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# Antibacterial Activity of Alkaloid Extracts and Active Constituents of Some Selected Plants against *Xanthomonas Campestris*

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**Abstract** The present investigation evaluates the antibacterial activity of alkaloid extracts of *Acacia catechu, A. ferruginea, Adenanthera pavonina, Albizia amara, A. saman, Breynia vitis-idaea, Senna spectabilis* and *Solanum indicum*, and bioactive compounds budmunchiamine-A isolated from *A. amara* and pithecolobine from *A. saman* against an important phytopathogen *Xanthomonas campestris*. The results revealed that alkaloid extracts of all plants showed significant antibacterial activity with zone of inhibition (ZOI), minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) ranged from 9.1 to 12.5 mm, 31.25 to 500 µg/ml and 125 to 2000 µg/ml, respectively. The active compounds isolated from alkaloid extract of *A. amara* and *A. saman* have been identified as budmunchiamine-A and pithecolobine, respectively. The active compounds budmunchiamine-A and pithecolobine showed concentration-dependent anti-*X. campestris* activity with ZOI, MIC and MBC ranged from 12.6 to 20.3 mm, 15.6 to 31.2 µg/ml and 62.5 to 125 µg/ml, respectively. The results revealed that alkaloid extracts of these plants as well as budmunchiamine-A and pithecolobine could be used as an alternative strategy for the management of diseases caused by *Xanthomonas* spp.

**Keywords:** Antibacterial activity, *Xanthomonas campestris*, alkaloid extracts, budmunchiamine-A, pithecolobine

## Introduction

Most bacteria belonging to the genus *Xanthomonas* are responsible for diseases on a large range of economically important crops, including monocots

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and dicots (Boureau *et al.,* 2013). *X. campestris* is one of the most agriculturally important pathogen causing a wide range of plants in the crucifer family (Brassicaceae), including vegetables, oilseed crops, and ornamental plants (Hayward, 1993; Silva *et al.,* 2002; Watt *et al.,* 2009; Bajpai *et al.,* 2011). This opportunistic pathogen invades host tissues through stomata, hydathodes, and wounds and eventually causes diagnostic V-shaped necrotic lesions at the foliar margin (Mansfield *et al.,* 2012). The use of pathogen-free seed, cultural practices and transplants has helped to manage black rot in many developed countries. However, the management of black rot is difficult in tropical environments, giving greater importance to development of other control measures. Rapid and effective control of the plant disease in crop cultivation is generally achieved by use of synthetic pesticides and antibiotics. However, these chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health (Babu *et al.,* 2007). Further, many reports stated that *X. campestris* has developed resistance to many antibiotics such as kanamycin, ampicillin, penicillin and streptomycin (Mandavia *et al.,* 1999; Mohana and Raveesha, 2006; Britto and Gracelin, 2011) The risk of developing resistance and the high cost–benefit ratio of synthetic pesticide uses, seriously hinders the management of diseases of crops and agriculture products. Hence, there is an urgent need to search for alternative eco-friendly strategies for the management of pathogenic *X. campestris* which is responsible for significant loss of agricultural commodities.

In recent decades, plants extracts have provided a valuable source for development of safe and eco-friendly antibiotics for the management of phytopathogenic bacteria including *Xanthomonas* spp. (Iwu *et al.,* 1999; Mohana and Raveesha, 2006; Kumaran and Karunakaran, 2007; Parekh and Chanda, 2007). Alkaloids are a structurally diverse group of over 12,000 cyclic nitrogen-containing compounds that are found in over 20% of plant species, which are important bioactive substances and have been reported for their various bioactivities (Medina *et al.,* 2001, Ahmad *et al.,* 2006, Roy and Chatterjee, 2010, Deng *et al.,* 2011). Considering these, we searched for plant alkaloids that are able to perturb the growth of *X. campestris* and identified active constituents from selected plants. In our laboratory, alkaloid extracts of

eight plants and, active molecules of *A. amara* and *A. saman* were screened against *X. campestris*.

Objectives: To evaluate the antibacterial activity of alkaloid extracts of eight plants and, active molecules of *A. amara* and *A. saman* against a phytopathogenic *Xanthomonas campestris*.

## Materials and methods

### *Chemicals and culture media*

The Mueller-Hinton Agar/Broth (MHA/MHB), neomycin and dimethyl sulfoxide (DMSO) were purchased from Hi-Media, Mumbai (India). All solvents, reagents and iodo-nitro-tetrazolium (INT) were purchased from Sisco Research Laboratory, Mumbai (India). Microtiter plates (96 wells) and serological pipettes were purchased from Axiva, New Delhi (India).

### *Plant materials*

Fresh disease free leaves of *Acacia catechu* (L.f.) Willd. (Fabaceae), *Acacia ferruginea* DC. (Mimosaceae), *Adenanthera pavonina* L. (Mimosaceae), *Albizia amara* (Roxb.) B.Boivin (Fabaceae), *Albizia saman* (Jacq.) Merr. (Fabaceae), *Breynia vitis-idaea* (Burm.f.) C. E. C. Fisch. (Phyllanthaceae), *Senna spectabilis* DC. (Fabaceae) and *Solanum indicum* L. (Solanaceae) were collected from different parts of southern Karnataka (India). The authenticated voucher specimens were deposited in the Herbarium of the Department of Microbiology and Biotechnology, Bangalore University, Bangalore (India).

### *Preparation of alkaloid extracts*

The alkaloid fraction from leaves of different plants was extracted following the procedure of Harborne (1998). Briefly, 50g of shade-dried powder of each plant material was repeatedly extracted with 200 ml of 10% acetic acid in ethanol separately. The mixture was then centrifuged at 4000rpm for 10 min and supernatant was concentrated to one quarter of the original volume using rotary flash evaporator. The crude alkaloid was precipitated by drop wise addition of concentrated $NH_4OH$ and the precipitation was collected by after centrifugation at 4000 rpm for 10 min. The residual solvent in the

collected alkaloid fraction was removed using a rotary flash evaporator. Dried alkaloid extracts of all eight plants were re-suspended in DMSO and subjected to antibacterial activity at desired different concentrations.

### *Isolation of bioactive compounds from alkaloid extracts of A. amara and A. saman*

The bioactive compounds from alkaloid extracts of *A. amara* and *A. saman* were isolated as described in our earlier report (Thippeswamy *et al.*, 2013). The isolated compounds were identified based on the comparison of infrared (IR), electrospray ionization-mass spectrometry (ESI-MS), $^{1}H$ and $^{13}C$ NMR data with the reported values (Wiesner *et al.*, 1968; Pezzuto *et al.*, 1991, 1992).

### *Antibacterial activity assay*

Antibacterial activity of alkaloid extracts of all eight plants, and active constituents of *A. amara* and *A. saman* was evaluated against phyto-pathogenic *X. campestris* by disc diffusion and broth-dilution methods. The test organism *X. campestris* (NCIM 2961) was obtained from National Collection of Industrial Microorganisms, Pune (India) and maintained on MHA. The zone of inhibition (ZOI) was determined by the disc diffusion method following the procedure of Ebrahimabadi *et al. (*2010). Briefly, sterile filter paper discs (6 mm) were individually impregnated with 20µl of two-fold diluted alkaloid extracts and bioactive compounds (0.95 to 4000 µg/disc), placed onto the pre-inoculated plates and incubated at 37℃ for 24hrs. DMSO served as a negative control, and two-fold diluted neomycin served as a positive control. The diameter of the ZOI was measured in millimetres (mm). Similarly, two-fold microbroth-dilution method was employed to determine the MICs and MBCs following the procedures of Hajji *et al. (*2010). Briefly, two-fold serial dilutions of alkaloid extracts and bioactive compounds with different concentrations (0.95 to 4000 µg/ml) were prepared in 96-well microtitre plate with MHB, inoculated with 15µl of *X. campestris* (inoculum size: $10^{8}$ cfu/ml) and incubated at 37℃ for 24hrs. After incubation, the inoculated plates were observed for the presence or absence of growth under the microscope (Olympus, CX 41, Japan). After macroscopic observation, a 10µl of broth was taken from the microtitre plate and streaked radially onto the MHA plates and incubated at 37℃ for

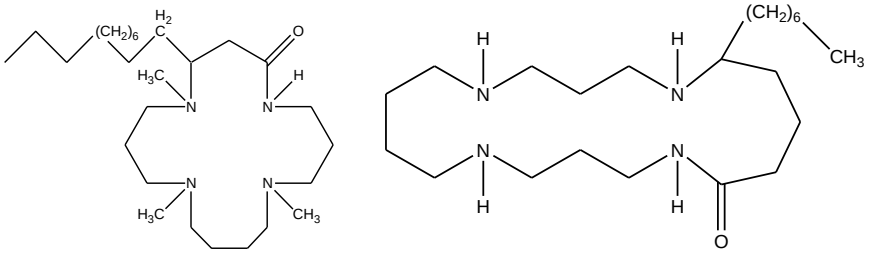
24hrs. The lowest concentration at which growth was inhibited that value was recorded as MIC and the complete absence of growth on the agar surface at the lowest concentration of the sample was defined as MBC (Hajji *et al.*, 2010). The phytotoxicity of alkaloid extracts of all the plants and active constituents was checked *in vivo* using maize seeds as a model system following the procedure of Thippeswamy *et al. (*2013). The maize seeds were treated with desired different concentrations of alkaloid extracts and bioactive compounds (125 to 4000 mg/kg) and untreated maize served as control.

### *Statistical analysis*

Values were expressed as Mean ± standard error. Analysis of variance (ANOVA) was performed, and the differences between values were tested for significance by Tukey's multiple comparison tests employing the SPSS 20 (IBM, USA) program.

## Results

Based on spectral analysis, the active compounds were identified as budmunchiamine-A (Figure 1A) from *A. amara* and pithecolobine (Figure 1B) from *A. saman* as reported in our earlier report (Thippeswamy *et al.*, 2013).




(A) (B)

**Figure 1.** Chemical Structures of budmunchiamine-A (A) and PC-pithecolobine (B).

The antibacterial activity of alkaloid extracts of all plants and the bioactive compounds budmunchiamine-A and pithecolobine were evaluated qualitatively and quantitatively (Table 1). The alkaloid extracts of *A. catechu, A. ferruginea, A. pavonina, A. amara, A. saman, B. vitis-idaea, S. spectabilis* and *S. indicum* showed significant antibacterial activity against *X. campestris* with ZOI, MIC and MBC values ranged from 9.1 to 12.5 mm, 31.25 to 500 µg/ml and 125 to 2000 µg/ml, respectively. Among the alkaloid extracts tested,

extracts of *A. saman, A. amara* and *S. spectabilis* showed the highest activity (MIC ≤ 62.5µg/ml), whereas extracts of *S. indicum, B. vitis-idaea* and *A. ferruginea* showed least activity (MIC ≥125 µg/ml).

**Table 1.** Antibacterial activity of alkaloid extracts of some plants against *X. campestris*.

| Alkaloid extracts/ active constituents | ZOI at 1000 µg/disc (mm) | MIC (µg/ml) | MBC (µg/ml) |
|---|---|---|---|
| *Acacia catechu* | 10.5 ±0.4 | 125 | 500 |
| *Acacia ferruginea* | 9.8 ±0.3 | 250 | 1000 |
| *Adenanthera pavonina* | 10.5 ±0.5 | 125 | 500 |
| *Albizia amara* | 11.8 ±0.7 | 62.5 | 250 |
| *Albizia saman* | 12.5 ±0.8 | 31.25 | 125 |
| *Breynia vitis-idaea* | 9.6 ±0.4 | 125 | 1000 |
| *Senna spectabilis* | 12.2 ±0.5 | 31.25 | 125 |
| *Solanum indicum* | 9.1 ±0.6 | 500 | 2000 |

Data given are the mean of four replicates ± standard error ($P \leq 0.001$). 6 mm diameter discs were used for antibacterial assay.

The active compounds budmunchiamine-A and pithecolobine also showed broad-spectrum and concentration-dependent bactericidal activity against *X. campestris* with ZOI, MIC and MBC values ranged from 12.6 to 20.3 mm, 15.6 to 31.2 µg/ml and 62.5 to 125 µg/ml, respectively. The bioactive compound pithecolobine (MIC 15.6 µg/ml) showed the highest activity than budmunchiamine-A (MIC 31.2 µg/ml). On comparative evaluation, the antibacterial activities of the bioactive compounds budmunchiamine-A and pithecolobine were comparable to synthetic neomycin. In phytotoxicity assay, no negative effects on seed germination and seedling growth were observed in maize treated with bioactive compounds, when compared to untreated maize. The seedling-vigour index in control, budmunchiamine-A and pithecolobine - treated maize seeds were 2370, 2530 and 2446 respectively.

**Table 2.** Antibacterial activity of active constituents of *A. amara, A. saman* and synthetic antibiotic neomycin against *X. campestris*

| Bioactive compounds | Zone of Inhibition (mm) | | | | | MIC (µg/ml) | MBC (µg/ml) |
|---|---|---|---|---|---|---|---|
| | 1000 µg/disc | 500 µg/disc | 250 µg/disc | 125 µg/disc | 62.5 µg/disc | | |
| Budmunchiamine-A | 12.6±0.5 | 10.1±0.4 | 9.4±0.5 | 7.5±0.2 | 7.0±0.2 | 31.2 | 125 |
| Pithecolobine | 20.3±0.6 | 18.1±0.4 | 14.5±0.5 | 11.6±0.1 | 9.1±0.7 | 15.6 | 62.5 |
| Neomycin | 16.8±0.4 | 16.1±0.6 | 15.7±0.4 | 14.3±0.7 | 13.6±0.4 | 15.6 | 31.2 |

Data given are the mean of four replicates ± standard error ($P \leq 0.001$). 6 mm diameter discs were used for antibacterial assay.

## Discussion

Management of bacterial diseases of plant remains difficult due to the limited availability of bactericides. Only a few synthetic chemicals are available, and their use is limited by poor efficacy in the field and their potential adverse effects to the environment. The use of antibiotics in plant protection is limited because of the possibility to select pathogen populations resistant to bactericides and the potential transfer of resistant genes to animal and human pathogenic bacteria (Iacobellis *et al.,* 2005). Despite serious side effects associated with the excessive use of synthetic pesticides, it remains play an important role in control of plant disease. The residuals of these synthetic pesticides are non-biodegradable and take a part in the food chain lead to various health problems in both animals and human beings. Testing antibacterial activity of plant extracts on phytopathogenic bacteria, especially *X. campestris* remains an area of intense interest. These are collectively necessitated to look for new safe and alternative agents from natural sources.

Green plants represent a reservoir of bioactive molecules and can provide valuable sources of natural pesticides (Harborne, 1998; Ahmad *et al.*, 2006). The obtained results revealed that the alkaloid extracts of all eight plants and bioactive compounds have been found effective in inhibiting the growth of *X. campestris*. To the best of our knowledge, the inhibitory activities of alkaloid extracts of all eight plants and bioactive compounds budmunchiamine-A and pithecolobine against *X. campestris,* have herein been reported for the first time. *In vitro* evaluation of the antibacterial activity of these plants against

phytopathogenic *X. campestris*, could add an approach to the management of diseases caused by *Xanthomonas* spp. in agriculture. Further investigations are necessary in order to support their mode of action and effects on other seed-borne pathogens.

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