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Ethnic differences in use, phytochemical screening and non-poisonous leaves of *Phyllanthus amarus* (Schum & Thonn.) in North of Benin

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Abstract
This study was to evaluate the traditional medicinal use of *Phyllanthus amarus* in Northern of Benin among three majority ethnic groups and process to phytochemical and toxicological analyses of leaves from three provenances. A semi structured questionnaire was addressed to the questioned people. Accompanied with a translator, the questions are individuals after having presented if necessary a sample of the species. Collected data are ethnobotanical regarding inner knowledge of different sociocultural groups of the study zone on what they use *Phyllanthus amarus* organs for. Ethnobanical surveys reveal that the IE values indicate the knowledge about *Phyllanthus amarus* are distributed in a uniform way among Bariba and Otamari. Peulh use *Phyllanthus amarus* for its food and veterinary properties, the Bariba for esoteric and fertilizing properties and the Otamari for its medicinal properties. The watery extract of *Phyllanthus amarus* leaves contains big families of chemical groups (alkaloids, tannins, anthocyanin, mucilage, heterosids, etc.) whose number and nature vary according ecological stations. As far as the toxicity study based on larvas survival is concerned, the watery and ethanolic extract of the leaves present a LC_{50} superior to 0.1 mg/ml no matter what the provenance of the plant is, they are non-toxic on the human cells.

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1. Introduction

1.1. Background

Poverty, the environmental erosion, degradation and the climate constitute the main factors related to sanitary determination in the developing countries. And yet the Rio Act (1992) on the environment and development on its principle 1 states that: the human beings are in the centre of preoccupations related to lasting development. They have right to a healthy and productive life in harmony with the nature. But unfortunately, the well-being, the major preoccupation of all the humanity hardly becomes a reality in developed countries as well as in the developing ones. Among the non-transmissible diseases, Africans suffer must from diabetes (Erasto et al., 2005) and we estimate its predominance at 44% of the world population in 2030 (Sarah et al., 2004; Etuk et al., 2010).

At present, in the west of Africa, Ebola virus has reemerged under a new form and makes many people. In view of this African disaster, traditional healers are the ones who firstly give the primary health care to fight again these diseases. In fact, their very intimate relationship with the plant and mainly their know-how constitute the origin and evolution of ethnopharmalogy. The plants virtues are knowledge which is passed on to from a generation to another (Adjanohoun et al., 1989; Klotoé et al., 2013). A study carried out of USA at Illinois University (Chicago) in 2001 showed that among the medicinal substances found on the market, 122 would come from plants (of 94 different species). Among these natural molecules,

80% were used for the same or similar objectives with the ones for which the plants were used in traditional medicine (Daniel, 2001). This shows that the use of plants is a part of tradition of all cultures and that the medical development of these practices is very interesting. In the developing countries, in the absence of modern medical system, the therapeutic use of these plants is very present (Tabuti et al., 2003). In Africa, medical plants constitute precious resources for the majority of rural population, who more than 80% use for the health care (Jiofack et al., 2003; Jiofack et al., 2010). The access to the health constitutes a real problem for the population because of high cost of imported medicines. In Benin, among the 232 species used by pregnant women for the treatment of diabetes (Djègo et al., 2011), we have *Phyllanthus amarus*, an herb of Euphorbiaceae family (Unander et al., 2005). Though many phytochemical and pharmacological studies were carried out on the species in Africa (Coulibaly et al., 2011) as well as in the world (Foo et al., 1992), it is be noted in Benin, no specific datum on the inner knowledge, the ethnobotanical and phytochemical aspects and different forms of the species development is available.

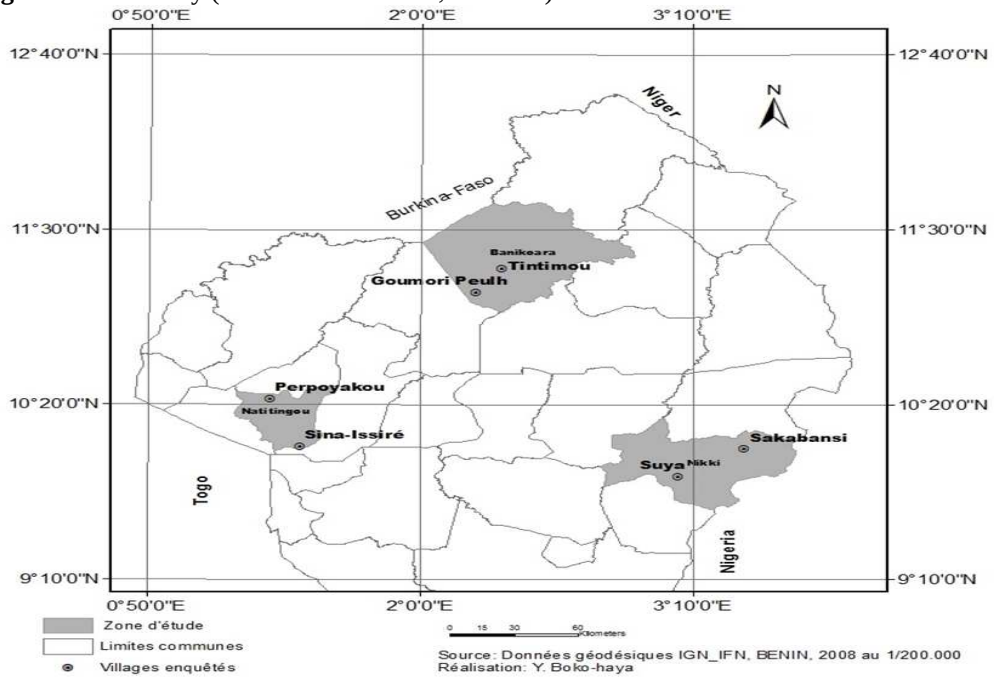
1.2 Objectives of research

This present study aims at evaluating the inner knowledge on *Phyllanthus amarus* use, identifying the different chemical groups characteristic of watery and ethanolic extracts of harvesting leaves per ecological station and evaluating in vitro the general toxicity of different watery and ethanolic extracts of *Phyllanthus amarus* larvas.

1.3 *Justification of the research*

Phyllanthus amarus is a medicinal plant on which there is no specific scientific data on inner knowledge, phytochemical aspect and different ways of promotion.

Figure 1: Area study (UTM zone 31 North, WGS 84)



2. Materials

2.1 Biological materials

The vegetable material that we used is composed of harvested *Phyllanthus amarus* leaves and authenticated at "l'Herbier National du Bénin". For the preliminary toxicity of *Phyllanthus amarus*, we used Saumur shrimps larvas (*Artemia salina*).

2.2 Sampling method

We choose the north of the country (Figure 1) where no similar previous study was carried out on vegetable species. We decide to choose three towns whose characteristic and geographical positions of housing are different. Thus, Natitingou, a mountainous region located in the West, Banikoara a little high region located in Centre of North and Nikki a non-mountainous region located in East were chosen. These tree towns also are the residential place of the three majority ethnic group of North-Benin: Bariba, Peulh and Otamari representing respectively 9.2%, 6.9% and 6.1% of Benin population (INSAE, 2013). In Bariba sociolinguistic group, we have three ethnics group (Boo, Boko and Bariba). In Fulani sociolinguistic

group, we have Gando and Fulfulde Fulani whereas Otamari is composed of Berba, Ditamari, Waama, Yendé, Gourmaché, Natimba, Betyodé and the Gagamba (INSAE, 2003). In each of these towns, two villages were chosen because of geographical location of the ethnic group on the one hand and their experiences in using medicinal plants on the other hand. For the sampling of the areas and surveys, a preliminary survey allowed us to question 75 people of both sexes randomly at the end of which 44% declared that they use the plant. Using Dagnelie formula (Dagnelie, 1998), N = $u^2_{1-\alpha/2}\, p(1-p)/d^2$ with $u^2_{1-\alpha/2} = (1.96)^2 \approx 4$ (for a normal distribution with α= 0.05) so N = $\frac{4p(1-p)}{d^2}$ where N represents the size of the sample. P the percentage of individuals having knowledge of the species (P = 0.04) and d the error margin on the considered parameters which is here 0.07. For the convenience reasons, we retained 198.

Per town, we based on the households using the plants on the one hand, in Natitingou on the traditional healers network, in Banikoara on the traditional healers association and in Nikki on the breeders association on other hand. 33 people (individuals were questioned in each of the following villages: Sinaïsséré and Perporiyakou (Natitingou), Tintinmou and Goumori (Banikoara) and Sakabansy with Suya (Nikki).

2.3 Method of data collection

For the ethnobotanical survey, it was carried out from August to October 2013; a semi structured questionnaire was addressed to the questioned people. Accompanied with a translator, the questions are individuals after having presented if necessary a sample of the species. The collected data are ethnobotanical regarding inner knowledge of different sociocultural groups of the study zone on what they use *Phyllanthus amarus* organs for. The leaves of *Phyllanthus amarus* were harvested in three different sites. In Natitingou, the leaves were harvested at Boriyouré at the foot of hills on stony ground on September 23 rd., 2013 in the morning from 8 to 11 a.m. The geographical coordinates of the station are N 10°19'11.1'' E 001°23'19.3''. In Banikoara, the leaves of the same species were harvested in maize farm at Sombikiragou on September 30 the, 2013 from 8 a.m. to 10 a.m. in the depression located on the clayey ground very favorable to agriculture. The geographical coordinates of the station are N 11°10'28.7'' E002°28'13.7''. As the samples harvested in Nikki occurred on October 4 the, 2013 at Goussounon Kperou from half past eight a.m. to 10 a.m. in soya farm on a sandy ground. The coordinates of the station are N 09°53'40.2'' E 003°08'45.6''. After having harvested the plants, their fresh samples were brought the same day at the laboratory where they were dried for from 10 to 14 days sheltered from the light within a confines at constant temperature (conditioned air). The dry leaves were then removed and reduced meticulously in powder with an electric waste disposal unit (mills) (FLOUR MILLS on Nigeria EL MOTOR N° 1827) and the obtained substance was filtered and stocked in a preservative for the analysis

Table 1: Uses and knowledge clue calculated for Phyllantus amarus

Clues	Calculation	Description
Interviewee diversity clue ID = U_x/U_t	ID, Number of use citations by a given informant (U_x) divided by the total number of uses (U_t)	Measures how many interviewees used a given species and how this knowledge is distributed among the interviewees
Interviewee equitability clue IE = ID/ID_{max}	IE, Interviewee diversity value (ID) divided by the highest diversity index value found (IDmax)	Measures the degree of homogeneity of the interviewees' knowledge
Consensual values for uses (CTU) CTU = (TU/*U*t)/*S*	CTU, number of times a given use is reported (TU) divided by the total number of uses (U_t). This value is then divided by the types of use separated within each category (S).	Measures the degree of concordance among interviewees with regard to the uses of a given species
Consensual values for plant parties CPP = P_x/P_t	CPP, number of times a given part of plant have been cited (P_x) divide par total number citations of all parts (P_t)	Measures the degree of concordance among interviewees according uses part of the plant.

2.4 Analysis of ethnobotanical data

The crude collected were captured in EXCEL spreadsheet and treated to make the statistic study easier. For the category of age and sex, we grouped together in each ethnic group the individuals/people in six groups: the male young people, the female young people, the male adult, the female adult, the male old people and the female old people. The young are people whose age is under 30, the one of adult is comprised between 30 and 60 and the one of the old people is above 60 (Assogbadjo et al., 2008).

For the quantitative analysis, different clues (Table 1) were calculated in order to measure the knowledge degree of people on whom the survey was carried out about *Phyllanthus amarus*. These measures are based on the diversity clue of questioned people (ID) and on the fairness clue (IE) (Byg et al., 2001; Monteiro et al., 2006).
A Factor Analysis of Correspondences was then carried out to describe the relationship existing between the how they used, used organs and the sociolinguistic groups on the one hand as well as the age and sex categories on the other hand with R Gui software (version 2.15.3).

2.5 Analysis of phytochemical data
2.5-1 Preparation of crude extracts
In this study two types of extracts: watery and ethanolic were prepared for the plant and for each area/town given a total of six extracts. Fifty (50 g) grams of each plant powder were put in solution in five hundred millilitres (500 ml) of solvent (distilled water for ethanolic and watery extracts at 90° and ethanolic extract). The mixture is left in steeping for 72 hours and the steeped product is filtered three times successively on absorbent cotton. Then the filtered product was evaporated at 40°C with the acid of with a rotavapor coupled with a water cooler and the residue is weighed for the determination of the yield.

2.5-2 Characteristics of chemical compounds
We found it necessary to make a quality analysis aiming at carrying out an experience the big chemical compound groups according to Houghton and Raman classical method (Houghton et al., 1998) based on the coloring and precipitation reactions. This analysis will allow us to have a global vision of the compounds groups responsible of eventual biological activities that the survey allocated to this plant.

2.6 Description of the toxicity test
The test is carried out according to Michael method (Michael et al., 1956) taking again by Vanhaecke (Vanhaecke et al., 1981) and by Sleet (Sleet et al., 1983). The *Artemia salina* eggs are incubated in sea water till the hatching of the young larvas (48 hours). We prepared a series of solutions of substances to be tested at progressive changeable/varied concentrations. A precise number of larvas is introduced in each solution. All the solution and the witness solutions (not containing active substance) are left under continuous agitation for 24 hours. The counting with microscope of the surviving larvas number in each solution helps to evaluate the toxicity of the solution. In the case where we notice death in the witness environment, the data are corrected by Abbott formula (Abbott, 1925)

% death = [(test – witness)/witness)] x 100

For our work, we used a colony of 16 larvas in each solution. The dose result data are transformed by logarithm and the LC_{50} is determined by linear decline. To appreciate the toxicity degree from LC_{50} values, correspondence table (Table 2) was referred (Mireille Mousseux, 1995).

Table 2: LC_{50} values

LC_{50}	Toxicity
$LC_{50} \geq 100$ µg/ml or 0,1mg/mL	- (non-toxic)
100µg/mL>$LC_{50} \geq$50µg/mL or 0,1mg/mL> $LC_{50} \geq$0,050mg/mL	+ (weak)
50µg/mL>$LC_{50} \geq$10µg/mL or 0,050mg/mL> $LC_{50} \geq$0,01mg/mL	+ + (moderate)
LC_{50}<10µg/mL or 0,01mg/mL	+ + + (high)

2.6-1 Preparation of solutions to be tested
A stock solution of each the six extracts is prepared by dissolving 200mg of extract in 4 ml of distilled water with a concentration of 500 mg/ml of the dissolution solvent. We proceeded then to ten (10) successive dilutions at half of the stock solution with sea water. The expressed concentration in mg/ml of diluted solution contained in testing tube numbered from 1 to 10 are respectively of 25; 12.5;6.25;3.125;1.563;0.781;0.195;0.098;0.049.
We sometimes proceed to a later dilution of stock solution for which the smallest quantity of tested concentration (0,049) kills more than half of the number of the larvas introduced and we take the test again to invalidate or validate the toxic character of the extract. Tests are repeated three times to be sure of the results obtained.

Table 3: Local names of *Phyllanthus amarus* according to different ethnic groups

GSL	Language	Local names
Otamari	Ditamari	Dori n'pueni
	Waama	Donna n'pueni, Donna m'poné
	Yendé	ouyonkouyonkou
	Gourmantché	Bousambou
	Natimba	Pèrèbigui alahaga
Bariba	Bariba	Biroubikou[1], bibigou[2] Birubinenku[3],
	Boo	Wèkpakpè
Peulh	Fulfudé	Rimatabétchél, Birmarukué, Gniyéi, Marmarkué[3], Danli[3], coudoubodoué[3]
	Gando	Bambowél, Birmaraboué, TchoGué, Dimirowélkpokpoouré
Others	Pila	Luna kawrr
	Nago	Eyi eyilolobé ounso

1= Natitingou, 2= Banikoara, 3= Nikki; GSL= Sociolinguistic Groups

3. Results

We notice the diversity of native/indigenous names given to the species according to the ethnic group and their geographical location (Table 3). This remark shows that the plant is well known by the different sociolinguistic groups.

3.1 Knowledge allocation

With the ethnic groups, the ID values, relatively near, show that *Phyllanthus amarus* users have almost the same level of knowledge of the species. The IE values indicate the knowledge about *Phyllanthus amarus* are distributed in a uniform way among Bariba and Otamari. With the Peulh, they don't have knowledge of the species. For the categories of age and sex, the female old people and male ones and then the male adult know more the plant (respectively ID= 0.20; IE= 0.48 and 0.46 then ID= 0.18; IE=0.42) of the species (Table 4).

Table 4: Fairness and diversity clues des of individuals

		ID	IE
		Mean	Mean
Ethnic groups	Bariba	0.16±0.14	0.39±0.19
	Otamari	0.16±0.14	0.39±0.19
	Peulh	0.20±0.14	0.47±0.19
	Total 1	0.17±0.01	0.41±0.18
Sex and age categories	Female Adult	0.16±0.15	0.39±0.06
	Male Adult	0.18±0.19	0.42±0.08
	Female Young	0.17±0.19	0.40±0.01
	Male Young	0.15±0.12	0.35±0.05
	Female Old	0.20±0.07	0.48±0.03
	Male Old	0.20±0.23	0.46±0.10
	Total 2	0.17±0.18	0.41±0.07

Interviewees number 198; Use number: 34; Use category: 7. ID = Diversity clue and IE= Fairness clue.

3.2 The ethnic group function

The AFC (Figure 2) showed that the axis 1 explains that 80.5% of the information are related to the use of *Phyllanthus amarus* whereas the axis 2 explains that 19.4%, so for the two axis 100% of the concerned information. So, on the axis 1, the Peulh use *Phyllanthus amarus* for its veterinary properties. On the axis 2, the Otamari use *Phyllanthus amarus* for its medical properties whereas Bariba use it for its magic and fertilizing properties. We can retain that the knowledge on *Phyllanthus amarus* varies from one ethic group to the other.

Figure 2: Use distribution of Phyllantus amarus according to sociolinguistic group in the axis system

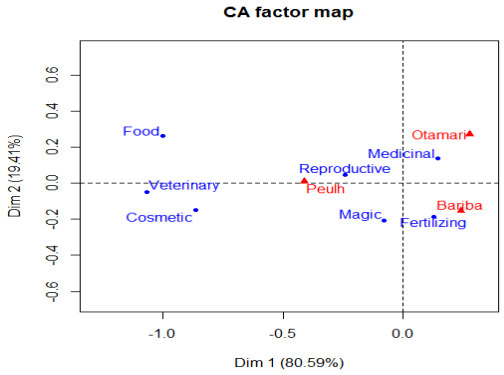
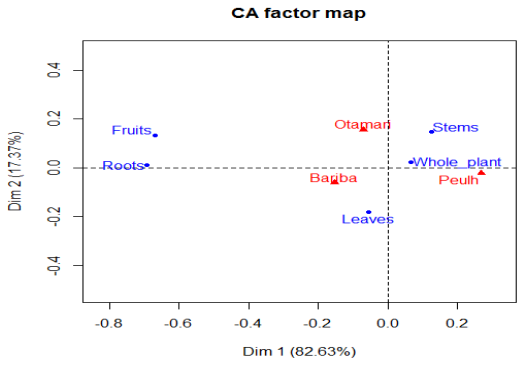


Figure 3 indicates that the axis 1 explains 82.6% of information related to *Phyllanthus amarus* organs users whereas the axis 2 explains 12.3% and for two axis 100% of information.

On the axis 1, the Peulh used weakly the roots and stems of *Phyllanthus amarus*, so Peulh don't have much knowledge related to roots and stems of the species. Otamari used weakly the leaves of *Phyllanthus amarus* on the axis 2. Knowledge related to organs use of *Phyllanthus amarus* varies according the ethnic group.

Figure 3: Use preference projection of Phyllantus amarus organs according sociolinguistic to ethnic groups in the axis system



3.3 Function of sex and age category

Figure 4 allows us to notice that axe 1 and 2 took into 80.5% of information related of *Phyllanthus amarus* use according the category of age and sex. In this way, on the axis 1 taking into account 53.5% of the information, the female old people (VF) use *Phyllanthus amarus* for its magic and cosmetic properties. On the axis 2 which took into account 26.9% of the information, the female adults use *Phyllanthus amarus* for its medicals properties whereas the male adults use regularly the plant for its food, reproductive and fertilizing properties. We notice that the women, adult and old ones use differently the plant where as the adult men and women use it also differently: the use of

Phyllanthus amarus vary according the category of sex and age.

Figure 4: Uses projection of P.amarus according to sex and age categories sociolinguistic group in the axis system

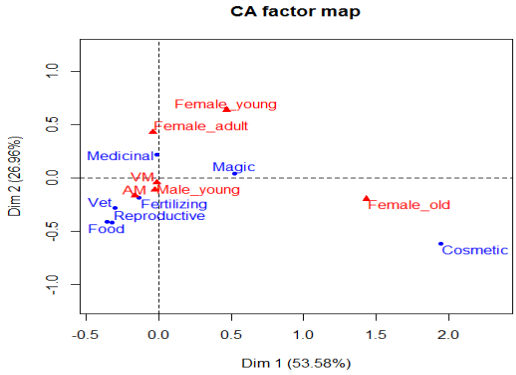
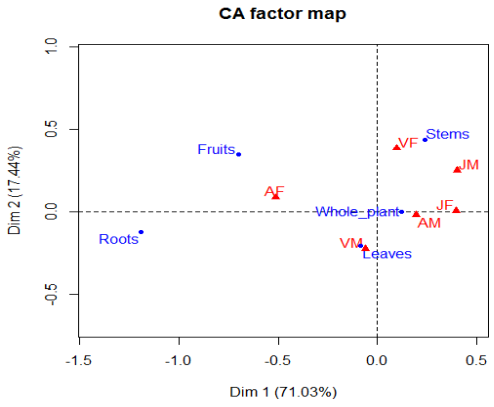


Figure 5 shows that the axis 1 explains 71% of information related to the use of *Phyllanthus amarus* organs whereas the axis 2 explains 17.4% and for the two 88.4% the concerned information. So, on the axis, the adult women use more the fruits and roots of *Phyllanthus amarus*: they have much knowledge of these organs of the plant. On the other hand, the men adult rarely use these organs, which show that their knowledge related to the use of the organs is weak. On the axis 2, the old women use strongly the stems and weakly the leaves of *Phyllanthus amarus*. We retained also that the use of *Phyllanthus amarus* varies from one sex and age category to the other.

Figure 5: Use preference projection of *Phyllantus amarus* organs according to sex and age categories in the axis system



3.4 Preservation method of Phyllanthus amarus

Traditional healers preserve the plant in form of powder (Figure 6). The leaves of *Phyllanthus amarus* dried are sprayed and sieved. The obtained powder is kept in dry cans of 25 liters sheltered from light.

Figure 6: *Phyllantus amarus*: Whole plant (a), drieded leaves (b), sifted and pulverized leaves (c)

3.5 The different uses of the plant

The organs of *Phyllanthus amarus* are used in the treatment of many affections. The Table 9 summarizes the therapeutic indications given by the users about each organ of the plant. As far as the use of *Phyllanthus amarus* as fertilizing is concerned, the leaves (or the whole plant) folded freshly (or the wet powder) are mixed on the eve with the grains (cotton, maize, beans…) before the seedling. The raw crushing of the whole plant with salt increases the productivity of livestock and assures them a good health on the one hand and allows the refractory mother to breastfeed its calf on the other hand; it is also the basis of precocious fertility powder stimulation and the increase of calf numbers of the cow. The whole plant can also be served as food for the bovines. The plant is also well used for its magic and medical properties. The leaves are used as memory help, the whole plant is used in the domains /of fields of luck, the cleansing of the woman whose child dies and the new born baby which is born with his foot forward; forecast, love, traditional fight but also as antidote and against enchantment.

3.6 Phytochemical screening

The leaves of *Phyllanthus amarus* are rich in varied and diverse according to the provenance (Table 5). They don't contain quinines, flavonoids, Leucoanthocyanes, cardio tonic heterosides, saponosides, steroids and cyanogenic derivatives. It contains gallic tannin, alcaloides, annthocyanes, mucilage, reducing compounds, reduced génines, O-heterosids and C-heterosids. We can conclude that these compounds are more frequent in the leaves of the studied plant. We also note that combined anthracenic (O-heterosides) are specific to the leaves of Goussounon Kperou (Nikki = F3); coumarines are specific to the leaves of Sombikiragou (Banikoara = F2) and Boriyouré

(Natitingou = F1); the catechic tannin to the leaves of Goussounon Kperou and Sombikiragou whereas the triterpénoïdes are specific to the leaves of Boriyouré and Goussounon kperou. Ten (10) secondary metabolites are present in the leaves of Goussounon kperou against nine (9) for the leaves of Boriyouré and Sombikiragou. The number and the nature of the metabolites vary from one ecological station to another. These present secondary metabolites possess/have diverse pharmacological mainly anti-oedema, anti-inflammation, antibacterial, antitumorus, healing could partly justify the use of the plant against diverse mentioned pathologies in the table 9.

Table 5: Secondary metabolites present in *Phyllantus amarus*

Chemicals compounds	F1	F2	F3	Total*
Alkaloïds	++	++	++	3
Anthocyanes	++	++	++	3
Combined Anthracenic : O-hétérosides	-	-	+	1
Free anthracenic	+/-	-	-	0
C- heterosides	++	+	++	3
Reduced compounds	++	++	++	3
Coumarines	+	+	-	2
Derivateds quinones	-	-	-	0
Derivted cyanogenic	-	-	-	0
Flavonoïdes	-	-	-	0
Cardiotonic heterosides	-	-	-	0
Leucoanthocyanes	-	-	-	0
Mucilage	++	++	++	3
O-hétérosides with reduced genins	+	++	++	3
Saponosids	-	-	-	0
Steroïds	-	-	-	0
Gallic tannin	++	++	++	3
Catechic Tannin	-	+	++	2
Triterpenoïdes	+	-	+	2
Total	9	9	10	

3.7 The yield of extraction

Fresh /cool water contains the high number the plant chemical principles no matter the place of the harvest. Thus the majority of molecules which contains the leaves of *Phyllanthus amarus* is polar. Moreover, the high yield (12.11%) obtained with the watery extracts of leaves coming from Nikki explains that the plant of this area contains probably some polar principles compared to the others areas (Table 6).

3.8 Larval toxicity test result

The figure 7 shows us that the shrimps larvas are sensitive to the tested extract according to the relationship of response-dose. We proceeded to the determination of the concentration which causes the death of 5% (LC_{50}) of the introduced lavas.

The table 7 summarizes the values of LC_{50} of the tested extracts according to the ecological station.

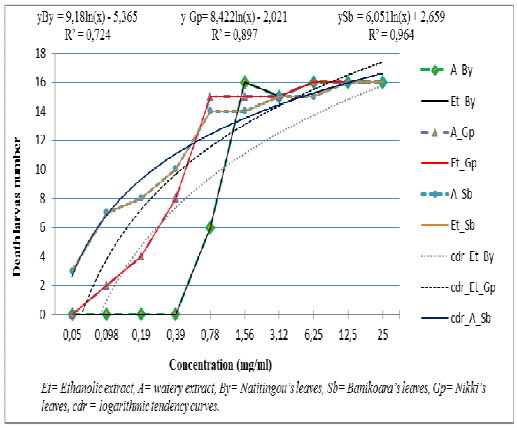
When we refer the Table 2 showing the correlation between the toxicity and the values of LC_{50}, we notice that the tested extracts (ethanolic and watery) have a LC_{50} superior to 0.1 mg/ml (toxicity limit) whatever the provenance of the plant is, so they are non-poisonous on *Artemia salina* larvas and in principle on the 9PS and 9KP human cells on the one hand and HT-29 on the other hand considering the correlation between the cytotoxicity on the shrimps larvas and on the human cells (Pelka et al., 2000; Carballo et al., 2002).

Table 6: Extracts yield recapitulative

Extracts	Yield (g/100g)			Variation Coefficient (CV)
	Sombikiragou	Goussounon kperou	Boriyouré	
Ethanolic	6.59	6.28	6.94	0.04
Watery	9.9	12.11	9.55	0.11

Table 7: LC_{50} values

Extracts	Linear equation	R^2Values	LC_{50}Values
A_ By	Y= 9.81 ln(X)-5.365	0.724	3.9 mg/ml
Et_By	Y= 9.81 ln(X)-5.365	0.724	3.9 mg/ml
A_Gp	Y= 8.713 ln(X)-2.283	0.895	3.25 mg/ml
Et_Gp	Y=8.422 ln(X)-2.021	0.897	3.28mg/ml
A_Sb	Y=6.051 ln(X) +2.659	0.964	2.41mg/ml
Et_Sb	Y=6.051ln(X) +2.659	0.964	2.41mg/ml



Et= Ethanolic extract, A= watery extract, By= Natitingou's leaves, Sb= Bamikoara's leaves, Gp= Nikki's leaves, cdr = logarithmic tendency curves.

4. Discussion

4.1 Inner knowledge of Phyllanthus amarus

The numerous users of *Phyllanthus amarus* show that the species has a great social importance for

local populations. The result are similar with the ones obtained by others authors (Schumann et al., 2012; Kristensen et al., 2003; Gustad et al., 2004; Assogbadjo et al., 2003; De Caluwe et al., 2009; Buchmann et al., 2010) on the baobab. Among the uses (Table 8), medical one is dominating (55%) because the plant would be efficient in the fight against some African diseases such as malaria and others metabolic diseases (diabetes, arterial, hypertension) according to the local populations (Cimanga et al., 2004; Fézan et al., 2008; Appiah et al., 2011). Most of the listed diseases are already enumerated in the literature. We note diseases such as fontanel (8.5%), the suppression of postnatal death (certainly related to an infection), backwardness of children as far as walking is concerned (2.85%), the healing of navel (2.85%) and the erection of problems (2.85%) which weren't implicitly in literature. However, the fertilizing use (22%) seems non negligible as the reproductive one (16%). For the fertilizing activity, *Phyllanthus amarus* would increase surely the biological activity of the pedological microorganism or would contains some mineral elements essential to a good agricultural yield. This last hypothesis seems more plausible with realized work (Okolo et al., 2012) showing that all the organs of the plant contains mineral salts in this case the leaves of *Phyllanthus amarus* contains in majority in the following concentrations (mg/g): calcium (3.477), potassium (2.550), sodium (1.450), manganese (0.214), magnesium (0.132), iron (0.084), copper (0.080) and zinc (0.052). The presence of calcium is responsible of the clay-wet complexes formation at the origin of the fertility of the soils. Moreover the use of the whole plant as far as the plant (Okolo et al., 2012). As far as the reproductive use is concerned the local breeders and some traditional healers think that the species is very active to finish with the bareness and stimulate fertility power. These properties could be justified by the presence of oestradiol in the roots and the barks (Mannan et al., 1973) but also by the fact the methanolic extract of *Phyllanthus amarus* leaves causes an significant rise of testosterone rate without affecting the ones of LH and FSH (Obianime et al., 2009). The improvement of these properties mainly the fertilizing one evoked in this study for the first time must be undertaken because it would contribute largely to the production of green fertilizer and the reduction/decrease of poverty at the planetary scale. The vary from one ethnic group to another, the Otamari use most the species for its medical properties, the Peulh for its veterinary properties whereas the Bariba use it for its fertilizing and magic properties. In fact the medical use of the plant by the Otamari is related to its antimalarial efficiency and to the gastric and enteric diseases and often related to libido and childhood. As the life of Peulh is highly related to their livestock, *Phyllanthus amarus* is considered as a miraculous plant for its multiple functions (food, veterinary and even reproductive) responsible for the good vital health and the increase of livestock in a short time. Unlikely, the Bariba, farmers in majority are more attach to fertilizing properties of *Phyllanthus amarus* for cotton and maize crops, this can justify the appellation "Grenier du Bénin" given to the town Banikoara.

Table 8: Consensual values for the use of Phyllantus amarus

Usages	Bariba	Peulh	Otamari	CTU
Medicinal	0,39	0,51	0,76	0,55
Veterinary	0,10	0,14	0,00	0,08
Food	0,00	0,12	0,14	0,08
Reproductive	0,31	0,08	0,08	0,16
Magic	0,45	0,10	0,08	0,21
Cosmetic	0,20	0,11	0,00	0,10
Fertilizing	0,51	0,07	0,08	0,22

4.2 Phytochemical screening

The presence of alkaloids shows that the decoction of the leaves of the plant is highly prescribed for the treatment of malaria (Okwu et al., 2006). Many others authors confirmed this antimalarial activity of the alkaloids (Malgras, 1992; Ntiejumokwu et al., 1990). These alkaloids would justify the anti-gastro intestinal spasms (Nacoulma et al., 1990) and have the plant used against stomachache. It was reported that the tannin present a weak antibacterial activity and anticryptococcuques activity (lavas), antiviral, anti-inflammatory, antihypertensive, anti-mutagenic, anti-tumoral and anti-diarrhea relatively high (Kolodziej et al., 1999; Bruneton, 1999). So we can understand the use of the plant for the treatment of diarrheas, fever, hypertension and hepatitis. The total absence of cardiotonic heterosids decreases highly risk of toxicologies related to the use of *Phyllanthus amarus*. These results corroborate the ones carried out on *Nauclea latifolia* (Smith) (Badiaga, 2011; Kaboré et al., 1995). The use of *Phyllanthus amarus* in the dermatology by the local population would be related to the presence of coumarine, known for its anti-oedema properties. These results are similar to the ones, (Bruneton, 2009).

4.3 Toxicity of Phyllanthus amarus leaves

The leaves of *Phyllanthus amarus* are non-toxic whatever their provenance is because all the extracts (ethanolic and watery) tested have LC_{50} superior to 0.1mg/ml, toxicity limit (Mireille Mousseux, 1995). The same result was obtained with ethanolic and watery extracts of the plant on albino rats (Shyamjith et al., 2011). However, *Phyllanthus amarus* possesses cytotoxic principles (Lira et al., 2014).

Table 1*: Diseases in which intervenes* **Phyllantus amarus**

Organs	Way of preparation	Use form	Treated diseases
Leaves	To crush with the fruit	massage the body 2/d until recovering	Body painfull
	To crush raw	Poultice whitewash the party	Fontanel et child diarrhoea
	To crush raw	Massage of the body	Child Fiver
	Decoction	Steam bath	Splitting headache
	To crush raw	aplasm + salt on the party	buccal extra wound
	To crush with fruit + salt to crush	drink by mother	buccal extra wound
	To crush with fruit	poultice whitewash the party	Breasts painfull
	Triturated leaves and mixed with milk butter of cow	To pass as pomade on the body	Dermatosis
	Watery herbal tea	Herbal tea to drink and to wash with for 7days and this 1 /d	myalgies
Roots	To grind dryly + others plants	One spoonful oral 4/month	Painful menstruating
	To grind dryly + others plants	One pinch in mushy 2-3/d	Erection problems
Stems	To crush roots and grind leaves	Roots sauce and leaves powder in mushy 4/d sur 3jrs	Bilharziasis
Whole plant	Raw and return into powder	oral pinch 3/d	Diarrhoea
	To grind raw and filter	3 spoonful of filtrate to drink daily	Gonorrhea
	To crush and infuse	Tisane and massage 3/d	Rib painfull
	Herbal tea in cheese water/ To grind	Drink one glass of beer /d before eating morning or tisane in mushy /Powder in sauce	Female sterility
	Decoction	Tisane to rinse body 1/d	Child asthenia
	To grind with black coal noir Herbal tea en 72h	Poudre dans la sauce 3/d pour une semaine ane 1/d pendant 7jrs	ostnatal mortality in a regular woman
	Herbal tea salt	Drink for one week	postnatal mortality of calfs of a cow
	Decoction	Tisane to drink1/d	
	Decoction/ filtrate	Oral tisane 2/d on 3days or drops on eyes	infull
	Decoction	tisane to drink unlimited	
	Decoction + peak milk	Tisane to drink unlimited	s B
	Decoction	Tisane to drink 2/d	
	Watery herbal tea	Tisane to drink	Spasmodic crisis with exit eyes to ocular glob
	Decoction	One glass of tisane 3/d	
	Decoction	Tisane	stone
	Decoction	oral tisane 2/d before eating	Headache
	Decoction	Tisane to take several times/d as drink	nsion
	Decoction	Tisane sert à laver 2/d on 7days	child walking late
	Decoction	Decoction oral 2/d	
	Decoction	Decoction to drink et lavage 2/d	Child dermatosis
	Decoction/ To grind	Tisane to drink et se laver 2/d ou poudre masse corps 3-4/d	ever
	Decoction	Tisane orale 2/d before eating	Stomachache
	To crush raw + salt (or lemon)	3 cuillerées de la tisane to drink2/d	sthenia
	To grind raw /Decoction	Poudre fine dans miel à laper ou dans bouillie 1/d	Ulcerous

	Decoction or raw + salt	Oral et washing the body	Vomiting
	Decoction	Tisane à se laver et gouttes dans oreilles 2/d	eth
	Decoction + mango bark	Mouthwash 3 times successively once day on 3days	iseases
	Decoction	Tisane to drink 3/d	Navel healing
	Decoction	Tisane to take 2-3/d on 2 days	
	Decoction	Tisane to take 2-3/d on 2 days	
	To grind raw and dry/herbal tea	Fine powder in boiled water 1/d	gulator

Conclusion

Phyllanthus amarus is a well-known plant to the Beninese located in the North who use it in seven (7) different domains: medicinal, magic, fertilizing, food, reproductive, veterinary and cosmetic because of its riches in active principles and its harmlessness. The plant can be an alternative solution in the fight against the diseases and largely participate to a decrease of poverty. It must be valued by Beninese and international authorities, scientists.

Research Highlights

The present study reveals that *Phyllanthus amarus* intervenes in seven different uses categories and treat thirty four diseases on the target groups. The Peulh use Phyllanthus amarus for its food and veterinary properties, the Bariba for esoteric and fertilizing properties and the Otamari for its medicinal properties. This plant is used to treat diseases affecting all the major body systems ranging from reproductive, nervous, circulatory to the digestive and excretory systems. Adult men use it in several ways as food, soil fertilizing and cow reproduction.

The results of this study confirm that ethanolic and watery extracts of *Phyllanthus amarus* are non-toxic on human cells. The plant is very rich in big compound groups whose number varies from a station to another.

Limitations

This research was limited only to three majority ethnic groups in North of Benin because of insufficient funds.

Recommendation

Based on the result obtained, sanitary authorities of Benin must attach a particular interest to endogenous research on medicinal plants as *Phyllanthus amarus* in order to promote traditional drug improved.

It's necessary to value in vitro on different strains, antibacterial activities of extracts. Identify, purify and isolate molecules from bioactive extracts non-toxic.

Authors' Contribution and Competing interests

B.Y.Y. conceptualizes the study and wrote the manuscript jointly and severally with O.C. who correct the manuscript on ethnobotanical level. H. A. carried out the phytochemical and toxicological analyses with the supervision of G.F. who read and correct the manuscript on this plan. All authors read and approved the final manuscript. There is no competing interests about this manuscript.

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