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Phytochemical Investigation of the N-Hexane and Chloroform Root Extracts of *Phyllanthus Floribundus* (Phyllanthaceae)

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Abstract:

Phyllanthus floribundus has been commonly used in traditional medicine, especially in western Nigeria; however, few reports have been made regarding its phytochemical constituents. The roots were collected, shade dried, exhaustively extracted with n-hexane and chloroform for phytochemical screening. The plant's root contained more polar compounds as indicated by the percentage yield; also, ketone and amide group as revealed by the functional group analysis. The thin layer chromatographic analysis of the crude n-hexane extract revealed three spots at varying retention factors - R_f : 0.30, 0.74, and 0.91; the chloroform extract also indicated three spots with retention factors - R_f : 0.34, 0.86, 0.96. Qualitative and quantitative phytochemical screening analyses on the roots n-hexane extract indicated alkaloids (0.60 mg/100 g), saponins (0.31 mg/100 g), and steroids (2.09 mg/100 g). In comparison, the chloroform root extract yielded alkaloids (1.33 mg/100 g), saponins (0.96 mg/100 g), and steroids (1.36 mg/100 g). The presence of important phytochemicals in the plant scientifically justifies its use in traditional medicine.

Keywords: Phytochemical, extract, chromatography, saponin, alkaloid, steroid, medicine, ketone, amide

1. Introduction

Plants serve as food and have also been used by humankind for shelter and medicinal purposes because they contain veritable nutrients, edible nuts, mushrooms, fibers, herbs, spices, gum, and fodders. They contain chemical compounds (metabolites) that attract insects (insect feed), protect plants from predators, etc. Medicinal plants are reservoirs of potentially useful chemical compounds that could serve as newer leads and clues for modern drug design. They have been used all over the world to help in relief from illnesses and many health issues. They are safe, less toxic, economical, and a reliable natural source of drugs worldwide (Vijyalakshmi and Ravindran, 2012). Thus folk medicine has been a major pathway for modern medicine through chemical and pharmaceutical screening.

Phyllanthaceae is a family of flowering plants in the eudicot order Malphigiales. It is most closely related to the family Picodendraceae (Wurdack and Davis, 2009). It comprises about 2000 species, which are grouped into 54 to 60 genera (Kathriarachchi, *et al.*, 2006). Phyllanthaceae has been reported to have antioxidant, anti-inflammatory, antihyperglycemic, and antihyperlipidemic effects. It has been used to treat hypertension, diabetes, hepatitis, urinary and sexual disorders, and other common ailments (Bharti, *et al.*, 2014).

Phyllanthus floribundus is a shrub found in the Euphorbiaceae (APG. Phyllanthaceae) family. In the western part of Nigeria, the plant is also called *Arunjeron*, *Arunyeran*, and *Eguneja* (Burkill, 1985). The leaf extract of *Phyllanthus floribundus* is used to treat malaria, and the fruits are given to children to cure jaundice (Poompachee and Chudapongse, 2011; Omulokoli, *et al.*, 1997). It has been reported to possess medicinal properties which have been helpful in traditional medicine. However, only a few reports exist on its phytochemical analysis. Herein, the phytochemicals found in *P. floribundus* are being reported.



Figure 1: Leaves of *Phyllanthus Floribundus*
(FUNAAB Nursery Unit, Abeokuta)

2. Materials and Methods

2.1. General Experiment

Thin-layer chromatography (TLC) was obtained on a pre-coated silica gel (60, 0.3 mm thick, Merck, England) coated aluminum foil plate and visualized in an iodine tank. All solvents used in this study were redistilled before use to ascertain their purities. Purity was ascertained by comparing the boiling points with the standards.

2.2. Collection and Extraction of Plant Roots

The root of *Phyllanthus floribundus* was collected from the FUNAAB Nursery Unit Abeokuta, Ogun State, Nigeria, in July 2018 and confirmed by the Head of the Nursery Unit. The roots were cut into small pieces, air-dried under shade, and pulverized using a grinding machine. Hot solvent extraction was carried out in an aspirator bottle fitted with extraction gadgets. 4.914kg of the pulverized sample was packed in the 10 L aspirator bottle and extracted sequentially using n-hexane and chloroform. Exhaustive extraction using n-hexane lasted for 17 hrs 37 minutes, while for chloroform, it lasted for 12 hrs 50 minutes. The extracts were concentrated using the simple distillation technique.



Figure 2: Extraction of the Pulverized Root of Using N-Hexane



Figure 3: Concentration of the N-Hexane Root of
Phyllanthus Phyllanthus Floribundus

2.3. Qualitative Determination of the Phytochemical Constituents of *P. floribundus*

2.3.1. Phytochemical Screening for Alkaloids

0.5 mL of each extract of *P. floribundus* was stirred with 5.0 mL of 1% aqueous HCl solution on a steam bath. 1.0mL of the filtrate was treated with a few drops of Meyer's reagent, and another 1.0mL portion was treated with Dragendoff's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extracted sample (Harbourne, 1973; Trease and Evans, 1989).

- Dragendoff's reagent: An orange precipitate indicates a positive test.
- Wagner's reagent: Brownish red precipitate indicates a positive test.

2.3.2. Phytochemical Screening for Steroids

0.5 g of the extract was dissolved in 2.0mL of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface indicates the presence of steroids (Trease and Evans, 1989).

2.3.3. Test for Saponins

5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube, and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously. The appearance of foam showed the presence of saponins (Rahman *et al.*, 2017).

2.3.4. Phytochemical Screening for Tannins

0.5 mL each portion of plant extract was stirred with 10 mL of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue-black, green, or bluish-green precipitate confirms the presence of tannins.

2.3.5. Phytochemical Screening for Cardiac Glycosides

Extract of *Phyllanthus floribundus* was dissolved in pyridine, and a few drops of 2 % sodium nitroprusside together with few drops of 20 % sodium hydroxide were added. A deep red colour which faded to brownish-yellow, indicates the presence of cardiac glycosides.

2.3.6. Phytochemical Screening for Flavonoids

A portion of the extract was dissolved in water and then filtered. The filtrate was then used for the following test. 2.0 mL concentrated HCl, and four pieces of magnesium turning were added to a portion of the filtrate. Production of pink or orange coloration is considered as a positive test for flavanol derivatives (Trease and Evans, 1989)

2.3.7. Phytochemical Screening for Reducing Sugar

0.1 g of the extract was dissolved in distilled water, and the solution was filtered. The filtrate was boiled in Fehling's solution A and B for 2 min. A red brick solution confirms the presence of reducing sugar (Trease and Evans, 1989)

2.4. Quantitative Determination of the Phytochemical Constituents of *P. floribundus*

2.4.1. Estimation of Alkaloids

0.5 g of the sample was dispersed in 10% acetic acid solution in ethanol in the ratio of 1:10 (10 %). The mixture was allowed to stand for 4 hours at 28°C and was then filtered with a filter paper. The filtrate was concentrated to one-quarter of the original volume by evaporation and was treated with three drops of concentrated ammonium hydroxide. The alkaloid precipitate was then received on a weighed filter paper, and it was washed with 1 % ammonia solution and oven-dried at 80°C (Harbourne, 1973).

2.4.2. Quantitative Determination of Saponins

1.0g of the sample was weighed into a 250 mL beaker, and 100 mL isobutyl alcohol was added to it. The mixture was shaken for over five hours to ensure uniformity in mixing, and it was filtered into a 100 mL beaker. 20 mL of 40 % magnesium carbonate was added to the filtrate which was mixed thoroughly and filtered to obtain a clear colorless solution. 1 mL of the colorless solution was pipette into a 50 mL volumetric flask, and 2 mL of 5 % FeCl₃ solution was added. It was then made up to the mark with distilled water. It was left to stand for 30 mins for the red blood colour to develop. The absorbance was read after the colour development at a wavelength of 380 nm (Brunner, 1994).

2.4.3. Quantitative Estimation of Steroids

1.0 mL of test extract of steroid solution was transferred into 10 mL volumetric flasks. Sulphuric acid (4.0N, 2.0 mL) and iron (III) chloride (0.5% w/v, 2.0 mL), were added, followed by 33 potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 mL). The mixture was heated in a water bath maintained at 70 ± 20°C for 30 mins with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Madhu, *et al.*, 2016)

3. Results and Discussion

3.1. Extraction of *PhyllanthusFloribundus*

The table below reports the weight, percentage yield, and colour of the n-hexane and chloroform root extracts of *P. floribundus*.

Item	Weight of Pulverized Root (kg)	Weight of Extracts after Concentration (kg)	Yield of Extracts (%)	Colour of Extracts
n-hexane extract	4.914	0.01871	0.381	Brown
Chloroform extract	4.304	0.01796	0.417	Dark Brown

Table 1: Extraction of the Roots of *Phyllanthus Floribundus* Using N-Hexane and Chloroform

Extraction of *P. floribundus* using chloroform resulted in a greater percentage yield of extract (0.417%) than extraction using n-hexane (0.381%). This implies that the roots of the plant contain more polar than non-polar compounds.

3.2. Functional Group Analysis of the Root Extracts of *PhyllanthusFloribundus*

The functional group analysis carried out on the root extracts of *P. floribundus* indicates ketone (CO) and amide (CONH₂) groups, as shown in table 2.

Test	n-hexane Extract	Chloroform Extract
Alcoholic solution of extract + 2,4-dinitrophenylhydrazine + dilute H ₂ SO ₄	Carbonyl is present. Aldehydes or ketones are suspected	Carbonyl is present. Aldehydes or ketones are suspected
Alcoholic solution of extract + Fehling solution A and B + warm	Ketone is confirmed	Ketone is confirmed
Alcoholic solution of the extract + NaOH solution + heat	Amide is present	Amide is present
0.5 mL of extract solution + ethanol + few drops of ferric chloride solution	Phenolic group is absent	Phenolic group is absent

Table 2: Functional Group Analysis of the Root Extracts of *PhyllanthusFloribundus*

Several medicinal compounds have been reported to contain the Carbonyl and amide group. For instance, Febrifugine and its synthetic derivative, Halofuginone, are quinazolinone alkaloids that contain a ketone group. They possess antimalarial properties and have been used against cancer, fibrosis, and inflammatory disease (Keller, *et al.*, 2012). Similarly, anesthetic drugs such as lidocaine, mepivacaine, and prilocaine contain the amide group (Gosnell, *et al.*, 2019). Thus, the presence of ketone and amide groups supports the therapeutic properties of *P. floribundus*.

3.3. Thin-Layer Chromatographic Analysis of the Crude N-Hexane Root Extracts of *PhyllanthusFloribundus*

Table (3) shows the thin layer chromatographic analysis carried out on the root extracts of *P. floribundus*. The analysis revealed three spots with R_f at 0.30, 0.71, and 0.91 for n-hexane; three spots with R_f at 0.34, 0.86, and 0.96 for chloroform, as shown in Fig II.

Plant Extract	Solvent Mixture	Adsorbent Used	Visualizing Medium	Number of Spots	Retention Factor
N-hexane extract	n-hexane: ethyl acetate (3:1; v/v)	Aluminum foil coated with silica gel	Iodine vapour	3	0.30, 0.74, 0.91
Chloroform extract	Chloroform: ethyl acetate (4:3; v/v)	Aluminum foil coated with silica gel	Iodine vapour	3	0.34, 0.86, 0.96

Table 3: Thin Layer Chromatography of the Crude N-Hexane Root Extract of *PhyllanthusFloribundus*

3.4. Qualitative Estimation of the Phytochemical Constituents of *P. Floribundus*

The qualitative analysis of the n-hexane roots extract of *P. floribundus* indicated the presence of alkaloids and steroids. In contrast, the chloroform roots extract revealed alkaloids, steroids, and cardiac glycosides (Table 4).

Phytochemicals	Phytochemical Test	n-hexane Root Extract of <i>P. floribundus</i>	Chloroform Root Extract of <i>P. floribundus</i>
Alkaloids	Dragendorff's reagent	+	+
	Wagner's Reagent	+	+
Steroids	Salvoski's test	+	+
Saponins	Frothing test	+	+
Tannins	Ferric chloride test	-	-
Cardiac glycosides	Legal test	-	+
Flavonoids	Shinoda test	-	-
Reducing Sugar	Fehling's solution test	-	-

+ =Present - = Absent

Table 4: Qualitative Determination of the Phytochemical Constituents in the Crude N-Hexane Root Extracts of *PhyllanthusFloribundus*

3.5. Quantitative Estimation of the Phytochemical Constituents of *P. Floribundus*

Table (5) revealed that the quantitative analysis of both root extracts yielded more steroids than alkaloids and saponins.

Sample	Steroids (mg/100g)	Alkaloids (mg/100g)	Saponins (mg/100g)
N-hexane Root extract of <i>P. floribundus</i>	2.09	0.60	0.31
Chloroform Root extract of <i>P. floribundus</i>	1.36	1.33	0.96

Table 5: Quantitative Determination of the Phytochemical Constituents in the Crude N-Hexane Root Extracts of *PhyllanthusFloribundus*

Medicinal plants used to treat coronary heart disease, ulcers, diabetes, high blood pressure, muscular degeneration, inflammation, infection, and psychotic diseases have been proved to contain steroids, alkaloids, and saponins (Premier, 2002). The presence of these phytochemicals – steroids, alkaloids, and saponins – in the roots of *P. floribundus* indicates its potential for medicinal purposes.

4. Conclusion

For the first time, herein lies the report of the phytochemicals present in the n-hexane and chloroform root extracts of *Phyllanthusfloribundus*. The quantitative and qualitative analyses indicated the presence of secondary metabolites but in low quantities. However, the higher percentage yield of the chloroform extract suggests that a more polar solvent should be used to extract more of the phytochemicals and active principles present in the plant. These research findings reveal the presence of important phytochemicals which confirm the plant's potency and use as an herbal drug; however, further research is necessary to identify the active compounds responsible for the therapeutic activity of *P. floribundus*.

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