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Comparative Phytochemical Analysis of the Methanol Extracts of the Roots, Stems and Leaves of Three *Phyllanthus* Species

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Authors' contributions

This work was carried out by author TMA under the supervision of authors SPB and SOA. This article written by author TMA from the work was edited by author SOA. All the authors support the manuscript for publication.

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ABSTRACT

This study was conducted to identify and compare the phytochemicals in the roots, stems and leaves of three *Phyllanthus* species: *Phyllanthus discoideus*, *Phyllanthus amarus* and *Phyllanthus muellerianus*. The phytohemicals were extracted from the parts of the three *Phyllanthus* species with methanol for qualitative and quantitative determination following standard procedures. The data obtained revealed the presence of alkaloids, flavonoids, tannins, saponins and phenols, although at varying levels in the various parts of the *Phyllanthus* species. Generally, most of the phytochemicals are of higher concentration either in the leaves or stems than in the roots of the *Phyllanthus* species, but phenols appeared to be very low in the various parts of these plants. The pooled data showed that, there were more tannins, saponins and phenols in the leaves than in the stems and roots, but more of alkaloids in the stems and flavonoids in the roots. The highest alkaloids, flavonoids, tannins and phenols were extracted from *Phyllanthus muellarianus* while the highest saponins was extracted from *Phyllanthus discoideus*. The study showed the presence of alkaloids, saponins, flavonoids, tannins and phenols in all the *Phyllanthus* species, but at varying

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concentrations in the various species and their parts. These differences in phytochemical levels would likely confer unique medicinal potentials on the various parts of each of these species.

Keywords: Phyllanthus species; phytochemicals; qualitative and quantitative screening.

1. INTRODUCTION

Phyllanthus is one of the largest genera in the family Euphorbiaceae of the flowering plants. Estimated number of the species in this genus varies widely from 700 to 1200 [1-3]. The *Phyllanthus* species are commonly found around all tropical regions of Africa, Asia, America, Australia and Europe. It has remarkable diversity of growth forms including annual and perennial herbs shrubs, climbers and floating aquatics. Despite their variety, almost all *Phyllanthus* species express a specific type of growth called "phyllantoid branching". *Phyllanthus* species are widely distributed in all tropical and sub tropical regions of the earth [4].

The medicinal significance of the genus *Phyllanthus* is largely attributed to the diversity of secondary metabolites in them and their broad therapeutics use in folk medicine. *Phyllanthus* are regarded as a source of new anti-viral compounds and showed the versatility of the genus [5]. A systematic review of 22 randomized clinical trial showed that, *Phyllanthus* species showed anti-viral activity and on liver biochemistry is chronic hepatitis B virus infection [6]. Bioactive principles like alkaloids, tannins, flavonoids, lignans phenols and terpenes have been isolated from various species of *Phyllanthus* and they showed antinociceptive activity [7]. In India, there has been an upsurge of interest in the *Phyllanthus* plants regarding their therapeutic potentials for the management of a number disease [8]. About 500 species of the plants are used for medicinal purposes and about 90 % of the medicinal plants provide raw materials for the herbal pharmaceuticals which are collected from wild habitats [4].

According to [9], the *Phyllanthus* plants are commonly used to expel kidney stones, support kidneys, increase urination, relieve pain, protect and detoxify the liver, reduce spasms and inflammation, kill viruses and bacteria, aid digestion, reduce fever and blood sugar, and cholesterol, treat malaria and prevent mutation. The bruised leaves and fruits of a *Phyllanthus* plant are applied by the Indians as a dressing to abscesses [10]. It is clear that, the medicinal value of *Phyllanthus* species and of plants in general, lies in the bioactive phytochemical constituents that produce definite physiological effect on the human body [11]. Some plants of the genus *Phyllanthus* have been reported to contain potential phytoconstituents like flavonoids, tannins, alkaloids and triterpenoids in earlier studies [6].

The use of these plants is however, not widespread in this region of the country possibly due to lack of information on their uses and components and information is scarce on the quantification of the phytochemicals in the different parts of the plants.

This study aimed at determining the phytoconstituents in the various parts of *Phyllanthus* species growing in the prevailing environmental conditions in the northern Guinea Savannah agro ecological zone of Nigeria, where the study was carried out. This will help to identify the species and parts of the plants from which higher quantities of the phytochemicals can be derived. Information obtained will be useful for pharmaceutical companies to initiate research and development programmes in an attempt to discover novel drugs from these plants.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Matured whole plants of *Phyllanthus amarus*, *Phyllanthus discoideus* and *Phyllanthus muellarianus* were collected from the premises of Ahmadu Bello University, Zaria. The plants were identified in the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The plants were washed, separated into roots, stems and leaves with knife. Each part was spread on clean table top and allowed to dry under the shade in the laboratory at room temperature around 25°C. The individual species parts were later pulverized using the pestle and mortar and stored in air tight labeled specimen bottles pending analysis which was carried out as described by [12].

2.2 Methanol Crude Extraction

From the powdered plant parts of each of the three *Phyllanthus* species 20 g was weighed into conical flask and moistened with 100 mls. of 70 % methanol and the flask's mouth was covered with aluminium foil and allowed to stand for about 4 hours. This was then transferred into the percolator and the 70% methanol was added until the material was saturated. Additional quantity of 70% methanol was added to form a shallow layer above the mass, and the percolator was closed. This mixture was allowed to macerate for 24 hours. The outlet of the percolator was opened, and the liquid was allowed to drip slowly into a container. The mass was pressed and the liquid squeezed out was added to the filtrates. The filtrates were placed in the water bath, evaporated and the methanol crude extracts were obtained.

2.3 Qualitative Phytochemical Screening

The Phytochemical examination was carried out on the methanol crude extracts of the roots, stem and leaves of the three *Phyllanthus* species using standard procedures described below to identify their constituents.

1. **Alkaloids test:** about 2 g of each of the extracts were dissolved individually in dilute hydrochloric acid and filtered.
   a. A few drops of the extracts (filtrates) were treated with Mayer reagent (Potassium Mercuric Chloride). The formation of yellow precipitate indicates the presence of alkaloids.
   b. A few drops of the extracts were treated with Dragendoff's reagent (Potassium Bismuth Iodide), and the formation of yellow precipitate confirmed the presence of alkaloids.
2. **Flavonoids test:** about 2 g of each of the crude extracts were treated with few drops of sodium hydroxide solution. The formation of intense yellow colour which becomes colourless on the addition of dilute acid indicates the presence of flavonoids.
3. **Tannins test**: Gelatin solution (1%) containing Sodium Chloride was added to 2 g of each of the crude extracts. Formation of white precipitate indicates the presence of tannins.
4. **Saponins test:** The froth test was carried out on each of the crude extracts. About 2 g of each of the extracts were diluted with 20 ml of distilled water in a graduated cylinder, and shaken for 15 minutes. The formation of about 1 cm layer of stable foam indicates the presence of saponins.
5. **Phenol test:** To about 2 g of each of the extracts were added 1ml of 10% Ferric Chloride ($FeCl_2$) solution and mixed together. The formation of blue precipitate indicates the presence of phenolic compounds.

The levels of these phytochemicals in the samples was indicated to be: + (present), ++ (deeply present), +++ (very deeply present), and – (absent), based on the degree of the expression of the expected colouration for each compound.

2.4 Quantitative Analysis of the Phytochemicals

2.4.1 Determination of alkaloids

About 2 g of the crude extracts were put into a 250 ml beaker, and 80 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The filtrates were concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added in drops until the precipitate was complete. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloids, it was dried and weighed.

2.4.2 Determination of saponins

To about 2 g of each of the crude extract in a conical flask was added 20 ml of 20% aqueous ethanol and it was heated over the water bath for 4 hours with continuous stirring at 55°C The filtrates were re-extracted with 200 ml of 20% ethanol and these were reduced to 40 ml over the water bath at 90°C. the concentrates were transferred into 250 ml separator funnels and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and re-purified, and 60 ml of n-butanol was added. This was washed twice with 10 ml of 5% aqueous sodium chloride. The solution was heated on the water bath, and then dried in the oven set at 35°C till a constant weight was obtained, after which the saponins contents was calculated.

2.4.3 Determination of tannins

To about 500 mg of the crude extracts was added 50 ml of distilled water in a 500 ml plastic bottle and shaken vigorously in a mechanical shaker for about one hour. This was filtered into a 50 ml volumetric flask and made to the mark. Then 3 ml of 0.1 M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferrocyanide was added to 5 ml of the filtrate in a test tube. The absorbance was measured at 120 nm wavelength within 10 minutes to get the total tannins. A standard was prepared using tannin acid to get 100 ppm and measured.

2.4.4 Determination of flavonoids

About 2 g of the crude extracts was re-extracted with 20 ml of 80% aqueous methanol at room temperature. It was filtered using Whatman filter paper No. 42 (125 mm). The filtrates were later transferred into crucibles and evaporated into dryness over a water bath weighed and the flavonoids was calculated.

2.4.5 Determination of phenolic compounds

About 500 mg of the crude extracts were dissolved in 100 ml of triple distilled water (TDW). Then, 1 ml of this solution was measured with pipette into test tubes and 0.5 ml 2 N of Folin – ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with triple distilled water, followed by vigorous shaking and finally allowed to stand for about 2 hours. The data was used to calculate the phenolic compounds using a standard calibrated curve obtained from various diluted concentrations of gallic acid.

The data obtained from the parameters above were subjected to analysis of variance (ANOVA) using SPSS package version 20. Duncan multiple range test (DMRT) was used to separate the means where there was significant difference.

3. RESULTS

The qualitative phytochemical screening of the crude extracts of the three *Phyllanthus* species revealed the presence of medicinally active constituents such as alkaloids, flavonoids, tannins, saponins, phenols etc. at varying intensities (Table 1). However, phenols were indicated to be absent in the roots, and leaves of all the *Phyllanthus* species. Most of the phytochemicals were shown to be present or deeply present, while only flavonoids indicated being very deeply present was shown only for the flavonoids in the roots of *Phyllanthus discoideus*, and alkaloids in the stems of *Phyllanthus muellarianus* (Table 1).

The data on the quantitative analysis of the phytochemicals in the crude extracts of *Phyllanthus discoideus* showed that, with the exception of saponins and phenols, the levels of the phytochemicals differ significantly with plant parts (Table 2). The data showed higher alkaloids, tannins and phenols content in the leaves, but higher flavonoids and saponins in the roots compared with that of other parts of *P. discoideus.*

Table 1. The Phytochemical screening (qualitative) of the methanol extracts of the three *Phyllanthus* species

Plant parts	Phytochemicals	*Phyllanthus* Species		
		P. discoideus	***P. amarus***	***P. muellarianus***
Roots	Alkaloids	+	+	++
	Flavonoids	+++	++	++
	Tannins	++	+	++
	Saponins	++	++	+
	Phenols	-	-	-
Stems	Alkaloids	+	+	+++
	Flavonoids	+	+	++
	Tannins	++	++	+
	Saponins	++	++	++
	Phenols	+	+	+
Leaves	Alkaloids	+	+	++
	Flavonoids	++	+	++
	Tannins	++	+	+
	Saponins	+	+	+
	Phenols	-	-	-

NB: +: Present, ++: Deeply present, +++: Very deeply present, - : Absent

Table 2. Quantitative determination of the phytochemicals in the methanol crude extracts of the three *Phyllanthus* species

Phyllanthus species	Phytochemicals (mg/g)	*Phyllanthus* parts		
		Roots	Stems	Leaves
P. discoideus	Alkaloids	0.16b	0.50a	0.50a
	Flavonoids	0.74a	0.60b	0.72a
	Tannins	0.11b	0.17a	0.18a
	Saponins	0.22a	0.17a	0.18a
	Phenols	0.05a	0.08a	0.12a
P. amarus	Alkaloids	0.43b	0.58a	0.53ab
	Flavonoids	0.58a	0.54a	0.22b
	Tannins	0.20b	0.26a	0.25a
	Saponins	0.05b	0.06b	0.20a
	Phenols	0.04b	0.08a	0.05ab
P.muellarianus	Alkaloids	0.48c	0.91a	0.76b
	Flavonoids	0.78a	0.71b	0.80a
	Tannins	0.34a	0.28b	0.29b
	Saponins	0.17a	0.09b	0.16a
	Phenols	0.09a	0.09a	0.10a

NB: Means with the same letters in each row under each species are not significantly different (P = 0.05), using DMRT

In *Phyllanthus amarus,* the levels of all phytochemicals differed significantly in the various parts of the plants (Table 2). The data showed that in *P. amarus,* there was higher alkaloids, tannins and phenols in the stem, higher flavonoids in the roots and higher saponins in the leaves than the other parts of the plant. The *P. amarus* stems had the lowest content of most phytochemicals (except flavonoids) compared with the other parts.

In *Phyllanthus muellarianus*, there was significant difference in the levels of the phytochemicals (except phenols) in the plants parts (P=0.05) (Table 2). The data showed that in *P. muellarianus,* there was higher content of tannins and saponins in the roots, higher flavonoids and phenols in the leaves and higher alkaloids in the stems compared with the other parts of the plant. The *P. muellarianus* stem had the lowest content of most phytochemicals (except alkaloids) compared with the other parts.

The analysis of variance of the pooled data on the quantitative analysis of the Phytochemicals of the methanol crude extracts of roots, stem and leaves of irrespective of *Phyllanthus* species, showed that, the methanol extracted more of tannins, saponins and phenols from the leaves than from the stems and roots, but more of alkaloids from the stems and flavonoids from the roots. The roots had the lowest content of most of the phytochemicals (except flavonoids and saponins) (Table 3).

4. DISCUSSION

The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins and phenols, in the plant parts (roots, stem and leaves) at varying intensities. This is of importance because these phytochemicals when obtained from natural sources can be used in manufacturing drugs. This is similar to the work of [13] on the phytochemical analysis of *Phyllanthus fraternus*, which also revealed the presence of medicinally active constituent such as tannins, alkaloids, terpenes, sterols and saponins. It has been reported that alkaloids, phenols and tannins are plant metabolites known for anti-microbial activity [14,15]. Studies conducted by [10] on the phytochemical constituents and anti-microbial studies of two South African *Phyllanthus* species showed the presence of secondary metabolites of medicinal interest. The results of the phytochemcial screening in this study are also being supported by the report of [16] on the phytoconstituents of fifteen *Phyllanthus* species. They discovered that, alkaloids, coumarins, flavonoids, phenols, glycosides, saponins are common compounds in all the plants parts (leaf, stem, root,) although at varying intensities. The presence of these phytochemicals provides explanation for the use of *Phyllanthus* species as anti-bacterial, anti-protozoan anti-malarial and anti-fungal agents [17]. The biosynthesis of secondary metabolites varies among plants, even in different organs of plants and their biosynthesis depends on the

Table 3. Quantitative analysis of the phytochemicals in the methanol crude extracts of the roots, stem and leaves of *Phytochemicals* species

Phytochemicals (mg/g)	*Phyllanthus* parts		
	Roots	Stems	Leaves
Alkaloids	0.36c	0.66a	0.60b
Flavonoids	0.70a	0.62b	0.58c
Tannins	0.22b	0.24a	0.24a
Saponins	0.15b	0.11c	0.18a
Phenols	0.06c	0.08b	0.09a

NB: Means with the same letter (s) in each row are not significantly different (P = 0.05), using DMRT.

environmental factors in which they grow [18, 19]. Intraspecific variation of phytochemcials was observed in the three species studied. The methanol extracts of the roots revealed the absence of phenols in the three species and very deep presence of flavonoids only in *Phyllanthus discoideus.* It has been observed that, inter-specific variation in phytoconstituents has been documented extensively among plants [20]. However, according to [21], genotype appeared to play an important role in affecting the total phenolic content and antioxidants activities in vegetable crops. He observed that, the differences in phenolic content between two green cabbage cultivars suggest that, cultivar could also be important factor in these species. The reports of [22,23] showed variations in anthocyanins, chlorogenic acids, antioxidant activity and phenolic compounds among blueberry cultivars. In *Phyllanthus discoideus* and *Phyllanthus muellarianus*, the concentrations of the phytochemicals was in this order: the flavonoids > alkaloids > saponins > tannins > phenols. However, in *Phyllanthus amarus*, it was in this order: alkaloids > flavonoids > tannins > saponins > phenols. These showed very low levels of phenols in these *Phyllanthus* species.

This is similar to the findings of [24], who researched on the phytochemcial screening of *Jatropha* species in the Niger Delta, Nigeria, and discovered that, among the five groups of phytochemicals investigated from the leaves, roots, seeds and stems, phenols were found to be lowest in concentration. In contrast with this, phytochemical investigations of some *Ficus* species revealed phenolic compounds as the major components in the different parts of leaves, stem wood, stem bark, root etc [25]. Also, according to [18], phenols are absent in the stems of *P. amarus, P. urinaria and P. odontadenius* or may be present in trace quantity but generally low in the leaves and roots. The differences in the concentrations of the phytochemicals in the different *Phyllanthus* species studied are likely to be due to differences in genotypes and in habitat and they are not likely to have the same level of medicinal potentials.

5. CONCLUSION

In conclusion, the study showed that, the methanol extracts of the three *Phyllanthus* species contain alkaloids, saponins, flavonoids, tannins and phenols and this justifies their use in traditional medicine for the treatment of diseases. There is variation in the phytohemical composition of the various parts of the three *Phyallanthus* species and this establishes the fact that, these plants parts may not have same medicinal potentials. Most of the phytochemicals were of higher concentration in the leaves, followed by the stems and lowest in the roots. It is recommended that, further studies be carried out to quantify the phytochemicals in other *Phyllanthus* species, in the Northern Guinea Savanna agro-ecological region of Nigeria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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