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RESEARCH ARTICLE

Screening of tubers of *Nelumbo nucifera* (water lilly) for lectin-like substances using hemagglutination assay

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SUMMARY

The tubers of water lily(*Nelumbo nucifera*)were collected from a tropical lake (Ossudu lake) and the methanol extract is screened for lectin-like substances using agglutination assay. The highest agglutination activity was found with human blood group B+ve with a maximum titre of 2^{11} among human group O and B+ve, goat and chick erythrocytes. To ascertain the molecular mass of the protein present in the extract, one-dimensional SDS-PAGE was performed using standard methods on the Bio-Rad Mini-Protean II system. The electrophoretic gel revealed that the crude extract of tuber of *Nelumbo nucifera* was found to possess a protein with a molecular mass of 19 kDa.

Key Words : *Nelumbo nucifera*, Hemagglutination, SDS-PAGE, Lectin

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Plant tubers are known to contain defense-related proteins such as chitinase and lectins which are of class of proteins, non-immune in origin and show a very specific interaction with carbohydrates. Different lectins bind with specific carbohydrate-containing compounds (e.g. polysaccharides, glycoproteins, and glycolipids), which can be free or bound in cell membranes (Frietas *et al.,* 1997). Another key characteristics of lectin is their ability to agglutinate erythrocytes, which provides an unambiguous indicator of their presence (Goldstein and Poretz, 1986) .The first biological activity to be recognised for lectins was their capacity to agglutinate erythrocytes, some lectins being blood group and/or subgroup specific (Horejsi and Kocourek, 1974).Though seeds are the richest source of lectins, these are also often quite abundant in vegetative organs such as roots, leaves, rhizomes and stems (Van Damme 1996). Lectins have been purified from tubers of *Trichosanthes kirilowii* (Yeung *et al.*, 1986a and b) . From the rhizome of *Smilacis glabrae*, a new flavanone (smitilbin) was isolated and evaluated for hypogycemic property (Chen *et al.,* 1999 and Fugunaka *et al.,* 1997). Two new lectins were purified from tubers of *Arisaema intermedium* Blume and *A. wallichiana* Hook (Kaur, 2005). Four major proteins designated DB1, DB2, DB3, and DB4 were isolated and characterized from the yam tuber *Dioscorea batatas*. (Gaidamashvili *et al.,* 2004) Lectins are also isolated from bulbs of liliaceae members including aloe (Koike *et al.,*1995), *Helianthus tubersosus* tubers (Suseelan *et al.*, 2000), *Tulipa gesneriana* bulb (Oda *et al.,* 1987) and Oda and Minami, 1986), *Trichosanthes kirilowii* (Yeung *et al.*,1986a and b) and tubers of *Eranthis hyemalis* (Oliver *et al.,* 2011). Presently an attempt has been made to screen tubers of *Nelumbo nucifera* for lectin like substances using hemagglutination assay.

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MATERIALS AND METHODS

Sample collection :

The tubers of water lilly (*Nelumbo nucifera*) were collected from a tropical lake (Ossudu lake) located at

Pondicherry and it was washed well in clean fresh water to removed the dirt and soil . It was then cut into pieces and dried in shade followed by oven drying for 2 hrs at 40 . For getting the solvent extract, the tubers were powdered and subjected to successive soxhlet extraction using methanol as solvent .

Preparation of red blood cells :

Following standards procedure, goat and chicken blood cells were obtained from the animal by venous puncture and human blood groups B+ve and O+ve were obtained from different donors. Approximately 4 ml of the blood was centrifuged at 5000rpm for 10 minutes at room temperature. The supernatant of the serum was discarded and pellets of erythrocytes were washed three times with cold PBS at pH7.4. It was then centrifuged and finally, 2% v/v of erythrocyte suspension was prepared to be used in agglutination assay (Fabregas *et al.,* 1986).

Assay for hemagglutinic activity :

The agglutination tests were made in V-Cooke Microtiter plates. Serial two-fold dilutions of the algal extracts in PBS were placed in wells and equal volumes of the 2 per cent erythrocyte suspension were added. The plates were gently shaken for 15 sec and then allowed to stand for 1 hr at room temperature before examination. The reciprocal of the highest dilution showing positive hemagglutination was recorded as the titration value (Fabregas *et al.*, 1986) which indicated the minimum amount of test extract, required for 100 per cent agglutination under the above assay conditions(Nair and Hasi, 2000).

Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) :

To ascertain the molecular mass of the protein present in the extract, one-dimensional SDS-PAGE was performed using standard methods on the Bio-Rad Mini-Protean II system. It is a discontinuous system with 12 per cent separating gel (pH 8.8) and 4 per cent stacking gel (pH 6.8) of size 7 cm x 10cm x 1 cm. Prior to electrophoresis, protein samples were re dissolved in laemmli buffer and boiled in the presence of dithiothreitol (DTT) for 5min at 100 C. For the molecular weight markers, sigma marker wide molecular weight range (Sigma) was used. The electrophoresis was performed at 10Ma per gel. The gels were stained with brilliant blue R concentrate (Sigma-Aldrich) for 30min and were de-stained in 50 per cent (v/v) methanol, 5per cent acetic acid (v/v) for 30min or until bands appeared(Ponpipol *et al.,* 2008) .

RESULTS AND DISCUSSION

A positive pattern which indicated agglutination was a uniform coating of the bottom of the well by erythrocytes while a negative pattern (indicating no agglutination) was a circular clump of erythrocytes surrounded by a concentric, clear zone of equal size to the blank. The presence of agglutinations was demonstrated in the extract against all the four types of erythrocytes tested however ,higher potential at the lowest dilution is in erythrocytes (RBC's) of B positive Human blood (Table 1). Such agglutination is quite obvious under microscope (40X) shown in Fig. 2. Further, SDS-PAGE electrophoresis studies on test extract showed a protein with 19 kDa molecular (Fig.3) is the type of protein present in the extract.

Table 1: Hemagglutinating activity profile

Source of erythrocytes	Highest dilution
Human blood (O+ve)	1:512
Human blood (B+ ve)	1:2048
Chick blood	1:4
Goat blood	1:4

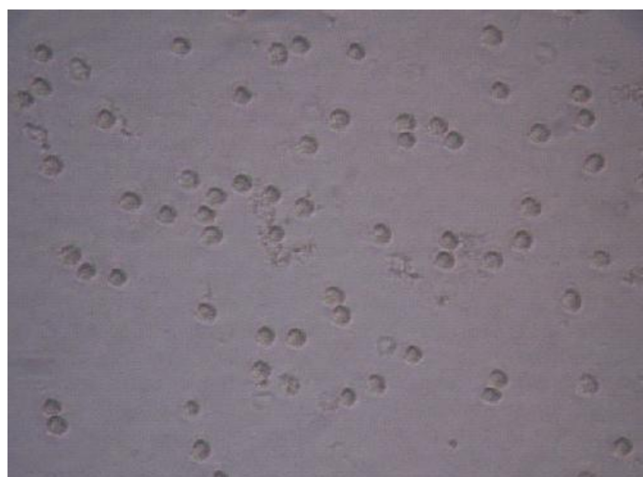

Fig.1: Control –Erythrocytes(RBCs)

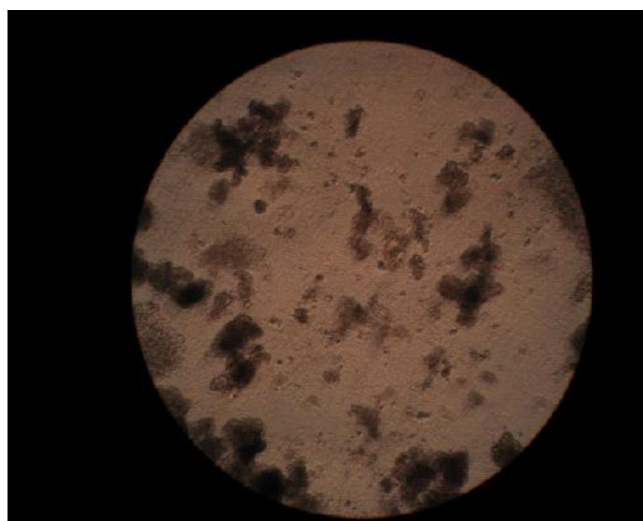

Fig. 2: Human blood:B+ve–agglutinated erythrocytes -RBCs (40X)

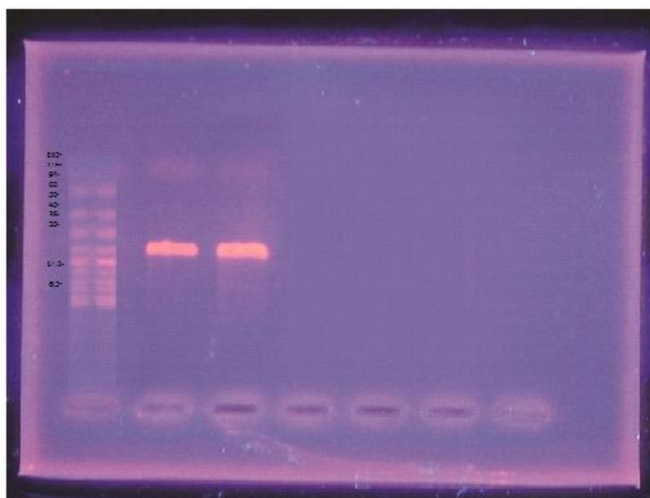

Fig. 3: SDS-PAGEelectrophoretic gel of extract

Lectins have been shown to be present in a variety of plant tissues spanning a wide range of taxonomic groups (Van Damme *et al.*,1996) and Sugar binding properties of lectin may reflect hemagglutinating activity towards different erythrocytes. Even now, the most popular procedure for the detection of lectins is the hemagglutination test using animal or human erythrocytes, or both, (Horejsi and Kocourek, 1974 and Goldstein and Poretz, 1986)

In the present study the methanol extract of tubers of water lily (*Nelumbo nucifera*), is subjected for its agglutinating property. The highest agglutination activity was found with human blood group B+ve with a maximum titre of 2^{11} among human group O and B+ve, goat and chick erythrocytes. The specificity for galactose and galactose containing sugars in tubers carrying the B antigen (galactose sugar determinant) than other boold groups (Frietas *et al.,* 1997). Similar studies in various other tubers have been done by Mo *et al.* (1999) in tuber of *Xanthosoma sagittifolium* and by Gaidamashvili *et al* . (2004)in tubers of *Dioscorea batatas* and by Kaur (2011) in tuber of ornamental plant *Caladium bicolor.* As stated by Frietas *et al.* (1997) the specificity of lectin type (type of sugars)present in the tubers of *Nelumbo nucifera* be the factor responsible for human erythrocyte lysis particularly of group B+ve. Besides, in the process of haemagglutination in which two distinct sites *i.e* type of carbohydrates on the surface of erythrocytes and the type of protein present in the tuber extract or test drug that binds the carbohydrates present in the binding sites,are of points to be pondered in view future research . Moreover, as rightly reported by Daman *et al.* (2005) absence of specific types of carbohydrate on the binding site may be the reason for no agglutination activity in the blood group O+ve and erythrocytes of goat and chick blood tested in the present study and vice versa for the highest agglutination in blood group B of human blood.

Further, studies to ascertain the type of protein present in the extract of tuber (in term of Molecular mass) through SDS-PAGE revealed that the crude extract of tuber of *Nelumbo nucifera* is found to possess a protein with a molecular mass of 19 KDa and ascertained that the agglutinatin might be due to protein with 19kDa. On comparing with previously reported lectin from tuber of *Saurotum venosum* lectin is 52kDa as determined by gel filtration chromatography (Daman *et al.*, 2005). Tubers of *Xanthosoma sagittifolium* lectin by SDS-PAGE, N-terminal acid sequencing, and gel filtration chromatography showed a heterotetramic protein composed of four 12kDa (Hanqing,1999). Similarly ,tubers of ornamental plant *Caladium bicolor*, lectin was 52kDa while subunit molecular mass was 13.1kDa, indicating the hetetetrameric nature of lectin (Kaur,2005) and also four major proteins of molecular masses 20kDa,31kDa,120kDa and 28kDa were isolated from the yam tuber *Dioscorea batatas*(Daman,2004). In view of these reports on presence of low molecular mass of lectin, it is presumed that the haemagglutinating property of tuber extract of *Nelumbo nucifera* might be due the protein with 19KD molecular mass present in the extract . Based on these findings it is understood that though it is a preliminary quantititave investigation, in view of future research, tubers of *Nelumbo nucifera* could be a better source of lectin /or lectin- like substances that has a potential carbohydrate binding capacity and could be used for the investigation of complex carbohydrate structures on cell surfaces and proteins, purification of carbohydrate-containing polymers, blood typing as well.

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