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Chemical Characterization of Crude Phenolic Leaf Extract of *Acalypha wilkesiana*

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ABSTRACT

Acalypha wilkesiana is a widely eaten vegetable, reportedly used traditionally in the management of hypertension, diabetes and as an antimicrobial agent, probably because of its rich phenolic composition. The phytochemical and phenolic profiles of the crude leaf extract of the plant were assessed using standard methods, and gas chromatography coupled to mass spectrometry (GC-MS) and flame ionization detector (GC-FID). Qualitative phytochemical screening showed the presence of alkaloids, phenols, flavonoids, cardiac glycosides, tannin, steroids and saponins. GC-FID analysis revealed the presence of dihydrocytisine, ammodendrine, spartein, hydroxylupanine, ribalinidine, anthocyanin, flavone, flavonones, aphylidine, proanthocyanidin, isolupanine, narigenin, sapogenin, cardiac glycoside, tannin, and cyanogenic glycoside in varying proportions. GC-MS analysis identified 14 constituents with major ones being 2,6-Octadienal, 3,7-dimethyl-, (Z) Citral (14.88 %), citral 2,6-Octadienal, 3,7-dimethyl-, (E) (16.9 %), cis-3-Hexenyl cis-3-hexenoate (10.59 %), trans-2,7-Dimethyl-4,6-octadien-2-ol (20.56 %) and 3,8,11-Trioxatetracyclo [4.4.1.0(2,4).0(7,9)] undecane (11.69 %). This study has shown that crude phenolic leaf extract of *A. wilkesiana* contains pharmacologically active compounds which support its traditional use in the treatment of oxidative stress related diseases and hence a potential candidate for drug discovery.

Keywords: *Acalypha wilkesiana*, phytochemicals, gas chromatography-mass spectrometry, Secondary metabolites.

1. INTRODUCTION

Medicinal plants have made great contributions to human health, which accorded plants as potential sources of novel drug compounds. They have been used as common agents for the treatment, management and prevention of many diseases in traditional setting, especially in Africa (Nwiloh *et al.*,2016). About 80% of the rural population in the developing world relies on traditional medicines for their health care (Igwe *et al.*,2021). This potential of plants have been ascribed to their rich content of a wide variety of secondary metabolites, such as tannins, saponins, alkaloids, flavonoids, etc (Nwiloh *et al.*,2016). Though, the presence of phytochemical compounds in medicinal plants has been reported in many literatures, yet advancement in scientific techniques gives room for more detailed profiling of these plants' chemical compositions.

Phytochemicals are grouped into several classes according to their chemical structure and biological activity. They have been widely applied for their medicinal properties as pure compounds, fruit and vegetable crude extracts. Phytochemicals such as flavonoids, phenolics, terpenoids, and

alkaloids have been investigated for their beneficial effect in various disease conditions (Boots *et al*., 2008).

Acalypha wilkesiana belongs to the family, Euphorbiaceae (spurge family). Its other names include *A. amentaceae* and *A. tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, beef steak plant and match-me-if-you-can (Ikewuchi *et al.,* 2010). The Hausas of Northern Nigeria call it "Jinwinini", while the Igbos and Yorubas of Southern Nigeria call it "Jiwene" and "Aworoso" respectively (Sofowora, 2013). Traditionally, in the Eastern Nigeria, parts of the plant have been reportedly used in the treatment of gastrointestinal disorders, fungal skin infections such as *Pityriasis versicolar*, *Impetigo contagiosa*, *Candida intetrigo*, *Tinea versicolor, Tinea corporis* and *Tinea pedis* and in the management of hypertension as well as diabetes (Ikewuchi *et al.,* 2008).

Understanding the chemical compositions of medicinal plants is important in drug discovery and design. The present study is aimed at quantitation of phytochemicals, and characterization of the bioactive compounds of crude phenolic leaf extract of *A. wilkesiana* by gas chromatography coupled with mass spectrometry (GC-MS) and (GC-FID) analyses. The study will elucidate the phyto-active compounds present in this plant that could be responsible for its numerous reported medicinal properties.

2. MATERIALS AND METHODS.

2.1 Plant materials:

Fresh leaves of *Acalypha wilkesiana* were obtained from Akabo in Ikeduru Local Government Area of Imo State, Nigeria. They were identified and authenticated by Prof. F.N. Mbagwu, a plant taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State Nigeria and deposited in the institution's herbarium with voucher number: IMSUH 286.

2.2 Preparation of extract:

Crude phenolic extract was prepared using soxhlet extraction method as described by Al-Owaisi *et al.* (2014) with little modifications. The fresh leaves of the plant were dried at room temperature (25^0C) and ground to a fine powder in a commercial mill. The powder (500g) was extracted with 1.5 L of 80% ethanol using a soxhlet extractor at 90 ^{0}C for 16 hours. The ethanol extract was partitioned between ethyl acetate and water, and the ethyl acetate soluble component containing the crude phenolic extract was recovered by distillation under reduced pressure at 49^oC in a Buchi rotavapour (Switzerland). The recovered extract was dried to solid form in vacuum desiccator, and stored in a freezer ($\leq 4^0$C) until needed.

2.3 Phytochemical analysis

2.3.1 Qualitative screening

Qualitative phytochemical screening of crude phenolic leaf extract of *A. wilkesiana* was carried out using standard methods (Trease and Evans, 1989; Odebiyi and Sofowora, 1978).

i. Test for alkaloids: One gram (1 g) of the extract was dissolved in 5ml of 10% ammonia solution and extracted with 15ml of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 ml of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests.

Mayer's reagent (Bertrand's reagent): Drops of Mayer's reagent was added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids.

Dragendorff's reagent: Two millilitres (2ml) of acidic solution in the second test-tube were neutralized with 10% ammonia solution. Dragendorff's reagent was added

and turbidity or precipitate was observed as indicative of presence of alkaloids.

ii. Test for the presence of phenols: In this test, 2ml of distilled water was added to 1g of sample and then some drops of 10% ferric chloride added. Formation of blue or green colour confirmed the presence of phenols.

iii. Test for flavonoids (Shibita's reaction test): One gram (1g) of the extract was dissolved in methanol (50%; 1-2ml) by heating, then metal magnesium and 5 - 6 drops of concentrated HCl were added. The solution when red was indicative of flavonols and orange for flavones.

iv. Test for cardiac glycosides: To 2 ml of the extract was added 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5ml of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride. This was transferred to the surface of 2ml concentrated sulph**uric acid** in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown coloration, respectively, which indicate the presence of cardiac glycosides.

v. Test for tannins (ferric chloride test): To 2ml of the aqueous solution of the extract was added a few drops of 10 % ferric chloride solution (light yellow). The occurrence of blackish blue colour showed the presence of gallic tannins and a green-blackish colour indicated presence of catechol tannins.

vi. Test for steroids (Salkowski's test): Half gram (0.5g) of the extract was dissolved in 10ml anhydrous chloroform and filtered, and concentrated sulphuric acid was carefully added so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

vii. Test for saponins (frothing test): Three millilitres (3ml) of the aqueous solution of the extract were mixed with 10ml of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5min. It was allowed to stand for 30min and observed for honeycomb froth, which was indicative of the presence of saponins.

2.3.2 Quantitative phytochemical analysis using GC-FID.

The crude phenolic extract was solubilised in 1000μl of pyridine and 200μl was transferred to a vial for analysis. The quantitation was according to the method of Ichihara and Fukubayashi (2010). The GC-FID phytochemical analysis was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280^{o}C with splitless injection of 2μl of sample and a linear velocity of 30cms^{-1}, Helium 5.0pa.s was the carrier gas with a flow rate of 40 ml min^{-1}. The oven operated initially at 200^{0}C. It was heated to 330^{0}C at a rate of 3^{0}C min^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320^{0}C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in μg/g.

2.4 Characterization of chemical compounds using GC-MS

Crude phenolic leaf extract of *A. wilkesiana* was analyzed with the help of GC-MS analyzer (GC Clarius 500 Perkin Elmer) as described by Selvamani and Balamurugan, (2015).

The carrier helium gas(99.999%) was used at flow rate of 1 ml per min in split mode (10: 1). The sample (8 µl) was injected to

column at 250 ºC injector temperature. Temperature of oven started at 80 ºC and was held for 2min and then was raised at rate of 10 ºC per min to 200 ºC without holding. Holding was allowed for 9 min at 280 ºC at program rate of 5 ºC per min. Temperature of ion source was maintained at 200ºC. The injector temperature was set at 230 ºC and detector temperature was set at 260 ºC. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operated in scan mode from 45 to 450 Da atomic mass units. A 0.5 seconds of scan interval and fragments from 45 to 450 Da was maintained. Total running time was 40 minutes.

The compounds in the GC-MS chromatogram of phenolic extract analysed were identified using mass spectrometry. The interpretation of mass spectra peaks of detected unknown compounds was done by matching with database of known component stored in database of National Institute of Standard and Technology (NIST). Major components were identified with authentic standards obtained from computerized libraries. The compound name, molecular formula, peak area, retention time and mass/charge ratio were ascertained. The relative percentage amount of each component was determined by matching its average peak area to the total area.

3. RESULTS.

3.1 Phytochemical screening of crude phenolic leaf extract of *A. wilkesiana*.

Result of qualitative phytochemical screening of crude phenolic leaf extract of *A. wilkesiana* revealed the presence of alkaloids, phenols, flavonoids and steroids. Other phytochemicals of interest present are the anti-nutrients cardiac glycoside, tannins and saponins (Table 1).

Table 1. Phytochemicals of crude phenolic leaf extract of *A. wilkesiana*

Phytochemicals	Result
Alkaloids	+
Phenols	+
Flavonoids	+
Cardiac Glycosides	+
Tannins	+
Steroids	+
Saponins	+

Key: + Present, - Absent

3.2 Phytochemical composition of crude phenolic leaf extract of *A. wilkesiana*.

Gas chromatography fitted with flame ionization detector (GC-FID) analysis of phytochemical content revealed the presence of dihydrocytisine (5.02 µg/ml), ammodendrine (6.17 µg/ml), spartein (9.56 µg/ml), hydroxylupanine (18.36µg/ml), ribalinidine (21.69µg/ml), anthocyanin (8.15µg/ml), flavone (9.70µg/ml), flavonones (6.30µg/ml), aphylidine (19.51µg/ml), proanthocyanidin (10.48µg/ml), isolupanine (2.42 µg/ml), and narigenin (4.56 µg/ml). Antinutrients present includes sapogenin (9.56 µg/ml), cardiac glycoside (1.58 µg/ml), tannin (3.54µg/ml) and cyanogenic glycoside(3.95µg/ml). Ribalinidine, hydroxylupanine and proanthocyanidin were the most abundant phytochemicals (Table 2).

Table 2: Phytochemical composition of crude phenolic leaf extract of *A. wilkesiana*.

Phytochemical components	Concentration (µg/ml)	Compound class	Biological activity
Dihydrocytisine	5.02	Alkaloids	Antimicrobial (Ezekiel *et al.*,2009), antiparasitic (Fernandez *et al.*,2010; Martinez-Peinado *et al.*,2020), antiplasmodial (Amlabu *et al.*,2018), antioxidative (Anokwuru *et al.*,2015) and insecticidal activities (Adeniyi *et al.*,2010; Oni *et al.*,2019).
Ammodendrine	6.17		
Spartein	9.56		
Hydroxylupanine	18.36		
Ribalinidine	21.69		
Aphylidine	19.51		
Isolupanine	2.42		
Anthocyanin	8.15	Flavonoids	Free radical scavenging (Alisi *et al.*,2014; Asiwe *et al.*,2018), antimalarial, anti-inflammatory, antioxidant, and antimicrobial (Serkedjieva and Ivancheva, 1999) agents, tumor suppressing and hepatoprotective effect (Knekt *et al.*,1997; Cox *et al.*,2000; Emejulu *et al.*,2014; Nwaoguikpe *et al.*, 2015).
Flavones	9.70		
Flavonones	6.30		
Proanthocyanidin	10.48		
Narigenin	4.56		

Table 2 To Be Continued…			
Tannins	3.54	Anti-nutrients	Exhibits antibacterial, antioxidants, antimicrobial, anti-inflammatory, antitumor, antivirus, antidiarrheal, antihaemorrhoid, and antimalarial activities (Buzzini *et al.*, 2008).Inhibition of enzyme activities, ability to bind to proteins and shrink them (Awulachew, 2022). Inhibiting the Na^+/K^+ pump, used in the treatment of congestive heart failure and cardiac arrhythmia, chelating agent (Egbuna and Ifemeje, 2015).
Cyanogenic glycoside	3.95		
Cardiac glycoside	1.58		
Sapogenin	9.56		

3.3 Chemical characterization of crude phenolic leaf extract of *A. wilkesiana*

In GC-MS analysis of crude phenolic leaf extract of *A. wilkesiana*, 14 constituents were identified using mass spectrometry (Table 3). The mass spectra peaks of the compounds were matched with those found in the National Institute of Standard and Technology (NIST) spectral database. The major constituents in the crude phenolic extract were quantified as 2,6-Octadienal, 3,7-dimethyl-, (Z) Citral (14.88 %), citral 2,6-Octadienal, 3,7-dimethyl-, (E) (16.9 %), cis-3-Hexenyl cis-3-hexenoate (10.59%), trans-2,7-Dimethyl-4,6-octadien-2-ol(20.56 %) and 3,8,11-Trioxatetracyclo [4.4.1.0(2,4).0(7,9)] undecane, (11.69 %).

Table 3: Chemical compounds in crude phenolic leaf extract of *A. wilkesiana*.

Peak No.	Retention time (RT)	Area (%)	Library/ID	Molecular Formula
1	7.067	14.88	2,6-Octadienal, 3,7-dimethyl-, (Z) Citral	$C_{10}H_{16}O$
2	7.691	16.9	Citral 2,6-Octadienal, 3,7-dimethyl-, (E)	$C_{10}H_{16}O$
3	9.103	10.59	cis-3-Hexenyl cis-3-hexenoate-	$C_{12}H_{20}O_2$
4	9.767	20.56	Trans-2,7-Dimethyl-4,6-octadien-2-ol	$C_{10}H_{18}O$
5	11.693	11.693	3,8,11-Trioxatetracyclo[4.4.1.0(2,4)- 0(7,9)]undecane,(1.alpha.,2.alpha.,4.alpha.,6. alpha.,7.beta.,9.beta)-	$C_8H_{10}O_3$
6	12.544	3.56	3-Hexene, 2-methyl-, (Z)-	C_7H_{14}
7	16.982	3.7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$
8	17.631	5.44	n-Hexadecanoic acid	$C_{16}H_{32}O$
9	18.809	4.97	cis-Vaccenic acid	$C_{18}H_{34}O_2$
10	18.972	2.81	6,11-Dimethyl-2,6,10-dodecatrien-1-o1	$C_{14}H_{24}O$
11	19.041	2.49	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{25}H_{46}O_2$
12	19.431	7.72	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$
13	19.605	2.36	Oleic acid	$C_{18}H_{34}O_2$
14	20.087	1.09	Citronellol	$C_{10}H_{20}O$

4. DISCUSSION

Phytochemicals found in fruits and vegetables are generally known for having protective health benefits to man and animals (Webb, 2013).Plant phytochemicals such as alkaloids have been reported to contribute multiple biological activities ranging from antimicrobial (Ezekiel *et al.*,2009), antiparasitic (Fernandez *et al.*,2010; Martinez-Peinado *et al.*,2020), antiplasmodial (Amlabu *et al.*,2018), antioxidative (Anokwuru*et al.*,2015) and insecticidal (Adeniyi *et al.*,2010; Oni *et al.*,2019) activities. Phenols are commonly found in plants and have diverse physiological functions, including anti-inflammatory, antioxidant and antimalarial activities (Ovenden *et al.*,2011).

Flavonoids, a large family of polyphenolic compounds having a wide spread occurrence in the plant kingdom are known free radical scavengers (Alisi *et al.*,2014; Asiwe *et al.*,2018; Igwe *et al.*, 2021), antimalarial, anti-inflammatory, antioxidant (Liu, 2013) and antimicrobial (Serkedjieva and Ivancheva, 1999) agents, and have been explored for tumour suppressing and hepatoprotective effect (Knekt *et al.*,1997; Cox *et al.*,2000; Emejulu *et al.*,2014; Nwaoguipke *et al.*,2015). Antiplatelet activity of red wine and grape juice reported in human and animal systems has been associated with synergy of flavonoids including anthocyanins (Asiwe *et al.*,2021). Tannins, which are polyphenols, are important for their physiological potentials. They have been reported to exhibit antibacterial, antioxidants, antimicrobial, anti-inflammatory, antitumor, antivirus, antidiarrheal, antihaemorrhoid, and

antimalarial activities (Buzzini *et al.*,2008). Saponins, from recent evidence seem to possess hypocholesterolemic, immunostimulatory and anticarcinogenic properties. In addition, they reduce the risk of heart diseases in humans (Gemede and Ratta, 2014).

The GC-MS profile of crude phenolic leaf extract of *A. wilkesiana* revealed the presence of a number of secondary metabolites that have therapeutic properties, ranging from antibacterial, antifungal, antiseptic, anthelmintic, anti-inflammatory, antihemolytic, anticancer, antioxidant, antiparasitic, antidiabetic, to wound-healing activities (Nitha *et al*.,2012). The quantified compounds in the crude phenolic extract with higher percentages in peak areas were trans-2,7-dimethyl-4,6-octadien-2-ol (20.56%), Citral 2,6-octadienal, 3,7-dimethyl- (E)(16.9%), 2,6-octadienal, 3,7-dimethyl-(Z) Citral (14.88 %), 3,8,11-Trioxatetracyclo[4.4.1.0(2,4).0(7,9)] undecane (11.69 %), cis-3-Hexenyl cis-3-hexenoate (10.59 %), 9,17-octadecadienal, (Z) (7.72 %) and n-hexadecanoic acid (5.44%).These compounds have previously been reported to have medicinal properties. The saturated fatty acid, hexadecanoic acid, has a wide range of activity, such as anticancer, antimicrobial, antioxidant, and antihaemolytic activities (Agoramoorthy *et al*.,2007; Wei *et al.,* 2011). Terpenes such as 2,6-octadienal, 3,7-dimethyl-(Z) exhibit antimicrobial activity, while monoterpenes and sesquiterpenes are active against bacteria and fungi (Yang *et al*.,2010).

5. CONCLUSION

The results of the present study on the phytochemical characterization of crude phenolic leaf extract of A. wilkesiana showed that the plant extract contained some phytoconstituents which are pharmacologically important. This plant represents a potential source of lead molecules with pharmacological activities for the development of new novel pharmaceutical products for treatment of diseases. Furthermore, appreciable presence of the identified compounds with biological activities justifies the traditional use of leaves of A. wilkesiana for the treatment of hypertension, diabetes, among others oxidative stress associated diseases. However, further studies into the isolation and identification of the individual bioactive compounds responsible for its therapeutic activity and the elucidation of their mechanism(s) of action are needed.

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