Scientific Research Publishing

Food and Nutrition Sciences, 2017, 8, 1085-1092
http://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Interaction of Glucose/Sucrose Binding Lectin Isolated from Nigeria Wild Bean with *E. coli* and *S. aureus*

O. A. Awoyinka[1], J. O. Awe[2], O. A. Omosebi[3], O. Osukoya[1], F. C. Oladele[1], B. A. Olofinbiyi[4], M. F. Asaolu[5]

[1]Department of Biochemistry College of Medicine, Ekiti State University, Ado Ekiti, Nigeria
[2]Department of Chemical Sciences (Biochemistry Unit), Afe Babalola University, Ado Ekiti, Nigeria
[3]Department of Science Laboratory Technology, Ekiti State University, Ado Ekiti, Nigeria
[4]Ekiti State University Teaching Hospital, Ado Ekiti, Nigeria
[5]Department of Biochemistry, Ekiti State University, Ado Ekiti, Nigeria
Email: olayinka.awoyinka@eksu.edu.ng

How to cite this paper: Awoyinka, O.A., Awe, J.O., Omosebi, O.A., Osukoya, O., Oladele, F.C., Olofinbiyi, B.A. and Asaolu, M.F. (2017) Interaction of Glucose/Sucrose Binding Lectin Isolated from Nigeria Wild Bean with *E. coli* and *S. aureus*. *Food and Nutrition Sciences*, **8**, 1085-1092.
https://doi.org/10.4236/fns.2017.812080

Received: October 24, 2017
Accepted: December 4, 2017
Published: December 7, 2017

Abstract

Lectin purified from wild underutilized local bean—*Otili, Feregede, Pakala* was comparatively characterized and further evaluated for interaction with gastrointestinal bacteria—*Esherichia coli* and *Staphylococcus aureus*. The purified lectin in all the bean samples showed to be glucose and sucrose binding. The hemagglutinating activity, was non selective to type of blood group (A, B, AB and O). Anti-bacteria interaction with *Escherichia coli* showed clear zone of inhibition of about 1.5 ± 0.5 mm with lectin from *Feregede* and *Otili* while there was slight agglutination with lectin from *Pakala*. *Staphylococcus aureus* sensitivity to the lectin extracted from *Otili* with clear zone of inhibition of 2.0 ± 0.5 mm was also found in the control chloramphenicol. However there was pronounced agglutination with lectin from *Feregede* and *Pakala* with *Staphylococcus auereus*. This may be a clear indication that lectin from local underutilized wild bean understudy will agglutinate and interact with a gram positive bacteria more than gram negative bacteria.

Keywords

Staphyloccus aereus, Lectin, Glucose, Gastrointestinal, Sucrose, Pathogenic

1. Introduction

Lectins are carbohydrate binding proteins of no immune origin that agglutinate cells or as carbohydrate-binding proteins other than antibodies [1] [2]. The pu-

rified Lectins have high demand in science, medicine and technology [3]. It has been recommended that these interactions may be physiologically applicable, since some of these molecules role as phyto-hormones [4].

Early studies suggested that lectins might be used to target the Gastro Intestinal tract infection [5] Montisci and his colleagues proposed the use of tomato lectin (TL) to target and bind to the luminal surface of the small intestine, that is the lectin would demonstrate bio-adhesion. This was further corroborated by Jimenez-catelanos *et al.* [6], when they exploit the property of bioadhesion to slow down the intestinal transit time of oral drugs and thus enhance their bioavailability. As a consequence of binding to mucosal cells, it was also transported across the mucosa *in vitro* in significantly higher amounts than other macromolecule [3] [7] [8]. The binding was inhibited by a number of sugars, which are specific binding targets of the lectin, confirming the true lectin nature of the interaction. Other lectins tested did, however, modify the intestinal transit profile: For example, kidney bean lectin (*Phaseolus vulgaris* agglutinin) led to delayed transit of the microspheres and a broad distribution down the small intestine after administration. Irache *et al.* [9] showed conjugated lectins, including TL, to polystyrene latex beads and demonstrated *in vitro* binding to intestinal segments and mucus. The same group had also made lectin-conjugated nanoparticles. For example, they conjugated a fructose specific lectin, to nanoparticles made from gliadin, and demonstrated binding of these particles to mucin. Recent progress in glycobiology has emphasized that numerous pathogens ranging from viruses and bacteria to pluricellular parasites use lectins as tools to recognize and bind to the oligosaccharides exposed by target cells and tissues [10].

The idea of using lectins as targeting molecules for cell specific drug delivery is both attractive and feasible, and has generated considerable interest. While some dietary lectins have been known to preferentially facilitates the growth of gut bacteria such as Escherichia coli and Lactobacillus lactis [8] [11], the pathogen surfaces have been known to bear a large number of oligoglucides that may be bound by specific lectins able to modulate the host infection leading to acute gastrointestinal symptoms including nausea, vomiting, irritation and over-secretion of mucus in the intestines causing impaired absorptive capacity of the intestinal wall [12] [13]. Against this backdrop the present study is intended to explore on our previous work [14] [15] [16] on the Nigerian wild underutilized beans in relation to activities of their lectin on gastrointestinal bacteria.

2. Materials and Method

2.1. Collection of Cultivar

Dry, wild bean—*Sphenostyles stenocarpa* (Otili), *Cajanus cajan* (Feregede), *Phaseolus lunatus* (Pakala), were sourced from the bush of Ado Ekiti environ. They were collected whole, dry and naked (*i.e.* without pods), with divers colors (off white, gold, navy blue, black and brown respectively). Authentication was carried at the herbarium of the Plant Science Department, Ekiti State University

Ado Ekiti before the dried seeds were pulverised.

2.2. Collection of Blood Samples

5 mL of blood samples of known blood groups A, B, AB & O were collected from individual. Each of sample was centrifuged at 1500*g for 5 minutes at room temperature. The red blood cells obtained were then washed by centrifugation at 1500*g for 5 minutes at temperature with 0.01 M phosphate-buffered saline (pH 7.2) This was repeated twice, after which the cells were mixed with 3% formaldyde in EDTA bottle and allowed to stir gently overnight, before it was centrifuged at 1500 g for 5minutes. The centrifuged red cells were then washed again as before, three times with 0.01 Mphosphate-buffered saline (pH 7.2) after which the cells were collected into a stopped bottle and 78.8 ml of 0.01 M phosphate buffered saline was added to make the cell 4% thereafter, it was stored in the fridge.

2.3. Isolation and Purification of Lectin from Wild Beans

The defatted samples were dissolved in water in ratio 1:20 An aliquot of it were separated and kept in refrigerator for carbohydrate analysis while the other part was centrifuged at 1500 revolution per minutes (rpm) for 30 minutes. The pellets were discarded and supernatant collected for ammonium sulphate precipitation as described by Trowbridge [17]. The precipitated protein were pulled together and dissolved in 240 ml of distil water, the resulting mixture was concentrated by ultra filtration (Millipore, India) at 1500 g for 30 minutes before dialyses against 0.15 M NaCl – 0.01 M $NaPO_4$ buffer for 24 hours. The dialysed sample was further purified by chromatography method 20 g of sephadex was dissolved in 50 ml of 0.15 MNaCl – 0.01 M $NaPO_4$ buffer to give a jelly mixture which was used to pack the column (8.5 × 1.5 cm) until it was well packed. Thereafter, the column was washed with the same buffer and loaded with dialyzed precipitated protein and eluted with 0.2 M glucose solution. The eluent were assay for lectin using hemagglutination activity as marker.

2.4. Assay for Lectin Activity

Agglutination of the red blood cells by the extracts and were obtained during purification stages was estimated as described by Bing *et al*, [18] A serial two-fold dilution of the lectin solution was mixed with 50 µl of a 4% suspension erythrocytes in phosphate buffered saline pH 7.2 at room of temperature (the erythrocytes of human blood group A, B, AB and O were fixed with 3% formaldehyde. The plate was left undisturbed for one hour at room of temperature in order to allow for agglutination of the erythrocytes to take place. The hem agglutination titer of the lectin expressed as the reciprocal of the highest dilution exhibiting visible agglutination of erythrocytes was reckoned as one hem agglutination unit, specific activity is the number of hem agglutination units per mg protein [19].

2.5. The *In Vitro* Sensitivity on the Bacteria Isolates

Two gastrointestinal tract microorganisms were collected from Ekiti State University Teaching Hospital Laboratory Ado Ekiti, Nigeria. The in-vitro-sensitivity of the isolated wild bean to the partial lectin was determined according to Baue *et al.,* [20]. Each isolated gastrointestinal tract pathogenic microorganism was inoculated at the center of a 9 cm Petri dish containing freshly prepared PDA and incubated at 27˚C for 24 hours four pieces 3 mm sterile paper discs were placed on the four cardinal points of growing culture at distance of 0.5. cm away from the boundary of the colony so as to give a square. Each of the four discs in *E. Coil* and *Staphyloccous aureus* culture was moistened with 5 µl of the partially purified lectin suspension. This procedure was performed for the 10 µl and 20 µl treatments of paper discs in a *E. Coil* and *Staphyloccous aureus* culture with the partially purified lectin. All cultures were treated in triplicates for each lectins treatment. A set of triplicate control plate were set up by impregnating the paper discs with 20 µl of chloramphenicol in one set and 5 µl of sterile distilled water in the other the plate was their incubated as stated above for 18 hours before the zone of inhibition was measured.

3. Results and Discussion

In **Table 1**, it was observed that throughout the process of purification, the specific activity generally increased. It was also observed that as the volume of red blood cell increase, the specific activity generally increases as shown in result. The time for blood agglutination got shorter as purification of lectin continues. Although there was a little deviation from these trends with ammonium sulphate having a lower specific activity and a longer hemagglutination time to that of crude, However as soon as ammonium sulphate was removed via dialysis the specific activity increased hence, it can be lectin gotten from affinity chromatography is still the best having highest hemaggluatination activity of 206 HU/mg at 800 µl volume. The data presented from this study showed that the extract of the seed of Nigeria wild bean contained level of hemagglutinating protein (lectin) Moreover the lectin from Nigeria wild bean agglutinated red blood cells nonspecifically which is typical of many lectin [21].

The inhibition studies (**Table 2**) to define the sugar specification of the crude extract (the phosphate buffered saline extract) of Nigeria wild bean showed that Maltose and Fructose had no effect on hemagglutination activity of lectin at lower concentration but at higher concentration there was hemagglutination activity of lectin which is in slight deferent with result given by Kuku *et al*, [21] as shown in **Table 2** where the lectin extracted from Nigeria wild bean was not inhibited at higher concentration of 800 mM. Galactose enhanced the hemagglutination activity of the lectin as the concentration increases as shown in the table 2, while Galactose inhibited activity of lectin extracted from Nigeria wild bean under study at concentration above 100 mM as reported by Kuku *et al* [21]. However, Ribose there was hemagglutination activity of lectin extracted from

Table 1. Purification profile of lectin.

Volume (µl)	25.0	50.0	100.0	200.0	400.0	800.0	Time taken for hemagglutination
Crude specific activity (HU/mg)	3.00	13.50	9.00	27.0	54.0	13.50	50 minutes
$(NH_4)_2SO_4$: Specific activity (HU/mg)	0.38	0.75	1.50	3.00	9.00	27.00	60 - 80 minutes
Dialyzed specific activity (HU/mg)	3.25	9.50	19.00	54.00	54.00	103.00	25 - 30 minutes
Affinity chromatography specific activity (HU/mg)	25.75	51.50	77.25	103.00	154.0	206.00	5 - 12 minutes

Table 2. Sugar inhibition study of hemagglutinating activity of Nigeria wild bean.

Sugar in mmol/I	800	400	200	100	50	25	PBS
Maltose	+++	+++	+++	–	–	–	
Fructose	+++	+++	+++	–	–	–	
Ribose	–	+	++++	++++	++++	++++	
Galactose	++++	++++	++++	++	++	++	
Xylose	+++	+++	+++	–	–		
Sucrose	+	++	++	++++	++++		
Glucose	+	++	++	++++	++++		

Note: +: hemaggluattinating activity; –: no hemaggluating activity, PBS: phosphate buffered saline.

Nigeria wild bean at lower concentration not beyond 200 mM and at higher concentration. The lectin activity extracted from Nigeria wild bean decreased in contrast to that by Alexander *et al.*, [22] while activity of the lectin was completely inhibited by Sucrose and Glucose with minimum inhibitory concentration at 800 mM similar to that observed by kaka *et al.*, [21]. The Phosphate buffer saline (PBS) reaction revealed that the lectin was non-glycosylated while hemagglutination inhibition studies carried out with purified Nigeria wild bean lectin revealed that the lectin is not inhibited by simple sugars but by glycoprotein. This is in general agreement with those found in previous studies [23] [24].

The isolation of lectin from the Nigeria wild bean investigated in this study is in line with a previous report Awoyinka *et al.*, [10]. Anti-bacteria interaction with *Escherichia coli* showed clear zone of inhibition of about 1.5 ± 0.5 mm with lectin from *Feregede* and *Otili* while there was slight agglutination with lectin from *Pakala*. *Staphylococcus aureus* sensitivity to the lectin extracted from *Otili* with clear zone of inhibition of 2.0 ± 0.5 mm was also found in the control chloramphenicol. However there was pronounced agglutination with lectin from *Feregede* and *Pakala* with *Staphylococcus auereus* (**Figure 1**). This study has shown that gastrointestinal tract pathogenic bacteria probably major phytopathogens and their spectrum of pathogenicity is dependent on the presence or absence of lectins. The ability of lectin from Nigeria wild bean to inhibit the

(a) (b) (c)

Figure 1. Result of effect of anti microbial activity. (a) Control; (b) *Staphylococcus aureus*; (c) *E. coli.*

gastrointestinal tract pathogenic bacteria suggests that it may play an important role in immobilizing invading microorganisms; particularly bacterial infection from gastrointestinal tract pathogens. In addition, the carbohydrate-binding site of Nigeria wild bean lectin may be vital in this activity, being responsible for the recognition of the bacteria. The wild bean used in this study may be a suitable candidate for the prevention and treatment of gastrointestinal tract pathogenic bacteria infections.

4. Conclusion

This study shown a clear indication that Nigeria wild bean can be used in treatment of *Escherichia coli* and *Staphylococcus aureus* notable for gastrointestinal tract pathogenicity in man.

Acknowledgements

The present project was partly funded by Tet Fund Individual Based Research grant 2016.

References

[1] Boyd, W.C. and Shapleigh, E. (1954) Specific Precipitating Activity of Plant Agglutinins (Lectins). *Science*, **119**, 419. https://doi.org/10.1126/science.119.3091.419

[2] Bruck, A., Abu-Dahab, R., Borchard, G., Schäfer, U. and Lehr, C.M. (2001) Lectin-Functionalized Liposomes for Pulmonary Drug Delivery: Interaction with Human Alveolar Epithelial Cells. *Journal of Drug Targeting*, **9**, 241-251 (Color Plates I-III). https://doi.org/10.3109/10611860108997933

[3] Naisbett, B. and Woodley, J. (1994) The Potential Use of Tomato Lectin for Oral Drug Delivery: 1. Lectin Binding to Rat Small Intestine *in Vitro*. *International Journal of Pharmaceutics*, **107**, 223-230. https://doi.org/10.1016/0378-5173(94)90438-3

[4] Kilpatrick, D.C. Pusztai, A., Grant, G., Graham, C. and Ewen, S.W (1985) Tomato Lectin Resists Digestion in the Mammalian Alimentary Canal and Binds to Intestinal Villi without Deleterious Effects. *FEBS Letters*, **185**, 299-305. https://doi.org/10.1016/0014-5793(85)80927-3

[5] Montisci, M.J., Dembri, A., Giovannuci, G., Chacun, H., Duchene, D. and Ponchel, G (2001) Gastrointestinal Transit and Mucoadhesion of Colloidal Suspensions of *Lycopersicon esculentum* L. and *Lotus tetragonolobus* Lectin-PLA Microsphere

Conjugates in Rats. *Pharmaceutical Research*, **18**, 829-837. https://doi.org/10.1023/A:1011044730790

[6] Jime'nez-Catelanos, M.R., Zia, H. and Rhodes, C.T. (1993) Muco-Adhesive Drug Delivery Systems. *Drug Development and Industrial Pharmacy*, **19**, 143-194. https://doi.org/10.3109/03639049309038765

[7] Bernkop-Schnurch, A., Gabor, F., Szostak, M.P. and Lubitz, W. (1995) An Adhesive Drug Delivery System Based on K99-Fimbriae. *European Journal of Pharmaceutical Sciences*, **3**, 293-299. https://doi.org/10.1016/0928-0987(95)00018-9

[8] Lanza, E, Hartman, T.J., Albert, P.S., Shields, R., Slattery, M. and Caan, B. (2006) High Dry Bean Intake and Reduced Risk of Advanced Colorectal Adenoma Recurrence among Participants in the Polyp Prevention Trial. *Journal of Nutrition*, **136**, 1896-1903.

[9] Irache, J.M., Durrer, C., Duchene, D. and Ponchel, G. (1994) Preparation and Characterization of Lectin-Latex Conjugates for Specific Bioadhesion. *Biomaterials*, **15**, 899-904. https://doi.org/10.1016/0142-9612(94)90114-7

[10] Awoyinka, O.A., Olajuyigbe, O.O., Anyasor, G.N., Osamika, O. and Adeniyi, M. (2013) Interaction of Lectins Isolated from Four Selected Local Vegetables on Gastrointestinal.

[11] Kik, M.J.L., Rojer, M.J., Mouwen, J.M.V., Koninkx, M., van Dijk, J.E. and van der Hage, M.H. (1989) The Interaction between Plant Lectins and the Small Intestinal Epithelium: A Primary Cause of Intestinal Disturbance. *Veterinary Quarterly*, **11**, 108-115. https://doi.org/10.1080/01652176.1989.9694207

[12] Naughton, P.J., Grant, G., Bardocz, S. and Pusztai, A. (2000) Modulation of Salmonella Infection by the Lectins of *Canavalia ensiformis* (Con A) and *Galanthus nivalis* (GNA) in Rat Model *in Vivo*. *Journal of Applied Microbiology*, **88**, 720-727. https://doi.org/10.1046/j.1365-2672.2000.01062.x

[13] Schmidt, M.A., Riley, L.W. and Benz, I. (2003) Sweet New World: Glycoproteins in Bacterial Pathogens. *Trends in Microbiology*, **11**, 554-562. https://doi.org/10.1016/j.tim.2003.10.004

[14] Awoyinka, O.A., Ileola, A., Imeoria, C.N. and Asaolu, M.F. (2016) Comparative Studies on Mineral and Scavenging Ability of Edible and Some Underexploited Wild Beans in Nigeria. *Open Access Library Journal*, **3**, e2318. https://doi.org/10.4236/oalib.1102318

[15] Awoyinka, O.A., Ileola A.O., Imeoria C.N., Olumakinde, S.T., Oladele, F.C., Asaolu, M.F. (2016) Functional Properties and Nutritional Quality of Some Wild and Edible bean in Nigeria. *Open Access Library Open Access Library Journal*, **3**, e2411.

[16] Awoyinka, O.A., Ileola, A.O., Imeoria, C.N., Tijani, T.D., Oladele, F.C., Asaolu, M.F. (2016) Comparison of Phytochemicals and Anti-Nutritional Factors in Some Selected Wild and Edible Bean in Nigeria. *Food and Nutrition Sciences*, **7**.

[17] Trowbridge, O. (1974) Isolation of a Homodimeric Lectin with Antifungal and Antiviral Activities from Red Kidney Bean (*Phaseolus vulgaris*) Seeds. *Journal of Protein Chemistry*, **20**, 367-375.

[18] Bing, D.H., Weyand, J.G. and Stavitsky, A.B. (1967) Heamagglutination with Aldehyde Fixed Erythrocytes for Assay of Antigens and Antibodies. *Proceedings of Society of Experimental Biology and Medicine*, **124**, 1166-1170. https://doi.org/10.3181/00379727-124-31953

[19] Wang, H., Gao, J. and Ng, T. (2000) A New Lectin with Highly Potent Antihepatoma and Antisarcoma Activities from the Oyster Mushroom *Pleurotus ostreatus*. *Bi-*

ochemical & Biophysical Resource Communication, **275**, 810-816. https://doi.org/10.1006/bbrc.2000.3373

[20] Bauer, A.W., Kirby, M.M., Sherris, J.C. and Turck, M. (1996) Antibiotic Susceptibility Testing by a Standardized Single Disc Method. *American Journal of Clinical Pathology*, **45**, 493-496.

[21] Kuku, A., Odekanyin, O., Adeniran, K., Adewumi, M. and Oloriade, T. (2009) Purification of A Mannose/Glucose-Specific Lectin with Antifungal Activity from Pepper Seeds (*Capsicum annum*). *African Journal of Biochemistry Research*, **3**, 272-278.

[22] Alexander, A., Bulgakov, O., Kyung-ll, P., Kwang-Sik, C. and Hee-Kyoung, L. (2004) Purification and Characterization of a Lectin Isolated from the Manila Clam *Ruditapes philippinarium* in Korea. *Immunity*, **16**, 487-499.

[23] Imberty, A., Wimmerova, M., Mitchell, E.P. and Gilboa-Garber, N. (2004) Structures of the Lectins from *Pseudomonas aeruginosa*: An Insight into the Molecular Basis for Host Glycan Recognition. *Microbes and Infection*, **6**, 221-228. https://doi.org/10.1016/j.micinf.2003.10.016

[24] Paiva, P.M.G., Gomes, F.S., Napoleon, T.H., Saz, R.A., Correia, M.T.S. and Coelho, L.C.B. (2010) Antimicrobial Activity of Secondary Metabolites and Lectins from Plants. *Applied Microbiology and Microbial Biotechnology*, **36**, 396-404.