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SEARCH FOR HAEMAGGLUTININS IN PLANT SEEDS- A PRELIMINARY REPORT

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Abstract: A search was conducted to detect the presence of haemagghutinins in the seed extracts of 22 plants. A ten per cent extract of the defatted seeds were prepared in phosphate buffered saline and the haemagglutination studies were carried out using human erythrocytes of A, B and O groups. Considerable lectin activity was detected in 10 seeds and the haemagglutination inhibition studies of these extracts revealed the sugar specificities of the lectins present in them. Eleven seeds did not show any haemagglutinating activity against the spectrum of erythrocytes tested.

1. Introduction

Lectins are sugar binding proteins or glycoproteins of non-immune origin which agglutinates cells and/or precipitate glycoconjugates (Goldstein et al 1980). The presence of two or more sugar binding sites on the molecule allows lectins to agglutinate celle by binding carbohydrate/glycoprotein surface molecules. Lectins are widely used in membrane studies of normal and cancer cells, studies of blood group substances, histochemical and immunohistochemical studies, purification of glycoproteins and polysaccharides and studies on lymphoblastogenesis (Lis and Sharon 1986). We have earlier reported the isolation and purification of lectin from the seeds of *Artocarpus hirsuta* and this lectin was found to be of use as a cheep mitogenic agent (Beena et al 1993). These findings prompted us to make an attempt to search for new lectins from indigenous plant seeds.

2. Materials and methods

Mature plant seeds were collected from localities in Kerala, dried, deskinned and ground to flours. The flours were individually defatted using petroleum ether (Boiling point 40-60^0 C). Twentyfive grams each of the defatted material was homogenised in a blender with 250ml portions of phosphate buffered saline (PBS 0.01M;PH 7.4) and kept at 4^{0}C overnight with constant stirring. The clear supernatant was collected after centrifugation for 30 minutes at 10,000xg and subjected to haemagglutination and sugar inhibition assays as described by Pueppke (1979) on WHO plates using PBS washed erythrocytes of human A, B and O groups. For the inhibition studies a spectrum of known mono and disaccharides were used. The haemagglutination titre was estimated visually after one hour of incubation as the reciprocal of the highest dilution of the

extract giving visible agglutination. The sugar specificity of the extracts was studied by mixing different sugar inhibitors with haemagglutinating units of the extracts before the addition of erythrocytes and determining the inhibition of agglutination.

3. Reslts and discussion

Table - 1. Taxonomical classification of plants

Sl. No.	Botanical Name	Common name	Family
1.	*Adenanthera pavonia*	Coral wood	Leguminosae
2.	*Syzygium cumini*	Black plum	Myrtaceae
3.	*Catheranthus roseus*	Nithyakalyani	Apocyanaceae
4.	*Cardiospermum haliccacabum*	Winter cherry	Sapindaceae
5.	*Ocimum sanctum*	Tulsi	Labiatae
6.	*Citrus sinensis*	Musambi	Rutaceae
7.	*Terminalia bellarica*	Thanni	Combretaceae
8.	*Prunus amygdalus*	Badam	Rosaceae
9.	*Tribulus terrestris L.*	Neringil	Zygophyllaceae
10.	*Myristica fragrens*	Nutmeg(Jathi)	Myristaceae
11.	*Guazuma ulmifolia*	Rudraksha	Sterculiaceae
12.	*Zizyphus Jujuba Lam.*	Jujube	Rhamnaceae
13.	*Phyllanthes niruri L.*	Keezharnelli	Euphorbiaceae
14.	*Pimpinella anisum*	The Anise	Umbelliferae
15.	*Strychnos potatorum*	Thettamparal	Loganinaceae
16.	*Sterculia foetida*	Thondi	Sterculiaceae
17.	*Tamarindus Indica*	Tamarind	Leguminosae
18.	*Helicterus isora*	Potum	Sterculiaceae
19.	*Psidium guajava*	Guava tree	Myrtaceae
20.	*Hydnocarpus wightiana*	Marotti	Flacourticaceae
21.	*Entada Pursaetha*	Gardal	Mimosae
22.	*Caesalpinia bonducella*	Kazhanji	Caesalpiniaceae

In the present study, attempts were made to detect the presence of lectin activity in the seeds of some plants locally available, some of which are used as food materials; while others are utilised in the indigenous system of medicine (Jain 1985). The taxonomical classification of plants, the seeds of which were screened for lectin activity is given in Table - 1 and the results of haemagglutination and haemagglutination inhibition assays carried out with crude extracts of these plant seeds is given in Table - 2. It is observed that out of 22 seeds screened, 11 seeds didn't show any haemagglutinating activity aganist human erythrocytes. The remaining 10 seeds showed haemagglutinating activity at varying concentration against different erythrocytes and the titres varied from 64 to 8192. The extracts of *Adenanthera pavonia, Syzygium cumini, Tamarindus Indica* and *Hydnocarpus wightiana* were found to be lactose specific whereas *Prunus amygdalus* showed galactose specificity. N-Acetyl-D-glucosamine proved to be the best inhibitory sugar of *Cardiospermum haliccacabum* and N-Acetyl-D-mannosamine for *Psidium guajava* extracts. In contrast, the haemagglutinating activity of *Strychnos potatorum, Pimpinella anisum* and *phyllanthes niruri* seed extracts were not inhibited by any of the sugars tested.

Table - 2. Haemagglutinating (HA) activity and sugar specificity of 10% extracts of defatted seed powders.

Sl. No.	Botanical name	HA titre			Specific
		A	B	O	Inhibitory sugar
1.	*Adenanthera pavonia*	4096	512	512	Lactose
2.	*Syzygium cumini*	2048	512	2048	Lactose
3.	*Cardiospermum haliccacabum*	256	512	256	N- Acetyl-D-glucosamine (Gluc-NAc)
4.	*Prunus amygdalus*	128	128	256	Galactose
5.	*Phyllanthes niruri*	128	2048	4096	*
6.	*Pimpinella anisum*	64	64	64	*
7.	*Strychnos potatorum*	256	1024	2048	*
8.	*Tamarindus Indica*	4096	2048	4096	Lactose
9.	*Psidium guajava*	256	512	8192	N-Acetyl D-mannosamine
10.	*Hydnocarpus wightiana*	512	1024	512	Lactose

* No inhibition obtained against glucose, fructose, galactose, lactose, mannose, sucrose, maltose, arabinose, xylose, N-Acetyl-D-glucosamine, N-Acetyl-D-mannosamine, N-Acetyl-D-galactosamine and fucose.

The sugar specificity gives a preliminary indication of the nature of lectin present in an extract. Once the best sugar inhibitor is found, the lectin can be purified using columns of immobilised sugar adsorbants. In light of the results of the present study, the isolation, purification and characterisation of some of these haemagglutinins are in progress.

References

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