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EVALUATION OF ANTIBACTERIAL ACTIVITY OF ALCOHOLIC CRUDE EXTRACT OF *ANTHOCEROS LONGII* - A BRYOPHYTE ON *XANTHOMONAS CITRI*

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In vitro study was conducted to find out the antiphytopathogenic property of *Anthoceros longii* (Steph.) methanolic extract against *Xanthmonas citri.* The number of colonies and inhibition zone of this bacterium enriched with nutrient agar medium were examined after 24 and 48 hours. Biomass and optical density of this bacterium under the influence of various concentrations of methanolic extract in Broth were also evaluated to find out antimicrobial property of this plant. Number of colonies and biomass decreased with increased concentrations of extract whereas inhibition zone increased respectively.

Keywords : Antiphytopathogenic; *Anthoceros longii;* Bryophyte; *Xanthomonas citri.*

Introduction

Plants grow in two well-defined habitats. These are the water and the land. In between extremes of these two habitats is a transitional zone. It is represented by the swamps and the areas where water and land meets. It may well be called the amphibious zone. Inhabiting the amphibious zone are the liverworts, hornworts and mosses which collectively constitute a group of non-vascular land plants called the bryophytes.

Several terpenoids and aromatic compounds were isolated from various liverworts (Hepaticeae) and antimicrobial activities and inhibitory activities of 5-lipoxygenase and Calmodilin studied[1]. The antibacterial activity of liverwort, *Lunularia crutiata* was studied. They evaluated the action of its acetone extracts against 13 bacterial strains. Inhibition of bacterial growth was compared with that of nacefotoxine, benzyl penicillin and tetracycline[2].

The extracted pigments of bryophytes exhibited antibiotic properties against gram-positive bacteria[3]. Antibacterial activity of the *Citrus limon* fruit juice by cup-plate diffusion method was evaluated against clinical isolates of drug resistant strains of *Salmonella typhimurium, Salmonella paratyphi, Shigella flexneri, Proteus mirabilis* and *Proteus vulgaris.* Almost all the isolates were found to be resistant to one or more antibiotics and chemotherapeutic agents. Fruit juice of *Citrus limon* was found to be effective against all clinical isolates and standard strains[4].

Antibacterial potency of methanol extracts of three green lower plants *Pneumatopteries afra, Platycerium bifurcatum* and *Nephrolepsis bisserata* was determined using agar clinical strains of *Escherichia coli, Staphylococcus aureus, Klebsiella spp*[5]. Phytoxic effects of crude aquoues extracts of three bryophyte, *P.articulatum, A.longii and F.bryoides* was carried out on *Agrobacterium tumifaciens* to unlock their antibiotic activity and revealed that *P.articulatum* exhibited more antibacterial activity followed by *A.longii and F.bryoide*[6]. The present study aims to explore antibacterial activity of some other bryophytes such as *Anthoceros longii* to investigate their potency against *Xanthomonas citri.* The aim of this study is to add some more plants to this list and to promote biocontrol methods.

Material and Method

Collection and storage of plants material : The plant material namely *A.longii* was collected from the natural habitat of Mt.Abu . The material was brought to the laboratory and was washed thoroughly with water to remove parts of other plants and soil adhered to rhizoids. It was washed with distill water and dryed in between blotting paper to remove extra moisture. The required quantities of materials was taken in pestel and mortar to prepare alcoholic crude extract. The remaining materials was wrapped in blotting paper and packed in polyethylene bags and stored in freeze to prevent from drying.

Test organism : The test organism, *Xathomonas citri* was cultured in nutrient agar tubes and broth in the laboratory.

The pure cultures were subcultured and serially diluted to obtain single colonies. Experiments were set up taking bacteria in microlitres.

Preparation of extract : 100g material of plant was taken in the pestel and mortar and crushed with 100ml of methanol and 900ml of distilled water repeatedly till the plant materials was properly crushed. The smooth pulps prepared were kept overnight so that all the water and alcohol soluble antibacterial substances of bryophyte dissolve. The extract was filtered through Whatsman filter paper. Then the extract was centrifuged at 5000 rpm for 30 minutes. The extracts so prepared were of 100000ppm. From these extracts different concentrations (10000ppm to 90000ppm) were prepared and stored in volumetric flask in freeze. Experiments were framed taking extracts in microlitres.

Preparation of medium : Nutrient agar medium and beef peptone agar medium of different pH ranging from 3 to 8.5 were prepared and autoclaved. The bacterium was then cultured over them in Petri dishes. It was observed that *Xanthomonas citri* grew best on 7.3 pH of nutrient agar.Therefore, nutrient agar having pH 7.3 was taken for this study.

Assay of antibacterial activity : The experiments were set in completely aseptic condition on laminar air flow bench. Four methods namely well and pour plate in Nutrient agar medium and optical density and biomass in Broth were adopted to assess the microbial growth under the influence of extract of *A. longii*.

In well method 10 µl of bacteria was spread over the agar film in Petri dish and well were made in it. In each well 25 µl of extract of different concentration (ppm) was poured and inhibition zone of growth was measured after every 24 and 48 hours.

In pour plate 10 µl bacterial culture, 25 µl extract of different ppm and medium were poured in Petri dishes. All these Petri dishes were sealed with parafilm and placed in an incubator at 28 °C for 24 and 48 hours.

In optical density 10 µl broth, 10 µl of bacterial culture and 25 µl of extract were poured in 100 ml conical flask and sealed with cotton plugs, aluminium foil and parafilm and incubated at temperature according to bacteria for 24 and 48 hours. After 24 and 48 hours optical density was taken by spectrophotometer in visible light at 600 wavelength.

In case of biomass as parameter 10 ml of broth 10 µl of bacterial culture and 25 µl of plant extract were poured in 100 ml conical flask and sealed with cotton plugs, parafilm and aluminium foil and incubated for 24 and 48 hours. After 24 and 48 hours, the test sample was centrifuged at 5000 rpm for 15 minutes. The centrifugate was decaned and bacterial mass was taken on pre-weighed butter paper and dried and weighed on the electronic balance.

Results and Discussion

Effect of methanolic crude extract of A. longii on Xanthomonas citri-

Solubility data and antibiotic spectra of the active plants indicated the occurrence of variety of antibiotic substances among bryophytes. The present work indicated that the number of colonies were maximum (198 and 205) in 10000 ppm while they were minimum (139 and 146) in 100000 ppm extract of *A. longii* after 24 and 48 hours (Fig.1). The persual data (Fig. 2) showed that zone of inhibition appeared to be highest (9 and 10 mm) in 100000 ppm whereas it was lowest (1.2 and 2 mm) in 10000 ppm extract after 24 and 48 hours collectively.

The effect of methanolic crude extract of *A. longii* against *Xanthomonas citri* advocated that the biomass of the bacterial growth (Fig. 4) appeared to be highest (0.00079 and 0.00082 µg) in 10000 ppm while it was found to be lowest (0.00064 and 0.00066) in 100000 ppm extract after 24 and 48 hours. Optical density of the bacterium was maximum (1.768 and 1.771) in 10000 ppm while it was minimum (1.743 and 1.747) in 100000 ppm extract of *A. longii* after 24 and 48 hours(Fig. 3).

The extracted pigments of bryophytes exhibited antibiotic properties against gram positive bacteria[3]. The present study observed that the growth of *Xanthomonas citri* was inhibited by bryophytes due to their antiphytopathogenic properties. Fruit juice of *Citrus limon* was found to be effective against all clinical isolates and standard strains.[4] The methanol extracts of three green lower plants *Pneumatopteries afra, Platycerium bifurcatum* and *Nephrolepsis bisserata* had antibacterial potency.[5] The results of present work suggested *A.longii* exhibited antibiotic activity due to antimicrobial substances inherited in it.

Phytoxic effects of crude aquoues extracts of three bryophytes, *P.articulatum, A.longii and F.bryoides* was carried out on *Agrobacterium tumifaciens* to unlock their antibiotic activity and revealed that *P.articulatum* exhibited more antibacterial activity followed by *A.longii and F.bryoides.*[6] The present study aims to explore antibacterial activity of some other bryophytes such as *A.longii* to investigate their potency against *Xanthomonas citri.*

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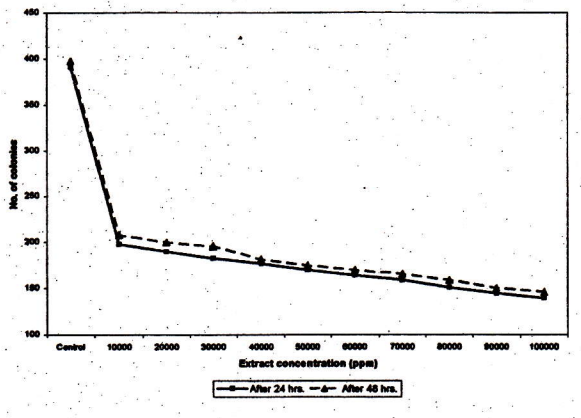

Fig. 1. Number of colonies of *Xanthomonas citri* after 24 and 48 hours in different concentrations of *A. longii* methanolic crude extract

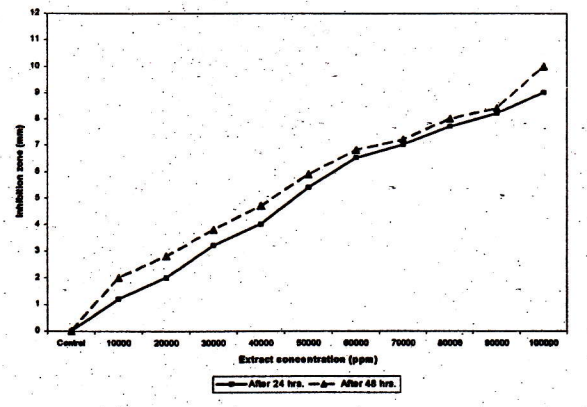

Fig. 2. Inhibition zone of *Xanthomonas citri* after 24 and 48 hours in different concentrations of *A. longii* methanolic crude extract

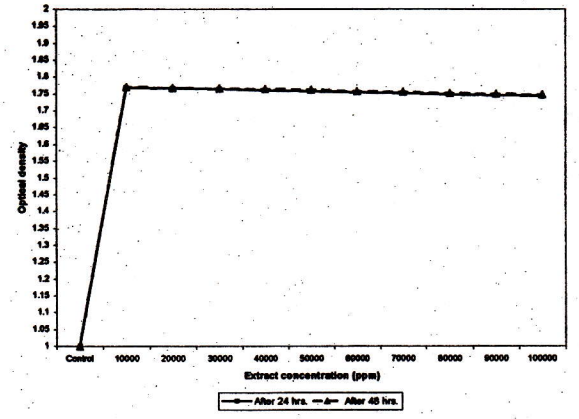

Fig. 3. Optical density of *Xanthomonas citri* after 24 and 48 hours in different concentrations of *A. longii* methanolic crude extract

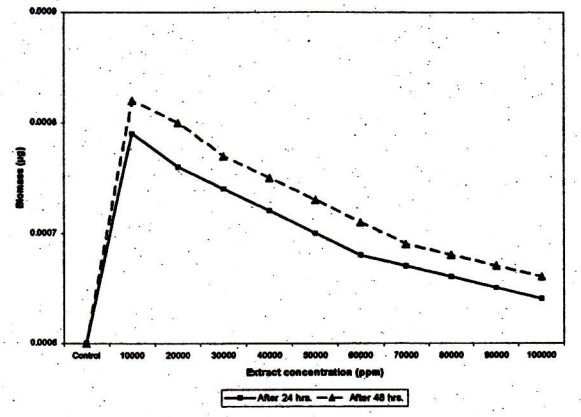

Fig.4. Biomass of *Xanthomonas citri* after 24 and 48 hours in different concentrations of *A. longii* methanolic crude extract

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