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Research Article

ANTI-BACTERIAL EVALUATION OF FEW SOUTH INDIAN MEDICINAL FLOWERS AGAINST PLANT PATHOGENIC *XANTHOMONAS* BACTERIA

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ABSTRACT

Xanthomonas campestris pv. *centellae* (*X.c.*pv.*c*) is a harmful phytopathogen which causes leaf spot disease on Centella plants (*Centella asiatica*). To control this harmful pathogen in biocontrol method, methanol and aqueous extracts of twenty south Indian flowers were evaluated for their antibacterial activity against the harmful pathogen which was isolated from the infected Centella plant in disc diffusion method in five different concentrations (5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml). Among these, the methanol and aqueous extracts of *Punica granatum, Carica papaya, Thevetia peruviana, Catharanthus roseus* and *Calotropis gigantea* showed significant inhibitory effect ($p<0.05$). Based on the MIC values, *C. papaya* (8µg/ml) and *C. roseus* (16µg/ml) were selected for biocontrol agents against *X.c.*pv.*c.*

Keywords: *Xanthomonas campestris* pv. *centellae, Centella*, Antibacterial activity and MIC.

INTRODUCTION

In agriculture 1/3 of the yearly harvest is destroyed by pests and loss due to this is expected to be nearly $300 billion per year[1]. To control the disease causing pathogens, numbers of synthetic pesticides and antibiotics were used by the formers. But pesticides cause environmental pollution and many unwanted effects in man. Incessant and extensive use of these synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity[2]. Many pathogenic microorganisms have acquired resistance to synthetic pesticides[3]. Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops and are reported to have developed resistance to ampicillin, penicillin and streptomycin[4]. 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[5]. A green plant represents a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides [6, 7, 8]. 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[9]. Flowers are the important parts of the plants. Some active compounds which are present in the flowers, exhibit medicinal properties as antibacterial, antifungal[10] and used to treat various kinds of health problems in the living organism[11, 12]. This led to screen *in vitro,* a large number of south Indian flowers of different plants as listed in Table 2 for antibacterial activity against important phytopathogenic *Xanthomonas*, with the ultimate aim of developing plant based formulations for plant disease management.

Hence the present investigation deals with the screening of methanol extracts of twenty south Indian medicinal flowers for their antibacterial activity against *X.c.*pv.*c.*

MATERIALS AND METHODS

Collection of plant materials

Fresh flowers of different medicinal plants were collected randomly from the region of south India and their identification was confirmed with the help of herbarium specimens in CBB library, St. Xavier's college, Palayamkottai. The flowers screened together with their families and their medicinal uses are given in Table 1. Fresh flowers were washed, shade dried and then powdered using the blender and stored in air tight bottles.

Table 1: Flowers of fourteen plant species selected for antibacterial activity

Plant species	Family	Local name (Tamil)
Mangifera indica L.,	Anacardiaceae	Maangaai
Annona squamosa L.,	Annonaceae	Seetha
Punica granatum L.,	Lythraceae	Mathulai
Carica papaya L.,	Caricaceae	Pappali
Psidium guajava L	Myrtaceae	Goyya
Chrysanthemum indicum L.,	Asteraceae	Sevanthi
Jasminum sambac (L.) Aiton	Oleaceae	Mallihai
Jasminum grandiflorum L.,	Oleaceae	Pichi
Rosa indica L.,	Rosaceae	Roja
Hibiscus rosa-sinensis L.,	Malvaceae	Sembaruthi
Prosopis juliflora (Sw.)DC	Fabaceae	Karuvali
Parthenium hysterophorus L.,	Asteraceae	Gajar ghas
Crossandra infundibuliformis (L.) Nees	Acanthaceae	Kanahambarum
Thevetia peruviana (Pers.) K. Schum	Apocyanaceae	Thanga arali
Azadiracta indica A Juss.	Meliaceae	Vembu
Cassia auriculata L.	Caesalpiniaceae	Aavarai
Caesalpinia pulcherrima (L) Sw.	Caesalpiniaceae	Mayilkontrai

Catharanthus roseus (L) G. Don	Apocyanaceae	Sudukatu malli
Calotropis gigantea L.	Asclepiadaceae	Erukku
Nerium oleander L.	Apocyanaceae	Sewarali

Methanol extraction

10 g of powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 42 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4° C [13].

Aqueous extraction

10 g of plant powder was added to 100 ml of distilled water and mixed well. After 42 hours the supernatant collected and concentrated to make the crude extract. It was stored at 4° C [13].

Bacterial strain

The culture of *Xanthomonas campestris* pv. *centellae* were isolated from infected *Centella* plant and maintained in nutrient agar slant at 4°C.

Antibacterial activity

The antibacterial activity of methanol and aqueous extracts of different flowers was tested in disc diffusion method[14]. Muller Hinton agar medium was seeded with 100µl of inoculum (1× 10^8 CFU/ml). The impregnated discs containing the test sample (5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml and 80µg/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 °C for 24 h to allow maximum growth of the microorganisms[14]. 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.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the crude aqueous extracts of *P.granatum, C. papaya, T. peruviana C. roseus* and *C. gigantea* were determined by using serial dilution technique[15]. 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[15]. Experiments were done in triplicate and repeated twice.

Relative Percentage Inhibition (RPI)

The relative percentage inhibition of the test extract with respects to positive controls (kanamycin and neomycin) was calculated by using the following formula [16, 17].

$$\text{Relative percentage inhibition of the test extract} = \frac{100\ X\ (x-y)}{(z-y)}$$

Where,

x : total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr2; where, r = radius of zone of inhibition.

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.

RESULTS

Antibacterial activity

To control this harmful phyto pathogen in biocontrol method, methanol and aqueous extracts of twenty different flowers as listed in Table.1 were evaluated for their antibacterial activity against the isolated harmful pathogen in disc diffusion method in five different concentrations (5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml). All the extracts showed marked inhibitory effects against the isolated bacteria (Table 2a and 2b). Among these, the methanol and aqueous extracts of *P.granatum, C. papaya, T. peruviana C. roseus* and *C. gigantea* showed significant inhibitory effect when compared with positive controls neomycin and kanamycin in 40µg/ml, 80µg/ml concentration. The test pathogen was more susceptible to the methanol extract of each plant material than the aqueous extract. The susceptibility of the test inoculum to the extract of individual plant material increased with increasing concentration of the extract. The highest susceptibility was recorded with the methanol extract of *C. papaya*, followed by the extracts of *P.granatum, T. peruviana, C. roseus* and *C. gigantea* and the least, being recorded with the aqueous extracts of *P. hysterophorus* and *C. infundibuliformis*. The ANOVA analysis of the data revealed that among the five samples *C. papaya* ($p<0.05$) showed highly significant activity against the tested pathogens (Table 2a and 2b). Tukey HSD analysis of the data revealed that *X.c.*pv.*c* was highly susceptible. Antibacterial activity of methanol and aqueous extract of *C. papaya* and *C. roseus* was highly significant when compared to Kanamycin and Neomycin (Table 3).

Minimum Inhibitory Concentration (MIC)

The MIC of methanol extract of *T. peruviana* was 128µg/ml against the tested pathogen *X.c.*pv.*c*. Then the MIC values of methanol extracts of *C. gigantea* and *P.granatum* were 32µg/ml and 64µg/ml against the isolated pathogen respectively. Similarly the MIC values of *C. papaya* and *C. roseus* were 8µg/ml and 16µg/ml against *X.c.*pv.*c* respectively. Hence it is concluded that the methanol extracts of *C. papaya* and *C. roseus* showed inhibition of bacterial growth even at low concentrations (Table 4). Among these three samples, the MIC value of *C. papaya* is the lowest against *X.c.*pv.*c*. Hence *C. papaya* shows significant ($p<0.05$) bactericidal activity compared to other samples. According to the results of antibacterial assay, the methanol extracts of *C. papaya* and *C. roseus* may be used as antibacterial agents against *X.c.*pv.*c* which affect plants.

Relative Percentage Inhibition (RPI)

The results of antimicrobial activity methanol extracts of flowers (80µg/ml) were compared with the positive control (kanamycin and neomycin) for evaluating their relative percentage inhibition (Table 5). For kanamycin the methanol extract of *C. papaya* exhibits maximum relative percentage inhibition against the test inoculum (138.02%) followed by *C. roseus* (131.84%), *C. gigantea* (124.46%) and *P.granatum* (118.85%) respectively. For neomycin the methanol extract of *C. papaya* exhibits maximum relative percentage inhibition against the test inoculum (133.51%) followed by *C. roseus* (127.54%), *C. gigantea* (120.57%) and *P.granatum* (114.97%) respectively.

Table 2a: Antimicrobial activity of methanol extracts of twenty flowers against *X.c*.pv.*c* in five concentrations

Plant species	5 µg/ml	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml
M. indica	10.33±0.57	11.33±1.15	12.30±0.82	13.30±0.47	13.56±0.57
A. squamosa	11.33±0.47	13.43±0.45	14.30±0.62	15.20±0.47	15.66±0.47
P. granatum	15.45±0.82	16.48±0.55	17.70±0.25	18.50±0.79	18.66±0.34
C. papaya	16.34±0.47	17.78±0.89	19.70±0.47	21.34±0.34	21.67±0.82
P. guajava	12.38±0.57	13.09±0.47	13.98±0.76	14.67±0.47	15.43±0.82
C. indicum	11.45±0.67	12.00±1.15	13.47±0.68	14.87±0.82	14.98±0.89
J. sambac	10.45±0.47	11.05±0.76	12.98±0.47	13.09±0.86	13.90±0.82
J.grandiflorum	12.34±0.47	13.67±0.57	14.54±0.23	15.67±0.57	15.85±0.54
R. indica	11.42±0.82	12.06±0.47	13.67±0.78	13.95±0.78	14.57±0.47
H.rosa sinensis	10.33±0.57	10.57±0.87	12.46±0.47	13.98±0.82	14.32±0.78
P. juliflora	10.33±0.57	11.33±1.05	12.70±0.82	13.90±0.47	13.96±0.57
P.hysterophorus	09.73±0.37	09.53±0.45	10.20±0.42	10.30±0.67	11.46±0.47
C.infundibuliformis	09.43±0.47	09.83±0.55	10.10±0.32	10.50±0.27	11.46±0.27
T. peruviana	15.34±0.47	16.77±0.82	17.30±0.47	18.34±0.34	18.67±0.82
A. indica	11.73±0.37	13.53±0.45	14.20±0.42	15.30±0.67	15.46±0.47
C. auriculata	11.35±0.67	12.30±1.00	13.41±0.68	14.37±0.42	14.28±0.82
C. pulcherrima	10.25±0.47	11.15±0.26	12.68±0.37	13.49±0.26	13.30±0.82
C. roseus	15.74±0.47	16.97±0.47	17.94±0.23	19.67±0.57	20.70±0.54
C. gigantea	14.42±0.82	16.66±0.47	17.67±0.78	18.95±0.78	19.57±0.47
N. oleander	10.33±0.57	10.57±0.87	11.46±0.47	12.98±0.82	13.32±0.78

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

Table 2b: Antimicrobial activity of aqueous extracts of twenty flowers against *X.c*.pv.*c* in five concentrations

Plant species	5 µg/ml	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml
M. indica	09.23±0.57	10.33±1.05	10.37±0.82	11.30±0.47	11.56±0.57
A. squamosa	08.33±0.47	09.43±0.47	10.30±0.62	11.20±0.47	11.66±0.47
P. granatum	11.45±0.82	12.48±0.55	12.70±0.25	13.50±0.79	14.66±0.34
C. papaya	12.34±0.47	13.78±0.89	14.70±0.47	16.34±0.34	17.67±0.82
P. guajava	10.38±0.57	11.09±0.47	11.98±0.76	12.67±0.47	12.73±0.82
C. indicum	09.45±0.67	10.00±1.15	10.47±0.68	11.87±0.82	11.98±0.89
J. sambac	07.45±0.47	08.05±0.76	10.98±0.47	11.09±0.86	12.90±0.82
J.grandiflorum	08.34±0.47	08.67±0.57	09.54±0.23	10.67±0.57	11.70±0.54
R. indica	06.42±0.82	07.06±0.47	08.67±0.78	08.95±0.78	09.57±0.47
H.rosa sinensis	07.33±0.57	08.57±0.87	09.46±0.47	10.98±0.82	11.32±0.78
P. juliflora	05.33±0.57	06.33±1.05	07.70±0.82	07.90±0.47	08.96±0.57
P.hysterophorus	04.73±0.37	05.53±0.45	06.20±0.42	07.30±0.67	09.46±0.47
C.infundibuliformis	09.43±0.47	09.83±0.55	10.10±0.32	10.50±0.27	11.46±0.27
T. peruviana	09.34±0.47	10.77±0.82	11.30±0.47	11.34±0.34	12.67±0.82
A. indica	08.73±0.37	09.53±0.45	10.20±0.42	12.30±0.67	13.46±0.47
C. auriculata	07.35±0.67	08.30±1.00	08.41±0.68	09.37±0.42	10.28±0.82
C. pulcherrima	06.25±0.47	07.15±0.26	08.68±0.37	09.49±0.26	10.30±0.82
C. roseus	11.74±0.47	12.97±0.47	13.94±0.23	14.67±0.57	15.70±0.54
C. gigantea	10.42±0.82	11.66±0.47	12.67±0.78	13.35±0.78	14.57±0.47
N. oleander	07.33±0.57	08.57±0.87	09.46±0.47	09.98±0.82	10.32±0.78

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

Table 3: Positive and negative controls used in antibacterial assay

Antibiotics	Type of control	Inhibition zone
Kanamycin(30µg/ml)	Positive	15.70±0.85
Neomycin (10µg/ml)	Positive	16.23±0.47
Aqueous (Blank)	Negative	0.00±0.00
Methanol (Blank)	Negative	0.00±0.00

Table 4: Minimum Inhibitory Concentration of the five methanol extracts of the flowers

Name of the samples	Methanol (µg/ml)
P. granatum	64.00±0.00
C. papaya	8.00±0.00
C. roseus	16.00±0.00
C. gigantea	32.00±0.00
T. peruviana	128.00±0.00

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

Table 5: Relative percentage inhibition methanol extracts (80µg/ml) of flowers compare to standard antibiotic – Kanamycin

Name of the samples	Kanamycin	Neomycin
M. indica	86.36	83.54
A. squamosa	99.74	96.48
P. granatum	118.85	114.97
C. papaya	138.02	133.51
P. guajava	98.28	95.06
C. indicum	95.41	92.29
J. sambac	88.53	85.64
J.grandiflorum	100.95	97.65
R. indica	92.80	89.77
H.rosa sinensis	91.21	88.23
P. juliflora	88.91	86.01
P.hysterophorus	72.99	70.60
C.infundibuliformis	72.99	70.60
T. peruviana	118.91	115.03
A. indica	98.47	95.65
C. auriculata	90.95	87.98
C. pulcherrima	84.71	81.94
C. roseus	131.84	127.54
C. gigantea	124.64	120.57
N. oleander	84.84	82.07

DISCUSSION

The aqueous, different solvent extracts and isolated constituents of seven higher medicinal plants viz., *Althea officinalis* L. (Malvaceae), *Origanum vulgare* Oregano (Lamiaceae), *Plantago lanceolata* L. (Plantaginaceae), *Polygonum bistorta* L. (Polygonaceae), *Satureja hortensis* L. (Lamiaceae), *Solanum dulcamara* L. (Solanaceae), and *Quercus robur* L. (Fagaceae) were screened in *in vitro* for anti-bacterial activity by cup diffusion method against important phytopathogenic *Xanthomonas* pathovars viz., *Xanthomonas axonopodis* pv. *malvacearum* (*X. a*. pv. *m*.), *Xanthomonas axonopodis* pv. *phaseoli* (*X. a*. pv. *p*.) and *Xanthomonas campestris* pv. *vesicatoria* (*X. c*. pv. *v*.) associated with angular leaf spot of cotton, common blight of beans and bacterial spot of tomato[18]. But in the present study an important part of the plants i.e. flowers are used as biocontrol agents. The methanol and aqueous extracts of flowers showed marked antibacterial activity when compared with the leaves of the plants. In general, gram-negative bacteria were more resistant to antibiotics than gram-positive bacteria[19, 20]. The resistance is due to the differences in their cell wall composition. But the present study revealed that gram-negative bacteria *X.c.*pv.*c* were more susceptible to the crude extracts. It may be due to the presence of broad spectrum of antibiotic compounds present in different parts of the flowers. Likewise many antibacterial studies were carried out against human pathogenic bacteria [21-24].

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new antimicrobial substances; hence in the present investigation the antibacterial activity of extracts of twenty different flowers has been demonstrated for the first time against phytopathogenic bacteria *X.c.*pv.*c.*

CONCLUSION

To control the pathogenic bacteria certain biocontrol activities (using different parts of the plant extracts) should be imported to the farmers.

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REFERENCES

1. Chandler J Cost reduction in SIT programmes using exosect auto dissemination as part of area wide integrated pest management. Inter J pest con 2005; 47(5): 257-260.
2. Campos A, Lino CM, Cardoso SM, Silveira MIN Organochlorine pesticide residues in European sardine, horse mackerel and Atlantic mackerel from Portugal. Food Add Contam 2005; 22:642-646.
3. White DG, Zhao S, Simjee S, Wagner DD, McDermott PF Antimicrobial resistance of foodborne pathogens. Micro infect 2002; 4:405.
4. 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. Ind Phytopathol 1999; 50:548-551.
5. Mahajan A, Das S Plants and microbes- Potential source of pesticide for future use. Pest inform 2003; 28:33-38.
6. Cowan MM Plant products as antimicrobial agents. Clin Micro Rev 1999; 12:564-582.
7. Newman DJ, Cragg GM, Snader KM The influence of natural products upon drug discovery. Nat Prod Rep 2000; 17:215-234.

8. Gibbons S Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochemistry Reviews 2005; 4:63-78.
9. Gottlieb OR, Borin MR, Brito NR Integration of ethnobotany and phytochemistry: dream or reality. Phytochem 2002; 60:145-152.
10. Patel SS, Verma NK, Chatterjee C, Gauthaman K Screening of *Caesalpinia pulcherrima* Linn Flowers for Analgesic and Anti-inflammatory Activities. Inter J App Res in Nat Prod 2010; 3:1-5.
11. Zahid Zaheer A, Ajinkya G, Konale S, Khuman patel A, Subur Khan J, Rana Ahmed Z, Comparative phytochemical screening of flowers of *Plumeria alba* and *Plumeria rubra.* A J Pharm Clin Res 2010; 3:88.
12. Zahid Zaheer A, Aniruddha P, Paithankar Sagar D, Deshpande A, Subur khan J, Rana Ahmed Z Comparative phytochemical screening of flowers and bark of *Spathodea campanulata.* In J App biol pharma tech 2011; 2:233-235.
13. Harbone JB Phytochemical Methods. London, Chapman and Hill; 1973.p.17.
14. Bauer AW, Kirby WMM, Sherries JC, Tuck M Antibiotic susceptibility testing by a standardized disc diffusion method. Amer J Clin Pathol 1966; 45:493-496.
15. Reiner R Antibiotics- An Introduction, F. Hoffman La Roche and Co., Basle, Switzerland; 1982.p.70.
16. Gaurav Kumar K, Karthik L, Bhaskara Rao KV In vitro anti-Candida activity of *Calotropis gigantea* against clinical isolates of Candida. J Pharm Res 2010; 3:539-542.
17. Ajay KK, Lokanatha RMK, Umesha KB Evaluation of antibacterial activity of 3,5-dicyano-4,6-diaryl-4-ethoxycarbonyl-piperid-2-ones. J Pharm and Biomed Ana 2003; 27:837-840.
18. Babu S, Satish S, Mohana DC, Raghavendra MP, Raveesha KA Anti-bacterial evaluation and phytochemical analysis of some Iranian medicinal plants against plant pathogenic *Xanthomonas* Pathovars. J Agri Tech 2007; 3(2):307-316
19. Paz EA, Lacy RN, Bakhtiar M The betalactum antibiotics penicillin and Cephalosporin in Prespective Hodder Stongton, London; 1995.p. 227.
20. Chowdhury AA, Islam MS Antibacterial activity of *Trema orientalis*. Dhaka University. J Phar Sci 2004; 3:115-117.
21. Sawhney SS, Painuli RM, Dolly Singh Antioxidant and Antimicrobial activity of *Phyllanthus emblica* for its application in treatment of ophthalmic disorders. Int J Pharm Pharm Sci 2011; 3(4): 129-132.
22. Archana Devi, Virender Singh, Bhatt AB *Invitro* antibacterial activity of Pomegranate and Daru (wild pomegranate) against dental plaque bacteria. Int J Pharm Pharm Sci 2011; 3(4): 182 184.
23. Grace Nirmala J, Narendhirakannan RT *In vitro* antioxidant and antimicrobial activities of grapes (*vitis vinifera. L*) seed and skin extracts - Muscat variety. Int J Pharm Pharm Sci 2011; 3(4): 242-249.
24. Surendra Kumar M, Rajeswari N, Astalakshmi Evaluation of antimicrobial activities of *Aristolochia indica* (Linn). Int J Pharm Pharm Sci 2011; 3(4): 271-272.