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RESEARCH ARTICLE

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Phytochemical and Mineral Analysis of Methanolic Extract of *Gossypium barbadense L.* (Cotton leaves)

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ABSTRACT

Medicinal plants contain biologically-active compounds which have the capacity to prevent, manage or cure various human and animal diseases. The critical point of decision on the possible potency and efficacy of a drug that is being developed depends on the bioactive components of the source plant. This study was designed to evaluate the phytochemistry and mineral element composition of G. barbadense. The phytochemical analysis of methanolic extract of Gossypium barbadense revealed the presence of alkaloid (3.82±0.17), flavonoid (2.80±0.18), total phenols (5.94±0.41), cyanogenic glycosides (18.07±0 .54) and saponins (7.28±0.19) in mg/100g. Anthraquinones and terpenoids were absent. The mineral element examination showed that iron (544.81 ± 0.0024), magnesium (447.05 ± 0.0051), zinc (13.56 ± 0.0011), calcium (17.76 ± 0.001) and potassium (3.321 ± 0.0027) were present.

Key words: Gossypium barbadense, phytochemical, mineral elements, magnesium, calcium

INTRODUCTION

The cotton plant is an annual herb belonging to the genus *Gossypium* of the *Malvaceae* family. It is a shrub-like herb that grows up to a height of 2 – 5 feet. The plant bears broad three-segmented greenish leaves, which are about 2 - 6 inches in length and emerge alternately on the stem. There are four *Gossypium* species, two diploids from Africa and Asia, *G. herbaceum L.*, *Gossypium arboreum L.*, and two tetraploids from Americas, *G. hirsutum L.* and *G. barbadense L.* [1]. *Gossypium barbadense L.* typically has a longer growing period, and produces smaller bolls that give a significantly low yield [1].

Gossypium barbadense L. also has some medicinal applications in emetics, veneral diseases, tumors, paralysis, epilepsy, convulsions, spasm, and cutaneous and subcutaneous parasitic infection [2]. It has antifungal properties and contains the chemical gossypol, making it less susceptible to insect damage [3-4]. It is also sometimes used as a male anti-fertility drug [5] and as a remedy for neonatal jaundice [6]. The leaves of *G. barbadense* are also reported to be useful in the treatment of hypertension and delayed or irregular menstruation [7]. In a preliminary report [8], gossypol which is an active constituent of *G. barbadense*, was reported to have an *in vitro* antimalarial activity against the human pathogen *Plasmodium falciparum* [9]. This study was, therefore, aimed at evaluating the phytochemistry as well as the mineral element composition of *G. barbadense.*

MATERIALS AND METHODS

Sample Collection
The cotton leaves were collected from Moduganari, area of Maiduguri, (Borno state, Nigeria) and authenticated at the Herbarium section of the Department of Biological Science, Ahmadu Bello University, Zaria, with a voucher no. 453.

Equipment / Chemicals/ Reagents
Equipment (and models of equipment) used in this research work includes Atomic Absorption Spectrophotometer (VARIAN AA240FS), Spectrophotometer (Jenway 6400), Centrifuge (Labofuge 300). Methanol were purchased from Sigma chemical Company, Paderborn - Germany. Other chemicals used where of analytical grade.

Preparation of Methanolic Extract
Leaves of the cotton were shade dried and ground to powder using mortar and pestle. Dried powdered plant material (500 g) was extracted with 2 L of methanol by cold extraction for 24 hours in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered and the filtrate concentrated by evaporation (at 45 ^{O}C).

Qualitative and Quantitative Phytochemical Screening
a. Qualitative Phytochemical Analysis
Test for Phenols
Ferric Chloride Test: Extracts (2g) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Terpenoids
Salkwoski's Test: To 0.5g of extract was dissolved in 2ml of chloroform and few drops of concentrated H_2SO_4 were added to form a lower layer. There was no reddish brown colour at interface indicates absences of a terpenoid.

Test for Anthraquinones
Borntrager's Test, Small portion of the extract was shaken with 10ml of benzene and filtered. 5ml of 10% of ammonia solution was added to the filtrate and stirred. There was no production of pink-red or violet colour indicates the absence of anthraquinones

Test for Alkaloids
Dragendoff's Test; Another 1 ml of extract solution was treated with a few drops of Dragendroff's reagent giving rise to an orange precipitate indicating the presence of Alkaloids [10].

Test for glycosides
Glycosides: The extracts were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycosides [11].

Cyanogenic glycoside: Extract (5 g) was mixed with 20 mL of water and heated in water bath with sodium picrate paper. A change from yellow to orange is positive for cyanogenic glycoside [12].

Test for Saponins
Froth test; The extract was dissolved in 3ml of ethanol and mixed with 10ml of distiled water in a test tube. The tube was shaked vigourously and allowed to stand for 30 min. Honey comb froth was observed [13].

Test for Flavonoids
Schinoda's Test; Small amount of magnisium chips were added to the 2ml of the extract solution followed by a few drops of concentrated hydrochloric acid. Appearence of an orange, pink or red colour indicate the presence of flavonoids.

b. Quantitative Phytochemical Determination
Determination of Total Polyphenol Contents: The total polyphenol content of the plant extracts was estimated using Folin-ciocalteu phenol reagent according to the method described by [14].

Alkaloid Determination: The alkaloid content of the plant sample was determined according to the method of [15].

Determination of Cyanogenic glycoside: Determination of Cyanogenic glycoside was carried out according to the method described by [16].

Saponin Determination: The saponin concentration was determined using the method described by [17].

Flavonoid Determination: The flavonoid concentration was determined according to the method of [18].

Quantitative Determination of Mineral Content of *G. barbadense* Leaves

A modified method of [19] was used. The samples in the powdered form were accurately weighed and digested in (4:1) mixture of nitric acid and perchloric acid. After digestion few drops of concentrated HCl was added. The solution was heated gently and then filtered. The residue was again subjected to digestion and filtrate was collected. The entire filtrate was diluted suitably with distilled deionized water. The dilute filtrate solution was used for analysis of elements of interest (Fe, Mg, K, Na, Zn). The principle was based on the fact that, radiation of a characteristics wave length from a hollow cathode lamp was passed through the flame and the absorbance reading which are proportional to the concentration of the element in question were recorded.

RESULTS

Phytochemical Contents of Methanolic Extract of G*ossypium barbadense* Leaves.

The result from tables 1 and 2 shows the qualitative and quantitative phytochemical composition of methanolic extract of *Gossypium barbadense* respectively. The qualitative phytochemical screening of *G. barbadense* showed the presence of alkaloid, flavonoid, total phenolic, cyanogenic glycoside and saponin, while anthraquinone and terpenoid were absent. The quantity of each phytochemical was determined in which the cyanogenic glycosides was found to be the highest followed by saponin and total phenolic. Alkaloid was found to have the least quantity.

Table 1: Qualitative Phytochemical Contents of Methanolic Extract of *Gossypium barbadense* (Cotton Leaves*)*

Phytochemicals	Chemical tests	Bioassay
Alkaloids	- Dragendorf's	+
	- Wagner's	+
Flavonoids	- Shinoda	+
	- Ferric chloride	+
Anthraquinones	- Borntrager's reaction	-
Total Phenolics	- Folin- Ciocalteu's	+
Cyanogenic glycosides	- Keller-Killani's	+
Terpenoids	- Salkowski's	-
Saponins	- Frothing	+

Key: '+' Present, '-' Absent

Table 2: Quantitative Phytochemical Contents of Methanolic Extract of Cotton Leaves (*Gossypium barbadense)* in mg/100g

Phytochemicals	1	2	3	Mean ± SD
Alkaloid	3.67	4.00	3.80	3.82±0.17
Saponin	7.22	7.49	7.13	7.28±0.19
Cyanogenic glycosides	18.61	17.56	17.88	18.07±0.54
Flavonoids	2.62	2.80	2.98	2.80±0.18
Total phenolics	6.03	6.30	5.49	5.94±0.41

Each value represents the mean ±SD from triplicate determination

Mineral Composition of Methanolic Extract of G. *barbadense*

The quantitative composition of mineral elements present in methanolic extract of cotton leaves (*Gossypium barbadense)* is shown in table 3. Iron and magnesium were found to be the predominant mineral with other minerals such as calcium, zinc and potassium all present.

Table 3: Quantitative Mineral Composition of Methanolic Extract of Cotton Leaves (*Gossypium barbadense)*

Elements	Composition in ppm (µg/cm)^3
Calcium	17.76 ± 0.001
Iron	544.81 ± 0.0024
Magnesium	447.05 ± 0.0051
Zinc	13.56 ± 0.0011
Potassium	3.321 ± 0.0027

Each value represents the mean ±SD from triplicate determination.

DISCUSSION

Preliminary phytochemical screening carried out in this study indicated that *G.barbadens* leaves contain flavonoid, phenols, alkaloids, glycosides, and saponins in its methanolic extract. Glycosides and saponins have been documented to significantly increase the proliferation abilities of bone marrow cells [20-21], anti-diarrheal effect [22]. Alkaloids are reported to have analgesic, antimicrobial and bactericidal effects [23-24] and may therefore combat infections and pathological condition. Phenolic compounds, which are widely distributed in plants, were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems [25-26]. Flavonoids are also recognized for their antioxidant properties [27-28]. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis and inflammation [29].

Magnesium (Mg) is an important cofactor in the activation of B-vitamins. Specifically, vitaminB_{12} and folic acid are important for final maturation of RBC's [30]; lack of which leads to diminished DNA and resulting in the production of larger than normal RBC. It may therefore be essential in the design of sickle cell disease drug. Iron (Fe) is an essential mineral element that has a direct stimulatory effect in erythropoiesis (hemoglobin synthesis). Calcium (Ca) is a reputed mineral element that builds and maintains bones. Osteoporosis is a well-recognized disease of the bone in which the bone becomes porous, breaks easily and heals slowly. Systemic deficiency of calcium is a major contributor to this disease. The potassium (K) content of *G. barbandense* may played a central role in homeostatic regulation by keeping a normal water balance between cell and body fluids. Although zinc (Zn) plays a significant role in carbohydrate and protein metabolism among other functions.

CONCLUSION

In conclusion, the significance of determining the bioactive components (phytochemicals and mineral elements) of plants cannot be overstated, as it will not only give scientific backing to traditional medicine, but will help pharmaceutical companies in the design and development of drugs.

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