

Quantitative Analysis of Phytochemical Compounds in the Cotton (Gossypium) Seed Extracts; an Important Commercial Crop Plant

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Abstract

Almost all the parts of the plant viz., bark, root, stem and seeds are known to have various medicinal properties. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials. According to the world health organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. In the present study the cotton plant was selected to determine the yield of phytochemicals quantitatively from the crude extracts of seeds. Cotton belongs to the family *Malvaceae*, genus *Gossypium*; popularly known as white gold is an important commercial crop in India. The plant has been found to possess several ethno medicinal uses. Scientific investigations have shown that cotton roots and seeds contain certain compounds that may be beneficial to the health, potentially for treating Cancer and HIV. The seed extracts were prepared by using acetone, chloroform, ethanol, ethyl acetate, methanol, petroleum ether and water. The yield of phytochemicals were calculated as weight/weight in all the seven solvents and are studied for seven phytochemical compounds. The maximum numbers of phytochemical compounds are soluble in methanol and ethyl acetate. Results revealed the presence of alkaloids, flavanoids, total phenols, tannins, saponins, terpenoids and glycosides in varying content; among the seven phytochemicals phenols are abundantly presents in cotton seed.

INTRODUCTION

Carbohydrates, proteins, fats and oils are utilized as food by man and animals. Other chemical compounds in plants apart from these listed above are phytochemicals. Such compounds usually exert peculiar, unique and specific active physiological effects responsible for their therapeutic and pharmacological functions. Activities of such naturally occurring compounds are generally responsible for changes, which are utilized to satisfy man's desires. Phytochemical studies afford relation and understanding of phytoconstituents, as much as possible conserving their bioactivities, and on how to standardize them; compared with the crude herbal methods that are not easily

standardized (Hamburger and Hostettman, 1991). These complex substances of diverse nature occur mostly in plant based foods; they are in very small amounts in grams or mg or $\mu\text{g/Kg}$ of samples. They do not add to body calorie and are numerous in types.

These phytochemicals are applied mostly for preventive and healing purposes. About 25% of prescribed drugs are obtained from phytochemicals in higher plants. Plants are safe means of obtaining drugs. About 250,000 higher plants have promising phytochemicals, half of which are located in tropical forests; 60% of these have their biological activities established, while about 15% of them have their phyto-compounds isolated and reported (Hamburger and Hostettman, 1991). Studies and researches into medicinal constituents of plants, involve qualitative and quantitative analyses. There is rationale behind each experimental work involving definite steps and processes; having in mind properties of compounds analysed in conjunction with procedures utilized. Also our desired active metabolite to be isolated and studied as interested lead compound, many times is in very complex mixtures of many unwanted and undesired materials (known as contaminants), which have close properties to our desired bioactive molecules.

Most of the techniques and procedures in phytochemical analyses are cumbersome and tasking, to have detailed understanding of phytoconstituents. If they are carefully followed one can achieve the aim of isolations, characterizations and better establish bioactivities of active metabolites (Hamburger and Hostettman, 1991 and Mendonca-filho, 2006). Phytochemical methods mainly involve Extractions, Purifications and Isolations of the active compounds in plants. Procedures are ways of carrying out the methods and techniques. There are numerous methods some specific for interested compounds aimed for, duly modified to meet the required aim and focus of work. There are daily modifications of techniques and procedures to suit individual purposes of having the phytochemical compound(s) of interest. It is important to say that some natural product may (to variable extent) or may not possess their pharmacological properties and activities when in isolation or in a mixture of compounds (synergy) in the natural setting in organism (Hamburger and Hostettman, 1991 and Mendonca-filho, 2006).

Cotton popularly known as 'White Gold' is an important commercial crop in India. It contributes about 30% of the country's export earnings. It provides employment to large section of society on farm, textile and allied industries. India stands first in global cotton acreage (13.17 m ha) and first in production (29.2 m bales), while the productivity is at 588 kg lint/ha among the major cotton growing countries. Cotton belongs to the family *Malvaceae*, genus *Gossypium* and comprises of 50 different species, of these only four species are cultivated in India. India is the only country in the world growing all the four cultivated species of cotton two diploids-*G.arboreum* and *G.herbaceum*; and two tetraploid *G.hirsutum* and *G.barbadense* which are considered as successfully exploiting heterosis through cultivation of intra and interspecific hybrids. Telangana and Andhra Pradesh are playing a key role in national efforts for stepping up of cotton production and productivity in India. The total area under cotton cultivation in both the states is around 11.3 lakh hectares with an annual production of about 30 lakh bales (1 bale=170 kg each). Out of this, about 12 percent is being grown under irrigated dry condition. All the cultivable species of *Gossypium* viz; *G.arboreum*, *G.herbaceum*, *G.hirsutum* and *G.barbadense* are grown in commercial proportion in both states (Current Cotton Scenario, 2016).

Cotton seed which remains after the cotton ginned is used to produce cotton seed oil. The plant has been found to possess several ethno medicinal uses. Cotton seeds feature in traditional medicine; in various forms, they are taken internally and applied externally to treat a range of conditions. Preparations containing cotton are notably used as a therapy for skin problems and injuries. For headaches, a drink is made from powdered cotton seeds and mixed with milk. Dysentery is also treated with an infusion of seeds. Spots and other skin conditions are treated using cotton seed or extracts from the leaves. In Western medicine, cotton is put to use in the form of dressings, bandages,

swabs and cotton wool. Scientific investigations have shown that cotton seeds contain certain compounds that may be beneficial to the health, potentially for treating Cancer and HIV.

The present work is aimed to analyse quantitative phytochemical compounds from cotton (*Gossypium*) seed extracts. These compounds have various activities such as anti-microbial, anti-bacterial, hemolytic and foaming activity (Feroz *et al.*, 1993). Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Gupta 2010). Most the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defence mechanisms against predation by microorganisms, insects and herbivores (Farnsworth and Morris, 1976). Several bioactive constituents have been isolated and studied for pharmacological activities. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather a few new drugs.

MATERIAL AND METHODS

The healthy and disease free cotton seed has been collected from the Regional Agricultural Research Station (RARS), Nandyal, Kurnool District, Andhra Pradesh, India. The collected seed material was washed thoroughly in tap water, shade dried in open air separately. Powder of the seed is obtained by grinding them mechanically. About 100 gm of seed powder soaked separately in 100 ml of different solvents like Methanol, Ethanol, Chloroform, Pet ether, Ethyl acetate, Acetone and Water in conical flasks and then subjected to agitation on a rotary magnetic shaker for about 72 hours. After three days the seed extracts were subjected to filtration, filtered with No 42 whatman filter paper separately. Concentrated extracts was preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4°C until required for further use. The extract was filtered under reduced pressure using rotary flash evaporator and subjected for further preliminary phytochemical tests (Chandrashekar *et al.*, 2013 a&b).

Quantitative Phytochemical Analysis

a. Estimation of Alkaloids

Alkaloid determination by using Harborne (1985) method. One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and it is covered and allowed to stand for 4 hours. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added in drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

b. Estimation of Flavonoids

One grams of plant sample was repeatedly extracted with 100 ml of 80% of various aqueous solvents at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah *et al.*, 2009).

c. Estimation of Total Phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five ml of the extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. Two ml of NH_4OH solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance was read at 505nm.

d. Total Tannins Content Determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5 mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

e. Determination of total saponins

The samples were ground and 20 grams of each were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated (Obdoni and Ochuko 2001)

f. Determination of total Terpenoids:

5 ml of aqueous extract of each plant sample is mixed with 2 ml of CHCl₃ in a test tube and 3ml of concentrated H₂SO₄ is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present. After evaporation the sample were dried in the oven to a constant weight; the total terpenoids content was calculated

g. Determination of total Glycosides:

1ml of concentrated H₂SO₄ is prepared in test tube 5 ml of aqueous extract from each plant sample is mixed with 2 ml of glacial acetic acid containing 1 drop of FeCl₃. The above mixture is carefully added to 1ml of concentrated H₂SO₄ so that the concentrated H₂SO₄ is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicate. Sample was dried in the oven then the total glycosides content constant weight was calculated.

RESULTS AND DISCUSSION

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant like bark, root, stem and seeds are known to have various medicinal properties. Phytochemistry is defined as the study of phytochemicals, these are chemicals derived from plants (Chakraborty and Brantmer, 1999). In a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and diseases. They also exhibit a number of protective functions for human welfare. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials. According to the world health organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individual medicines have compounds derived from medicinal plants. Therefore, such plants should be investigated to understand their properties such as safety and efficiency (Das and Ventalchalam, 1999).

Cotton seed which remains after the cotton ginned is used to produce cotton seed oil. The seed have been found to possess several ethno medicinal uses. The present study was carried out on the seed extracts of cotton and the presence of active phytochemical constituents in various solvents and the results are mentioned in Table-1 and Figure-1. The quantitative estimation of phytochemical constituents revealed that the various phytochemical constituents present in the seed extract are as the content of the Alkaloid is 4.9 W/w in ethanol and water extracts. While flavanoids and total

phenols have shown their maximum content in all solvent extracts from 3.9 W/w and 5.3 W/w respectively. On the other hand, the content of carbohydrates are maximum in ethanol and methanol extracts as 3.5 W/w. A key observation is that total tannins are maximum in acetone extract as 4.8 W/w. Furthermore, saponins and total glycosides content are maximum in ethanol and methanol extracts as 4.6 W/w and 4.6 W/w respectively. Finally, terpenoids are extracted maximum in ethanol extract as 5.1 W/w. The presence of alkaloids, flavanoids, phenols, tannins, saponins, terpenoids, carbo hydrates and glycosides in varying content; among the seven phytochemicals phenols are abundantly present in cotton seed extracts.

Table 1: Quantitative phytochemical analysis of cotton seed extracted with different solvents

S.No.	Phytochemical Content in Seed (W/w)	Name of the Solvent						
		Ethanol	Methanol	Acetone	Chloroform	Ethyl Acetate	Petroleum Ether	Water
1	Alkaloids	4.9	4.7	4.5	4.3	3.3	4.7	4.9
2	Flavonoids	4.3	4.6	2.7	2.9	2.9	0.9	4.4
3	Total Phenols	4.6	5.2	4.6	4.8	4.8	3.9	5.3
4	Total Tannins	4.1	4.7	4.8	4.5	4.5	4.5	4.7
5	Saponins	4.6	4.6	4.2	3.3	3.3	3.6	3.6
6	Terpenoids	5.1	4.8	4.7	4.1	4.1	2.8	2.7
7	Total Glycosides	4.3	4.6	3.8	0.5	0.5	0.8	2.5

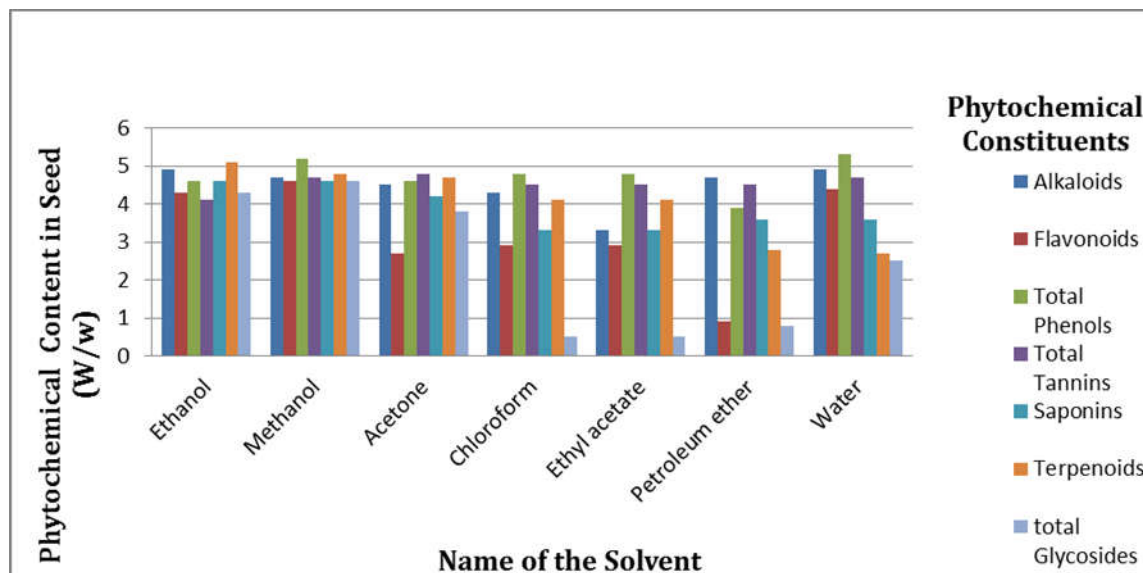


Figure 1: Graphical representation of quantitative phytochemical analysis of cotton seed extracted with different solvents

CONCLUSION

Cotton seeds are an important source certain bioactive compounds. The yields of phytochemicals were calculated as weight/weight in all the seven solvents and are studied for seven phytochemical compounds. Alkaloids, flavanoids, phenols, tannins, saponins, terpenoids, and glycosides in varying content; among the seven phytochemical constituents phenols are abundantly presents in cotton seed

and clearly shown that the more number of phytochemical compounds are maximum soluble in methanol and ethyl acetate. The quantity of phytochemicals appears to be high from the methanol, ethanol followed by the water extracts. Some phytochemical constituents are found to be in very less quantity in chloroform, ethyl acetate and petroleum ether; indicates that the methanol and ethanol extracts make a better source for the isolation and quantification of the phytochemical constituents under study.

The work so far achieved on cotton seeds extraction also sets the source of future studies on the particular constituents of seed extracts are characterized and assessed for their biological activities simultaneously. The information obtained from the bioassay and chemical analyses give full description of the bioactive compound, and afford easy and appropriate detection of specific targeted bioactive metabolite(s). It is becoming clear that traditional systems of medicine have become a topic of universal reputation. Cotton plant could be one of them particularly because of its widespread use for its abundant therapeutic properties. The phytochemical compositions and biological activities need to be well understood and the data gathered so far for cotton plant and its seed extracts aim at achieving that goal.

CONFLICT OF INTEREST/ AUTHOR CONTRIBUTIONS

Dr.N.Lakshmi Bhavani designed the work, R.Chandrashekhar performed studies and Dr.Bhavani Ram helped the analytical data interpretations. All the authors reviewed the manuscript.

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