Antimicrobial Activity of Some Indian Herbs Against Plant Pathogens

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Abstract: Antimicrobial activity of 25 %, 50 %, 75 % & 100 % alcohol extract of *Aloe vera* and *Cissus quandrangularis* has been evaluated against *Xanthomonas compestris* and *Pseudomonas fulva* in Nutrient agar, *Aspergillus flavus* and *Aspergillus niger*, in Potato Dextrose Agar. *Aloe vera* extract showed excellent antimicrobial activity against all the test organisms, and in particular *Asperigillus niger*. Also among the tests conducted in *Cissus quadrangularis*, best result was observed with *Aspergillus niger*. In 25 % concentration, *Cissus quadrangularis* showed the highest 16 mm antimicrobial zone against *Aspergillus niger*. In 50 % concentration, *Aloe vera* showed the highest 14 mm antimicrobial zone against *Aspergillus niger*. In 75 % concentration *Cissus quandrangularis* showed the highest 16 mm antimicrobial zone against *Xanthomonas compestris* and in 100 % concentration, *Cissus quadrangularis* showed the highest 22 mm antimicrobial zone against *Pseudomonas fulva.* Among all the results obtained, the maximum of antimicrobial zone formation was obtained with 50 % and 75 % extracts of *Aloe vera* against *Pseudomonas fulva*, with 10 mm of antibacterial zone and with 25 % extract of *Cissus quadrangularis* against *Aspergillus niger*, with 16 mm of antifungal zone.
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INTRODUCTION

Plant pathogens such as *Xanthomonas compestris*, *Pseudomonas fulva*, *Aspergillus flavus* and *Aspergillus niger* are widely distributed in nature, causing disease epidemics in plants with huge losses in yield of crops as well threatening to wipe out and entire species such as was the case with "Dutch Elm Disease" and could occur with "sudden oak death." An epidemic of potato late blight, caused by *Phytophthora infestans*, led to the Great Irish Famine and the loss of many lives (1).

The bacterium *Xanthomonas compestris* causes a variety of plant diseases. It is used in the commercial production of a high molecular weight polysaccharide, Xanthan gum, that is an efficient viscosifier of water and that has many important uses, especially in the food industry. *Pseudomonas fulva* is an Gram-negative environmental bacterium (2), originally isolated from rice and commonly associated with rice plants, grains and paddy fields (3). Based on 16s rRNA analysis, *Pseudomonas fulva* has been placed in the *Pseudomonas putida* group (4).

Aspergillus niger causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys*, species of which have also been called "black mold" (5). Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins (6). Recent evidence suggests some true *Aspergillus niger* strains do produce ochratoxin A (7).

Aspergillus flavus, is a common mold in the environment, and can cause storage problems in stored grains. It can also be a human pathogen, associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic, and nasoorbital infections. Many strains produce significant quantities of aflatoxin (8), a carcinogenic and acutely toxic compound, which is one of the aetiological agents for hepatocellular carcinoma (9). *Aspergillus flavus* spores are allergenic too.

Plant disease epidemiology is often looked at from a multi-disciplinary approach, requiring biological, statistical, agronomic and ecological perspectives. Biology is necessary for understanding the pathogen and its life cycle. It is also necessary for understanding the physiology of the crop and how the pathogen is adversely affecting it. Agronomic

practices often influence disease incidence for better or for worse. Commonly the elements of an epidemic are referred to as the "disease triangle": a susceptible host, pathogen, and conducive environment (10).

For disease to occur all three of these must be present. Below is an illustration of this point (Fig. 1). Where all three items meet there is disease. The fourth element missing from this illustration for an epidemic to occur, is time. The host might out-grow susceptibility as with high-temperature adult-plant resistance (11), the environment changes and is not conducive for the pathogen to cause disease, or the pathogen is controlled through a fungicide application for instance.

This shift in susceptibility greatly affects our ability to successfully treat diseases. Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80 % of the world population rely on botanical preparations as medicines to meet their health needs. Herbs are generally considered safe and proved to be effective against certain ailments (12). They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of herbs has been gradually increasing in developed countries also.

In the present study, we have evaluated the antimicrobial effect of the extracts of two widely available herbs in India, *Aloe vera* and *Cissus quandrangularis* against two different bacterial and fungal plant pathogens, *Xanthomonas compestris*, *Pseudomonas fulva* and *Aspergillus flavus*, *Aspergillus niger* respectively.

MATERIALS AND METHODS

Microorganisms

Xanthomonas compestris, *Pseudomonas fulva* and *Aspergillus flavus*, *Aspergillus niger* were the pathogenic microorganisms included in the study. All the cultures were obtained in pure form from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of Herbal Extracts

The freshly harvested herbs were obtained from the local market. The herbs were cleaned, descaled when necessary, and washed in sterile distilled water. In order to obtain the herbal extracts, about 100 g of washed herb were crushed with mortar and pestle. The extracts were sieved through a fine mesh cloth and sterilized using membrane filter (0.45 - micron sterile filter). This extract was considered as the 100 % concentration of the extract.

The concentrations, 75 %, 50 % and 25 % were also made by diluting the concentrated extract with appropriate volumes of sterile distilled water.

Antibacterial sensitivity testing using filter paper method

Filer paper discs of 7mm diameter were prepared and sterilized. Using an ethanol dipped and flamed forceps, these discs were aseptically placed over nutrient agar plates for testing of the anti-bacterial activity and over potato dextrose agar for testing of the anti-fungal activity with the respective bacterial and fungal test organisms (13). One hundred microlitres of the various herbal extracts (100 %, 75 %, 50 %, 25 %) were aseptically transferred to these discs. The plates were incubated in an upright position at 37 ºC for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. Inhibition zones with diameter less than 12 mm were considered as having no antimicrobial activity. Diameters between 12 and 16 mm were considered moderately active, and these with >16mm were considered highly active.

All the media used in the present investigation were obtained from Hi-Media Laboratories Ltd., Mumbai, India.

Results and Discussion

Among the two herbs tested, against the plant pathogens, all showed antimicrobial activity. The result of the antimicrobial activity against the tested pathogens are reported in the below:

Table 1: Antimicrobial activity of different concentrations of Herbal Extracts

Pathogenic Organisms	Diameter of Inhibition Zone in mm against various concentrations of Herbal Extract							
	Aloe vera				*Cissus quandrangularis*			
	25 %	50 %	75 %	100 %	25 %	50 %	75 %	100 %
Xanthomonas compestris	-	-	-	1 mm	-	1 mm	16 mm	2 mm
Pseudomonas fulva	8 mm	10 mm	10 mm	1 mm	-	1 mm	1 mm	22 mm
Aspergillus niger	-	14 mm	12 mm	14 mm	16 mm	1 mm	-	12 mm
Aspergillus flavus	4 mm	7 mm	10 mm	12 mm	5 mm	5 mm	7 mm	10 mm

In 25 % concentration, *Aloe vera* showed the highest of 8mm antibacterial zone against *Pseudomonas fulva* and the highest of 16 mm antifungal zone against *Aspergillus niger*. In 50 % concentration, *Aloe vera* proved to be the best showing the highest of 10 mm antibacterial zone against *Pseudomonas fulva* and the highest of 14 mm of antifungal zone against *Aspergillus niger*. In 75 % concentration, *Xanthomonas compestris* showed the highest, 16 mm of antibacterial zone and 12 mm of antifungal zone against *Aspergillus niger*. And in 100 % concentration, a 22 mm of antibacterial zone was recorded with *Pseudomonas fulva* and a highest of 14 mm with *Aspergillus niger*. Among all the results obtained, the maximum of antimicrobial zone formation was obtained with 50 % and 75 % extracts of *Aloe vera* against *Pseudomonas fulva*, with 10 mm of antibacterial zone and with 25 % extract of *Cissus quadrangularis* against *Aspergillus niger*, with 16mm of antifungal zone.

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