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Research Article

ANTIMICROBIAL PROPERTY, PHYTOCHEMISTRY AND NUTRITIONAL PROFILES OF *GOSSYPIUMHIRUSITIUM*

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ABSTRACT

The leaves of a malvacae-*Gossypiumhirusitium* was subjected to proximate analyses and amino acid analyses using standard procedures. Determination of amino acid profile consisted of hydrolysis of proteins to constituent amino acids followed by the quantitative estimation of the amino acids in the hydrolysate. Crude protein, fibre, ash, nitrogen free extracts (fat) and moisture contents were determined by AOAC methods. Anti-nutritional composition and antibacterial properties of the plant was determined using standard procedures. Chromatogram peaks of the hydrolysate showed that the leaf of the plant contains all essential amino acids that would avert risk associated with amino acid deficiencies in a diet. The nutritional analysis of the plant showed a high level of protein (36%). The crude fibre (26%) and ash (22%) are high and may aid bowel movement and increase mineral contents respectively. The quantitative phytochemical analysis revealed the presence of Alkaloid (0.89%), Oxalate (0.14%), Cyanogenic glycosides (1.02mg/100ml), Phytic acid (5.53mg/100ml) and Tannin (0.35mg/100ml). The antimicrobial activity of the plants tested on *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli* and *Pseudomonas aeruginosa* revealed that the plant had a low activity on most of the organisms at a low concentration but the activity was better at higher concentrations. Although this plant is cultivated for its fibre, it is now indicated to contain amino acids to consumers which use its leaves for tea for phytotherapy. It is therefore concluded that the leaf of *G. hirusitium* is a complete protein and regular consumption of the leaf and its concoctions would boost amino acid levels in the body and fight against some invading pathogenic bacteria.

INTRODUCTION

Gossypiumhirsutum is a plant under the family Malvacae (Smith 1999). This family is primarily grown as fibre crop. It is harvested as 'seed cotton' which is then 'ginned' to separate the seed and lint. The long 'lint' fibres are further processed by spinning to produce yarn that is knitted or woven into large volume of textile products-fabrics. The ginned seed is covered in short, fuzzy fibres, known as 'linters'. These must be removed before the seed can be used for planting or crushed for oil, and are also used in a variety of products including foods. *Gossypiumhirsutum* species is considered the most important of cotton yielding plants, providing the bulk of the commercial cottons. (Taiye *et al*., 2011). Cotton is currently the leading plant fibre crop worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries including Nigeria (Smith 1999).

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Despite its use as a fibre crop, this plant has been in use in traditional medicine (Taiye *et al*., 2011), for several infectious diseases however, their active principle(s) has not been elucidated. Mucilaginous teas of fresh or roasted seeds of *G. hirsutum* have been used for bronchitis, diarrhoea, dysentery, and hemorrhage including an antiviral activity against a number of enveloped viruses, including the AIDS virus (Vander Jagt*et al*., 2000). The plants have also been reported to have an antiparasitic activity (Sotelo *et al*., 2005). Human consumption of cotton seed meal is reported mainly in Central American countries and India where it is used as a low cost, high quality protein ingredient (Franck 1989; Ensminger*et al*. 1990). Amino acids are monomers of protein that plays a major role in maintaining the integrity of so many body structures as well as playing an important role in metabolism. They are classified into essential and non-essential amino acids. Essential amino acids are obtained from nutrition and non-essential amino acids are synthesized by the body. Figure 1 shows the essential and non-essential fatty acids.

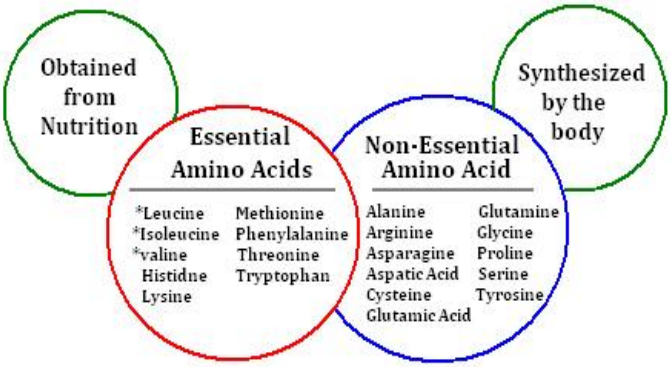

Figure 1. Essential and non-essential amino acids

This research is poised to analyse the leaf of *Gossypiumhirusitium* for its antimicrobial activity, phytochemistry and nutritional composition.

MATERIALS AND METHODS

Sample collection

The leaves of *Gossypiumhirusitium*were obtained from Onitsha, an eastern part of Nigeria located at 6.1667° N, 6.7833° E, and identified by Dr. Paul Obi, a Pharmacognocist at the main market in Anambra State, Nigeria. The sample was packaged and transported to the laboratory for analyses.

Antimicrobial susceptibility testing

Clinical cultures (24h) of *Salmonellatyphi*, *Escherichiacoli*, *Shigelladysentariae* and *Pseudomonas aeruginosa* obtained from the Anthony Van Leuwenhoek research laboratory at Nekede, Imo State were used for the antimicrobial susceptibility testing using the agar well diffusion technique (Cheesbrough, 2009). The turbidity of the inoculums were equivalent to 0.5 McFarland's standard. Each organism was inoculated into the Mueller Hinton agar using the spread plate method. Sterile cork borers were used to produce wells into a seeded Mueller Hinton agar. The different concentrations of the plant extract were introduced into each well at a volume of 0.1ml. The resulting plate was incubated at a temperature of 37^0C for 24 hrs. The zones of inhibition were measured using a metre rule to the nearest mm.

Qualitative phytochemical analysis: Qualitative phytochemical analysis of petroleum ether, methanol, benzene, chloroform and aqueous extracts of *G. hirusitium* was conducted following the standard procedures (AOAC, 2006).

Quantitative phytochemical analysis: The phytochemicals which are present in the methanol extract of *G. hirositium* was determined and quantified by standard procedures.

Phytic Acids (Phytate) Determination

The method adopted was as described by Harbone (1980). The plant material was extracted and was made up to give up to 3-30 mgml-1 phytate solution. 0.5ml of extract was pipetted into a test tube filled with a ground glass stopper. Into 1ml of Ferric solution was added, the tube was covered with the stopper and fixed with a clip. The tube was heated in a boiling water bath for 30 minutes. Within the first 5 minutes, care was taken that the tube remained well stoppered. The set up was covered in ice water for 15 minutes. It was then allowed to adjust to room temperature. Once the tube has reached room temperature, about 2ml of 2, 2-Bipyridine solution was added to the test tube and mixed. The bipyridine reacted with the iron phytate and the color changed with time. The absorbance was measured after 1 min at 519 nm against distilled water.

Determination of total tannins

The tannin content of the plant were determined by the method described in Munro and Basir (1969).Five hundred milligram (500 mg) of the sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker.
This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Determination of Alkaloids

The gravimetric methods of Harbone (1980) were adopted. A given weight (5.0g) of each sample was weighed out and dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hours before filtering. The filtrate was evaporated to one quarter (¼) of its original volume. Then a concentrated NH_4OH was added drop-wise to precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper. The precipitate in filter paper was dried in an oven at 60^oC for 30 minutes and reweighed. The weight of alkaloids is normally determined by weight difference.

The percentage alkaloids were calculated thus:

$$percentage\ alkaloid = \frac{W_2 - W_1}{W}$$

Where W = weight of sample
W1 = weight of empty filter paper
W2 = weight of paper plus precipitate

Determination of Cyanogenic Glycosides

The alkaline picrate method was used according to Harbone (1980). Five grams (5.0 g) of the sample was ground to paste. The paste was dissolved in 50 ml distilled water in a corked conical flask and allowed to stay overnight. The extract was filtered and the filtrate used for the cyanide determination. Alkaline picrate solution was prepared by dissolving 1g picrate and 5 g sodium carbonate in a volume of minimally warm water and then made up to 200 ml with distilled water. Exactly 1 ml of sample filtrate was put into a test tube and corked firmly. This was followed by addition of 4 ml of alkaline picrate and incubated in a water bath for 5 minutes. After colour development (reddish brown colour), the absorbance read at 490 nm using a Spectrophotometer against a blank solution which contained only 1ml distilled water and 4ml alkaline picrate solution.

Determination of oxalates

The oxalate was determined by the method described in Munro and Basir (1969). The oxalate was extracted with dilute HCl at

50^0C and treated with ammonium hydroxide and glacial acetic acid and then further treated with $CaCl_2$ solution. Precipitated calcium oxalate which was solubilized with hot dilute H_2SO_4 was titrated against $KMnO_4$ as equivalent to 2.2 mg of Oxalate.

Nitrogen determination

A small amount, (200mg) of ground sample was weighed, wrapped in whatman filter paper (No. 1) and put into the Kjeldhal digestion flask. Concentrated sulfuric acid 10ml was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), Copper sulphate ($CuSO_4$) and selenium sulphate (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put into the Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (100ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then treated with standardized 0.01N HCl to grey coloured (James, 1995; Teferra *et al*., 1997; Chang, 2003).

$$Percentage\ Nitrogen = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

where:

$a = titre\ of\ the\ digested\ sample;\ b = titre\ value\ of\ the\ blank\ sample;$
$V = volume\ after\ dilution(100ml);\ W = weight\ of\ dried\ sample\ (mg);$
$C = Aliquot\ of the\ sample\ used(10ml);\ 14 = nitrogen\ constant\ in\ mg$

Determination of amino acid profile

The amino acid profile in the known sample was determined using methods described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the technicon sequential Multi-sample Amino acid Analyser (TSM).

Defatting the sample

The sample was defatted using chloroform methanol mixture of ratio 2:1. Four grams (4g) of the sample was put into extraction thimble and extracted for 15 hours in the soxhlet extraction apparatus (AOAC, 2006)

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7ml of 6NHCl was added and Oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105^0C ± 5^0C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humus. It should be noted that tryptophan is destroyed by 6NHCl during hydrolysis. The filtrate was then evaporated to dryness at 40^0C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which are kept in the freezer.

Loading of the hydrolysate into TSM analyzer

The amount loaded was between 5 to 10 microlitre. This was dispended into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes.

Method of calculate amino acid values from the chromatogram peak

An integrator attached to the analyzer calculates the peak area proportion to the concentration of each of the amino acids. Alternatively, the net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height with the width at half height.

The Norleucine Equivalence (NE) for each amino acid in the standard mixture was calculated using the formula

$$NE = \frac{Area\ of\ norleucine}{Area\ of\ each\ amino\ acid}$$

A constant S_{std} was calculated for each amino acid in the standard mixture:

$$where\ S_{std} = NE_{std} \times molecular\ weight\ \times \mu MAA_{std}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the formula

$$Concentration\left(\frac{g}{100g}protein\right) = NH \times W@NH/2 \times S_{std} \times C$$

$$where, C = \frac{dilution \times 16}{smple\ wt(g) \times N\% \times 10 \times Vol.\ loaded} \div NH \times W(nleu)$$

where NH = net height; W = width @ half height and nleu = Norleucine

Proximate analysis

The moisture, crude protein, crude fat, total ash and crude fibre contents of the sample was determined using Standard methods of the Association of Official Analytical Chemists (AOAC, 2006). Moisture content was determined by heating 2.0g of each fresh sample to a constant weight in a crucible placed in an oven maintained at 105 EC. The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogen x 6.25) was determined by the Kjeldahl method, using 2.0g samples; crude fat was obtained by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. Ash was determined by the incineration of 10.0g samples placed in a muffle furnace maintained at 550°C for 5h. Crude fibre was obtained by digesting 2.0g of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550°C for 5h. Moisture content was determined by heating 2.0g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. Each analysis was carried out in duplicates.

RESULTS

The amino acid analysis quantified proteins (expressed in g/100g of protein) revealed that the plant contained both essential and non essential amino acids (Table 1).

The antimicrobial activity of the plants tested on *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli* and *Pseudomonas aeruginosa* revealed that the plant had a low activity on most of the organisms at a low concentration but the activity was better at higher concentrations. The plant had the least activity on *Salmonellatyphi* with a zone of inhibition of 12mm at

Table 1. amino acid analyses of *Gossypiumhirusitium*

1	2	3	4	5	6=(2×4×5×C)
Amino acid	Net height	NH/2(mm)	Width@NH/2 (mm)	S_{std}	Concentration: g/100g protein
Lysine	69	34.5	4	13.00	3.73
Histidine	30	15	4	15.18	1.89
Ammonia					
Arginine	29	14.5	18	9.23	5.01
Aspartic acid	143	71.5	3.5	8.53	8.87
Threonine	61	30.5	3.5	7.59	3.37
Serine	44	22	3.5	7.46	2.39
Glutamic acid	66	33	6	12.15	10.00
Proline	14	7	7	13.99	2.85
Glycine	74.5	37.25	4	5.78	3.58
Alanine	52	26	4	9.14	3.95
Cystine	6.5	3.25	7	9.10	0.86
Valine	66.5	33.25	3	9.29	3.85
Methionine	24	12	2.5	10.03	1.25
Isoleucine	41	20.5	3.5	8.72	2.60
Leucine	110	55	3.5	7.51	6.01
Norleucine	70	35	5.5		
Tyrosine	7	3.5	10	15.28	2.22
Phenylalanine	23.5	11.75	8	10.51	3.96

Wt. of sample hydrolyzed=0.8811g; Dilution= ×5; %N (fat free) =3.27.

Table 2. Antimicrobial activity of *Gossypiumhirusitium*

Concentration of extract (mg/ml)	Diameter of zone of inhibition (mm)			
	Salmonella typhi	*Shigella dysenteriae*	*Escherichia coli*	*Pseudomonas aeruginosa*
250	12	16	14	17
125	4	9	8	*16*
62.5	No inhibition	8	8	*11*
31.625	No inhibition	No inhibition	4	*3*
Ciprofloxacin (50)	21	16	20	*29*
Ampiclox (25)	27	34	28	*23*
MBC	250	125	125	125

Table 3. Quantitative phytochemical composition of plant extract

Alkaloid (%)	Oxalate (%)	Cyanogenic Glycoside(mg/100ml)	Phytic acid (mg/100ml)	Tannin (mg/100ml)
0.89	0.14	1.02	5.53	0.35

The proximate analysis depicts that the plant contains 26% crude fibre, 10% moisture, 22% ash, 6% fat and 36 percent crude protein. The crude protein was the highest with a percentage of 36% compared to fat (6%) which was the least (Fig 2).

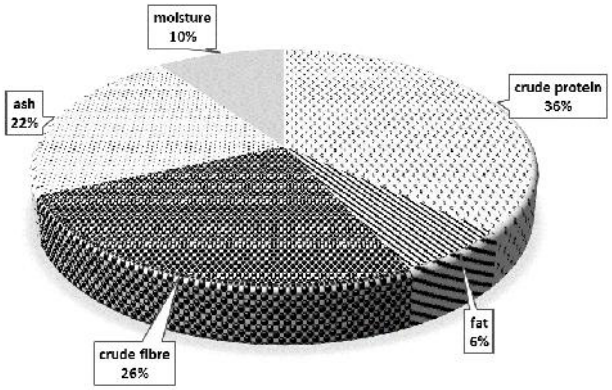

Figure 2. proximate composition of *Gossypiumhirusitium*

250mg/ml but also had the best activity on *Pseudomonas aeruginosa* with a zone of inhibition of 17mm at 250mg/ml (Table 2). The plant had a low activity compared to the conventional beta-lactam antibiotic (Ampiclox) and the fluoroquinolone (Ciprofloxacin). The quantitative phytochemical analysis shown in Table 3 revealed the presence of Alkaloid (0.89%), Oxalate (0.14%), Cyanogenic glycosides (1.02mg/100ml), Phytic acid (5.53mg/100ml) and Tannin (0.35mg/100ml).

DISCUSSION

The proximate composition or the leaf of *G. hirusitium*shown in Figure 2 reveals high levels of protein, fibre and ash. High proteins (36%) indicate high nutritional qualities, and could serve as supplement in animal feeds and human diet. The high protein content of the leaf of some plants had been reported (Braide*et al*., 2010). Crude fibre is high and this could aid bowel movement and prevent gastrointestinal problem.

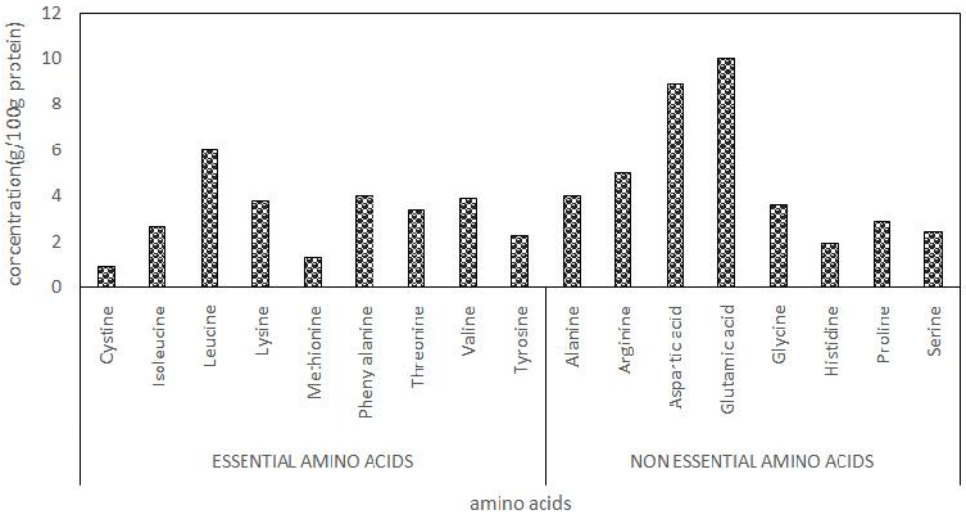

Figure 3. essential and non-essential amino acids present in *Gossypiumhirusitium*

The ash content is high indicating a high mineral content. The amino acid analysis contents compares well with the WHO profile, indicating protein of high biological values, thus would support growth of children and tissues maintenance in adults. In addition, since the product is rich in protein, it can be used as an alternate source of protein and as a protein complements in developing countries confronted with problems of malnutrition resulting from high cost of conventional protein foods. The plant contains all the essential amino acids in adequate amount (Table 1). The importance of amino acids in the synthesis of protein and its quality had been reported (Wardlaw and Kessel, 2002; Delvin, 2006. Wardlaw and Kessel (2002) also reported on the importance of protein in the regulation and maintenance of the body.

Essential and nonessential amino acids are hydrocarbons derivatives consisting of nitrogen and oxygen. These simple molecules play an important role in the human body and metabolism (Hosomi*et al.*, 2011; Young and Ajami. 2000; Wu *et al.*, 2009; Tomporowski, 2009). The 22 known amino acids, essential and nonessential, affect a broad range of physical and mental processes. Recent studies have witnessed the discovery that amino acids are cell signalling molecules as well as being regulators of gene expression and the protein phosphorylation cascade (Wu *et al.*, 2009). Dietary supplementation with one or a mixture of these amino acids may be beneficial (Hosomi*et al.*, 2011; Young and Ajami. 2000; Tomporowski, 2009) for ameliorating health problems at various stages of the life cycle (e.g., fetal growth restriction, neonatal morbidity and mortality, weaning associated intestinal dysfunction and wasting syndrome, obesity, diabetes, cardiovascular disease, the metabolic syndrome, and infertility) (Wu *et al.*, 2009).

Although the plant has the essential and non-essential amino acids, its consumption has been proven to be detrimental. Gossypium seeds have been reported to have detrimental effects on humans as well as some other monogastric animals. The antinutritional properties of the leaf of the plant had been demonstrated with animals fed with the cotton seed product (Eisele, 1986; Blom*et al.*, 2001). This is because cotton seeds contain a polyphenolic compound called gossypol. Cotton (*Gossypiumhirsutum* L.) and related species have pigment glands located throughout the plant in which this compound is also present (Adams *et al*., 1960).

Gossypol, a compound initially isolated from *Gossypiumhirsutum*L. (Malvacea) seeds have been studied mainly in relation to its reversible antifertility effects in men (Sotelo *et al*., 2005), diverse pathogenic agents, such as *Trypanosomacruzi*(Abe *et al*., 2004), *Plasmodium falciparum* (Tripathi*et al*., 2004), *Edwardsiellaictaluri*(Yildirim-Aksoy*et al*., 2004). Gossypol, cyclopropenoid fatty acids and other related compounds, are an integral part of cotton's self-defense mechanism and protect the plants from pests and possibly some diseases (Bell and Stipanovic, 1977; Hedin *et al*., 1992; Jenkins and Wilson, 1996). *Gossypiumhirusitium* had the least activity on *Salmonellatyphi* with a zone of inhibition of 12mm at 250mg/ml but also had the best activity was recorded on *Pseudomonas aeruginosa* with a zone of inhibition of 17mm at 250mg/ml. The plant had a low activity compared to the conventional beta-lactam antibiotic (Ampiclox) and the fluoroquinolone (Ciprofloxacin) (Table 2).

The quantitative phytochemical analysis revealed the presence of Alkaloid (0.89%), Oxalate (0.14%), Cyanogenic glycosides (1.02mg/100ml), Phytic acid (5.53mg/100ml) and Tannin (0.35mg/100ml). The presence of alkaloids in low quantity contributes to the low activity on microorganisms. Alkaloids have a wide range of applications. They are reported to have analgesic, antimicrobial and bactericidal effects (Stary, 1998; Okwu and Okwu, 2004) and may therefore combat infections and pathological condition. Phenolic compounds and oxalates, 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 (Wang *et al*., 2000; Stanner*et al*., 2004). Comparing the results with the phytochemical analysis of methanolic extract of *Gossypiumbarbadense* (another species of Gossypium) the plant had been reported to contain 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 (Muhammad *et al*., 2014).

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