Scientific Journals Scientific Journal of Pure and Applied Sciences (2013) 2(9) 323-331 ISSN 2322-2956 doi: 10.14196/sjpas.v2i9.982

Contents lists available at Sjournals Scientific Journal of

Pure and Applied Sciences

Journal homepage: www.Sjournals.com

Original article

Phytochemical, proximate and elemental analysis of acalypha wilkesiana leaves

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ARTICLEINFO

ABSTRACT

Article history: Received 11 August 2013 Accepted 22 August 2013 Available online 29 September 2013

Keywords: Acalypha wilkesiana Qualitative phytochemicals Ethanol extract Aqueous extract Diuretic plant Medicinal herbs Proximate composition Elemental composition

Acalypha wilkesiana, commonly called Irish petticoat, is native to the south pacific islands and belongs to the family Euphorbiaceae. Apart from its use as a vegetable, the plant is also used in traditional medicine as a diuretic plant and is eaten in the management of hypertension. In this study, three samples (ethanol extract, aqueous extract and dried powder) of Acalypha wilkesiana leaves were analyzed for the presence of phytochemicals according to standard methods. Apart from flavonoids, steroids, anthraquinones and phytate, all the phytochemicals tested for are present in the three samples. This qualitative analysis showed the presence of flavonoids only in the ethanol extract and powdered leave, steroids only in the ethanol extract, anthraguinones and phytate in the agueous extract and powdered leave only. Proximate analysis revealed the presence of ash [4.16% (aqueous extract),13.98% (ethanol extract) and 13.65% (powdered leaves)], moisture [66.0% (aqueous extract), 30.89% (ethanol extract) and 12.0% (powdered leaves)], total lipid [1.75% (aqueous extract), 4.25% (ethanol extract) and 2.20% (powdered leaves)], as well as fiber, crude protein and energy, while elemental analysis revealed the presence of sodium [0.0086% (aqueous extract), 0.024% (ethanol extract) and 0.015% (powdered leaves)], potassium [0.49% (aqueous extract), 1.36% (ethanol extract) and 1.44% (powdered leaves)], as well as chloride and calcium. The various phytochemical compounds detected are known to have

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beneficial use in industries and medical sciences, and also exhibit physiological activity.

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1. Introduction

The use of plant, plant extract or plant-derived chemicals to treat diseases; topical, subcutaneous and systemic, has stood the test of time (Oladunmoye, 2006). Medicinal herbs are plants which contain substances that can be used for therapeutic purpose, of which are precursors for the synthesis of drugs (Sofowora, 1984). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. (Kumar et al, 2009). Plants are known to contain food and mixture of extraordinary chemicals, some of which act beneficially to human and others may have detrimental effects (Ngbede et al, 2008). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Iniaghe et al., 2009). Also, there has been little or no report of any form of microbial resistance during the use and administration of herbal medicines (Stephen et al., 2009). More importantly in Africa, particularly West Africa, new drugs are often beyond the reach of the poor such that up to 80% of the population use medicinal plants as remedy against infections and diseases (Kirby, 1996; Hostettmann and Marston, 2002). 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. The plant has been reported to contain sesquiterpenes, monoterpenes, triterpenoids and polyphenols (Akinde, 1986). Acalypha wilkesiana has antimicrobial and antifungal properties (Akinde, 1986; Alade and Irobi, 1993; Adesina et al .2000; Ogundaini, 2005; Oladunmoye, 2006). Apart from its use as a vegetable, the plant is also used in traditional medicine as a diuretic plant and is eaten in the management of hypertension. Relatively few studies have mentioned the phytochemical, proximate and elemental composition of Acalypha wilkesiana leaves. Thus, the aim of this study is to determine the phytochemical, proximate and elemental composition of Acalypha wilkesiana leaves with a view to evaluating its medicinal and possible nutritional 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. This was designated the powdered leaves.

2.2. Preparation of ethanol extract

100g of the powdered leaves was soaked in 400ml 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

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

2.4. Determination of tannins

Into 10ml of freshly prepared 10% KOH in a beaker, 0.5g of extract/sample was added and shaken to dissolve. A dirty precipitate observed indicated the presence of tannin (Williamson et al, 1996).

2.5. Determination of saponins

0.2g of the extract/sample was shaken with 5ml distilled water and then heated to boiling. Frothing showed the presence of saponins. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (soluble) (Sofowora, 1993).

2.6. Determination of flavonoids

0.2g of the extract/sample was dissolved in dilute NaOH and HCl was added. A yellow solution which turned colourless was observed (Trease and Evans, 1989).

2.7. Determination of steroids

One milliliter of the sample was dissolved in 10ml of chloroform and equal volume of conc. H2SO4 was added by the sides of the test tube. The upper layer turned red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Kumar et al, 2009).

2.8. Determination of terpenoids

Five milliliter of each extract/sample was mixed in 2ml of chloroform, and conc. H2SO4 (3ml) was carefully added to form a layer. A reddish brown coloration at the interface showed positive results for the presence of terpenoids (Sofowora, 1993).

2.9. Cardiac glycosides

Hundred milligram of extract/sample was dissolved in 1ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under-layered with 1ml of conc. H2SO4. A brown ring obtained at the interface indicated the presence of deoxysugars, characteristic of cardenolides (Odebiyi and Sofowora, 1978).

2.10. Determination of anthraquinones

Five milliliter of the extract/sample solution was hydrolyzed with diluted H2SO4, extracted with benzene and 1ml of dilute ammonia was added to it. Rose pink colouration suggested the positive response for anthraquinones (Kumar et al, 2009).

2.11. Determination of alkaloids

Three grams of extract/sample was stirred with ethanol containing tartaric acid. The filtrate was poured into a beaker and Mayer's reagent was added. Precipitation in the beaker indicated the presence of alkaloids (Banso and Ngbede, 2006).

2.12. Determination of terpenoids

Ten milligrams of the extract/sample was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of conc. H2SO4. Formation of reddish violet colour indicated the presence of triterpenoids (Kumar et al, 2009).

2.13. Determination of oxalate

This was determined by the method of Oke (1966). 2g of the sample was weighed and digested with 10ml of 6M HCl for 1hr. It was then filtered and made up to 250ml with H2O in a volumetric flask. The pH was adjusted with NH4OH solution until the colour of the solution changed from salmo pink to faint yellow, indicative of the presence of oxalate.

2.14. Determination of phenols

Total polyphenols were determined according to the Folin–Ciocalteau reagent method (Singleton et al, 1999).

2.15. Determination of crude protein

(ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, AOAC OFFICIAL METHOD 2003). The crude protein in the samples was determined by the semi-micro Kjeldahl, technique. This consists of three techniques of analysis namely Digestion, Distillation and Titration.

Determination of total lipid, dry matter and moisture, energy, ash and fibre: These were done using the AOAC OFFICIAL METHODS, 2003.

2.16. Determination of chloride

Chloride determination was done by Argentometric titration method (Harris, 2003). 50ml of the sample was titrated with standard solution of 0.02M AgNO3 at pH between 7 and 10, using potassium chromate as indicator. The change from yellow to perceptible red colour marked the end point of the reaction.

 $Cl (mg/L) = \frac{A \times M \times 70,900}{Vol. of sample}$

Where A = Mass of Silver nitrate

M = Molar mass of Silver nitrate

Determination of potassium, sodium and calcium: Potassium (K), Sodium (Na) and Calcium (Ca) were determined using the Flame Photometer.

2.17. Statistical analysis

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

3. Results

This study on Acalypha wilkesiana leaves, as shown in Table 1, revealed the presence of tannins, phenols, saponins, cardiac glycosides, alkaloids, oxalate and terpenoids in the ethanol and aqueous extract, as well as in the powdered leave. Apart from flavonoids, steroids, anthraquinones and phytate, all the phytochemicals tested for are present in the three samples. This qualitative analysis showed the presence of flavonoids only in the ethanol extract and powdered leave, steroids only in the ethanol extract, anthraquinones and phytate in the aqueous extract and powdered leave only.

Table 1

Qualitative	Phytochemical	Constituents of	f Acalypha [.]	wilkesiana Leaves
Quantative	I IIV LUCIICIIIICAI	Constituents of		

Phytochemical Constituent	Aqueous extract	Ethanol extract	Powder	
Tannin	+	+++	++	
Phenol	+	++	++	
Saponin	+	+	+	
Flavonoid	-	+	+++	
Cardiac Glycoside	+	+	++	
Alkaloids	+	+++	++	
Oxalate	+	+++	+++	
Steroids	-	++	-	
Terpenoids	+	+	+	
Anthraquinones	+	-	++	
Phytate	+	-	+	

Key: + = Present

- = Absent

(+ = low, ++ = moderate, +++ = high)

Proximate analysis of Acalypha wilkesiana leaves (Table 2) revealed the presence of ash, moisture, total lipid, fiber, protein and energy. The aqueous extract was shown to contain the highest amount (%) of moisture, while the ethanol extract contained the highest amount (%) of ash, total lipid and energy, and the powdered leave, the highest amount (%) of fiber and protein. These differences are, however, significant (p < 0.05)

Nutrient	Aqueous Extract	Ethanol Extract	Powder
Ash (%)	4.16 ± 0.03^{a}	13.98 ± 0.01^{b}	13.65 ± 0.29^{b}
Moisture (%)	66.0 ± 0.87^{a}	30.89 ± 0.63^{b}	$12.0 \pm 0.29^{\circ}$
Total Lipid (%)	$1.75 \pm 0.03^{\circ}$	4.25 ± 0.03^{b}	$2.20 \pm 0.12^{\circ}$
Fiber (%)	0.03 ± 0.01^{a}	0.21 ± 0.01^{b}	$0.68 \pm 0.02^{\circ}$
Protein (%)	1.86 ± 0.12^{a}	6.45 ± 0.17^{b}	$11.84 \pm 0.66^{\circ}$
Energy (KJ mol-1)	0.02 ± 0.00^{a}	9.45 ± 0.06^{b}	$1.92 \pm 0.01^{\circ}$

Proximate Constitue	ents of Acalypha	wilkesiana	Leaves.

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

Elemental Composition of Acalypha wilkesiana Leaves.			
Element	Aqueous extract	Ethanol extract	Powder
Na+ (%)	0.0086 ± 0.000^{a}	0.024 ± 0.001^{b}	0.015 ± 0.001^{b}
K+ (%)	$0.49 \pm 0.03^{\circ}$	1.36 ± 0.03^{b}	$1.44 \pm 0.02^{\circ}$
Cl - (mg/L)	$2410.6 \pm 3.00^{\circ}$	2481.5 ± 0.58^{b}	$7444.5 \pm 1.15^{\circ}$
Ca2+ (%)	0.32 ± 0.01^{a}	1.91 ± 0.01^{b}	$1.79 \pm 0.01^{\circ}$

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

Elemental analysis of the leaves of Acalypha wilkesiana revealed the presence of sodium (Na+), potassium (K+), chloride (Cl-) and calcium (Ca2+). As shown in Table 3, comparatively, the aqueous extract contained the least amount of these elements. The ethanol extract contained, significantly (p < 0.05), the highest amount of sodium and calcium, while the powdered leaves contained, significantly (p < 0.05), the highest amount of potassium and chloride.

4. Discussion

Table 2

Table 3

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. This study on Acalypha wilkesiana leaves, as shown in Table 1, revealed the presence of tannins, phenols, saponins, cardiac glycosides, alkaloids, oxalate and terpenoids in the ethanol and aqueous extract, as well as in the powdered leave. Apart from flavonoids, steroids, anthraquinones and phytate, all the phytochemicals tested for are present in the three samples. This qualitative analysis showed the presence of flavonoids only in the ethanol extract and powdered leave, steroids only in the ethanol extract, anthraquinones and phytate in the aqueous extract and powdered leave only. Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of Acalypha wilkesiana, while Akinde (1986) reported that the plant contains sesquiterpenes, monoterpenes, triterpenoids and polyphenols. The various phytochemical compounds detected are known to have beneficial use in industries and medical sciences, and also exhibit physiological activity (Sofowora, 1993).

Evidence from records show that the benefit of these compounds detected in Acalypha wilkesiana leaves are as follows; It was reported that certain tannins are able to inhibit HIV replication selectively and are also used as diuretics (Kunle and Egharevba, 2009; Doss et al, 2011). Thus, the diuretic effect of the plant (Acalypha wilkesiana leaf) as well as its possible use in the management of HIV infection may be connected to its tannin content. In medicine, saponin is used in the management of hypercholesterolaemia and hyperglycemia, as an antioxidant, anti-cancer, anti-inflammatory and for weight loss e.t.c. 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.

They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity, thus indicating the enormous benefits associated with Acalypha wilkesiana leaves. 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), may be beneficial in the management of hypertension since it has direct effects on the contractions of the cardiac muscles. Cardiac glycosides are used therapeutically mainly in the treatment of cardiac failure, due to their anti-arrhythmic effects. 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 increases the amount of Ca2+ 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. Anthraguinones, also called anthracenedione or dioxanthracene 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. The plasma cholesterol-lowering properties of plant sterols have been known since the 1950s (Pollak, 1953). 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. Preparations of plant containing alkaloids and their extract, and later pure alkaloids have long been used as psychoactive substances. Thus, apart from the plant being able to manage hypertension and cardiovascular diseases, it can also be used as a source of precursors for the synthesis of psychoactive drugs. In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca2+) and iron (II) (Fe2+) 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. 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. 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.) (Morozumi et al., 2006; Hossain et al., 2003). 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. As indicated in the elemental analysis, the high amount of calcium in Acalypha wilkesiana leaves may have a balancing/moderating effect on the effect of oxalate, as it may chelate with it and form calcium oxalate that will precipitate out of the gut. Hence, the plant (leaves) may be eaten or used in the management of hypertension and cardiovascular diseases without the fear of complicating any existing kidney stone, gout or rheumatoid arthritis. Phytic acid might be beneficial in small doses and might have anticancer effects. From epidemiological data, foods with high phytate content, as indicated in Acalypha wilkesiana leaves, are not associated with increased risk for several chronic diseases. Overall, it can be seen (from Table1) that comparatively, ethanol is a better extraction solvent than water and the plant (Acalypha wilkesiana) can be seen as a potential source of useful drugs.

Proximate analysis of Acalypha wilkesiana leaves (Table 2) revealed the presence of ash, moisture, total lipid, fiber, protein and energy. The aqueous extract was shown to contain the highest amount (%) of moisture, while the ethanol extract contained the highest amount (%) of ash, total lipid and energy, and the powdered leave, the highest amount (%) of fiber and protein. These differences are, however, significant (p < 0.05). Ash content is the total amount of the mineral elements present in the plant (leaves). These mineral elements are essential for tissue functioning and are a necessity in human nutrition. The high content of ash of the leaf of this plant is a reflection of the mineral contents preserved in the plant, which suggests a high deposit of mineral elements in the leaves. The moisture content of the dried powder (12.0%) is relatively high when compared with values from literatures. The moisture content of any food is an index of its water activity (Olutiola et al., 1991), and is used as a measure of stability and the susceptibility to microbial contamination (Uraih and Izuagbe, 1990). This implies that dehydration would increase the relative concentrations of the other food nutrients (Table 2) and improve the shelf-life/durability of A. wilkesiana leaves. Total lipid, as shown, is highest in the ethanol extract. Dietary fats (or total lipid) help in increasing the palatability of food by absorbing and retaining flavours (Antia et al., 2006). Fat also serves as a useful buffer towards a host of diseases. Vitamins A, D, E, and K are fat-soluble, which implies they can only be digested, absorbed, and transported in conjunction with fats. Fats are also sources of essential fatty acids,

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an important dietary requirement. The high content of fat in Acalypha wilkesiana leaves, thus, suggests its importance in animal nutrition. The fiber content of the leaves, as indicated in Table 2, is relatively low. Epidemiological evidences suggest that increased fiber consumption may contribute to a reduction in the incidence of certain diseases including colon cancer, coronary heart disease, diabetes, high blood pressure, obesity, and various digestive disorders (SACN, 2008). Comparatively, the aqueous extract contained the least while the powdered leaf contained the highest amount of fiber. These, however, may not be adequate as more of the leaves may have to be consumed for full nutritional benefits. Dietary fiber has been associated with alterations of the colonic environment that protect against colorectal diseases. Among the theories on colonic carcinogenesis are those that involve increased concentrations of bile acids and their metabolites, alterations in colonic pH, low Ca2+, elevated NH3 and long-chain fatty acid concentrations, and alterations in bacterial profiles. Fiber may also provide protection by increasing fecal bulk, which dilutes the increased colonic bile acid concentrations that occur with a high-fat diet (Dillard and German, 2000), thus, the likely benefits derivable from the consumption of this plant. The high protein content of this plant (highest in the powdered leaf) suggests that a 100g serving of A. wilkesiana can provide about 7-19'% of the recommended dietary allowance (RDA). Proteins demonstrate numerous biological functions such as enzymes, regulator of metabolism, as antibodies and component of complement system. Plasma proteins maintain the osmotic pressure of plasma. They transport hormones, vitamins, metals and drugs often serving as reservoirs for their use. Within an organism, energy is responsible for growth and development of a biological cell or an organelle of a biological organism. Energy is thus often said to be stored by cells in the structures of molecules of substances such as carbohydrates (including sugars), lipids, and proteins, which release energy when reacted with oxygen in respiration. The high amount of energy in A. wilkesiana leaves as depicted in the ethanol extract shows the availability of high amount of transferable energy. Any living organism relies on an external source of energy-radiation from the Sun in the case of green plants; chemical energy in some form in the case of animals—to be able to grow and reproduce. The daily 1500–2000 Calories (6–8 MJ) recommended for a human adult are taken as a combination of oxygen and food molecules, the latter mostly carbohydrates and fats, of which glucose (C6H12O6) and stearin (C57H110O6) are convenient examples. The food molecules are oxidized to carbon dioxide and water in the mitochondria.

Elemental analysis of the leaves of Acalypha wilkesiana revealed the presence of sodium (Na+), potassium (K+), chloride (Cl-) and calcium (Ca2+). As shown in Table 3, comparatively, the aqueous extract contained the least amount of these elements. The ethanol extract contained, significantly (p < 0.05), the highest amount of sodium and calcium, while the powdered leaves contained, significantly (p < 0.05), the highest amount of potassium and chloride. Generally, the amount of Na+ in the plant is the least, while the amount of Cl-, the highest. But Ca2+ and K+ are present in almost the same amount. Sodium is an essential nutrient. It is the principal cation of extracellular fluid and a major determinant of intravascular fluid volume. An abrupt and sustained increase in dietary salt intake expands asymptotically the extracellular fluid space by inducing thirst and water drinking and causing, through its osmotic action, an internal redistribution of fluid from the intra- to extracellular compartment. Evidence from many sources suggests a possible relationship between excess salt ingestion and human hypertension (Scott et al, 2012; Jody et al, 2012; Tanika et al, 2013; Hector et al, 2013). This however, may not be a problem with the consumption of the leaves of A. wilkesiana, as it contains little amount of Na+. By reducing extracellular volume and blood volume, the natriuretic effect of potassium is generally considered to be an important component of its antihypertensive effect. The plant (leaf) which contains high amount of K+ and low amount of Na2+ is a potential source of useful diuretic drugs since the effects of sodium can be countered by potassium. Hypertensive cardiovascular damage is accelerated by salt loading but counteracted by dietary potassium supplementation, thus suggesting the medicinal use of the plant. Calcium, present in relatively high amount in this plant, is an important component of a healthy diet and a mineral necessary for life. In the electrical conduction system of the heart, calcium replaces sodium as the mineral that depolarizes the cell, proliferates the action potential. In cardiac muscle, sodium influx commences an action potential, but during potassium efflux, the cardiac myocyte experiences calcium influx, prolonging the action potential and creating a plateau phase of dynamic equilibrium. This suggests the importance of this plant in the maintenance of action potential in the heart. Long-term calcium deficiency can lead to rickets and poor blood clotting and in the case of menopausal women, it can lead to osteoporosis, in which the bone deteriorates and there is an increased risk of fractures. While a lifelong deficit can affect bone and tooth formation, over-retention can cause hypercalcemia (elevated levels of calcium in the blood), impaired kidney function and decreased absorption of other minerals. In view of the effects of excess of this mineral element (calcium), caution may be taken in the consumption or use of this plant to avoid over-retention of this element in the blood. Though, this may not be a problem as the plant (leaves) has been shown to contain high amount of oxalate, which may chelate the possible excess calcium and form calcium oxalate that will precipitate out of the gut.

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 and nutrients.

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