



QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING OF *Acalypha wilkesiana* HOFFMANNI (GREEN ACALYPHA)



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Abstract: *Acalypha wilkesiana* Hoffmanni is a medicinal plant used in herbal clinical practice in Nigeria and this is due to the combined effects of the phytochemical components it contains. This study was carried out to determine the qualitative and quantitative phytochemical constituents according to prescribed standard methods. The extraction was done with soxhlet extractor using hexane and ethanol solvents respectively. The resulting crude extracts were concentrated in a rotary evaporator. The result indicated glycoside, terpenoid, alkaloid, saponin, steroid, phenolic and eugenol for hexane extract while glycoside, terpenoid, alkaloid, saponin, flavonoid, tannin and eugenol were present in the ethanol extract. The quantitative phytochemicals indicated total phenolic content of 0.44%, total saponin content of 0.50%, total flavonoids content of 0.91% total tannin content of 0.33% and alkaloids content 5.00% for hexane extract while the ethanol extract has total phenolic content of 1.14%, total saponin content of 0.58%, total flavonoids content of 1.01% total tannin content total of 0.45% and alkaloids content 8.33%. The phytochemicals present suggests that the plant contains bioactive chemical constituents useful for pharmacological formulation.

Keywords: *Acalypha wilkesiana*, phytochemicals, alkaloids, phenolics, flavonoids

Introduction

Medicinal plants have been used to treat infectious diseases for many years worldwide leading to a growing interest in the development of drugs of plant origin (Gupta *et al.*, 2010). Although hundreds of plant species have been screened for their phytochemical constituents and tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandin, *et al.*, 1985). *Acalypha wilkesiana*, (family-Euphorbiaceae) is an ornamental plant distributed all over the world especially in Africa and. It is commonly called beefsteak plant, copperleaf, fire dragon and copper leaf green (Aigbokhan, 2014). Its native name is "Aworoso" (Yoruba) in Nigeria and it is applied as ornamental and medicinal plant. It is propagated by stem cuttings throughout the year (both rainy and dry season) and it develops as evergreen shrub spreading with upright branches which originate near the base and grows up to 3.1 m tall (Atef, 2010). From the reports of Imaobong and Uwakmfon (2019), leaf extracts of *A. wilkesiana* revealed a high presence of tannins and glycoside, saponin, flavonoids, Phylobatanins and glycosides (reducing sugar) and slight presence of alkaloids and cardiac glycosides. While Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of *Acalypha wilkesiana*, while Adesina *et al.* (2000) reported gallic acid, collagen, geranin, quercetin and Kaempferol in the leaves.

Reported medicinal application of the plant indicated that the leaf extract has antibacterial and antimicrobial activity. The leaf decoction is used traditionally for the treatment of gastrointestinal disorders and fungal skin infection particularly *Impetigo contagiosa* and *Tinea versicolor* which affect the back, chest and axillae of many babies in Nigeria (Akinde, 1986). In recent times, there has been much push by the scientific community for the use and exploration of medicinal plants towards drug discovery for the treatment and management for various diseases. Hence, the present study is aimed at the preliminary investigation of qualitative and quantitative phytochemical screening of extract of *Acalypha wilkesiana*.

Materials and Methods

Collection of plant

The plant leaves were collected from Ugbowo community in Benin City, Edo state Nigeria and was identified by Prof. J. F. Bamidele, a taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

Extraction

With the use of soxhlet extractor apparatus, 100 g of the pulverised plants were first extracted using 600 ml of hexane solvent, after which the extract was collected and the residue was spread out on large filter papers and the solvents were allowed to dry off under a fume hood. After drying, it was weighed. 89 g of the residue was again extracted using 500ml ethanol solvent in a soxhlet extractor apparatus. Both crude extract were respectively concentrated in a rotary evaporator (Model RE, 200. USA).

Qualitative phytochemical screening of the extracts

Each extracts were tested for the presents of glycosides, steroids, terpenoids, alkaloids, saponins, flavonoids, tannins, phenolics and eugenols using standard procedures by Sofowora (1993); Trease and Evans (1989) and Odebiyi and Sofowora (1978).

Test for glycosides: 1 ml of each extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was backed with 1ml of conc.H₂SO₄. A brown ring indicated the presence of glycoside.

Test for saponins: ½ g of each extract was shaken with water in a test tube and observed for frothing. The standard used was Saponin rein weiss (supplied by Merck).

Test for flavonoids: 2 ml of each extract was boiled in 10ml of distilled water after which it was filtered. 10% Lead acetate solution was added in few drops. A yellowish precipitate indicated a positive result.

Test for phenolic compounds: 1 ml of the plant extract was added to 5 ml of 90% ethanol. In addition, one drop of 10% FeCl₃ was added. A pale yellow colouration indicated a positive result.

Test for tannins: 10ml of distilled water was added to 2ml of the extract and boiled for 5minutes after which it was filtered. Ferric chloride (FeCl₃) solution was added to about 2 drops of the filtrate; formation of a bluish precipitate is required for hydrolysable tannin.

Test for eugenols: 5 ml of 5% KOH solution was added to 2 ml of each extract and stirred together for proper mixing. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale yellow precipitate indicates a positive result.

Test for steroids: To 0.5 g of each extract, 2 ml of acetic anhydride was added in 2 ml of dilute H₂SO₄. A color change from violet to blue or green indicates the presence of steroids.

Test for terpenoids (Salkowski test): 5 ml of each extract was mixed in 2 ml of chloroform and 3 ml of conc. H₂SO₄ was added gradually down the side of the inner wall of the test tube to form a layer. A reddish brown coloration of the inter-phase is required for the presence of terpenoids.

Test for alkaloids: Picric acid was used to test for alkaloids. About 1ml each of the plant extracts was transferred into and 2 ml of picric acid is added. A yellowish precipitate is a positive test.

Quantitative phytochemical screening

Determination of total phenolic contents

The amount of total phenolics in each extracts were determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modification using tannic acid as a standard.

About 1.0 ml of extract solution (250 ug/ml) was added in a test tube. Then, 1.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 min, 15.0 ml Na₂CO₃ (20 %) was added and allowed to stand for 2 h. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer. The total phenolic content was determined as Ug of Gallic acid equivalent (GAE) using an equation obtained from the standard tannic acid calibration graph.

Determination of alkaloids content

The total alkaloid content was measured using the method described by Harborne (1973). 5 g of the extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 h. 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 precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analyzed in triplicates.

$$\text{Alkaloid (\%)} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Determination of flavonoid content

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) Ilahy *et al.* (2011). Thirty-microliter aliquots of the each extracts were used for flavonoid determination. Samples were diluted with 90 µl methanol, 6 µl of 10% Aluminum chloride (AlCl₃), 6 µl of 1 mol/l Sodium acetate (CH₃CO₂Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (Ug QE/g).

Determination of total saponins content

Estimation of total saponins content was determined by the method described by Makkar *et al.* (2007), based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) was added. Then 2.5 mL of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 570nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples. The values were expressed as PPM.

Determination of tannins content

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77 - 80°C for 1hour and shaken. The extract was quantitatively filtered using a double layered Whatman No. 1 filter paper and 20 mL of

distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na₂CO₃ were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbances as well as samples were read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

Statistical analysis

The statistical analysis was carried out using student's t-test. P values of less than 0.05 were considered to be statistically significant. And the results are presented as Mean±SEM.

Results and Discussion

The results of the qualitative and quantitative phytochemical screening are shown in Tables 1 and 2, respectively. The qualitative phytochemical indicated that glycoside, terpenoids, alkaloids, saponins and eugenols, steroids and phenolics were present in the hexane extracts while flavonoids, tannins, glycoside, terpenoids, alkaloids, saponins and eugenols were present in the ethanol extracts.

Table 1: Qualitative phytochemical constituent of *Acalypha wilkesiana* extracts

Chemical components	Hexane extract	Ethanol extract
Glycosides	+	+
Steroids	+	-
Terpenoids	+	+
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Phenolics	+	+
Eugenols	+	+

+ = Present- = Absent

Table 2: Quantitative phytochemical constituents of *Acalypha wilkesiana* extracts

Phytoconstituents (%)	Hexane extract (Mean ± SEM)	Ethanol extract (Mean ± SEM)
Total phenolic content	0.44±0.01	1.14±0.02
Total saponin content	0.50±0.00	0.58±0.00
Total flavonoids content	0.91±0.01	1.01±0.01
Total tannin content	0.33±0.03	0.45±0.02
Total alkaloids content	5.0±0.00	8.33±0.00

The quantitative phytochemical indicated that the hexane extract had total phenolic content of 0.44%, total saponin content of 0.50%, total flavonoids content of 0.91% total tannin content total of 0.33% and alkaloids content 5.00% while the ethanol extract contains total phenolic content of 1.14%, total saponin content of 0.58%, total flavonoids content of 1.01% total tannin content total of 0.45% and alkaloids content 8.33%. On comparison with ethanol extract in the work of Imaobong and Uwakmfon (2019), the qualitative estimation of bioactive compounds in the different leaf extracts of *A. wilkesiana* showed that the leaves were rich in alkaloids, saponins, flavonoids, tannins, cardiac glycosides, phytosteroids, steroids and triterpenes. Thus the result of qualitative phytochemical screening in this work was found to be similar. However, close value of 8.33 was obtained in this work when compared to 8.32 obtained by Imaobong and Uwakmfon (2019) for total alkaloids with respect to ethanol extract. The total saponin, total tannins,

total phenolics and total flavonoids were not similar. According to Thakur et.al, 2020, phytochemicals have great antioxidant benefits and the give immense health benefits to the consumers. For example, Alkaloids which is one of the major phytochemicals found in this plant have pharmacological effect and are used as medication and recreational drugs. They are mind altering thus the saying “potent brains are not strengthened by milk but by alkaloids” (Odesanmi et al., 2009). And Flavonoids are very important phytochemicals in plants because of their prominent role in pigmentation and protection from different external agents such as insects and mammals (Harborne et al., 2000).

Conclusion

The study has indicated the presence of important phytochemicals like alkaloids, saponins, flavonoids and phenolics which are useful pharmaceutical agents for drug formulation.

Conflict of Interest

The authors declare no conflict of interest.

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