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Medicinal Potential of *Acalypha wilkesiana* Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: *Acalypha wilkesiana*, commonly called Irish petticoat, is native to the south pacific islands and belongs to the family Euphorbiaceae. The plant has antimicrobial and antifungal properties and in traditional medicine, the leaves are eaten as vegetables in the management of hypertension, being a diuretic plant. This study was conducted to determine some phytochemical (quantitative) constituents of *Acalypha wilkesiana* leaves, with a view to evaluating its medicinal potentials.
Method and Design: The samples (ethanol extract, aqueous extract and dried powder) of *Acalypha wilkesiana* leaves were analyzed for the presence of phytochemicals according to standard methods.
Results: Quantitative analysis of these phytochemicals in the leave extracts (aqueous or ethanol) and powder of this plant revealed the presence of medicinally active constituents like saponins (0.44% in the aqueous extract, 0.22% in the ethanol extract and 0.23% in the powdered leaves), cardiac glycosides (0.031% in the aqueous extract, 0.073% in the ethanol extract and 0.099% in the powdered leaves), alkaloids (0.92% in the aqueous extract, 3.20% in the ethanol extract and 2.62% in the powdered leaves) and oxalate (2.4% in the aqueous extract, 16.2% in the ethanol extract and 18.6% in the powdered leaves). Other phytochemicals found were tannins, phenols, steroids, anthraquinones, flavonoids, phytate and terpenoids.
Discussion and Conclusion: The various phytochemical compounds detected are

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known to have beneficial use in industries and medical sciences, and also exhibit physiological activity. The plant (*Acalypha wilkesiana*) studied here can be seen as a potential source of useful drugs.

Keywords: Acalypha wilkesiana; quantitative phytochemicals; ethanol extract; aqueous extract; diuretic plant; medicinal herbs.

1. INTRODUCTION

Medicinal herbs are plants which contain substances that can be used for therapeutic purposes, of which are precursors for the synthesis of drugs. Since ancient times, phytotherapy has been used as folk medicine to treat various diseases. An herbal medicine is any medicinal product that contains as active ingredient, aerial or underground parts of plants, or other materials or combinations thereof whether in the crude state or as plant preparations [1]. Herbal medicines are the mainstay of about 75–80% of the world population, mainly in developing countries, for primary health care because of better cultural acceptability regarding compatibility with the human body and less side effects [2,3,4,5]. About 30% of modern conventional drugs are derived from plant sources [6]. *Acalypha wilkesiana*, commonly called Irish petticoat, is native to the south pacific islands and belongs to the family Euphorbiaceae. It is a plant of great ornamental value due to its showily colored foliage and is widely cultivated in the tropical and subtropical countries. In traditional medicine, the leaves of this diuretic plant are eaten as vegetables in the management of hypertension in Southern Nigeria. A lot of research work has been carried out on some medicinal herbs and they have been found to have definite action on the nervous, circulatory, respiratory digestive and urinary systems; as well as the sexual organ, the skin, vision, hearing and taste [7]. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants [8]. Medicinal plants are of great importance to the health of individuals and communities. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body [9,10]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [11,12]. However, plants used in traditional medicine are still understudied. *Acalypha wilkesiana* is frequently used in traditional medicine, exclusively or as a major constituent of many herbal preparations for the management or treatment of hypertension. This study was therefore conducted to determine some phytochemical (quantitative) constituents of *Acalypha wilkesiana* leaves, with a view to evaluating its medicinal potentials.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh *Acalypha wilkesiana* leaves were obtained from local gardens within Benin City and authenticated at the department of Plant Biology and Biotechnology, University of Benin, Benin City. The leaves were properly washed, air-dried and ground into fine powder.

2.2 Preparation of Ethanol Extract

100 g of the powdered leaves was soaked in 400 ml of ethanol (95%) for 72 hours (3 days), with occasional stirring using a magnetic stirrer to ensure proper mixture of the vessel content. The content was then filtered using a sintered funnel, (which is equivalent to four folds of bandage or sheet of cheese cloth). The extract (filtrate) was then concentrated using rotary evaporator. This was then weighed and used for the analysis.

2.3 Preparation of Aqueous Extract

100 g of the powdered leaves was soaked in 400 ml of distilled water for 72 hours (3 days), and treated as described above.

2.4 Preparation of Powdered Leaves

The dried powdered leaves were prepared as described above. The powdered leaves was weighed and also used for the analysis.

2.5 Quantitative Determination of Phytochemicals

The samples (ethanol extract, aqueous extract and dried powder) of *Acalypha wilkesiana* leaves were analyzed for the presence of alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, flavonoids, phlobatanins, terpenoids, phytosterols, phenols and oxalate, according to standard methods.

2.6 Determination of Oxalate

This was determined by the method of Oke (1966) [13]. About 2 g of the sample was weighed and digested with 10ml of 6M HCl for 1hr. It was then filtered and made up to 250 ml with H_2O in a volumetric flask. The pH was adjusted with concentrated NH_4OH solution until the colour of the solution changed from salmo pink to a faint yellow colour. 10 ml of 5% $CaCl_2$ solution was added to the precipitate, the insoluble oxalate. This was centrifuged at 2500 rpm and filtered. The residue or pellets was dissolved in 10mL of 20% (v/v) H_2SO_4, filtered and made up to 300 ml. An aliquot of 125 ml of the filtrate was taken and heated to near boiling point. This was titrated against 0.05 M of standardized $KMnO_4$ solution to give a faint pink colour which persisted for 30 s.

The redox reaction is as given below,

$$2MnO4^- + 5C_2O_4^{2-} + 16H^+ \rightleftharpoons 2Mn^{2+} + 8H_2O + 10CO_2$$

2.7 Determination of Alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne [14]. Two (2) g of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4hrs at 28ºC. It was later filtered using whatman No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a

weighed filter paper, washed with 1% ammonia solution dried in the oven at 80ºC. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

2.8 Determination of Flavonoids

This was determined according to the method of Harborne [14]. 5 g of the sample was boiled in 50 ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample [15,16].

2.9 Determination of Tannins

The method of Swain (1979) [17] was used. 0.2 g of the sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80ºC for 1 hr and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 mL volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. 1 ml of sample extract was pipette into 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 mL of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1 ml of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm.

Percentage tannin was calculated using the formula [15,18]:

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x 10,000}}$$

2.10 Determination of Saponin

The Spectrophotometric method of Brunner (1984) [19] was used for saponin analysis. 1 g of the sample was weighed into a 250 ml beaker and 100 ml Isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Thereafter, the mixture was filtered through using a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of Magnesium carbonate added. The mixture obtained with saturated $MgC0_3$ was again filtered [15] through a Whatman No 1 filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. 0-10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% FeCl solution [15] as done for 1 ml of the sample above. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm.

Percentage saponin was calculated using the formula: [15]

$$\text{Saponin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x 10,000}}$$

2.11 Determination of Total Phenols

Total polyphenols were determined according to the Folin–Ciocalteau reagent method [20]. Two-hundred microlitres (200 µl) of extracted sample, in triplicate, were added to 1 ml of 0.2 N Folin–Ciocalteau reagents and 0.8 ml of 7.5% sodium carbonate solution, mixed well and allowed to stand for 30 min at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV–Vis spectrophotometer (Shimadzu UV-1601). Quantification was based on the standard curve [15] generated with 100– 400 mg/l of gallic acid.

2.12 Determination of Anthraquinones

Fifty (50) milligram of the sample was soaked in 50 ml 0f distilled water for 16 hours. This suspension was heated in water bath at 70ºC for 1hr. After the suspension was cooled, 50ml of 50% methanol (MeOH) was added and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450nm and compared with a standard solution containing 1mg/100mL alizarin and 1mg/100 ml purpurin with the absorption-maximum 450nm [21].

2.13 Determination of Steroids

This was determined by the method described by Okeke and Elekwa [22]. Five (5) g of each sample was dispersed in 100 ml of freshly distilled water and homogenized in a laboratory blender. The homogenates were filtered and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). 2 ml of the eluents were put in test tubes and mixed with 2ml of chloroform. 3ml of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added to cool. Standard sterol solution was prepared and treated as described above. The absorbances of standard and prepared samples were measured in a spectrophotometer at 420 nm.

2.14 Determination of Terpenoids (Salkowski Test)

Five milliliter of each extract/sample was mixed in 2 ml of chloroform, and conc. H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface showed positive results for the presence of terpenoids [23,14,24].

2.15 Determination of Cardiac Glycosides (Keller Killiani Test)

A hundred milligram of extract/sample was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of conc. H_2SO_4. A brown ring obtained at the interface indicates the presence of deoxysugars, characteristic of cardenolides [25,26,27,28].

2.16 Statistical Analysis

Data are Mean ± SEM of three independent determinations. Statistical Analysis was by student t-test at $p<0.05$ using SPSS 17.0

3. RESULTS

Data represents Mean ± S.E.M (n = 3). Means with different letter superscripts, across rows, are significantly different ($p<0.05$).

Quantitative analysis of the leaf extracts (aqueous and ethanol) and powdered sample, showed that there were significant differences in phytochemical compositions ($p<0.05$). The highest amount of saponins was found in the aqueous extract, while the ethanol extract contained the highest amount of tannins, phenols, alkaloids, steroids and terpenoids. The powdered leaves contained the highest amount of flavonoids, cardiac glycosides, oxalate, anthraquinones and phytate.

4. DISCUSSION

Relatively few studies have mentioned the phytochemical constituents of *Acalypha wilkesiana* leaves. The present study carried out on *Acalypha wilkesiana* leaves revealed the presence of medicinally active constituents. The phytochemical constituents of the leaves investigated are presented in Table 1. Oladunmoye [29] reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of *Acalypha wilkesiana*, while Akinde [30] reported that the plant contains sesquiterpenes, monoterpenes, triterpenoids and polyphenols. These were however, qualitative determination and not quantitative, which is the objective of this study. Quantitative analysis of these phytochemicals in the leave extracts (aqueous or ethanol) and powder of this plant (Table 1), showed that the aqueous extract contains the highest amount (%) of saponins, while the ethanol extract contains the highest amount (%) of tannins, phenols, alkaloids, steroids and terpenoids, and the powdered leave contains the highest amount (%) of flavonoids, cardiac glycosides, oxalate, anthraquinones and phytate. The various phytochemical compounds detected are known to have beneficial use in industries and medical sciences, and also exhibit physiological activity [23].

Table 1. Quantitative phytochemical constituents of *Acalypha wilkesiana* leaves

Phytochemical	Aqueous extract	Ethanol extract	Powder
Tannin (%)	0.08 ± 0.01^{a}	0.92 ± 0.01^{b}	0.62 ± 0.01^{c}
Phenol (%)	0.05 ± 0.01^{a}	0.26 ± 0.01^{b}	0.25 ± 0.01^{b}
Saponin (%)	0.44 ± 0.02^{a}	0.22 ± 0.01^{b}	0.23 ± 0.02^{b}
Flavonoid (%)	Nd	0.18 ± 0.01^{a}	1.84 ± 0.03^{b}
Cardiac Glycoside (%)	0.031 ± 0.001^{a}	0.073 ± 0.001^{b}	0.099 ± 0.001^{c}
Alkaloids (%)	0.92 ± 0.01^{a}	3.2 ± 0.17^{b}	2.62 ± 0.02^{b}
Oxalate (%)	2.4 ± 0.12^{a}	16.2 ± 0.12^{b}	18.6 ± 0.35^{c}
Steroids (%)	Nd	3.65±0.02	Nd
Terpenoids (%)	0.92 ± 0.01^{a}	1.21 ± 0.02^{b}	1.10 ± 0.02^{c}
Anthraquinone (%)	2.5 ± 0.17^{a}	Nd	4.5 ± 0.23^{b}
Phytate (%)	0.002 ± 0.00^{a}	Nd	0.01 ± 0.00^{b}

Note: Nd = Not detected

Tannins are effective in protecting the kidneys; hence the leaf may have protective effect on the kidney, a major organ in the regulation of homeostasis. They have been used for immediate relief of sore throats, diarrhea, dysentery, haemorrhage, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds. Tannins can cause regression of tumors that are

already present in tissues, but if used excessively overtime, they can cause tumors in healthy tissues. It was also reported that certain tannins are able to inhibit HIV replication selectively and are also used as diuretics [31]. Thus, the diuretic effect of the plant (*Acalypha wilkesiana* leaf) may be connected to its tannin content. Saponins class of natural products, in research use, involves their complexation with cholesterol to form pores in the lipid bilayer of cell membranes, e.g in red cell (erythrocyte) membranes where complexation leads to red cell lyses (haemolysis) in intravenous injection [32]. In medicine, it is used in the management of hypercholesterolaemia and hyperglycemia, as an antioxidant, anti-cancer, anti-inflammatory and for weight loss e.t.c. It is also known to have anti-fungal properties [33]. Hyperglycemia and hypercholesterol are major risk factors in the development of hypertension and cardiovascular diseases. The presence of saponins in this plant (leaves) indicates its possible beneficial effects in the management of these conditions.

Flavonoids (both flavonols and flavanols) are most commonly known for their anti-oxidant activity *In vitro*. The leaves of *Acalypha wilkesiana*, rich in flavonoids, may serve as a source of anti-oxidants which are useful in protecting against damage by free radicals. Although physiological evidence is not yet established, the beneficial effects of fruits, vegetables and tea or even red wine have sometimes been attributed to flavonoids compounds rather than to known micronutrients, such as vitamins and dietary minerals [34]. The increase in antioxidant capacity of blood seen after the consumption of flavonoid-rich foods is not caused directly by flavonoids themselves, but most likely is due to increased uric acid levels that results from metabolism of flavonoids. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity, thus indicating the enormous benefits associated with *Acalypha wilkesiana* leaves.

Hundreds of distinct steroids are found in plants, animals and fungi. The steroid biosynthetic pathways, in animals, are common targets for anti-biotic and other anti-infective drugs. Plant steroids are known to be important for their cardiotonic activities as well as their insecticidal and anti-microbial properties. The cardiotonic activities of steroids, present in high amount in the plant (leaves), are beneficial in the management of hypertension since it has direct effects on the contractions of the cardiac muscles. They have also been reportedly used in nutrition, herbal medicine and cosmetics [35]. Plant terpenoids, present in appreciable amount in *Acalypha wilkesiana* leaves, are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for anti-bacterial, anti-neoplastic, and other pharmaceutical functions. The steroids and sterols in animals are biologically produced from terpenoids precursors. Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants, like *Acalypha wilkesiana* (leaves), but also in some animals. Cardiac glycosides are used therapeutically mainly in the treatment of cardiac failure, due to their anti-arrhythmic effects. These are caused by the ability to increase cardiac output by increasing force of contraction and allowing more time for ventricular filling. Cardiac glycosides are known to work by inhibiting Na^+/K^+ pump. This causes an increase in the level of sodium ions in the myocytes which then lead to a rise in the level of calcium ions. This inhibition increase the amount of Ca^{2+} ions available for contraction of the heart muscles which improves cardiac output and reduces distention of heart; thus are used in the treatment of congestive heart failure and cardiac arrhythmia, which is one of the major benefit associated with the use of this plant (*Acalypha wilkesiana* leaves) in traditional medicine.

Anthraquinones, also called anthracenedione or dioxanthracene is an aromatic organic compound, found in *Acalypha wilkesiana* leaves. This compound is an important member of the quinine family. Derivatives of 9, 10-anthraquinone includes many important drugs (collectively called anthracene-diones), which suggests the use of the leaves in preparation of important drugs. They include; laxatives, anti malarias, anti neoplastics (used in the treatment of cancer). Natural anthraquinones derivatives tend to have laxative effects. Prolonged use and abuse leads to melanosis coli [36,37].

Most of the known functions of alkaloids are related to protection. Presence of alkaloids in some plants prevents insects and chordate animals from eating them. Besides, such alkaloid related substances as serotonin; dopamine and histamine are important neurotransmitters in animals. The presence of alkaloids in the leaves of *Acalypha wilkesiana* indicates its use as a source of substances that are precursors of neurotransmitters. These neurotransmitters function in the transmission of signals in the nervous system, which has direct effect on the contraction of blood vessels in the cardiovascular system. The effects of these alkaloids (present in the leaves of the plant) on the cardiovascular system, helps in the management of cardiovascular diseases and hypertension. Many alkaloids are still used in medicine, usually in the form of salts. Many synthetic and semi-synthetic drugs are structural modification of the alkaloids, which were designed to enhance or change the primary effect of the drug and reduce unwanted side effects. Preparations of plant containing alkaloids and their extract, and later pure alkaloids have long been used as psychoactive substances. Thus, apart from the plant (*Acalypha wilkesiana* leaves) being able to manage hypertension and cardiovascular diseases, it can also be used as a source of precursors for the synthesis of psychoactive drugs. There are, however, alkaloids that do not have strong psychoactive effect themselves, but are precursors for semi-synthetic psychoactive drugs.

Phenols, also found in *Acalypha wilkesiana* leaves, are versatile precursors to a large collection of drugs, most notably aspirin but also many herbicides and pharmaceutical drugs. Phenol is also used as an oral anesthetic/analgesic in products such as Chloraseptic or other brand name and generic equivalents, commonly used to temporarily treat pharyngitis. Phenol cools and numbs skin on contact, kills germs, and reduces the risk for infection in minor skin irritations. It is also caustic, which makes it suitable as an exfoliant. It has been used medically for over 100 years, for these and other applications. In large doses, phenol is highly toxic, but when properly used, it remains a valuable chemical for medical and surgical use. Natural phenolic compounds play an important role in cancer prevention and treatment. Phenolic compounds from medicinal herbs (such as *Acalypha wilkesiana* leaves) and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways [38,39]. These benefits may be derived from the use of *Acalypha wilkesiana* leaves.

In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. These oxalates can form larger kidney stones that can obstruct the kidney tubules. An estimated 80% of kidney stones are formed from calcium oxalate [40]. Those with kidney disorders, gout, rheumatoid arthritis, or certain forms of chronic vulvar pain (vulvodynia) are typically advised to avoid foods high in oxalic acid [41,42]. The high

amount of oxalate in *Acalypha wilkesiana* leaves may pose problem for those with gout, rheumatoid arthritis or kidney disorders, taking the plant (leaves) for either hypertensive condition or cardiovascular diseases. Methods to reduce the oxalate content in food are of current interest [43]. In studies with rats, calcium supplements given along with foods high in oxalic acid can cause calcium oxalate to precipitate out in the gut and reduce the levels of oxalate absorbed by the body (by 97% in some cases.) [41,42]. Thus, supplementing the herbal preparation from this plant (leaves) with calcium may be beneficial, as it forms calcium oxalates which will precipitate out in the gut. Phytic acid (phytate) found in *Acalypha wilkesiana* leaves might be beneficial in small doses and might have anticancer effects. From epidemiological data, foods with high phytate content are not associated with increased risk for several chronic diseases. The interaction of intracellular phytic acid with specific intracellular proteins has been investigated *In vitro*, and these interactions have been found to result in the inhibition or potentiation of the physiological activities of those proteins [44,45]. The best evidence from these studies suggests an intracellular role for phytic acid as a cofactor in DNA repair by nonhomologous end-joining [44]. Other studies using yeast mutants have also suggested intracellular phytic acid may be involved in mRNA export from the nucleus to the cytosol [46,47]. Phytic acid may be considered a phytonutrient, providing an antioxidant effect. As a food additive, phytic acid is used as the preservative E391.

Overall, it can be seen (Tables 1) that comparatively, ethanol is a better extraction solvent than water. This may be due to the fact that most of the phytochemicals are more soluble in ethanol than in water. Hence, they are more readily extracted by ethanol than water. The plant (*Acalypha wilkesiana*) studied here can be seen as a potential source of useful drugs.

5. CONCLUSION

Evident from the benefits of these compounds detected in *Acalypha wilkesiana* leaves, the plant (*Acalypha wilkesiana*) studied here can be seen as a potential source of useful drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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