JOURNAL OF BIOMEDICAL RESEARCH & ENVIRONMENTAL SCIENCES
issn: 2766-2276
SUBJECT AREA(S): BIOMEDICINE | BIOLOGY
DOI: 10.37871

RESEARCH ARTICLE

Phytochemical Evaluation and Acute Toxicity Study of Ethanol Leaf Extract of *Acalypha wilkesiana*

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ABSTRACT

Increased curiosity on natural plant products has been raised due to problems of cost, unavailability, and after-effects of countless synthetic drugs. Worrisome, many plant-derived formulations lack phytochemically or toxicological screening. Hence, this study phytochemical and elemental screened the ethanolic leaf extract of *Acalypha wilkesiana* and as well as determining acute toxicity in adult male Wistar rats. The leaves were obtained in Benin City, Nigeria. Ethanol extraction was carried out on leaves and the extract was subjected to proximate, qualitative, and quantitative phytochemical screening and elemental analysis. Acute toxicity was determined on 12 adult male Wistar rats following Lork's method. Proximate analysis revealed a high presence of carbohydrate, ash, fiber, and moisture. The qualitative and quantitative evaluation showed the abundance of alkaloids (68.7 ± 0.120%), flavonoids (34.7 ± 0.001%) and minute (<1mg/g) saponins, tannins, phenol, and terpenes. The extract contain nutritive (vitamin E = 1.184 ± 0.055µg/g; vitamin A = 0.0066 ± 0.003µg/g; vitamin C = 0.046 ± 0.037µg/g) and anti-nutritive (oxalates = 229.780 ± 16.93mg/100g; cyanide=0.162 ± 0.006 mg/100g; phytate = 0.131 ± 0.01mg/100g) elements. The elemental evaluation showed an abundance of potassium, sodium, and chloride with traces of cadmium and lead and the absence of manganese and copper. There was no sign of acute toxicity or mortality at an extract dose of 5000mg/kg. These findings indicate the ethanol leaf extract of *A. wilkesiana* as a rich source of phytochemicals and major macro elements and high safety at 5000mg/kg dose. Considering the several components in the leaves extract, *Acalypha wilkesiana* leaf might be pharmacological significant for the biological system.

BACKGROUND

Herbal medicine or botanical medicine or phytomedicine sometimes referred to as herbalism which is the use of herbs for their therapeutic or medicinal values; refers to using plants seeds, flowers, roots for the medicinal purpose [1]. It is the oldest form of health care known to mankind. About 80% of the world population particularly in developing countries depend on herbal medicines for primary health care because of better cultural acceptability, safety, efficacy, potent, inexpensive, and lesser side effects [2]. It is now frequently been considered that plant drugs are less toxic compared with synthetic drugs [3]. In most of the world today medicinal plants are increasingly used as contraceptives, abortifacients, emmenagogues or oxytocic [4], antihypertensive [5], hypoglycemic [6-8], hypolipidemic [7], antibacterial and antifungal [9-11]), treatment of skin diseases [12], One of such plant is *Acalypha wilkesiana*.

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DOI: 10.37871/jbres1302

Submitted: 23 August 2021

Accepted: 27 August 2021

Published: 30 August 2021

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OPEN ACCESS

Keywords

- Plant-derived medicines
- *Acalypha wilkesiana* leaf
- Proximate analysis
- Phytochemical screening
- Acute toxicity

BIOLOGY GROUP

BIOMEDICINE | BIOLOGY

VOLUME: 2 ISSUE: 8

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How to cite this article: Iyamu AO, Akpamu U, Iyamu KU. Phytochemical Evaluation and Acute Toxicity Study of Ethanol Leaf Extract of *Acalypha wilkesiana*. J Biomed Res Environ Sci. 2021 Aug 30; 2(8): 715-720. doi: 10.37871/jbres1302, Article ID: JBRES1302, Available at: https://www.jelsciences.com/articles/jbres1302.pdf

Acalypha wilkesiana (A. wilkesiana) (other names include *A. amentacea* and *A. tricolor*) with common names like "copperleaf", "Joseph's coat", "fire dragon", "match me if you can" belongs to the family *Euphorbiaceae* (spurge family) [13,14]. The Yorubas of Southern Nigeria calls it "aworoso" while the Hausas of Northern Nigeria call it "Jinwinini" [15]. According to Al-Attar [14], the plant is native to Fiji and nearby islands in the South Pacificans. It is a popular outdoor and indoor spreading evergreen shrub with branches that tend to originate near the base and propagated by stem cuttings at any time of the year. It grows up to 3.1 m tall, with leaves (12.7-20.3 cm long) that are alternate, elliptic to oval, serrate and multi-coloradans small inconspicuous flowers (10.2-20.3 cm) that hangs in catkin-like racemes beneath the foliage [14].

A. wilkesiana is used in the traditional medicines for the treatment of bacterial and fungal skin infections, neonatal jaundice, and gastrointestinal disorders [16] and as antimicrobial [17], antihypertension [18], and anticarcinogenic properties [19] as well as exhibit antiparasitic and analgesic properties [20]. The methanolic leaves extract of *A. wilkesiana* has been documented to exhibit hypoglycemic activities and positively affected the hematopoietic system, the integrity of liver and kidney functions, and improved the lipid profile [21].

With the discovery that most plant extracts and products have significant antioxidant activity their significance in the treatment of several diseases is now been investigated. For this reason, Scartezzini and Speroni, documented that herbal plants are now considered useful means for the prevention, and amelioration of many disorders and complications [21,22]. Although ethnobotanical information indicates the use of over ten of thousand plants and plant products and formula as traditional remedies for the treatment of several diseases and Al-Attar [11] reported that many of these plants do not have scientific scrutiny. Not much pharmacological research has been carried out on *A. wilkesiana* despite its importance in traditional medicine [23]. Therefore, this study aims to evaluate the phytochemical and elemental component of the ethanolic leaves extract of *Acalypha wilkesiana* and determine the acute toxicity in adult male Wistar rats.

MATERIALS AND METHODS

Collection, processing, and extraction of plant materials

Samples of *Acalypha wilkesiana* leaves were collected from Benin City in Edo State, Nigeria, and authenticated at the Herbarium Unit of Forestry Research Institute, Ibadan, Oyo State, Nigeria. The leaves were plucked, sorted, air-dried for 7 days, and then pulverized and packaged in polyethylene airtight bags. 200g of the powder leaf was added into a container containing 1.5L of 70% ethanol and used to prepare the ethanolic extract as described in Majekodunmi and Nubani with few modifications [24].

Extract screening

Proximate analysis was carried out using the method and procedure described in the Association of Official Analytical Chemists [25]. Qualitative and quantitative phytochemical screening was done using the method of Harborne [26] and Trease and Evans [27], to identify active constituents and simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, sterols, phenols, glycosides and reducing sugars. Anti-nutrients constituents were determined following standard methods. The phytate content was as described by Lucas and Markaka [28], oxalate as described by Munro [29], and hydrogen cyanide and haemagglutinin as in Boyle and Shepiengh [30]. Elemental contents were determined by atomic absorption spectrometry, flame photometry, and spectrophotometry according to the method of AOAC [25].

Experimental animals

Twelve adult male Wistar rats with an average weight of 145-160g were obtained from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma. The rats were housed at room temperature on a 12-hour dark-light cycle and acclimatized for 14 days with *ad libitum* access to food (rat chow; Vital Feed Nig. Ltd, Jos, Nigeria) and water.

Acute toxicity study

Oral acute toxicity (LD_{50}) study was carried out as the method described by Lorke with few modifications in two stages [31]:

- **Stage I:** Nine rats were divided into 3 groups (A1, B1, and C1) of 3 rats each. Groups A1, B1, and C1 received 10, 100 and 1000 mg/kg body weight of ethanolic leaves extract of *A. wilkesiana* respectively. With no death recorded, the experiment proceeded to state two.

- **Stage II:** Three rats were divided into three groups (A2, B2, and C2) and treated as followed; Groups A2, B2, and C2 received 1500, 3000 and 5000 mg/kg body weight of ethanolic leaves extract of *A. wilkesiana* respectively. In each stage, the animals were observed hourly for the 1st 6 hours and then 6 hourly for the next 18 hours for changes in physical and behavioral signs and mortality.

Analysis

The obtained data were analyzed for Mean ± SD and presented in suitable tables and figures.

RESULTS

Phytochemical evaluations

Figure 1 presents the proximate analysis of the leaf extract of *A. wilkesiana* to contains abundant carbohydrates, ash, crude fiber, and moisture, and little crude fat and protein. Qualitative and quantitative phytochemical

screenings of leaf extract of *A. wilkesiana* are presented in tables 1,2 respectively. There were the presence of alkaloids, flavonoids, tannins, phenol, terpenoids, saponins, and glycosides as well as reducing sugar (Table 1) and abundance of alkaloids (68.7 ± 0.120%), and flavonoids (34.7 ± 0.001%) while saponins and tannins were between 1 and 0.5 mg/100g with a little number of terpenes, steroids, and glycosides (Table 2).

Nutritive and anti-nutritive evaluation

The nutritive and anti-nutritive components are shown in table 3. Nutritive components were more of vitamin E (1.184 ± 0.055 µg/g) and low levels of vitamin A (0.0066 ± 0.003 µg/g) and C (0.046 ± 0.037 µg/g). Anti-nutritive analysis showed the presence of oxalates (229.780 ± 16.93 mg/100g), cyanide (0.162 ± 0.006 mg/100g ± SD) and phytate (0.131 ± 0.01 mg/100g ± SD).

Elemental composition evaluation

The elemental composition of the Leaf extract of *A. wilkesiana* is presented in table 4 and showed the abundance of potassium (406.08 mg/kg), sodium (118.06 mg/kg), and Chloride (41.18 mg/L) with a trace amount of magnesium (7.29 ppm) and zinc (0.5541ppm) but absent of manganese and copper. There were traces of cadmium (4.67ppm) and lead (0.25ppm) in the extract of *A. wilkesiana*.

Acute toxicity study

Table 5 shows the acute toxicity (LD_{50}) of the ethanolic leaf extract of *A. Wilkesiana* in adult male Wistar rats. All the oral doses of the extract of *A. wilkesiana* administered showed no signs of toxicity and no death occur at the highest dose of 5000 mg/kg body weight. All the rats displayed normal behavioral, neurological, autonomic, and physical signs.

DISCUSSION

The study of the chemical constituents and the active principles of medicinal plants have acquired a lot of importance all over the world (1). The proximate analysis of the leaf extract of *A. wilkesiana* in the present study revealed the extract to be carbohydrate, ash, crude fiber, and moisture-rich but diminutive in crude fat and protein. There was the presence of alkaloids, flavonoids, tannins, phenol, terpineol, saponins, glycosides, and reducing sugar in the qualitative phytochemical screening of the extract while quantitative analysis revealed the abundance of alkaloids, flavonoids with a small number of saponins and tannins, and traces of terpenes, steroids, and glycosides. In line with

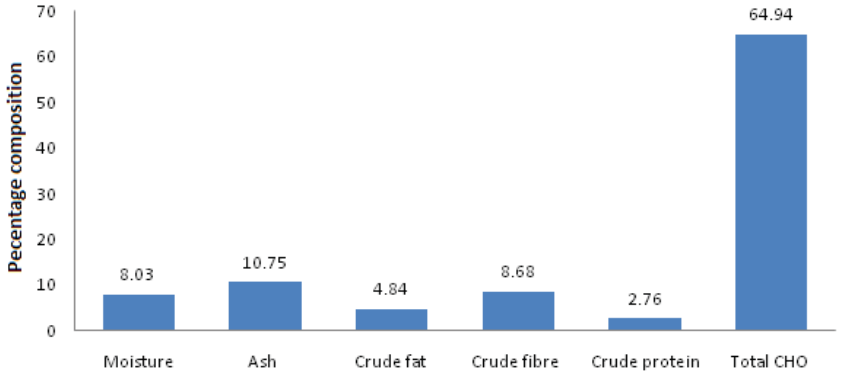


Figure 1 The proximate analysis of the ethanolic leaves extracts of *A. wilkesiana* (CHO = carbohydrate).

Table 1: Qualitative analysis of the phytochemical compositions of the ethanolic leaves extract of *A. wilkesiana*.

S/N	Phytochemicals	Bioavailability
1	Steroids	+
2	Terpenoids	+
3	Saponins	+
4	Flavonoids	++
5	Glycosides	+
7	Alkaloids	+++
8	Total phenolics	++
9	Total tannins	++
10	Reducing sugar	+

Key: + = low, ++ = moderate and +++ = high

Table 2: Quantitative compositions of the phytochemical analysis of the ethanolic leaves extract of *A. wilkesiana* (Values are amount ± standard deviation).

S/N	Phytochemical	Quantitative value
1	Alkaloids (%)	68.7 ± 0.120
2	Saponins (mg/100g)	0.864 ± 0.016
3	Flavonoids (%)	34.7 ± 0.001
4	Total tannins (mg/100g)	0.562 ± 0.023
5	Terpenes (µg/g)	0.221 ± 0.147
6	Steroids (µg/mg)	0.27 ± 0.021
7	Total glycosides (g%)	0.391 ± 0.015
8	Reducing sugar (g%)	0.672 ± 0.083

Table 3: Nutritive and anti-nutritive components of the ethanolic leaves extract of A. wilkesiana (Values are amount ± standard deviation).

Vitamin type	Content (µg/g)
Vitamin A	0.0066 ± 0.003
Vitamin C	0.046 ± 0.037
Vitamin E	1.184 ± 0.055
Antinutrient	Concentration in extract (mg/100g)
Phytate	0.131 ± 0.01
Oxalate	229.780 ± 16.93
Cyanide	0.162 ± 0.006
N = 3	

Table 4: Elemental compositions of the ethanolic leaves extract of *A. wilkesiana* (Values are mean ± SD).

S/N	Element	Concentration
1	Sodium	118.06 (mg/kg)
2	Potassium	406.08 (mg/kg)
3	Chloride	41.18 (mg/L)
4	Magnesium	7.29 (ppm)
5	Manganese	Nil
6	Copper	Nil
7	Zinc	0.5541 (ppm)
8	Cadmium	4.67 (ppm)
9	Lead	0.25 (ppm)

these findings, Odoh, et al. [32] have reported alkaloids and terpineol to be abundant with the lowest glycosides in the methanolic root extract of *A. wilkesiana*. El-Khateeb, et al. [21] has also documented methanolic *A. wilkesiana* leaves extract to reveal the presence of alkaloids, flavonoids, saponins, and tannins, and Ikewuchi, et al. [15] has obtained similar phytoconstituents from the leaves of *A. wilkesiana*. However, Fonkoua, et al. [33] reported aqueous and hydroethanolic extracts of *A. wilkesiana* leaves to be rich in bioactive compounds like phenols, tannins, and flavonoids. These variations in phytochemicals in *A. wilkesiana* extracts may be due to variation in the location where they were obtained, solvent and method of extractions, as well as arts of the plant used.

Also shown in the present study is the rich elemental composition of the leaf extract of *A. wilkesiana* which showed the abundance of potassium, sodium, and Chloride. Of a problem is the presence of toxic heavy metals like cadmium and lead; although traces but long high intake may raise health concerns. The presence of several phytochemicals, nutritive and elemental constituents in the leaf of *A. Wilkesiana* are indicative of the plant's nutritious and pharmacological significance. For example, carbohydrates and their derivatives are known to take part in the immune system, fertilization, pathogenesis, blood clotting, and development [34]. Alkaloids are pharmacologically useful as a local anesthetic, CNS stimulant, analgesic [35]. Tannins on the other hand have been reported to inhibitor microbial proliferation and possess antibacterial anti-parasitic, anticancer and improve wound healing properties [36-41]. Saponins also have been documented to have antibacterial activities and expectorant properties and these abilities support their use in upper respiratory tract infection. The presence of flavonoids in the leaf extract is also very important because flavonoids act as natural modifiers of biological response and have anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer potentials [27,42,43].

On the elemental significance, macro elements like sodium, potassium, and calcium are known to regulate fluid balance and influence cardiac output while restriction of sodium intake or increase Potassium intake exert an antihypertensive effect [44,45]. Calcium ions play important physiological and biochemical processes like neuromuscular

Table 5: Acute toxicity of the ethanolic leaves extract of Acalypha Wilkesiana in adult male Wistar rats.

Groups	Number of rats	Ethanolic Leaves extract of *A. wilkesiana* (mg/kg body weight)	Mortality	Percentage mortality	Other symptoms
Phase 1					
A1	3	10	0	0%	No sign
B1	3	100	0	0%	No sign
C1	3	1000	0	0%	No sign
Phase 2					
A2	1	1500	0	0%	No sign
B2	1	3000	0	0%	No sign
C2	1	5000	0	0%	No sign

excitability, blood coagulation, secretary processes, the proper extracellular fluid concentration of calcium and phosphate ions are required for bone mineralization [45,46]. Elements such as iron, zinc, and manganese are essential for enzyme reactions as co-factors [46]. It may be possible therefore that the extract of *A. wilkesiana* might be useful in the treatment of several diseases considering its several phytochemicals and elements with health benefits.

Acute toxicity studies are conducted to ascertain the safety of plant extracts and Ashafa and Olunu noted that toxicity studies of herbal extract in animals are commonly used to assess potential health risks in humans that can cause intrinsic adverse effects of chemical compounds or plant extracts [47]. In the present study, the highest dose of 5000mg/kg was safe in the adult male Wistar rats and there was no sign of toxicity or death. In agreement with this study, Iniaghe, et al. [48] documented that at an administered dose of 10,000 mg/kg body weight of aqueous leaf extract of *A. wilkesiana*, all rats displayed normal behavioral, neurological, and autonomic profiles.

Conclusively, this study showed that the ethanolic leaves extract of *A. wilkesiana* is a rich source of phytochemicals and major macro elements that can be pharmacological significant for the biological system. Also shown in this study is the safety of this extract at doses as high as 5000mg/kg. However, the need for chronic toxicity study with this extract is needed and their potentials in several disease conditions are recommendations for further studies.

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How to cite this article: Ghosh S. To Flip or Not to Flip is No Longer the Question - It is How to Flip Effectively. J Biomed Res Environ Sci. 2021 Aug 28; 2(8): 713-720. doi: 10.37871/jbres1301, Article ID: JBRES1301, Available at: https://www.jelsciences.com/articles/jbres1301.pdf