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EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF PARTIALLY PURIFIED LECTIN FROM TUBERS OF *XANTHOSOMA VIOLACEUM*

Anitha N. and Sathisha G. J.*

Department of Postgraduate Studies and Research in Biochemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga - 577 451, Karnataka, India.

***Corresponding Author: Dr. Sathisha G. J.**
Department of Postgraduate Studies and Research in Biochemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga - 577 451, Karnataka, India.

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ABSTRACT
The present work describes the antimicrobial and antioxidant activities of a partially purified lectin from tubers of *Xanthosoma violaceum* (XVL). XVL was partially purified by ammonium sulphate precipitation followed by dialysis. Partially purified lectin was assayed for antibacterial activity against bacteria *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The antibacterial activity was studied using agar well diffusion method. Further, antioxidant activity was performed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method, iron chelating and superoxide radical scavenging activity. XVL exhibit antibacterial activity against *Escherichia coli*, *Salmonella typhimurium* and *Bacillus subtilis* but not towards *Pseudomonas aeruginosa.* The results of antioxidant activity of XVL were expressed in terms of percentage of inhibition. Therefore, XVL found to be a promising antibacterial and antioxidant agent which can be used as a natural medicinal product.

KEYWORDS: *Xanthosoma violaceum*, antimicrobial, antioxidant, lectins, tuber.

1. INTRODUCTION

Lectins are glycoproteins of nonimmune origin differ from enzymes[1], bind specific mono or oligosaccharide[2] and agglutinate cells.[3] They are initially isolated in plants so referred as hemagglutinins or phytoagglutinins. These proteins are widely distributed in nature starting from microorganisms, plants and animals.[4,5] Being abundantly found in seeds, plant lectins are also present in other parts of plants like leaves, rhizomes, bulbs, tubers etc. In recent years lectins have attracted great research interest due to their novel sugar specificities and various biological activities like cell agglutination, antitumor, immunomodulatory, antiproliferative, insecticidal, fungicide, antibacterial and antifungal.[6,9] Lectins are responsible for the plant defense and specific protein-carbohydrate interaction mediators within the plant cells.[10]

Antimicrobial agents have been used to treat diseases caused by bacteria and fungi. Owing to the multidrug resistance towards the present antibiotics by the microorganisms and the problems of emerging infectious diseases have made it inevitable to search for new antimicrobials of plant origin. Natural products which are part of our daily diet serve as the best candidates for discovering new antibacterial drugs. Plant lectins with potent antibacterial and antifungal activities have been isolated from various plants.[11,12] Lectins exert their antibacterial activity through their interaction with glycoconjugates present on bacterial cell surfaces, such as peptidoglycans, lipopolysaccharides and teichoic acids.[13,14] Antifungal activity of lectins occurs through an interaction with the fungal cell wall, which is composed of chitin, glucans and other polymers.[15]

Accumulating research evidences demonstrate that oxidative stress plays a major role in the development of several chronic diseases such as different types of cancer, cardiovascular diseases, arthritis, diabetes, autoimmune and neurodegenerative disorders and aging. Antioxidants play an important role in biological systems, including cell signalling pathways and defence against oxidative damage. Antioxidant are the substance that delays the oxidation of biomolecules and prevents damaging effects caused by reactive oxygen species(ROS).[16] ROS are produced as a product of normal cellular functioning, excessive amounts can cause deleterious effects on DNA, RNA and protein molecules, which may contribute to the physiology of aging and may be involved in many human diseases, such as atherosclerosis, cancer, diabetes and cardiovascular and neurological diseases, such as Parkinson's and Alzheimer's disease.[17,18] Though internal antioxidant defence systems, either enzymes (superoxide dismutase, catalase and glutathione peroxidase) or other compounds (lipoic acid, uric acid, ascorbic acid, α-tocopherol and glutathione), are available in the body, external sources of antioxidants are needed, as internal defence system

may get overwhelmed by excessive exposure to oxidative stress. Food antioxidants are important for human nutrition by decreasing the oxidative damage to lipids, proteins and nucleic acids induced by free radicals.[19] Recently research has been increased considerably in finding naturally occurring antioxidants for use in foods or medical products to replace synthetic antioxidants, owing to their adverse side effects.[20] Many bioactive compounds including the lectins from plants have been reported to possess strong antioxidant properties.[21,24]

In addition to the main role as an energy contributor, tubers provide a number of desirable nutritional and health benefits such as antioxidative**,** hypoglycaemic, hypocholesterolemic, antimicrobial and immunomodulatory activities.[25] In our continuous search for the bioactive compounds from plant *Xanthosoma violaceum*, an edible tuberous plant belongs to Araceae family, responsible for antibacterial and antioxidant activities, we designed the present work to evaluate the antibacterial and antioxidant activities of a partially purified XVL.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the chemicals required for antimicrobial and antioxidant activity were purchased from Himedia Pvt ltd, India.

2.2 Isolation of lectin

Xanthosoma violaceum tubers were peeled, cut and homogenised using 50 Mm phosphate buffer, pH 7.2 containing 154 Mm NaCl (PBS) (w/v 1:10) and stirred overnight at 4^{o}C. The extract was filtered and centrifuged at 10,000rpm for 20min at 4^{o}C. Subsequently, the supernatant was fractionally precipitated with ammonium sulphate at 60% saturation. The content was stirred with magnetic stirrer at 4^{o}C overnight. After centrifugation, the pellet was dissolved in minimal volume of PBS and dialysed against water. Dialysed sample was used for protein estimation, antimicrobial and antioxidant assay.

2.3 Antibacterial activity

2.3.1 Test organisms

The microorganisms used in this study were *Escherichia coli* (ATCC 25922), *Salmonella typhimurium (*ATCC 27853), Bacillus *subtilis* (ATCC 6633) and *Pseudomonas aeruginosa* (ATCC Number 27853). All bacterial strains were maintained on nutrient agar medium and stored at 4°C. These cultures were obtained from the Department of Microbiology, Kuvempu University. For determining the antibacterial activity nutrient agar media was prepared. For 100mL of the given media 4g nutrient agar, 2g of dextrose was used. The media and the petriplate were then autoclaved.

2.3.2 Preparation of the Inoculum

An inoculum of size 10^8 Colony forming units per milliliter (cfu/mL) of each of the isolates was prepared according to the method described by Bauer *et al.*[26] This was prepared by suspending loopful of inoculum from the stock into different labeled test tubes, each containing 10mL of the nutrient broth. A total of 3 test tubes were used for each test organisms and were incubated for 24 hours at 37^{o}c. The resultant cultures were then diluted with fresh nutrient broth in order to achieve optical densities corresponding to10^8 cfu/mL.

2.3.3 Antimicrobial susceptibility test

Partially purified XVL and standard (Streptomycin) at a concentration of 1mg/mL were used for antimicrobial activity on the test organisms using modified cup plate method described by Collins *et al.*[27] The nutrient agar plates were prepared and 0.002mL of each of the bacterial suspension corresponding to $1x10^8$ colony forming unit (CFU/ml) was spread evenly. The plates were then air dried for a period of 5min and cups were bored in each solid media using sterile cork borer (number 3). Two concentrations of lectin were tested (25 µg and 50 µg). Lectin solutions of different concentrations were introduced into each of the aseptically bored holes. The control was the buffer used to dilute the lectins (50 mM PBS, pH 7.2). The plates were then incubated for 24hrs at 37^{o}C. The assay was performed in triplicate. Average inhibition zone diameters around the wells were measured in mm. 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 such as streptomycin.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al.*[28] The different concentrations of partially purified XVL were added to 3ml of 0.1mM ethanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30min at room temperature in dark. Changes in absorbance of samples were measured at 517nm. A control reading was obtained using ethanol instead of the extract. Ascorbic acid was used as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula and the results are expressed as IC_{50}, which is the amount of antioxidant necessary to decrease the initial concentration by 50%.

$$\% \text{ Inhibition} = ((A_0-A_1)/A_0 \times 100)$$

Where, A_0 = absorbance of the control (without test samples) and A_1 = absorbance of test samples.

2.4.2 Fe^{2+} chelating activity

The chelation of ferrous ions by extracts was estimated by method of Dinis *et al.*[29] To the different concentrations of partially purified XVL, 0.05ml of 2mM

$FeCl_2$ was added. After 30 seconds, 0.1ml of 5 mM ferrozine was added. Ferrozine reacted with divalent iron to form stable magenta complex species that were very soluble in water. After 10 minutes at room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562nm. EDTA was used as a standard metal chelating agent. The chelating activity of the extract for Fe^{2+} was calculated using the following formula and the results are expressed as IC_{50}.

% Inhibition = $((A_0-A_1)/A_0 \times 100)$

Where, A_0 = absorbance of the control (without test samples) and A_1 = absorbance of test samples.

2.4.3 Superoxide radical scavenging assay

The activity was evaluated using nitro blue tetrazolium (NBT) reduction method using a slightly modified method of Nishimiki *et al.*[30] All the reagents were prepared in phosphate buffer (pH 7.4). 1ml of NBT (156 µM), 1 ml of NADH (468 µM) and 2 ml of partially purified XVL at different concentrations were added to each test tube. The reaction was initiated by adding 100µl of phenazine methosulfate solution (60 µM) and incubated at 25°C for 5 min followed by the measurement of absorbance at 560 nm against blank. Ascorbic acid was used as the standard. Free radical scavenging activity of the extract was calculated using the following formula and the results are expressed as IC_{50}.

% Inhibition = $((A_0-A_1)/A_0 \times 100)$

Where, A_0 = absorbance of the control (without test samples) and A_1 = absorbance of test samples.

3.1. RESULTS AND DISCUSSION

3.1 Antimicrobial activity

The results of the antibacterial activity of the partially purified lectin tested in this study was shown in Table 1. The results demonstrate that, XVL exhibit remarkable antibacterial activity against *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis* and not towards *Pseudomonas aeruginosa*. The antibacterial activity of the standard antibiotic is shown to be higher than that of the XVL for all the tested organisms. Various studies have shown that plants are rich in antinutritional compounds like lectins possess antimicrobial activity against number of microorganisms.

Table 1: Comparison of antibacterial activity of XVL with standard antibiotic.

Microorganism	Diameter of zone of inhibition in mm			
	XVL		Streptomycin	
	25 µg	50µg	25µg	50µg
Escherichia coli (ATCC Number 25922)	2.3	2.7	3.1	3.5
Salmonella typhimurium(ATCC Number 14028)	1.3	1.9	2.7	3.3
Bacillus subtilis(ATCC Number 19659)	0.8	**1.5**	3.0	3.4
Pseudomonas aeruginosa(ATCC Number 27853)	-	-	2.5	2.9

Antibacterial activity of the lectins is an interesting and important topic of study because of the abundant prevalence of pathogenic microorganism 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.

3.2 Antioxidant activity

3.2.1 DPPH radical scavenging activity

DPPH was used to determine the proton scavenging activity of XVL at different concentrations were measured along with standard ascorbic acid and their percentage inhibition values are shown in **Fig 1**. The IC_{50} value for lectin was found 68.44 ± 0.45µg/ml compared to that of standard (82.93 ± 0.19 µg/ml).

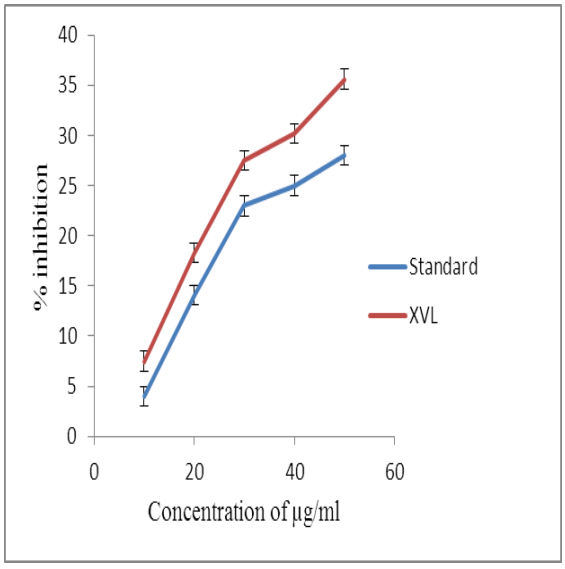

Fig 1. DPPH radical scavenging activity of XVL.

3.2.2 Fe^{2+} chelating activity

Fe2+ chelating activity of XVL at different concentrations were measured along with standard EDTA and their percentage inhibition values are shown in **Fig 2**. The IC_{50} value for lectin was found 46.02 ±

0.58µg/ml compared to that of standard (40.16± 0.45µg/ml) shown in fig 2.

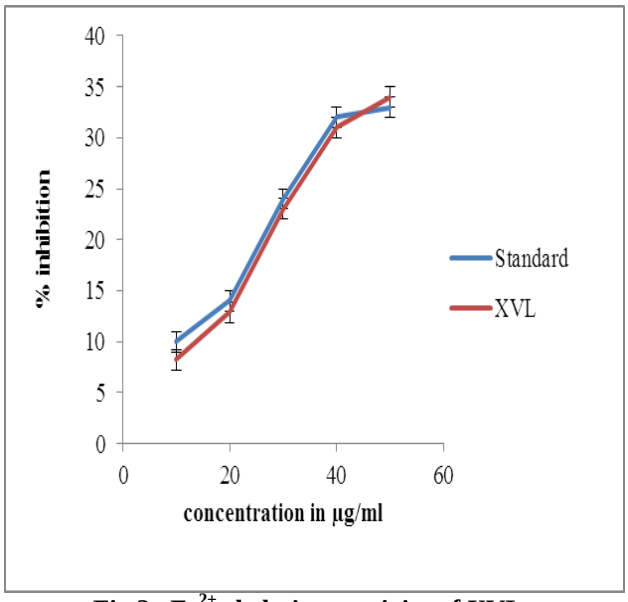

Fig 2. Fe^{2+} chelating activity of XVL.

3.2.3 Superoxide radical scavenging activity

The superoxide radical scavenging activity of partially purified XVL at different concentrations was measured and their percentage inhibition values are presented in **Fig.3**. The IC_{50} value for lectin was found to be 54.85±0.989µg/ml compared to that of standard (44.75± 0.707µg /ml).

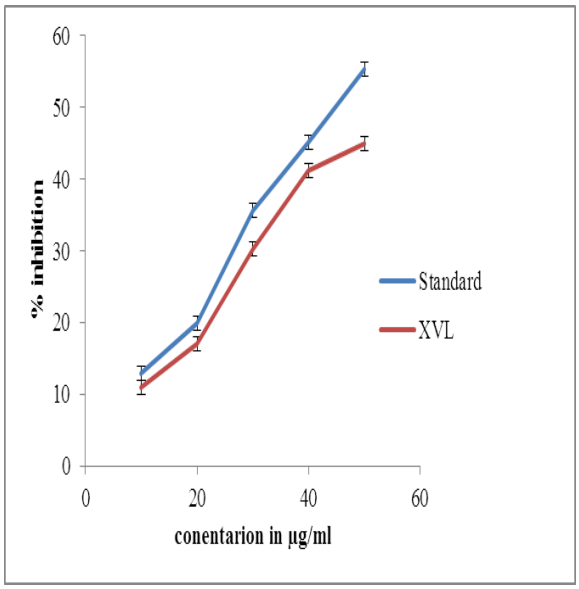

Fig 3. Superoxide radical scavenging activity of XVL.

Although mechanism of action of antimicrobial activity has not yet been elucidated in detail, the present data confirm the *in vitro* antibacterial activity of XVL against pathogenic bacteria. It may be due to interaction of lectin with carbohydrates on cell wall of bacteria or that the proteins form a channel on cell membrane and the cell dies as a result of leaking of cellular contents.[31] Many of the lectins showing antibacterial activity was reported earlier.[32,33] These results of XVL point out that future applications from plants can be of great importance for clinical microbiology and possible therapeutic applications. A water soluble lectin with potent antioxidant activity was isolated from *Moringa oleifera* seeds.[34] Many antioxidants from plant sources have been identified as free radical or active oxygen scavengers.[35] Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods.[36] To the best of our knowledge, antibacterial and antioxidant activities of XVL is described here for the first time. Inspite of its antibacterial and antioxidant activities, in the future the XVL could be used for blood typing, bacterial typing and other biotechnological applications. Hence further work can be continued for exploring its medicinal value as well as its other biomedical uses.

4. CONCLUSION

The evaluation of antibacterial and antioxidant activity results clearly demonstrates that XVL from the edible tubers possesses the significant antibacterial and antioxidant activities. The present investigation showed that partially purified XVL exhibit potent antibacterial activity towards pathogenic gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*), probably due to their specific interaction / binding to sugars that are present in the LPS of those bacteria. XVL being active against several gram-negative pathogenic microorganisms could be used as an antimicrobial agent from animal and plant infections. In conclusion, these results point out that future finding of lectin applications from tuber can be of great importance for clinical microbiology and possible therapeutic applications.

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