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Phytochemical screening to validate the ethno botanical importance of *Acalypha wilkesiana* leaves

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Abstract
The objective of the present study was to find out the presence of bioactive compounds in the ethanol and acetone extracts of *Acalypha wilkesiana* leaves, a common medicinal plants by both qualitative and quantitative methods, using standard procedures. The results of the preliminary phytochemical screening of the different extracts indicated the presence of alkaloids, saponnins, flavonoids, tannins, cardiac glycosides, phytosteroids, steroids and triterpenes. However, carbohydrates, anthraquinones and coumarins were not detected in any of the extracts. Quantitative estimation of the percentage crude yields of chemical constituents of the different plant extracts studied revealed that cardiac glycosides were the major phytochemical constituent present in highest percentage followed by tannins, while the lowest yield was recorded for saponins in both extracts. The presence of these phytochemical constituents in the different extracts of *Acalypha wilkesiana* studied validates the ethno pharmacological uses of this plant in the treatment of different illnesses.

Keywords: Bioactive compounds, *Acalypha wilkesiana*, quantitative estimation, phytochemical screening, cardiac glycosides

Introduction
In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [1]. According to [2], herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care. This is so because plants are endowed with the phytochemical compounds such as terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [3-5]. It is reported that secondary metabolites in plants contribute to flavour and colour [6]. Studies have shown that many of these bioactive compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [7-9]. An advantage of natural bioactive molecule is that they have a milder side effect on the body in comparison to chemically synthesized drugs [10]. The therapeutic actions of plants are unique to particular plant species or groups as the combinations of secondary metabolites in a particular plant are often taxonomically distinct [11-12]. It is important to know the composition of phytochemical constituents, thus knowing the type of biological activity which might be exhibited by the plant [13]. *Acalypha wilkesiana* is one of several medicinal plants used in Nigeria and it has various ethnobotanical uses.

Acalypha wilkesiana 'godseffiana' Muell Arg belongs to the family Euphorbiaceae. It is commonly known as copper leaf, Joseph coat, fire dragon and beef steak. It is found all over the world most especially in the tropics of Africa, America and Asia. It is native to Fiji and nearby islands in the South Pacific and its common in Mauritius. It is a popular outdoor plant that provides colour throughout the year, although it is also grown indoors as a container plant [14]. It is propagated by stem cuttings at any time of the year. Under ideal conditions, it grows as a spreading evergreen shrub with upright branches that tend to originate near the base and can get up to 3.1 m tall with a similar spread. It has leafs (12.7- 20.3 cm long) that are alternate, elliptic to oval, serrate and multicoloredans small inconspicuous flowers (10.2-20.3 cm) that hangs in catkin-like racemes beneath the foliage [15]. Phytochemical analysis of the ethanol leaf extracts of *A. wilkesiana* revealed a high presence of tannins and glycoside,

a moderate presence of saponin, flavonoids, Phylobatanins and glycosides (reducing sugar) and slight presence of alkaloids and cardiac glycosides [16]. *Acalypha wilkesiana* juice or boiled decoction was reported to be used in the treatment of gastrointestinal disorders, hypertension, malaria and fungal skin infection such as pityriasis versicolar, impetigo contagiosa, candida intetrigo, Tinea versicolor, Tinea corporis and Tinea pedis [17-18]. In Southern Nigeria, the leaves of this plant are eaten as vegetables in the management of hypertension [19]. The antioxidant, anti-plasmodial, hepatoprotective, antiemetic, antimicrobial, hematotoxic hyponatremic, antidiabetic, analgesic, anti-inflammatory, antipyretic and antiulcer activities of extracts of *A. wilkesiana* have been reported [20-27].

Materials and Methods

Collection and Extraction of Medicinal plants

Fresh leaves of *A. wilkesiana* were collected from Ufeh Street, Federal housing estate, Uyo, Akwa Ibom State. The plant was identified and authenticated by Mr Etefia, department of Pharmacognosy and Natural Medicine, University of Uyo. The leaves were thoroughly washed under running tap water to remove debris and the leaves were air dried under shade at room temperature for 14 days. The dried samples were pulverized to powder using electric blender and stored in polythene bag. Approximately 8kg of the powdered plant material was extracted by cold maceration method with ethanol and acetone and left for 72 hours with intermittent shaking. The plant extracts was filtered and then concentrated using rotary evaporator at 40 ^{o}C, and each extract was transferred into well labelled sterile glass vials and stored at 4 ^{o}C before use.

Qualitative Phytochemical Analysis of Plant Extract.

The leaf extract (ethanol and acetone) were screened for the presence of various phytoconstituents such as alkaloids, flavonoids, cardiac glycosides, carbohydrates, saponins, tannins, steroids, anthraquinones, phytosteroids, coumarins and triterpenes using the standard procedures as described by [28-30].

Test for Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Dragendorff's Test: 1ml of the filtrate was treated with few drops Dragendorff's reagent. Formation of orange brown precipitate indicated the presence of alkaloids.

Mayer s test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicated the presence of alkaloids.

Test for Tannins

Ferric chloride test

0.5g of each extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicated that the presence of tannins.

Test for saponins

Frothing test: About 0.5mg of each extract was shaken with five ml of distilled water. Formation and persistence of frothing showed the presence of saponins.

Test for cardiac glycosides

Salkowski's test: 0.5g of each extract was dissolved in 2 ml of chloroform. Concentrated H_2SO_4 (3ml) was carefully added down the side of the tube to form a layer. An appearance of reddish brown colour in the interface was indicated the presence of steroidal aglycone portion of cardiac glycosides.

Keller –Killiani test: 0.5g of each plant extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underplayed with 1 ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardenolites.

Test for anthraquinones

Borntragers test: About 0.5g of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicated the presence of anthraquinone.

Test for carbohydrates

Molisch's test: Take 0.5g of each extract was mixed with Molisch's reagent. Concentrated sulphuric acid was added along the side of the tubes to form layers. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour at the interface indicated the presence of carbohydrates in the sample extract.

Fehling's test: Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. A brick red coloured precipitate of cuprous oxide forms, if reducing sugars present

Test for Coumarins

1ml of 10% sodium hydroxide solution was added to 1ml of the plant extract. Formation of yellow colour indicated the presence of coumarins.

Test for steroids

To 1 ml of the leaf extract, 2 ml of chloroform and 1 ml of Sulphuric acid (H_2SO_4) were added. Formation of reddish brown ring at interface indicates the presence of steroids.

Test for phytosterols

Liebermann – Burchard's test

0.5g of extract was dissolved in 2ml of acetic anhydride and conc. H_2SO_4 was added slowly along the sides of the test tube. Formation of blue green colour indicated the presence of phytosteroids.

Test for flavonoids

Shinoda test: Few pieces of magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added to 5 ml of each extract. Formation of pink colour was taken as evidence for the presence of Flavanoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated that the presence of flavonoids.

Quantitative Estimation of Phytoconstituents

The phytochemicals which are present in the ethanol and acetone extract of *A. wilkesiana* was determined and quantified by standard procedures.

Determination of total alkaloids

5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

10g of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed.

Determination of Total saponins

20g of plant sample was 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 ºC. 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 ºC. The concentrate was transferred into a 250 ml separating 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 extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage.

Determination of total tannin content.

The sample extract (1 ml) was mixed with Folin-Ciocalteau's reagent (0.5 ml), followed by the addition of saturated Na_2CO_3 solution (1 ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 min at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm, using tannic acid solution as a standard.

Determination of total glycosides content.

For determination of cardiac glycosides, 10ml of each extract was mixed with 10 ml freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH) and allowed to stand for an hour. The mixture was diluted with 20 ml distilled water and stirred. Cardiac glycosides develop an orange –red colour with Baljet's reagent. The intensity of the colour obtained against blank at 495 nm was determined using a UV visible spectrophotometer. The difference between the test and the blank was taken for calculation.

Results and Discussion

Table 1: The percentage yield of different extracts of *A. wilkesiana* and colour of extracts

Solvents	Yield (%)	Colour
Ethanol	7.0	Dark Green
Acetone	9.0	Dark Green

Table 2: Results of preliminary qualitative phytochemical screening of leaf extracts of *A. wilkesiana*

S/N	Phytochemical test/ chemical constituent	Test	Observation of	Plant extracts
			Ethanol extract	Acetone extract
1	Alkaloids	Dragendorff's test Mayer's test	+++ +++	+++ +++
2	Saponnins	Froth test	+++	+++
3	Flavonoids	Shinoda test Lead acetate test	+++ +++	+++ +++
4	Carbohydrates	Molisch's test Fehling's test	- -	- -
5	Tannins	Ferric chloride test	+++	+++
6	Cardiac glycosides	Salkowski's test Keller –Killiani test	++ +	++ +
7	Anthraquinones	Borntragers test	-	-
8	Coumarins	NaOH test	-	-
9	Phytosteroids	Liebermann – Burchard's test	++	+
10	Steroids	-	+	+
11	Triterpenes	-	+	+

Table 3: Quantitative estimation of phytoconstituents results in *A. wilkesiana*

Extracts	Alkaloids (%)	Saponins (%)	Tannins (%)	Flavonoids (%)	Glycosides (%)
Acetone	15.16	1.51	42.48	30.16	56.76
Ethanol	8.32	4.01	27.18	38.17	43.82

Table 1 depicts the percentage yield of ethanol and acetone extracts. From the results obtained, acetone gave the highest yield. This is may be due to the fact that acetone dissolves many hydrophilic and lipophilic components [31].

Phytochemical screening is a preliminary step in the characterization of plant products. Preliminary screening for presence or absence of a particular class of compound is qualitative in nature and form a robust base for the quantitative estimation of bioactive components and qualitative separation of pharmacologically active chemical compounds [32-33].

Bioactive natural products have enormous economic importance as specialty chemicals as they can be used as drugs, lead compounds, biological or pharmaceutical tools, feedstock products excipients and nutraceuticals [34]. The ethanol and acetone extracts of *A. wilkesiana* were screened for the following secondary metabolites; Alkaloids, Saponnins, Flavonoids, Carbohydrates, Tannins, Cardiac

glycosides, Anthraquinones, Coumarins, Phytosteroids, Steroids and Triterpenes. All the extracts were subjected to further analytical tests for the quantification of phytochemical compounds and the results obtained are presented in Table 2 and 3 respectively. Qualitative estimation of bioactive compounds in the different leaf extracts of *A. wilkesiana* showed that the leaves were rich in alkaloids, saponnins, flavonoids, tannins, cardiac glycosides, phytosteroids, steroids and triterpenes. However, carbohydrates, anthraquinones and coumarins were not detected in any of the extracts. Among these compounds alkaloids, phenolic compounds, flavonoids, saponins and tannins are important secondary metabolites and are responsible principles for medicinal values of the respective plant [35]. The phytochemical content of *A. wilkesiana* in this study was found to be similar to that obtained by other authors [36, 21, 37, 25, 26, 16, 20]. Although the quantity of the bioactive compounds present differed, showing the different partitioning abilities of the different solvents used. Quantitative analysis was done to check the percentage of major bioactive compounds present in the different leaf extracts of *A. wilkesiana.* Results of the quantitative analysis revealed significant levels of phytochemical constituents present in the leaf as evident in the qualitative data. For both extracts, the results of the quantitative analysis followed this trend; cardiac glycosides > tannins > flavonoids > alkaloids > saponins.

Cardiac glycosides, also known as cardiotonic steroids, are a group of natural steroidal compounds that contain an unsaturated lactone ring in their structure and have the ability to inhibit the Na+/K+-ATPase pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscle, improves cardiac output and reduces distension of the heart. Some cardiac glycosides are used in cardiology for the treatment of cardiac congestion and some types of cardiac arrhythmias [38-41].

Anticancer effects of cardiac glycosides have been reported in several types of carcinoma, including breast cancer. Cardiac glycosides have been reported to induce apoptosis and disrupt numerous cellular processes vital to tumour growth such as inhibition of proliferation, angiogenesis, cell migration and invasion [42].

The abundance of tannins and flavonoids in the leaves of *A. wilkesiana* is also indicative of its potential antioxidant effect, which suggests that the plant may be very useful as an antibacterial, anti-inflammatory, antiallergic, antiviral, diuretics and astringent properties [43 – 46].

Conclusion

Phytochemical screening of the leaf part of *A. wilkesiana* revealed the presence of alkaloids, saponnins, flavonoids, tannins, cardiac glycosides, phytosteroids, steroids and triterpenes, which are important secondary metabolites. However, carbohydrates, anthraquinones and coumarins were not detected in any of the extracts. The phytochemical constituents of *A. wilkesiana* have validated its ethnopharmacological uses in the successful treatment of different illnesses.

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