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Hemolytic and Antimicrobial Activities Differ Among Saponin-rich Extracts From Guar, Quillaja, Yucca, and Soybean

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Abstract Hemolytic and antibacterial activities of eight serial concentrations ranged from 5-666 μg/mL of saponin-rich extracts from guar meal (GM), quillaja, yucca, and soybean were tested in 96-well plates and read by enzyme-linked immunosorbent assay plate-well as 650 nm. Hemolytic assay used a 1% suspension of chicken red blood cells with water and phosphate buffered saline as positive and negative controls, respectively. Antibacterial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli* were evaluated using ampicillin and bacteria without saponin-rich extract as positive and negative controls, respectively. The 100% MeOH GM and commercial quillaja saponin-rich extracts were significantly the highest in both hemolytic and antibacterial activities against all bacteria at the same concentration tested. Soybean saponin-rich extract had no antibacterial activity against any of the bacteria at the concentrations tested while yucca saponin-rich extract had no antibacterial activity against the gram-negative bacteria at the concentrations tested. GM and quillaja saponin-rich extracts were hemolytic, while yucca and soybean saponin-rich extracts were not hemolytic at the concentrations tested. No saponin-rich extract source had antibacterial activity against *S. typhimurium* or *E. coli* at the concentrations tested. Both GM and quillaja saponin-rich extracts exhibited antibacterial activity against *S. aureus*. Saponin-rich extracts from different plant sources have different hemolytic and antibacterial activities.

Keywords Bacteria · Guar meal · Hemolytic · Quillaja · Saponin · Soybean · Yucca

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Introduction

Development of antimicrobial compounds remains an interesting and important area of research due to recent bans of a wide range of prophylactic antibiotics used in animal feed [1]. Concerns about bacterial resistance to antibiotics and subsequent restricted use of antibiotics for animal production applications have made development of the compounds a necessity. In the last decade, several antimicrobial-like compounds have been suggested as antibiotic alternatives for prevention and treatment of pathogenic diseases. These alternatives include dietary use of probiotics, prebiotics [2], and organic acids [3]. Non-traditional treatments such as chlorate [4] and bacteriophages [5] also have been recently evaluated.

Among these original candidates, chemical structure compounds are originating from plants such as quillaja, yucca, and soybean. So, these plants produce saponins which have several beneficial effects on animal health [6, 7]. Guar, *Cyamopsis tetragonoloba* L. (syn. *Cyamopsis psoraloides*) or cluster bean is a drought-tolerant annual legume grown primarily for guar gum (galactomannan polysaccharide) that has many industrial and food processing applications. Guar meal (GM) is a combination of hull and germ fractions that is produced as a by-product of guar gum manufacture. Guar is also reported to contain 13% dry matter (DM) curd triterpenoid saponin [8] and, thus, contains potentially useful bioactive compounds.

Saponins are glycoside compounds composed of a fat-soluble nucleus (aglycone) that is either a triterpenoid (C-30) as in soybean, alfalfa, quillaja, and guar or alkaloid steroid (C-27) as in yucca, tomato, and oats. One or more side-chains of water-soluble sugars (glycone) are attached through ester linkages to the aglycone nucleus at different carbon sites [9]. Saponins have several biological effects, among them antibacterial [6] and hemolytic [10] activities. Not all saponins have the same biological activities. While some saponins are beneficial, others are considered harmful to animal performance.

No research has evaluated the hemolytic and antibacterial activities of saponin-rich extract of GM nor compared its biological activities with different saponin-rich extract sources in a dose-dependent manner. Therefore, this study was conducted to isolate saponin-rich guar meal extracts and to evaluate these extracts for hemolytic and antibacterial activities. A minimal inhibitory concentration (MIC) assay compared antibacterial activity of saponin-rich GM extract with three common commercial saponin-rich extract sources (quillaja, yucca, and soybean) in a dose-dependent manner.

Materials and Methods

Commercial preparations of saponin-rich yucca extract containing 30% steroid saponin (Ultra Bio-Logic Inc. Rigadu, Quebec, Canada), saponin-rich quillaja extract containing 8.0–10% triterpenoid saponin (Sigma®, Saint Louis, MO, USA), saponin-rich soybean extract containing 95% pure triterpenoid saponin (Organic Technologies, Coshocton, OH, USA) were purchased, and 100% MeOH saponin-rich extract were prepared as described by Hassan et al. [11].

Hemolytic Activity Assay

Hemolytic activity assays were performed as previously described by Hassan et al. [11] with some modifications. A cell suspension of 1% chicken red blood cells in phosphate

buffered saline was used and each treatment group resulted from 12 individual values (three replicates of two plates/replicate with two columns/plates).

Antibacterial Activity Assay

The MIC assays were used as a measure of antibacterial activity of saponin-rich extract sources as previously described by Hassan et al. [11] with some modifications. A single gram-positive bacterium (*Staphylococcus aureus* ATCC 49525) and two gram-negative bacteria (*Salmonella typhimurium*, NCIM 2719; *Escherichia coli*, ATCC 933) were used. A stock solution of each saponin-rich extract was prepared by dissolving 25 mg of saponin-rich extract/mL of tryptic soy broth and each treatment group resulted from 18 individual values (three replicates of three plates/replicate with two columns/plates).

Statistical Analysis

Each treatment group resulted from 12 individual values (n=3, three replicates of two plates/replicate with two wells/plates) for hemolytic activity and from 18 individual values (n=3, three replicates of three plates/replicate with two wells/plates) for the antibacterial activity assay. The mean of a saponin-rich extract at each concentration was compared to the negative control group within a row to establish significant differences among treatment groups in hemolytic and antibacterial activities.

The MIC for each treatment was recorded at the lowest concentration which inhibited growth compared to the negative control group. Data obtained were subjected to one-way analysis of variance and were expressed as mean ± standard error of mean (SEM) using the general linear model program (SPSS 14.0, SPSS Inc., Chicago, IL, USA). Treatment means were separated ($P \leq 0.05$) using Duncan's multiple range test [12].

Results and Discussion

The 100% MeOH saponin-rich extract yielded approximately 1.55±0.15% DM of the original weight of GM extracted. In this study, the hemolytic and antibacterial activities of the 100% MeOH saponin-rich extract were compared with three other common commercial saponin-rich extract sources (quillaja, yucca, and soybean) in a dose-dependent manner. Oleszek [13] noted that saponin-rich extract purified by graded MeOH concentrations showed that eluants up to 40% MeOH removed carbohydrates and some phenolics, eluants containing 50–60% MeOH removed only the bisdesmoside saponins, while 70–80% MeOH removed monondesmoside saponins. This phenomena may contribute to the hemolytic and antibacterial properties of the presumed saponin structures isolated by the 100% MeOH saponin-rich extract rather than 20% and 60% MeOH saponin-rich extracts reported by Hassan et al. [11].

This study agrees with Choi et al. [14] who reported that not all saponins have hemolytic activity. The 100% MeOH saponin-rich GM extract and commercial saponin-rich quillaja extract exhibited significantly higher hemolytic activity than both commercial yucca and soybean saponin-rich extracts at the same concentrations tested (Table 1). Commercial saponin-rich quillaja extract was 100% hemolytic with substantial hemolysis occurring even at the lowest dose (0.01 mg/mL). The 100% MeOH saponin-rich GM extract exhibited similar hemolytic activity until concentrations were ≤0.031 mg/mL. Yucca and soybean saponin-rich extracts showed hemolysis only at the highest concentration (1.0 mg/mL).

Table 1 Hemolytic activities of 100% MeOH saponin-rich GM extract separated by reverse phase-high performance liquid chromatography (RP-HPLC) and solutions prepared from three commercial saponin-rich extracts of quillaja, yucca, and soybean.

Saponin extract	Positive control[a]	Negative control[b]	Saponin-rich extract source			
			GM	Quillaja	Yucca	Soybean
mg/mL			Optical density (OD[c])			
1.000	0.42±0.01[c]	1.05±0.02[a]	0.52±0.02[c]	0.38±0.04[c]	0.65±0.07[b]	0.65±0.02[b]
0.500	0.42±0.01[b]	1.05±0.02[a]	0.48±0.01[b]	0.39±0.05[b]	1.00±0.02[a]	1.00±0.01[a]
0.250	0.42±0.01[b]	1.05±0.02[a]	0.48±0.01[b]	0.39±0.06[b]	1.00±0.02[a]	1.00±0.03[a]
0.125	0.42±0.01[b]	1.05±0.02[a]	0.47±0.01[b]	0.41±0.06[b]	1.02±0.03[a]	1.00±0.03[a]
0.062	0.42±0.01[b]	1.05±0.02[a]	0.47±0.01[b]	0.41±0.06[b]	1.03±0.03[a]	1.02±0.03[a]
0.031	0.42±0.01[b]	1.05±0.02[a]	0.54±0.01[b]	0.41±0.07[b]	1.03±0.03[a]	1.03±0.03[a]
0.016	0.42±0.01[b]	1.05±0.02[a]	1.01±0.01[a]	0.46±0.07[b]	1.04±0.03[a]	1.03±0.04[a]
0.008	0.42±0.01[c]	1.05±0.02[a]	1.01±0.02[a]	0.68±0.07[b]	1.04±0.04[a]	1.04±0.03[a]

[a–c] Means (± SEM, $n=3$) within a row that do not share a common superscript are significantly different ($P \leq 0.05$).

[a] Water replaced saponin solution to serve as a positive control since this treatment results in 100% hemolysis.

[b] Phosphate buffered saline replaced saponin solution to serve as a negative control since this treatment results in 0% hemolysis.

[c] Lower values are associated with increased cell lysis.

Hemolytic activity has been used by researchers to follow the isolation of saponins. It is the simplest and the fastest bioassay employed to detect and quantify some saponins in plant material [10, 14, 15].

Our results were in disagreement with Birk et al. [16] who reported hemolytic activity of soybean saponins. Takechi and Tanaka [17] noted hemolytic rates of the steroid saponins were greater than the hemolytic rates of the triterpenoid saponins, and Santos et al. [18] reported that steroid saponins had higher hemolytic activity than triterpenoid saponins. On the other hand, our results agree with Jenkins and Atwal [19] who reported hemolytic activity of quillaja, and Gestetner et al. [20] who reported that soybean saponins, which are a mixture of soyasapogenol glycosides, were not hemolytic.

Some research reported that hemolytic activity of saponins is attributed to the affinity of their aglycone moiety to sterols within membranes, particularly cholesterol [21]. Other research reported that hemolytic activity is decreased by removing the glycone moiety of the saponin [18], which is inconsistent with reports that the higher the hemolytic activity, the higher the ratio of aglycone to sugar [22].

Saponins possessing two or more sugar side-chains show less hemolytic activity than saponins containing one sugar side-chain [23]. The monodesmoside saponins with glucose

attached at C-3 show higher hemolytic activity than monodesmoside saponins with glucuronic acid at the same position [15].

The guar saponins tested in this study were not pure and their molecular structures are unknown at this time. Curl et al. [8] reported that the primary saponin in guar was a bisdesmoside triterpenoid with a molecular weight of 1,452. The stereochemistry of the saponin related to chain composition and length appears to be very important in conferring activity on the saponin molecule [21]. As number of the monosaccharide units attached to the hydroxyl group at C-3 on the aglycone [23] increases up to four to six sugar units, hemolytic activity increases [24]. Also, hemolytic activity of saponins increases with decreasing numbers of polar groups on the aglycone moiety [25].

Gestetner et al. [22] noted that alfalfa saponin, with the highest R_f value on silica gel showed the strongest hemolytic activity. The active groups on the aglycone and acylation of saponins also affect hemolytic activity. Neutral and acidic triterpenoids along with the acyl saponins are less hemolytic than the ester saponins [21]. Laurence et al. [26] noted that ethanol acylated triterpenoid saponins isolated from stem bark of *Harpullia austro-caledonica* showed 100% hemolysis of a 10% suspension of sheep erythrocytes. Deacylated quillaja saponins, which differ only in the absence of one glucose residue, showed decreased hemolytic activity [27].

Some studies report that hemolytic activity is the result of saponin's effects on cell membrane permeability by either forming pores in membranes [14], altering sodium–potassium and calcium–magnesium ATPase activity [14], or insertion of the hydrophobic saponin nucleus into the lipid bilayer [28].

The MIC is the lowest concentration of antibiotic or saponin that can inhibit growth of a microbe. A general summary of MIC values for saponin-rich extract solutions against *S. aureus*, *E. coli*, and *S. typhimurium* (Table 2) are compiled from more detailed data presented in Tables 3, 4, and 5. The 100% MeOH saponin-rich GM and commercial saponin-rich quillaja saponin extract were more effective than commercial saponin-rich yucca and soybean extracts which showed very weak or no antibacterial activity against any bacteria at the concentrations tested (Tables 2, 3, 4, and 5).

The MIC against *S. aureus* were 3.13, 0.10, and 12.5 mg/mL for the 100% MeOH saponin-rich GM extract, commercial saponin-rich quillaja, and yucca extracts, respectively (Tables 2 and 3). The MIC concentrations against *S. typhimurium* were 0.78 and 0.10 mg/mL

Table 2 The MIC[a] of antibacterial activity of 100% MeOH saponin-rich GM extract separated by RP-HPLC and solutions prepared from three commercial saponin-rich extracts of quillaja, yucca, and soybean against *S. aureus*, *S. typhimurium*, and *E. coli*.

Bacterium	Saponin-rich extract source			
	GM MIC (mg/mL)	Quillaja	Yucca	Soybean
S. aureus	3.13	0.10	12.5	ND[2]
S. typhimurium	0.78	0.10	ND	ND
E. coli	1.56	0.10	ND	ND

The means of MIC values of each saponin source against different bacteria show in the same column.

[a] Minimal inhibitory concentration IC, the lowest concentration of antibiotic, or saponin inhibits growth of a microbe.

[b] ND, MIC assay did not show antibacterial activity at the concentrations tested.

Table 3 Antimicrobial activities of 100% MeOH saponin-rich GM extract separated by RP-HPLC and solutions prepared from three commercial saponin-rich extracts of quillaja, yucca, and soybean against against *S. aureus*.

Saponin extract	Positive control[a]	Negative control[b]	Saponin-rich extract source			
			GM	Quillaja	Yucca	Soybean
mg/mL			Optical density (OD[C])			
12.5	0.25±0.08[d]	1.14±0.01[a]	0.51±0.02[c]	0.87±0.05[b]	0.88±0.01[b]	1.05±0.04[a]
6.25	0.51±0.17[e]	1.14±0.01[a,b]	0.68±0.03[d]	0.97±0.04[c]	1.08±0.01[b,c]	1.23±0.02[a]
3.13	0.54±0.18[c]	1.14±0.01[a]	0.98±0.03[b]	0.96±0.05[b]	1.11±0.02[a,b]	1.19±0.02[a]
1.56	0.56±0.19[c]	1.14±0.01[a]	1.12±.03[a]	0.97±0.05[b]	1.15±0.02[a]	1.17±0.02[a]
0.78	0.57±0.19[c]	1.14±0.01[a]	1.15±0.02[a]	0.95±0.05[b]	1.15±0.02[a]	1.15±0.02[a]
0.39	0.56±0.19[c]	1.14±0.01[a]	1.18±0.02[a]	0.93±0.05[b]	1.13±0.02[a]	1.12±0.02[a]
0.20	0.72±0.11[c]	1.14±0.01[a]	1.21±0.02[a]	0.94±0.05[b]	1.14±0.02[a]	1.13±0.02[a]
0.10	0.86±0.07[b]	1.14±0.01[a]	1.13±0.01[a]	0.91±0.05[b]	1.11±0.01[a]	1.08±0.02[a]

[a–e] Means (± SEM, n=3) within a row that do not share a common superscript are significantly different ($P \leq 0.05$).

[a] Contained only ampicillin at 0.01-1.0 mg/mL.

[b] Contained neither ampicillin nor saponin.

[c] Lower values of optical density were indicative of decreased bacterial growth and increased antibacterial activity.

Table 4 Antimicrobial activities of 100% MeOH saponin-rich GM extract separated by RP-HPLC and solutions prepared from three commercial saponin-rich extracts of quillaja, yucca, and soybean against against *S. typhimurium*.

Saponin extract	Positive control[a]	Negative control[b]	Saponin-rich extract source			
			GM	Quillaja	Yucca	Soybean
mg/mL			Optical density (OD[C])			
12.5	0.05±0.01[d]	1.22±0.02[a]	0.54±0.02[c]	0.93±0.01[b]	0.92±0.01[b]	1.15±0.02[a]
6.25	0.05±0.01[d]	1.22±0.02[a]	0.68±0.01[c]	1.07±0.02[b]	1.10±0.03[b]	1.28±0.01[a]
3.13	0.05±0.01[d]	1.22±0.02[a]	0.98±0.03[c]	1.00±0.01[c]	1.10±0.01[b]	1.23±0.01[a]
1.56	0.06±0.01[d]	1.22±0.02[a]	1.05±0.01[c]	0.99±0.02[c]	1.15±0.01[b]	1.21±0.01[a,b]
0.78	0.06±0.01[e]	1.22±0.02[a]	1.09±0.01[c]	1.00±0.02[d]	1.15±0.01[b]	1.18±0.01[a,b]
0.39	0.15±0.05[c]	1.22±0.02[a]	1.10±0.01[a]	0.98±0.02[b]	1.15±0.01[a]	1.16±0.01[a]
0.20	0.44±0.07[c]	1.22±0.02[a]	1.10±0.01[a,b]	0.97±0.01[b]	1.14±0.01[a,b]	1.14±0.01[a,b]
0.10	0.80±0.03[c]	1.22±0.02[a]	1.14±0.02[a]	0.94±0.03[b]	1.21±0.02[a]	1.18±0.02[a]

[a–e] Means (± SEM, n=3) within a row that do not share a common superscript are significantly different ($P \leq 0.05$).

[a] Contained only ampicillin at 0.01-1.0 mg/mL.

[b] Contained neither ampicillin nor saponin.

[c] Lower values of optical density were indicative of decreased bacterial growth and increased antibacterial activity.

Table 5 Antimicrobial activities of 100% MeOH saponin-rich GM extract separated by RP-HPLC and solutions prepared from three commercial saponin-rich extracts of quillaja, yucca, and soybean against *E. coli*.

Saponin extract	Positive control[a]	Negative control[b]	Saponin-rich extract source			
			GM	Quillaja	Yucca	Soybean
mg/mL			Optical density (OD[c])			
12.5	0.05±0.01[d]	1.11±0.02[a]	0.43±0.03[c]	0.97±0.04[b]	1.03±0.02[a,b]	1.06±0.02[a,b]
6.25	0.05±0.01[d]	1.11±0.02[a]	0.57±0.02[c]	1.04±0.02[b]	1.14±0.01[a]	1.14±0.01[a]
3.13	0.06±0.01[d]	1.11±0.02[a]	0.82±0.02[c]	0.94±0.01[b]	1.14±0.01[a]	1.13±0.00[a]
1.56	0.16±0.02[c]	1.11±0.02[a]	0.93±0.01[b]	0.92±0.01[b]	1.14±0.01[a]	1.12±0.01[a]
0.78	0.38±0.04[c]	1.11±0.02[a]	1.00±0.01[a,b]	0.89±0.01[b]	1.12±0.01[a]	1.12±0.01[a]
0.39	0.64±0.03[c]	1.11±0.02[a]	1.03±0.01[a]	0.91±0.02[b]	1.09±0.02[a]	1.11±0.01[a]
0.20	0.81±0.01[c]	1.11±0.02[a]	1.06±0.01[a]	0.90±0.02[b]	1.08±0.01[a]	1.11±0.01[a]
0.10	0.80±0.01[b]	1.11±0.02[a]	1.08±0.03[a]	0.88±0.01[b]	1.04±0.01[a]	1.06±0.01[a]

[a–d] Means (± SEM, $n=3$) within a row that do not share a common superscript are significantly different ($P \leq 0.05$).

[a] Contained only ampicillin at 0.01-1.0 mg/mL.

[b] Contained neither ampicillin nor saponin.

[c] Lower values of optical density were indicative of decreased bacterial growth and increased antibacterial activity.

for the 100% MeOH saponin-rich GM extract and commercial saponin-rich quillaja extract, respectively (Tables 2 and 4), and 1.56 and 0.10 mg/mL for the 100% MeOH saponin-rich GM extract and commercial saponin-rich quillaja extract against *E. coli*, respectively (Tables 2 and 5). Commercial saponin-rich soybean extract did not show antibacterial activity against *S. aureus, S. typhimurium*, and *E. coli* while commercial saponin-rich yucca extract did not show antibacterial activity against either *S. typhimurium* or *E. coli* at any concentration tested in this study.

In general, many saponins are considered natural antimicrobial compounds that function as part of plant defense systems [29]. Several studies reported antibacterial activity of dietary saponins in ruminant animals at different levels against different types of bacteria. For example, adding yucca extracts to a ruminant animal's diet negatively affected cellulolytic bacteria without any effect on amylolytic bacteria [30]. Also, saponin-rich yucca extract showed little or no antibacterial activity against gram-positive *S. aureus* and *Lactobacillus* spp., and gram-negative *E. coli* [31]. In another study [32], adding saponin-rich yucca powder or extract to ruminant animals' diets showed more antimicrobial activity against gram-positive bacteria (*S. aureus*) than gram-negative bacteria (*E. coli*).

Some saponins exhibit antimicrobial activity against gram-positive bacteria and no activity against gram-negative bacteria as in the saponin-rich yucca extract tested in this study. The same observation was reported by Tanaka et al. [31] who noted that the saponin fraction isolated from soapnut pericarps (*Sapindus mukurossi*, which grows abundantly in China and Japan) showed moderate antibacterial activity against gram-positive bacteria, while no activity was observed against gram-negative bacteria. Conversely, saponins isolated from orchid tree (*Bauhinia variegata* L.) bark exhibited greater antibacterial activity for gram-negative bacteria than gram-positive bacteria at concentrations ranging from 2.5-10 mg/mL [29]. Kuete et al. [33] reported significant antibacterial effects of

saponin-rich methanolic extracts from the stem bark of African *Tridesmostemon omphalocarpoides* locally known as "Babama".

Sen et al. [6] reported that commercially produced quillaja (*Quillaja saponaria*) and yucca (*Yucca schidigera*) saponins showed different antibacterial activities against *E. coli*, suggesting that saponins from various commercial sources differ in their biological activities. This is most likely due to different extraction procedures which produce saponins with different chemical structures. In this study, commercial saponin-rich quillaja and yucca extracts exhibited antibacterial activity against *S. aureus* and *E. coli* at different concentrations.

Saponin extraction procedures undoubtedly affect the antibacterial activity of the isolated saponins. Rakhimov et al. [34] and Morrissey and Osbourn [29] noted that fat-free content extracts from the Hong Kong orchid tree (*B. variegata* L.) bark were more active than high-fat extracts against gram-positive bacterial strains such as *S. aureus.* Against gram-negative bacterial strains such as *E. coli*, the fat-free extracts exhibited either similar or less activity than the high-fat extracts.

Results obtained in this study showed that the saponin-rich GM extract was more active against gram-positive *S. aureus* than gram-negative *E. coli* and *S. typhimurium.* These findings agree with the results obtained by several studies reporting that saponins showed antibacterial activity against gram-positive bacteria more than gram-negative bacteria [7, 32]. This may be due to the degradation of saponins by glucosidase enzymes produced in higher concentrations by gram-negative bacteria than by gram-positive bacteria.

The MIC values of the 100% MeOH saponin-rich GM extract was exactly the same as reported by Hassan et al. [11] against *S. aureus*, *E. coli*, and *S. typhimurium*. The specific mode of action for the antibacterial activity of saponins against both gram-negative and gram-positive bacteria is not yet clear. One report suggests that the aglycone moiety of the saponin is the antibacterial determinant [7], and suggesting that the sugar moiety is not critical for antimicrobial efficacy, while another study [35] reported that saponins hydrolyzed by bacterial enzymes to its corresponding aglycone resulted in decreased antibacterial activity. As with hemolytic activity, the antibacterial activity of saponins likely is affected by many factors such as the aglycone, number, position, and chemical structure of sugar side-chains [34]. Soybean, GM, and quillaja saponins are triterpenoid, while yucca is a steroid saponin. The predominant guar saponin has two sugar side-chains, one attached at C-3 and another at C-29 [8], while the predominant quillaja saponin [36] has two sugar side-chains at C-3 and C-28. Yucca can be found both as monodesmosides with one sugar side-chain attached at C-3 and bidesmosides with two sugar side-chains attached at C-3 and C-26 positions [9]. Soyasaponin is primarily classified into two main groups (groups A and B). Group A soyasaponins are bidesmosidic with two sugar side-chains attached at C-3 and C-22, while group B soyasaponin is monodesmosidic with one sugar side-chain attached at C-3 (Wei and David) [37]. While saponins may share similar sugar attachment sites, the sugar side-chains themselves vary in type, number, sequence, and chain length. Quillaja saponin contains quillaic acid as the central aglycone attached with glucuronic acid, rhamnose, hexose, and a fatty acyl chain in varying ratios [36], while soyasaponin I has a carboxylic group on the glucuronic acid aglycone [15].

In summary, the 100% MeOH saponin-rich GM extract yielded approximately 1.55±0.15% DM of the original GM weight. Saponin-rich extracts from different plant sources have different antibacterial and hemolytic activities. The 100% MeOH saponin-rich GM extract and commercial saponin-rich quillaja extract were significantly higher in both hemolytic and antibacterial activities against all bacteria tested than commercial saponin-rich yucca and soybean extracts at the same saponin concentration. Saponin-rich soybean extract had no

antibacterial activity against any of the bacteria at the concentrations tested while saponin-rich yucca extract had no antibacterial activity against the gram-negative bacteria at the concentrations tested. Hemolytic activity can be a predictor of antibacterial activity with different levels of some saponin-rich plant extracts such as guar and quillaja saponins while not for other saponins such as soybean and yucca saponins.

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