



Preliminary Study on Phytonutrient Analysis of Gossypium Seeds

Krishnaveni M^{*1}, Dhanalakshmi R²

^{*1}Assistant Professor, Department of Biochemistry, Periyar University, Salem, Tamilnadu, India.

²M.Phil Student, Department of Biochemistry, Periyar University, Salem, Tamilnadu, India.

*Corresponding author's E-mail: krishnavenim2011@gmail.com

Accepted on: 24-08-2014; Finalized on: 31-10-2014.

ABSTRACT

Cotton is a leading plant fiber crop worldwide, grown in temperate and tropical regions of 50 countries. Cotton seed is valuable food stuff for cattle. For the present study, the aqueous extract was prepared from Gossypium seeds and subjected to qualitative analysis for its phyto-constituents, quantitative analysis for its nutrients, yield in percent, fluorescence study, and behavior of Gossypium seed powder from aqueous extract. The results obtained showed positive results for carbohydrate, alkaloids, glycosides, saponins, tannins, proteins, amino acids, fixed oil etc. The carbohydrate content was found to be 32.66 ± 2.30 mg/g, protein content was 04.84 ± 0.15 mg/g, amino acid content was 01.06 ± 0.23 mg/g. The recovery percent of the extract was 10.50, no fluorescence was observed, presence of alkaloid, steroid, flavonoid, protein was observed with different chemicals. From the present study, it is confirmed that, aqueous extract of Gossypium seed was found to contain chemicals which are therapeutic in value.

Keywords: Extract, Gossypium, Nutrients, Phytoconstituents, Seed, Water.

INTRODUCTION

Malvaceae is a family, comprises 243 genera and containing 4225 species of herbs, shrubs, trees. Gossypium (cotton) is economically most important plant¹ comprises of around 50 species.² The genus name is derived from Arabic word goz means a soft substance.³ Cotton was first cultivated in 5th millennium BC⁴ by Indus valley civilization. It is grown mainly for fiber. The short cellulose fibers are used for the making of coarse yarns and many cellulose products. The outer coverings of seed are used as roughage in ruminant animal feed and oil extracted from seed kernel is used for cooking, salad dressing due to its flavor stability. The remaining cake is added as a supplement in cattle and swine feed. Chemical analysis of cotton seed meal showed lignin, cellulose, amino acids, proteins, Polyphenolic compounds⁵ containing carboxyl, hydroxyl, methyl groups and anionic, cationic moieties which offer therapeutic effects such as antimicrobial, anti-inflammatory, anticancer, antioxidant activities and pain reliever. Hence, an initiative has been taken to analyze qualitatively and quantitatively the nutrient content of Gossypium seeds and various other parameters to know in detail about its properties and also to validate the compounds present.

MATERIALS AND METHODS

Sample collection

The sample-Dry Gossypium seeds were purchased from shops at Krishnagiri, Krishnagiri District, Tamil Nadu, India. The purchased seeds were cleaned thoroughly and ground to powder for further use.

Aqueous extract preparation

Aqueous extract was prepared by dissolving 15g of Gossypium seed powder in 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20 minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage yield and the remaining marc left was extracted with water for qualitative analysis.

Phytochemical analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures^{6,7} fluorescence analysis,⁸ behavior of drugs powder with different chemical reagents.⁹

Test for carbohydrate

Molisch's test

To the extract added few drops of alcoholic alpha naphthol solution, few drops of concentrated sulphuric acid along the sides of test tube. Positive result gives purple or violet colored ring at the junction.

Fehling's test

To the extract added equal amount of Fehling's A and B solution, heat the tubes in a boiling water bath. Brick red precipitation of cuprous oxide is formed, if reducing sugar is present.

Benedict's test

To the extract add Benedict's reagent, the tubes were heated in a boiling water bath. Red precipitation indicates positive result.



Test for alkaloids**Wagner's test**

To the extract add few drops of iodine solution in potassium iodide. Reddish brown precipitate shows positive result.

Hager's test

To the extract add few drops of saturated solution of picric acid. Yellow color precipitation signifies positive result.

Test for steroids and sterols**Libermann-Burchard test**

To the extract add 2ml chloroform, 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid. Bluish red to cherry red color in chloroform layer shows positive result.

Salwoski test

To the extract add few drops of chloroform, concentrated sulphuric acid. Bluish red to cherry red color.

Test for Glycosides**Legal test**

To the extract added pyridine, sodium nitroprusside. Positive result shows pink red colour.

Baljet test

To the extract add picric acid. Appearance of orange color signifies positive result.

Test for saponins**Foaming test**

Foams produce when the extract is shaken with water.

Test for flavonoids**Shinoda test**

To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.

Zinc hydrochloride test

To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.

Test for tannin and phenolic compounds**Ferric chloride test**

To the extract add ferric chloride. Formation of greenish black color shows positive result.

Potassium dichromate test

To the extract add potassium dichromate solution. Positive result is confirmed by a formation of brown precipitate.

Gelatin test

To the extract add 1% gelatin solution containing 10% sodium chloride gives white precipitation.

Test for protein and amino acids**Biuret test**

To the extract added 4% sodium hydroxide, few drops of 15% copper sulphate gives purple colour.

Ninhydrin test

Bluish violet color forms when a solution of ninhydrin and extract mixture was heated.

Heat test

Protein coagulation shows positive result when test solution is heated on a boiling water bath.

Test for fixed oil**Copper sulphate test**

Blue colour forms when the extract is mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Quantitative analysis of phytonutrients

Total carbohydrates,¹⁰ proteins,¹¹ aminoacids¹² were performed according to the standard prescribed methods.

Estimation of carbohydrate

The total carbohydrate was estimated by Anthrone method. 1mg of Gossypium seed powder was hydrolyzed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cool to room temperature. After neutralizing, the contents were centrifuged and 0.1ml of supernatant was used for the analysis. To the sample add 4ml of anthrone reagent and the contents were heated in a boiling water bath for 8 minutes. The tubes were cooled and read at 630nm using spectrophotometer Shimadzu-Model-UV 1800. The standards were developed with glucose. Standard graph plotted was used to find out the concentration of glucose present in the hydrolyzed extract.

Estimation of protein

The total protein was estimated by Lowry's method. To 0.1ml of extract add 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of folin ciocalteau reagent (diluted in the ratio of 1: 2) was added after 30minutes of incubation, the contents were read at 660nm using spectrophotometer Shimadzu-Model UV 1800. The standards were developed with Bovine serum albumin. Standard graph plotted was used to calculate the concentration of protein present in the extract.

Estimation of amino acid

The amino acid present was estimated by Ninhydrin method. To 0.1ml of sample added 1ml of Ninhydrin



solution dissolved in ethanol. Cover the test tube with a piece of paraffin film to avoid the loss of solvent due to evaporation. With gentle stirring, allow the contents to react at 80-100°C for 4-7 minutes. Cool the test tubes and the color developed was read at 570nm. Tyrosine was used for developing standards. From the standard graph obtained, the amino acid content in the extract was calculated.

Statistical Tool

Each experiment was carried out in triplicate and the results are given as the Mean \pm Standard deviation. The Mean and Standard deviation (S) was calculated by using

Table 1: Percentage yield of Gossypium seed aqueous extract

Name of the seed powder	Weight taken for extraction	Initial weight of the beaker (gm)	Final weight of the beaker (gm)	Weight of the extract powder (gm)	Recovery (%)
Gossypium	15g in 200ml water	180.3303	181.9064	1.5761	10.50

The percentage recovery of the aqueous extract obtained was calculated and expressed in Table 1. The percent recovery was found to be 10.50 from 15gm seed powder. The total weight of the extract was 1.5761.

Analysis of seed powder for its behavior

Table 2: Behavior of Gossypium seed powder with different chemical reagents

Tests	Observation	Inference
Powder + Picric acid	Yellow color	Presence of alkaloid
Powder + Conc. H ₂ SO ₄	Reddish brown color	Presence of steroids
Powder + Iodine solution	Pale brown colour	Absence of starch
Powder+ Aqueous 5% KOH	Pale brown color	Presence of anthroquinone
Powder + NaOH	Yellow colour	Presence of flavonoid
Powder + Aqueous AgNO ₃	White precipitate	Presence of protein

The results of Gossypium seed powder behavior is shown in Table 2. The behavior of seed powder with different chemicals showed positive result for alkaloid, steroid, anthraquinone, flavonoid, protein.

Fluorescence Analysis

Table 3: Fluorescence analysis of aqueous Gossypium seed extract

Name of the extract	Day light	UV light
Aqueous	Light purple color	No fluorescence

The results of fluorescence analysis are depicted in Table 3. The Gossypium seed powder extracted with water was pale purple in color when observed in day light. The same when viewed under UV light, no fluorescence was observed.

the following formula: Mean = Sum of x values / n
(Number of values), $s = \frac{\sqrt{\sum(x-M)^2}}{n-1}$

RESULTS AND DISCUSSION

The results obtained are shown in Table 1 to Table 5. The yield was tabulated in Table 1. The results obtained for the analysis of seed powder with different chemical reagents are shown in Table 2. The results of fluorescence analysis of aqueous Gossypium seed extract is presented in Table 3. Phytochemicals assessed qualitatively are expressed in Table 4. Table 5 shows the results of nutrients analyzed.

Table 4: Qualitative analysis of phytochemicals in aqueous Gossypium seed extract

Name of the test	Results
Test for carbohydrate:	
a) Molisch's test	+++
b) Fehlings test	+
c) Benedicts test	++
Test for alkaloids:	
a) Wagners test	+
b) Hagers test	++
Test for steroids and sterols	
a) Libermann - Burchard test	-
b) Salwoski test	+
Test for Glycosides	
a) Legal test	++
b) Baljet test	+++
Test for saponins	
Saponin test	++
Test for flavonoids	
a) Shinoda test	+
b) Zinc hydrochloride test	-
Test for tannin and phenolic compounds	
a) Ferric chloride test	+
b) Potassium dichromate test	++
c) Gelatin test	-
Test for protein and amino acids	
a) Biuret test	++
b) Ninhydrin test	+++
c) Heat test	+
Test for fixed oil	
a) Copper sulphate test	+++

+ Slight changes, ++ Moderate, +++ Stronger reactions

Phytochemical analysis

The results of phytochemical analysis are shown in Table 4.



Results showed positive result for carbohydrate, alkaloids, steroids, glycosides, saponin, flavonoid, tannin, phenolic compound, protein, amino acids, fixed oil. (Table 4)

Phytonutrient Analysis

The phytonutrients estimated were tabulated in Table 5.

Table 5: Phytonutrients in aqueous *Gossypium* seed extract

Phytonutrients	Calculated nutrient content (mg/g)
Total carbohydrate	32.66 ± 2.30
Total protein	04.84 ± 0.15
Amino acids	01.06 ± 0.23

Values are Mean ± SD for three experiments

The total carbohydrate content observed was 32.66 ± 2.30mg/g carbohydrate, likewise, the total protein, amino acid content was found to be 04.84 ± 0.15mg/g albumin, 01.06 ± 0.23mg/g tyrosine. Here, the carbohydrate content was higher when compared to total protein and amino acid content. The *Gossypium* seeds have been reported for its phyto-constituents, fatty acid profile through GC-MS analysis, antimicrobial studies,¹³ and also for its antioxidant activities by Krishnaveni et.al.¹⁴

CONCLUSION

Present study revealed the recovery percent, seed behavior, fluorescence nature, phytochemical, nutrient analysis. These results confirmed that it contains carbohydrate, protein, amino acids both qualitatively and quantitatively. From the results obtained, it can be concluded that *Gossypium* seeds can be used as a natural remedy for many ailments.

Acknowledgement: The author wishes her thanks to Honorable Vice-chancellor Dr. C. Swaminathan Avl and Registrar Dr. K. Angamuthu Avl, Periyar University, Salem for their administrative support and excellent infrastructure facilities provided and also thank Dr. V. Raj, Professor and Head, Department of Chemistry, Periyar University, Salem for his help.

REFERENCES

- Berry PE, Malvaceae: Encyclopedia Britannica Online, Encyclopedia Britannica Inc, 2012.
- Wendel JF, Brubaker C, Alvarez I, Cronn R, Stewart JM, Evolution and natural history of the cotton genus, In Peterson AH, editor, Plant Genetics and Genomics: Crops and Models, 2009.
- Gledhil D, The names of plants, 4th edition, Cambridge: Cambridge University Press, 2008, 182.
- Senchina DS, Alvarez I, Cronn RC, Liu B, Rong J, Noyes RD, Andrew H Paterson, Rod A Wing, Thea A Wilkins, Jonathan F Wendel, Rate variation among nuclear genes and the age of polyploidy in *Gossypium*, Mol Bio Evol, 20, 2003, 633-643.
- Vollmann, Rajcan, In oil crops Ist edition, Springer, Berlin, Germany, 2009, 14-25.
- Harborne JB, Phytochemical methods, 2nd edition, Chapman and Hall, New York, 1984.
- Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy, 3rd edition, Nirali Prakashan, Pune, 1995.
- Kokoshi CL, Kokoshi RJ, Sharma FJ, Fluorescence of powdered vegetable drugs under UV radiation, J Am Pharm Assoc, 47, 1958, 715-717.
- Chase CR, Pratt RJ, Fluorescence of powder drugs with particular reference to development of a system of identification, J Am Pharm Asso, 38, 1949, 324-331.
- Hedge JE, Hofreiter BT, In: Carbohydrate chemistry, 17 Eds. Whistler RL, Be Miller JN, Academic press, New York, 1962.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with folin phenol reagent, J Biol Chem, 193, 1951, 265-275.
- Yemm EW, Cocking EC, Ricketts RE, The determination of amino acids with ninhydrin, Analyst, 80, 1955, 209-214.
- Krishnaveni M, Dhanalakshmi R, Nandhini N, GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of *Gossypium* seeds, International Journal of pharmaceutical sciences review and research, 27, 2014, 273-276.
- Krishnaveni M, Dhanalakshmi R, Nandhini N, Antioxidant activity of *Gossypium* seeds, International journal of pharmaceutical sciences review and research, 27, 2014, 339-342.

Source of Support: Nil, Conflict of Interest: None.

