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Comparative Phytochemical Constituents and Pharmacognistic Importance of the Vegetative Organs of Some *Phyllanthus* Species in South Eastern Nigeria

Awomukwu D. A.[1,*], Nyananyo B. L.[2], Onukwube N. D.[3], Uka C. J.[4], Okeke C. U.[4], Ikpeama A. I.[5]

[1]Department of Biology/Microbiology, Abia State Polytechnic, P.M.B. 7166, Aba, Abia State, Nigeria
[2]Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria
[3]Department of Chemistry/Biochemistry, Abia State Polytechnic, P.M.B 7166, Aba, Abia State, Nigeria
[4]Department of Botany, Nnamdi Azikiwe Univerisity, Awka, Anambra State, Nigeria
[5]National Root Crop Research Institute, Umudike, Umuahia, Abia State, Nigeria

Abstract An assessment and investigation into six species of *Phyllanthus* namely *P. amarus* Schum and Thonn, *P. urinaria* Linn., *P. odontadenius* Mull-Arg., *P. niruroides* Mull-Arg., *P. mullerianus* (O. Ktze) Excel and *P. discoideus* (Baill) Mull-Arg. belonging to the family of *Euphorbiaceae* were carried out with the aim of identifying and quantifying the bioactive components of these medicinal plants. The alkaloid, flavonoid, saponin, phenol and tannin contents of the vegetative parts of these plants viz. the leaves, stems and roots were screened and compared. Result from the phytochemical analyses showed that alkaloids, tannins, flavonoids and saponins were all present in the leaves, stems and roots of the studied species. Phenols were, however only absent in the stems of *P. amarus*, *P. urinaria* and *P. odontadenius* and roots of *P. urinaria* but present in the leaves, stems and roots of the rest species. All the species investigated contained appreciable amount of alkaloids, tannins and flavonoids and saponins ranging from (0.14 ± 0.06% - 3.78 ± 0.51%), (0.15 ± 0.03% - 1.82 ± 0%), (0.13 ± 0.03% - 1.59 ± 0%) and (0.14 ± 0.01% - 1.74 ± 0.01%) respectively. Phenols present ranged from (0.08 ± 0% - 0.52 ± 0%). These analytical results suggest the plants have a significant role in phyto-medicine. The importance of these plants was discussed in line with the role they play in ethno-medicinal life of the people.

Keywords *Phyllanthus* species, Phytochemical constituents, Medicinal properties

1. Introduction

In many countries around the world, *Phyllanthus* species are used in folk remedies [1]. The genus *Phyllanthus* has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites [2]. The name "*Phyllanthus*" means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf other common names includes gripe weed, stonebreaker, leaf flower and among others [3]. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these substances are alkaloids, glycosides, tannins, flavonoids, saponins, phenols, oils and many others [4]. These are very useful in economic botany, medicinal chemistry and pharamacognosy, some drugs have been obtained from natural sources while some may be prepared by the modification of the natural ones [5]. According to Rahila *et al.* [6], there is need for the local herbs to be evaluated for phytochemicals so as to determine the potential of indigenous sources of medicines.

Nigeria is one country rich in raw and useful herbs from which important drugs could be prepared or agent which serve as starting products for the potential synthesis of drugs [7]. Most of these plants used for traditional medicine are equally consumed by humans in Nigeria, but their medicinal values are not determined. The biosynthesis of secondary metabolites varies among plants, even in different organs of plants and their biosynthesis depends on the environmental factors in which they grow. Intra-specific variation in phytoconstituents has been documented extensively among the plants [8] [9]. According to Thyagarajan *et al.* [10] plant extracts from *Phyllanthus* have beneficial effects on liver functions. Mehrata *et al.* [11] and Uander and Blumberg [12] showed, using in vitro studies that *P. amarus* extracts (polar fractions) also have antiviral activity and are a potential remedy for hepatitis –B viral infection. Since the extract of *Phyllanthus* has a long history of use in tropical countries in indigenous medicine for the treatment of liver ailment, they were examined during several researches.

* Corresponding author:
xdanny18@yahoo.com (Awomukwu D. A.)
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The importance of medicinal plants has been elucidated by Edeoga *et al.* [13] [14] and their importance in the pharmaceutical industry. These medicinal plants have been underutilized in orthodox medicine but have confirmed to be used worldwide in the pharmaceutical, food, cosmetics and perfume industries [15].

Alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological effect on animals [5] and show considerable pharmaceutical activity [16]. Alkaloids are stimulants acts and by prolonging actions of several hormones which require phosphodiestrerase [17] though are poisonous to cattle [18].

Tannins are useful in medicine because of their astringent properties. Tannins and Alkaloids are known to have anti-herbivore defense functions in plants [19]. Thus, the presence of tannins and alkaloids in medicinal plants could be serving as a deterrent to grazers [5]. Herbs that contain tannins are recommended for a wide range of treatments including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, stomach problems and inhibition of active oxygen and are commonly recommended as diuretics, anti-diarrheas and haemostatic [20].

Saponins are glycosides widely occurring in a variety of plants and are characterized by their bitter taste and foaming in aqueous solution. They prevent disease invasion of plants by parasitic fungi [21]. Steroidal saponins from various studies indicate their importance and the interest in pharmacy due to their relationship with such compounds such as sex hormones especially in development of the female contraceptive pills [22]. In medicine, it is used to some extent as an expectorant and emulsifying agent [23].

Flavonoids are the commonest phenolic constituents having 15-compounds generally distributed throughout the plants kingdom [19]. Some flavonoids have antibacterial function with gram-positive species more sensitive to isoflavanones, than their negative counterpart. Flavours are related to flavonoids and they promote particular tastes to prepared foods. The presence of flavonoids in plants have shown some effects like antibacterial, antiviral, antitoxin, antioxidant, anti-inflammatory anti-carcinogenic activities [24]. Isoflavanones, isoflavans, isoflavonones are extremely fungal pathogens [25]. They act as allelochemicals widely used in insecticides and in treatment certain physiological disorders and disease control.

Phenols are synthesized via the shikimic acids pathway. Phenolic compounds are known to have anti-fungal and anti-microbial effects. Phenolic compounds believe to be active ingredients in the herbicide found up as well as some other commercial herbicide formulation [2].

Several chemical investigations have been conducted where the structures of most of the phytochemicals were determined by UV, IR, Mass and NMR spectroscopy. Following the current research in phytochemicals, the secondary metabolites present in *Phyllanthus amarus* are alkaloids, flavonoids, hydrolysable tannins, major lignans, polyphenols [1] [26] [27] but no work has compared the chemical constituents of other species of *Phyllanthus* in Eastern Nigeria. Some of these species are *P. urinaria* Linn., *P. mullerianus* (O. Ktze), *P. discoideus* (Baill) Mull–Arg., *P. niruroides* Mull–Arg., and *P. odontadenius* Mull-Arg. [28]. It is in attempt to fill the gap on our present knowledge that this work was done. Thus, this study investigates the fundamental scientific bases for the use of these taxa by contributing to the list of plants used for ethnobotanical and medicinal purposes and to qualify and quantify the crude chemical constituents present in the plant species. The work will also give clues in aiding other scientist who may use these plants for other purposes, such as nutritional and pharmaceutical rather biological studies. This may also review works done by other scientist.

2. Materials and Methods

Collection of plant materials

Mature plants of the six species *P. amarus, P. urinaria, P. niruroides, P. odontadenius, P. mullerianus* and *P. discoideus* were collected from different locations of Eastern Nigeria by various investigators as in Table 1. Only healthy, fresh and succulent parts of the plants were collected. The six specimens were identified and authenticated at the Herbaria of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike and the Department of Botany, Nnamdi Azikiwe University, Awka. Herbarium specimens were also studied at the various institutions as well making reference to the Flora of West Tropical Africa by Hutchinson and Dalziel [29].

Table 1. Collection sites of the six *Phyllanthus* species studied

SPECIES	SITES OF COLLECTION
Phyllanthus amarus	Along the school fence, Abia State Polytechnic, Aba.
Phyllanthus urinaria	Field around National Root Crop Research Institute, Umudike.
Phyllanthus odontadenius	Road side along National Root Crop Research Institute, Umuahia.
Phyllanthus niruroides	Science Village, Nnamdi Azikiwe University, Awka.
Phyllanthus discoideus	Near the Herbarium Building, Nnamdi Azikiwe University, Awka.
Phyllanthus mullerianus	Near the Herbarium Building, Nnamdi Azikiwe University, Awka.

Phytochemical Determination

Preparation and analysis

The plant materials were air-dried and milled into uniform powder using Thomas-Willey milling machine. The aqueous extract of each sample was determined by soaking 50g of dried powdered sample in 10ml of distilled water for 12hours. The mixtures were filtered using Whatman filter paper and excess water removed by concentration by boiling the extract to 100℃ for 10 minutes.

Table 2. Ethnobotanical uses of the studied *Phyllanthus* species around the world

SPECIES	LOCATION	AILMENT & TREATMENT/ PROPERTIES AND ACTION
Phyllanthus amarus	Aruba	Blood purifier.
	Bahamas	Appetizer, Cold, Fever, Flatulence, Flu, Stomach ache, Vermifuge.
	Barbados	Arbortificant.
	Cuba	Edema and Malaria.
	India	Appetizer, Asthma, Bronchial infections, Diuretic, Dyspepsia, Fever, Jaundice, Liver diseases, Itches, Skin ulcer, Sores, Swellings.
	Indonesia	Colic, Cough, Diuretic, Eye disease (external), Kidney diseases, Stomach ache, Tooth ache, Venereal diseases.
	Island of North Caicos	Fever, Prevention of intestinal worms.
	Jamaica	Diabetes, Dysentery, Diuretic, Edema, Gonorrhea, Jaundice, Stomach ache.
	Nigeria	Diabetes, Dysentery, Malaria, Prevention of intestinal worms, Stomach aches.
	Trinidad	Diuretic, Venereal diseases.
Sources: [1] [2] [26] [28] [30] [31] [32] [33] [34] [35][36] [37] [38]		

SPECIES	LOCATION	AILMENT & TREATMENT/ PROPERTIES AND ACTION
Phyllanthus urinaria	Indo-china, Malaysia, Indonesia	Jaundice, Diarrhea, Dysentery, Dropsy, Gonorrhea, Menorrhea, Children's cough, Appetizer, Kidney-Trouble, Syphilis, Tumors, Diaphoresis, Abortion.
	India	Fish poison.
Sources: [28] [39]		

SPECIES	LOCATION	AILMENT & TREATMENT/ PROPERTIES AND ACTION
Phyllanthus odontadenius	Ghana	Hiccup, Paralysis, Epilepsy, Convulsion and Spasm
Sources: [28] [39]		

SPECIES	LOCATION	AILMENT & TREATMENT/ PROPERTIES AND ACTION
Phyllanthus niruroides	Cote d'Ivoire	Diuretic, Purgative, Jaundice, Enema, Bronchitis in infants, Cough, Fever pain, Oedema, Sore-throat,
	Tanzania	Hookworm expeller.
Source: [40] [41] [42]		

SPECIES	LOCATION	AILMENT & TREATMENT/ PROPERTIES AND ACTION
Phyllanthus discoideus	West Africa	Purgative, Aphrodisiac, Tooth-ache, Stomach-ache, Fever.
	Central Africa	Post-partum pain, boil, anthelmintic.
	Malawi	Inflammation, Lumbar pain.
	Tanzania	Malaria, Schistosomiasis, Gonorrhea, Diarrhea, Hard abscesses.
	Democratic Republic of Congo	Stomach and Kidney problems, Parturition.
	Sudan	Cough, Wound, Ulcers, Ear-ache, Poison antidote Blennorrhoea, Ophthalmia.
Sources: [39] [43] [44] [45]		

SPECIES	LOCATION	AILMENT & TREATMENT/ PROPERTIES AND ACTION
Phyllanthus mullerianus	Cameroon	Tetanus, Wound infection.
Source: [46]		

Qualitative Determination of the Chemical Constituents

Freshly prepared ground samples are chemically tested for the presence of chemical constituents using standard procedures [47]. Water or ethanol extracts are commonly used.

Test for alkaloids

One mL of extract of the sample was shaken with 5.0ml of 2% HCl on a steam bath and filtered. To 1.0mL of the filtrate

was treated with Wagner's Reagent (Iodine in Potassium-Iodine solution) and observed for reddish brown precipitate [47].

Test for tannins

To 1.0mL of extract was added an equal volume of bromine water. The formation of a greenish to red precipitate was taken as evidence for presence of condensed tannins [47].

Test for flavonoids

To 1.0mL of extract 1.0ml of 10% lead acetate was added. The formation of yellow precipitate is taken precipitate for flavonoids [47].

Test for saponins

One mL of extract was boiled with 5.0ml of distilled water for 5 minutes and decanted while still hot. The filtrate is used for the test. 1.0ml of the filtrate was diluted with 4.0ml of distilled water, shaken vigorously and observed on standing for stable froth [47].

Test for phenols

To 1.0mL of extract was added 1.0ml of 10% ferric chloride. The formation of a greenish-brown or black precipitate or colour is taken as positive for phenolic nucleus [47].

Quantitative Determination of the Chemical Constituents

Alkaloid determination

5g of the sample were weighed into a 250mL beaker and 200mL of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the extract was concentrated using a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the preparation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [48] [49].

Tannin determination

500 mg of the sample was weighed into 100 mL plastic bottle. 50 mL of distilled water was shaken for one hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipette out into a tube and mixed with 3 mL of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured [50].

Flavonoid determination

100g of the plant sample were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed [51].

Saponin determination

The samples were ground. 20g of each plant samples were dispersed in 200 mL of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55℃. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90℃. The concentrate was transferred into a 250 mL separator funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage [48].

Phenol determination

For the extraction of the phenolic component, the fat free sample was boiled with 50 mL of ether for 15 minutes. 5 mL of the extract was pipette into a 50 mL flask, and then 10 mL of distilled water was added, 2 mL of ammonium hydroxide solution and 5 mL of the extract was pipette into a 50 mL flask, and then 10 mL of distilled water was added, 2 mL of ammonium hydroxide solution and 5 mL of concentration amyl alcohol were also added. The samples were left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths [48] [49].

3. Results

The qualitative analysis and the quantitative estimation of the percentage crude chemical constituents in *Phyllanthus* spp studied are summarized in Table 3 and Chart 1-5. Alkaloids were very highly present in the leaves of *P. niruroides*. They were found to be highly present in the leaves of *P. amarus* and *P. odontadenius*, stems of *P. niruroides* and in the leaves, stems and roots of *P. mullerianus* and *P. discoideus*. Furthermore, they were found to be fairly present in the leaves, stems and roots of *P. urinaria*, stems and roots of *P. amarus* and *P. odontadenius* and in the roots of *P. niruroides.*

The leaves, stems and roots of *P. mullerianus* and *P. discoideus,* leaves of *P. amarus*, *P. odontadenius* and *P. niruroides* all have tannins and flavonoids highly present in them. Tannins and flavonoids were also found to be fairly present in the stems and roots of *P. amarus*, *P. urinaria* and *P. niruroides.*

However, saponins were very highly present in the leaves of *P. niruroides* and highly present in the stem. The leaves, stems and roots of *P. mullerianus* and *P. discoideus* were found to have saponins highly present in them but the leaves, stems and roots of *P. amarus*, *P. urinaria* and *P. odontadenius* were all fairly present in saponins.

Results of the phenols show clearly that phenols were

fairly present in the leaves, stems and roots of the studied *Phyllanthus* species but were found absent in the roots of *P. urinaria* and only present in the leaves. Phenols were also found to be totally absent in the stems of *P. amarus, P. urinaria* and *P. odontadenius* respectively though may only be contained in trace quantity.

Table 3. Qualitative analysis and percentage of the investigated crude phytochemical constituents in the studied *Phyllanthus* species on dry weight basis

TAXA		ALKALOIDS (%)	TANNINS (%)	FLAVONOIDS (%)	SAPONINS (%)	PHENOLS (%)
	Leaf	++ (1.36±0.51)	++ (1.24±0.14)	++ (1.52±0.04)	+ (0.80±0.00)	+ (0.08±0.00)
P. amarus	Stem	+ (0.28±0.06)	+ (0.19±0.01)	+ (0.24±0.03)	+ (0.16±0.03)	-
	Root	+ (0.51±0.03)	+ (0.36±0.03)	+ (0.38±0.00)	+ (0.25±0.01)	+ (0.26±0.00)
	Leaf	+ (0.86±0.03)	+ (0.77±0.00)	+ (1.36±0.01)	+ (0.19±0.04)	+ (0.09±0.00)
P. urinaria	Stem	+ (0.14±0.03)	+ (0.20±0.03)	+ (0.43±0.03)	+ (0.35±0.01)	-
	Root	+ (0.35±0.03)	+ (0.18±0.01)	+ (0.84±0.01)	+ (0.35±0.03)	-
	Leaf	++ (1.05±0.04)	++ (1.46±0.03)	+ (0.86±0.01)	+ (0.73±0.00)	+ (0.25±0.00)
P. odontadenius	Stem	+ (0.18±0.16)	+ (0.15±0.01)	+ (0.13±0.00)	+ (0.25±0.04)	-
	Root	+ (0.54±0.04)	+ (0.28±0.00)	+ (0.36±0.00)	+ (0.40±0.01)	+ (0.17±0.00)
	Leaf	+++ (3.78±0.02)	++ (1.82±0.00)	++ (1.45±0.00)	+++ (2.36±0.03)	+ (0.52±0.00)
P. niruroides	Stem	++ (1.45±0.06)	+ (0.24±0.03)	+ (0.30±0.01)	++ (1.74±0.03)	+ (0.16±0.00)
	Root	+ (0.63±0.01)	+ (0.21±0.01)	+ (0.34±0.03)	+ (0.20±0.00)	+ (0.28±0.00)
	Leaf	++ (1.93±0.04)	++ (1.69±0.00)	++ (1.51±0.01)	++ (1.63±0.01)	+ (0.35±0.00)
P. mullerianus	Stem	++ (1.45±0.16)	++ (1.31±0.01)	++ (1.13±0.01)	++ (1.26±0.03)	+ (0.23±0.01)
	Root	++ (1.20±0.04)	++ (1.18±0.03)	++ (1.16±0.03)	++ (1.14±0.00)	+ (0.16±0.00)
	Leaf	++ (1.92±0.03)	++ (1.75±0.01)	++ (1.59±0.01)	++ (1.69±0.04)	+ (0.30±0.01)
P. discoideus	Stem	++ (1.41±0.03)	++ (1.34±0.03)	+ (1.15±0.01)	++ (1.28±0.01)	+ (0.25±0.00)
	Root	++ (1.29±0.03)	++ (1.22±0.00)	++ (1.05±0.00)	++ (1.09±0.03)	+ (0.29±0.00)

Results are mean of five determination dry weight basis ± standard deviation
(2.00% - ~ = Very highly present; 1.00 – 1.99% = Highly present; 0.01 – 0.99% = Fairly present; 0.00% = Absent)
KEY
+ + + = Very highly present
+ + = Highly present
+ = Fairly present
- = Absent

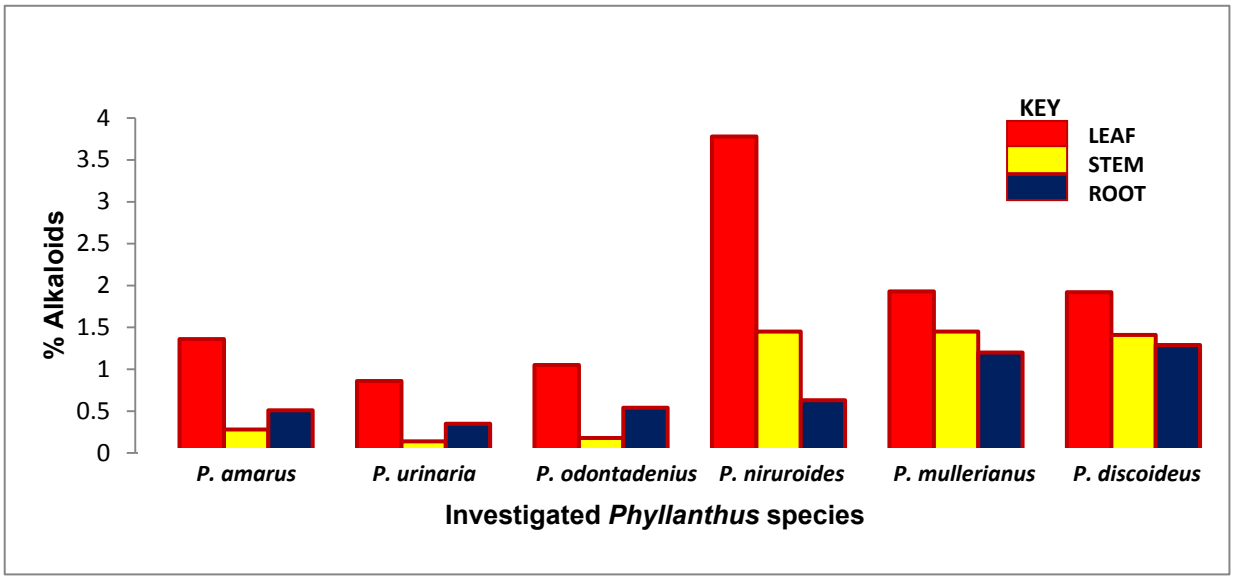

Chart 1. Percentage alkaloids of the screened *Phyllanthus* species

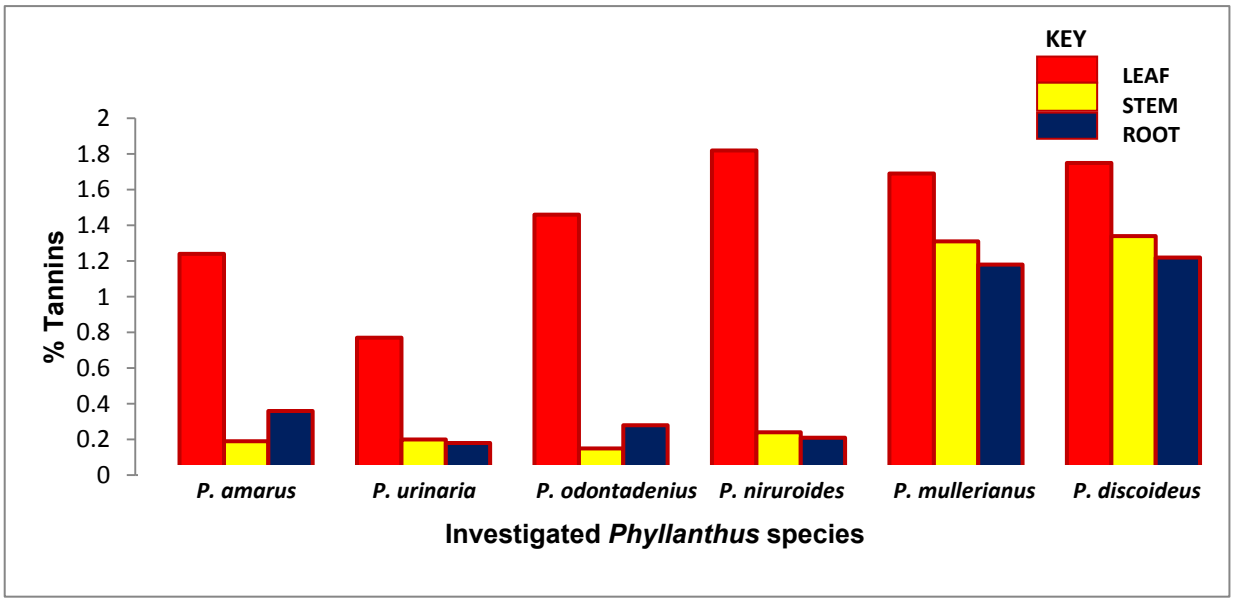

Chart 2. Percentage tannins of the screened *Phyllanthus* species

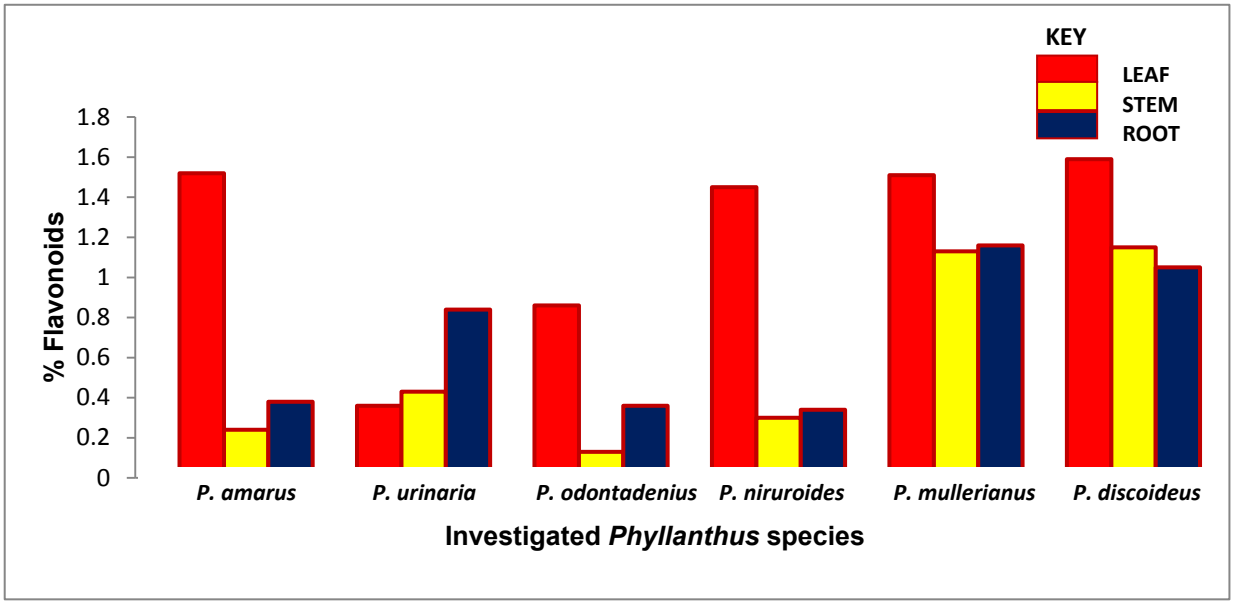

Chart 3. Percentage flavonoids of the screened *Phyllanthus* species

From the present investigation on the percentage crude phytochemicals, the leaves of the studied species contained the highest concentration of the alkaloids, tannins, flavonoids, saponins and phenols in general. The leaves of *P. niruroides* contained the highest percentage of crude alkaloids (3.78±0.02%), tannins (1.82±0.0%), saponins (2.36±0.03%) and phenols (0.52±0.0%) except in flavonoids (1.59±0.01%) where the leaves of *P. odontadenius* ranked the highest.

The stems of the *Phyllanthus* spp contained appreciable amount of the crude chemical constituents but yield recorded were minimal. Form this result in general; the stems of *P. discoideus* contained the highest percentage of crude tannins (1.34±0.03%), flavonoids (1.15±0.01%) and phenols (0.25±0.0%) while the stems of *P. mullerianus* contained the highest percentage of crude alkaloids (1.45±0.16%). The stems of *P. niruroides* also recorded the highest percentage of saponins (1.74±0.03%).

The roots of the studied *Phyllanthus* spp also recorded notable amounts of the crude chemicals. Comparing the roots of the studied species, *P. discoideus* contained the highest percentage of crude alkaloids (1.29±0.03%), tannins (1.22±0.0%) and phenols (0.29±0.0%). On the other hand the roots of *P. odontadenius* recorded the highest yield of alkaloids (0.54%) and saponins (0.40%). The roots of the *P. mullerianus* recorded the highest yield of flavonoids (1.16±0.03%) and saponins (1.14±0.0%).

In general, the plants produced high yield alkaloids

ranging from (0.14 ± 0.06% - 3.78 ± 0.51%), tannins (0.15 ± 0.03% - 1.82 ± 0%), flavonoids (0.13 ± 0.03% - 1.59 ± 0%) and saponins (0.14 ± 0.01% - 1.74 ± 0.01%). This could explain why they are recommended for a wide range of treatments.

4. Discussion

The occurrence of bioactive ingredients and the quantitative estimation of the percentage crude yield of chemical constituents of the Phyllanthus species studied therefore suggest that the leaves, stem and roots were rich in alkaloids, flavonoids, tannins and saponins except for phenols that were contained in trace amount in the leaves and roots and totally absent in some of the stems of the studied species. They were known to show medicinal activity [7].

In recent times, phytochemicals are promoted for the prevention and treatment of many health conditions [52] as well as their physiological activity which facilitate natural healing with little or no side effects. The presence of these phytochemicals provide scientific explanation of the long use, recommendation and activities of Phyllanthus species as, antibacterial, anti fungal, anti diarrhoea, anti dysentery, anti protozoan and anti malarial [28].

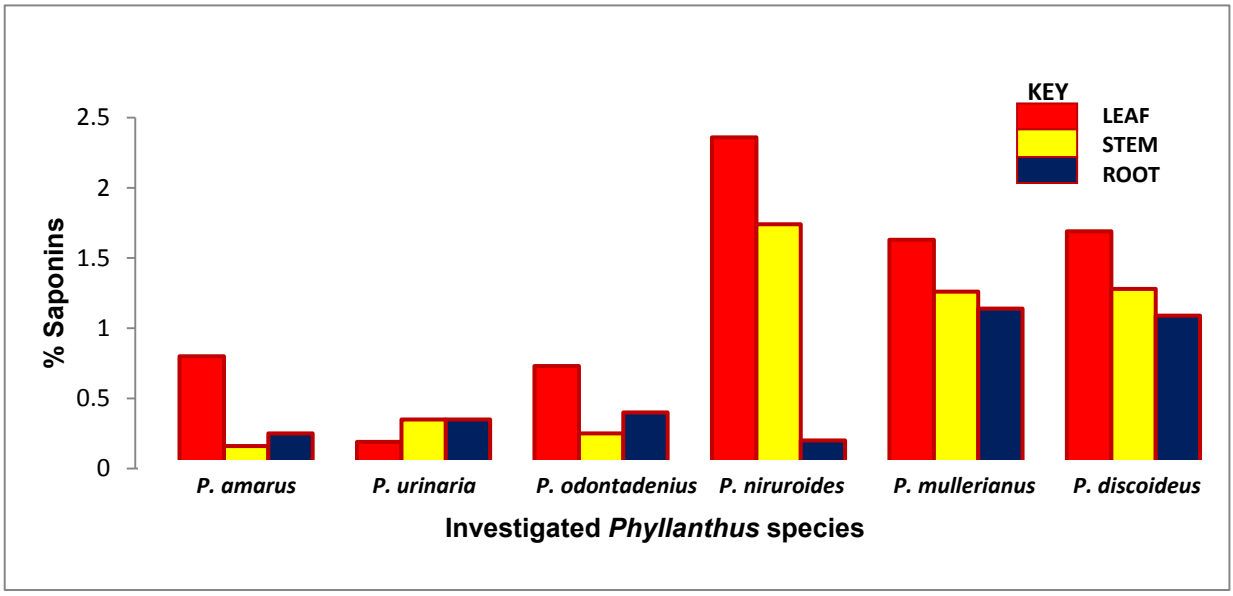

Chart 4. Percentage saponins of the screened *Phyllanthus* species

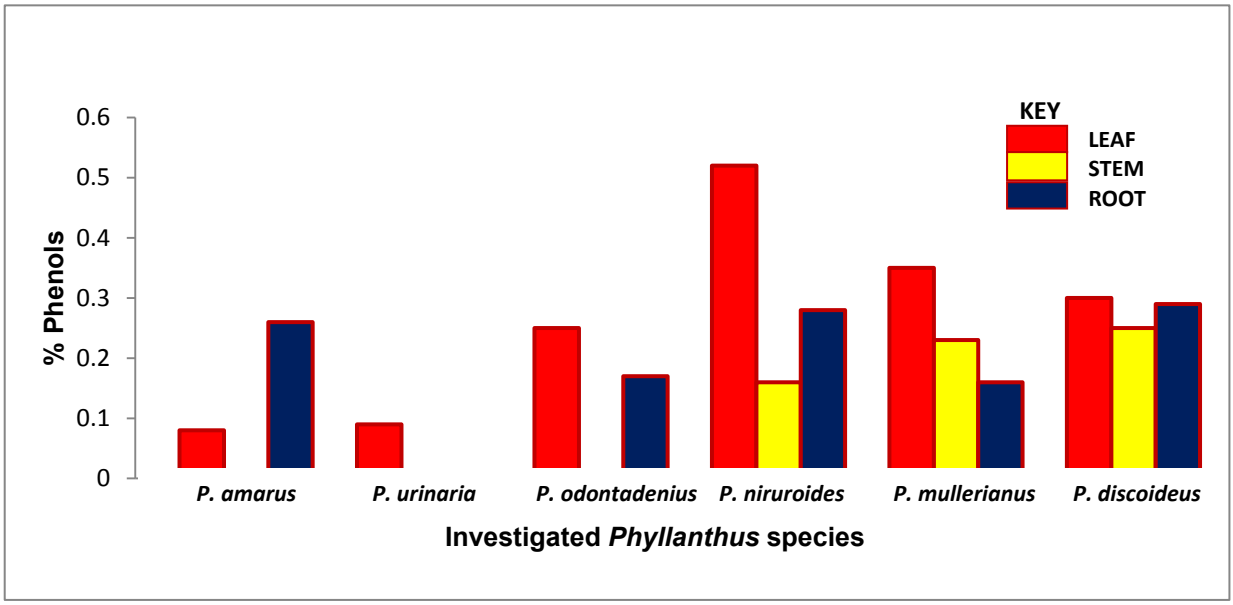

Chart 5. Percentage phenols of the screened *Phyllanthus* species

The leaves of P. niruroides had the highest percentage of alkaloids (3.78%) followed by the leaves of P. mullerianus (1.93%). The presence of alkaloids agrees with the opinion of Houghton et al. (1996) who noted there were securine, eipubbialine an isobubbaline types of alkaloids. Alkaloids are known to have a powerful effect on properties and animal physiology [5]. They are used in medicine especially the steroidal alkaloids and also show considerable pharmacological activity [16]. This explains the reason why they could not be used to feed livestock because the presence of alkaloids makes it poisonous to cattle [18]. Alkaloids are known to play some metabolic roles and control development in living system. They also have protective functions in animals and are used in medicine. Phyllanthus species are known for their potent pharmacological properties [53]. Pure isolated plant alkaloids and their synthetic derivatives are known for their analgesic, anti plasmodic and anti bacterial effects [54]. This may be the reason why the aerial parts of P. amarus are used in herbal medicine to treat malaria, stomach ache, liver ailment, kidney stone and gall bladder infection. This may also explain why the infusion of leaves from P. odontadenius is used in the treatment of paralysis, epilepsy, convulsion and spasm. Furthermore, the alkaloid contents in P. discoideus may be the reason why the plants have been recorded to have aphrodisiac effect, treatment of toothache, malaria, fever, ear-ache and lumbar pain [43] [45]. In recent years, attention has been focused on alkaloids with anti-tumorous effect [55]; this may be why leaves of P. urinaria are used in the treatment of tumors and related ailments.

The leaves of P. niruroides also contained the highest percentage yield of tannins (1.82%) followed by the leaves P. discoideus (1.75%). The presence of tannins in these plants may also be the reason why most animals do not graze on the plant. This supports the opinion of Harborne [19], who pointed out that tannins have anti-herbivore function in plants and inhibit pathogenic fungi [5]. Also, tannins and alkaloids have been documented to show anti-herbivore defense function in plant [56] [57] and this could explain why the leaves of the studied Phyllanthus species are hardly grazed by herbivores. Rather, the plants show strong herbicidal potential as they repel fleas, moths and other insects [58]. In the past, it was thought to counteract poison. Thus, the presence of tannins and alkaloids in these plants could be serving as deterrence to grazer. The presence of hydrolysable tannin or pyrogallol tannins [26] in P. amarus has proved why it is recommended for a wide range of treatment including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, diuretics, diarrhoea, haemostatic, dysentery and menstrual disorder [59]. This can explain why some extracts from the roots can be used for constipation and treatment of jaundice [35], and also why the leaves of P. niruroides and P. discoideus are widely used as purgatives, treatment of jaundice, stomach ache, kidney problems, oedema, ulcer and as a poison antidote [39] [40] [41] [44]. Tannins are known to be organic substances of diverse composition with pronounced astringent properties that promote the healing of wounds and inflamed mucous membranes [55] [60]. This may be the reason why P. mullerianus is used for wound infection and tetanus (Webster, 1994). High levels of tannin in diet have reported to cause growth depression. Tannins also have the potential to complex divalent ions such as Zinc, Iron and Copper etc resulting in their unavailability [60] and have been reported to form complexes with digestive enzymes thus reducing the digestibility of proteins in foods [61].

The leaves of P. discoideus recorded the highest percentage of flavonoids (1.59%) followed by the leaves of P. amarus (1.52%) although flavonoids are contained in reasonable amount in all the investigated parameters of the medicinal plants. This may be the reason why they have anti-bacterial and anti-malarial function [25] [32]. Flavonoids are known to perform very many finctions such as anti oxidant, anti allergic, anti carcinogenic, anti microbial, hepatoprotective and anti viral abilities [62]. The appreciable flavonoid contents of P. amarus is significant enough, and therefore supports the pharmacological implications shown by the plant especially in the treatment of hepatitis –B viral infection, diabetes, kidney disorders, flu, phlegm, asthma, influenza [1]. Also, the high quantity of flavonoids in P. discoideus may also prove the fact why it is used in the treatment of cough, fever, gonorrhoea, diarrhoea and ophthalmia [43]. Flavonoids are related flavours. This may also explain why the plant decoction work as an appetizer and also used in herbal teas after labour in Suriname [33] [34] [36]. Flavonoids can act as metal chelator in the cells and selectively reduce or kill cancer cells with high influx of ion [33]. Likewise, Phyllanthus teas containing flavonoids may have health promoting properties. The presence of flavonoids help to prevent platelets sickness and hence platelets aggregation [63].

The leaves of P. discoideus not only contain the highest alkaloids and tannins, it also contained the highest percentage of saponins compared to other plants, having 2.36% respectively. The low percentage of crude yield of saponins in P. urinaria agreed with the opinion of Houghton et al. [32] who noted that saponins are in trace quantity in the plant species. Saponins are often referred to as "natural detergent" because of their foamy natural and have anti carcinogenic properties, immune modulation activities and regulation of cells proliferation as well as health benefit such as inhibition of the growth of cancer cells and cholesterol lowering activity [64]. The presence of saponins may be the reason why the leaves of P. urinaria are used as a fish poison in India, Indo-China, Malaysia and Indonesia [28]. Saponins serve as natural antibiotics which help the body to fight infections and microbial invasion [54]. Saponins have been recorded to prevent disease invasion of plants by parasitic fungi and has shown to affect body and liver weight, urine, plasma, faecal output and liver cholesterol concentration [41]. This can be attributed to the fact why P. amarus is used as an ingredient for diabetes, liver ailment, bladder and kidney disorder, cramps and uterus complaints (with other herbs) [33]. The high level of saponins in P. niruroides and P.

discoideus may also be the reason why they are used as worm expellers and anthelminthics in the treatment of schistomiasis and related ailments [41]. Saponins are also used in the manufacture of shampoos, insecticides and various drug preparation and synthesis of steroid hormones [65]. This can be attributed to the reason why the decoctions are used in herbal baths after labour in Suriname due to the presence of saponins and flavonoids [33] [34] [36]. Saponins are known to make bronchial secretion more liquid, reduce the congestion of the bronchi and ease coughing [54]. This may also be the reason why P. amarus is used in herbal medicine for treatment of flu and asthma in combination with other herbs [34]. P. amarus plant is thus a very useful ingredient in treatment of bronchial infection [26]. The occurrences of steroidal saponins from various studies indicate the importance and the interest of saponins in pharmacy due to their relationship with such compounds that act as sex hormones especially in development of the female contraceptive pills. This may also be attributed to the fact why P. discoideus is used during parturition, treatment of blennorrhoea and post-partum pain [44]. Other characteristics of saponins include haemolytic activity, cholesterol binding properties and bitterness [32].

Generally Phyllanthus species have low phenolic contents with the highest percentage yield recorded in the leaves of P. odontadenius (0.52%) followed by the leaves of P. mullerianus (0.25%) respectively. Phenols are recorded to be absent in the stems of P. amarus, P. urinaria and P. odontadenius or may be contained in trace quantity but generally low in the leaves and roots. The presence of phenolic compound in the plant proves that they have anti microbial and anti fungal effect [24]. Also plants that contain phenol could be used as anti inflammatory, immune enhancers and hormone modulators [66]. Phenols are also known to have the ability to block specific enzymes that cause inflammation and to prevent disease [60]. The presence of phenols in the leaves of P. discoideus may be attributed to the reason why extracts from it can be used for the treatment of inflammation in Malawi [45]. Phenolic compounds are well known potential phytotoxins [67] and exist as free forms, esters or as glycoside when combined with sugars. Such compounds contribute to the bitter taste, flavours and colour of foods [57].

5. Conclusions

The present research on phytochemicals in *Phyllanthus* species studied has not only revealed the medicinal potential of these plants as employed by indigenous people but also their attempt to phytochemically characterise the taxa as it is commonly used in traditional medicine among Nigerians. Further studies should be carried out in order to isolate and identify, characterize and elucidate the structures of these bioactive principles and ace their potential usefulness as pharmaceutical raw material in the production of drugs. It is known and used by people for ethnomedicine though traditionally. However, the presence of various phytochemicals supports its medicinal use. The anti microbial activities of this plant against indigenous pathogenic organisms is worthy of investigation as only a few records of such exist at the moment and to assess the efficacy of these plants for the treatments of diseases as claimed by traditional healers. The diversity of chemical constituents level of toxicants and toxicity and physiology of these plants investigated also requires further investigation. This will help the users of the extracts of the *Phyllanthus* species studied to be sure of the compounds of the plants they use and at the same time guard against any toxicant that could be concealed in different parts of the medicinal plants so as to ascertain the safety of their consumption.

REFERENCES

[1] Foo, L. Y., 1993, Amarulone, A novel cyclic hydrolysable tannin from Phyllanthus amarus. Natural Products Letters, 3, 45-52.

[2] Heyde, H., 1990, Medicjin planten in Suriname. (Den dresi wiwiri foe Sranan). “Medicinal Plants in Suriname”. Uity Stichting Gizondheidsplante Informaite (SGI) Paramaribo, p. 157.

[3] Cabieses, F., 1993, Apuntes de medicinal trational. La racionalizcion de lo irracinal. “Notes on Traditional Medicine”. Consejo Nacional de Ciencia Y Technologia CONCYTEC, Lima-Peru, pp. 414.

[4] Veeramuthu, D., Muniappan, A. and Savarimuthu, I., 2006, Antimicrobial activity of some ethnobotanical plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine, 6: 35.

[5] Edeoga, H. O. and Eriata, D. O., 2001, Alkaloid, tannin and saponin contents of some medicinal plants. Journal of Medicinal Aromatic Plant Sciences, 23: 344-349.

[6] Rahila, T., Ruklsandra, N., Zaidi, A. A., Schanishila, R., 1994, Phytochemical screening of medicinal plants belonging to Euphorbiaceae, Pak. Verr., 14: 160-162.

[7] Sofawara, A., 1993, Medicinal Plant and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, p. 289.

[8] Johnson, K. S., Scribber, J. M. I., 1994, Geographical variation in plants allelochemicals of significance to insect herbivores. In: Ananthakrishnan, T. N. (ed.) Functional dynamics of phytophagous insects, Oxford and IBH Publishing Co., Lebanon, NH: 7-31.

[9] Chew, F. S. and Rodman, J. E., 1979, Plant resources for chemical dense. In: Rosenthal, G. A., Jerzen, D. H., (ed) Herbivores: Their interaction with secondary plant metabolites. Acad. Press, New York, pp. 271-307.

[10] Thyagarajan, P., Subrananian, S., Thirunalasundasi, T., Venkabeswaran, P. S. and Blumberg, B. S., 1998, Effect of Phyllanthus amarus on chronic carriers of hepatitis-B virus, The Lancet, 2: 764-766.

[11] Mehratra, R., Rawat, S., Kulshreshtha, D. K., Goyal, P., Patnik, G. K. and Dhawan, B. N., 1991, In vitro effect of Phyllanthus amarus on hepatitis-B virus. Indian Journal of Medicinal Research, 93: 71-73.

[12] Uander, D. W. and Blumberg, B. S., 1991, In vitro activity of Phyllanthus species (Euphorbiaceae) against the DNA polymerase of hepatitis virus; effect of growing environment and inter and intra-specific differences. Economy Botany, 45: 225-242.

[13] Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O., 2003, Minerals and nutritive values of some Nigeria medicinal plant. J. Med. Aromatic Plant Sci., 25: 1010-1015.

[14] Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O., 2005, Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4 (7): 685-688.

[15] Robber, J. M. and Speedie, T. V., 1996, Pharmacognosy, Pharmacobiotechnology. Williams and Wilkins, Baltimore, pp 1-14.

[16] Davis, P. H. and Heywood, V. H., 1963, Principles of Angiosperm Taxonomy, Oliver and Boyd, Edinburgh.

[17] Chukwu, T., 2000, Anti-nutritional factors in some selected indigenous species, Unpublished project work, Dept. of Biochemistry, Abia State University, Uturu, pp 8-15.

[18] Holm, L., Del, Y., Holm, E., Panchon, T. and Herberger, T., 1997, World Weeds: National Histories and Distribution, John Wiley and Sons Inc., New York.

[19] Harborne, J. B., 1988, Introduction to Ecological Biochemistry 3rd edn., Academic Press London., pp. 10-15.

[20] Zhu, M., Phillipson, T. D., Greengrass, P. M., Bowney, J. and Cai, T., 1997, Plant polyphenols: biological active compounds of non-selective binders to protein, Phytochemistry, 44: 441-447.

[21] Igile, G. O., Olezzek, W., Jurzysta, M. and Burda, S., 1994, Flavonoids from Venonia amygdalina and their anti oxidant activities, Journal of Agriculture, Food and Chemistry, 42: 2445-2446.

[22] Okwu, D. E., 2003, The potentials of Ocimum gratissimum, Pengularia extrensa and Tetrapleura tetraptera as spice and flavouring agents. Nig Agric. J., 34:143-148.

[23] Edeoga, H. O. and Ikem, C. I., 2001, Tannins, saponins and calcium oxalate crystals from Nigeria species of Boerhavia L. (Nycoaginaceae), South African Journal of Botany, 68: 386-388.

[24] Huang, M. T. and Ferraro, T., 1992, Phenolic compounds in food and cancer prevention in phenolic compounds in food in the effects of health II. In: Huang, M. T., H.O.C.T., C. T. (Eds): Acs Symposium Series 507; American Chemical Society, Washington D.C., pp. 8-34.

[25] Dakoro, F. D., 1995, Plant flavonoids: biological molecules for useful exploitation, Aust. J. Plant Physical., 22: 87-99.

[26] Foo, L. Y. and Wong, H., 1992, Phyllanthusin D, an unusual hydrolysable tannin from Phyllanthus amarus. Phytochemistry, 31(2): 211-713.

[27] Foo, L. Y., 1995, Amariinic acid related ellangitanins from Phyllanthus amarus. Phytochemistry, 31(2): 711-713.

[28] Burkill, H. M., 1994, The Useful Plants of West Tropical Africa, vol.2, Families E-I, Royal Botanic Garden, Kew, pp. 605.

[29] Hutchinson, J. and Dalziel, M. D., 1963, Flora of West Tropical Africa, vol. 2. Crown Agents, London, UK.

[30] Chevalier, A., 2000, Encyclopaedia of Herbal Medicine: Natural Health, 2nd edition, Dorlin Kindersley Book, USA, p. 336.

[31] Gill, L. S., 1992, Ethnomedicinal Uses of Plants in Nigeria, University of Benin Press, Nigeria, p. 276.

[32] Houghton, P. J., Woldermarian, T. Z., O'Shea, S. and Thyagarajan, S. P., 1996, Two securinega type alkaloids from Phyllanthus amarus, Phytochemistry, 43: 715-717.

[33] May, A. I., 1982, Surinaams Kruidenboek. Sranan Oso Drei. "Surinameses Book of Herbs" Uitgeeverji Vaco, Paramaribo-Suriname, p. 80.

[34] Titjari, Z. K., 1989, Fraunivi-encyclopedia Foe da Natoera Presi – Fasi. Gezinsleruiden bock van de Natwurgenesivijzen. Natwurgenesivijzen uit her Zonnige Suriname. "Family Herb Book of Cures, Natural Cures of Sunny Suriname", Amsterdan, p. 419.

[35] Tjoung-Ayoung, G., 1989, Het gebruik van medicinal plauten door de Javaanse bevolkingsgroep in Suriname. "The Use of Medicinal Plants by the Javanese Community in Suriname" Institut voor de Opleiding van Leraren Paramaribo, p. 196.

[36] Sedoc, N., 1992, Afro-Surinaamse naturgeneeswijzen. Bevaltende Meer dan tweehounderd meest gebruikelijke geneeskrachige kruden. "African-Surinannse natural cures containing more than two hundred most useful healing curative herbs", p. 224.

[37] Wessel-Boer, J. G., Hekking, W. H. A. and Schulz, J. P., 1976, Fa Joe Kan tak' mi no moi. Inleiding in flora en vegetative van Suriname; Deel I en II "Why do say that I am not beautiful. Introduction to the Flora and Vegetation of Suriname; Part I and II" Natuurgids serie B. No. 4., Stinasu Paramaribo.

[38] Wong, W., 1976, Some Folk Medicinal Plants from Trinidad, Econ. Bot., 30: 103-142.

[39] Watt, J. M. and Beyer-Brandwijk, B., 1962, The Medicinal and Poisonous Plants of Southern and Eastern Africa, Ed. 2., Livingstone Edinburgh and London.

[40] Banquet, A. and Debray, M., 1974, Plantes Medicinales de la Cote d'Ivoire, Traw Doc. O.R.S.T.O.M 32, pp. 85.

[41] Haedi, F., 1964, Die Eingeborenen. Heilpflanzen des unlanga dislike tanganyikeas (osta-erika), In: F. Haerdi, J. Kerharo and J. G. Adam, Arikanshes Heilpflanzen/Plantes Medicinales Africanes, Besel, p. 96.

[42] Kerharo, J. O. and Banquet-Brabdwijik, W., 1950, Plantes Medicinales of Toxiquees de la Cote d'Ivoire, Hante-velra Vigot Freres, Paris.

[43] Keay, R. W. J., 1989, Trees of Nigeria: A revised version of Nigerian trees (1960, 1964) by Keay, R. W. J., Onochie, C. F. A. & Stanfield, D. P. Clarendon Press, Oxford, United Kingdom, p. 476.

[44] Kerharo, J. O. and Adam, J. G., 1974, La Pharmacopie

Senegalese traditionelle, Plants medicinales et Toxiques, Paris, France: Vigot Freres.

[45] Mensah, J. L., Gleye, J., Moulis, C. and Fouraste, I., 1988, Alkaloids from the leaves of Phyllanthus discoideus. Journal of Natural Products, 51(6): 1113-1115.

[46] Webster, G. L., 1994, Classification of the Euphorbiaceae, Annals of the Missoure Botanical Garden, 81(1): 3-32.

[47] Trease, G. E. and Evans, W. C., 1989, Pharmacognosy II, 2nd edn, Braillier Tiridel and Macmillian Publisher, London.

[48] Harborne, J. B., 1973, Textbook of Phytochemical Methods, Chapman and Hall Ltd., London, pp. 49-188.

[49] Obadoni, B. O. and Ochuko, P. O., 2001, Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, Global J. of Pure and Applied Sciences, 8: 203-208.

[50] Van-Burden, T. P. and Robinson, W. B., 1981, Formation of complexes between protein and tannic acid, J. Agric. Food Chem., 11: 77.

[51] Boham, A. B. and Kocipai, A. C., 1994, Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulum and V. calycinium, Pacific Science, 48: 458-463.

[52] www.phytochemicals.com.

[53] Peter, B. K., Leland, J. C., Sara, W., James, A. D. and Huang, L. B., 1999, Natural Products from Plants, C. R. C. Press, N. W. Florida, pp. 2, 30, 60.

[54] Okwu, D. E., 2005, Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants, International Journal of Molecular Medicine and Advanced Sciences, 1: 375-381.

[55] Frantisek, S., 1998, The Natural Guide to Medicinal Herbs and Plants, Tiger Books International, PLC, Twickenham, UK, p. 14.

[56] Steven, J. F., Hart, T., Han, R. C., Van-Elema, E. T., Uander, E. M., Wilderboer, M. Zwaving, J. H., 1995, Distribution of alkaloids, tannins in the Clussiaceae, Biochem. Syst. Ecol., 23: 257-276.

[57] Omaye, S. T., 2004, Food and Nutritional Toxicology, C. R. S. Press, USA, pp. 184-215.

[58] Gordon, L. A., 1980, A Colatry Herbal Deron, England, Webb and Bower Publishers Group Ltd, Singapore.

[59] Heyde, H., 1998, Suriname Planten als Volksmedicijn. "Suriname Plants as Folk Medicine", GRANMA-MK, Paramaribo-Suriname, p. 33.

[60] Okwu, D. E., 2004, Phytochemicals and vitamin contents of indigenous species of South Eastern Nigeria, Journal of Sustainable Agriculture and Environment, 6: 30-37.

[61] Amelio, S. O. F., 1999, Botanical: A Phytochemical Disk Reference, C.R.C Press, Washington D. C.

[62] O'Neil, L., Kim-Standage, W. S., Hughes, G. B. and Murray, K. B., 2000, In: Watson, R. R., 2000, Ed. Vegetables, Fruits and Herbs in Health Promotion, C. R. C. Press, USA.

[63] Okwu, D. E. and Emenike, I. N., 2006, Evaluation of the phytonutrients and vitamin contents of Citrus fruits. International J. of Molecular Med. and Advanced Science, 2(1): 1-6.

[64] Jimoh, F. O. and Oladji, A. T., 2005, Preliminary studies on Pilostigma thonningii seed: proximate analysis, medicinal composition and phytochemical screening, African Journal of Biotechnology, 4(12): 1439-1442.

[65] Edeoga, H. O., Omosun, G. and Uche, I. C., 2006, Chemical composition of Hyptis suaveolens and Ocimum gratssimum hybrids from Nigeria, Afri. J. Biotech., 5 (10): 892-895.

[66] Okwu, D. E. and Omodamino, O. D., 2005, Effects of hexane extract and phytochemical content of Xylopia aethiopica and Ocimum gratissimum on uterus of guinea pig. Bio. Research, 3: 40-44.

[67] Manar, A., Al-Charachetechi, F. and Modallel, N., 2006, Biological activity and anti mutagenicity of water soluble phytotoxins from Artemisia herba Alba, Pakistan J. Biol Sci., 9(9): 1974 -1978.