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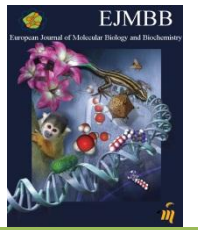

STUDY ON POTENTIAL BIOCONTROL AGENT - *ANGIOPTERIS EVECTA* (FORST) HOFF. AGAINST *XANTHOMONAS CAMPESTRIS*

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ABSTRACT

A phytopathogenic bacterium, *Xanthomonas campestris* play on vital role in plants which causes the plant diseases all around the world. 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. To control the disease causing pathogens, number of synthetic pesticides and antibiotics are used by the farmers. Pesticides have made great contribution for quick and effective management of plant diseases and microbial contaminations in several agricultural commodities. But pesticides cause environmental pollution and many unwanted effects in man. 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. Pteridophytes show medicinal utility and many of them are being used from ancient time. The rich diversity of Indian medicinal ferns has been evaluated for their antimicrobial properties, and this may have proved beneficial for mankind. All the parts like rhizome, stem, fronds, pinnae and spores contain antimicrobial and medicinal potency. *Angiopteris evecta* is a medicinal fern. Hence, the aim of this study is to evaluate the biocontrol potential of the petroleum ether, chloroform, benzene, methanol and aqueous extracts of fronds of *A. evecta* against *X. campestris.* The methanol extracts of the ferns gave successful result against the tested bacteria. Phytochemical analysis of all the extracts revealed that antibacterial activity is due to the presence of alkaloids, flavonoids and phenolic compounds. According to the results of MIC (Minimum Inhibitory Concentration) values, *A. evecta* could be used as potential biocontrol agent for the management of pathogenic bacteria, *X. campestris* which is known to cause diseases on many vegetables and cash crops.

INTRODUCTION

Xanthomonas campestris is a very important kind of phytopathogenic bacteria, which causes the plant diseases all around the world. Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops [1]. Among the Pathovars *Xanthomonas campestris* is very dangerous. 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. 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 [2].

Pteridophytes are one of the oldest land plant groups on earth and constitute a vast group of vascular

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cryptograms. Pteridophytes are also show medicinal utility and many of them are being used medicinally from ancient time [3]. Traditionally people used pteridophytes as medicine and anti-bacterial agents. Hence the present investigation is focused to control the phyto pathogen in eco-friendly methods through screening the antibacterial activity of five solvent extracts of *A. evecta* against *Xanthomonas campestris.*

MATERIALS AND METHODS

Collection of plant material

Healthy, disease free fronds of the *A. evecta* were collected from Kothayar and their identification was confirmed with the help of herbarium specimens in XCH (Xavier's College Herbarium), St. Xavier's college.

Preparation of plant extracts

The collected plant samples were thoroughly washed, shade dried and then powdered with the help of a blender. 50 g of the powder was extracted successively with 250 ml of petroleum ether, benzene, chloroform, methanol and distilled water using a Soxhlet extractor for 8 h at a temperature of 50-60^{o}C (not exceeding the boiling point of the solvent). All the extracts were concentrated and preserved in airtight bottle until further use.

Phytochemical analysis

Phytochemical analysis of benzene, chloroform and methanol extracts of the selected plants was conducted following the standard procedure [4].

Bacterial strain

The culture of *Xanthomonas campestris* isolated from diseased plant was maintained in nutrient agar slant at 4^{o}c.

Antibacterial activity

The antibacterial activity of five solvent extracts of the selected fern was tested in disc diffusion method [5].

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the benzene, chloroform and methanol extracts of *A. evecta* was determined by using serial dilution technique [6].

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< 0.005 were considered statistically significant. Mean and standard deviation were also calculated using the Microsoft Excel sheet, Office Edition 2007.

RESULT AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical analysis of the benzene, chloroform and methanol extracts of *A. evecta* showed the presence of steroids, triterpinoids, alkaloids, phenolic compounds, flavonoids and tannins (Table 1).

Antibacterial activity

The ANOVA analysis of the data revealed that among the five solvent extracts, methanol extract of *A. evecta* ($p<0.005$) showed highly significant activity against the tested pathogen (Table 2). Tukey HSD analysis of the data revealed that *X. campestris* was highly susceptible to methanol extract. Antibacterial activity of methanol and benzene extract of *A. evecta* was highly significant ($p<0.005$) compared to Kanamycin and Neomycin (Table 3).

Minimum Inhibitory Concentration (MIC)

The MIC value of methanol extract of *A. evecta* was 10µg/ml against *X. campestris*. Then the MIC value of benzene extract was 18µg/ml. Similarly the MIC value of chloroform extract was 34µg/ml against *X.campestris.* Hence it is concluded that the methanol extract of *A. evecta* showed inhibition of bacterial growth even at low concentrations (Table 4). Among these five solvents, the MIC value of methanol extract is the lowest against *X.campestris*. Hence the methanol extract of the selected fern *A. evecta* shows significant ($p<0.005$) bactericidal activity compared to other solvents.

Very less work has been done on the antimicrobial activity of pteridophytes, yet ethanobotanical importance of these plants have been investigated and studied by various authors. They have been found for their biological activity [7]. Antibiotic activity of pteridophytes has been studied [8] while the antiviral activities of crude extracts of some pteridophytes have also been analyzed [9]. Here the selected pteridophytes showed the significant antibacterial activity against the plant pathogens. In general, gram-negative bacteria were more resistant to antibiotics than gram-positive bacteria [10,11].

The resistance is due to the differences in their cell wall composition. But the present study revealed that gram-negative bacteria were more susceptible to the ferns extracts. It may be due to the presence of broad spectrum of antibiotic compounds present in different parts of the ferns.

Table 1. Phytochemical analysis of three solvent extracts of *A. evecta*

Compounds	Benzene	Chloroform	Methanol
Steroids	+	+	+
Triterpinoids	+	-	+
Reducing sugars	-	-	-
Sugars	-	-	-

Alkaloids	+	+	+
Phenolic compounds	+	+	+
Flavonoids	+	-	+
Catechins	-	-	-
Saponins	-	-	+
Tannins	-	-	+
Anthroquinones	-	-	-
Amino acids	-	-	-

Table 2. Antibacterial activity of *A. evecta* against *Xanthomonas campestris*

Name of the Solvents	Concentrations (µg/ml)		
	20	**40**	**80**
Petroleum ether	11.3±0.4	12.3±0.7	10.0±0.8
Benzene	20.3±1.2	23.6±0.8	21.0±0.8
Chloroform	14.6±1.2	19.6±0.4	15.0±0.4
Methanol	26.3±0.8	30.3±0.8	27.6±0.8
Water	05.6±1.7	09.0±0.8	07.0±0.4

Data given are mean of three replicates ± standard error, $p < 0.005$

Table 3. Zone of inhibition in positive and negative controls

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

Data given are mean of three replicates ± standard error, $p < 0.005$

Table 4. MIC Values of three solvents extracts of *A. evecta*

Name of the solvents	*X. campestris*
Benzene	18µg/ml
Chloroform	34µg/ml
Methanol	10µg/ml

CONCLUSION

It is hoped that this study would lead to the establishment of some compounds from the selected fern that could be used to formulate new and more potent antimicrobial agents of natural origin for the treatment of *Xanthomonas* in plants.

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