in high altitude and those living in the lowest zones. Anyway, in high altitude they often treat yellow fever, gonorrhea, but those of low lands treat common malaria, typhoid fever and amibias. The chi-square shows 14.8 at the degree of liberty, dl (11) and the p-value (0.2) higher to the critical value of 0.05.

Diseases of digestive system

The diseases of digestive system are treated conjointly by the Bantus of high and low altitude. Figure 7 illustrates the convergence which exists between the two groups. The table shows that the Bantus are specialists in taking care of diseases, as they live in high or low altitude. Among the diseases of the digestive system, the Bantus living in the low altitude specialize in treating diarrhea, gastrite and gastric ulcers. The test of Freidman has given a chi-squared score of (17.9), dl (11) and p-value (0.08 > 0.05). This makes us to conclude that there are no significant differences in treating diseases of the digestive system by the Bantus of low and high altitude in Beni and Lubero territories.

Diseases of genito-urinary system

Treating diseases of the genitor-urinary system is very frequent in Bantu popular traditional medicine. It is noticed that there are significant differences even inside the same village. The Figure 8 visualizes well the situation as presented in the Bantus villages of high and low altitude of Beni and Lubero territories. The analysis of data of the crossed table of the diseases of the genito-urinary system shows a net difference of the villages of high altitude, which are positively linked to the two factorial axes for the care of prostates, dysmenorrheal;

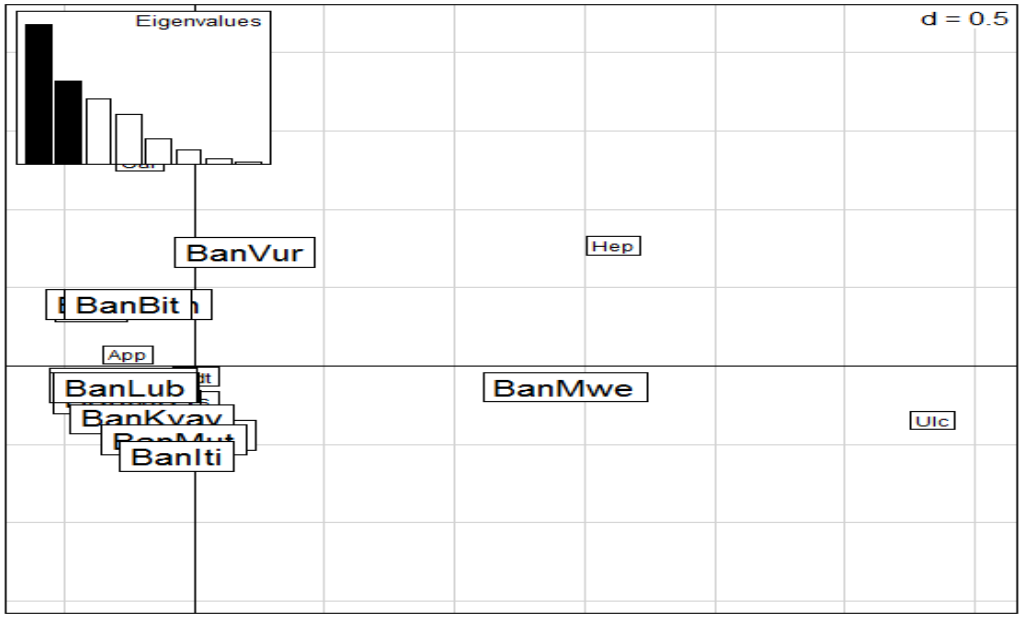

Figure 7. Representation of AFC of the crossed table of diseases of the digestive system-villages of low and high altitude of Beni-lubero.

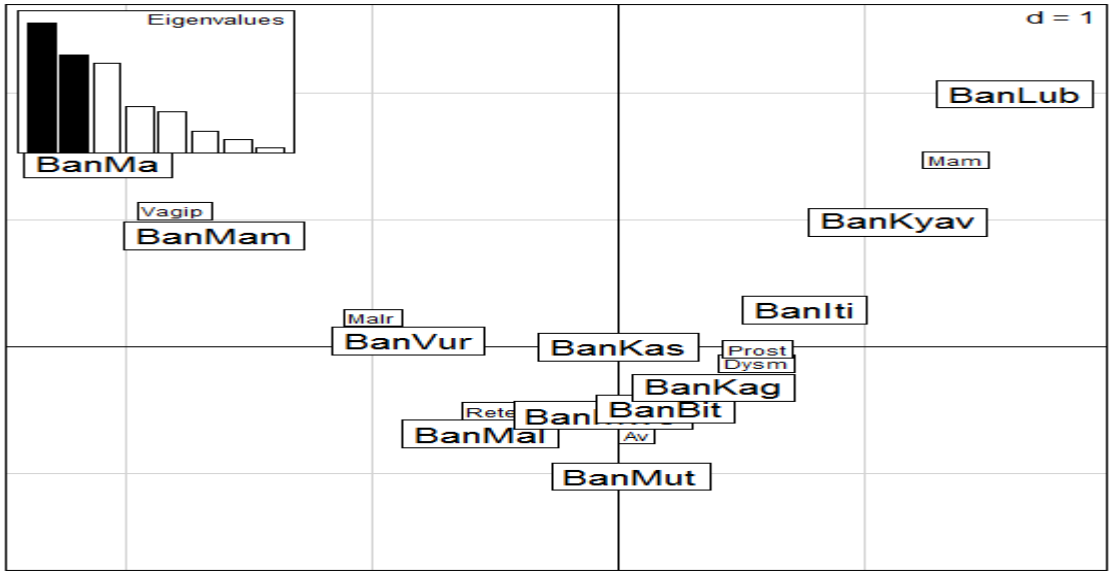

Figure 8. Representation of AFC of diseases of the genitor-urinary system treated by the Bantus of high and low altitude.

but the villages of low altitude seem to be very far from the axes and negatively correlated with the latter.

Diseases of the respiratory system

The diseases of respiratory ways are treated separately in the villages. The specialties based on the situation of the villages above the sea level are less pronounced. Figure 9 which presents the analysis of the crossed table of villages and the respiratory diseases illustrates well this distribution. The test of Friedman applied to the data of the two blocks taken separately did not show significant differences. The expressed Chi-square is, respectively (χ^2 = 3, dl = 4 and P-value = 0.5) for the villages of low altitude (χ^2 = 2, dl = 3 and p-value = 0.57). For the villages of high altitude, the P-value of 0.42 superior to 0.05 has been obtained in comparing the two blocks. This makes us to conclude that they have strong similitude in treating diseases of the respiratory system.

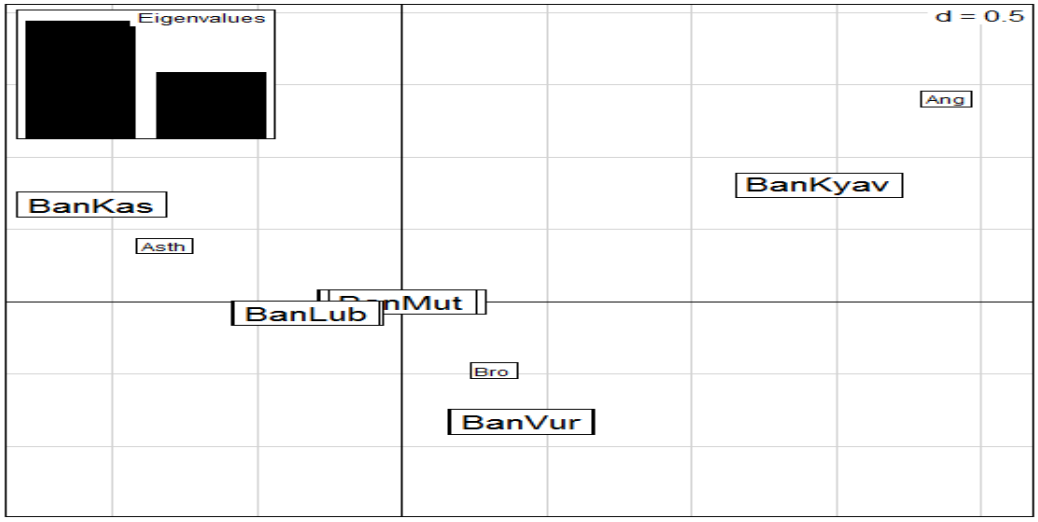

Figure 9. Representation of AFC of diseases of respiration through the villages.

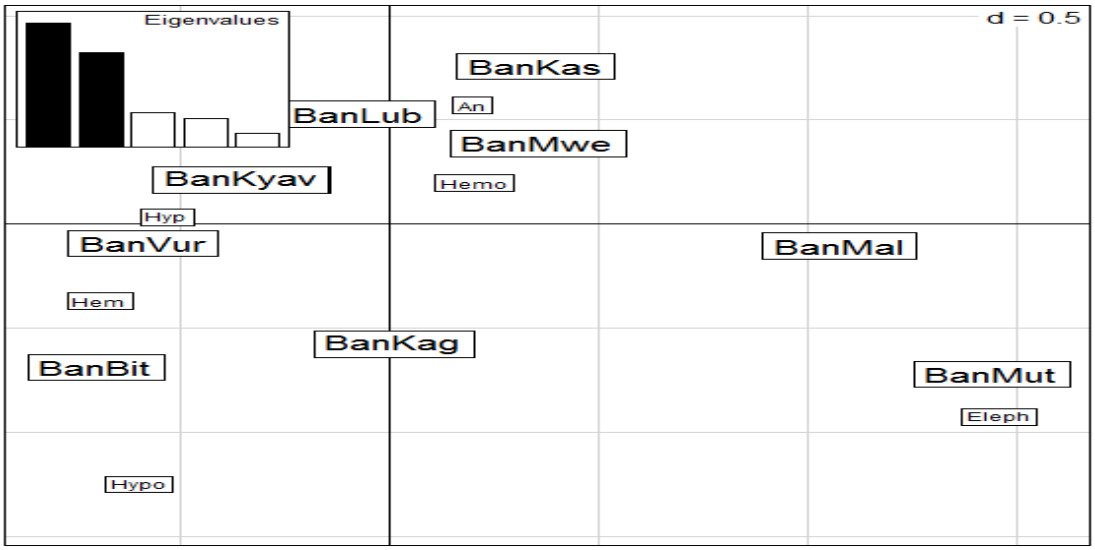

Figure 10. Representation of AFC of Bantu villages of low and high altitude in the care of blood diseases.

Blood diseases

The table of analysis of the following correspondence shows the liaison which exists between the Bantu of low altitude and those of high altitude in treating blood diseases (Figure 10). The Bantus of high altitude are positively correlated with the principal axis, namely Kasugho and Lubero for treating anemia, whereas the villages of kyavinyonge and Vurusi belonging to two different blocks are correlated negatively with the second axis for treating hypertension. Most of the villages of low altitude treat blood diseases independently. The test of χ^2 of Friedman or (16.8), dl = 11 gives a p-value (0.1) superior to the critical value of 0.05. This confirms a strong similitude between Bantus of low and high altitudes.

Diseases of the nervous system

The diseases of the nervous system are many and uniformly distributed in the villages of high and low altitude in Beni and Lubero territories (Figure 11). Kasugho and Kagheri villages of high altitude are correlated negatively with the first factorial axis for otitis, whereas the two epilepsies (E. little bad Epilepsy and Epilepsy) are treated conjointly by the Bantus of low and high altitude, who are more specialized in treating diseases of the nervous system.

Affinities between used species and recurrent diseases

The dendrogram hereafter summarizes the matrix that

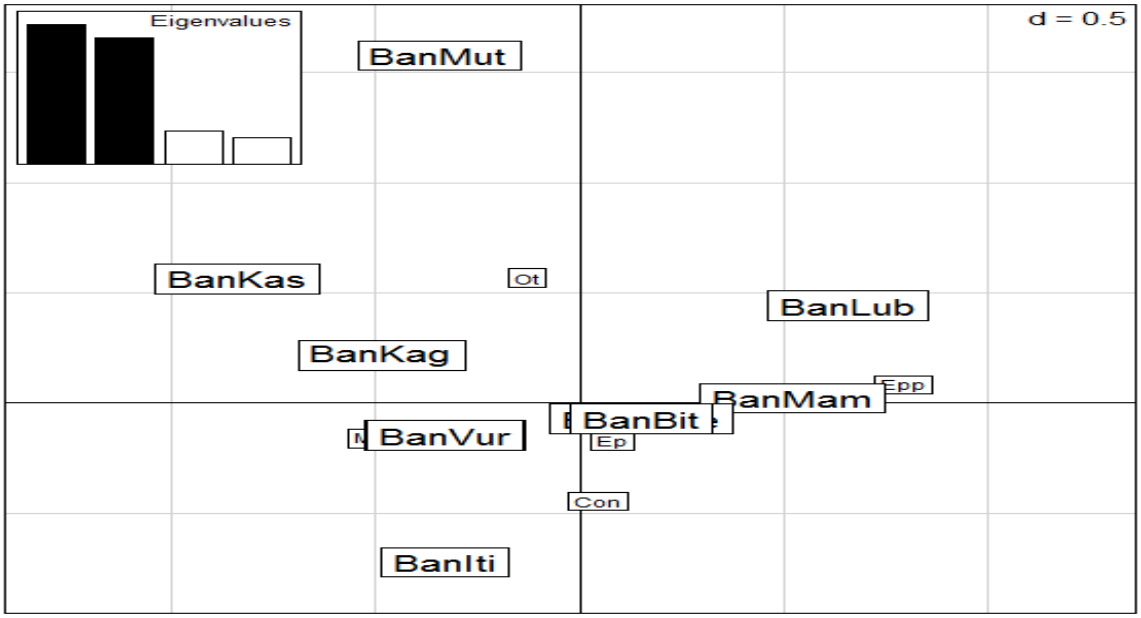

Figure 11. Representation of AFC of care of diseases of the nervous system by the Bantus of villages of low and high altitude

constitutes 12 groups of entertainers of the surveyed villages, 15 vegetal species used for preparing the recipes for treating 10 different diseases and 3 recurrent diseases (cited by at least 20% of the surveyed participants). The classification based on the method of Ward allowed us to distinguish 11 classes at the level of the final knots (100% of similarity), which consist of 7 mixed classes of the Bantus of high and low altitude and 3 exclusive classes of Bantus of low altitude. From 60% of similarity, two mixed classes of high and low altitudes are drawn, whereas at the inferior level (10%) of similarity, two classes are drawn from which consists one exclusive class of high and low altitude. Figure 12 presents the classes of vegetal species used against recurrent diseases.

The Bantus living in the villages of low and high altitude use more than 60% of the same plants to treat recurrent diseases. This is confirmed by the inter-groups which predominate on the dendrogram, in accordance with exclusive classes of Bantus of high and low altitude. Particularities are noticed in the preferences of use of such or other species to cure a given disease. In high altitude, the species of *Carica papaya* and *Bidens pilosa* are mostly used to treat malaria and the species of *Conyza sumatrensis* are mostly used against diarrhea. The test of Chi squared of Wallis has shown a significant difference in treating malaria and diarrhea between the two entities (χ^2 = 21.3, dl = 9, p-value = 0.011 < 0.05 and χ^2 = 21.7, dl = 9, p-value = 0.0097 < 0.05).

In low altitude, the species of *Solanum aculeastrum* is mostly used against hernia, but also intervenes in treating a series of diseases in high altitude. The test of Chi square of Wallis has shown a great difference in the use of plants against hernia (χ^2 = 25.7, dl = 9, P-value = 0.0022 < 0. 05). The global analysis of the results shows that the Nande Bantus of low altitude use a great number of the same plants as the Nande Bantus of high altitude. Anyway, they combine them differently against diseases. This brings us to invalidate partially our hypothesis that stipulates that “The Nande Bantus living in the villages of high altitude in Beni and Lubero territory use different plants from those used by the Nande Bantus of zones of low altitude”.

In order to raise the correspondences which exist in the use of species against recurrent diseases, we have done multi-varied analysis by the hierarchical classification presented in Figure 13. The analysis of the crossed table of most used species and recurrent diseases by the hierarchical classification of the method of Ward, using the squared of the Euclidian distance has come to 8 classes. At the level of 100% of correspondence of species used, 2 classes are seen: hand *Aloe vera* and *Bidens pilosa* against diarrhea and poisoning and hernia and poisoning. At the second level of the correspondence or at 95% of similarity of species used against recurrent diseases, only one class is drawn: *C. sumatrensis* and *Erythrina abyssinica* against diarrhea and malaria. At 92% correspondence, one class composed of *C. papaya*, *Citrus limon*, *S. aculeastrum* against poisoning is drawn. Whereas at 90% correspondence, *Allium cepa* and *Allium sativum* form a class against diarrhea and hernia. From 85% correspondence, 3 classes are formed: a class of plantago palmate, *C. sumatrensis* and *E. abyssinica* against malaria and cough; a class of *C. papaya, C. limon, S. aculeastrum, A. cepa* and *A. sativum* against poisoning at the level of 80% correspondence. Lastly or

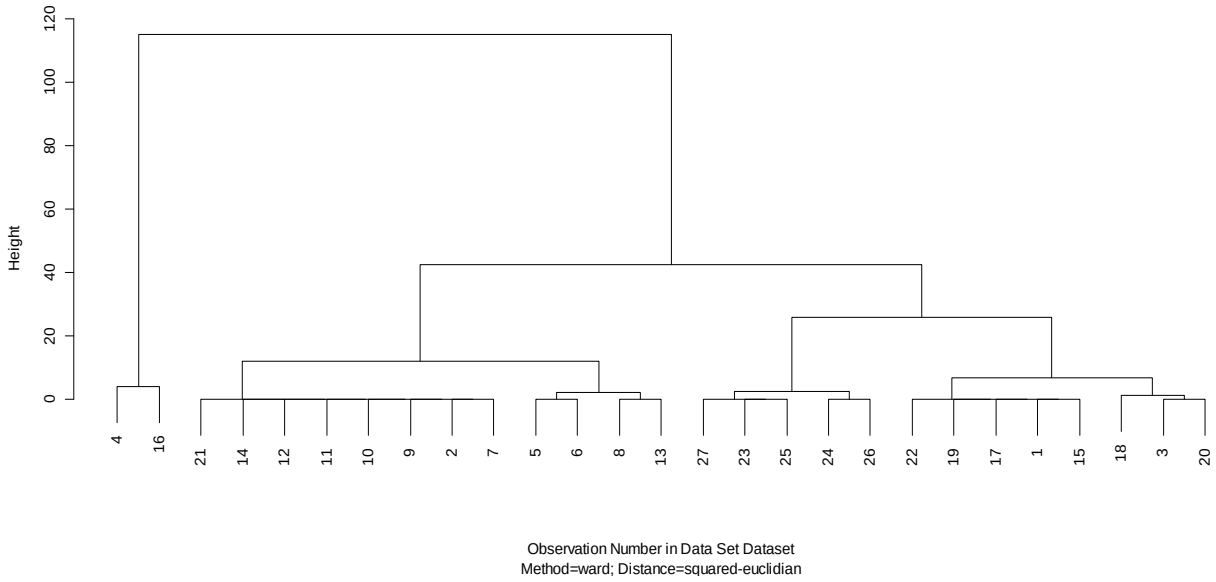

Figure 12. Classification of villages according to vegetal species used in popular traditional medicine(Légende: 1= *Allium cepa*- Kas , 2 = *Allium sativum*- Lub , 3 = *Aloe vera*- Vur , 4 = *Bidens pilosa* - Kas, 5 = *Bidens pilosa*- Iti, 6 = *Carica papaya*- Mal , 7 = *Carica papaya*-Mam, 8 = *Carica papaya* - Kyav, 9= *Carica papaya*-Bit, 10 = *Carica papaya*-Vur, 11= *Carica papaya*- Kas, 12= *Carica papaya*-Kag, 13 = *Carica papaya*-Lub, 14 = *Citrus limon*-Bit, 15 = *Conyza sumatrensis*- Kas, 16 = *Conyza sumatrensis*-Kag, 17 = *Conyza sumatrensis*-Kyav, 18 = *Conyza sumatrensis*- Mwe, 19= *Conyza sumatrensis*-Mut, 20= *Conyza sumatrensis*-Bit, 21= *Erythrina abyssinica*- Mal, 22 = *Plantago palmata*-Kag, 23= *Solanum aculeastrum*- Ma, 24= *Solanum aculeastrum*- Mam, 25= *Solanum aculeastrum*- Bit, 26 = *Solanum aculeastrum*-Vur, 27= *Solanum aculeastrum*- Kas

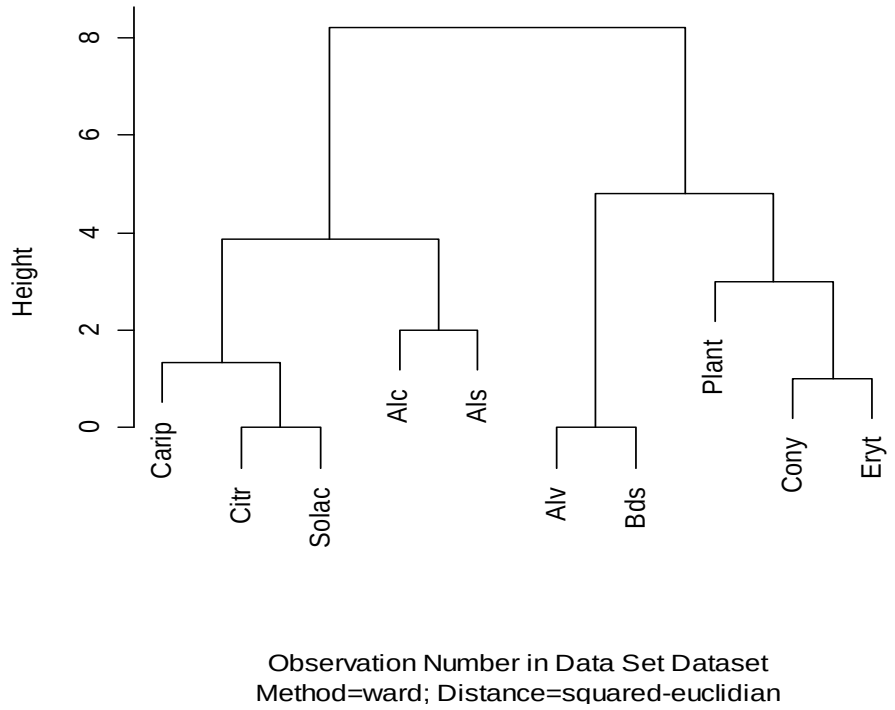

Figure 13. Dendrogram of convergence of use of plants against recurrent diseases.

under-classes is drawn*: A. vera* and *B. pilosa* against diarrhea and poisoning and *Plantago palmate*, *C. sumatrensis* and *Erythrina abyssinica* against malaria and cough.

Table 1. Vegetable species which treat at least 10 different diseases

Species	Family	Biotope	Number of diseases	Number of villages where plants have been cited	
				High land	Low land
Allium cepa	Liliaceae	Garden	18	5	2
Allium sativum	Liliaceae	Garden	26	5	2
Aloe vera	Xanthorrhoeaceae	Garden	20	6	4
Bidens pilosa	Asteraceae	Fallow	14	3	4
Carica papaya	Caricaceae	Garden	19	4	5
Citrus limon	Rutaceae	Garden	26	5	6
Conyza sumatrensis	Asteraceae	Fallow	15	4	3
Crassocephalum montuosum	Asteraceae	Fallow	13	5	3
Dichrocephala integrifolia	Asteraceae	Fallow	12	3	2
Elaeis guineensis	Arecaceae	Garden	11	4	1
Erythrina abyssinica	Fabaceae	Fallow	10	4	3
Persea americana	Lauraceae	Garden	10	4	5
Plantago palmata	Plantaginaceae	Fallow	13	5	2
Ricinus communis	Euphorbiaceae	Garden	23	5	5
Solanum aculeastrum	Solanaceae	Fallow	16	3	4

DISCUSSION

The Nande Bantus who live in villages of high and low altitude present many similitudes in the use of medicinal plants in Beni and Lubero territories. The global analysis of the results shows that the Bantus of low altitudes use a great number of the same plants as the Nande Bantus of high altitudes, but combine them differently against diseases. The preparation mode of recipes, the pharmaceutical forms and the administration modes of formulas do not differ significantly between the Bantus of high and low altitudes. Decoction, distillation and triturations are by far the preparation methods commonly used by Bantu Nande, while oral remains the most popular mode of administration. Similar observation was made in the Sanan people in Burkina Faso (Zerbo et al., 2000).

The treating of recurrent diseases such as diarrhea, malaria, hernia, poisoning and cough throughout the villages shows great similarities. The leaves of *B. pilosa,* for preparing decoction, are used against diarrhea. This same plant has been signaled for the same use in Congo Brazzaville (Adjanohoun and Ake Assi, 1988., Tra Bi et al., 2008). The extraction of juice or a decoction of the leaves and young flowers of *Bidens pilosa* to which is added a few drops of *A. vera* juice is also used to treat poisoning. The similar use of *Bidens pilosa* was reported in Rwanda (Rwangabo, 1993).

The decoction of skins of *E. abyssinica*, as well as the leaves of *Plantago palmata* taken through rectal or oral way against malaria in the Bantus villages of the two territories has been also signaled in the traditional medicine of Rwanda for the same use (Karagomba, 2007; Rwangabo, 1993 and James et al., 1997). The exudates of the leaves of *A. vera* mixed with Citrus juice and the extract of *A. cepa* obtained by maceration of Citrus juice has been signaled in other countries of the African continent (Schmelzer et al., 2008). *Gloriosa superba* species used for leaf decoction against cough in the two territories has been also signaled in Ivory Coast (Schemelzer et al., 2008).

The juice obtained from the maceration of slices of garlic bulbs and onion with lemon juice used against hernia in both territories is known to have anti tumor properties, this mixture causes superficial inflammation to resolve inflammation deeper (Gomez, 2003). This property of the mixture of garlic, onion and lemon justifies its use against hernia, which is defined as a tumor provoked by the output of an organ or an organ outside the cavity. The garlic and onion juice frequently used in many villages in Beni and Lubero against cough and Asthma have been reported for the same diseases (Gomez, 2003; Pamplona, 2000) and against bronchitis and several pulmonary infections (Kasali, 2013; Kabangu, 1990). The similar usages of plants in some countries of Africa are shown in Table 2.

Conclusion

The results of our investigations show that Nande Bantus living in areas of high and low altitude in Beni and Lubero territories use the same plants to treat diseases in traditional medicine. Belonging to a same ethnic group and the share of a common history seem to play more role than other factors in the use of medicinal plants. The global analysis shows that the Bantus living in villages of low altitudes use many plants which are the same as the

Table 2. Use of medicinal plants by the Nande Bantu of high and low altitude in Beni and Lubero territories and the similar usages in other countries of Africa.

Disease	Species	Used parts	Familly	References
	Acacia sp	Peel	Fabaceae	-
	Allanblackia stanerana	Peel	Clusiaceae	-
	Albizia ferruginea	Peel	Fabaceae	-
	Allium cepa	Bulbs	Liliaceae	-
	Allium sativum L.	Bulbs	Liliaceae	Kasali et al. (2013) and Pamplona (2000)
	Aloe vera	Leaves	Asphodelaceae	-
	Alstonia boonei De Wild.	Leaves	Apocynaceae	-
	Azadiracta indica	Leaves	Meliacea	-
	Bidens pilosa L.	Leaves	Asteraceae	Adjanohoun et al. (1988) and Trabi et al. (2008)
	Camelia sinensis	Leaves	Theaceaa	James (1997)
	Cassia floribunda	Leaves	Fabaceae	Balagizi et al. (2007)
	Cassia occidentalis	Leaves	Fabaceae	-
	Coffea canephora var robusta	Seed	Rubiaceae	-
	Conysa sumatrensis(Retz)E.h Walker	Leaves	Asteraceae	Pamplona (2000)
	Cyathea maniana	Leaves	-	-
	Combretum platyplerum	-	Combretaceaea	-
	Gallium aparine	Plant	Rubiaceae	-
	Gynura scandens O.Hoffm.	Leaves	Asteraceae	-
Diarrhea	*Harungana montana Spirlet.*	Leaves	Clusiaceae	Balagizi et al. (2007)
	Impatiens sp	Leaves	Balsaminaceae	-
	Ipomoea batatas (L.) Lam.	Leaves	Convolvulaceae	-
	Jatropha curcas L.	Leaves	Euphorbiaceae	Schmelzer et al. (2008) and Balagizi et al. (2007)
	Leucas martinisensis(Jack) R.Br.	Leaves	Lamiaceae	Rwangabo (1993)
	Mangifera indica L.	Leaves	Anacardiaceae	-
	Manihot esculenta		Euphorbiaceae	-
	Musa paradisiaca	Fruits	Musaceae	Balagizi et al. (2007)
	Musa sapientum L.	Rhizom	Musaceae	-
	Musa sp	-	-	-
	Passiflora edulis Sims.	Leaves	Pacifloraceae	-
	Persea americana Mill.	Leaves	Lauraceae	-
	Phaseolus vulgaris	Leaves	Fabaceae	-
	Plantago palmata Hook. F.	Leaves	Plantaginaceae	Schmelzer (2008), Rwangabo (1993) and Kasali et al. (2014)
	Psidium guajava L.	Leaves	Myrtaceae	Adjanohounet al. (1988), Trabi et al. (2008) and Kasali et al. (2014)
	Sida acuta Burm.	Leaves	Malavaceae	-
	Solanum tuberosum	Tuber	Solanaceae	-
	Tetradenia riparia(Hoscht)Codd	Leaves	Lamiaceae	Rwangabo (1993)
	Urtica dioïca	Leaves	Urticaceae	-
Empoisonment	*Allium sativum*	Bulbs	Liliaceae	-
	Aloe vera	Leaves	Asphodelaceae	-

Table 2. Cont'd

	Alstonia boonei	-	Apocynaceae	-
	Ananas comosus Merr.	Fruits	Bromeliaceae	-
	Anthocleista schweinfurthii	Peel	Loganiaceae	-
	Bidens pilosa	Leaves	Asteraceae	Rwangabo (1993)
	Carduus nyasanus	Leaves	Asteraceae	-
	Carex erecta	Leaves	Cyperacea	-
	Carica papaya L.	Leaves	Caricaceae	-
	Centella asiatica (L.) Urb.	Leaves	Apiaceae	-
	Chenopodium ambrasoïdes	Leaves	Chenopodiaceae	-
	Citrus limon (L) Burm.	Leaves	Rutaceae	-
	Crassocephallum montuosum	Leaves	Asteraceae	-
	Desmodium adscendens	Leaves	Fabaceae	-
	Fagara macrophylla	Peel	-	-
	Gynura scandens O.Hoffm	Leaves	Asteraceae	-
	Impatiens sp	Fruits	Balsaminaceae	-
	Indigofera arrecta Hochst.ex A Rich	Fruits	Fabaceae	Rwangabo (1993)
	Ipomoea batatas	Leaves	Convolvulaceae	-
	Senecio manii	-	Asteraceae	Rwangabo (1993)
	Leucas martinicensis	Leaves	Lamiaceae	Rwangabo (1993)
	Microchlosa pyrifolia	Leaves	Asteraceae	Balagizi et al. (2007)
	Musa sapientum	Fruits	Musaceae	-
	Cyphostema adenocaule	-	-	-
	Fleutherine bulbosa	Bulbs	Iridaceae	-
	Plantago palmata Hook. F.	Leaves	Plantaginaceae	Rwangabo (1993)
	Psidium guajava	Leaves	Myrtaceae	-
	Ricinus communis	Seed	Euphorbiaceae	-
	Sida acuta Burm. F.	Leaves	Malvaceae	-
	Sida rhombifolia L.	Leaves	Malvaceae	Rwangabo (1993)
	Solanum aculeastrum	Fruits	Solanaceae	-
	Zingiber officinalis	Tuber	Zingiberaceae	-
Hernia	*Achyrenthes aspera L.*	Plant	Amaranthaceae	-
	Ageratum conyzoides L.	Leaves	Asteraceae	-
	Albizia grandibracteata(Taub.)	Leaves	Fabaceae	-
	Allanblackia stanerana	Peel	Clusiaceae	-
	Allium cepa	Bulbs	Liliaceae	-
	Allium sativum	Bulbs	Liliaceae	-
	Allium sativum	-	Liliaceae	-
	Anthocleista schweinfurthii Gilb.	Peel	Loganiaceae	Schmelzer (2008)
	Capsicum frutescens L.	Fruits	Solanaceae	-
	Carduus nyassanus (S.Moore) R. E. Freis	Leaves	Asteraceae	-

Table 2. Cont'd

	Cassia occidentalis	Leaves	-	-
	Cissampelas mucranata A.Rich	-	Menispermaceae	Balagizi et al. (2007)
	Citrus limon(L) Burm.	Fruits	Rutaceae	-
	Colocasia anticorum	Stem	Araceae	-
	Crassocephalum montuosum	Leaves	Asteraceae	-
	Cyathea maniana (Hook.) Tryon	Sap	Cyatheaceae	-
	Cymbopogon citratus	Root	Poaceae	-
	Cymbopogon citratus	Leaves	Poaceae	-
	Vernonia sp	Leaves	Asteraceae	-
	Melinis minutiflora	Leaves	Poaceae	-
	Mikania cordata (Burn.f.) B.L. Robinson	Leaves	Asteraceae	-
	Microglossa pyrifolia(Lam.)	-	Asteraceae	-
	Musa sp	-	Musaceae	-
	Persea americana Mill.	-	Lauraceae	-
	Plantago palmata	-	Plantaginaceae	James (1997)
	Poliscia fulva (Hiern.) Harms	Leaves	Araliaceae	-
	Portulaca oleracea L.	Plant	Portulacaceae	-
	Rauvolfia vomitoria Afzel.	Peel	Apocynaceae	Schemalzer (2008)
	Ricinus communis	Leaves	Euphorbiaceae	-
	Solanum aculeastrum Dunal	Fruits	Solanaceae	-
Malaria	*Bidens pilosa L.*	Leaves	Asteraceae	Balagizi et al. (2007)
	Capsicum frutescens	Fruits	Solanaceae	-
	Cassia occidentalis L.	Leaves	Fabaceae	Balagizi et al. (2007) and Trabi et al. (2008)
	Colocasia sp	Leaves	Araceae	-
	Conysa sumatrensis (Retz) E.h. Walker	Leaves	Asteraceae	-
	Crassocephalum montuosum (S.Moore) Milne- Redh.	Leaves	Asteraceae	-
	Cyathea maniana	Leaves	-	-
	Cymbopogon citratus(DC)Stapf	Leaves	Poaceae	Balagizi et al. (2007) and Trabi et al. (2008)
	Erythrina abyssinica	Peel	Fabaceae	Rwangabo (1993)
	Eucalyptus citriodora	Leaves	Myrtaceae	-
	Eucalyptus globolus	Leaves and Peel	Myrtaceae	-
	Eucalyptus saligna	Leaves	Myrtaceae	-
	Eulesina corocana	Seed	Poaceae	-
	Fagara macrophylla(Oliv.) Engl.	Peel	Ruetaceae	-
	Gloriosa superba L.	Tuber	Colchicaceae	Schmelzer et al. (2008)
	Gynura scandens O. Hoffm.	Leaves	Asteraceae	-
	Hygrophila auriculata	Leaves	Acanthaceae	Balagizi et al. (2007)
	Laportea sp	Leaves	Urticaceae	-
	Moringa oleifera Lam.	Leaves	Moringaceae	-
	Musa sapientum L.	Flowers	Musaceae	-

Table 2. Cont'd

	Musanga cercropioïdes	Peel	Euphorbiaceae	-
	Ocimum gratissimum	Leaves	Lamiaceae	Balagizi et al. (2007)
	Pilea sp	Leaves	Urticaceae	-
	Piper guineense Schum.& Thonn.	Leaves	Piperaceae	-
	Piper capense L. f.	Leaves	Piperaceae	-
	Plantago palmata	Leaves	Plantaginaceae	Balagizi et al. (2007)
	Psidium guajava L.	Leaves	Myrtaceae	-
	Rubus adaeus	Leaves	Rosaceae	-
	Senna dydimobotria (Fresen)	Leaves	Fabaceae	Schemelzer (2008)
	Senna elata	Leaves	Fabaceae	-
	Senna occidentalis	Leaves	Fabaceae	-
	Spathodea campanulata P.Beauv.	Peel	Bignoniaceae	-
	Spilanthes mauritiana(Rich et Pers) DC	Leaves	Asteraceae	Rwangabo (1993)
	Thitonia diversifolia Gray.	Leaves	Asteraceae	-
	Vernonia amygdalina Del.	Leaves	Asteraceae	Rwangabo (1993), Kasali et al. (2013) and Ngbolua et al. (2011)
	Achyrenthes aspera	Plant.	Amarantaceae	-
	Allium sativum	Bulbs	Aliaceae	Gomez (2003) and Pamplona (2000)
	Allium cepa	Bulbs	-	Gomez (2003) and Paplona (2000)
	Aloe vera	Resin	Asphodelaceae	Schmelzer et al. (2008)
	Bidens pilosa L.	Leaves and Fruits	Asteraceae	Kasali et al. (2013) and Trabi et al. (2008)
	Bryophyllum pinnatum Kurz.	Leaves	Crassulaceae	-
	Carica papaya L.	Root, Flower, peel	Caricaceae	-
	Citrus limon (L) Burm.	Fruits	Rutaceae	Kasali et al. (2013) and Kasali et al. (2014)
	Cinchona succirubra	Leaves	-	-
	Coffea canephore var arabica	Leaves	Rubiaceae	-
	Cyathula uncinulata	Leaves	-	-
	Dichrocephala integrifolia (L. f.) o. Ktze.	Leaves	Asteraceae	-
Cough	*Elaeis guineensis Jacq.*	Seed	Arecaceae	-
	Eucalyptus globolus ssp (F.J) Muell.	Leaves	Myrtaceae	Rwangabo (1993) and Kasali et al. (2014)
	Gloriosa superba L.	Tuber	Colchicaceae	Schmelzer et al. (2008)
	Hisbiscus surattensis L.	Leaves	Malavaceae	-
	Kalanchoe crenata(Andr) Haw.	Leaves	Crassulaceae	Trabi et al. (2008)
	Lantana camara L.	Flower	Verbenaceae	Kasali et al. (2014)
	Lantana trifolia	Leaves	Verbenaceae	-
	Lapoprtea aestuans (L.)	Leaves	Urticaceae	-
	Maesa lanceolata Gilg.	Leaves	Myrsinaceae	-
	Maesa lanceolata Gilg.	Fruits	Myrsinaceae	-
	Mangifera indica L.	Leaves	Anacardiaceae	-
	Markhamia luthea (Benth.) K.Schum.	Leaves	Bignoniaceae	Rwangabo (1993)
	Momordica foetida Schumach.	Leaves	Cucurbitaceae	-

Table 2. Cont'd

Musa sapientum L.	Sap	Musaceae	-
Myrica salicifolia	Peel	Myricaceae	-
Persea Americana Mill.	Leaves	Lauraceae	Kasali et al. (2014)
Plantago palmata	Leaves	Plantaginaceae	-
Rauvolfia vomitoria Afzel.	Leaves	Apocynaceae	-
Vernonia sp	Leaves	Asteraceae	-
Rumex bequaertii De Wild	Leaves	Polygonaceae	-
Senna occidentalis	Leaves	Fabaceae	-
Spathodea campanulata P. Beauv.	Leaves	Bignoniaceae	-
Tagetes erecta L.	Leaves	Asteracea	-
Tagetes minuta	Leaves	Asteraceae	-
Tetradenia riparia (Hochst) codd	Leaves	Lamiaceae	Rwangabo (1993)
Zingiber officinalis	Tuber	Zingiberaceae	James (1997)

Bantus living in the villages of high altitudes, but in different combination against diseases. Preparation methods of recipes, pharmaceutical forms and routes of administration of recipes did not differ significantly between two groups; however Nande Bantus of high altitude prefer more decoctions, while those living in lowland villages mostly use distillation and triturating. The leaves, skins and stems are the parts most commonly used. This increases the vulnerability of the coveted species that are in most cases collected in the wild. Vulnerability studies of the exploited species, good intensive production methods or preparation and analysis of the active ingredients should be held in both territories to safeguard the heritage of great value for the autochthone population as well as for the whole nation.

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Conflict of interest

Authors have not declared any conflict of interest.

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