COMPARATIVE STUDY ON THE ANTIMICROBIAL NATURE OF Bt COTTON LEAF EXTRACT AND TWO OTHER MEDICINAL PLANTS (Acalypha indica L. AND Tridax procumbens L.)

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Abstract
Plant system consists of a broad range of allochemicals which are found to act against various microorganisms. Transgenic Bt cotton (BG II) has Bt toxin Cry - protein in addition to naturally present phytochemicals. Objective of this investigation was to determine the antibacterial nature of Bt cotton leaf extract against soil bacterial isolates as compared to other two selected medicinal plants – Acalypha indica L., and Tridax procumbens L. Acetone and methanolic extracts of leaves were tested against soil bacterial cultures by Disc diffusion method. Comparatively, Bt cotton extract exhibited more antimicrobial effect than the other two herbs under investigation.

Key words: Bt cotton, Antimicrobial nature, Acalypha indica and Tridax procumbens.

1. Introduction
Our ancestors depended on the plants for cure and medication against various ailments. Even today, plant materials serves as the potential sources of drugs. Plants release hundreds of different volatile compounds and some of these allochemicals could directly detrimental to herbivores (Ted et al., 1995; Raguso, 2004). The antimicrobial nature of the plant extracts is regarded due to the occurrence of these phytochemicals (Khaleequer et al., 2012; Maldhure, 2015; Chaturvedi and Nag, 2015; Chekun et al., 2016; Pardeshi and Bhiungade, 2016; Zahidin et al., 2017; Beck et al., 2018; Al-Snati, 2018). Gossybiurn hirsutum is widely cultivated for its valuable fiber. Bt cotton (BG II) has inbuilt ability to produce insecticidal Cry - protein. Expression of Bt toxin protein was found to be reflected in the profile of volatile allelochemicals (Bhat et al., 2011; Parimala et al., 2013). The present investigation is an attempt to determine whether this transgenic property provides any significant antimicrobial potential more than the other common medicinal herbs.

2. Materials and Methods
Fresh leaves of Acalypha indica L. (Euphorbiaceae), an herb distributed throughout India and other tropical regions of the world and Tridax procumbens L. (Asteraceae) commonly
known as Vettukkaaya - thalai considered as a
gregarious weed, distributed throughout the
tropics and sub tropics were collected from herbal
garden of Madurai Kamaraj University, Madurai. *Gossypium hirsutum* L., a genetically modified Bt-
cotton *RCH 659 BG II* (BG II) variety propagated
by seeds in earthen pots were used for sample
collection. The collected leaves were air - dried
and powdered. Using two different solvents
(Acetone and Methanol) leaf extracts were
prepared with help of soxhlet apparatus which
were filtered and stored for further analysis.
Qualitative analysis for phytochemicals present in
the leaf extracts were determined as described by
Sofowora (1993), Trease and Evans (1989) and
Harbone (1973).

A) Carbohydrates (Molish’s Test)

One ml of extract was taken in a test tube
and 2 ml of Benedict’s reagent was added to the
solution. Formation of a reddish precipitate within
three minutes shows the presence of carbohydrates
in the extract.

B) Tannins (Ferric chloride Test)

Two ml of extract was taken in a clean test
and 1 ml of ferric chloride solution was
added. Appearance of bluish black color indicates
the presence of tannins in the extract.

C) Flavanoids (Lead Acetate Test)

One ml of 10 % of Lead Acetate solution
was added to the 1 ml of extract in a test tube.
Appearance of yellow color indicates the presence
of flavanoids in the extract.

D) Alkaloids (Wagner’s Test)

One ml of Wagner’s reagent was added to
the 1 ml of extract. Appearance of reddish brown
indicates the presence of alkaloids in the extract.

E) Volatile Oil

One ml of extract was taken in a test tube
and 5 ml of alcohol (1 ml of volatile oil is
dissolved in 5 ml of ethanol) and then 1 drop of
ferric chloride was added to the tube. Appearance
of pale green color indicates the presence of
Volatile oil in the extract.

F) Glycosides (Keller killiani test)

Ten ml of extract was mixed with 100 ml
of 50 % H₂SO₄. Then, the mixture was heated in
the boiling water bath for 15 minutes. Then, 10 ml
of Fehling solution was added and boiled. Appearance of red precipitate indicated the
presence of glycosides in the extract.

G) Saponins

One ml of extract was added with water
and shaken. Formation of foamy lather indicates
the presence of saponins in the extract.

The microorganisms isolated from the soil
taken from Herbal garden, Madurai Kamaraj
University, Madurai were used. The Serial dilution
method was done to isolate microorganisms from
soil. Five isolates were selected. The isolated
colonies were pure cultured and sub-cultured in
Nutrient media. The isolates were maintained in
appropriate medium for further studies. The
isolated cultures were subjected to various
biochemical tests (Bigham, 1994).

Antibacterial activity assay by Disc diffusion
method

Petriplates were prepared by pouring 20 ml
of Muller Hinton Agar. The inoculum was spread
on the top of the solidified media and allowed to
dry for 10 minutes. Five mm of sterile discs were
incorporated in 75 μl, 150 μl, 300 μl and 600 μl of
plant extracts (5 mg/disc). The discs were
completely saturated with the extract and allowed
to dry and then applied it over the media which
was swabbed with the bacterial culture and the
plates were left for 30 minutes at room
temperature to allow the diffusion of the extract
before their incubation for 24 hours at 37 °C. The
inhibition zone diameters were measured in
millimeters. The pure solvents of Acetone and
Methanol were used as negative control. The
diameters of the zone of inhibition of negative
controls and positive controls were also measured
in millimeters.
3. Results and Discussion

Biochemical Properties of Soil Bacterial Isolates

Biochemical studies revealed that isolates III and IV showed positive results in the tests carried out, whereas isolate I was positive to three tests (Citrate utilization, Triple sugar iron test and Catalase test). Data collected clearly indicated that each isolate is different from other (Table - 1).

Nature of leaf Extracts

Methanol extract of *Acalypha indica* showed negative result to saponin (Table - 2). Bt cotton leaf extract from both solvents were devoid of tannin (Table - 3) Acetone and methanol leaf extract of *Tridax procumbens* is lacking glycosides and saponins. Additionally in acetone extract tannin was recorded negative (Table - 4).

Antimicrobial Nature of leaf extracts

The Acetone leaf extract of *Acalypha indica* showed greater antibacterial activity and was more effective only at the higher concentration. This extract was more effective against isolate 5 and isolate 1 which followed by isolate 4. And there were absence of zone formation at the concentrations of 75 µl (Fig.1). The Methanolic leaf extract of *Acalypha indica* showed greater antibacterial activity and was effective only at higher concentration 600 µl. This extract was more effective against all the 5 isolates. There were absence of Zone formation at the concentrations of 75 µl and 150 µl (Fig.2). The Acetone extract of *Gossypium hirsutum* was active against six bacterial species. The extract was more effective against isolate 5, which was followed by isolate 3. Low antibacterial activity were found in isolates 1 and 2 and there were absence of zone observed in isolates 1, 2 and 4 in low concentration (75 µl) (Fig.3). The Methanolic extract of *Gossypium hirsutum* was more effective against isolates 1, 2 and 5 at higher concentrations which was followed by isolate 2 and 3. At low concentration 75 µl and 150 µl showed low antibacterial activity (Fig.4). The Acetone leaf extract was effective against isolate 5 in higher concentrations followed by isolate 1 and 2. At low concentrations in 150 µl only antibacterial activity showed only in isolate 2 other isolates showed absence of antibacterial activity (Fig.5). The Methanolic extract of *Tridax procumbens* showed effective antibacterial activity in all the concentrations against isolate 5 followed by isolate 4. In other isolates absence of antibacterial activity was observed (Fig.6).

Medicinal properties of *Acalypha indica* L. (Murugan and Saranraj, 2001; Govindarajan et al., 2008; Tariq et al., 2015; Darshini, 2015; Chekun et al., 2016; Zahidin et al., 2017), *Tridax procumbens* (Malhure, 2015; Pardeshi and Bhiungade, 2016; Beck et al., 2018), *Gossypium herbaceum* (Al-Snati, 2018), *G. hirsutum* (Khaleequer et al., 2012; Chaturvedi and Nag, 2015; Al-Snati, 2018) were studied by many earlier workers. Leaf extract of both solvents (Acetone and Methanol) of all the three plants, generally showed high zone of inhibition in all bacterial isolates at higher concentration (600 µl). Isolates II and III were not inhibited by *Acalypha indica* acetone extract even at higher concentration, whereas Isolate I was inhibited only at 600 µl concentration. *Tridax procumbens* acetone leaf extract was inhibitory only at 300 µl concentration for isolates II, III and V. Isolate IV did not show any inhibition even at 600 µl of *T. procumbens* acetone extract. On the contrary Bt cotton leaf acetone extract could inhibit isolate II and V even in very low concentration (75 µl), likewise methanolic extract inhibited the growth of isolates III, IV and V at very low concentration (75 µl).
### Table - 1: Biochemical Test result for bacterial isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Indole</th>
<th>MR Red</th>
<th>VP Test</th>
<th>Citrate Utilization Test</th>
<th>TSI Test</th>
<th>Catalase Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate 1</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Isolate 2</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Isolate 3</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Isolate 4</td>
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<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Isolate 5</td>
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<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
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</tbody>
</table>

### Table - 2: Phytochemical composition of *Acalypha indica* leaf extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Acetone</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

### Table - 3: Phytochemical composition of *Gossypium hirsutum* leaf extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Acetone</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
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<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavanoids</td>
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<td>+ve</td>
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<tr>
<td>Alkaloids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
<td>+ve</td>
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</tbody>
</table>

### Table - 4: Phytochemical composition of *Tridax procumbens* leaf extracts

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Acetone</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavanoids</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Volatile oil</td>
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<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>
Figure – 1: Antibacterial activity of Acetone leaf extract of *Acalypha indica* against soil microorganisms (Disc diffusion method)

Figure – 2: Antibacterial activity of Methanol leaf extract of *Acalypha indica* against soil microorganisms (Disc diffusion method)

Figure – 3: Antibacterial activity of Acetone leaf extract of *Gossypium hirsutum* against soil microorganisms (Disc diffusion method)
Figure – 4: Antibacterial activity of Methanol leaf extract of *Gossypium hirsutum* against soil microorganisms (Disc diffusion method)

Figure – 5: Antibacterial activity of Acetone leaf extract of *Tridax procumbens* against soil microorganisms (Disc diffusion method)

Figure – 6: Antibacterial activity of Methanol leaf extract of *Tridax procumbens* against soil microorganisms (Disc diffusion method)
4. Conclusion

The present study indicated that comparatively soil bacterial isolates growth was inhibited by Bt cotton leaf extracts than other two herbal extracts.

5. Reference


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