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Comparative analysis of the antibacterial activity of some phytolectins

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ABSTRACT

The aim of this work was to analyse the comparative effects of the antibacterial properties of partially purified lectins from the seeds of *Artocarpus heterophyllus (jack fruit), Canavalia ensiformis* (*jack bean*), *Lens culinaris* (lentil) and *Pisum sativum* (pea) against the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* The lectins were isolated by partial purification using ammonium sulphate precipitation and dialysis. The antimicrobial activity was studied using agar well diffusion method. The results showed that the Jack fruit lectin had a potent anti-bacterial activity against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* whereas Pea and jack bean lectin were found to be effective bacteriostatic agents which reduced the growth of bacteria and lentil lectin showed the least antibacterial activity. A comparison of the antibacterial activity of phytolectins with conventional antibiotics namely ampicillin and tetracycline was also carried out. Studies revealed that the antibacterial activities of the conventional antibiotics are higher than that of the plant extracts at the same concentration in accordance to literature.

***Key Words*:** *Artocarpus heterophyllus, Canavalia ensiformis*, *Lens culinaris, Pisum sativum*, lectin.

INTRODUCTION

The proteins present in the seeds of plants with the ability to bind and agglutinate cells were identified during the last century and such proteins were called phytohemagglutinins because of their ability to agglutinate red blood cells (Lis and Sharon, 1986). Broadly lectins are a class of proteins of non-immune origin that bind to carbohydrates without modifying them. Initially the term lectin was confined to soluble, multivalent proteins capable of agglutination and was restricted to proteins of plant origin (Joseph and Priya, 2011). However, now the term lectin is used in a broad sense to denote all types of carbohydrate-binding proteins that do not catalyze reactions with their ligands. Their bioactivity extends from simple anti microbial action to complex anti tumour properties (Conley and Kabara, 1973). Natural products therefore serve as pure compounds or as standardized plant extracts for the development of new drug leads (Nowshin *et al*., 2012).

Many seeds contain a considerable amount of lectin. Plant lectins are relatively soluble and can be easily extracted. Lectin extraction is usually carried out by different methods including diffusion in aqueous solution and ammonium sulphate precipitation. Lectins can be extracted from plant tissue with water or buffer solutions with controlled pH for maintaining their hemagglutination activity. The stability of lectins is dependent on the time and temperature used for the extraction process. Lectins present in a protein mixture can be separated and isolated by column chromatography where separation occurs due to differential migration of proteins adsorbed to the matrix (Zocatelli *et al*., 2003). Plants have enormous ability to synthesize aromatic substances, most of which include phenolics or their oxygen-substituted derivatives. It comprises of secondary metabolites which help in plant defensive mecha-

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nisms that offer protection against insects, herbivores and microorganisms (Rios and Recio, 2005).

There are many important reasons to screen for novel alternative antimicrobial substances from natural sources mainly plants (Ankri and Mirelman, 1999). The toxicity and side effects of the drugs presently used in health care and medicine being a major area of concern. The generation of drugs in plenty from natural sources with more efficacy, low cost of production and low or negligible side effects has become a prime focus of the pharmacological industry (Newman and Cragg, 2007).All over the world and also in the developing countries, most infectious bacterial diseases have resulted in the death of individuals. These organisms include Gram positive and Gram negative bacteria like different species of *Staphylococcus, Bacillus, Pseudomonas and Salmonella* which cause severe infections in humans (Livermore, 2000). The development of synthetic antibiotics have been successful in eliminating these organisms to an extent but pose the limitations like development of drug resistance by microorganisms (Arima and Danno, 2002), high cost and adverse side effects on the host.

The continuous emergence of novel infections and the microorganisms developing resistance make already existing antibiotics less effective. In such scenarios, natural products which are a part of our daily diet serve as the best candidates for new antibacterial drug discovery (Sunil *et al*., 2012).

MATERIALS AND METHODS

Source of material

Seeds of *Canavalia ensiformis, Lens lentil, Artocarpus heterophyllus* and *Pisum sativum* were collected locally and used for the isolation of lectins. The present study was conducted at the Centre for research and advanced studies, Mount Carmel College, Bangalore.

Isolation of lectins from *Pisum sativum* & *Lens culinaris*

Fifty grams of seeds were soaked overnight in 1X Tris buffer. It was homogenated in 10 mL Tris buffer, filtered and centrifuged for one hour at 10,000 rpm. The supernatant was collected and ammonium sulphate was added to the sample with constant stirring to a concentration of 80% saturation and kept overnight on magnetic stirrer. The precipitate thus formed was collected by centrifugation as above, dissolved in 2 mL of Tris buffer and then dialyzed extensively against three changes of Tris buffer (pH 7.2). The dialyzed protein was stored at 4°C for further use (Moreira *et al*., 1983).

Table 1: Protocol for evaluation of MIC by broth dilution method.

Amount of lectin extract/ml	Amount of medium (ml)	Total volume (ml)	Concentration of lectin extract in final solution (mg/ml)
0.1	9.9	10	0.1
0.2	9.8	10	0.2
0.3	9.7	10	0.3
0.4	9.6	10	0.4
0.5	9.5	10	0.5
0.6	9.4	10	0.6
0.7	9.3	10	0.7
0.8	9.2	10	0.8
0.9	9.1	10	0.9
1.0	9.0	10	1.0

Isolation of lectin from *Artocarpus heterophyllus*

Fifty grams of the seeds were de-skinned and soaked overnight in 10 mL of phosphate buffer saline at 4°C. It was homogenated in 10mL phosphate buffer saline and the homogenate was filtered and centrifuged for one hour at 10,000 rpm. The supernatant was collected and ammonium sulphate was added to the sample with constant stirring to a concentration of 40% saturation and kept overnight. The precipitate thus formed was collected by centrifugation as above, dissolved in 2ml of PBS and then dialyzed extensively against three changes of PBS (pH 7.2). The dialyzed protein was stored at 4°C for further use (Kabir,1995).

Isolation of lectin from *Canavalia ensiformis*

Fifty grams of defatted, finely ground seeds of *Canavalia ensiformis*, were suspended in 0.15M NaCl and stirred overnight in the cold. The suspension was homogenized and the filtered. The filtrate was then centrifuged for one hour at 10,000 rpm and the residue discarded. The supernatant solution was made 30% saturated with ammonium sulphate by gradual addition of the solid salt. The mixture was stirred at room temperature for one hour and the precipitate was collected by centrifugation as above, dissolved in distilled water and dialyzed in cold

Table 2: Antibacterial activity of the lectin extracts on the test organisms and comparison of antibacterial activity of lectin extracts with conventional antibiotics.

Microorganisms	Diameter of zone of inhibition (mean ± SD)					
	Artocarpus heterophyllus lectin	*Canavalia ensiformis* lectin	*Lens culinaris* lectin	*Pisum sativum* lectin	Ampicillin (25µg)	Tetracycline (25µg)
S. aureus	22.33±1.31	16.26±0.72	8.77±0.22	11.6±0.42	33	40
B. subtilis	21.16±1.42	14.96±0.93	7.36±0.45	9.96±0.41	24	32
E. coli	19.17±1.68	11.5±1.08	8.12±0.27	10.03±0.26	28	35
P. aeruginosa	18.66±1.35	12.06±1.10	9.56±0.55	11.36±0.48	27	34

against several changes of distilled water, and finally against 1M NaCl (Moreira and Perrone, 1977).

Test organisms

The organisms used in this study were *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*. The organisms were cultured in the Centre for research and advanced studies, Mount Carmel College, Bangalore.

Antimicrobial Activity Screening

The partially purified lectins were screened for their antibacterial activities by the standard Disc-Diffusion Method (Perez *et al.,*1990) by measuring the diameter of the inhibitory zones in mm using a concentration of 25µg/15µl of each of the lectins. Mueller Hinton Agar medium was used for determining antibacterial activity. The diameters of the zones of inhibitions of the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc such as ampicillin and tetracycline. The plates were incubated for 24 hours at 37°C. One well containing extractant serves as control in each plate. The plates were examined for zones of inhibition, that indicate the degree of susceptibility of the test organism.

Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method

Using sterile pipettes exact amount of extract was added as indicated in the Table 1 to obtain a final volume of 10 ml. The tubes were then inoculated with 0.05 ml of the standardized culture and further incubated at 37°C for 24 hours and observed for any microbial growth in form of turbidity. The test procedure was repeated to check the reproducibility of the results. The lower concentration that inhibited the microbial growth was taken as the Minimum Inhibitory Concentration (MIC). Ampicillin was used as reference standard (Thiem and Grosslinka, 2003; Islam *et al.*, 2008).

Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by sub-culturing test solutions which showed no detectable growth i.e. no turbidity after 24hours incubation onto fresh nutrient agar and incubated further for 24 hours to determine the MBC of the extract required to kill the organism. The failure of the test organism to grow on the plate after incubation indicated a bacteriostatic effect, while the plates that do not show growth after incubation indicated a bactericidal effect (Poole, 2001).

RESULTS AND DISCUSSION

The results of the anti bacterial activity of partially purified lectin extracts from the seeds of *Artocarpus heterophyllus* (jack fruit), *Canavalia ensiformis (*jack bean), *Lens culinaris* (lentil) *and Pisum sativum* (pea) tested in this study is shown in Table 2. As per the obtained results, *Artocarpus heterophyllus* lectin extract showed greater antibacterial activity compared to the other extracts. The *Artocarpus heterophyllus* lectin extract showed the highest activity against *B. subtilis* and *P. aeruginosa*, followed by *E. coli* and least on *S. aureus. All* the plant lectin extracts used in the study showed antibacterial activity at 1mg/ml. The least anti bacterial activity was exhibited by *Lens culinaris* lectin. Table 2 also shows comparison of the antibacterial effectiveness of the lectin extracts of plant samples with the activity of some conventional antibiotics namely Ampicilin and Tetracycline of same concentration. The antibacterial activities of the conventional antibiotics are shown to be higher than that of the lectin extracts.

Table 3 and 4 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the lectin extracts against

Table 3: MIC of the lectin extracts against concentration.

Microorganisms	MIC (mg/ml)			
	Artocarpus heterophyllus lectin	*Canavalia ensiformis* lectin	*Lens culinaris* lectin	*Pisum sativum* lectin
S. aureus	1	1	1	1
B. subtilis	1	1	1	1
E. coli	1	1	1	1
P. aeruginosa	1	1	1	1

Table 4: MBC of the lectin extracts against concentration.

Microorganisms	MBC*			
	Artocarpus heterophyllus lectin	*Canavalia ensiformis* lectin	*Lens culinaris* lectin	*Pisum sativum* lectin
S. aureus	+	-	-	-
B. subtilis	+	-	-	-
E. coli	+	-	-	-
P. aeruginosa	+	-	-	-

*+: Killing effect, -: no killing effect

concentration. *Artocarpus heterophyllus* lectin extract showed killing effect against all the microorganisms used whereas the other three lectin extracts were only able to inhibit the growth of the organisms but did not exert a killing effect on the test organism. This suggests that the *Artocarpus heterophyllus* lectin was bactericidal and the other lectin extracts were bacteriostatic in nature. Various studies have shown that plants that are rich in antinutritional compounds like lectins possess antimicrobial activity against a number of microorganisms.

Antimicrobial activity of lectins is an interesting and important topic of study because of the abundant prevalence of pathogenic microorganisms in the environment. Plant sources can be used to reduce the pathogenic effect of such microorganisms as they are eco-friendly and do not cause toxicity to the environment.

CONCLUSION

The study has showed that Jack fruit lectin is a potent antimicrobial agent as it shows antibacterial activity against *B. subtilis, E. coli, S. aureus* and *Pseudomonas.* Pea, jack bean and lentil lectin are effective bacteriostatic agents which help to decrease the growth of bacteria. This property of jackfruit lectin is helpful in medical and research as it is easily available and can also be purified efficiently. The failure of some of the lectin extracts to exert antibacterial effect on the test organism is not enough to conclude that the extract does not contain substances that can exert antibacterial activity against the test organism because the potency and activity of the extracts majorly depends on the method used for isolation and purification. In conclusion the antibacterial activity of the *Artocarpus heterophyllus* lectin could be enhanced if the component is completely purified. The isolated plant lectin extracts therefore serve to be a potential source of novel herbal drug for treating infectious diseases caused by some clinical pathogens.

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