ECO-FRIENDLY BIOCONTROL MEASURES FOR *XANTHOMONAS* INFECTION ON VEGETABLE CROPS

A. John De Britto*[1] and D. Herin Sheeba Gracelin[1]

[1] Plant Molecular Biology Research Unit, Post Graduate and Research Department of Plant Biology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.

Summary

Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops and are reported to have developed resistance to kanamycin, ampicillin, penicillin and streptomycin. This seriously hinders the management of diseases of crops and agriculture products. To control these bacteria farmers used many synthetic pesticides. But pesticides have made great contribution for quick and effective management of plant diseases and microbial contaminations in several agricultural commodities. Many *Xanthomonas* Pathovars have acquired resistance to synthetic pesticides. Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the management of pathogenic microorganisms. Hence the present study focused to control the phyto pathogen in eco friendly method using green plant extracts. Different parts of a medicinal plant namely *Lannea coromandelica* was screened for their antibacterial activity on *X. campestris*. The methanol and aqueous extracts of the leaves and fruits of the plant exhibited significant inhibitory effects against the tested bacteria. Hence in future the selected plant may be used as biocontrol agent to control *Xanthomonas* infection on vegetable crops.

Key words: Pesticides, biocontrol, eco-friendly, *X. campestris* and *Lannea coromandelica*

***Corresponding Author:** Dr. A. John De Britto
Dept. of Plant Biology and Biotechnology
St. Xavier's College (Autonomous)
Palayamkottai - 627 002
Tamil Nadu, India
Tel: 0091- 462- 4264374, Fax: 0091- 462-2561765
E-mail: bjohnde@gmail.com

Introduction

Xanthomonas is a very important kind of phytopathogenic bacteria, which causes the plant diseases all around the world. The hosts of this genus include atleast 124 monocotyledonous and 268 dicotyledonous plants, among which the rice bacterial blight, cabbage black rot disease, and citrus blight disease are the most serious diseases, which cause a big economic impact on agricultural production every year. Chemical control has been proved efficient and economical in controlling blight disease.

However, increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (4, 16). Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops (10). Among the Pathovars *Xanthomonas campestris* is very dangerous. Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the management of pathogenic microorganisms (9).

A green plant represents a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (3, 6, 12). Reports are available on the use of active agents from higher plants, in place of chemical fungicides, that are non-phytotoxic, more systemic and easily biodegradable (7). *Lannea coromandelica* is one of such important plants which belong to the family Anacardiaceae well distributed in India. It is commonly called as Odiar, Gumphini in South India. All the parts of the plant (leaves, bark, root, flowers, and fruits) have high medicinal values (11). Many investigations on the chemistry of the plant have been done. They revealed the plant contains majorly Polyphenols including Flavonoids and Tannins, Terpenoids, Gums, and Polysaccharides. These phytochemicals may exhibit potential antibacterial activity against the harmful phyto pathogen *Xanthomonas campestris*. Hence the present investigation is focused to control the phyto pathogen in eco-friendly methods through screening the antibacterial activity of different parts of *Lannea coromandelica* against *Xanthomonas campestris.*

Materials and methods

Collection of plant materials

Fresh plant and plant parts were collected randomly from the region of Tirunelveli, India. Fresh plant material was washed; shade dried and then powdered using the blender and stored in air tight bottles.

Extraction of plant materials

Aqueous extraction

10 g of plant powder was added to 100 ml of distilled water and mixed well. After 24 hours the supernatant collected and concentrated to make the crude extract. It was stored at 4℃ (8).

Methanol extraction

10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4℃ (8).

Phytochemical analysis

Phytochemical analysis of methanol extracts of different parts of *L. coromandelica* was conducted following the procedure of (2).

Antibacterial assay

Xanthomonas campestris (MTCC No. 2286) was procured from the Institute of Microbial Technology (IMTECH), India. The antibacterial activity of methanol and aqueous extracts of different parts of *L. coromandelica* was tested in disc diffusion method following the procedure of (1). Muller Hinton agar medium was seeded with 100µl of inoculum (1×10^8 CFU/ml). The impregnated discs containing the test sample (100µg/ml) were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Kanamycin 30µg/disc, Neomycin 10µg/disc) and blank discs (impregnated with solvent) were used as positive and negative control. The plates were then incubated at 37℃ for 24 h to allow maximum growth of the microorganisms (1). The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and mean of the three experiments was recorded.

Minimum Inhibitory Concentration (MIC)

The MIC of the aqueous and methanol extracts of different parts of the selected plant was determined by serial dilution technique as described by (14). 1 mg/ml of the sample solutions of all the extracts were prepared using Dimethyl Sulfoxide (DMSO). In this technique a large number of test tubes were used and each of the test tubes was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. Then these test tubes were inoculated with the selected organisms (inoculum contains 1×10^6 cells/ml) followed by incubation at 37° C for 24 hours to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC). Another three test tubes containing medium, medium and sample, medium and inoculum were used as control. Bacterial growth observed was only in test tubes (solution content was cloudy) containing medium and inoculum and the other two were clear showing no growth (14). Experiments were done in triplicate and repeated twice.

Statistical analysis

All data were expressed as mean ± SD. Statistical analyses were evaluated by one-way ANOVA followed by Tukey HSD test. Values with $P< 0.05$ were considered statistically significant.

Result and discussion

Phytochemical analysis

The preliminary phytochemical analysis of the leaves, bark, flowers and fruits of *L. coromandelica* showed the presence of steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins (Table 1).

Antibacterial activity assay

Aqueous extract

Antibacterial activity of aqueous extracts of all the parts of the plant are presented in Table 2. Highly significant antibacterial activity was observed in fruits of the selected plant followed by, leaves, flowers and bark respectively against the tested pathogen. The tested pathogen *X. campestris* was highly susceptible.

Solvent extract

The ANOVA analysis of the data revealed that the four parts of *L. coromandelica* ($p<0.05$) showed highly significant activity against the tested pathogens (Table 2). Tukey HSD analysis of the data revealed that *X.campestris* was highly susceptible to methanol extracts than aqueous extracts. Antibacterial activity of methanol and aqueous extract of fruits of *L. coromandelica* was highly significant when compared to Kanamycin and Neomycin.

Minimum Inhibitory Concentration (MIC)

The MIC methanol extracts of fruits of the selected plant was 16µg/ml against *X. campestris*. Then the MIC value of leaves was 32µg/ml against the microorganisms. Similarly the MIC value of bark was 64µg/ml against *X.campestris*. The MIC value of flowers of *L. coromandelica* was 64µg/ml. Hence it is concluded that the methanol extracts of fruits and leaves of *L. coromandelica* showed inhibition of bacterial growth even at low concentrations (Table 3). Among these four parts, the MIC value of fruits of the selected plant is the lowest against *X.campestris*. Hence the fruit shows significant ($p<0.05$) bactericidal activity compared to other parts.

(15) reported the anti – phytopathogenic activity of crude and methanol extract of leaves, stem bark, seed and dry fruit of *Terminalia thorelli*, against four phyto pathogens. (5) evaluated the antibacterial potentiality of hot aqueous and methanol solvent extract of mature leaves of *Polyalthia longifolia* against six reference bacteria. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (13). Hence the present investigation is focused to screen the antibacterial activity of leaves, bark, flowers and fruits the medicinal plant *Lannea coromandelica* against *Xanthomonas campestris.*

Conclusions

According to the results of antibacterial assay, the methanol extracts of fruits and leaves of *L. coromandelica* might be used as biocontrol antibacterial agents against *X.campestris* which affect plants.

Table 1: Phytochemical analysis of methanol extracts of selected plant parts

Compounds	Leaves	Bark	Flowers	Fruits
Steroids	+	+	+	+
Triterpinoids	+	_	_	+
Reducing sugars	+	_	_	+
Sugars	+	_	+	+
Alkaloids	+	+	+	+
Phenolic compounds	+	_	+	+
Flavonoids	+	+	+	+
Catechins	_	_	_	_
Saponins	_	_	_	_
Tannins	+	_	+	+
Anthroquinones	_	_	_	_
Amino acids	_	-	_	+

Table 2: Antibacterial activity of different parts of selected plant (zone of inhibition in mm)

Samples	Methanol extract	Aqueous extract	Neomycin	Kanamycin
Leaves	20.30±0.47	17.00±1.00	17.00±0.82	8.00±1.60
Bark	11.60±1.25	8.00±0.82		
Flowers	15.45±0.82	12.66±0.47		
Fruits	29.30±0.47	19.33±0.57		

Data given are mean of three replicates ± standard error. $P < 0.05$

Table 3: MIC Values of methanol extracts different parts of the selected plants (µg/ml)

Name of bacteria	MIC Values
Leaves	32.00±0.00
Bark	64.00±0.00
Flowers	64.00±0.00
Fruits	16.00±0.00

Results are mean from three sets of experiments, each set in triplicate ± SD, $p < 0.05$

Acknowledgement

The authors are grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for financial support (Ref. No: 38(1260)/10/EMR-II 17/05/2010).

References

1. Bauer AW, Kirby WM, Sherries JC and Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. American Journal of Clinical Pathology 1966: 45: 493-496.
2. Brinda P, Sasikala B and Purushothaman KK. Pharmacognostic studies on *Merugan kilzhangu*, BMEBR 1981: 3(1) 84 – 96.
3. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999:12: 564-582.
4. Cuthbertson AGS and AK Murchie. Economic spray thresholds in need of revision in Northern Irish Bramley orchards. *Biological News* 2005: 32:19.
5. Ghosh A, Das BK, Chatterjee SK and Chandra G. Antibacterial potentiality and phytochemical analysis of mature leaves of *Polyalthia longifolia* (Magnoliales: Annonaceae). The south Pacific Journal of Natural Science 2008: 26: 68-72.
6. Gibbons S. Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochemistry Reviews 2005: 4: 63-78.
7. Gottlieb OR, Borin MR and Brito NR. Integration of ethnobotany and phytochemistry: dream or reality. Phytochemistry 2002: 60: 145-152.
8. Harbone JB. Phytochemical Methods. *London: Chapman and Hill* 1973:17.
9. Mahajan A and Das S. Plants and microbes- Potential source of pesticide for future use. Pesticides information 2003: 28:33-38.
10. Mandavia MK, Gajera HP, Andharia JH, Khandar RR, Parameshwaram M. Cellwall degradation enzymes in host pathogen interaction of Fusarian wilt of chicken pea: Inhibitory effects of phenolic compounds. Indian Phytopathology 1999: 50:548-551.
11. Merlin Franco F and Narasimhan D. Plant names and uses as indicators of knowledge patterns. Indian Journal of Traditional Knowledge 2009: 8(4): 645.
12. Newman DJ, Cragg GM and Snader KM. The influence of natural products upon drug discovery. Natural Product Reports 2000:17: 215-234.
13. Rastogi RP and Mehrotra BN. Glossary of Indian Medicinal Plants. National Institute of science communication, New Delhi, India 2002**:** p. 20-25.
14. Reiner R. Antibiotics- An Introduction, F. Hoffman La Roche and Co., Basle, Switzerland 1982: p. 70.
15. Shirsat RP. Screening of Anti-Phytopathogenic Activity of *Terminalia thorelii.* Ethnobotanical leaflets 2008: 12:538-541.
16. Singh HP, DR Batish RK and Kohli. Allelopathic interactions and alleloc-hemicals: New possibilities for sustainable weed management. Cri. Rev. Plant Science 2003: 22: 239-311.