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Lectins Gal/GalNAc from medicinal plants extracts with gastrointestinal activity have effects on the biological activity of *Escherichia Coli* O157H7

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Abstract

In this work we characterized lectins from different gastrointestinal medicinal plant extracts (*Hyptis mutabilis; Zebrina pendula; Salvia officinalis; Mentha piperita; Melissa officinalis; Ruta chalepensis; Psidium guajava; Foeniculum vulgare; Chenopodium ambrosoides; Marrubium vulgare; Matricaria chamomilla; Heteroteca indulis)*. We found by hemaglutination test (HA) that all protein plant extracts (PE) recognized all analyzed blood types A, B, and O determinant antigens, but highly specificity to type B, which means Gal recognized. The results shown that majority proteins from all PE migrate around 14 to 250 kDa, and all has a common pattern bands around 14 to 21 kDa. In blood type B and A the principal sugars recognized by PE were principally Gal, GalNAc, Gal N, Neu5Ac and GlcNAc, suggesting the presence of at least RIP, Gal/GalNAc o CHI type of lectins. In addition to this, most the PE inhibited *E. coli* O157H7 hemagglutination with the same specificity in all 3 blood types A, B and O, better that their HA activity, and also PE from *Hyptis, Manrrubium, Zebrina, Mint and Foeniculum* shown highly activity in *E. coli* O157H7 agglutination, suggesting a recognition to the bacteria with exception of *Salvia, Chenopodium* and *Ruta* that were cytotoxic to it. Adhesion to cells HELA was not detected because most PE in the presence of the bacteria were toxic to cells. For the first time we detected that most plant PE have lectin activity to Gal and acytaled sugars that recognized highly group B and inhibited *E. coli* O157H7 hemagglutination and induces toxicity and it is agglutination also they recognized xylose sugar, suggesting proteoglycan participation in those mechanisms.

Lectins, *E. coli* O157H7, Medicinal plants, Gal/GalnAc type lectins

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Introduction

The *E. coli O157H7* is the microbial agent involved in hemorrhagic enteritis diarrheic disease and, as other enteropathogenic *E. coli*; it has a fimbrial 1 adhesin, and adherent factors, such as intimin, Shiga-like toxin I (SLTI) and II (SLTII). All virulent and pathogenic factors involved in this disease were characterized by the hemolytic uremic syndrome developed in about 2 to 7% of the cases. This disease also affects children from one to five years old, and specially, those under one year (82%).With a mortality range from 10% to 30%, causing damage, specially, chronic renal failure, hypertension, microangiopathic anemia, and thrombocytopenia. In Argentina and Chile, the HUS is one of the main causes of acute renal failure in children under 4 years old [1-5].

E. coli 0157H7 is epidemiologically important since it is acquired through the consumption of uncooked meal from swine and cattle. Recently in Chile, report pigs as the main source representing a public health problem, because, strains isolated from fecal samples either healthy or with disease, displayed a *E. coli* 0157H7 adherence factor (EAF) than in bovines [6]. *E. coli* 0157H7 is not a public health problem in México.

Several studies shown that *E. coli* normally inhabits in mammals intestines, including humans. However, some highly pathogenic bacteria causing severe diarrhea and many of them are *E. coli* varieties; enterotoxigenic, enteropathogenic, enteroaggregative, enteroinvasive and enterohemorrhagic [6]. The pathogenicity depends on many adhesive and virulent factors, such as capsules, fimbriae, toxin production and the presence of adhesins that allow adhesion in the intestinal surface. Most of them are associated with the presence of plasmids, bacteriophages and bacteria chromosomal genes [7-18].

The recognition between bacteria and epithelial cells usually occurs in the presence of glycosidic residues in the membrane capsules, in receptors or proteins that act as lectins, such as bacterial fimbriae, EAF and colonization or invasion factors of epithelial cells. The case of type 1 fimbriae which recognizes mannose and participates in the colonization, and penetration of the epithelial cells at the bottom of the intestine, before enteropathogenic *E. coli* and *E. coli* 0157:H7.

Type 1 fimbriae can also agglutinate red blood cells (they are called type P fimbriae in the presence of uropathogenic *E. coli*, through the recognition of α–D-Gal (1-4)-β-D-Gal sugar residues[10-13, 19]. Another important group of adhesin is type S fimbriae, which recognizes α-sialyl-(2, 3)-β-Gal associated with glycoproteins from the extracellular matrix such as laminin. It has been reported that in enterotoxigenic *E. coli* several fimbriae factors shown lectin activity involved in adherence, through Man, GlcNac, GalNAc or sialic acid (Neu5Ac) sugar recognition and their genes are located either in the chromosome or in plasmids[15]. Also, bacterial toxins such as Shiga toxin and Shiga like Toxin produced by enterohemorrhagic strains of *E. coli*, are classified as lectin type 2 RIP´s.

The role of lectins in microbial pathogenesis has been extensively studied [10, 17, 19-26] and these active compounds from natural sources may prevent several process like microbial pathogenesis, toxin adhesion, bacterial agglutination, growth and cell death such as apoptosis and necrosis [26-27]. Lectins have been an important tool for oligosaccharide characterization as well as isolation of cellular populations in studies in cell regulation and also as potentially antineoplastic drugs [28]. In this work, we characterized the effects of lectins from several PE from gastrointestinal medicinal plants on the biological activity of *E. coli 0157H7* and identified the sugar involves in that recognition.

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Materials and Methods

Plants

Plants with gastrointestinal effects used in this work were previously reported in Mexico by Lozoya [29] and described in table 1. All plants were bought at the local market and classified comparatively with a taxonomic guide from the National Ecology Institute (INECOL).Scientific and common names: *Hyptis mutabilis* (tropical bush mint or tapon), *Zebrina pendula* (dayflower) *Salvia officinalis* (sage) *Chenopodium ambrosoide*,(wormseed), *Heteroteca indulis (*arnica), *Mentha piperita* (mint), *Matricaria chamomilla* (chamomilla), *Melissa officinalis* (melissa), *Foeniculum vulgare* (fennel) *Marrubium vulgare* (horehound) *Psidium guajava* (guava), *Ruta xalapensis* (rue)

Plant proteins extract (PE)

A total of 100g of each plant was incubated in 200 ml of petroleum ether per gram for 30 min at room temperature. After delipidation, the samples were macerated in 250 ml of phosphate buffer solution containing 10 mM of phosphate, plus 150 mM of sodium chloride at pH 7. Macerated samples were filtered using gauze, then centrifuged for 10 min at 700 rpm. To the clear supernatant, several drops of trichloroacetic acid at 10% to precipitate proteins were added, and then centrifuged for 15 min at 7000 rpm (Survall super T 21); finally, a double volume of cool acetone was also used to precipitate proteins. Each pellet was washed twice in distilled water to eliminate acid excess, and the pellets were solubilized in phosphate buffer, then aliquots (text refereed as PE) of 10 ml were stored at 0ºC.

Quantification of carbohydrates and proteins

Protein quantification was done by the modified Lowry procedure, using 400 µg/ml of bovine albumin as standard. The sugar concentration was quantified by the Smith method, 400 µg/ml of glucose was used as standard.

SDS-PAGE gels

Samples (50 µg/protein well) were applied to 10 and 15% SDS-Polyacrylamide gels according to Laemmli procedure, with or without mercaptoethanol. Gels were stained with Coomasie Brillant Blue R250. To determine molecular weight of the bands, pre-stained standards of Gibco and cross-linked hemoglobin were used, and the resulting bands were analyzed with Digital MSD 40 Science 1D program from Kodak.

Hemagglutinating activity

Erythrocytes from groups A, O, and B from healthy human donors were collected in heparinized syringes, and washed 5 times in 10 mM of sodium phosphate diluted in 150 mM of sodium chloride pH 7.4. Finally, a suspension of 5% of red cells was prepared to test lectin activity through hemagglutination (HA) activity as Coutiño [22] described. The hemagglutinating capacity of the extracts was evaluated using 25 µl of extracts mixed with 25 µl of 5% red cells suspension from groups A, B and O, and added with 50 µl of phosphate buffer (10 mM sodium phosphate and 150 mM sodium chloride pH 7.4).

Then, double dilution was performed on microplates, in order to obtain the hemagglutination titer after one hour incubation at room temperature or, 30 min at 35ºC, the hemagglutination titer was expressed as the highest dilution showing detectable agglutination. The hemagglutinating units were expressed as the minimum concentration of the protein necessary to obtain agglutination (HA units) and the specific activity of hemagglutinating is the number of HA units per mg of protein.

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Inhibition of hemagglutination by sugars

The specificity of the lectin was determined by HA competition assay using sugar standards as references: Monosaccharides: GalNac (N-acetyl-D-galactosamine), Gal (Galactose), Fuc (L-fucose), Glc Nac (N-acetyl D glucosamine), Man (mannose), Glu (glucose), Neu5Ac (neuraminic or sialic acid), Xyl (Xylose), GalN (Galactosamine) and GlcN (glucosamine) at 1 mM (Sigma Chemicals, Saint Louis, Missouri). Double serial dilutions using the last titer or 50 µg from each PE that can produce HA was incubated for 30 min. with 25 µl of 2% of red cells suspension. The HA inhibition was determined by the lost of agglutination and by OD values at 650 nm, based on control.

Bacterial growing cells

The *E. coli* 0157:H7 bacteria was cultured in Eosin Methylene blue agar (EMB) for 24 hrs at 37ºC. Then, colonies were taken out and placed in 3 ml of PBS, 1 ml of this solution was placed EMB agar plates, and incubated for 48 hrs at 37ºC (1 OD 650 nm or at 2 on the Marfan scale). Finally, this culture was diluted in order to obtain 2% of bacteria solution that was used by *E. coli* hemagglutination and agglutination assays.

Hemagglutinating activity of *E. coli* 0157:H7

The method used for hemagglutinating activity was according to Evans [30] A total of 25 µl of *E. coli* 0157:H7 solution at 2% were mixed with 25 µl of erythrocytes at 2%, and added with PBS, in order to complete a volume of 100 µl in plates of 96 cells. Then the plates were incubated at room temperature, the bacterial agglutination was identified directly by adding to each sample some drops of acridine orange stain to identify the ring of the agglutination without microscopy (corroborated by Nikon stereoscopic microscopy observation). Spectrophotometer values from the Spectra Max at 625 nm were used, the value of OD changes when bacterial agglutination exists.

Hemagglutination activity assay and inhibition by sugars

This assay was according to Evans [30] and the sugar-binding lectin specificity was identified by competition experiments using serial double dilutions with 1 mM of sugar standards.

Hemagglutination activity Assay and inhibition by lectins

A total of 25µl of *E. coli* 0157:H7 solution at 2% was mixed with 25 µl of erythrocytes at 2%, and with 50 µg/ml of protein extract in a final volume of PBS phosphate buffer solution. The inhibition of *E. coli* hemagglutination activity was detected as described above. The inhibition could indicate that lectins from extract compete for the same carbohydrate, which *E. coli* recognized in the red cells attachment.

Specific Activity of plant extracts in agglutination of *E. coli* 0157:H7

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 then diluted in order to obtain $4.8x10^6$ cells/mL. After, were placed 50µl/well and then 50 µl of PE. Double dilution was performed and then was incubated for 30 min at room temperature. The bacterial agglutination was identified as described above.

Activity from plant PE in the adhesion of *E coli* 0157H7 and their toxicity to 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; they were kept in a moist atmosphere with 5% of CO_2 in a BEM Dulbecco medium with 10% of bovine fetal serum (Difco) without antibiotics). Three T75 confluent flasks were tripsinized for sowing about 500,000 cells in cover slips, 3 per Petri dish, for 24 hrs. of incubation in a medium free of antibiotics.

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Subsequently, they were treated with 400µg/mL of proteins from the different extracts in the presence of 480,000 bacteria/mL, incubated for 2 hrs washed 3 times with PBS and fixed. The data reported are from a microscopic analysis of the cover slips, in which cells with adhering and agglutinated bacteria were analysed and the total number of HELA cells per cover slip were counted. Table 6 (A y B).

The same procedure was reported with the HELA cells in Petri dishes fixed washings with PBS and after that, they were incubated with 400µg/mL of proteins from the different extracts in the presence of 480,000 bacteria/mL, incubated for 2 hrs and washings with PBS. Subsequently nutritive agar was added to the cells in Petri dishes and they were incubated for 24 hrs, after which the units forming colonies (UFC) were counted.

Results

Characterization and identification of lectins in plants PE

The content of proteins vs carbohydrates are resumed in the Table 1 and shown low quantities of carbohydrate vs proteins in all PE. The results showed lectins activity in most of the PE measured by HA assay in blood type A, B or O, excluding chamomille PE (Figure 1). PE from tropical bushmint has the higher HA capacity in blood type B with 204, 800 units and 800 units in blood type O vs arnica with 4697 units of HA in blood type A (Figure 1).

Characterization of sugar-binding lectins

The tropical bushmint PE showed the higher specificity by Gal recognition in the blood type B. However, the other PE shows more than one titer, which suggests a different type of lectin or a monomeric form with different specificity (Table 1). Our results shown that all sugars inhibited at 15 µM HA caused by PE, (see Table 2).

In contrast, sugars displaying higher HA inhibition activity were Gal and derivatives GalNAc and GalN in 10 of the 11 PE in blood type A and Neu5Ac for blood type B and O in 5 of 12 PEs; Man, in 6 of 12 PE in blood type B; xylose, only inhibited in A and O blood type in 5 of 12. In unwashed erythrocytes GlcNAc and Neu5Ac induce hemolysis.

Molecular weight characterization of the PE

The majority of PE displays common bands, between 20-14 to 58 kDa (Table 3). Some of them presented bands with high molecular weight above 100 kDa, which may correspond to super-molecular aggregates. Few proteins migrated a similar molecular weight that the protein albumin and around of 30 kDa.

Characterization of *E. coli* 0157:H7 hemagglutination

According to Table 4, we found almost an equal affinity between *E. coli* 0157H7 and all blood types A (titer 1:32 to 1:512), B (titer 1:1 to 1:256) and O (titer 1:1 to 1:256), these data indicate that the higher affinity was with blood type A. In addition to this, *E. coli* 0157H7 HA competition assay with sugar standards demonstrated the participation with high affinity in the tree groups of Man, Glc, Fuc and Xyl in the membrane recognition between bacteria and red cells and with less affinity in the group A Gal and derivates, in the group O Neu5A and in the group B Glc and derivates.

Bacterial hemagglutination is mediated by fimbrial type 1, although in *E. coli* O157H7 has not been demonstrated and literature data are controversial. However, our results suggest that Man Glc, Fuc and also Xyl could be involved in bacteria adherence to red cells, as well as other fimbriae, such as P frimbriae which recognized Gal and GalNAc.

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Inhibition of *E. coli* 0157H7 hemagglutination by lectin from PE

All PE inhibit *E. coli* 0157H7 hemagglutination, but only in blood type B displayed effective inhibition because also presented high HA specific activity. The most important PE for inhibited the HA of *E. coli*, were from *labiatae* family; mainly, those that showed the highest HA activity in blood type B, such as tropical bush mint, dayflower, sage and mint (Table 5). Also we report that greater part of PE showed the same specific activity in the inhibition of *E. coli* hemagglutination in all blood types (A, B and O) which suggest a bacterial recognition probably to P fimbrae.

Effect of lectins in *E.coli* 0157H7 agglutination

Our results shown that most of PE that inhibited *E. coli* hemagglutination have a high activity in *E. coli* 0157H7 agglutination (Table 5); this is confirmed by lectin activities and similar sugar recognitions (Table 2, 4). Such as the case of PE from tropical bush mint, horehound; tropical bush mint, and dayflower which present a specific activity above of 10000 units of *E. coli* agglutination, in comparison with PE from sage which present high inhibition of *E. coli* 0157H7 hemagglutination but low activity in *E. coli* 0157H7 agglutination (Table 5).

The PE from rue causes a poor agglutination (14 units of *E. coli* 0157H7 agglutination), however our outcomes evidenced high cytotoxic effect and crystal formation determined by microscopy observation with both sage and rue PE. It is necessary to consider that the greater part of the PE showed high HA in blood type B, which means Gal and GalNAc recognition also and hemolytic protection effect against Neu5Ac and GlcNAc sugars in unwashed blood samples.

Activity from fruits PE in the adhesion of *E. coli* 0157H7 bacteria and their toxicity to HELA cells

In about 100 HELA cells analysed most PE after 2 hrs in the presence of the bacteria were highly toxic (100-70%), such as rue, chamomile, dayflower, fennel, sage, melisa arnica, and in the case of mint, guava, wormseed, tropical bushmint and horehound were less toxic around 30% similar to the toxicity to the cells of the bacteria per se (Table 6). The number of bacteria on the cells that were identified, with arnica, chamomile fennel and wormseed was around 20-30 bacteria/per cell, similar to the control, in the others PE were around 5-15 bacteria /per cell and in the case of sage, rue and horehound, bacteria were not identified because only crystals were find.

Discussion

As expected, we detected lectin activity in all PE and suggest that at least two or more types of lectins recognized glycoside determinants in the membranes of the erythrocytes such as Gal, Gal NAc, Man, Gluc, GlucAc, and Neu5Ac. Also, all PE showed a better recognition of blood type B, suggesting the presence of lectins, mainly related to Gal or it is derivates as GalNAc, GalN may be a Gal/GalNAc type lectin; even those PE from different families.

The PE with high specificity were Tropical bush mint, dary flower, sage, wormseed, arnica, mint, and chamomile, which inhibited *E. coli* 0157H7 hemagglutination activity in all blood types A, B and O, as well as *E. coli* 0157H7 agglutination. Those data suggest that recognition may occurs by some antigenic determinant in the microorganism or common sugars in erythrocytes, such as Neu5Ac, Gal present in TN, M antigenic in the case of erythrocytes, [31] or Gal/GalNAc and Neu5Ac antigens from bacteria [30,31].

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According to the results from the agglutination of *E. coli* 0157H7, lectins from PE shown more specificity to some bacterial antigen determinants such as fimbrae type1 (Man), S (Neu5Ac) or P, (Gal/GalnAc) because they are involved in adhesión [22,32,33]. When we explored the sugar recognition specificity of lectins from bacteria, erythrocyte or PE, we found that is required different quantities of sugars for HA and inhibition of *E. coli* 0157H7 hemagglutination.

The quantity of each sugar varies significantly depending on blood type and PE. We propose these results towards two mechanisms of adhesion: the first one, those responsible for carbohydrate domain recognition (CDR) in the lectins and the glycosidic signal, which means, a protein-sugar interaction that could be involved in trigger some catalytic activity that can remove the glycosidic signal and be more specific; second one, those that are not well known or established, for example, adhesion interaction between glycosidic chains in both erythrocytes and lectins, either from the microorganism or PE.

This sugar-sugar interaction could be done by hydrogen bridge between the hydroxyls (OH), that requires greater sugars quantities to break this molecular binding as compared to low amount needed in the CDR protein-sugar interaction. Also, the effects of some secondary metabolites such as phenolic derivates (flavonoids and lignans) that contain large number of OH, could compete with the sugars involved in adherence between bacteria and the host tissue and act as natural antiadhesive [33].

Although sugar-sugar interaction could be product of unspecific binding and may require some kind of orientation about OH^- atoms of sugars involved, may be why high molar concentrations of sugars such as Glc, Xyl Man and Fuc showed an inhibition of hemagglutination *E. coli* 0157H7 in blood groups 3.

This may apply mannose and glucose sugars strongly were recognized in most PE in blood type B and involved in lectin activity, HA, hemagglutination inhibition and agglutination of *E. coli* 0157H7, give us a better understanding of the results. The mechanism of adhesion used by bacteria could be explained by the high specificity of PE to inhibit *E. coli* 0157H7 hemagglutination in all blood types and by *E. coli* 0157H7 agglutination. The hemagglutination specificity for blood type B, the similar inhibition of *E. coli* 0157H7 HA in blood types A, B or O and also the agglutination of *E. coli* 0157H7 by the PE, suggest basically a recognition to a determinant antigenic from the microorganism, such as lipopolyssacharide (LPS), O antigen or, fimbrial type 1 or P or S [32,20,33], and also to some common sugar from the erythrocytes such as Neu5Ac; Gal present in TN and M antigenic in erythrocytes[32].

However, the effect of the PE in the E coli activities, principally P Fimbrae type P are involved, because plants PE´s that inhibited the EcAg and the EcHA recognized Gal / GalNAc.
In this work we use the PE from rue as negative control, because it is particularly used as abortive, but few reports revealed their gastrointestinal activity, however we show that rue and sage PE were highly cytotoxic to bacteria and HELA cells, this may explain the low *E. coli* 0157H7 agglutination observed in those PE, basically it was watching crystals formation.

The cytotoxic effect may involve amine sugars recognition by rue and sage PE, such as GlcN and GalN, besides Neu5Ac lectins, present in bacterial fimbriae associated to porins [3,10] or through RIP type lectins with highly toxic effect, both with 30 kDa band [27, 26, 34,35]. Concerning to HA activity from PE of plants, most of them have lectin which recognized with the same specificity glycosides determinant from group A, B and O, which also involved in their activity in the inhibition of the *E. coli* O157H7 HA.

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On the contrary PE from tropical bushmint, dayflower showed highly *E. coli* O157H7 agglutination, and fennel and horehound showed only be more specific to it that means that have a lectins that recognized better the *E. coli* O157H7, and respect the sugar involved in those activities, probably are acylated derivates sugars (GalNAc, Neu5Ac, GlcNAc), also for the first time that Xyl is founded involve in those activities. About protein band pattern, most of the PE showed a common bands, all PE showed a 14 kDa band that may be Monocot lectin and 17-18 kDa band possibly a (Chi) type lectin [36,37].

Rue and dayflower showed different bands, around 30 kDa and PE from sage, melissa, arnica, mint and wormseed displayed bands of 30-40 kDa, these may be a RIP type lectin, or some lectin derivates without cytotoxic effect, such as B-rycin reported for *Sambucus nigra*[34].

We found proteins similar to albumin migration in PE from melissa, guava and sage also with high molecular weight over 100 to 257 kDa, probably molecular aggregates a consequence of the extraction conditions mainly to low salt. Our results suggest in the majority of the PE at least two or three types of lectins with bands pattern around 14, 18, 20 and 28 kDa; a molecular weight that may belongs to Monocot (Man), Chi (GlcNAc), B-ricin or RIP (GalNAc/Neu5Ac) lectins [34-37].

Which are implicated in some of the activities studied in *E. coli* O157H7 enterohemorrhagic such as hemagglutination, agglutination and toxicity, probably RIP, CHI and Gal/GalNAc type lectins are the implicated by it is toxic effect en HELA cells that could be necrotic apoptotic effect and also lectins that recognized GalN and GlucN in the toxicity to bacteria, and these toxic effect do not allowed determinates cells adhesion.

Ribosome inactivating proteins lectins recognized GalNAc/Neu5Ac, are involved in apoptosis as most PE from plants has a cytotoxic effect in HELA cells. and also recognized Gal/GalNAc, probably are RIP lectin and the cytotoxic effect basically depend in a Gal recognizment through the apoptotic death FAS/CD95 receptor, those death receptor galectin depend on calcium and is associated with the independent caspase-3 of mitochondria inducing apoptosis in lymphocyte also this second apoptotic pathway (extrinsic pathway) triggered necrosis after the binding to death receptors, may induces activation of proteolytic enzymes such as, caspases; that in turn activate endonucleases, all mediated by bcl-2 proteins, which regulate mitochondrial permeabilization processes and induce apoptosis or necrosis because granzymes proteases that induced the pore formation and lysis are also activated through death FAS/FasL receptor (38). The pore formation may be also activated by the GlcNAc recognition by Chi type lectins because chitin recognition also is involved in apoptosis (38,39,40).

The high cytotoxic effect of plants PE to HELA cells and to *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 be Chi, Gal/GalNAc or RIP type lectin (27,34,38,39,40). However, it most possible to be dealing with leguminosea type lectins too, because they recognize several complex sugars and also Xyl lectin, as xylose was found to participate in HA and in the EcHA and probably in *E. coli* agglutination and toxicity to HELA cells, this suggests proteoglycan participation in adhesive and toxicity mechanisms (41).

The use of teas or tizanas from plants can give protection against some pathogenic factors of enterohemorrhagic *E. coli* O157H7 [25] and can prevent diseases, and others, such as viral infections.

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Previous reports has shown that Man, GlcNAc lectins are selective and powerful inhibitors of human deficiency virus and cytomegalovirus replication *in vitro*[42,43]. In addition, several enterobacteria such as *Vibrio cholerae, Shigella and Salmonella* share antigenic determinants and pathogenic factors with *E. coli*.[18,22, 44]. The use of PE above mentioned may counteract the effects of those bacteria as well as the *E. coli* 0157H7. In Mexico the common use of alternative medicinal plants could be a reason why in México, *E. coli* O157H7 is not a public health problem as it is in the USA or some Latin-American countries such Argentine and Chile that consume uncooked swine meat and cattle which are also *E. coli* O157H7 reservoir [6].

Our and other studies support the use of lectins as active compounds to control bacterial infections and there are useful markers in order to study cellular growth, also can be used in malignancy diagnostic, in prognostic and tumor treatment because type RIP I and II lectins which have apoptotic or necrotic death[26,27,28, 34,38,39,45].

However, we must be discrete using plants and other natural sources for treatments, because in the case of lectins they recognize specifically red cell groups and agglutinate them or even caused hemolysis of red cell. This may cause not only an immunological response, but also, a renal disease. We consider that each treatment or preventive care must be customized considering lectins capacity to recognize some sugars present in specific blood type but also specific for sex, because in preliminary studies in PE from mango (*Mangifera indica*) (Maria Carmen Arenas 2005)[46], and other PE from *Eichornia crassipens and Lemna minor* (no published Data) was detected lectins from those that are specific to sex recognition for group A and O.

Finally, in the almost 100 years of lectins studies our studies summarized to it was shown, that they play an important role as recognition molecules in cell-molecule, and cell-cell interactions in a variety of biological systems (47) even it is importance in molecule-molecule interaction which are usefully to understand cell regulation process such as necrotic and apoptotic by the FAS receptor and lectins type RIP and CHI [38,40,46].

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 traditional Ethnomedicine knowldege[29], some of the bioactives found in these fruits, plants and marine organism are secondary metabolites such as alkaloids, flavonoids, phenolic compounds , 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.

This Ethnomedicine knowledge is very important to maintain, for treatments and curing of current ailments, especially for the extensive and abusive use of antibiotics and synthetic chemicals that has brought an increase of bacteria resistant to drugs, and an increase in neoplastic processes (28, 48,49,50).

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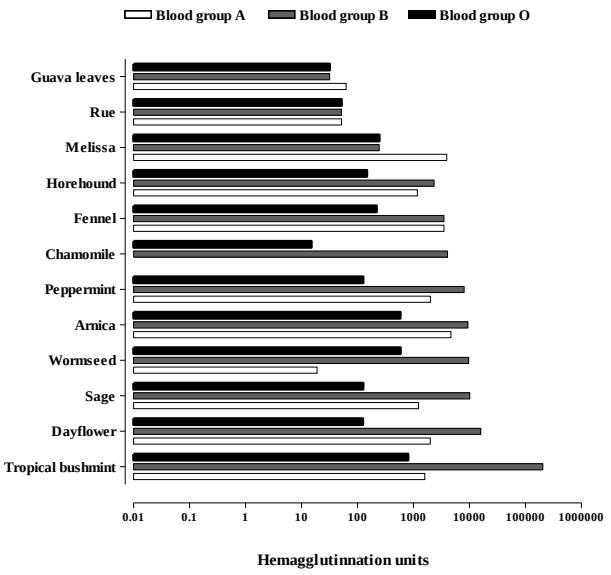

Figure 1 Characterization of hemagglutination activity from PE in each blood type. Hemagglutination activity (HA) of PE was carried out as described in material and methods in different male blood type A, B or O. The HA is reported as HA units (titer x dilution factor)/protein concentration. The agglutination of erythrocytes, based on a control, was taken as a positive control

Annexes

Scientific name (Common name)	Family	Uses	Protein (mg/mL)	Carbohydrate (µg/mL)
Hyptis mutabilis (Tropical bush mint)	*Lamiaceae or Labiataceae*	I	0.50	0.06
Zebrina pendula (dayflower)	*Commelinaceae*	I, II	0.32	0.12
Salvia officinalis (sage)	*Lamiaceae*	II, III	0.505	0.17
Chenopodium ambrosoides (wormseed)	*Chenopodiaceae*	III	2.11	0.15
Heteroteca indulis (arnica)	*Compositae or Asteraceae*	III	2.18	0.355
Mentha piperita (mint)	*Lamiaceae or Labiataceae*	I, II, III, IV	0.635	0.125
Matricaria chamomilla (chamomilla)	*Compositae or Asteraceae*	II, III, IV	2.52	0.085
Melissa officinalis (melissa)	*Lamiaceae or Labiataceae*	III	0.65	0.198
Foeniculum vulgare (fennel)	*Umbelliferae*	III	1.46	0.365
Marrubium vulgare (horehound)	*Lamiaceae or Labiataceae*	II, IV	2.18	0.54
Psidium guajava (guava)	*Myrtaceae*	I, II,III	1.26	0.42
Ruta xalapensis (rue)	*Rutaceae*	II,III, IV	0.775	0.12

Table 1 Plants classifications and concentration of proteins and carbohydrates. Classification of plants was according to the ethnobotanical studies and by surveys by IMSS COPLAMAR, Javier Lozoya, 1987. I, antidiarrheal; II, antidysenteric; III, antiparasitic; IV, antiamoebic. Protein and carbohydrates quantification as described in material and methods

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Common name	Protein titer (mg/mL)	Blood type	Sugars type concentration
Tropical bushmint	0.005	A B O	Xyl, GalNAc (15µM) Man, Glc, GalNAc, Fuc (15µM) Gal, GlcNac (15µM)
Sage	0 .05	A B O	Gal, GlcNAc, GalNAc, GalN (31µM) Man, Glc, GalNAc (15µM), Neu5Ac (15µM) Xyl, (31µM)
Wormseed	0.13	A B O	Glc ,Gal (15 µM), GlcNAc (31µM) Glc, Fuc, Gal, (15µM) Glc, Man (15µM) GlNAc and Xyl (31µM)
Arnica	0.017	A B O	Xyl, GalNAc (62µM) GalNAc, Neu5Ac (62µM) Gal (125µM)
Mint	0.079	A B O	GalNAc, GlcN, Neu5Ac, Man and GalN (15µM) Glc, Man GlcN, Fuc (15µM) Fuc, GlcNAc, GalNAc Neu5Ac and Xyl (15µM)
Chamomile	0.078	A B O	ND Fuc, Neu5Ac (15µM) Fuc (15µM)
Fennel	0.36	A B O	Man, Glc,GlcN, Xyl (15µM) Man, Glc, GalNAc, Fuc (15µM) Man GalNAc (15µM)
Dayflower	0.01	A B O	Man, GlcNAc, GalNAc, GalN (31µM) Neu5Ac (31µM) Neu5Ac (31µM)
Horehound	0.55	A B O	Man, Gal,GlcN,GlcNac,GalNAc, Neu5Ac and Xyl (15µM) Gal, GlcNAc (15µM) Man, GlcNAc, GalNAc, Neu5Ac and Xyl (15µM)
Melissa	0.65	A B O	Gal, GalN, Neu5Ac (15µM) Man, GlcNAc GalNAc, Glc, GalN (15µM) Man and Fuc (15µM)
Guava leaves	1	A B O	Glc (15µM) Neu5Ac, Man, Glc, and Gal (15µM) Man, Gal, Xyl, Fuc, GalN and Neu5Ac (15µM)
Rue	0.775	A B O	GlcN (31µM) Man.Glc, GalNAc, GalN, GlN , Neu5Ac (15µM) Neu5Ac (15µM)

Table 2 Common Sugars that inhibiting the HA caused by PE in each blood group. The lectins specificity was determined by HA competition with sugar standards at 0.015 at 1mM and with double serial dilutions using titer with lower protein concentrations from each PE that induces HA (see material and methods)

EXTRACT	Relative molecular weight (kD)								
Horehound	257						25	18	17
Sage		160	98	71	47	30, 34, 37	20	18,17	14
Melissa		164	89	69		35	20, 23	18, 16	14
Tropical bushmint	257						20	18,17	15
Mint	238		112		58	33	20, 28	18	
Arnica	245	167	112	88	53	32, 34, 41	20	18,	16
Chamomile	250						20	18,17	15
Wormseed	246				56	39, 43	21, 20	17	14
Fennel						46	20	18	17
Rue						30	22,20,	19	
Dayflower	264				58		21	19, 18	14
Guava leaves	264			60			21	19	17
Weight marker (GIBCO)	194	120	87	64	52	39	26	21	15

Table 3 Protein electrophoretic mobility from PE. Proteins from PE were resolved in 10% SDS-PAGE as described in material and methods. The results were obtained with autoanalyzer and Digital MSD 40 Kodak Program using standard proteins pre-stained weight marker(GIBCO)

Sugars	Type A	Type B	Type O
Neu5Ac	125 µM	1 mM	15 µM,
GlcNAc	15 µM, 250 µM	15µM	62 µM,10mM
GalNAc	125 µM, 10mM	31µM	250 µM, 1mM
Gal	250 µM, 15 µM,	250	62 µM, 250 µM
Gal N	15 µM, 125 µM	µM,10mM	62 µM,
Glc	250 µM	250 µM,	15µM, 250 µM,
GlcN	250 µM , 1mM	15 µM,	,
Xyl	1mM, 10 mM	1mM, 10mM	62 µM, 125 µM,
Fuc	1mM, 10 mM	1mM	10 mM
Glc	1mM	1mM	1mM, 10 mM
Man	1mM, 10mM	1mM	1mM
		1mM	1mM, 10 mM
			ND

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

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Common name	HA group B (HA units)	*E. coli* 0157:H7 HA inhibition (specific activity)	*E. coli* 0157:H7 agglutination (specific activity)
Tropical bushmint	***204800***	**102400** A,B,O	**26841**
Dayflower	**16000**	**16000** A,B,O	**14755**
Sage	**10139**	**10137** A,B,O	265
Mint	**8063**	**8063** A,B,O	**7367**
Melisa	246	7877 A,B,O	2897
Rue	52	6006 A,B	14
Guava leaves	32	4063 A,B,O	667
Fennel	3507	3505 A,O	**6918**
Wormseed	**9706**	2426 A,B,O	129
Horehound	2347	2349 A,B,O	**19033**
Chamomile	4063	2031 B	603
Arnica	9706	1174 A	1280

Table 5 Effect of PE in the HA of blood group B, in E. coli HA Inhibition and E. coli agglutination. Hemagglutinating units was calculated as (titer x dilution factor)/protein concentration. The Specific activity of E.coli 0157:H7 HA inhibition was (titer) minimum for perceiving E.coli 0157:H7 HA x dilution factor (g of protein from PE). Specific activity of PE of E.coli 0157:H7 agglutination was calculates as E.coli 0157:H7 agglutination x dilution factor (40)/(g of protein from PE)

Samples extracts	Cytotoxic Effect (A)	Number of bacteria present after 2 hours(B)	Number of bacteria present after 24 hours(C)
Rue	100	0 crystals	0
Chamomile	90	25	5 small colonies
Dayflower	83	5	Dot colonies
Fennel	88	20	Dot colonies
Sage	80	0 crystals	Dot colonies
Melisa	80	5	Dot colonies
Arnica	70	30	0
Wormseed	35	20	Dot colonies
Tropical bush mint	30	15	0
Mint	20	5	1 big and dot colonies
Guava	15	10	Dot colonies
Horehound	5	O crystals	0
Cells with bacteria	25 and 1% anuclei cells	30	5 regular colonies
cells	10	0	0

Table 6 Effect of the PC on adhesion of HELA cells in E.coli 0157:H7 presence. A; % of toxicity of HELA cells in the presence of bacteria and PE for 2 hrs. B; % of bacterias showing adhering or internalized after 2 hrs in the presence of PE. C;% of bacteria colonies after 2 hrs in the presence of PE in HELA and two washings with PBS, nutritive agar was added to the cells and they were incubated for 24 hrs, after which the units forming colonies (UFC) were counted.

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