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Research Article

Antimicrobial Efficacy of *Gossypium hirsutum* L. (*Bt* and Non-*Bt*) Phytochemical Extracts: The Most Widely Cultivated Species of Cotton in the World

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Abstract: The phytochemical analysis of *Gossypium hirsutum* was carried out using standardized methods for the evaluation of active compounds and provided opportunities for use in the preparation of new drugs. *Bacillus thuringiensis* (*Bt*) and non-*Bacillus thuringiensis* (non-*Bt*) cotton species are picked for the current study and during this, different solvents such as acetone, ethanol, methanol, pet-ether, chloroform, water used for phytochemical and antimicrobial screening from cotton leaves and seeds in the year 2013. The phytochemical analysis found the presence and absence of flavonoids, phenols, glycosides, saponins, amino acids and tannins, alkaloids, anthraquinones, steroids, terpenoids, sugars respectively. The investigation on the antibacterial efficacy against pathogenic strains of bacteria has been conducted and the highest inhibitory effect is present in leaf chloroform extract of non-*Bt* cotton against *Pseudomonas fluorescens*, whereas leaf methanol extract of *Bt* cotton was found to be most potent against *Escherichia coli*. Ethyl acetate and chloroform extracts have been effective against the remaining bacterial strains. Water extracts have a great inhibiting effect on all fungal pathogens tested. Results suggest that some samples may contain various pharmacologically active compounds with moderate antimicrobial properties.

***Keywords*:** *Bt* and non-*Bt* cotton, *Gossypium hirsutum*, phytochemical analysis, antimicrobial activity, inhibitory effect

1. Introduction

According to World Health Organization [1-4], interest in traditional herbal medicine has increased significantly in many countries, over the past two decades. The higher plants are the source of new remedies and the potential to cure a huge number of disorders; still, they are mostly underutilized as well as unexplored [5]. Among the assessed (250,000-500,000) plant species, only a tiny fraction of phytochemical exploration has been carried out, and even smaller fractions give into pharmacological screening [6]. Even now, different drugs from higher plants stay to occupy a significant position in modern medicine. Globally, a minimum of 122 drugs are taken out from higher plants and modified further unnaturally for marketable reasons [7, 8]. Plants are a rich basis of effective anti-microbial managers which are medicinally used in diverse nations for the production of potential drugs [9, 10].

The cotton plant is an annual herb belonging to the genus *Gossypium* of the family Malvaceae and it grows up to a height of 2-5 feet. The plant is put up with broad three-segmented greenish leaves, which are about 2-6 inches in length

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and emerge alternately on the stem. There are four main *Gossypium* species across the world, among those two diploids (*Gossypium arboreum* L.; *Gossypium herbaceum* L.) from Africa and Asia, and two tetraploids (*Gossypium barbadense* L.; *Gossypium hirsutum* L.) from America. In past reports of *G. barbadense* L., gossypol is an active compound that has *in-vitro* antimalarial activity against the human pathogen *Plasmodium falciparum* [11, 12]. Typically, *G. barbadense* has a longer growing period and produces smaller cotton bolls that give a significantly low yield lower than that of *G. hirsutum* [13]. *G. hirsutum seed* is a source of edible oil and it has several medicinal applications in emetics, venereal diseases, tumours, paralysis, epilepsy, convulsions, spasm as well as cutaneous and subcutaneous parasitic infections [14, 15]. It has anti-fungal properties and contains the chemical gossypol, which makes the plant less susceptible to insect damage [16, 17]. It is also used as a male anti-fertility drug [18, 19] and a remedy for neonatal jaundice [20]. The leaves are reported to be useful in the treatment of hypertension and delayed or irregular menstruation [21].

Bacillus thuringiensis (*Bt*) is a bacterial species which lives in soil and makes some toxic proteins for insects. The genes responsible for the production of such toxic proteins are introduced in genetically modified *G. hirsutum* species. *Bt* and non-*Bt G. hirsutum* species are chosen for the current interesting study and aimed to evaluate the antimicrobial activity of phytochemical extracts. Considering the enormous potentiality of medicinal plant sources, a systematic approach has been taken for phytochemical screening and investigated the antimicrobial activity in *G. hirsutum* leaf and seeds.

2. Materials and methods

Bt and non-*Bt G. hirsutum* leaf and seed samples were used for phytochemical and *in-vitro* antimicrobial activities. For simplicity, *Bt G. hirsutum* is referred to as *Bt* cotton and non-*Bt G. hirsutum* is referred to as non-*Bt* cotton. The healthy *Bt* and non-*Bt* cotton seeds were acquired from the local seed shop, Hyderabad in the year 2013. Disease-free seeds are separated, labelled, and washed carefully in running tap water. Then, the seeds were rinsed with double distilled water and air-dried for further use under the shadow. Next, the seeds are powdered to a fine texture in a grinder. The fresh and infection-free cotton leaves were collected from the botanical garden of Osmania University, Hyderabad. Samples were labelled and properly washed under running tap water. Then, the samples were rinsed in double distilled water and dried in the shade for 7 days in the open air. The dried cotton leaves were pulverized by an electric grinding machine and stored in a desiccator. The seed and leaf samples were prepared for phytochemical analysis using standard methods. One gram powdered samples were kept in different solvents, such as methanol, ethanol, chloroform, pet-ether, acetone, and double distilled water in conical flasks. Next, the flasks were placed on a rotary shaker for around 72 hours. The supernatant was collected and filtered with Whatman No 42 filter papers. Concentrated extracts were stored at 4 °C in sterile airtight bottles. Qualitative phytochemical screening of cotton crude extracts was carried out through the methods given by Lozoya et al. [22], Karthikeyan et al. [23], and Morsy [24]. Different coloured precipitates and solutions were formed in the following tests (Figure 1).

For testing phenols, samples were treated with an equal volume of ferric chloride solution in a test tube and mixed well. A bluish-green colour solution was formed. For testing the presence of amino acids, the samples were treated with a few drops of ninhydrin in a test tube and mixed well. After a few seconds, a purple colour development appeared. For testing the presence of terpenoids, equal volumes of sample, chloroform and acetic anhydride were added to a test tube. Then, a two-fold volume of sulfuric acid was added carefully and reddish colouration was observed. For testing alkaloids, filtrate from each sample was mixed with an equal volume of 2N hydrochloric acid in a water bath and filtered for further use. To the filtrate, a few drops of Mayer's reagent were added and mixed well. A cream colour precipitate was observed in the solution.

To detect the presence of tannin, a few drops of 1% lead acetate solution were added to each sample and mixed well. The yellow colour precipitate was perceived in the test tube. For testing reducing sugars, the sample extracts were treated with a few drops of Fehling's solution and boiled for 15 minutes. A brick-red precipitate was developed in the solution. To detect the presence of monosaccharides, the sample extracts were mixed with equal volumes of Barfoed's reagent and heated in a water bath for a few minutes. The brown precipitate formation was observed in the solution. Flavonoids were tested by adding a few drops of dilute sodium hydroxide solution to each sample and intense yellow colour development was observed in the solution. It became colourless by adding a few drops of dilute acid. For saponins, each sample was boiled with double distilled water and shaken vigorously for about 1 minute. The test tube

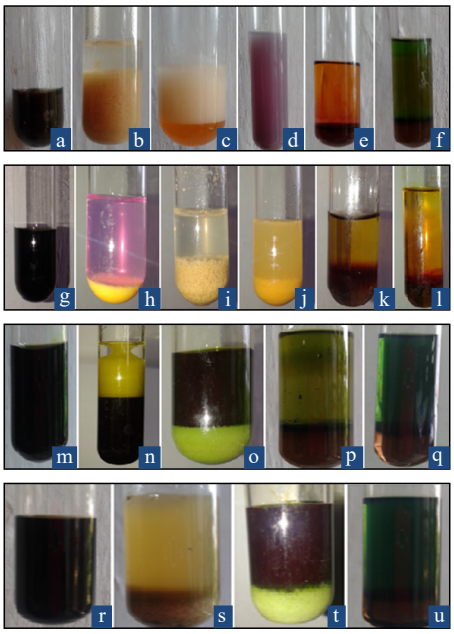

Figure 1. Different seed extracts of *Bt* and non-*Bt* cotton: (a) Phenols, (b) Flavonoids, (c) Saponins, (d) Amino acids, (e) Terpenoids, (f) Glycosides, (g) Phenols, (h) & (i) Flavonoids, (j) Alkaloids, (k) Terpenoids, (l) Glycosides; Different leaf extracts of *Bt* and non-*Bt* cotton: (m) Phenols, (n) Saponins, (o) Flavonoids, (p) Glycosides, (q) Steroids, (r) Phenols, (s) & (t) Flavonoids, (u) Steroids

allows for settling and lathering was observed.

Glycosides were detected by mixing with a few drops of glacial acetic acid, ferric chloride, and concentrated sulphuric acid to each sample through the side wall of the test tube. At the junction of the two layers' reddish-brown colour appeared and the upper layer appeared as bluish-green colour. For testing the presence of steroids, the sample was dissolved in 10 times the volume of chloroform. Concentrated sulfuric acid of the same volume was added through the side wall of the test tube. Upper red layer and yellow with green fluorescence lower layers appeared in the tube. To test the presence of anthraquinones, the sample extract was boiled with a double volume of sulfuric acid and filtered while hot. To the filtrate, chloroform was added and shaken well. The chloroform layer was pipette out and transferred to another test tube. Then, diluted ammonia was added and the resulting solution was observed for colour changes.

Antibacterial activity was assessed with the disc diffusion method given by Murray et al. [25]. The extract that has been mentioned was tested and activity assessed against four pathogenic bacterial strains that are *Bt*, *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. For that, about 20 µL of strain was spread onto 20 mL of agar plates by using a sterile cotton swab, and the surface of the medium was allowed to dry for around 3 minutes. Sterile filter paper discs (5 mm in diameter) soaked in different test extracts (100 µL disc) were placed on the surface of inoculated agar plates. Then, the plates were incubated at 37 °C for 24 hours. After that, microbial growth was determined by the diameter of the inhibition zone (mm) and measured using a transparent scale. All extracts were analysed in triplicate and the mean values were considered for final calculations. Agar well diffusion assay was used for antifungal activity [26] and to test fungi, *Colletotrichum capsici*, *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Rhizoctonia solani* inoculated into freshly prepared potato dextrose agar (PDA) plates. Then, the wells were made with 6 mm in diameter on the inoculated plates using a sterile cork-borer and each well was loaded with extracted compounds of cotton leaves and seeds. The plates were incubated at room temperature for 4 to 5 days. After 5 days, the zone of inhibition was measured in millimeters.

3. Results

Specific tests were carried out on each group of phytochemicals, amongst the tested extracts polar solvents such as acetone and methanol were a good source than others followed by chloroform and water. After adding reagents

formation of different coloured solutions indicates the presence of different phytochemicals such as bluish-green colour solution for phenols, purple colour for amino acids, and reddish colouration for terpenoids. The appearance of various coloured precipitates was taken as a positive test for different phytochemicals like cream for alkaloids, yellow for tannins, brick red for reducing sugars, and brown coloured precipitate for monosaccharides.

When the base solution was added to the plant extract, an intense yellow colour formation was perceived and it became colour with the addition of a few drops of dilute acid. This shows the presence of flavonoids. Frothing formation was indicative of positive for saponins. The appearance of various layered colours in the same test tube and the formation of rings between the layers indicate the existence of glycosides and steroids.

The test results of *Bt* cotton leaf extracts were screened by different tests and extracted maximum occurrence of saponins, phenols, steroids, glycosides and flavonoids from chloroform, acetone, pet-ether, and methanol respectively. But they are completely absent in extracts of ethanol and water (Table 1). In Table 2, non-*Bt* cotton leaf extracts revealed the presence of phenols, steroids, flavonoids, and monosaccharides. The phytochemical screening results of *Bt* cotton seed extracts illustrated the presence of saponins, phenols, tannins, glycosides, flavonoids, terpenoids, and amino acids (Table 3). The quantitative estimation of phytochemicals showed that the seeds of non-*Bt* cotton contain a significant amount of alkaloids, phenols, glycosides, flavonoids, and terpenoids content (Table 4). Under this study, phenols and flavonoids are common in all the tested samples. The leaf and seed extracts (*Bt* and non-*Bt*) revealed the presence of flavonoids, phenols, glycosides, saponins, terpenoids, and amino acids, commonly from methanol, acetone, chloroform, pet-ether, and water but comparatively absent in ethanol. The phytochemicals like alkaloids, tannins, anthraquinones, amino acids monosaccharides, and sugars were not found in leaf and seed extracts of both the cotton verities. Among the tested phytochemicals, steroids and terpenoids were absent in seed and leaf extracts respectively.

Specifically, saponins in chloroform, phenols in acetone, steroids in pet-ether, flavonoids as well as glycosides in methanol of seed extracts illustrated better results than all other solvents. A similar kind of phytochemicals was observed in *Bt* and non-*Bt* cotton, however, the appearance of test colouration is a little bit darker for the *Bt* variety. This might be due to the amount of the constituents in *Bt* cotton seed extracts is greater than non-*Bt* cotton seed extracts. Anthraquinones, tannins, alkaloids, and sugars were not observed in many of the tested samples. May be due to the presence of these phytochemicals in seed extracts they have antimicrobial, antifungal, anti-diabetic, and anti-cancer activities.

Table 1. Phytochemical substances of *Bt* cotton leaf extracts

Phytochemicals	Methanol	Ethanol	Chloroform	Pet-ether	Acetone	Water
Alkaloids	–	–	–	–	–	–
Amino acids	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–
Flavonoids	+	–	–	–	–	–
Glycosides	+	–	–	–	–	–
Monosaccharides	–	–	–	–	–	–
Phenols	–	–	–	–	+	–
Reducing sugars	–	–	–	–	–	–
Saponins	–	–	+	–	–	–
Steroids	–	–	–	+	–	–
Tannins	–	–	–	–	–	–
Terpenoids	–	–	–	–	–	–

+ Presence of phytochemical, – Absence of phytochemical

Table 2. Phytochemical substances of *Bt* cotton leaf extracts

Phytochemicals	Methanol	Ethanol	Chloroform	Pet-ether	Acetone	Water
Alkaloids	–	–	–	–	–	–
Amino acids	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–
Flavonoids	+	–	–	–	–	–
Glycosides	–	–	–	–	–	–
Monosaccharides	–	–	–	–	–	+
Phenols	–	–	–	–	+	–
Reducing sugars	–	–	–	–	–	–
Saponins	–	–	–	–	–	–
Steroids	–	–	–	+	–	–
Tannins	–	–	–	–	–	–
Terpenoids	–	–	–	–	–	–

+ Presence of phytochemical, – Absence of phytochemical

Table 3. Phytochemical substances of *Bt* cotton seeds

Phytochemicals	Methanol	Ethanol	Chloroform	Pet-ether	Acetone	Water
Alkaloids	–	–	–	–	–	–
Amino acids	–	–	–	–	–	+
Anthraquinones	–	–	–	–	–	–
Flavonoids	+	–	–	–	–	–
Glycosides	–	–	+	–	–	–
Monosaccharides	–	–	–	–	–	–
Phenols	–	–	–	–	+	–
Reducing sugars	–	–	–	–	–	–
Saponins	–	+	–	–	–	–
Steroids	–	–	–	–	–	–
Tannins	–	–	–	–	–	–
Terpenoids	–	+	–	–	–	–

+ Presence of phytochemical, – Absence of phytochemical

Table 4. Phytochemical substances of non-*Bt* cotton seeds

Phytochemicals	Methanol	Ethanol	Chloroform	Pet-ether	Acetone	Water
Alkaloids	–	–	–	–	–	+
Amino acids	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–
Flavonoids	+	–	–	–	–	–
Glycosides	–	–	+	–	–	–
Monosaccharides	–	–	–	–	–	–
Phenols	–	–	–	–	+	–
Reducing sugars	–	–	–	–	–	–
Saponins	–	–	–	–	–	–
Steroids	–	–	–	–	–	–
Tannins	–	–	–	–	–	–
Terpenoids	–	–	–	–	+	–

+ Presence of phytochemical, – Absence of phytochemical

Initial screening has shown that *Bt* and non-*Bt* cotton extracts are a good source of natural phytochemicals. The pharmacological actions of a plant cannot be ascertained by the results of phytochemical studies so antimicrobial activity against pathogenic bacteria and fungi was used for evaluation. The investigation revealed the effectiveness of all the extracts against the selected pathogens. Different samples extracted from *Bt* and non-*Bt* cotton (leaf and seed) measured varying degrees of growth inhibition on different bacteria and fungi at different concentrations. The wide zones of inhibitory activity of non-*Bt* leaf chloroform extract were obtained against *P. fluorescens*, whereas *Bt* leaf methanol extract was found to be most potent against *E. coli*. Ethyl acetate and chloroform extracts were active against all the test bacterial strains and only water extracts gave positive results against all the tested fungal pathogens and the zone of inhibition ranges from 2-8.5 mm. Extracts isolated from leaf samples showed the most effective activity than seed extracts under study. All of the fungi used in the experiment were insensitive to the extracts could be attributed to their unique cell wall structure. In contrast to bacteria, fungi are eukaryotic and have rigid cell walls comprising of chitin as well as polysaccharides and a cell membrane composed of ergosterol. Hence, fungal infections are mostly resistant to antibiotics used in the treatment of bacterial infections.

4. Discussion

Plant-based compounds often referred to as phytochemicals are prominent bioactive molecules, which are widely discovered in plants. The common phytochemicals consist of essential oils, alkaloids, polyphenols, steroids, terpenoids, glycosides, saponins, flavonoids, and so on. These bioactive compounds are well-known for their pharmacological effect against various diseases including cancers, jaundice, cough, bronchitis, diarrhea, asthma, heart diseases, brain abnormalities, and diabetes mellitus. The phytochemical substances in the leaves of *G. hirsutum* are used as traditional medicine for the treatment of infertility and contraceptive in males and females respectively. Muhammad et al. [27] carried out the preliminary phytochemical screening from methanolic extracts of *G. barbadense* leaves.

The presence of different phytochemicals in acetone, methanol, pet-ether, chloroform, ethanol, and hot water extracts of seeds of *Bt* and non-*Bt* cotton have been previously reported [28]. Nevertheless, our study is the first and foremost to report on qualitative comparative analysis of seed and leaf extracts of *Bt* and non-*Bt* cotton variety and their antimicrobial activity. A similar type of phytochemical screening and observations were reported by other workers in different plants viz., *Ocimum basilicum* - leaves [29], *Monechma ciliatum* - seeds [30], and *Aegle marmelos* - leaves [31]. *Bt* and non-*Bt* cotton leaf and seed extracts are pharmacologically more active as a result of the synergistic effects of different components present in the whole extracts [32]. The leaf extracts of *A. marmelos* showed antibacterial

potential against three gram-positive bacteria (*Bacillus cereus*, *Staphylococcus epidermidis*, and *S. aureus*), and two gram-negative bacteria (*Enterobacter aerogens* and *Klebsiella pneumoniae*) [31]. The antimicrobial activity of *Ocimum* leaf extract was found to be effective against *S. aureus*, *Streptomyces* species, *Salmonella* species, *Shigella* species, and *Pseudomonas aureginosa* reported by Sanni et al. [29]. The antibacterial activity of five green vegetables from methanol extracts was found active against all the test bacterial strains reported by Bhat and Al-Daihan [33]. Evaluation of the antimicrobial activity based on the zone of inhibition was done for different plant extracts *Allium cepa* [34]; *Mentha longifoli*a [35]; *Nerium oleander* L. and *Nicotiana tabacum* [36]. Bioactive compounds accumulated in plants as secondary metabolites. Concentration of these bioactive compounds found in plant cells differs depending on the part of the plant, growth phases, seasons and climates. Leaves are one of the major sources for accumulation and are highly constructive to plants [37, 38]. Most of the secondary metabolites such as flavonoids, saponins, tannins, steroids, and alkaloids are phytoprotectants also essential to cell growth, replacement, and bodybuilding [39]. Considerable interest in the potential medicinal value is attributed to the presence of chemical substances that can produce definite physiological actions on the human body with immune system stimulation and detoxification activities [40]. Considering the above views, it should be prominent that *G. hirsutum* phytochemicals are capable of antibacterial and antifungal properties.

5. Conclusions

The test results of both *Bt* and non-*Bt* cotton extracts showed phenols and flavonoids as common phytochemicals in a maximum amount. The wide inhibitory zones formed with non-*Bt* leaf chloroform extracts were obtained against *P. fluorescence*, whereas *Bt* leaf methanol extract was found to be most potent against *E. coli*. Ethyl acetate and chloroform extracts were active against all the tested bacterial strains and only water extracts gave positive results against all the tested fungal pathogens. Extracts from leaf samples are the most effective than seed extracts. However, all these phytochemical leaf and seed extracts have good and moderate antimicrobial properties. This study may justify the use of the plant by trade-medical practitioners.

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Conflict of interest

The authors declare no competing financial interest.

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