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COMPARISON STUDY OF THE ANTIMICROBIAL ACTIVITY OF SEED PROTEIN EXTRACTS FROM FOUR MEDICINAL PLANTS AGAINST *XANTHOMONAS OXANOPODIS* VER. *PUNICAE*.

Shantaveera Swamy H. M*, Vijay Kumara Swamy H. V and Preeti Upadhya

Department of Plant Biotechnology, University of Agricultural Science, GKVK, Bangalore, Karnataka, India, 560065.

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***Correspondence for Author**
Shantaveera Swamy H. M
Department of plant Biotechnology, University of Agricultural Science, GKVK, Bangalore, Karnataka, India, 560065.

ABSTRACT

The present study was conducted to evaluate the efficacy of antimicrobial peptides from four medicinal plants, i.e, *Glycine max*, Crotalaria *juncea*, *Adenanthera pavonina* and *Lawsonia inermis* against *Xanthomonas oxanopodis* pv. *punicae* which is an obligatory parasite of Pomegranate - *Punica granatum*. The extraction was carried out in Potassium phosphate buffer, Sodium phosphate buffer and Sodium acetate buffer. The antimicrobial activities of these plants were determined by microbiological technique using disc diffusion method against *Xanthomonas*. All the extracts exhibited antibacterial activity against *Xanthomonas* and the stronger antibacterial activity was observed by *Crotalaria juncea* extract against *Xanthomonas* with 31±1.5mm zone of inhibition. Our results indicated that the *Crotalaria juncea* is a rich source for antimicrobial peptides. The detach of Vance length of antimicrobial activity of the crude extraction from medicinal plants and presented in this paper.

Keywords: Antimicrobial activity, medicinal plants, *Xanthomonas oxanopodis* pv. *punicae*.

INTRODUCTION

A large group of Antimicrobial compounds known to the “natural antibiotics" are active against a large spectrum of microorganisms, including bacteria and filamentous fungi in addition to protozoan and metazoan parasites.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for

natural therapeutics. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and compounds. Therefore, such plants should be used as standard to the better understanding of their properties, safety and efficiency (Ellof, 1998).

Thousands of indigenous plants are used for medicinal treatment of ailments since prehistoric times (Capasso, 1998). Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen substituted derivatives such as tannins. In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds (Tapsell, 2006). The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. This system of medicine caters to the needs of nearly seventy percent of rural population. In addition to these there is demand for medicinal plants from pharmaceutical industries.

Microbial infections pose a health problem throughout the world and plants are a known source of antimicrobial agents (Adonis *et al*, 2000). Medicinal plants contain active compounds which can be used as an alternative to cheap and effective herbal drugs against common infections (Kareru *et al*, 2008). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). At present pathogenesis related plant proteins have been generally classified in accordance with their functional role in the formation of host plant immunity. On the other hand, considerable attention of researchers is attracted to a specific class of plant polypeptides capable of exerting antimicrobial effect. The list of bactericidal and fungicidal plant proteins is being updated continuously. These are relatively small polypeptides (molecular weight, from 2 to 9 kDa) of similar structure (Molina *et al*, 1993; Koo *et al*, 1998; Tarchevskii *et al*, 2001). According to one of the hypotheses molecules of antimicrobial peptides interact with the bacterial membrane giving rise to the formation of a trans-membrane cluster (probably, an ion channel) resulting in decrease in the membrane potential value and subsequent cytolysis (Taran *et al*, 2002).

The present study was conducted to compare the antimicrobial activity from four different seeds of the plants *Glycine max, Crotalaria juncea, Adenanthera pavonina, Lawsonia inermis* against *Xanthomonas oxanopodis* pv. *punicae.* This obligatory pathogen is reported to be the reason behind 50 to 100% crop loss of Pomegranate (*Punica granatum*) in India. In Karnataka specially pomegranate growing regions like Tumkur and Shira has have reported 100% crop loss in 2010 and caused huge yield and market loss. Since pomegranate and its allied products have a worldwide market and spreading of this decease can cause huge damage as there is no chemical counter measures are reported to prevent or control the decease. So it is the need of the hour to solve this critical problem before it reaches other parts of the world. The present work had been carried out in Laboratory, under DBT, plant Biotechnology, University of Agricultural Sciences. The objectives of this research were to evaluate the potential of plant extracts on standard microorganism strain, and investigate the synergistic effects of extracts with antimicrobial activity.

MATERIALS AND METHODS

Bacterial strain

Bacterial strain, *Xanthomonas oxanopodis* pv. *punicae* collected from the Department of plant Pathology, University of Agricultural Sciences, Bangalore.

Medicinal plants

The seeds were selected on the basis of review and ethno pharmacologic effects, these seeds were collected from the Central Western Ghats of karnataka, some from North Cañara, Karnataka, India.

Extraction of Antimicrobial Proteins

Antimicrobial proteins and peptides were extracted using 10mM of potassium phosphate buffer (pH 7.0), 10mM of sodium phosphate buffer (pH 7.0) and 3mM of sodium acetate buffer (pH 5.2). The buffers were prepared and seeds of selected medicinal plants were grinded using buffers and extract was collected in 10ml tube. This extract was further centrifuged at 10,000 rpm at 4^{o}C for 20 minutes. The crude extract isolated was saturated with 65% ammonium sulphate. Centrifuged the crude extracts and collected with supernatant and residues were re suspended in buffer. Now the resuspended residues were eluted using gel filtration column and collected in 1.5mL tubes. These samples were subjected to Bradford assay (Bradford M., 1976).

Culture Medium and Inoculum Preparation

Nutrient agar was used to test antibacterial activity of seeds of different plant extracts against *X. oxanopodis* pv. *punicae*. These cultured slants were incubated at 37°C for bacterial growth for 2-3 days. Nutrient broth was autoclaved at 121°C for 15 minutes. A loop full from pure culture of a bacterial strain was mixed in the medium after cooling the flask and then placed in the shaker at 37°C for 24 hours. Inoculum for the strain also prepared and stored at 4°C.

Assay by Disc Diffusion Method

Nutrient agar was dissolved in 100 ml of distilled water and autoclaved at 121°C for 15 min, allowed it to cool prior to transfer in to sterile Petri plates and then added were Inoculum mixed thoroughly and then poured it into the Petri plates and allowed it to solidify. After this, small filter paper (Whatman filter paper) discs were laid flat on growth medium and 100 µL of extract/protein was poured on each disc. Three replicates were prepared from each sample. The Petri plates were then incubated at 37°C for bacteria for 24 hours. The extracts having antimicrobial activity, inhibited the microbial growth, the inhibition and the clear zones were formed. The zone of inhibition was measured in millimeters using zone reader (Huynh *et al*. 2001).

RESULTS AND DISCUSSION

Plant seed extracts have control over the growth of *Xanthomonas oxanopodis* pv. *punicae* as potential antimicrobial compounds. Treatment of infectious disease of bacterial blight caused by this pathovariety of microbe seems possible in future using more standardized application methods.

Amount of protein and its antimicrobial activity after extraction with different types of buffers

The extracts of seeds of different medicinal plants were screened for antibacterial activity. The determination of antibacterial activity in disc diffusion method and the zone of inhibitions were also measured. Zone of inhibitions varied among the samples. Some of the samples had moderate antibacterial activity (11-19 mm); strong antibacterial activity (21-31 mm); highly antibacterial strong activity (31-41 mm). Negative results of some plants indicate that either plants had no active compound or it has very low concentration or activity. The results showed that different buffers have different protein extract ability percentages for different seeds (Table-1). The highest percentage of protein (4.68 µg/100mL) extracted by potassium phosphate was observed in *Adenanthera pavonina* and the lowest

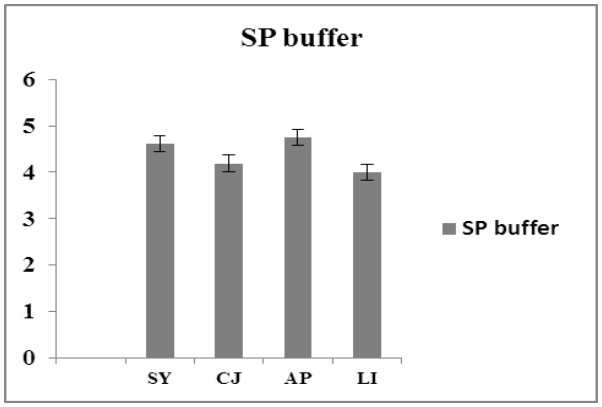
(4.10µg/100mL) was found in *Lawsonia inermis*. In sodium phosphate buffer, the highest percentage was of *Glycine max* and *Adenanthera pavonina* (4.75µg/100mL) and the lowest was *Lawsonia inermis* (4.00µg/100mL). In sodium acetate the highest percentage was of *Glycine max* and *Adenanthera pavonina* (5.52µg/100mL) whereas the lowest percentage was of *Crotalaria juncea (*4.10µg/100mL). Overall comparison of three buffers systems showed that sodium acetate buffer had maximum protein extract ability and the highest protein concentration was found in *Lawsonia inermis* whereas the lowest percentage was observed in by Sodium phosphate buffer.

Table 1. Comparison of protein concentration (µg/100mL) in crude extract by different buffers

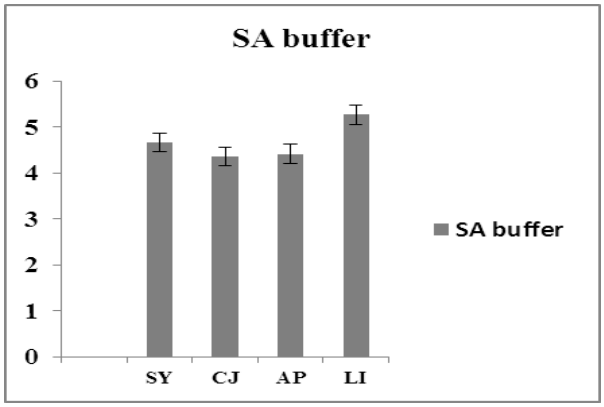
Sl. No	Plants	Potassium phosphate buffer (µg/100mL)	Sodium phosphate buffer (µg/100mL)	Sodium acetate buffer (µg/100mL)
1	*Glycine max*	4.56	4.61	4.67
2	*Crotalaria juncea*	4.32	4.19	4.36
3	*Adenanthera pavonina*	4.68	4.75	4.42
4	*Lawsonia inermis*	4.10	4.00	5.27

Antibacterial activity of different crude extracts prepared in potassium phosphate buffer against *Xanthomonas oxanopodis* pv. *punicae* strain was studied. The extract prepared in potassium phosphate buffer shown results. The highly strong activity was observed in the seed extracts of *Crotalaria juncea* against *Xanthomonas oxanopodis* pv. *punicae*.

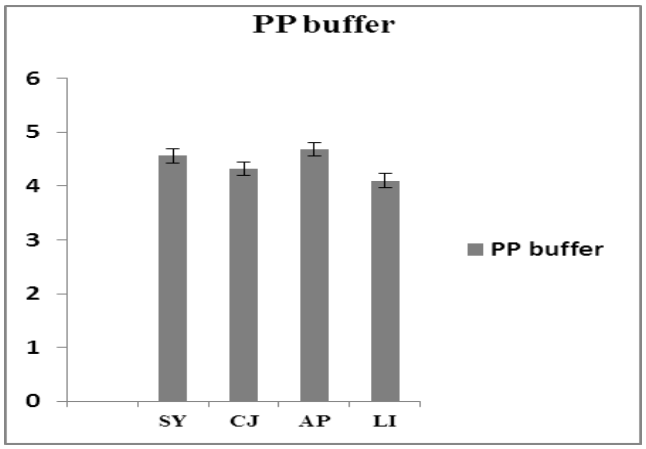
Antibacterial activity of crude extracts prepared in sodium phosphate buffer against *Xanthomonas* strain was studied. There has been found a highly strong activity of *Crotalaria juncea* extracted in sodium phosphate buffer against *Xanthomonas oxanopodis* pv. *punicae*. The least activity was found in *Lawsonia inermis.*



Potassium phosphate buffer



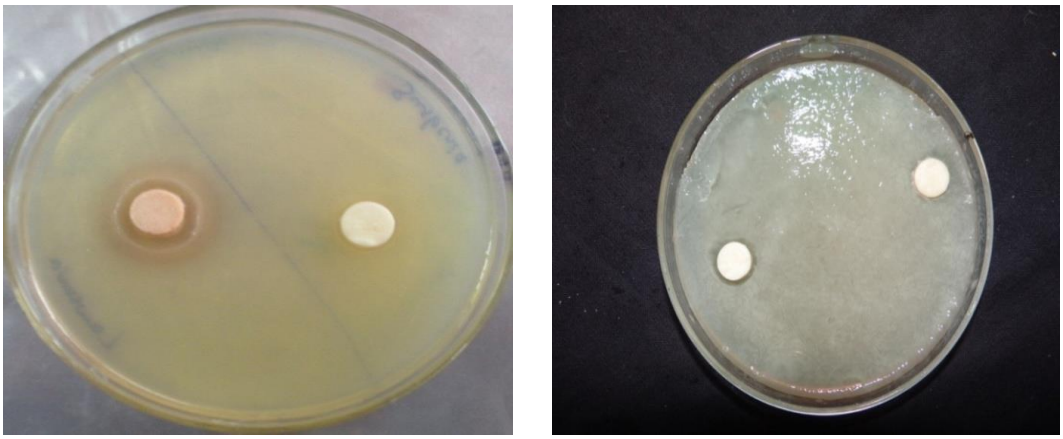
sodium phosphate buffer



Potassium phosphate buffer

SY- soybean, CJ- Crotalaria *juncea*, *AP- Adenanthera pavonina*, *LI -Lawsonia inermis*

Figure 1. Extension of zone of bacteria growth inhibition around filter papers with proteins extracted by different buffers in mm.



Crotalaria juncea Vs *Xanthomonas sp.* *Glycine max Vs Xanthomonas*

Figure 2. Antimicrobial activity of protein crude extracted in potassium phosphate buffer against the selected bacterial strain *Xanthomonas oxanopodis* pv. *punicae.*

CONCLUSION

Plant seed extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. These phytochemicals can be used in phytosanitation or prophylaxis of the bacterial blight caused by *Xanthomonas oxanopodis* pv. *punicae* by further standardization of protocals.

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