Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.6, 2015

Phytochemical, Proximate and Mineral Analyses of the Leaves of *Gossypium hirsutum* L. and *Momordica charantia* L.

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Abstract

The leaves of *Gossypium hirsutum* L. and *Momordica charantia* L. were analysed for phytochemical, proximate and mineral compositions. The qualitative phytochemical screening from the two plants revealed the presence of alkaloids, saponins, flavonoids, tannins and cardiac glycosides whereas terpenoids and steroids were absent. Subsequent quantification analysis revealed that *G. hirsutum* contained 12.20± 0.28% alkaloids, 2.63±0.04 % saponins, 11.90±0.4 % flavonoids, 2.73 mg/100g tannins and 1.62±0.00 mg/100g total phenol. *Momordica charantia* contained 13.60±0.00 % alkaloids, 2.30±0.00 % saponins, 7.20±0.00 % flavonoids, 1.37±0.00 mg/100g tannins and 0.74 ±0.00mg/100g total phenol. Proximate analysis revealed that carbohydrate had the highest percentage in *G. hirsutum* and *M. charantia* (46.66±0.31% and 57.92± 0.04 % respectively). Crude protein had the lowest percentages of 2.70±0.01% and 2.46 ± 0.03% in *G. hirsutum* and *M. charantia*. Mineral analysis revealed that potassium had the highest concentration of 38.61 mg/100g in *G. hirsutum* and 32.84mg/100g in *M. charantia*. The least concentration of minerals in *G. hirsutum* was sodium (3.37mg/100g) while magnesium (5.88mg/100g) recorded the least concentration in *M. charantia*. The high carbohydrate contents in both plants might justify the potentials of the plants as good source of energy.

Keywords: Phytochemical, *Gossypium hirsutum*, *Momordica charantia*, Proximate, Mineral analyses.

1. Introduction

The use of plants as sources of drugs, vegetables and foods cannot be underestimated (Hammer *et al*. 1999 and Sofowora 2008). Virtually all plants are medicinal hence they serve as raw materials for synthetic drugs (Sofowora 1993, 2008; Nair *et al*. 2005). Akinmoladun *et al*. (2007) noted that the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological action on the human body. These bioactive substances which can be present in all plant parts include saponins, tannins, flavonoids, alkaloids (Sofowora 1993).

G. hirsutum known as upland cotton belongs to the family Malvaceae. It is a perennial shrub that grows to approximately 1.5- 2 m and has the potential to develop leaves, stem, flowers, fruits and seeds all at the same time. The leaves have long petiole, cordate, weakly 3-5 lobed, the lobed are broadly triangular to ovate, acute to acuminate. The flowers are large and showy with cream to pale yellow petals, sometimes with red spot at the base of the petals. The capsules have 3-5 valves with a smooth surface and many black gland dots. This yield white or brown lint with seeds embedded. The seed vary from black and smooth to green with tightly adhering fuzz (Howard 1989 and Liogier 1994). Kannan *et al*. (2009) reported that *G. hirsutum* fruits contained complex antibiotic compounds which cured various diseases like cancer, cardiovascular and digestive diseases. Also, Gossypol obtained from *G. hirsutum* seeds have been reported by Sotelo *et al*. (2005) to have reversible antifertility effects in men. Fasola *et al*. (2011) confirmed that water extracts of *G. hirsutum* leaves have shown promising but differential in- vitro antiviral activities and recommended that application of the extracts could help in the treatment of yellow fever infections.

Momordica charantia known as bitter lemon belongs to the family Curcurbitaceae. *Momordica charantia* is a tropical and sub-tropical vine. Its leaves are simple usually palmately, 5-7 lobed alternate with yellow flowers. The plant has male and female flowers borne on separate plants. The fruits of the plant have a distinct warty exterior, oblong shaped and green in colour while fully ripe fruits turn orange (Kumar *et al*. 2010). They reported further that when *M. charantia* is taken continuously for some period it has the ability to substitute the insulin in the body. It contained steroids called charantin that effectively control sugar level in the body. *M. charantia* extracts was reported to inhibit cancer and tumour (Cunnick *et al*. 1990). It is an anti- HIV agent

(Bourinbair & Lee- Huang 1995), anti-obesity (Umesh *et al*. 2005) and antimicrobial agents (Sankaranarayan & Jolly 1993).

2. Materials and Methods

2.1 Collection and sample preparation

Fresh leaves of *Gossypium hirsutum* and *Momordica charantia* were obtained from Iworoko Ekiti in Irepodun / Ifelodun Local Government Area of Ekiti State Nigeria, a town situated at about 3 km from the campus of Ekiti State University, Ado- Ekiti. The samples were identified and authenticated in the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti. The leaves were air dried at room temperature (25-30^{0}C) for two weeks. The air-dried leaves were ground into powdery form and stored in air tight containers. The samples were taken to the laboratory of Chemistry Department, University of Lagos for analysis.

2.2 Phytochemical Screening

2.3 Qualitative Analysis

Test for Alkaloids: About 0.5 g of each plant sample was stirred with 5 ml of 1% hydrochloric acid on a steam baths and filtered using 10 cm Whatman filter paper. 1ml of the filtrate was treated with a few drops of Dragendorffs reagent, (Bismult nitrate + Conc. HCL). A change in the colour of the sample from brown to deep brown or black indicates the presence of alkaloids in the extracts being evaluated.

Test for Saponins: The ability of saponins to produce frothing in solution was used as screening test for saponins. About 0.5 g of each plant sample was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins (Obadoni & Ochuko 2001).

Test for Tannins: About 5 g of each portion of plant sample was stirred with 100 ml of distilled water, filtered and a test drop of 0.1% of Ferric chloride reagent was added to the 20 ml of filtrate. The appearance of a blue black green or blue green or blue green precipitate was taken as evidence for the presence of tannins (Trease & Evans 1989).

Test for Flavonoids: About 5 ml of dilute ammonia solution was added to 0.5 g of plant sample, followed by the addition of concentrated H_2SO_4. A yellow colouration formed indicates the presence of flavonoid which disappeared later on standing (Sofowora 1993).

Test for Cardiac Glycosides (Keller-Killiani test): About 0.2 g of plant sample was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. 1ml of concentrated H_2S0_4 was then added. A brown ring obtained at interface indicates the presence of desoxy- sugar characteristics of cardenolides. A violet ring appeared below the ring while in the acetic layer; a greenish ring formed just above the brown ring and gradually spread throughout this layer (Trease & Evans 1989).

Test for Terpenoids: About 0.5 g of plant sample was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 were carefully added to form layer. A reddish brown coloration at the interface shows the presence of terpenoids.

Test for Steroids: About 2 ml of acetic anhydride was added to 0.2 g of plant sample, with 2 ml of concentrated H_2SO_4. A colour change from violet to blue or green in some of the samples indicates the presence of steroids (Harborne 1993).

Test for Phenol: About 1g of finely ground sample were soaked in 25 ml of 2% of HCL for 1 h and then filtered through 10 cm Whatman No. 1 Filter paper. 5ml of each plant extract was mixed with 1ml of 0.30% Ammonium thiocyanate solution and few drops of ferric chloride solution. A brownish yellow colour indicates the presence of phenol.

2.4 Quantitative Analysis

Further quantification of the phytochemical constituents in the samples was determined using the standard procedures of Harbone (1993), Obadoni & Ochukwo (2001), Boham & Kocipal (1994).

2.5 Proximate Analysis

The proximate analysis of the samples was carried out according to the standard procedures of Association of Official Analytical Chemist (AOAC 2000). The moisture content was determined by drying 10.0g of the powdered in an oven at 105^0C until a constant weight was obtained; Crude protein was calculated by Nitrogen determination using Macrokjeldahi method and conversion of nitrogen to protein by the factor of 6.25 (AOAC 2005). Crude fiber was determined by successive digestion of the defatted samples with 0.26N Sulphuric acid and 0.23N Potassium hydroxide solutions. Crude fat was obtained by exhaustively extracting 5.0 g of the sample in a Soxhlet apparatus using petroleum boiling range 40- 60^0C on the extract**.** Ash content was determined by incineration of 10.0 g in a muffle furnace maintained at 550^0C for 5 h. Carbohydrate content was obtained according to Onwuka (2005). Available carbohydrate was calculated as follows:

% Available carbohydrate = 100 – (% moisture + % Ash + % protein + % fiber).

2.6 Determination of Mineral Analysis

The mineral constituents (phosphorus, calcium, magnesium, potassium, and sodium) in the samples were analysed with the use of Atomic Absorption Spectrophotometer (AAS). About 1g dried sample was weighed into crucible placed in muffle furnace at 550^0C for 1 h. The ashed sample was dissolved in hot 10% HCl and HNO_3 (ratio 3: 1) and diluted to 100 ml standard flask with distilled water. The solution was read with Atomic Absorption Spectrophotometer (AAS). The readings were calculated in milligram per 100 g.

2.7. Statistical analysis. The results obtained were subjected to Standard Deviation to compare the means.

3.0 **Results**

Table1: Qualitative analysis of *Gossypium hirsutum* and *Momordica charantia* leaves

Sample	Alkaloids	Saponins	Tannins	Cardiac glycosides	Flavonoids	Terpenoids	Steroids
G. hirsutum	+	+	+	+	+	-	-
M. charantia	+	+	+	+	+	-	-

Key = + (present), - (absent)

Table 2: Quantitative analysis of *Gossypium hirsutum* and *Momordica charantia* leaves

Parameters	*Gossypium hirsutum*	*Momordica charantia*
Alkaloids (%)	12.20 ± 0.28	13.60 ± 0.00
Saponins (%)	2.63 ± 0.64	2. 30 ±0.00
Flavonoids (%)	11.90 ± 0.14	7. 20 ± 0.00
Tannins (mg/100g)	2.73 ± 0.00	1.37 ± 0.00
Total phenol (mg/100g)	1.62 ± 0.00	0.74 ±0.00

Table 3: Proximate composition of *Gossypium hirsutum* and *Momordica charantia* leaves

Parameters (%)	*Gossypium hirsutum*	*Momordica charantia*
Moisture content	15.04 ± 0.01	13.08 ± 0.05
Ash	18.72 ± 0.13	14.71 ± 0.00
Crude fat	6.57 ± 0.04	5.83 ± 0.01
Crude fiber	8.31 ± 0.04	6.01 ± 0.01
Crude Protein	2.70 ± 0.01	2.46 ± 0.03
Carbohydrate	48.66 ± 0.31	57.92 ± 0.04

Table 4: Mineral composition of *Gossypium hirsutum* and *Momordica charantia* leaves

Parameters (mg/100g)	*Gossypium hirsutum*	*Momordica charantia*
Phosphorus	29.05 ± 0.01	24.36 ± 0.00
Calcium	31.69 ± 0.01	22.36 ± 0.01
Magnesium	8.32 ± 0.00	5.88 ± 0.00
Potassium	38.61 ± 0.00	32.84 ± 0.00
Sodium	3.37 ± 0.00	6.58 ± 0.01

The results of phytochemical screening of *G. hirsutum* and *M. charantia* are presented in Tables 1 and 2. The results revealed the presence of alkaloids, saponins, cardiac glycosides, and flavonoids in both plants. However, terpenoids and steroids are absent. The quantitative phytochemical analysis revealed that *G. hirsutum* contained 12.20 ± 0.28% alkanoids, 2.63 ± 0.04% saponins, 11.90 ± 0.4% flavonoids, 2.73 ± 0.00%, tannins, 1.62 ± 0.00% phenols while 13.60 ± 0.00%, 2.30 ± 0.00%, 7.20 ± 0.00%, 1.37 ±0.00%, 0.74 ± 0.00% were recorded for alkaloids, saponins, flavonoids, tannins and phenols respectively in *M. charantia*. Tables 3 and 4 showed the nutritional value of the leaves of the two plants. *G. hirsutum* contained moisture content of 15.04 ± 0.01%, ash (18.72 ± 0.13%), crude fat (6.57 ±0.04%), crude protein (2.70 ± 0.01%), and carbohydrate (48.66 ± 0.31%) while *M. charantia* contained moisture content (13. 08 ± 0.05%), ash (14.71 ±0.00%), crude fat (5.83 ± 0.01%), crude fiber (6.01 ± 0.01%), crude protein (2.46 ± 0.03%) and carbohydrate (57.92 ± 0.04%) (Table3). The result of the mineral analysis (Table 4) shows that *G. hirsutum* leaves has high phosphorus content (29.05mg/100g), calcium (31.69mg/100g), potassium (38.61mg/100g) and moderate quantity of sodium (3.37mg/100g) while the leaves of *M. charantia* equally have high quantity of phosphorus (24.36mg/100g), calcium (22.36mg/100g), potassium (32.84mg/100g) with low concentration of sodium (6.58mg/100g) and magnesium (5.88mg/ 100g).

4. Discussion.

The presence of phytochemicals such as alkaloids, saponins, cardiac glycosides, flavonoids and tannins in the leaves of *G. hirsutum* and *M. charantia* reported in this study suggest their uses as medicinal plants. Several authors such as Gills, 1992; Banso & Adeyemi, 2007; Temitope & Omotayo, 2012, Oyeyemi *et al*. 2014 had also reported the medicinal properties of these secondary plant metabolites. The phytochemical profile obtained in this study for *M. charantia* was similar to the reports of Annapoorani and Manimegalai (2013) and Santhi *et al*.

(2011) but differs from their reports by the absence of terpenoids and steroids in *M. charantia*. The presence of alkaloids in the leaves of both plants, in considerable quantity, suggests analgesic, anti-inflammatory properties and an increase in the potential for disease resistance and stress (Gupta 1994). Sodipo *et al*. (2000) reported that saponins lower the cholesterol level, acts as immune booster and anti- carcinogenic agent. However, it was also reported that high level of saponins may cause gastro- enteritis (Awe & Sodipo 2001). The presence of low quantity of saponins in the two plants confirmed that their consumption could not have detrimental effect on human body. Tannins are known to have antiviral, antifungal, antibacterial and anti- tumour properties (Andzouana & Mombouli, 2012) hence the presence of tannins in *G. hirsutum* and *M. charantia* strongly supports their use in wound treatments, tumours, malaria, virginal discharge (Sofowora 2006 and Taylor 2005). Appreciable quantity of flavonoids, 11.90% and 7.20% are noted in *G. hirsutum* and *M. charantia* respectively. This inferred that the leaves of the plants have biological functions such as anti-oxidant, free radical and anti-tumour (Farquar 1996). In addition, this supports the anti- inflammatory activity of these plants (Okwu 2004) hence their uses in the treatment of wounds, haemorrhoids, liver inflammation, rheumatism and pains. Cardiac glycosides acts on the heart muscles and increase renal flow (diuresis) (Temitope & Omotayo 2012).

The proximate analysis shows that the leaves of the two plants have low moisture content when compared to some common leafy vegetables consumed in Nigeria such as *Celosia argentea* (80%), *Amaranthus cruentus* (86%) by Mensal *et al*. (2008) and *Vernonia calvaona* (37.67%) by Igile *et al*. (2013). However, the leaves have higher values than what was reported for *Gnetum africanum* (9.18%) and *Telfairia occidentalis* (8.64%) by Dike (2010). The low moisture content helps to prevent the leaves from spoilage by microorganism (Adeyeye & Adejuyo 1994). The ash content values of the leaves of *G. hirsutum* and *M. charantia* (18.72% and 14.71% respectively) are favourably compared to the values reported for *M. charantia* leaf (15.42%) by Bakare *et al*. (2010). However, the values were higher than the values 5.54% and 6.14% for *Urera trinervis* and *Hippocratea myriantha* respectively reported by Andzouana and Mombouli (2012). The high ash content is an indication of the level of inorganic minerals and organic matter present in the leaves.

The crude fat content of *G. hirsutum* (6.57%) and *M. charantia* (5.83%) were lower than 15.00% in *Costus afer* and 11.00% in *Cedrela odorata* reported by Asekun *et al*. (2013) but higher than the values reported in spinach leaves, *Cnidoscolus acontifolius* leaves and *Amaranthus hybridus* leaves by Nwaogu *et al*. (2000). The fat content of the leaves were low and it can therefore be recommended as part of weight reducing diets. Gordon & Kessel (2002) reported that low fat foods reduce cholesterol level and obesity. The leaves of the two plants investigated contained appreciable amount of crude fiber of 8.31% and 6.01% for *G. hirsutum* and *M. charantia* respectively. These values were comparable to 8.61% reported for *Amaranthus hybridus* (Akubugwo *et al*. 2007) and 7.63% for *Vernonia calvaona* reported by Igile *et al*. (2013). The crude fiber quantity in these leaves is desirable because adequate consumption of dietary fiber may aid digestion. Fiber softens stools and therefore prevents constipation (Ayoola & Adeyeye 2010). Dietary fiber is also important for lowering serum cholesterol level and reduces the risk of diseases such as coronary heart diseases, hypertension, diabetes and breast cancer (Ishida *et al*. 2000). The crude protein content obtained in the study was high compared to 1.98% reported for *Securinega virosa* leaves (Danlami *et al*. 2012) but considerably low compared to some other vegetables like *Ocimum gratissimum*, *Genetum africanum* and *Vernonia amygdalina* (Dike 2010). The observed values for carbohydrates in *G. hirsutum* (48.66%) and *M. charantia* (57.92%) were high suggesting that the leaves can serve as food. These values were high compared to carbohydrate values obtained in some vegetables such as *U. trimervis* 6.07% (Uzama *et al*. 2012) and 11.55% for *Mucuna poggei* (Oko *et al*. 2012). However, the carbohydrate content values were favourably compared with 54.71%, 55.75%, 59.70%, 53.30% and 54.72% for *Morinda lucida*, *Landolphia hirsuta*, *Elaeis guineensis*, *Pterocarpus soyanxii* and *Vernonia amygadalina* respectively (Dike 2000).

The mineral content in the leaves revealed potassium with the highest values of 38.61mg/ 100g and 32.84mg/100g in *G. hirsutum* and *M. charantia* respectively. The findings agreed with the previous work of Afolabi *et al*. (1995) who stated that potassium is most abundant mineral in Nigerian agricultural products. Potassium plays important roles in diuretics, regulating heart functions (Mclvor *et al*. 1987) and also regulates water and ionic balance in the blood and tissues (NRC 1989). The presence of phosphorus in high quality was notable as it plays a vital role in normal kidney functioning and transfer of nerve impulse. The calcium content was high when compared to the values found in twelve commonly consumed leafy Nigerian vegetables as reported by Mensal *et al*. (2008). Calcium is noted for growth and maintenance of bones, teeth and muscles (Dosumu 1997; Turan *et al.* 2003). In addition, Olayiwola *et al*. (2009) reported calcium to be useful in formation of blood, intracellular and extracellular fluids within and outside body cells. The magnesium content of the leaves as recorded in this study were 8.32mg/100g and 5.88mg/100g in *G. hirsutum* and *M. charantia* respectively which were relative lower than 56.05mg/100g reported by Oko *et al*. (2012) for *M. poggei* leaves

but higher than 2.56mg/100g for *Diospyros mespiliformis* (Hassan *et al*. 2004). Magnesium is an activator of co-enzymes in carbohydrates and protein metabolism (Ahmed & Chandhary 2009). Magnesium also protects and manages high blood pressure and cardiovascular diseases (Rude 1998 and Vormann 2003). The presence of sodium in moderate quantity could be useful in lowering blood pressure. Sodium and potassium found in the intracellular and extracellular fluid helps to maintain electrolyte balance and membrane fluidity (Ahmed & Chandhary 2009).

5. Conclusion

In conclusion, the leaves of *G. hirsutum* and *M. charantia* contained chemical groups, which contribute to the medicinal usefulness in ameliorating various diseases and ailments. The proximate and elemental analyses afford them interesting nutritional properties. The plant leaves can contribute to the nutritional and energy requirements of man.

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