

Assessment of the Phytochemical, Proximate and Elemental Contents of *Cassytha Filiformis* (Love-Vine) Whole Plant from South-Eastern Nigeria

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Abstract

In Nigeria, *Cassytha filiformis* plant is used as both medicine and nutritional supplement because of its variety of secondary metabolites, proximate analytes and mineral contents. The present study is aimed to analyze the phytochemical compositions of the whole plant of *Cassytha filiformis* in four solvent extracts as well as to analyze its proximate and elemental contents. Phytochemicals were determined using known and standard procedures. The analysis of the proximate compositions of *C. filiformis* whole plant was carried out using Association of Official Analytical Chemists (AOAC) methods, the composition of carbon in the plant sample was determined using gravimetric method while other elemental compositions were determined using flame atomic absorption spectroscopic (AAS) method. Phytochemical screening confirmed that the plant possess major classes of secondary metabolites such as flavonoids, alkaloids, terpenoids, steroids, phenolic compounds, cardiac glycosides, cyanogenic glycosides, oxalate, phytate and tannins which expressed anti-nutritional properties of the plant. The proximate analysis findings proved that *C. filiformis* has greater proportions of crude protein ($10.850 \pm 0.495\%$), crude fibre ($7.803 \pm 1.510\%$), crude fat ($2.392 \pm 0.348\%$), carbohydrates ($59.564 \pm 1.913\%$), moisture content ($10.948 \pm 0.607\%$) and ash content ($8.444 \pm 0.168\%$). The results also revealed rich sources of elements in increasing order Al (0.021 ± 0.003 mg/kg) < Pb (0.133 ± 0.000 mg/kg) < Cu (0.268 ± 0.007 mg/kg) < Zn (0.473 ± 0.008 mg/kg) < Fe (1.259 ± 0.076 mg/kg) < Ca (5.237 ± 0.036 mg/kg) < K (6.083 ± 0.042 mg/kg) < Mg (6.944 ± 0.070 mg/kg) < Na (7.057 ± 0.039 mg/kg) < C (12.813 ± 0.110 mg/kg) which proved the good nutritional value of the plant. This research would help to formulate pharmaceutical medications and feed supplement.

1. Introduction

Plant extracts are potential sources of phytochemicals, proximate analytes and elements being used as medicines for the treatment of wide range of ailments and diseases as well as rich sources of food nutrients due to their low cost and easy accessibility [1–4]. Medicinal plants play vital roles in the development of new drugs and effective health care in both the developed and developing countries [5]. The isolation of phytochemicals from plants finds use in traditional medicine. Traditional medicine use plant extracts to provide healthcare for over 80% of the world's population, especially in the developing world [4, 6–8]. Various plant extracts has the ability to supply nutrients during the period of food scarcity. Carbohydrates, protein, fats, water, fibre and minerals constitute our body composition and chemistry and are of great nutritional values [1, 3, 4, 2, 7].

Macro minerals such as Ca, Mg, K, Na etc. are needed in higher concentrations while micro minerals which include Zn, Fe, Cu, Al etc. are required in smaller concentrations. Micro minerals are involved in many metabolic activities and vital enzymatic processes. Consumption of these minerals in the right proportions lowers specific risks of diseases [8, 2, 1]. Zn, Fe and Cu are essential heavy metals while Pb is a toxic heavy metal. The deficiency or lack of these minerals in the required quantity affects the growth and immunity of plants and humans. Fe, Zn, Cu & Pb are used for making pigments, paint, paper, cement, and rubber, metal alloys etc. Various agencies, for example WHO (World Health Organization) and FAO (Food and Agriculture Organization) have set permissible limits of these elements/minerals in drinking water, food substances and plants [9, 10].

Cassytha filiformis L. is a leafless, vine-like, twinning, climbing, autoparasitic and plant-hyperparasitic seed-producing plant in the family Lauraceae. It is a perennial vine with small scales as replacement for the leaves. Its common names are dodder laurel, woe-vine, love-vine and seashore dodder while its scientific name is *Cassytha filiformis* Linn. The flowers are borne in small panicles. The fruit is small, fleshy and berry-like, bearing a single spherical seed. *C. filiformis* parasitizes a wide variety of mainly woody plant hosts such as citrus, avocado, mango, nutmeg, cloves etc. it clings to woody plants for physical support, nutrition and water [11]. *C. filiformis* is used for

suppression of lactation after stillbirth. The juice made from the crushed vine of *C. filiformis* is taken four weeks before giving birth in order to ease labour pains, quicken labour time and lubricate the birth canals. It is used to treat jelly fish stings in Fiji and for the treatment of cancers [11, 12]. It is used in the treatment of jaundice. Men use it as love magic. It is used as dyes in dyeing industry. Alcoholic and aqueous extracts of *C. filiformis* possess diuretic activity. *C. filiformis* has in vitro cytotoxic properties in Wistar rats [13]

Research into natural product plants with pharmacological properties is vital. This could lead to the development of new forms of pharmaceuticals and other therapeutics. The objective of the current investigation is to assess the anti-nutritional and nutritional compositions of the whole plant of *C. filiformis*. This is in order to verify and administer the plant as a safe medication and as a good source of food nutrients for feed supplement.

2. Materials and methods

All the chemicals and reagents employed in this research are of analytical grade.

2.1 Sample collection, preparation and processing

The whole plant of *C. filiformis* were collected from an acacia plant in a farm located at Ukpo, Dunukofia Local government area, Anambra State in Eastern Nigeria. The plant was identified and authenticated by Mrs Uzoamaka Anthonia Emezie, the Chief Laboratory Technologist of the Department of Chemistry, Nnamdi Azikiwe University Awka.

The whole plant of *C. filiformis* was washed with clean water thrice and dried indoors under open air for 3-4 days at room temperature. Furthermore, the plant was subjected to oven-drying for about 4–5 minutes to ensure proper drying. Then the plant was ground into powdered form and stored in a tight bottle before use for determination of phytochemicals, proximate contents and mineral compositions

2.2 Extraction of phytochemicals from the plant

About 200g of the ground sample of the whole plant was dissolved in 1000ml each of ethanol, acetone, diethyl ether and distilled water and was allowed to react in a temperature-controlled shaking water bath at 60°C for 30 minutes and then placed in the refrigerator for 72hr. Chess cloth and Whatman filter paper was used to filter the extract to get filtrates of the respective solvents. The filtrates were then used for the determination of the phytochemicals [4, 14].

2.3 Phytochemical assay

Qualitative phytochemical analysis for alkaloid, flavonoid, tannins, cardiac glycosides, saponins, resins and terpenoids were carried out using known and standard procedures. Alkaloids were determined using Wagner's reagent test and Meyer's reagent test. Flavonoids were determined using Ferric chloride test, Lead acetate test and Sodium hydroxide test. Tannins were estimated by Acid test and Lead acetate test. Resins were extracted with 15ml of ethanol and 20ml of distilled water. Saponins were determined by the Frothing test and Emulsion test. Terpenoids were estimated using chloroform and concentrated H₂SO₄. Cardiac glycosides were determined using 50% H₂SO₄ and Fehling's solutions A and B [4, 15]. Quantitative phytochemical analysis was carried out with Gas chromatography equipped with a flame ionization detector (GC-FID) using a RESTEK 15 metre MXT-1 Column (15m x 250µm x 0.15µm).

2.4 Proximate Analysis

The analysis of the proximate composition of *C. filiformis* whole plant, for moisture, ash, crude fat, crude fibre, protein and carbohydrates, was carried out using the Association of Official Analytical Chemists (AOAC) methods, 2016 [2, 4, 10, 6].

The moisture content was evaluated by drying the plant sample in an oven at $105 \pm 2^\circ\text{C}$ till constant weight was achieved according to the AOAC procedure. Ash content was determined by incineration of the plant for 20hr at 550°C according to AOAC procedures. Nitrogen (N) content was determined using micro-kjeldahl method with respect to AOAC procedures and then protein content was calculated as $\%N \times 6.25$. Crude fat content was analysed using the soxhlet method following the AOAC methodology. The total carbohydrates content was calculated by the differential method as $100 - (\% \text{ protein} + \% \text{ moisture} + \% \text{ ash} + \% \text{ fat} + \% \text{ fibre})$ following AOAC methods. Crude fibre was determined by loss in weight after incineration of the plant sample following AOAC methodology. All plant samples were analysed in two replicates [10, 14].

2.5 Elemental analysis

The ashed plant samples were first digested with nitric acid and perchloric acid and the filtered aliquots were used for the determination of the elements. Potassium, sodium, magnesium, calcium, aluminium, zinc, iron, copper and lead were determined by flame atomic absorption spectrophotometry using a Varian 220FS Spectra AA apparatus (Varian USA) while carbon was determined by gravimetric method [4, 10, 16].

2.6 Data analysis

All determinations were conducted in two replicates and the data presented as mean $\pm$ standard deviation. Microsoft excel 2013 package was used to represent the percentage compositions of the proximate analytes with bar charts.

3. Results and discussion

3.1 Phytochemical assay

The results of the phytochemical analysis of the whole plant of *C. filiformis* in the four solvent extracts were presented in Tables 1 and 2. Phytochemical contents confer antimicrobial properties on plants. Qualitative phytochemical screening in Table 1 showed the presence of alkaloids, flavonoids, tannins, resins, saponins, cardiac glycoside and terpenoids in the four solvent extracts with resin absent in the diethyl ether extract. Considering the quantitative phytochemical profiling in Table 2, Spartein ($20.0734 \pm 4.0385\text{ppm}$) was present in the highest amount in the ethanol extract. Tannin ($11.0588 \pm 0.0031\text{ppm}$) was highest in abundance in the acetone extract. Spartein ($15.2649 \pm 2.1234\text{ppm}$) was highest in concentration in the diethyl ether extract. Phytate ($17.6169 \pm 2.3343\text{ppm}$) was highest in amount in the water extract. Proanthocyanin ($0.3761 \pm 0.5318\text{ppm}$) was lowest in the concentration in the ethanol extract. Ammodendrine ($0.5578 \pm 0.5578\text{ppm}$) was present in the lowest quantity in the acetone extract. Ephedrine (1.7848 ± 2.5241) was least in amount in the diethyl ether extract. Proanthocyanin ($0.3160 \pm 0.4469\text{ppm}$) was least in abundance in the water extract [4, 9, 14, 16].

Cardiac glycoside, anthocyanin, ribanilidine, naringenin, spartein, cyanogenic glycoside, steroids, kaempferol, phytate, flavone, oxalate, saponin and ephedrine were all present in each of the four solvent extracts. Dihydrocysteine and aphyllidine were absent in both the ethanol and water extracts but present in the acetone and diethyl ether extract. Linamarin, naringin, epicatechin, resveratrol and rutin were absent in both the acetone extract and diethyl ether extract but present in ethanol and water extracts. Flavonones was absent in diethyl ether extract

but present in the ethanol, acetone and water extracts. Catechin and tannin were absent in the water extract but present in the ethanol, acetone and diethyl ether extracts [4, 10].

Polyphenols and flavonoids are known to exhibit anti-microbial, anti-virus, anti-inflammatory, anti-tumour, anti-ulcer, anti-oxidative, anti-platelet aggregation, anti-allergic and anti-hepatic toxicity effects [1, 4, 3, 7]. Amongst the phytochemicals present in the plant sample, flavonoids had the highest content [17]. Flavonoids due to its anti-oxidant activity prevents cancer and cardiovascular disease [18]. Steroids which are present in appreciable but safe and non-toxic concentrations in the plant sample can serve as fertility agents [4, 3]. Saponins are used in cosmetics (lipstick), shampoo, emulsions and photographic films. Saponins helps in controlling cholesterol in the body by preventing reabsorption into the body. They have anti-protozoa, antifungal and antibacterial properties [19]. High saponin levels in the body leads to dysentery and diarrhoea [4]. Alkaloids as one of the most effective pharmacological bioactive component in plants exhibit analgesic, antispasmodic and bactericidal properties [1, 4, 3]. Ephedrine is known for many centuries for its use against asthma [20]. Terpenoids act as chemical signalling agents between plants and other living organisms. They are used in perfumery in earliest time [21]. They act as an attractant to pollinators and allelopathic agents or as defence chemicals against predators and pathogens. Some of the terpenes can act as substrates for enzymatic processes [20]. Tannins are used in the manufacture of ink. They precipitate proteins and alkaloids from solution [21].

Table 1
Qualitative phytochemical profile of the whole plant of *C. filiformis*

Parameters	Ethanol extract	Acetone extract	Diethyl ether extract	Water Extract
Alkaloids	++	++	++	++
Flavonoids	++	++	++	++
Tannins	++	+	+	++
Resins	++	+	-	++
Saponins	+	+	+	++
Cardiac glycoside	+	+	+	+
Terpenoids	+	+	+	++
Key				
++ = Highly present				
+ = Lowly present				
- = Absent				

Table 2
Quantitative phytochemical profile of the solvent extracts of the whole plant of *C. filiformis*

Component	Molecular formula	Class of secondary metabolite	Ethanol extract (ppm)	Acetone extract (ppm)	Diethyl ether extract (ppm)	Water extract (ppm)
Proanthocyanin	C ₃₀ H ₂₄ O ₁₂	Flavonoids	0.3761 ± 0.5381	0.0000 ± 0000	0.0000 ± 0000	0.3160 ± 0.4469
Linamarin	C ₁₀ H ₁₇ NO ₆	Quinoline alkaloids	4.3442 ± 1.4800	0.0000 ± 0000	0.0000 ± 0000	6.1907 ± 2.4815
Naringin	C ₂₇ H ₃₂ O ₁₄	Flavonoids	8.0785 ± 0.5314	0.0000 ± 0000	0.0000 ± 0000	9.7475 ± 0.0275
Cardiac glycoside		Cardiac glycosides	5.0431 ± 0.0466	8.0091 ± 0.1803	5.8274 ± 2.2372	6.3418 ± 0.3552
Anthocyanin	C ₁₅ H ₁₁ O ⁺	Flavonoids	3.8488 ± 0.0035	7.6168 ± 0.0168	5.8747 ± 0.4147	10.7107 ± 0.2866
Ribalinidine	C ₁₅ H ₁₇ NO ₄	Alkaloids	2.1369 ± 0.3906	5.0800 ± 0.0199	7.8026 ± 5.7795	9.6249 ± 1.8599
Naringenin	C ₁₅ H ₁₂ O ₅	Flavonoids	2.0773 ± 0.0011	2.6925 ± 0.0047	5.0532 ± 2.0727	5.8172 ± 0.1811
Sparteine	C ₁₅ H ₂₆ N ₂	Alkaloids	20.0734 ± 4.0385	9.2009 ± 0.1092	15.2649 ± 2.1234	6.5161 ± 0.2476
Rutin	C ₂₇ H ₃₀ O ₁₆	Flavonoids	2.1958 ± 3.1055	0.0000 ± 0.0000	0.0000 ± 0.0000	13.7279 ± 0.2533
Cyanogenic glycoside		Cyanogenic glycosides	12.1703 ± 6.6984	1.4433 ± 0.0110	9.7360 ± 1.1044	16.6739 ± 0.6850
Flavanones	C ₁₅ H ₁₂ O ₂	Polyphenols/Flavonoids	3.0552 ± 1.4402	5.3398 ± 0.0019	0.0000 ± 0.0000	4.0304 ± 0.1116
Steroids	C ₂₆ H ₄₀ O ₈ S ₂ Na ₂	Steroids	10.2220 ± 2.1135	3.1753 ± 0.0056	7.4844 ± 1.6898	12.8435 ± 0.1933
Kaempferol	C ₁₅ H ₁₀ O ₆	Flavonols/ Flavonoids	2.6686 ± 0.0231	4.4740 ± 0.0262	5.7374 ± 0.2589	7.8484 ± 0.2005
Epicatechin	C ₁₅ H ₁₄ O ₆	Flavonoids	6.6594 ± 0.0434	0.0000 ± 0.0000	0.0000 ± 0.0000	7.9833 ± 0.3461
Phytate	C ₆ H ₆ O ₂₄ P ₆ ⁻¹²	Phytate	2.1554 ± 3.0482	5.4627 ± 1.6550	3.4279 ± 4.8477	17.6196 ± 2.3343
Flavone	C ₁₅ H ₁₀ O ₂	Flavonoids	4.6745 ± 1.4052	3.6350 ± 0.0310	6.0474 ± 0.4265	5.4314 ± 0.2950
Oxalate	C ₂ O ₄ ²⁻ / (COO) ²⁻	Oxalate	10.2702 ± 0.0473	1.5062 ± 0.1182	3.3570 ± 1.5144	9.9960 ± 1.4902
Catechin	C ₁₅ H ₁₄ O ₆	Flavonoids	2.4574 ± 0.5588	3.5242 ± 0.0514	1.9065 ± 2.6879	0.0000 ± 0.0000
Resveratrol	C ₁₄ H ₁₂ O ₃	Phenolic compounds	2.4197 ± 1.3194	0.0000 ± 0.0000	0.0000 ± 0.0000	3.8200 ± 0.0982

Component	Molecular formula	Class of secondary metabolite	Ethanol extract (ppm)	Acetone extract (ppm)	Diethyl ether extract (ppm)	Water extract (ppm)
Tannin	C ₇₆ H ₅₂ O ₄₆	Tannins	1.8824 ± 0.1706	11.0588 ± 0.0031	5.5733 ± 2.6805	0.0000 ± 0.0000
Sapogenin	C ₂₇ H ₄₄ O ₂	Saponins/ Sapogenins	9.6836 ± 0.2502	1.5222 ± 0.0514	4.0194 ± 0.6183	5.7336 ± 0.0481
Ephedrine	C ₁₀ H ₁₅ NO	Alkaloids	8.2679 ± 0.1771	1.7832 ± 0.0031	1.7848 ± 2.5241	14.4885 ± 1.3006
Flavan-3-ol	C ₁₅ H ₁₄ O ₂	Flavonoids	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	10.6711 ± 0.0115
Dihydrocytisine			0.0000 ± 0.0000	3.1571 ± 0.0031	5.0718 ± 1.3130	0.0000 ± 0.0000
Aphyllidine	C ₁₅ H ₂₂ N ₂ O	Quinolizidine alkaloid	0.0000 ± 0.0000	1.0852 ± 0.4081	2.7298 ± 0.3891	0.0000 ± 0.0000
Proanthocyanidin	C ₃₁ H ₂₈ O ₁₂	Polyphenols/ Flavonoids	0.0000 ± 0.0000	2.4346 ± 0.0124	5.1467 ± 0.6413	0.0000 ± 0.0000
Ammodendrine	C ₁₂ H ₂₀ N ₂ O	Piperidine alkaloid	0.0000 ± 0.0000	0.5578 ± 0.0605	2.8642 ± 0.7677	0.0000 ± 0.0000

3.2 Proximate contents

Proximate compositions revealed that the carbohydrate content ($59.564 \pm 1.913\%$) has the highest value followed by moisture content ($10.948 \pm 0.607\%$), protein content ($10.850 \pm 0.495\%$), ash content ($8.444 \pm 0.168\%$), fibre content ($7.803 \pm 1.510\%$) and then crude fat ($2.392 \pm 0.348\%$) with the least value as presented in Table 3 and Fig. 2 [4, 9, 10]. Ash, fibre, moisture, protein and carbohydrate contents occurred in significant amounts [14, 16]. The fat content is very low suggesting that *C. filiformis* is highly nutritive. Carbohydrates has the highest value and thus serves as a good source of energy. Crude fibre helps in the absorption of trace element in the gut and in the excretion of undigested food substances through the bowels as well as prevents diverticulosis [22, 4, 15]. High total ash value signifies high mineral content [4, 17]. High moisture content implies easy deterioration of the plant sample by microorganisms while low moisture content indicates long shelf-life for the plant sample. [22, 3, 6, 7]. Very low amounts of protein was found in the whole plant sample indicating that it cannot provide the recommended level of protein [17, 15].

Table 3
Proximate contents of the whole
plant of *C. filiformis*

Content	Composition (%)
Crude fibre	7.803 ± 1.510
Crude fat	2.392 ± 0.348
Ash	8.444 ± 0.168
Moisture	10.948 ± 0.607
Protein	10.850 ± 0.495
Carbohydrate	59.564 ± 1.913

3.3 Elemental composition

The elemental profile of the plant sample are presented in Table 4. K, Na, Mg, Ca and C were present in significant amounts while heavy metals such as Zn, Cu, Pb and Fe were present in insignificant concentrations and therefore do not show any toxic effect. The presence of these elements in *C. filiformis* whole plant gives it nutritional benefits. The concentrations of the elements fell within the permissible limits by WHO and FAO, 1984 except that of magnesium which exceeded the acceptable limit [10, 9, 16, 14]. Iron in the body boosts the immune system, controls temperature, develops cognition and metabolizes energy. It is also utilized in the natural chemical production of haemoglobin and myoglobin. Deficiency of iron results in anaemia [4, 23]. Calcium is needed for the formation of strong bones and teeth. It is also needed for muscle contraction and blood clotting [4, 15]. Magnesium is required in the synthesis of DNA and RNA during cell multiplication. It contributes to nerve and heart function, the release of insulin and the decrease of blood pressure by the dilation of arteries and by the prevention of abnormal heart rhythm. Magnesium deficiency in animals causes irritability, convulsion and even death [4, 15]. Zinc constitute the component of antioxidative enzyme and is essential and beneficial for sexual development and growth in man [4, 23]. Although aluminium forms major constituent of foods, cosmetics and most part of the body, it has no nutritive benefits but is rather toxic in high levels. High levels of magnesium prevents toxic effect of aluminium in the body [4, 2]. Sodium is a major composition of the extracellular body fluid. Together with potassium, it conducts impulses and allows the contraction of muscles. Sodium facilitates the absorption of nutrients such as glucose and aminoacids in the small intestine. However, high concentrations of sodium leads to high blood pressure and hypertension [4]. The collective presence of Ca, Mg and K results in the reduction of hypertension and high blood pressure. Consumption of right proportions of minerals like Na, K, Mg, Ca, Cu and Zn lowers specific risks of diseases [18]. The heavy metals analyzed are present in highly insignificant amounts and did not exceed the allowable limits, hence have no detrimental effect on health. Iron, zinc, copper and lead are used for making pigments, paint, paper, cement, rubber, metal alloys etc. [9].

Table 4
Elemental Compositions of the whole plant of *C. filiformis*

Element	Composition (Mg/kg)	FAO/WHO Limit (Mg/kg)
Iron	1.259 ± 0.076	20
Potassium	6.083 ± 0.042	-
Sodium	7.057 ± 0.039	-
Magnesium	6.944 ± 0.070	2
Zinc	0.473 ± 0.008	27.4
Calcium	5.237 ± 0.036	-
Lead	0.133 ± 0.000	0.43
Copper	0.268 ± 0.007	3.00
Aluminium	0.021 ± 0.003	-
Carbon	12.813 ± 0.110	-

4. Conclusion

Natural drugs derived from plants are preferred to synthetic drugs because they are safer and better for the health of humans. The present assessment has shown the phytochemical, proximate and mineral compositions of the whole plant of *C. filiformis*. The phytochemical profile in different solvent extracts presents *C. filiformis* as a plant with very good potential for medicinal use and in the possible treatment of various diseases. The proximate and elemental compositions, with both good ash value and low moisture content all falling within the acceptable limits, also contribute to the use of the plant in drug formulation. The plant is a rich source of micronutrients and macronutrients giving its good nutritional values. There is an ongoing investigation on the antimicrobial and spectroscopic properties of the whole plant of *C. filiformis*.

Declarations

Author contribution declaration

All authors contributed to the conceptualization, design and implementation of the research, formal analysis of the results and the writing of the original manuscript.

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We state that there is no conflict of interests among the authors.

Data availability declaration

All data associated with this work are included within this article and with supplementary information.

Ethic declaration

This work is original and has not been published elsewhere in any form or language, either partially or fully. No data or text by others were presented as if they were the authors' own.

Consent to participate declaration

Not applicable to this study.

Consent to publish declaration

Not applicable to this work.

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Figures

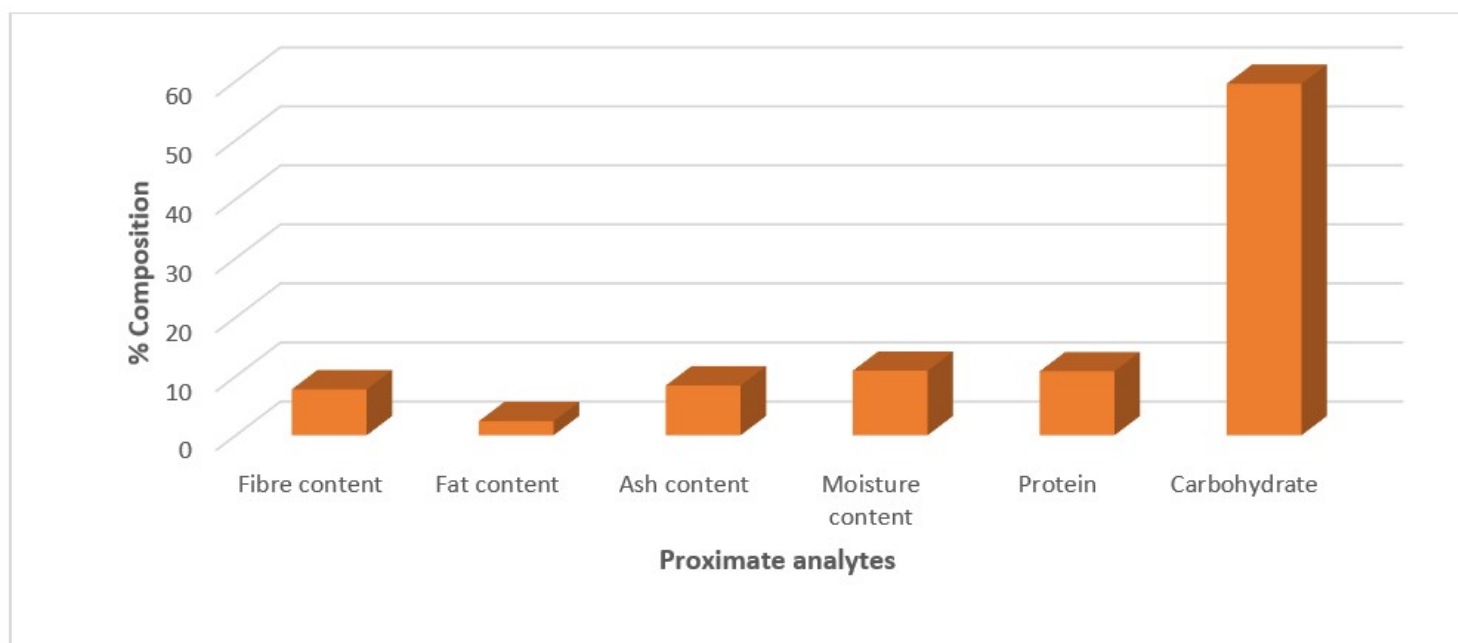


Figure 1

Bar chart representing the percentage composition of proximate analytes in the plant sample



Figure 2

Cassytha filiformis whole plant