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Phytochemical Screening and Anti-Bacterial Activity of Methanolic Leave Extract of *Phyllanthus muellerianus*

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ABSTRACT

In this research, phytochemical screening and antibacterial properties of *Phyllanthus muellerianus* were investigated to confirm the potency of the plant on the basis of ethno medicinal significance. The percentage yield of crude methanolic leave extract of *Phyllanthus muellerianus* was 12.58% while the phytochemical screening of crude methanolic leave extract of *Phyllanthus muellerianus* revealed the presence of anthraquinone, carbohydrates, tannins, alkaloids, flavonoids, cardiac glycosides, saponin and lipids. The result of the antibacterial activities showed that the crude methanolic leave extract of the plant exhibits high potency against all the tested organisms. These properties above may be the reason *Phyllanthus muellerianus* plant has been reported to be useful in the treatment of several ailments.

Introduction

The genus, phyllanthus contain over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Taylor, 2003). *Phyllanthus muellerianus* is a deciduous or evergreen shrub. It has straggling stems from the base with a climbing habit and sometimes becomes more tree-like in habit. It has the potential to grow up to 12 metres tall (PROTA, 2015). *Phyllanthus muellerianus* is widely used to treat intestinal troubles, severe dysentery, constipation, stomach ache, jaundice, urethral discharges and as wound dressing (Burkill, 1995). The leaf extract of *Phyllanthus muellerianus* is used as a vapour bath to treat toothache and venereal diseases (Arbonnier, 2004).

Phyllanthus muellerianus has been widely used as an herbal remedy in different parts of the world. In Guinea, the leaves of the plant are boiled with palm fruits and served to women undergoing labor. In Ghana, the root is cooked with maize meal and used to treat chronic dysentery, and in Togo, Cote d'Ivoire, and Zambia, the roots and leaves of the plant are boiled and administered to children to treat eruptive fever (Fowler, 2006). In Sierra Leone and southern Nigeria, the fresh juice of the plant is used to treat infections of the eye and skin diseases (Fowler, 2006). The fresh leaves of the plant can also be crushed and applied to wounds and the decoction can be used as purgative for bronchitis and for relieving urethral discharges (Fowler, 2006; Siram *et al.*, 2004). The antibacterial activities of the plant have been demonstrated in the leaf and stem bark against some selected bacteria (Doughari and Sunday, 2008; Katsayal and Lamal, 2009).

In view of the medicinal significance of *Phyllanthus muellerianus*, this work was carried out investigate the antibacterial activities of this plant in the North Central part of Nigeria. The chemical constituents of the plant extract will also be examined.

Experimental

Materials and Methods

The solvent used which include menthol, acetone, chloroform, petroleum ether and diethyl ether were redistilled before use.

Collection of Plant

The leaves of the plant were collected within University of Ilorin Environment near Chemistry Laboratory Permanent Site (main campus). The plant was identified and authenticated at the herbarium unit of the Department of Plant Biology University of Ilorin, Ilorin Nigeria and a voucher specimen was deposited. The leaves were air-dried for two weeks.

Extraction of Plant

The leaves of the plant were chopped into smaller pieces using mortar and pestle. The cold extraction was carried out using methanol as the organic solvent. 6.852g of the powered leaves was weighed and successively extracted with two litres of methanol for a period of 2 days. The crude extract was decanted, filtered and concentrated to dryness on the water bath.

Phytochemical Screening

Phytochemical screening was carried out on the methanolic extract using standard methods to detect the presence of various chemical constituents as described below:

Test for Anthraquinone: 2 ml of dissolved extracts was

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measured and concentrated to dryness. 3 ml of petroleum ether and 2 ml of NH_3 was added to the extract. The presence of red colour at the aqueous phase of the extract was taken as an evidence for the presence of anthraquinone while the absence of red colour indicated the absence of anthraquinone in the extract (Kumar *et al.,* 2007).

Test for Carbohydrates: A small portion of the methanolic extract was put in a test tube and 2 ml of H_2O was added to dissolve it, 6 drops of Molisch's reagent was added and 1 ml of conc. H_2SO_4 was also added. A brownish ring formation at the center of the 2 phase in the test tube indicates the presence of carbohydrate (Harborne, 1973).

Test for Tannins: A small portion of the methanolic extract was taken out in a test tube and 2 ml of H_2O was added to dissolve the extract, 3 drops of $FeCl_3$ was added and heated. The presence of a blackish green colour indicated the presence of cathecol tannin (Kumar *et al.,* 2007; Parekh and Chanda, 2007).

Test for Alkaloids: 1 ml of dissolved methanolic extract was measured and concentrated to dryness, 2 ml of 2 % conc. HCl was added and divided into 2 portions; to the first portion, 3 drops of dragendorff's reagent was added. The appearance of orange-red precipitate confirmed the presence of alkaloids. To the second portion, 3 drops of mayer's reagent was added and the appearance of a creamy precipitate confirmed the presence of alkaloids (Kumar *et al.,* 2007).

Test for Flavonoids: A small portion of the extract was added into a test tube and 2 ml of H_2O was added to dissolve the extract and 1 ml of lead acetate was added. The appearance of a creamy or yellowish-white precipitate indicates the presence of flavonoids (Kumar *et al.,* 2007).

Test for Cardiac glycosides: 1 ml of the extract was dissolved in 2 ml of H_2O and filtered into another test tube. 1 ml of glacial acetic acid was added to the filterate in a water bath to prevent explosion, after which 1 ml of $FeCl_3$ was added followed by 1 ml of conc. H_2SO_4. A green-blue colouration confirmed the presence of cardiac glycosides (Parekh and Chanda, 2007).

Test for Saponin: 1 ml of the methanolic extract was dissolved in 2 ml of distilled water and shake well with gentle heating. The appearance of persistence frothing confirmed the presence of saponin (Parekh and Chanda, 2007).

Test for Reducing Sugar: 1 ml of the methanolic extract was dissolved in 2 ml of H_2O and filtered into another test tube, 3 drops of Fehling's solution A & B was added and heated gently. A brick-red precipitate confirmed the presence of reducing sugar (Akinyemi *et al.,* 2005).

Test for Lipids: 1 ml of the extract was dissolved in 2 ml of distilled water, 3 drops of Sudan solution was added to the extract and a brick-red precipitate was taken as the confirmation of fats (Dahiru *et al.,* 2006).

Test for Steroids: A small portion of the methanolic extract was dissolved in 3 ml of chloroform, 2 ml of acetic anhydride and 1 ml of conc. H_2SO_4 was also added to the extract. A deep green colouration confirmed the presence of steroids (Kumar *et al.,* 2007).

Test for Coumarin: 3 ml of the extract was evaporated to dryness and 2 ml of warm distilled water was added. The extract was divided into 2 portions. The first portion was observed under UV visible light and the presence of intense florescence confirmed the presence of Coumarin. To the second portion, dilute NH_3 was added for a deep and clear intense florescence (Kumar *et al.,* 2007).

Anti-bacterial activity study

i) Determination of Zones of Inhibition of the Extract on Bacterial Isolates

Sterile Mueller Hinton agar was prepared and poured into each Sterile Petri dishes of equal size and allowed to solidify. Gentamicin was used as the control. The surface of this Sterile Mueller Hinton agar plate was streaked with the pure culture of the standardized bacterial cell suspension (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Salmonella paratyphi*, *Proteus mirabilis*, *Shigella sonnei*, *Lactobacillus casei*, and *Escherichia coli*). A cork borer (4 mm in diameter) was sterilized by flaming and used to bore hole at the center of the agar plate and the standardized bacteria was spread across the plate. The holes were filled with 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml of the leave extract of the plant. The plates were allowed to stand for one hour for pre-diffusion of the extract and incubation was done at 37^0C for 24 hours. At the end of the incubation period, the diameter of the clear zone of inhibition was measured and recorded in millimeter (Hassan *et al.,* 2003: Selvamohan and Sandhya, 2012).

ii) Determination of Minimum Inhibitory Concentration (MIC)

The initial concentration of the plant extract (200 mg/ml) was diluted using double fold serial dilution by transferring 1 ml of the plant extract (stock solution) into 1 ml of methanol to obtain 100 mg/ml concentration. The above process was repeated several times to obtain other dilutions: 50 mg/ml, 25 mg/ml and 12.5 mg/ml (Ofokansi et al., 2008; Ofokansi et al., 2012). Having obtained different concentrations of the extract, 0.5 ml of the extract of each concentration was aseptically transfer into 9 ml of sterile nutrient broth in test tube and allowed to diffuse for 30 minutes before adding 0.5 ml of the standardized bacterial inoculums. Incubation was done at 370C for 24 hours. The growth of the inoculums in the broth was indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organism were taken as the Minimum Inhibitory Concentration (MIC). Control was set up as nutrient broth and sterile plant extract.

Results and Discussion

(i) Percentage Yield

The percentage yield of the methanolic extract was calculated as follows:

Methanolic extract

Weight of the plant /Theoretical =276.852 g

Weight of empty bottle = 10.699 g

Weight of empty bottle + extract = 45.533 g

Weight of methanolic extract/ Experimental

= (45.533 – 10.699) g

= 34.834 g

$$\text{Percentage yield of methanolic extract} = \frac{\text{Experimental}}{\text{Theoretical}} \times 100\ \%$$

$$= \frac{34.834\ \text{g}}{276.852\ \text{g}} \times 100\ \%$$

= 12.58 %

The methanolic extract showed that the polarity of the solvent used has significantly influenced the quantity of the crude extract obtained from the plant that is, the more polar the solvent, the higher the extract to be obtained.

Table 2. shows the diameter of zones of inhibition of the methanolic leave extract of *Phyllanthus muellerianus* on the test organisms (bacterial isolates from Ileum of chicken).

Isolates	Zone of inhibition (mm) at different concentrations of crude methanolic extract				Gentamicin Control
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	10 mg/ml
Pseudomonas aeruginosa	18	15	13	9	37
Klebsiella pneumonia	19	17	15	12	36
Lactobacillus acidophilus	25	21	19	12	35
Enterobacter aerogenes	22	20	16	8	36
Bacillus subtilis	19	12	8	7	37
Salmonella paratyphi	13	12	11	10	35
Proteus mirabilis	22	20	16	13	37
Shigella sonnei	22	20	14	10	36
Lactobacillus casei	21	18	17	12	36
Escherichia coli	25	23	18	12	35

KEY: < 10 = Resistant; 10 – 15 = Moderately resistant; > 15 = Sensitive

(ii) Phytochemical Screening

Table 1. shows the summary of the phytochemical screening of the crude methanolic leave extract of *Phyllanthus muellerianus*.

Test	Inference
Anthraquinone	+
Carbohydrates	+
Tannins	+
Alkaloids	+
Flavonoids	+
Cardiac glycosides	+
Saponin	+
Reducing sugar	-
Lipids	+
Steroids	-
Coumarin	-

Table 1 shows the results of the phytochemical test on the leave extract that of *Phyllanthus muellerianus*. The chemical screening reveals the presence (+) of anthraquinone, carbohydrates, tannins, alkaloids, flavonoids, cardiac glycosides, saponin and lipids while reducing sugar, steroids and coumarin were absent (-). This result is consistent with the work of Olalekan et al., 2020 (on *Phyllanthus muellerianus*) for tannins, flavonoids, cardiac glycosides and saponins but different for anthraquinone, alkaloids and steroids. Cardiac glycosides which is present in this plant are often used to treat severe heart failure and atrial fibrillation that can occur with congenital heart defects (Newman *et al.*, 2008). Saponins present in this plant has several benefits which include; helping to inhibit the growth of cancer cells, serving as cholesterol lowering agent (Shi *et al.*, 2004). They also serve as an immune booster and as a sweetener. Flavonoids have been investigated and reported to be synthesized by plants in response to microbial infection and have been shown to have antibacterial activities (Kujumgiev *et al.*, 1999; Gorniak *et al.*, 2019). Flavonoids is also known to provide colour, texture and taste present in varieties of fruits and vegetables such as citrus fruits and juices, berries, apples, tea, red cabbage and various botanicals including *Phyllanthus muellerianus* plant which is known as a good source of nutrients and play preventive roles against diseases in the body (Pietta, 2000). The presence of tannins in this plant demonstrated its activity against bacteria (Akiyama *et al.*, 2001).

(iii) Anti-bacterial Activity

Table 3. shows the Minimum Inhibitory Concentration (MIC) of the methanolic leave extract of Phyllanthus muellerianus on the bacterial isolates.

Isolates	Concentration (mg/ml)
Pseudomonas aeruginosa	100
Klebsiella pneumonia	100
Lactobacillus acidophilus	25
Enterobacter aerogenes	50
Bacillus subtilis	100
Salmonella paratyphi	100
Proteus mirabilis	50
Shigella sonnei	50
Lactobacillus casei	25
Escherichia coli	25

Table 2 revealed that the crude extract shows moderate to high activity against all the selected bacteria. This supports the report that bacteria vary widely in the degree of their susceptibility (Emeruwa, 1982; Prescott *et al.*, 2008). The extract was found very active against most of the bacteria even at a very low minimum inhibitory concentration (MIC). For example, at 25 mg/ml the extract was active against *Escherichia coli, Lactobacillus acidophilus* and *Lactobacillus* while the extract was moderately active against other bacteria at higher MIC values (Table 3). This result supported the findings of Katsayal and Lamal (2009). Methanolic leave extract of *Phyllanthus muellerianus* has potential to be used as inhibiting agents against bacterial infections caused by bacteria because they exhibited high potency against all the test organisms.

Conclusion

Phyllanthus muellerianus plant revealed the presence of anthraquinone, carbohydrates, tannins, alkaloids, flavonoids, cardiac glycosides, saponin and lipids which helps to protect the body against diseases. Crude methanolic leave extract of *Phyllanthus muellerianus* has exhibited antibacterial activity against all the isolated bacterial organisms. This study therefore justifies the ethno-medicinal uses of *Phyllanthus muellerianus* for the treatment of infectious diseases caused by these organisms.

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