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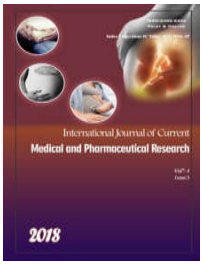

MEDICINAL GASTROINTESTINAL FRUITS, HAS XYL LECTIN ACTIVITY WHIT EFFECT ON THE BIOLOGICAL ACTIVITY OF ESCHERICHIA COLI O157H7

Dra Rocío Coutiño-Rodríguez., M en C Héctor Giles-Ríos., Mirna Pérez Cornelio., Dra María del Carmen Arenas., QFB Mirna Pérez Cornelio., Aron Mosqueda., Manuel S. Luzanía Valerio., MSP Enrique Colar G and Dra Edit Rodríguez,

Instituto de Salud Pública Universidad Veracruzana

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ABSTRACT

We characterized lectins from different gastrointestinal medicinal fruits proteic extracts (PE) such as pumpkim, orange, mango, avocado, plum, coconut, papaya, peach, pomegranate guava, mango. We found by hemaglutination test (HA) that all fruits PE recognized determinant antigens in blood types A, B, and O, but highly specificity to type A, particularly coconut, mango, guava and pomegranate, through the HA inhibition with sugar competition tests, most of them has a recognition to GlcNAC, Fuc, Man, GlcN, GalNAc and Xyl, particularly in group A. Band patterns in 10% SDS PAGE gels shown a common pattern bands around 14 to 28 kD and other bands about 30 to 60kD, also coconut, mango, guava and avocado showed bands that run as albumin, but the majority showed bands no so homogenous up to 100 kD.

The participation of PE on Escherichia coli O157H7 activities, such as inhibition of the E. coli hemaglutinacion (IEcHA), agglutination (EcAg), growth (EcG) and adhesion to cell HELA, was founded that IEcHA was enhanced in group B and O by mango squash, pomegrate, pumpkin, avocado, peach, guava and plum PE, and also are those that shown most EcAg activity, suggesting a recognition to the bacteria principally papaya.

The presence of the bacteria in cells HELA reduced it is growth in about 10 to 25% and induced anuclei cells 2 to 5%, only pumpkin PE increased it around 10% . Most PE at 300 µgr/mL in the presence of the bacteria, were toxic to cells HELA around 50%. At that concentration most PE shown a 30% reduction in EcG, however plum, mango squash, avocado and pumpkin increased it about 25%, and in the test of adhesion to cells HELA these favored bacteria growth with a slight increment of colonies around 7-12 compared to 6 formed in controls on the cells HELA. The results indicate that PE from medicinal fruits with lectin activity are participated in the activities of EHEC studied, where GlcNAC, Fuc, Man, GlcN, GalNAc and Xylose are involved, suggesting proteoglycan participation and Xyl lectins, too.

INTRODUCTION

Lectins are a well-known class of multivalent carbohydrate binding proteins of non-immune origin which recognizment sugars in very specific way, in plants principally, and fruits lectins have been classified in several types according to the kind of sugar they recognize and from the family they belong to their types and characteristics being as follows: 1-Chitin–binding type lectins mainly recognize GlcNac and derivatives, with homonomers of 18 kD or multimeric shapes (1), 2.- Monocot type, recognize Man, are homotetramerous with a basic unit of 12 Kd, some tetrameric, octameric shapes with basic subunits of 28 kD (2) 3.- Others, recognize GalNAc, like the tulip lectin, are heterotetramerous made up of 33 and 36 kD polypeptides, as the amaranthus lectin (3), 4.- The leguminous type lectin recognize chains of complex carbohydrates: Man, Glc, GalNac, GlcNac, Fuc, and Gal, with a basic unit of 30 kD (3), 5.- The RIP type lectins (ribosome inactiving protein), they behave like N glycosidase and poly-nucleotide-adenosine glycosidase (PAG), recognizing Gal/GalNac or Gal/Neu5Ac, and normally are dimmers or tetramerous with 30 to 37 Kd basic unit (3,4,5).The role of lectins in microbial pathogenesis has been extensively studied (6-10), some reports are about the identification of the lectins in medicinal plants and fruits having gastrointestinal uses, and their actions on biological mechanisms, particularly in the enterohaemorrhagic Escherichia coli serotype O157H7 (EHEC) (9-10) and enteropathogenic E coli (EPEC). EHEC can cause serious infections ranging in symptoms from diarrhea and haemorrhagic colitis to life-threatening

Corresponding author:* **Dra Rocío Coutiño-Rodríguez
Instituto de Salud Pública Universidad Veracruzana.

complications including haemolytic uremic syndrome, also can be can be fatal and morbidity long-term. EHEC in USA was recognized as an emerging human pathogen in two outbreaks of haemorrhagic colitis in 1982 and since then it has been associated with many food-borne outbreaks worldwide the pasteurization of milk and cooking of beef prevent food-borne transmission (11). Bacteria pathogenicity mainly depends on various adhesive and virulent determinants, such as capsules, fimbriae, toxin production and the presence of adhesins that allow them to adhere to the intestinal surfaces (12-18).The pathogenic determinants of bacterial virulence, are establish through specific recognition occurring between them and the epithelial cells and will be their targets for colonization or invasion. Such recognitions generally occur with the participation of glycosidic residues in the membrane capsules or in the receptors and of proteins that function as lectins. Bacterial fimbriae, are involved in the colonization and invasion of epithelial cells, mainly by the type 1 fimbriae which recognize mannose, type P fimbriae which join to residues of α–D-Gal (1-4)-β-D-Gal, present in the receptors of the epithelial cells (7,12-19), participate in the colonization and penetration of the epithelial cells and to agglutinate red blood cells (15, 16-18); Another important group implicated in pathogenicity are S-type fimbriae, which recognize α-sialyl-2,3 -β-Gal and are associated with extracellular matrix glycoproteins, such as laminin, and a species of intimin type adhesins that play a role important role in the adhesion of EHEC and EPEC (9,10).

Also Neu5Ac or syalic acid plays important role in the adhesive mechanisms of various bacteria such as Bordetella pertussis, Mycobacterium tuberculosis, Helicobacter pylori and EPEC, EHEC and enterotoxigenic E coli, as well have been widely studied GlcNac, in the adhesion of amoebic trophocytes, and fungi to cells; the Gal in the ganglosides receptors recognition by cholerae toxin and in the globocerebroside receptors for the vero-like cytotoxins from E. coli, and the participation of Gal, Gal/GalNAc type lectins or Galectins in the immune response, cancer, and in the apoptotic, necrotic cytotoxicity cell. It is also known the protective role of monocot lectins against infections by retrovirus and cytomegalovirus (20-23) in that way lectins are powerful tools in many biomedical applications (24-29).Lectins presents in medicinal fruits may be the bioactives that interferes in adhesion mechanism and could prevent bacteria, virus and fungi infections and parasites. The aim of this work is identified lectins present in extract proteic and determinate their participation on EHEC biological activities.

MATERIALS AND METHODS

Fruits from medicinal uses (Lozaya 1987) (30) such as *Curcubita máxima* (pumpkim), *Sechium edule (chayote)*, *Mangifera indica* (mango), *Citrus sinensis* (orange), *Persea americana* (avocado), *Spondias mombin* (plum), *Cocos nucifera* (coconut), Carica papaya (papaya), *Prunus pérsica* (peach), *Punica granatum (*pomegranate*)*, and *Psidium guava*(guava), were bought in the market and were not classified with the taxonomic guide from the Ecology Institute. Chayote and Guava were used as negative and positive controls. Monosacharides: GalNac (N-acetyl-D-galactosamine), Gal(galactose), Fuc(L-fucose), GlcNAc(N-acetyl-D-glucosamine), Man(Mannose), Mal(Maltose), Glc(Glucose), Neu5Ac(neuroaminic or syalic n-acetyl acid), Xyl(Xylose), GalN(galactosamine), GlcN(Glucosamine), TCA (Trichloracetic acid), were bought from Sigma Chemicals, Saint Louis, Missouri.

Fruits proteins extract (PE)

Procedure was followed, Coutiño 2001, Giles 2017, only modified by using double volume of cool acetone to precipitate proteins and the pellet of fruit´s proteins (text refereed as PE) was solubilized in phosphate buffer, the aliquots of 10 mL were stored at 0ºC.

Quantification of Carbohydrates and Proteins

The protein concentration from the PE was quantified by Lowry 1951 procedure (31), utilizing 400 µg/mL of bovine albumin as standard. The sugar concentration was quantified by the Smith 1956 method (32), utilizing 400 µg/mL of glucose as standard.

SDS-page

Gels at 10% were prepared without mercaptoethanol, 50µgr/mL of protein from each PE were added. To determine molecular weight, pre-stained standards (Gibco) were used after stained with coomassie. The resulting bands were analyzed with the Kodak 40 program Digital photo-documentation Sciences 1D.

Hemagglutinating activity in PE

Lectin activity through hemagglutinaton activity (HA), was evaluated by modified Coutiño2001 procedure, using microplates with 50 µL of 5% red cell suspension from all groups, in 150 µL of phosphates buffer (PBS), followed were added 50 µL of PE using the serial double dilution procedure. HA titer obtained after 1 hour is expressed as the highest dilution showing detectable cell aggregation (HA). Lectin activity was reported in units of hemaglutination for each blood (UHA) as the minimum concentration of protein necessary to obtain HA [9].

Inhibition of hemagglutination by sugars

The procedure was similar to that for HA, except that 1mM sugar standards were used as competitive inhibitors for HA, in double serial dilutions was mixed with a lectin solution at using the lowest concentration of proteins from PE by which HA also could be detected, and incubated for 30 min before the 5% suspension of red cells was added.

Bacterial cells E. coli 0157:H7

E. coli 0157:H7 (referred as *E. coli*) was maintained as was previously described by Coutiño 2001(9, 10). The culture was diluted in order to obtain bacteria at 2% solution that was used for *E. coli* hemagglutination (EcHA), inhibition of EcHA (IEcHA), agglutination (EcAg) and growth (EcG) assays

Hemagglutinating activity of E. coli

The method used for EcHA was according to that described by Evans in 1980(33) and modified by Giles (10). EcHA was identified directly by adding to each sample some drops of acridine orange stain to identify the ring of the HA and corroborated by Nikon stereoscopic microscopy observation.

Inhibition of EcHA for lectins

Lectins present PE were used as competitive inhibitors for EcHA. Inhibition EcHA mixture contained 25µl of *E. coli* solution at 2% mixed with 25 µL of erythrocytes at 2%, and with 50 µg/mL of PE in a 150 µL final volume of PBS

phosphate buffer solution. The IEcHA could indicate that lectins from extract compete for the same carbohydrate signal between bacteria and red cells attachment.

E. coli agglutination

E. coli agglutination (EcAg) by lectins was carried as followed, bacteria were grown in a culture broth at one OD on a scale from 1 to 625 nm or 2 on the Marfan scale and diluted in order to obtain $4.8x10^6$ cells/mL. After, were placed 50µl/well and then 50 µl of fruits PE. Double dilution was performed and then was incubated for 30 min at room temperature. The bacterial agglutination was identified by the ring formation.

E. coli growth

The percentage of bacterial growth was calculated in one million of bacteria/mL that were sown in a nutritive medium by triplicate and were exposed to 0 and 300µg/mL of different PE and then 625 nM OD was determined at 0 and 24 hrs after treatment compared with the OD values of a standard growth curve.

E. coli adhesion on HELA cells

The HELA cells were donated by Dr. Alfonso Gonzalez Noriega from the Institute of Biomedical Research of the National Autonomous University of México. In each experiment were sowing about 500,000 HELA cells for each cover slips collocated 3 per Petri dish, for 24 hrs. in a BEM medium. Subsequently they were treated with 300µg/mL of PE from the different extracts and in the presence of 480,000 bacteria/mL, incubated for 2 hrs, washed 3 times with PBS and fixed, and analysed by microscopy. The data corresponded to the percentages in relation to controls of the total number of HELA cells counted per coverslide (A) and the cells with have adhered or agglutinate bacteria (B),Adherence, also was done with the HELA cells in Petri dishes after fixed and washing that, they were incubated with 300µg/mL of different PE in the presence of 480,000 bacteria/mL, were incubated for 2 hrs and were washed with PBS. Subsequently nutritive agar was added to Petri dishes and they were incubated for 24 hrs, after which the units forming colonies (UFC) were counted Table 6 (C).

RESULTS

The most common and cosmopolitan fruits with gastrointestinal activity, basically antidiarreic based on ethnobotanical studies at national level by Lozoya 1987 (Table 1) were chosen, Pisidium were studied as positive control because previously has been studied when standardized the lectins identification (9).

Fruit PE

The extraction of proteins was closely as possible to physiological conditions, and the protein precipitation was carried out by using double volume of cold acetone because it is not necessary to dialyze or filter after precipitating proteins, and secondly, because it does not interfere with secondary metabolites, mainly tannins present in plants and pigments in some fruits; whereas in the case of PE, due to the presence of glycoproteins, it is not convenient to use as method the TCA (9), because increases the formation of aggregates, the only inconvenience to use cold acetone is that it also precipitates phosphates; therefore we need to wash several times with saline solution and to resuspend in this in order not to increase the amount of phosphate, and it is also necessary to use the appropriate controls, to see if phosphate does not interfere in results.The fruit PE present a higher proportion carbohydrates than proteins, corroborated that the proteins detected in most fruits are mainly glycoproteins (9,10,24), contrary to that saw in from medicinal plants extracts (10) that has less carbohydrates.

Table 1 Classification of the plants and fruits studied according to ethno-botanical studies and surveys by IMSS COPLAMAR, Javier Lozaya, 1987: Scientific and common name, Family, uses (I antidiarrheic, II antidysenteric, III antiparasitic, IV antiamoebic.), and C/P proportion from the PE

Scientific name (common name) fruits:	Family	Uses	Carbohydrates / proteins (c/p) Relation
Cocos nucifera (coconut)	Arecaceae	II, III	10.95
Mangifera indica (mango)	Anacardiaceae	I	1.57
Curcubina máxima (pumpkin)	Cucurbitaceae	III	5.04
Punica granatum (pomegranate)	Punicaceae	I, II, III	2.91
Prunus persica (peach)	Rosaceae	II,	11.55
Psidium guajava (guava)	Myrtaceae	III	1.02
Persea americana (avocado)	Laureacea	I, III	5.71
Spondias mombin (plum)	Anacardiaceae	I, II	6.08
Carica papaya (papaya)	Caracaceae	III	67.4
Citrus sinensis (orange)	Rutaceae	I	26.24
Sechium edule (mango squash)	Curcubitacea	-	3.58

Hemagglutinating activity (HA) of the fruits crude PE

Most PE have lectin activity with more specificity to group A, principally up for the coconut to 1,029,145 UHA and mango 602,353 UHA, much higher than in group O for mango 588.238UHA followed for pumpkin 288,045 UHA and guava 35,617 UHA, and for group B up to 1,004 UHA for peach and 455 UHA for avocado. The UHA in group A and O was higher than in B group; this means that fruits have lectins to recognize GlcNAc and Fuc, but not Gal or maybe recognize another Gal glicosidic determinant no related to the group B. Those results may be due to their high sugar concentration; as they are crude extracts, and there is a mixture of proteins and glycoproteins that compete with the glycosides signals from the erythrocyte, and may also have recognition among them and with another glycosides present in others surface antigens like N, TN and M, and sialyl lactosamines of the H antigen common in the groups A, B and O (9,10).

Lectin hemagglutination inhibition by suggars

The specificity of the lectins was done by inhibition of the HA (IHA) by competition with the series of serial dilutions of sugars standard and conducted using the lowest concentration of proteins from the PE by which HA also could be detected.
Table 2 shows the results, but before we must mention that Neu5Ac and GlcNAc at 1mM in both groups, O and A, in unwashed erythrocytes and without PE caused lysis, also after washing in group A, GalNAc and Xyl in presence of plum, guava, avocado, chayote, pumpkin and pomegrate PE caused hemolysis, probably those suggars in lymphocytes activated a protease, haemolysin, or esterase or also these could be modify the binding site in the erythrocythes. Results shown that PE have more than one lectin that recognize not only the glycosidic determinant for groups A, B and, but other sugars common in them involved in HA. Few sugars were capable of

inhibiting at low concentration as was reported with plant extracts [10], the majority inhibited at concentrations much higher, possibly due to the presence of competing glycoproteins in the PE which interfere in the results, in one of the two adhesion mechanisms, such as a sugar-sugar or sugar-protein interaction through hydrogen bridges between hydroxides (OH), electrostatic Van der Walls which probably requires a greater o lower concentration of sugars to break this molecular binding, also these results could be from unspecific binding for the amount of carbohydrates in PE, which may cause the induction or liberation of an protease or glycosidase.Coconut PE shown the presence of lectin with highly recognized to GalN in blood group A, inhibited at 620 µM. It is important to point out the need for making serial dilutions, in order to handle equivalent doses because one may be working in prezone and postzone, providing false negatives, because en the case of guava the results showed a 35,617.5 UHA and was inhibited with 310 µM of Gal in the O group, and previously in those group Gal at 1M does not inhibited the HA, although to obtain very similar results 30,971 UHA [9].
Xyl was highly recognize by PE in group B and O (Table 4) but not in group A, similar as was reported in plants extracts [10]. This suggests either of two possibilities: (1) the presence of Xyl in the glycoproteins erythrocyte membrane and the presence of a lectin in PE with recognition to Xyl in it, or (2) the existence of a Xyl lectin in the erythrocyte membranes and the presence of glycoproteins containing Xyl in PE. However, Xyl in group A at I mM caused hemolysis with some PE.

Molecular Weights Characterization of the PE from fruits

The analysis of gels in photo-documentation, indicating that some of PE such as guava, mango, pomegrate and coconut PE have 3 to 4 common bands, all of them below 30 kD, more precisely between 14kD and 21 kD, similar the ones was found in plants PE [10], probably those band indicate the presence of a family of lectins with a common ancestor of phylogenetic interest, which has been preserved due to some common biological activity. Although coconut, mango, guava, plums and avocado PE, showed a homogeneous band close to 68-75kD, similar to albumin from bovine, albumin is a protein that join with several chemical compounds such as sugars (Table 3). And in pumpkin, mango and coconut showed bands around 25 -38kD.

Most fruits PE, the bands were no so homogeneous, around 60 to 200kD molecular weight possibly by the sugar concentration could be patterns of multiplicity forms of supramolecular aggregates and also the bands that run very close together, could be isomorphic with different degrees of glicosidation, because lectins could carried sugars, and some of them could be a leguminosae type lectins with a recognition to complex sugar.In previously reports with plum and mango PE, no lectin activities were identified, because the work was performed only with the O group, and no serial dilutions were done, where these PE have a better recognition to group A, as shown in table (3), also has been detected a differential recognition with respect to sex basically between the group O and A (9, 24, 37).

For the molecular weight and the sugars recognizment, PE may have Chi (GlcNAc, 18 kD); Mono Cot (Man, 12 kD); RIP (Gal/GalNAc, 30 to 35 kD), Si (Neu5AC,), Xyl (Xyl around 14.4 or 28.8-kD) type lectins; and Leguminosae (complex sugars), with monomer around 18kD and with a multiplicity of these up to 60kD (3,4).

Table 2 Activity from fruit p-ext´s. in the Hemaglutination of A,B and O blood groups (HA) and is expressed as Units of HA (UHA) the minimum concentration of protein necessary to obtain HA. The HA competition with sugar was done with standards of at 1 mM and with double serial dilutions using the lowest protein concentration from fruit PE capable of producing HA and was expressed with the lowest titer (as micromoles µM)) of sugar availed to inhibit HA.

Scientific	UHA/mgr protein	Blood type	Sugars type concentration
Plum	16,113	A	A Gal (620 µM)
	221B	B	B Fuc (30 µM) Glc 60 µM
	1,007.1	O	O all sugar (310 µM)
Pomegrate	240,375	A	A GalNAc (310 µM)
	96	B	B Xyl,Neu5Ac,GlcNac,GalNAc (30 µM)O all suggars (310 µM)
	15,023.5	O	
Pumpkin	288,045	A	A Glc, Fuc, Gal (62µM)
	175.7	B	B Xyl, Neu5Ac, GalNAc, GlcNac (30 µM)
	288,045	O	O all suggars (310 µM)
Avocado	27,779	A	A Man,Gal (620 µM)
	455	B	B Xyl , GlcN (30 µM)
	11,394.9	O	O all suggars (310 µM)
Peach	207,204	A	A Xyl ,GlcNAc (620 µM)
	7,249	B	B Neu5Ac, GlcN, GlcNAc (30 µM)
	454	O	O all suggars (310 µM)
Coconut	1.029,146		A GlcN (620 µM)
	334		B GlcNAc, GlcN, GalNAc (30 µM)
	11	A	O all suggars (310 µM)
	397	B	
	58	O	A Man, GlcNAc (125 µM)
Orange	4,568.4		B Fuc, GlcNAc, GalNAc, Neu5Ac, GlcN (30 µM)
			O all suggars (310 µM)
Papaya	10	A	A GlcNAc (125µM)
	83	B	B Xyl, GlcNAc (30 µM)
	8,533	O	O all suggars (310 µM)
Mango	602,353	A	A Gal (620 µM)
	43	B	B Glc GalN (30 µM)
	5880.23	O	O all suggars (310 µM)

Table 3 Pattern of bands detected in plant and fruit PE in 10% SDS gels. The results shown came from the autoanalyzer with the Digital MSD 40 Kodak Program. Relative weights were obtained according to protein Albumin Stock (68-132kD), and prestained GIBCO Stock .

Extract	Relative molecular weight (kd)								
Peach	205	188	135						
Avocado	265	194		70					
Orange			149		58	41			
Papaya		193	153						
Pumpkin		183	120		58	38	34		
Chayote		188	137						
Plum	207	195		84					
Coconut		185	156	67			27	20	
Mango	270	194		70			26	18	15
Guava		194		68			21	19	17
Pomegranate	207		133		56		21		
Weight marker (GIBCO)	194	120	87	64	52	39	26	21	15

It is important to point out in our experience that the fruits PE, they can lose or gain activity compared with plants PE which are more stable, after several months of refrigeration with hardly any contamination; contrary with fruits PE that are easily contaminated (due to their high sugar content), also they reduced activity which suggests a degrading process of proteins, probably the glycosidation degree is associated. It is well-known that the average life of glycoproteins in a cell membrane and those circulating in serum is determined by

their glycosidation patterns (35), although there are no reports regarding the existence of this process in plants.

E. coli hemagglutination (EcHA)

The characterization of the EcHA, was previously done in all blood groups (A,B and O) based in the lectin-type structures and glycosidic components (adhesins or fimbriae) involved in bacteria and eritrocythes recognition through IEcHA and the results corroborated that fimbrae type I, type P, S and also Xyl are participating (Table 4) [9,10, 36].

Table 4 Sugars that inhibit HA of *E. coli* 0157:H7. The HA assay was as described in material and methods. The standard sugars were diluted (10mM to 1 M), double serial dilutions was done to get the minimum amount of sugar able to inhibit the *E. coli* HA from (9,10)

Sugars	Group A (echa Title 0.41)	Group B (echa Title 0.2)	Group O (echa Title 0.2)
Neu5Ac	125 µM	1 mM	15 µM, 62 µM,10mM
GlcNAc	15 µM, 250 µM	15µM	250 µM, 1mM
GalNAc	125 µM, 10 mM	31µM	62 µM, 250 µM
Gal	250 µM, 15 µM,	250 µM,10mM	62 µM,
Gal N	15 µM, 125 µM	250 µM,	15µM, 250 µM,
Glc	250 µM	15 µM,	62 µM, 125 µM,
GlcN	250 µM , 1mM	1mM, 10mM	10 mM
Xyl	1mM, 10 mM	1mM	1mM, 10 mM
Fuc	1mM, 10 mM	1mM	1mM
Glc	1mM	1mM	1mM, 10 mM
Man	1mM, 10mM	1mM	ND

Table 5 Compared the effect of PE in the HA of blood group AB 0, in E. coli HA Inhibition and E. coli agglutination. The Specific activity of E. coli HA inhibition or Units of IEHA was (titer) minimum for perceiving E. coli HA x dilution factor (g of protein from PE). Specific activity of PE on the E. coli agglutination or Units of EcAg was calculates as E.coli agglutination x dilution factor (40)/(mg of protein from PE).E. coli 0157:H7 grown one million of them /mL were sown in a nutritive medium in triplicate; they were exposed to 0 and 300µg/mL of different PE and the OD was determined at 560 or 625 nM at 0 and 24 hrs, results are expressed as porcent of inhibition or increase in the OD relation from 0 to 24hrs.

common name	E. coli 0157:H7 EcHA inhibition (Units IEHA)	E. coli 0157:H7 agglutination (Units of EcAg)	E. coli 0157:H7 Grown inhibition
Plum	4,321 O	2,014	15+
Guava	2,040 O	1,936	25
Chayote	2,008 AB	16	25+
Pomegrate	1,406 B	1,407	27
Pumpkin	1,380 BO	1,379	10+
Avocado	1,077 BO	114	30+
Peach	906 B	113	17
Coconut	667 A	164	21
Orange	382 B	6	23
Papaya	233 A	1,379	30
Mango	57 B	1,484	25

The presence of Xyl has been reported in proteoglycan structures from laminins probably from cells (10) and in N-glicoproteins , so this might be the reason for the participation in the HA, and EcHA, and seems to plays an important role in the hemolysis and toxicity in group A.Thus probably determining the differences in the hemagglutination of the bacteria in group A (EcHA) that was twice greater than in group B and O, possibly the content of those sugars, varies or perhaps the glicosidic determinant is involved, and facilities the adhesion, as well as other components from the erythrocytes sensible in bacteria colonization, such as in the case from adults with diarrhea, were isolated several serotypes of enterotoxigenic *E. coli* possess an the colonization factor antigen(CFA) and was found a particularly mannose-resistant hemagglutination only in human group A erythrocytes (19).

Table 6 Percentage of Cytotoxic and adhesion effect of the fruits PE on the cells HELA in the presence of *E. coli* 0157:H7,. A.- Citotoxicty % of toxicity of HELA cells in the presence of bacteria and PE for 2 hrs. B.- Adhesion % of cells showing adhering or internalised bacteria after 2 hrs in the presence of PE. and C.- Number of colonies formed on fixed HELA

Samples extracts	Cytotoxic Effect to cells HELA (A)	Porcent cells HELA with bacteria present in after 2 hours (B) Agglutination	Number of bacteria present after 24 hours (C)
Pomegranate	85	9 -	10 big colonies
Peach	77	3 -	Small colonies
Plum	65	10 ++	12 regular colonies
Pumpkin	60 and 10 anuclei cells	6 ++	7 regular colonies
Orange	60	11 -	2 big colonies
Mango	56	9 +	-
Chayote	50	4 +	Small colonies
Coconut	50	12 +	4 big colonies
Papaya	45	1 +++	-
Guava	40	3 +++	Dot colonies and few small colonies
Avocado	20	8 +	8 regular colonies and dot colonies
Cells with bacteria	25 (1% anuclei cells)	3 -	
cells	8		6 regular colonies
cells	10	0	0

E coli hemagglutination inhibition by lectins from PE

The most fruit PE has lectins that recognize some of the sugars involved in EcHA (10), it is be expected that them to compete for the sugars thus involved, thereby inhibiting the EcHA (IEcHA).

The Table 5 shown that PE has high HA in group A, and low specificity to inhibit EcHA, contrarily to detected in plants PE that has highly HA and IEcHA in group B. The only PE with more IEcHA in the group B were pomegrate, pumpkin, avocado, peach, and in in group O, plum and guava, those results with guava as positive control corroborate the reported previously (9), which means that plants PE has a lectin with a better recognizment to *E. coli*, and is common with the glycosidic determinant of group B the Gal (10), whereas the fruits PE recognize a similar sugar or different from the group B determinant but which is common with some antigenic determinant of the microorganism such as GlcNAc, Gal NAc. Fruits PE (Table 4,5) that were most effective in the HA and in IEcHA are those that recognize Gal, Neu5Ac Man, Xyl and GlcNAc, those sugars are involved in the bacteria-erythrocyte adhesion (EcHA), Said recognition could be to the erythrocytes or to bacteria and particularly in the case of papaya, pomegrate, guava, is through to the bacteria.

E coli Agglutination by lectins

The Table 5, show, few PE have effect on the agglutination of *E coli* (EcAg) and clearly corroborated that most of PE that IEcHA in group B and O have activity in EcAg, such as; plum, guava, papaya, pomegrate, and pumpkin PE around 2,000 to 1,500 EcAg units. Chayote, as negative controls, did not

agglutinate the bacteria (20 EcAg), and also shows little capacity to HA and IEcHA in those groups. The ability to EcAg again suggests their recognition of lectin activity capable of recognizing glicosidic antigenic determinants in the bacteria similar or different to be the ones involved in the IEcHA.

Again the PE that recognize Gal, GlcNAc, Neu5Ac, Xyl and Glc at 15 μM (Table 2 and 4), favoured the EcAg. It is know that *E. coli* pathogenic determinants are shares with others many enterobacteria such as Fimbrial, but also M, LPS, antigens containing Gal and GlcNAc in their external membranes, a fact that may depend on said recognition (9,10).

E coli Growth and lectins

Table 5 show the effect of PE on growth obtained from the differences in the OD values at 560nm, between 0 and 24 hrs, from the bacterial cultures treated with 0 and 300 μg/ml of PE. The papaya, pomegrate, mango and guava PE inhibited growth about 25% to 40%, and peach, orange, and coconut by only 10 to 23%, on the other hand pumpkin, plum, avocado and chayotePE, shown and increased growth by about 10% and 30%, perhaps the amount of protein or sugars presents in those are participated.Probably in those results Chi type lectins that have a cytotoxic effect on fungi and insect (1) and an apoptotic citotoxicity in human monoblastic leukemia U937 cells (38) are involved because most PE (mango, guava and pomegrate) that showed some bactericidal effects recognized GlcNAc, GalNAc and showed an protein band of 15kD -20kD.

E coli adhesion to HELA cells and lectins

We attempted to corroborate the inhibiting activity of the PE on the adhesion of EHEC to erythrocytes cells by using a model closer to epithelial cells, in this case, HELA cells. In an attempt to determine whether the different fruits PE also inhibit the adhesion of EHEC to HELA cells (Table 6).

The adhesion was clearly not be determined; first because also the bacteria were internalised, and few cells contained bacteria around 10% to 30% very closely as the control 10%; and second, due to the cytotoxic effect of the bacteria to HELA cells about 25%, these later results corroborated the production of cytotoxin by the bacteria, in is cytotoxic effect. However pomegrate, peach, plum, pumpkin and orange PE in the presence of the bacteria were highly cytotoxic to cells HELA around 60 to 85%, and most of them recognized acylated sugars.The foregoing results suggest strongly that the fruits PE in the bacteria presence possess cytotoxic activity on cells HELA similarly as Rue, Salvia PE (10) and based on the types of sugars they recognize and the band pattern in gels, they may have one or more lectins such as RIP type, Chi type, Si (Neu 5Ac), or even legumynosea type, whose has been attributed cytotoxic activity. In order to see adhesion in fixed HELA cells, after 24 hrs, 7 colonies were detecting in the control and around 8 to 12 in pomegrate, plum, and avocado PE: Those fruits PE that slightly favoured the bacteria adhesion and internalization also favoured EcG and EcAg, and also could be a reason of its toxicity to HELA cells.

DISCUSSION

The study of the lectins that fall within the branch of glycobiology is a discipline of great interest for all the advances and applications that have been seen in the regulation of biological functions that is why the most important universities have been involved in the studies and investigation thereof. More than 30 biotech and pharmaceutical companies around world are currently doing research particularly on glyconutrients, because such proteins and peptides that have glycans attached to them, is of increasing importance for science as diverse as the development of pharmaceutical drugs, research. Ethnomedicine, is an integral part of the culture and the interpretation of health by indigenous populations in many parts of the world. Mexico has an ancestral tradicional Ethnomedicine, some of the bioactives in fruits and plants are secondary metabolites such as alkalides, flavonoids, tannins etc, and another could be primary metabolites such several proteins with lectin activity and inclusive peptides, vitamins, minerals and carbohydrates which are involved in the therapeutic activities (9,36, 39,40).The pharmacological activity attributed to fruits and even plants used in medicine traditional knowledge seems to dependent of those bioactives present in most living organism (9,39,40).

Lectins participation in physiological processes in animals is well studied, such as recognition and elimination of glycoproteins of the circulatory system, host pathogen interactions, the elimination of aging cells, tumor and microorganisms, cell–cell interactions in the immune system, they are important molecules in the recognition of several biological process been involved in cellular signalling, and protein targeting to cellular compartments, that allows the differentiation, the organogenesis, lymphocyte migration and as determining factors in the metastasis and malignancy (27-29, 40).The aim of this work is to study the presence of lectins on Medicinal fruits used as gastrointestinal activities through that inhibit adhesive mechanism, basically through hemaglutination procedures.

The HA procedures is a very useful and trutsworthy for the identification of lectins even the results are consistently between groups, as we shown with the positive control Psidium PE which in previously reports performed with another group O blood donor, the results were very similar to these, where the HA activity was 30,971, EcHA inhibition of 3,871 and EcAg of 1,936(9), compared with 35,617.4 HA inhibition EcHA 2,040 and the EcAg was the same 1,936 like was expect. However in this work the identification of lectins was done in the three blood groups (A,B,O), because behave different some of them are most specific's to one group as we found with coconut, plum, and mango for group A, inclusive are different recognition for sex reason why previously were not identification the presence of lectins in group O (9, 37).
Previous reports show that Gal, Fuc, Xyl and acylated sugars are implicated, in the *E coli* activities, (9,10) in that way, the results indicate that medicinal fruits that have lectin activity with a recognizment those sugars they have similar effect on *E. coli* activities. However, the degree of glycosidation of fruit PE, may interferes to those activities, probably due to the ability of the sugars to establish hydrogen bonds or electrostatic bonds that allow to compete between them to inhibits or induce the agglutination and adhesion of the bacteria to the red or HELA cells and in toe internalization to the cells exerting a more toxic, as was observed with pomegrate, plum, peach and pumpkin PE, that showed more capacity to induce EcAg and adhesion to the cells HELA, were also more toxic to HELA cells.

The high cytotoxic effect of fruit PE to HELA cells and low effect on *E coli* activities may be due to a synergistic effect of more than two lectins involved in the different effects, which for the sugars recognizment and for the pattern of bands could

also be Chi, Glc or RIP type lectin. However, it most possible to be dealing with leguminosea type lectins, and also Xyl lectin, because xylose again was found to participate in HA and in the EcHA (9,10) and probably in EcAg and toxicity to HELA cells, this suggests proteoglycan participation in adhesive and toxicity mechanisms in group A.

Reports from the fungus Xylaria hypoxylon shows activity of Xyl lectin with show as a single band of 14.4 kDa or 28.8-kDa depending the procedure to identification (41), which probably for the molecular weight may also related with Monocot and Chi lectins because complex sugars present in most cells membranes have a glicosidic core linked to Asgaragine -2 GlcNAc, 3 Glc and 9 Man, where in several position Man is replaced by Xyl, and in all of the known structures, p-D-xylose is 1-2-linked to p-D mannose of the trimannosyl-N,N'-diacetylchitobiose and when a-L-fucose is present at the asparagine-linked N-acetyl-Pglucosamine, then there are differences in the sites of its attachment, namely, al-3- or al-6-linked, depending on the biological origin (42).

Most Xylose glycoproteins belong of N-linked oligosaccharides chains are integral parts of certain plant and animal glycoproteins and have shown so far in lectins, enzymes, storage proteins, and protease inhibitors. On the other hand, the expression of xylose-binding proteins (XBPs) or Xyl lectin exhibit highly potent antiproliferative activity toward tumor cell lines, and a potent anti-mitogenic action on mouse splenocytes (43), and in some tissues such as hepatic cells, XBPs were up or down-regulated significantly in activated hepatic stellate cells (HSCs) and the precision alteration of the XBPs is associated with pathological changes in HSCs during liver fibrosis/cirrhosis which may provide pivotal information needed to discover potential glycan-binding protein-related biomarkers for diagnosis of liver fibrosis/cirrhosis and for development of new anti-fibrotic strategies (43).

The role of Xylose glycoproteins in host–pathogen interaction has been reported in fungi infections, and recently, mushroom and other fungal lectins have attracted wide attention due to their antitumour, antiproliferative and immunomodulatory activities (40,41)

Our results indicate that Xyl is participating in HA of group B and O, but in group A in the presence most fruit PE, at 1 mM Xyl produce hemolysis with exception of coconut, peach, papaya, and orange. These results might also determine the susceptibility and sensitivity of red groups to viral and bacteria infections and their risk of developing cancer, and also to developmental allergic process, particulary A gruop.

On the other hand, coconut, mango, peach, orange and avocado PE, such showed recognition to GalN, GlcN, in group A or B were toxic to HELA cells, and presented bands of around 58-70kD, with the exception of peach possibly those proteins could be responsible for that cytotoxicity to cells and also to bacteria reason why these GlcN and GalN suggars were significant with the toxicity of HELA cells and with the EcAg. Fruits PE that recognized acylated sugars were showed significant values with EcAg and with the toxicity of HELA cells, but not with IEcHA and with HA as was Xyl.

Nevertheless the degree of glycosidation and even the elaboration of the PE could be determinant in the electrophoretic mobility, since only analyzing the results reported for mango pulp they has been found around the recognition of sugars and the patterns of bands, in other reports similar to this work, were reported in the mango pulp that presented necrosis and apoptosis activity and the presence of lectins with a recognition to Gluc, Gal in the group O and with a pattern of bands of 75, 34 and 20 kD (24) in addition the band around 20 kD highly glycosidated could be 18kD and be responsible for necrotic effect; another unpublished previous report also showed in the pulp of the mango a recognition in the group O and A to: GalN, GlcN besides Gal Glc and Fuc where was showed a differential recognition by sex and a pattern of bands of 250, 149, 54, 24 and 18 and 15 kD (37), and in other studies report the presence of two proteins of 24 and 28 kD with lectin activity and GlcNAc recognition (24). Whereas a study in mango leaves detected the presence of several bands of 50, 30, 27 and 15 kD, and the 30 kD band has a Glc, GlcNAc and GalNAc recognition (24), our study found on the mesocarp of mango the presence of one or more lectins with recognition in groups A, B and O to Gal GalN, Glc, and a pattern of bands of 270, 194, 70, 26, 18 and 15 kD, and an effect in the EcAg (1,484 UAg) and a cytotoxic activity in HELA cells around 56%. In these work corroborate in mango PE the presence of lectins with same sugars recognition and molecular weights, very similar to that reported previously. Probably the differences in the pattern of bands and sugars recognition could be by the degree of glycosylation. The band between around 70-75kD could be albumin band and may be involved in the cytotoxic effect, because albumin is involved in transport metals, metabolites and sugars, such also could be the ones that recognize GlcN, GalN responsible for the cytotoxic effect on HELA cells.The results support their gastrointestinal uses, specifically as antidiahrreic, because when were compared it was founded significance relation with the results of EcAg and with the EcHA.

Lectins present in those fruits PE with medicinal used in the treatment of gastrointestinal ailments might well be the active principle that inhibits adhesion, agglutination and other factors implicated neutralities of infectious process of this bacteria and other bacteria that share pathogenic determinants, with other enterobacterias affecting public health, such as *Vibrio cholerae, Salmanoella thyphimurim, Shigella flexnerii* , among other gastrointestinal bacterias, but also fruit PE, for it is cytotoxic effect to HELA cells, could prevents grown of cells transformation, such as a 15kD lectin thype Chi from mushroom Kurokawa which shows that inhibited proliferation of human monoblastic leukemia U937 cells (38) and Xyl lectin with antiproliferative activity (41) and Gal lectin with apoptotic/necrotic effect (24). The results allowed give a support to one of the strategic in Public Health recommendations, the consume of fruits because, they not only preventing infectious disease but also chronic degenerative diseases such as cancer, where immune response is involved such as the inflammation and also it is well known the role of glycosidic determinants in many biological processes (27-29).

However these effects you must manage very carefully because are going to be differential depending on the blood group that as shown with the lectin of the coconut and plum PE specifically agglutinates to the blood group A as well pumpkin and guava to group O and B respectively. On the other hand some lectins in fruits and plants protein extracs recognize and agglutinate at level of the sex in A and O group

such as in *Mangifera indica* and *Eichornia crassipens* and *Lemna minor* PE (39), and other lectins in some blood groups such may cause hemolysis and maybe involved in renal disease and allergic processes. In Mexico, EHEC is not a public health problem as in other countries, which have gone through several outbreaks including EE. UU (11), possibly the blood group is related, because this bacteria has more recognition to A group, but we also believe that it is also due to the fact that Mexican people frequently drink teas from medicinal plants, which has a Gal/GalNAc lectins which are more effective against this bacterium (10); Such practice could act preventively, however, in the case of EPEC, whose adherence is mainly through Fuc, the lectins that recognize Fuc are found commonly in fruits, where the population susceptible to infection, and also to chronic diseases such as cancer, because of their economic situation, they do not consume fruits.

It has been propose the consume of fruits in order to maintain the health, because fruits are sources of vitamins and minerals that have been demonstrated are involved in several diseases (38), but also fruit consumption provides bioactive such as secondary and primary metabolites as lectins able to control cell growth, specially the malignant and therefore could be used as a preventive and perhaps even curative of tumors, but we have to be very careful in the use of fruits and plants as treatment because as mention before they specifically recognize the red groups by agglutinating them and in some occasions it may induce hemolysis and this can cause an immune response and also rennal diseases, and it is necessary that for treatment or prevention have to be considered individually the ability of lectins to recognize and agglutinate the specific A, B and O group type, but as was seem are also specific for the recognition at sex level and possibly at Rh level antigen, because they may have different glicosidic antigens.

CONCLUSION

The pharmacological activity attributed to fruits and even plants used in traditional knowledge seems not only to be dependent on secondary metabolites, as it has been extensively described, but also on several proteins with lectin activity present in the crude protein extracts, as it was found in this work, that fruits PE that recognized A and O group were able to EcHA, to inhibit EcHA, EcG and induce adhesion, and toxicity to cells HELA, but from our experience plant PE that recognized group B, are more effective in those activities tested in *Escherichia coli* suggests participation of Gal and derivates rather than that GlcNAc and Fuc where GlcNAc and Xyl lectins are related more to HELA toxicity.

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