



## Proximate Analysis and Phytochemical Constituents of *Acalypha hispida* Leaves

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### ABSTRACT

The proximate analysis and phytochemicals constituents were of the leaves of *Acalypha hispida* were determined. The result indicated that it contained moisture (11.02%), crude fat (6.05%), total ash (10.17%), crude protein (13.17%), crude fibre (10.36%) and carbohydrate (48.65%). The phytochemical composition of the aqueous extract of *Acalypha hispida* leaves include phenolic, glycoside, flavonoid, steroids, phytobatanin, saponin and hydroxylenraquenone. These results suggest that the *Acalypha hispida* leave contains nutrients and mineral elements that may be useful in nutrition. The presence of some phytochemicals like saponins and flavonoids might be responsible for its the medicinal value of the plant in its therapeutic use.

**Keywords:** Proximate analysis, Phytochemicals constituents, Aqueous extract.

### INTRODUCTION

The contribution of medicinal plants in the traditional system of medicine for curing diseases has been documented. Nowadays increased scientific interest and consumer demand have promoted the development of herbal products as dietary supplements. In view of renewed interest, oriental herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21<sup>st</sup> century. It has been reported that whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals (Singh and Sharma, 2010). Both the deficiency and excess of essential micronutrients and trace toxic metals may cause serious effects on human health.

The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history, but has increased substantially in the last decades. However, the safety of their use has recently been questioned due to the reports of illness and fatalities. (Singh and Sharma, 2010). WHO recommended that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further, it regulates maximum permissible limits of toxic metals like lead which amount to 1.0 and 10 mg/L, respectively. Medicinal herbs are easily contaminated during growth, development and

processes. After collection and transformation into dosage form the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers (Singh and Sharma, 2010).

Plant constituents, as the word implies, are the individual chemicals from which plants contain. These constituents are organic in nature and synthesized in plants by the activity of individual cells. The process by which these complex organic chemical constituents are formed, utilizing simple substances and enzymes are known as biosynthesis.

Several compounds, which are pharmaceutically and medicinally important, are derived from plant sources. However, the medicinal value, depends on the nature of its constituents. This is known as active principle or active constituent. Active constituents are those chemical substances which are solely responsible for therapeutic activity of plants. A

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large number of theories have been proposed as to why these compounds are formed in plants. It is likely that many of them are synthesized as part of chemical defense system to protect the producing organism.

On the other hand, chemical constituents present in plants that do not possess any definite therapeutic value are known as inactive constituents. The formation of the various active and inactive constituents of plants involves various metabolic pathways. Hence, the inactive plant constituents are termed primary plant metabolites, whereas the active plant constituents are termed secondary plant metabolites.

Primary plant metabolites are simple molecules or polymers of simple molecules synthesized by plants. They do not possess therapeutic activity as such, but are essential for the life of plants and contain high-energy bonds. These are used in for the biosynthesis of secondary metabolites e.g. Carbohydrates, proteins, lipids and nucleic acids.

Secondary metabolites on the other hand, are complex organic molecules biosynthesized from primary plant metabolites in plant cells (Fig. 1). They are unique to a plant or group of plants, generally possess therapeutic activity, neither essential for plants life nor contain high energy bonds. These are usually stored in vacuoles. Secondary metabolites are classified as: alkaloids, glycosides, tannins, phenolic compounds, volatile oils, terpenoids, saponins, steroids, resins and bitter principles. These are used as medicine, food, flavors, colours, dyes, poisons and perfumes etc. It is estimated that a quarter of prescription drugs contains at least one chemical originally identified from plants. (Ahmed, 2007).

Toward the end of the 20<sup>th</sup> century, epidemiological studies and associated meta-analyses suggested strongly that long-term consumption of diets rich in plant foods offered some protection against chronic diseases, especially cancer (Wallstrom *et al.*, 2000). Because uncontrolled production of free radicals was thought to be significantly implicated in the etiology of cancer (Guyton and Kensler, 1993). These observations focused attention on the possible role of radical scavenging and radical suppressing nutrients

and non-nutrients in explaining the apparent benefit of such diets (Weisburger, 1991).

Herbal medicine is based on the premise that plants contain natural substance that can promote health and alleviate illness (Graig, 1999). Herbs refer to not only the herbaceous plant but also to bark, roots, leaves, seeds, flowers and fruits of trees, shrubs and woody vines. The trypanocidal activity of the natural compounds berberine and harmaline, both documented as being trypanocidal have been reported (Edward, 2007).

In ethno-medical practices, the root and flower decoction is used for kidney ailments and as a diuretic leaf poultice is used as a cure for leprosy. The decoction of leaves and flowers are taken internally as lownative and for treatment of gonorrhoea. The bark is used as expectorant and for asthma (Iwu *et al.*, 1999; Kafaru, 2000; Sofowora, 2008). Previous work done on the leaves of *Acalypha hispida* revealed the presence of phenolics, flavonoid, glycosides, steroids, saponins, phylobatannins and hydroxyanthraquinones (Imagbe *et al.*, 2009; Okorondu *et al.*, 2009).

The antifungal, antibacterial, antiulcer and anti-tumor properties of extracts of leaves of *Acalypha hispida* have been established (Ejechi and Soucey, 1999; Adesina *et al.*, 2000; Guherrez-Hugo *et al.*, 2002).

## METHODOLOGY

### Sample Collection and Preparation.

The plant was obtained from Ibrahim Badamasi Babangida University, Lapai, main Gate, Niger State, Nigeria and was identified at the Herbarium section of the Department of Biological Sciences, of Ahmadu Bello University, Zaria, as *Acalypha hispida*. The leaves was air dried for two weeks and ground to uniform powder using wood type of mortar and pestle. The aqueous extract of sample was prepared by soaking 100grams of dried powder sample in 500ml of distilled water for 72 hours. The filtrate was used for phytochemical screening. Proximate analysis was carried out on dried sample of the *acalypha hispida* leaves (AOAC, 1990; Sofoworo, 2008).

### Reagents.

All reagents used were of analytical reagent grade. Distilled water was used in the

preparation of all aqueous solutions. All solutions were stored in amber colour bottles.

### **10% Sodium Hydroxide Solution**

The solution was prepared by dissolving 10g of sodium hydroxide pellet in 100cm<sup>3</sup> volumetric flask and made up to the mark with distilled water.

### **5% Ferric Chloride Solution**

The solution was prepared by dissolving 5g of ferric chloride in 100cm<sup>3</sup> of volumetric flask and made up to the mark with distilled water.

### **50% H<sub>2</sub>SO<sub>4</sub> Solution (V/V).**

The solution was prepared by diluting 50cm<sup>3</sup> of Conc. H<sub>2</sub>SO<sub>4</sub> in 100cm<sup>3</sup> volumetric flask and made up to mark with distilled water.

### **10% HCl solution (V/V)**

The solution was prepared by diluting 10cm<sup>3</sup> of Conc HCl in 100cm<sup>3</sup> volumetric flask and made up to mark with distilled water.

### **Mayer's Reagent**

13.35g of mercuric chloride was dissolved in 60ml of distilled water to give solution A. 5g of potassium iodide was dissolved in 20ml of distilled water to make solution B. The two solution (A and B) were mixed and made up to 1000cm<sup>3</sup> with distilled water in a 1000cm<sup>3</sup> volumetric flask

### **Wagner's Reagent**

1.27g of sublimed solution of iodine and 2g of potassium iodide was in 20cm<sup>3</sup> of distilled water in a beaker. This was transferred into 1000cm<sup>3</sup> volumetric flask and made up to mark with distilled water.

### **Phytochemical Screening of Extract**

Chemical tests was carried out on the aqueous extract and on the powder specimens using standard procedures to identify the constituents as described by Sofoworo, 1993; Trease and Evans, 1989; Harborne, 1973).

### **Test for Flavonoid:**

3cm<sup>3</sup> aliquot of the filtrate was added to 1 cm<sup>3</sup> of 10% NaOH sodium hydroxide, if a yellow color is developed this indicates the possible presence of flavonoid compounds (El-Oleyi *et al.*; 1994, Harbone, 1998).

### **Test for Tannins**

Ferric chloride solution 5% was added drop by drop to 2-3cm<sup>3</sup> of the extract and the colored changed to blue-black, indicating the presence of tannins (Harbone, 1998; Trease and Evans, 1978).

### **Test for Saponin**

10cm<sup>3</sup> of the extract was placed in a test tube and mixed with 5cm<sup>3</sup> of distilled water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Harbone, 1998).

### **Test for Glycosides**

2.5cm<sup>3</sup> of 50% H<sub>2</sub>SO<sub>4</sub> is added to 5cm<sup>3</sup> of the extracts in a test tube. The mixture was heated in boiling water for 15 minutes. Cooled and neutralized with 10% NaOH, and 5cm<sup>3</sup> of fehling's solution added and the mixture allow to boiled. A brick-red precipitate was observed which indicate the presence of glycosides (Harbone, 1973).

### **Test for Alkaloids**

About 2cm<sup>3</sup> of each extract was stirred with 2cm<sup>3</sup> of 10% aqueous hydrochloric acid. 1cm<sup>3</sup> was treated with a few drops of Wagner's reagent and a second 1cm<sup>3</sup> was treated similarly with Mayer's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids (Harbone, 1973).

### **Test for Steroids (Salkowski)**

This was carried out according to the method of Harbone 1973. 5cm<sup>3</sup> of the extract was dissolved in 2cm<sup>3</sup> of chloroform and 2cm<sup>3</sup> of sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interface indicates the presence of a steroidal ring.

### **Test for Hydroxylanthraquinones**

5cm<sup>3</sup> of each plant extract is shaken with 10cm<sup>3</sup> benzene, and 5cm<sup>3</sup> of 10% ammonia solution was added. The mixture was shaken and the presence of a pink, red or violet color in the ammoniacal (lower) phase indicates the presence of anthraquinones.

### **Test for Phylobatannis**

Deposition of red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phylobatannin.

### Proximate Analysis

Proximate analysis involves the estimation of the main components of food (moisture content, crude lipid, crude protein, fat, ash and crude carbohydrate) using standard methods of analysis.

### Determination of Moisture Content

The method of AOAC (1990) using hot air drying oven was used. Empty clean dish was dried in an oven at a temperature of 105°C for one hour and cooled in a desiccator. Two grammes of the samples was put into the dish and heated in an oven for 24 hours. The dish was then removed from the oven, cooled in a desiccator and weighed. Moisture content was then calculated using equation below.

$$(\%) \text{ Moisture content} = \frac{\text{Loss in weight}}{\text{Sample weight}} \times 100$$

### Determination of Ash Content

The method of AOAC (1990) was adopted to determine the percentage of ash content. Two grammes each of dried samples were weighed into a pre-heated and cooled crucibles and incinerated in a muffle furnace (Lenton, England) at 550°C for six hours. The ash was then cooled in a desiccator and weighed. Ash content was then calculated using equation below.

$$(\%) \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

### Determinaion of Crude Fibre

Two grammes of the ground sample was weighed and placed in 1litre conical flask containing 200cm<sup>3</sup> of 1.25% H<sub>2</sub>SO<sub>4</sub> and boiled gently for thirty minutes. The content was filtered and the residue was scrapped back into the flask with spatula. 200cm<sup>3</sup> of 1.25% NaOH was added and allowed to boil gently for 30 minutes. The content was filtered and washed thoroughly with hot distilled water. The precipitate was rinsed once with 10% HCl and twice with

ethanol. The content was allowed to dry and the residue was scrapped into a crucible and dried overnight at 105°C in a hot oven. It was then removed and cooled in a desiccator. The sample was then weighed and ashed at 600°C for ninety minutes in a furnace. This was finally cooled in a desiccator and weighed again (AOAC, 1990).

The percentage crude fibre was calculated using equation below.

$$(\%) \text{ Crude fibre} = \frac{\text{Loss of weigh on ignition}}{\text{Weight of sample}} \times 100$$

### Determination of Crude Lipid

The method of AOAC (1990) using Soxhlet extractor was adopted for crude lipid determination. Two grammes each of the dried samples were weighed into a porous thimble, and it is mouth covered with cotton. The thimble was then placed in an extraction chamber which was suspended above a receiving flask containing petroleum ether (B.P 40-60°C). The flask was heated on hot mantle and the oil extracted. The extraction continued for eight hours after which the thimble was removed from the soxhlet and the apparatus was reassembled and heated on water bath or solvent recovery. The flask containing the crude oil was then disconnected, cleaned up and placed in an oven at 100°C for thirty minutes. The flask was then cooled in a desiccator and weighed

The percentaged crude lipid content was then calculated using equation

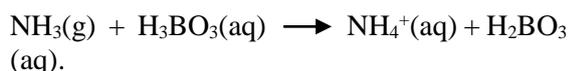
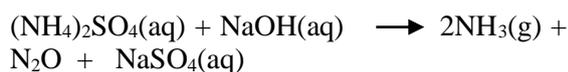
$$(\%) \text{ crude oil lipid} = \frac{\text{Weight of oil extracted}}{\text{Weight of sample}} \times 100$$

### Determination of Crude Protein

The method of AOAC (1990) was adopted using Micro-Kjeldahl apparatus. The technique was based on the transformation of protein nitrogen and other nitrogen compounds other than nitrate and nitrite into ammonium sulphate by acid digestion with strong acid usually, Conc. H<sub>2</sub>SO<sub>4</sub>. The stages involved are:



The ammonia present in the digest with the help of sodium hydroxide was distilled out and collected into a receiving flask containing boric acid indicator which changes colour from pink to green.



The nitrogen content was then estimated by titrating the content in the receiving flask with standard acid ( $\text{H}_2\text{SO}_4$ ).



The amount of  $\text{H}^+$  consumed in the reaction is equivalent to the amount of nitrogen present in the sample.

Although the assumption is not entirely valid. The protein contained in plant sample in term of nitrogen content is from 13-18%.

### Procedure

Two grams of the dried sample was weighed into kjeldahl digestion flask and a catalyst mixture was added ( $\text{NaSO}_4$ ,  $\text{CuSO}_4$  and Selenium oxide in 10:5:1 ratio) followed by  $10\text{cm}^3$  of concentrated tetraoxosulphate (iv) acid. The content in the flask was then heated in the Kjeldahl digestion until the digestion is completed (approximately one and half hour). The flask was cooled, the contents diluted with  $10\text{cm}^3$  distilled water, filtered into a  $100\text{cm}^3$  volumetric flask and made up to the mark with distilled water.  $10\text{cm}^3$  of the aliquot was taken into the digestion flask and  $20\text{cm}^3$  of 45%  $\text{NaOH}$  solution was added. The content was diluted to about  $200\text{cm}^3$  with distilled water and distilled using Micro-kjeldahl distillation apparatus (AOAC, 1990).

The distillate was receiving into receiving flask containing  $10\text{cm}^3$  boric acid solution indicator. After the distillation, the distillate was titrated with standardized 0.01M  $\text{HCl}$  to the end point. The blank was also determined in the same way with out the sample. crude protein is then calculated using the equation below:

$$\text{Crude protein (\%)} = \frac{\text{TV} \times \text{C} \times 0.0014 \times \text{V}_1}{\text{W} \times \text{V}_2} \times 100$$

Where

TV = Titre value of the acid

C = Concentration of acid used.

$\text{V}_1$  = Volume of the distilled water used for diluting the digest

$\text{V}_2$  = Volume of the aliquot used for titration

W = Weight of the sample used

F = Protein multiplication factor = 0.0014

### Determination of Carbohydrate (By Difference).

The method of James (1995) was used where the total amount of carbohydrate in the sample was obtained by calculation using percentage weight difference. This involved subtracting the percentage sum of the food nutrients, % crude protein, % crude lipid, % crude fibre, and % ash from 100% dry weight.

Percentage carbohydrate was calculated using equation below:

$$\text{Carbohydrate \%} = 100 - (\text{crude protein} + \text{crude lipid} + \text{crude fibre} + \text{ash})$$

## RESULT AND DISCUSSION

### Proximate Composition

The Proximate Composition of *Acalypha hispida* Leaves are shown in the table below.

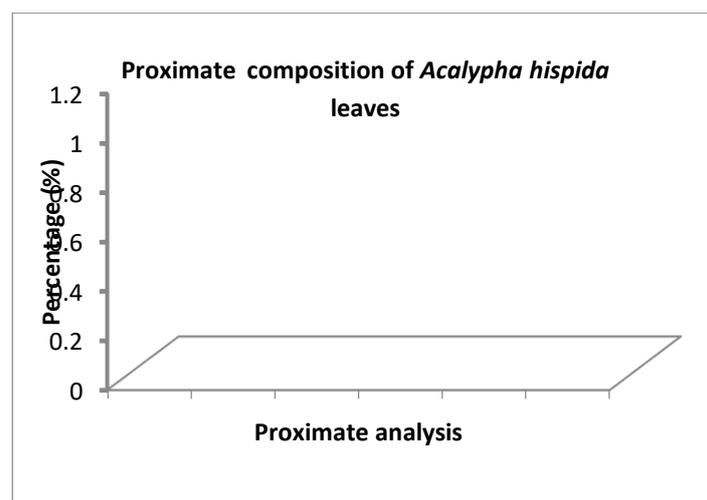


Figure 2: Proximate Composition of *Acalypha hispida* leaves

The result obtained from proximate analysis of leaves of *Acalypha hispida* establishes that they can be ranked as carbohydrate rich leaves due to their relatively high carbohydrate content when compared with the other components of the leaves. The low moisture content of leaves would hinder the growth of micro organisms and the storage life would be high (Adeyeye and Ayejuyo, 1994). The crude protein content of the leaves of *Acalypha hispida* is 13.78% and it compares favourably with *Heinsia crinita* (14.7%), but it is relatively low when compared with *Amarantus candatus* (20.59%) (Etuk *et al.*, 1998; Akindahunsi and Salawu, 2005), cassava leaves (*Manihot utilisima*), 24.88%, *Piper*

*Guineenses* 29.78% and *Talinum triangulare* 31.00%, which are said to be highly rich in protein (Akinda-hunsi and Salawu, 2005). The value for Ash content is 10.17%. This requires investigation to ascertain the species of mineral element as they are essential for tissue functioning and a necessity in daily requirement for human nutrition. The ash content of the leaves is lower than that of some leafy vegetable commonly consumed in Nigeria such as *Talinum triangulare* (20.05%), but is higher than that for other vegetables such as *Ocimum gratissimum* (8.00%) and *Hibiscus esculentus* (8.00%) (Akindahunsi and Salawu, 2005). The high ash content is a reflection of the mineral content preserved in the food materials. The result therefore suggests a high deposit of mineral elements in the leaves (Anta et al., 2006). The value of the crude fat for the leaves of the *Acalypha hispida* is moderate when compared to those of *Talinum triangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthus hybridus* (4.80%), *calchorus africanum* (4.20%), (Ifon and Bashir, 1979); (Akindahunsi and Salawu, 2005). Dietary fat function in the increase of palatability of food by absorbing and retaining flavours (Antia et al., 2006). A diet providing 1 -2 % of its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging (Anti et al., 2006). The crude fibre content of 10.36% is high when compare with *Talinum triangulare* (6.20%), *Piper guineeses* (6.40%), *Corchorus olitorius* (7.0%), bitter leaves (*vernonia amygdalina*), 6.5% (Akindahunsi and Salawu, 2005). Non-starchy vegetables are the richest sources of dietary fibre and are employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Agostoni et al., 1995; Saldanna, 1995).

### Phytochemical Constituents

The preliminary phytochemical constituents of the aqueous extract of *Acalypha hispida* leaves revealed the present of phenolic, glycoside, flavonoid, steroid, phytoabattannin, saponin and hydroxylantraquinones. In the concentration shown in the table.

Table 1: Phytochemical Constituents of Aqueous Extract of *Acalypha hispida* Leaves.

Extract constituent	Concentration
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Alkaloids	-
Tannin	-
Phenolic	+
Glycoside	+
Flavonoid	+
Steroids	+
Phytoabattannin	+
Saponin	+
Hydroxylantraquinone	+

Key: + = Detected - = Not detected

The phytochemical constituents of the *Acalypha hispida* studied showed that the leaves were rich in phenolic, glycoside, flavonoid, steroids, phytoabattannins, saponins, and hydroxylantraquinones. These are known to exhibit medicinal activity as well as physiological activity (Sofoworo, 1993). The absence of alkaloid in *Acalypha hispida* in this study is inconsistent with the opinion of Musa et al; (2000) who reported the presence of alkaloids in the leaves of *Acalypha hispida*. Flavonoids have been shown to have antibacterial, anti-inflammatory, antihistaminic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilatory activity (Alan and Miller, 1996). Steroids and phytoabattannins were detected in *Acalypha hispida*. Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). Various studies have shown that saponins although non-toxic can generate adverse physiological response in animals that consume them. They exhibit cytotoxic effect and growth inhibition against a variety of cell making them have anti-inflammatory and anticancer properties. They also show tumour inhibiting activity on animals (Akindahunsi and Salawu, 2005). From the above result, the leaves may serve as a constituent of human diet supplying the body with minerals, protein and energy. The presence of secondary plant products in the leaves of *Acalypha hispida* that are biologically important e.g. saponins and flavonoid contributes to its medicinal value, thus, they can be potential sources of useful drugs.

### Conclusion

The use of medicinal plants in therapeutic or as a dietary supplement, date back to ancient

times. Determination of the presence of heavy metal is equally important since most

medicinal herbs are easily contaminated during growth and development processes.

From the result of the analysis, it revealed that the *Acalypha hispida* leaves have therapeutic uses and equally contain nutrients and mineral elements useful in nutrition.

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