Antimicrobial potency of five south Indian ferns against *Xanthomonas campestris*

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ABSTRACT

The objective of the present study is to evaluate the antimicrobial potency of five solvents (petroleum ether, benzene, chloroform, methanol and water) extracts of five medicinal ferns *Pteris biaurita* L., *Lygodium flexiuosam* (L.) Sw., *Hemionitis arifolia* (Burm.F.) T. Moore, *Actinopteris radiata* (J. Koenig ex Sw.) Link and *Adiantum latifolium* Lam. against the gram negative plant pathogenic bacteria *Xanthomonas campestris*. Antimicrobial activity, Minimum Inhibitory Concentration (MIC) and Relative Percentage Inhibition (RPI) value of the extracts against *X. campestris* were screened according to the standard procedures. Among the twenty five tested extracts, methanol and benzene extracts of *P. biaurita* showed significant inhibition ($p<0.05$) against the tested bacteria. Based on the results of MIC (8µg/mL) and RPI (199.36%) values, it could be concluded that *P. biaurita* can be used as potential plant for the management of *X. campestris* which is known to cause leaf spot disease on many vegetables and cash crops particularly *Centella asiatica.*

Key words: Antimicrobial activity, *Pteris biaurita, Xanthomonas campestris*

INTRODUCTION

Xanthomonas is an important phytopathogenic bacterium which is involved in leaf spot diseases on many crop plants including cotton, pepper, tomato, beans, paddy, rice etc. (Mohana and Raveesha, 2006). In pepper, beans, cabbage and rice cultivation *Xanthomonas* caused bacterial spot and black rot diseases resulting in significant losses estimated at about 10- 20%, 10%, 40% and 5-30% respectively. The losses caused by this bacterium were different every year. But during some years, the damages were extremely higher as a result of favorable climatic conditions, warm and rainy summer (Opina and Exconde, 1971; Saettler, 1989; Mitrev *et al*., 1999; Massomo, 2002). The eradication of *Xanthomonas* pathovars was achieved so far with acid compounds e.g., HCl, acetic acid, copper compounds or chlorine derivatives and heat treatments with a certain efficacy (Raghavendra *et al*., 2006). Even though these methods are effective, copper compounds and chlorine derivatives cannot be used in crops for human consumption (Campos *et al*., 2005). Hence, alternatives from biological sources would be highly useful in the management of these pathogens in an ecofriendly way.

The five selected ferns *Lygodium flexiuosam, Actiniopteris radiata, Pteris biaurita, Adiantum latifolium* and *Hemionitis arifolia* have medicinal properties (Upreti *et al*., 2009; Rout *et al*., 2009; Mithraja *et al*., 2012). The antibacterial studies of these medicinal plants were already investigated against some common human pathogenic bacteria (Dalli *et al*., 2007; Wills and Asha, 2009). Due to the presence of medicinal properties and bactericidal effects against human pathogens the above plants were selected. Hence the present study analyzed the antibacterial activities of these ferns against *X. campestris.*

MATERIALS AND METHODS

Collection and preparation of plant extracts

Healthy, disease free leaves of *Pteris biaurita, Lygodium flexiuosam, Hemionitis arifolia, Actinopteris radiata* and *Adiantum latifolium* were collected randomly from the region of Southern Western Ghats (latitude - N 8^0 35'and longitude - E 77^0 25') and their identification was confirmed

with the help of herbarium specimens in Xavier's College Herbarium, St. Xavier's College, Palayamkottai. These samples were used for the preparation of aqueous and different solvent extracts. Thoroughly washed leaves were shade dried for 2 weeks and then powdered with the help of a blender (Preethi® eco chef heavy duty mixer grinder). 25g of the powder was extracted successively with 250 mL of petroleum ether, benzene, chloroform, methanol and distilled water using a Soxhlet extractor (SUNBIM®, India.) for 48 hrs (50°C). All the extracts were concentrated using distillation unit by complete evaporation and preserved in airtight bottles (100 mL) until further use.

Antimicrobial assay

Bacterial strain: *Xanthomonas campestris* (MTCC No. 2286) was procured from the Institute of Microbial Technology (IMTECH), India and was used to examine the antibacterial activity. The microorganism was maintained at 4°C on nutrient agar slants (HIMEDIA® - M001).

Antibacterial activity: The antibacterial activity of five solvent extracts of ferns was tested in disc diffusion method (Bauer *et al*., 1966). Muller Hinton agar medium (HIMEDIA®- M173) was seeded with 100 µl of inoculum (1×10^8 CFU/mL). The impregnated discs containing the test sample (20 µg/mL, 40 µg/mL and 80 µg/mL) were placed on the agar medium seeded with *X. campestris*. Standard antibiotic discs (Kanamycin 30 µg/disc, Neomycin 10 µg/disc) and blank discs (impregnated with respective solvents) were used as positive and negative control. The plates were then incubated at 37°C for 48 hrs to allow maximum growth of *X. campestris*. The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. Experiments were done in triplicate and the mean of the experiments was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the methanol and benzene extracts of *P. biaurita, L. flexiuosam, H. arifolia*, *A. radiata* and *A. latifolium* were determined by using serial dilution technique (Reiner, 1982). The methanol and benzene extracts were selected based on their efficient bactericidal activity against *X. campestris*.1 mg/mL of the stock solutions of the extracts were prepared using Dimethyl Sulfoxide (DMSO). In this technique a large number of test tubes (ten tubes for each extract) were used and each of the test tubes was filled with 1 mL of sterile nutrient broth media (HIMEDIA® - M002) and graded doses (2 µg-128 µg) 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 48 hrs to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. Another three test tubes containing nutrient broth medium, nutrient broth medium+sample, nutrient broth medium+inoculum were used as control. Bacterial growth observed was only in test tubes (solution content was cloudy) containing nutrient broth medium+inoculum and the other two were clear showing no growth Experiments were done in triplicate.

Statistical analysis

The data were analyzed using the analysis of variance (ANOVA) and the mean values were compared by using the Tukey multiple range tests ($p<0.05$) using statistical package for social science (SPSS) package 7.5 version.

RESULTS AND DISCUSSION

Antibacterial activity

The ANOVA analysis of the data revealed that among the five samples methanol extracts of *P. biaurita* ($p<0.05$) showed highly significant activity against the tested pathogen (Table 1). Tukey HSD analysis of the data revealed that *X. campestris* was highly susceptible to methanol extracts compared with other extracts. Antibacterial activity of methanol and benzene extract of *P. biaurita* was highly significant compared to Kanamycin (P=0.0132; F=64.000; df=2.6) and (P=0.01106; F=20.000; df=3.3) Neomycin.

Minimum Inhibitory Concentration (MIC)

Table 1. Antibacterial activity of five solvent extracts of five ferns against *Xanthomonas campestris* in three concentrations (µg/mL)

Solvents	Concentration (µg/mL)		
	20	40	80
P. biaurita			
Pet ether	11.3±0.9	15.6±0.4	12.3±0.8
Benzene	23.3±0.2**	27.6±0.8**	26.0±0.8**
Chloroform	13.6±0.2	18.6±0.4**	16.0±0.4*
Methanol	25.3±0.8**	31.3±0.8**	26.6±0.8**
Aqueous	5.6±0.7	8.0±0.8	6.0±0.4
L. flexiuosam			
Pet ether	06.3±0.4	08.3±0.4	07.0±0.8
Benzene	13.6±0.2	18.6±0.4**	16.0±0.4*
Chloroform	07.6±0.9	12.3±0.8	11.3±0.8
Methanol	15.6±0.4	18.3±0.2**	18.0±0.8**
Aqueous	4.3±0.2	6.3±0.9	5.3±0.4
H. arifolia			
Pet ether	07.3±0.4	08.0±0.8	07.4±0.2
Benzene	12.0±0.7	17.3±0.6**	13.6±0.2
Chloroform	05.6±0.4	09.3±0.4	07.3±0.2
Methanol	07.3±0.2	10.0±0.8	08.4±0.8
Aqueous	8.0±0.4	9.0±0.8	7.0±0.7
A. radiata			
Pet ether	10.3±0.4	11.3±0.7	10.0±0.8
Benzene	13.6±0.2	25.0±0.2**	15.3±0.4
Chloroform	15.0±0.4	15.3±0.4	10.6±0.9
Methanol	23.3±0.2**	26.3±0.4**	22.3±0.4**
Aqueous	8.0±0.4	9.6±0.9	4.0±0.7
A. latifolium			
Pet ether	05.3±0.6	13.0±0.8	07.3±0.2
Benzene	14.3±0.7	16.3±0.4**	14.0±0.2
Chloroform	10.6±0.8	16.0±0.8*	14.0±0.8
Methanol	14.0±0.4	16.3±0.2**	15.3±0.4
Aqueous	5.6±0.7	8.0±0.8	6.0±0.4
Positive control			
Kanamycin(30µg/mL)	15.7±0.8		
Neomycin (10µg/mL)	16.2±0.4		

Values are expressed as percentage mean ± SE (n = 6). SE=0.58 Tukey HSD significance (P<0.05); *statistically significant to Kanamycin P=0.0132, F=64.000 df=2.6; ** Statistically significant to Kanamycin and neomycin P=0.01106, F=20.000, df=3.3; Inhibition zone expressed in mm

Among these five samples, the MIC value of the *P. biaurita* was the lowest against *X. campestris* (08.00 and 16.00 µg/mL for methanol and benzene respectively) when compared to *L. flexiuosam* (64.00 and 128.00 µg/mL for methanol and benzene respectively), *H. arifolia* (128.00 and 256.00 µg/mL for methanol and benzene respectively), *A. radiate* (16.00 and 32.00 µg/mL for methanol and benzene respectively) and *A. latifolium* (64.00 and 128.00 µg/mL for methanol and benzene respectively). Hence *P. biaurita* showed efficient bactericidal activity compared to other samples. According to the results of antibacterial assay, the methanol extracts of *P. biaurita* might be used as antibacterial agent against *X. campestris* which severely affect plants. The antibacterial efficiency of *Hemionitis arifolia* was tested *in vitro* against infectious disease causing pathogens such as *Enterobacter aerogens, Klebsiella pnemoniae, Salmonella paratyphi A, Ralstonia eutropha, Salmonella typhi, Salmonella paratyphi B, Staphylococcus aureus, Bacillus cereus, Bacillus substilis, Bacillus sphericus, Bacillus sterothermophilus* and *Micrococcus luteus* (Bindu *et al*., 2012). Naik and Jadege (2010) screened the bactericidal activity of *Actiniopteris radiata* against human pathogenic bacteria such as *Escherichia coli, Shigella, Pseudomonas aeruginosa, Vibrio cholerae, Proteus vulgaris, S. typhi, B. subtilis, K. pnemoniae,* and *S. aureus*. The antibacterial activity of *Adiantum latifolium* was tested against *S. aureus*, *E. coli* and *P. aeruginosa* (Babu *et al*., 2012). In the above studies the ferns showed efficient bactericidal activities against human pathogens. But in the present study the selected fern extracts were tested against the plant pathogen *X. campestris*. Among the ferns *P. biaurita* showed significant bactericidal activities. It may be due to the presence of phytochemicals in it. The phytochemicals such as alkaloids, steroids, flavonoids, terpenoids, phenolic compounds and tannins have protective or disease preventive properties (Martins *et al*., 2001). The phytochemical analysis of *P. biaurita* showed the presence of steroids, alkaloids, flavonoids, triterpinoids, phenolic compounds, tannins and saponins (Britto *et al*., 2012). Hence due to the presence of the phytochemicals referred to above *P. biaurita* showed significant antibacterial activities. The results of the present investigation is successful in identifying the antibacterial activity of selected medicinal ferns which will

help in further identifying the nature of the bioactive principle and its solubility, isolation and characterization of the active principle responsible for the activity.

ACKNOWLEDGEMENT

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

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Manuscript history

Received : 22.02.2012

Revised : 15.10.2012

Accepted : 24.11.2012