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(RESEARCH ARTICLE)

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Antimicrobial potential of aqueous extracts of selected plant species against crown gall disease

Anusha Rani Yavvari, N Haseeda, J Reddy Kumari, M Mounika, Ankanna S, Venugopal A, Vijaya T and Nagalakshmi Devamma M *

Department of Botany, Sri Venkateswara University, Tirupati 517502.

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Abstract

This study investigates the antimicrobial potential of aqueous extracts from selected plant species (*Lantana camara, Mentha spicata, Chromolaena odorata, Hyptis suaveolens, and Anisomeles malabarica*) against Agrobacterium tumefaciens, the causal agent of crown gall disease. The plant extracts were prepared using the Soxhlet and decoction methods, and their phytochemical composition was qualitatively analyzed. The antimicrobial activity of the extracts was evaluated through in vitro and in vivo methods. Results indicated that *Lantana camara, Mentha spicata, and Chromolaena odorata* extracts effectively suppressed the growth of A. tumefaciens, while Hyptis suaveolens and Anisomeles malabarica did not exhibit significant antimicrobial activity. This study suggests the potential of *Lantana camara, Mentha spicata, and Chromolaena odorata* as natural alternatives for controlling crown gall disease.

Keywords: Crown gall disease; Agrobacterium tumefaciens; Antimicrobial activity; plant extracts; *Lantana camara; Mentha spicata; Chromolaena odorata*

1. Introduction

Crown gall is a widespread disease affecting numerous woody and herbaceous plants, caused by the bacterium Agrobacterium tumefaciens[1]. The disease leads to the formation of tumors or galls on various plant parts, causing significant economic losses in agriculture[2]. Agrobacterium tumefaciens modifies the genetic material of host cells, transferring part of its Ti-plasmid DNA into the host cell DNA, leading to uncontrolled cell proliferation and tumor formation[3]. The control of crown gall disease is challenging, and there is a need for effective and eco-friendly control strategies[4]. Plant-derived compounds have emerged as promising alternatives for disease management due to their antimicrobial properties[5]. The present study aims to evaluate the antimicrobial potential of aqueous extracts from five selected plant species (*Lantana camara*, *Mentha spicata*, *Chromolaena odorata*, *Hyptis suaveolens*, and *Anisomeles malabarica*) against Agrobacterium tumefaciens

2. Material and methods

2.1. Plant Material Collection and Preparation

The leaves of the selected plant species, namely *Hyptis suaveolens*, *Lantana camara*, *Chromolaena odorata*, *Mentha spicata*, and *Anisomeles malabarica*, were collected from Tirupati, Andhra Pradesh, India. The leaves were then dried, pulverized and subjected to extraction.

* Corresponding author: Nagalakshmi Devamma M

Figure 1 Drying, Grinding and Weighing

2.2. Extraction Methods

Two extraction methods were used to obtain plant extracts: Soxhlet extraction, primarily used for extracting non-thermolabile compounds, and the decoction method, traditionally used for water-soluble and thermostable compounds.

- **Soxhlet Extraction:** 15 grams of the powdered plant material was placed in a filter paper thimble and then placed in a Soxhlet apparatus. Ethanol, a common solvent for extracting plant compounds, was placed in a round-bottom flask and heated. The ethanol vapours rose and condensed in a condenser. The condensed ethanol dripped into the thimble containing the plant material, extracting soluble compounds. As the ethanol level in the thimble rose, it siphoned back into the flask, carrying the extracted compounds. This process was repeated to ensure efficient extraction. After extraction, the ethanol was removed by distillation, leaving the crude extract.
- **Decoction Method:** In this method, the crude plant material was boiled in water within an open-type extractor. The ratio of crude drug sample to water was 1:4 or 1:16. The mixture was boiled, reducing the volume to one-fourth of the original, and the concentrated extract was then strained or filtered.

Figure 2 Soxhlet Extraction Method

Figure 3 Decoction Method

2.3. Qualitative Phytochemical Analysis

The plant extracts were subjected to preliminary phytochemical screening to detect the presence of various secondary metabolites. Standard methods were employed to identify the following compounds:

2.3.1. Alkaloids

- **Wagner's Test:** Extracts were treated with Wagner's reagent (iodine in potassium iodide). A reddish-brown precipitate indicates the presence of alkaloids.

2.3.2. Flavonoids

- **Lead acetate test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.

2.3.3. Steroids

- **Acetic Anhydride Test:** Extracts were treated with acetic anhydride and sulfuric acid. A color change from violet to blue or green indicates steroids.

2.3.4. Terpenoids

- **Salkowski Test:** Extracts were treated with two ml of chloroform and concentrated sulfuric acid. Brown colour in the lower layer indicates the presence of terpenoids.

2.3.5. Anthraquinones

- **Borntrager's Test:** About five mg of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

2.3.6. Phenols

Ferric Chloride Test: Extracts were treated with ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.3.7. Saponins

Foam Test: Extracts were shaken vigorously with water. Persistent foam indicates the presence of saponins.

2.3.8. Tannins

- **Ferric Chloride Test:** Extracts were treated with ferric chloride solution. A dark green or bluish black colour indicates the presence of tannins.

2.3.9. Carbohydrates

- **Benedict's Test:** Extracts were treated with Benedict's reagent and heated. A color change and precipitate indicate reducing sugars.

2.4. Protein and Aminoacids

- **Biuret Test:** Extracts were treated with NaOH and copper sulfate. A violet color indicates proteins.

2.4.1. Oils and Resins

Extracts were applied to filter paper. A transparent appearance indicates oils and resins.

2.5. Antimicrobial Activity Assays

The antimicrobial activity of the plant extracts were evaluated through *in vitro* and *in vivo* methods.

2.5.1. In Vitro Method

The antimicrobial activity of the plant extracts was evaluated through an *in vitro* assay using the agar diffusion method. An *Agrobacterium tumefaciens* culture was uniformly spread on MacConkey agar. Wells were impregnated with 50 µL of the plant extracts at a concentration of 100 mg/mL and placed on the agar surface. The plates were incubated at 28°C for 24-48 hours. The zone of inhibition was measured in millimeters to assess the effect of the plant extracts on the growth of *Agrobacterium tumefaciens***.**

2.5.2. In Vivo Method:

Agrobacterium tumefaciens was inoculated into carrots by introducing 100 µL of the bacterial inoculum at a concentration of 10^8 CFU/ml into a wound created in each carrot. Gall development in the carrots was observed after two weeks, with measurements taken of gall size and number. Plant extracts were applied to the inoculated carrots to assess their ability to control gall formation, and the concentration and application method of the extracts were recorded.

- **Control:** Carrots inoculated with A. tumefaciens but without plant extract treatment were included as a control.

2.6. Gram Staining

Gram staining was performed to confirm the identity of A. tumefaciens. A sample was heat-fixed on a slide, stained with crystal violet for one minute, treated with Gram's iodine for one minute as a mordant, decolorized with acetone or alcohol for about three seconds, counterstained with safranin for one minute, and observed under a microscope.

3. Results and discussion

3.1. Phytochemical screening

Table 1 Phytochemical screening

S. No	*Hyptis suaveolens*	*Lantana camara*	*Mentha spicata*	*Anisomeles malabarica*	*Chromolaena odarata*
Alkaloids	++	-	+	++	+
Flavonoids	+	+	++	++	+
Steroids	-	+	-	-	-
Terpenoids	+	-	+	+	+
Anthraquinones	+	-	-	-	-
Phenols	+	++	+	+	+
Saponins	+	+	+	+	+
Tannins	-	+	+	+	+
Carbohydrates	-	+	-	-	-
Proteins and Aminoacids	-	-	+	-	-
Oils and Resins	-	-	+	+	+

3.2. In Vitro Antimicrobial Activity

The disk diffusion method revealed varying zones of inhibition against A. tumefaciens:

Table 2 Zone of Inhibition of selected Plants

S.No	Name of the Plant	Zone of Inhibition
1	*Lantana camara*	1.5
2	*Mentha spicata*	1.0
3	*Chromolaena odarata*	0.5
4	*Hyptis suaveolens*	0
5	*Anisomeles malabarica*	0

The study revealed that extracts from *Lantana camara*, *Mentha spicata*, and *Chromolaena odorata* exhibited antimicrobial activity against *A. tumefaciens*, while extracts from *Hyptis suaveolens* and *Anisomeles malabarica* did not show significant inhibition. This variation can be attributed to the differences in the phytochemical composition of each plant species. The larger inhibition zones observed for *Lantana camara*, *Mentha spicata*, and *Chromolaena odorata* extracts suggest a higher concentration of bioactive compounds effective against *A. tumefaciens*. These compounds may include various secondary metabolites known for their antimicrobial properties. Conversely, the lack of significant inhibition by *Hyptis suaveolens* and *Anisomeles malabarica* extracts indicates a lower concentration or absence of effective antimicrobial compounds.

Figure 4 Control

Figure 5 Media + A.tumefaciens

Figure 6 Media+selected plant species extract

Figure 7 Inoculation of media containing *A.tumefaciens* into the carrot

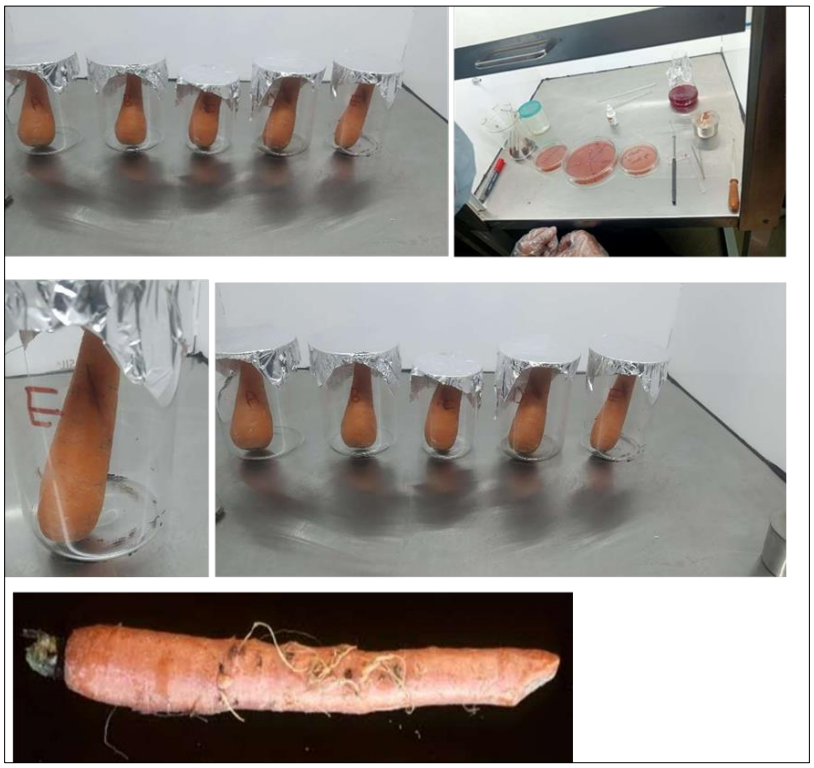

Figure 8 Carrots showing growth of *A.tumefacians*

The variations in antimicrobial activity are likely due to the presence or absence of secondary metabolites. Plant secondary metabolites are a diverse group of organic compounds that are not directly involved in the normal growth, development, or reproduction of plants. These metabolites often play a crucial role in plant defense against herbivory and microbial pathogens. Many secondary metabolites possess antimicrobial properties, and their presence and concentration in plant extracts can determine the extract's effectiveness against bacteria like *A. tumefaciens*.

In summary, the differences in antimicrobial activity observed among the plant extracts can be attributed to variations in their secondary metabolite composition. Extracts with higher concentrations of antimicrobial secondary metabolites, such as those from *Lantana camara*, *Mentha spicata*, and *Chromolaena odorata*, demonstrated greater inhibitory effects on *A. tumefaciens*.

Table 3 Growth of *A.tumefaciens*

S.No	Carrot with extracts	Growth of *A.tumefaciens*
1	Carrot with *A.tumefaciens*	Present
2	Carrot with *Lantana camara*	Absent
3	Carrot with *Mentha spicata*	Absent
4	Carrot with *Chromolaena odarata*	Absent
5	Carrot with *Anisomeles malabarica*	Present
6	Carrot with *Hyptis suaveolens*	Present

3.3. *In Vivo* Antimicrobial Activity

The *in vivo* experiments using carrot models confirmed the results obtained from the in vitro tests:

- Carrots inoculated with A. tumefaciens alone showed gall formation after two weeks.
- Carrots treated with Lantana camara, Mentha spicata, or Chromolaena odorata extracts after inoculation with A. tumefaciens showed no gall formation.
- Carrots treated with Hyptis suaveolens or Anisomeles malabarica extracts after inoculation with A. tumefaciens still developed galls, indicating the ineffectiveness of these extracts against the pathogen.

3.4. Gram Staining

The gram-negative A. tumefaciens appeared as red, rod-shaped microorganisms.

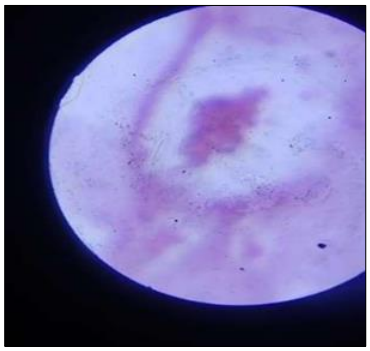

Figure 9 Microscopic view of *Agrobacterium tumeficiens*

4. Conclusion

In conclusion the findings of this study demonstrate the suppressive growth of crown gall on bacterium A.tumefaciens by Lantana camara, Mentha spicata, Chromolaena odorata. In carrot we also observe suppressive growth of crown gall disease causing A.tumefaciens.The plant extract of selected plants species, extraction method done by water to reduce the chemical use in fields and reduce the economically less to farmers and ecofriendly.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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