STABILITY OF SUMAC (*RHUS CORIARIA*) POLYPHENOLIC EXTRACT DURING SIMULATED GASTROINTESTINAL DIGESTION AND COLONIC FERMENTATION

A Dissertation

by

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ABSTRACT

The effects of Sumac (*Rhus coriaria*) polyphenolic extract were evaluated against common pathogenic and probiotic bacteria. The bacteria were individually treated with free sumac (free Su) and encapsulated sumac (NPSu) polyphenols. It was hypothesized that the encapsulated particles would have a higher engagement and interaction potential with the chemical constituents within the bacteria cell wall structure. The factors influencing the disruption of the membrane and eventually death of the bacteria were investigated. To assess the antibacterial properties attributed to sumac polyphenols, three Gram-positive and three Gram-negative bacteria were chosen and treated with free Su and NPSu ethanolic extracts. The antibacterial activity against all tested bacteria was increased as the concentration of polyphenols elevated; however, the samples exhibited different antibacterial properties. While free sumac extract showed a higher bacterial growth inhibition property against Gram-positive bacteria compared to that of Gram-negative bacteria, the encapsulated extract exhibited an opposite trend and behavior. The antibacterial activity in encapsulated samples was also higher against all tested bacteria compared to that of free samples. The stability and release profile of encapsulated samples were evaluated under different pH conditions to simulate the pH in the oral, gastric, and intestinal environments. The stability of encapsulated particles was increased with lowering the pH values. The stability was tested using a membrane dialyzing assay followed by UV-V analysis. pH 2.5 exhibited the highest stability compared to other pH conditions being tested. The samples were also exposed to different gastric juices with similar chemical compositions as the human gastrointestinal tract. In addition to the gastrointestinal chemically simulated conditions, the samples were used as treatments for probiotic

bacteria as a representative of human gut microbiota. The results indicated that the *Lactobacillus plantarum* strain was able to produce low molecular weight tannin metabolites. However, the production of metabolites was higher when the tested bacteria were treated with nanoparticle sumac extracts. Encapsulation of sumac polyphenolic extracts may lower the rate of tannin degradation during simulated gastrointestinal digestion. The Pluronic-based nanoparticles' lower stability to the pH prevailing intestinal compartment may dissociate the chemical binding between galloylated compounds with Pluronic polymers.

DEDICATION

This work is dedicated to my wonderful wife and best friend, Helen, whose unconditional love and support are the basis for my accomplishments.

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Contributors

This work was supervised by a dissertation committee consisting of Professors Stephen Talcott (Advisor), Gary Acuff (Home Department), Mustafa Akbulut (Outside Department), and Susanne Talcott (Home Department).

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CHAPTER I

INTRODUCTION

According to the World Health Organization definition, antibiotics are the low molecular weight chemical compounds that can be used against bacterial infections [1]. The bacterial responses to such compounds may change over time causing antibiotic resistance that can become harder to overcome in future treatment interventions. In contrast to synthetic antimicrobial drugs, cleaners, and therapeutics naturally-occurring antimicrobial agents have a broader application against different microbes, including parasites, viruses, and fungi. Food safety is considered as a major concern for food industries and consumers. Human may consume contaminated food that has been directly or indirectly exposed to animal and/or animal wastes. Some of the known routes for contamination are animal parts after slaughtering, animal excrement, and fruits and vegetables previously contaminated by animal waste [2]. Drug resistance developed by pathogenic microorganisms due to the indiscriminate use and side effects of synthetic drugs has also drawn attention towards plant extracts and biologically active compounds isolated from plant species used in herbal medicine.

Bioactive components of sumac fruit have been investigated, and more than 200 chemical constituents are identified [3]. Sumac dried fruit powder is commercially available and frequently used in Mediterranean cuisine as a spice. polyphenolic extract can be in high demands as a potential replacement for commercial antibiotics. Sumac polyphenols can be released during food processing, such as fermentation, brewing, macerating, or boiling in hot water [4-5]. The chemical composition of sumac fruit extract consists of diverse hydrophobic and hydrophilic constituents. The chemical profile of the fruit also includes appreciable protein, oil, and pigments, including

anthocyanins and carotenoids, since the seeds are commonly incorporated into typical sumac powders used commercially [6]. Sumac has been used in traditional herbal medicines for the treatment of diarrhea, dysentery, ulcer, hemorrhoids, hemorrhages, wound healing, hematemesis, hemoptysis, leucorrhea, sore throat, ophthalmia, conjunctivitis, diuresis, animal bites, poison, pain, and liver disease as well as antimicrobial, abortifacient, and as a stomach tonic [7].

Sumac polyphenolic extracts from ripe and unripe fruits have shown growth inhibitory properties against Gram-positive and Gram-negative species [8]. While *E. coli* has exhibited the highest resistance against the treatment compared to that of *Staphylococcus aureus*, *Salmonella enteric*, *Bacillus cereus*; however, sumac ethanolic extract has shown an appreciable minimum inhibitory concentration (MIC of 0.78%) antibacterial activity against all tested microorganisms.

The consumption of sumac polyphenols-rich tannins may also modulate the human gut bacterial ecosystem by reducing the number of harmful bacteria while increasing the number of beneficial bacteria [9]. The predominant phyla present in the human gut microbiome are identified as Firmicutes and Bacteroidetes represent almost 90% of the intestinal bacterial flora [10-11].

The use of antibiotics not only reduces the total number of microorganisms within the human microbiome but also alters the ratio of abundance of some microbial populations [12]. As mentioned above, the drug resistant bacteria can be transmitted to human gut microbiota and cause intestinal infections. The drug resistance is developed through the action of resistance genes residing in human gut microbiota [13]. These genes become a part of the human gut microbiome and can be further directly or indirectly transferred to the other pathogenic bacteria granting them an extra protection against the environment. The gut microbiota community can resemble its antibiotic

pretreatment composition; however, some members of the community may never be repopulated again [14].

Due to the susceptibility of bioactive compounds to environmental conditions, the use of nanotechnology to create nanoparticles and entrap phytochemicals within the structures of coating materials has increased. Encapsulation can enhance the absorption of a target bioactive compound in the gastrointestinal tract [15]. Encapsulation of target polyphenols has also exhibited increased stability of the phenolic compound compared to that of the non-encapsulated compound. Microencapsulation has been exhibited to slow degradation of the phenolic compounds during simulated gastrointestinal digestion and colonic fermentation [16].

Hydrolysable tannins are determined as the types of tannins found in *Rhus coriaria* fruit; this study investigated the bacterial growth prevention/promotion effects attributed to these compounds. The presence of gallic acid in sumac extract may be attributed to the initial free gallic acid present in sumac extract. However, the amount of gallic acid may also be a function of the amount of hydrolysable tannin present in the sample. The hydrolysable tannins may further be converted to gallic acid upon hydrolysis [17]. There was no comparison study elucidating the effect on sumac polyphenols on both pathogenic and human gut microbiota. Therefore, the aim of these studies was to evaluate the antibacterial potency of sumac polyphenolic extracts (free and nanoparticle with tannase enzyme and without) against pathogenic bacteria. Also, the stimulatory effect of sumac extract rich in tannins in growth and survival on healthy gut microbiota as well as the stability and suitability of all samples in simulated gastrointestinal tract (GIT) were assessed. The objectives of this study were to:

- Analyze the antibacterial activities of sumac and investigate the impact of sumac extract treatments on Gram-positive and Gram-negative bacteria.
    - Aim 1: To assess the inhibitory effect of different sumac extracts against foodborne pathogens.
    - Aim 2: To evaluate the effect of sumac ethanolic extract on the susceptibility of Gram-positive bacteria compared to that of Gram-negative bacteria.
- Assess the impact of the sumac polyphenolic extract encapsulated with Pluronic® F127 on the growth inhibitory behavior of pathogenic bacteria.
    - Aim 1: To evaluate the impact of size distribution and surface charge values of the sumac nanoparticle on its physicochemical characteristic.
    - Aim 2: To elucidate the potential effect of encapsulated sumac polyphenols on the growth inhibitory behavior against tested pathogenic bacteria.
- Evaluate the chemical stability and assess the fate of sumac extract polyphenols after consuming free and nanoparticle sumac polyphenolic extract in an *in vitro* simulated human gastrointestinal digestive model and during their reciprocal interaction with probiotic bacteria.
    - Aim 1: To assess the stability of free and nanoparticle polyphenolic extract in an *in vitro* gastrointestinal digestive system
    - Aim 2: To assess the impact of the encapsulation process on the release profile of the targeted sumac gallolyl derivatives.

- Aim 3: To determine the effect of bacterial enzymatic activity on the dissociation of hydrolysable tannins and the production of metabolites from sumac polyphenolic extracts upon fermentation.

CHAPTER II

LITERATURE REVIEW

History of Sumac

The name “sumac” has an Arabic background that comes from the definition of the red color, which is the ultimate color of the ripening fruits. After harvesting the dark red fruits, sumac is typically dried and ground into fine to coarse powders. When used in foods, sumac may be served by either sprinkling the dry powder over foods, directly adding it into foods, or steeping it into a sweetened beverage similar to lemonade. Even though the sumac powder is usually added directly into food, the application of the fruit powder has been broadened from a popular spice mixture in traditional Arab cuisine called “za’atar to apply in cosmetic products, to consume as drug for medicinal purposes, to use in the leather industry. This provides a significant interest in conducting different studies on sumac [18]. Sumac is the name for a type of shrub that has been reported to grow worldwide due to its adaptation and tolerance to harsh environmental conditions. The height of the plant can rise to 1-3 m with a specific type of feather-like leaves made of 9-15 leaflets on opposite sides of a central bar. The tiny greenish-white flowers will make single seed dark red fruits that have been the subject of many research studies. The scientific name for sumac is *Rhus coriaria* which is a genus from the Anacardiaceae family therein, some well-known fruits (drupes) such as mangos and cashews are also included as members of the family. The genus *Rhus* contains more than 250 subgroup species that can be found even in nonagricultural lands providing them a unique property of growing wild and domestic in different geographic regions mostly in Middle Eastern countries [19].

Phenolic Compounds Chemical Classification

While primary metabolites such as proteins, nucleic acids, carbohydrates, and lipids are essential and available based on the physiological state, the secondary metabolites are not inherently considered as vital compounds for the survival of the living organisms, yet, they are essential in nature ecology. Based on their chemical structure, secondary metabolites are divided into five subgroups including: nitrogen-based compounds (such as alkaloids, non-proteinogenic amino acids, lectins, and cyanogens), isoprenoids (such as terpenes and steroids), polyketides, fatty acids, and phenols [20].

More than 8000 phenolic compounds have been identified in different plant species (**Figure 1**). These polyphenolic compounds are all developed from a common backbone that is identified as either intermediate phenylalanine, tryptophan, or their precursor shikimic acid [21]. Phenolic compounds are primarily categorized into two major categories, including flavonoids and non-flavonoids compounds. The flavonoids are then divided into six subgroups including anthocyanins, flavanols, flavanones, flavonols, flavones, and isoflavones). On the other hand, non-flavonoid compounds are divided into five subgroups: phenolic acids, xanthones, stilbenes, lignans, and tannins [22].

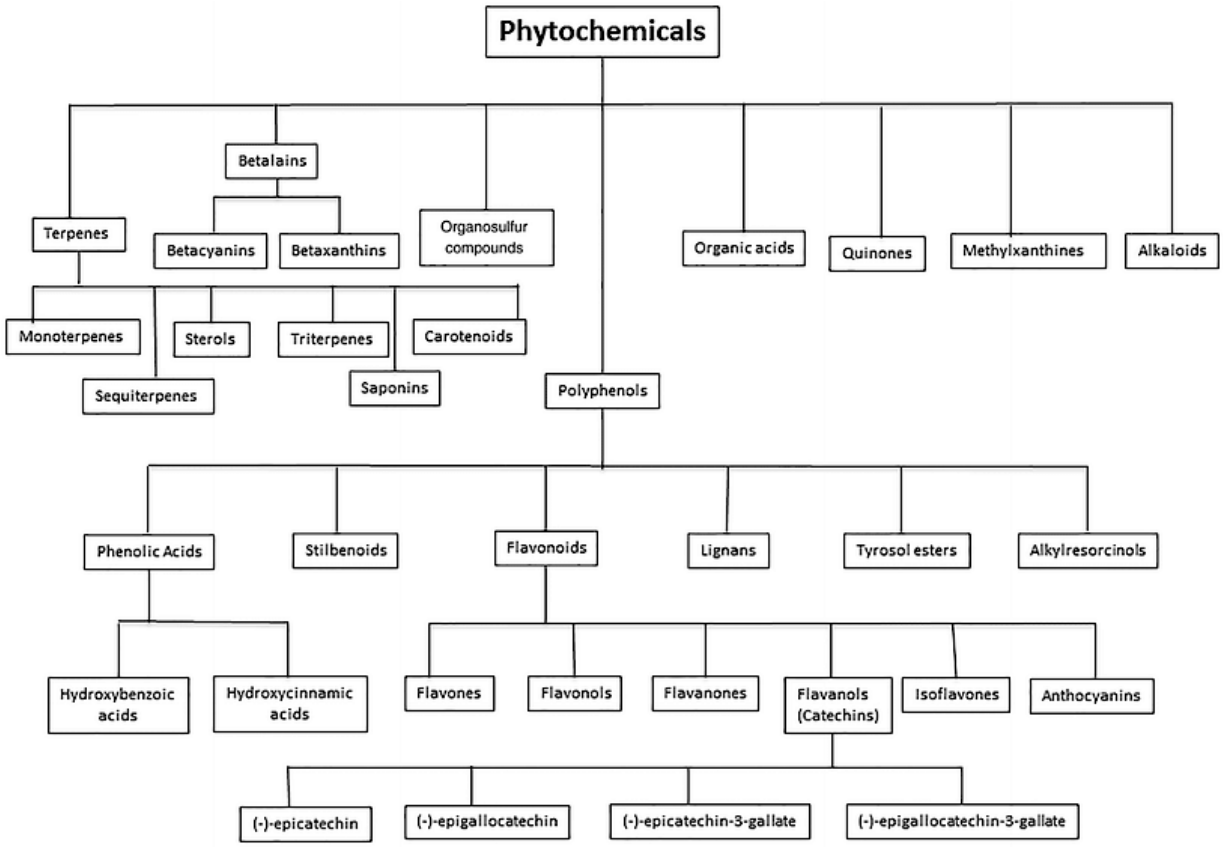


Figure 1. Classification of plant phytochemicals- Polyphenols are among the largest group of the family- Adopted from Susana Almeida et al., 2016

Plant polyphenols are varied based on their chemical structure complex ranging from simple species such as phenolic acids to highly polymerized compounds such as tannins. Although they usually exist in conjugated forms with up to two sugars residues linked to hydroxyl groups, the direct linkages of the sugar to an aromatic carbon are also identified [23].

The Potential Role of Fruit and Vegetables in Health

The health benefits attributed to naturally occurring compounds from fruits and vegetables' chemical structures have been supported by many studies. These are a wide range of chemical components that naturally occur in plants [24-26]. However, the high diversity of such compounds in fruits and their possible interaction with other food components makes it almost impossible to determine an overall net chemical reaction but instead associating particular mechanisms of action to the principal compounds in each food. It is likely that no one particular compound is solely responsible for the benefits associated with a food. For instance, the ability of fruits and vegetables to reduce the risk of chronic disease has mainly been associated with their total polyphenol constituents [27].

The world has a growing tendency for fast and processed foods has not only negatively impacted the domestic resources and ingredients availability to be used in traditional foods, but also changed the eating habits and ultimately consumers' lifestyle and overall health. There are only so many food resources available that can meet certain levels of global food demands. Adherence to the classical "Mediterranean Diet" has gradually declined due to globalization and the widespread use of modern technologies in food production as well the adoption of a more "Western" lifestyle. Mediterranean diet is known and defined as a healthy diet traditionally rich in olive oil, assorted fruits, vegetables, cereals, legumes, nuts along with medium levels of fish, poultry, and red wine as well as lower consumption of dairy products, red meat, and sweets [28]. What has distinguished the Mediterranean diet from a western diet is not only the high amount of plant-based protein in the med diet compared to the high animal protein content of a western diet,

but also the high amounts of low glycemic carbohydrates such as fiber, antioxidants such as polyphenols, and unsaturated fatty acids [29].

The Mediterranean Diet has been long recommended as a healthy diet not only among consumers, but also nutritionists who have been studying the best lifestyle and eating habits of healthy people. The diet is basically included but not limited to plant-based energy source nutrients and fresh fruit and vegetables. In addition, the amount of protein and fat from animal sources have been limited while healthy fats and whole-grain are recommended. Many types of seeds, nuts, and spices have been used as ingredients in this diet [30]. The popularity of Mediterranean and Middle Eastern foods is associated with the taste and palatability of the diet itself and incredible diversity within the ingredients used for these foods that provide a wide range of food choices to consumers. Herbs and spices are significant components of the Mediterranean diet which is suggested to have multiple beneficial bio-mechanisms that promote the consumers's health and wellbeing of the consumers [31]. Green Med Diet, as a new, greener version of the traditional Mediterranean diet, is promoting the replacement of red meat with plant-based proteins. A Mediterranean diet incorporated with higher amount of polyphenols and lower consumption of meat has shown a higher beneficial cardiometabolic effects compared to that of the regular Mediterranean diet [32].

The Potential Market of Sumac and its Role in Health

There are many health benefits attributed to the consumption of sumac, including the treatment of different disorders such as obesity-associated metabolic syndromes [3]. Sumac is also known to possess DNA protective, non-mutagenic, chondroprotective, antifungal, antibacterial, antioxidant, anti-ischemic, vasorelaxant, hypoglycemic, xanthine oxidase inhibition, vascular smooth muscle

cell migration inhibition, and hepatoprotective properties [33]. The antioxidant and antibacterial properties of sumac fruit polyphenols, along with its availability and cultivability, have made sumac a ubiquitous ingredient for different industries. Consumption of one gram of sumac per day for six weeks has shown a statistically significant decrease in weight, waist circumference, and Body Mass Index (BMI) among participants [34]. Sumac is not consumed alone or as a replacement for other foods. Instead, it has always been consumed as an additional food ingredient and different food products in a diet [35]. The consumption of sumac with other food matrices may affect the integrity and wholesomeness of the diet due to sumac's complex chemical composition and possible interactions with food constituents.

Polyphenolics: Synthesis Nomenclature and Properties

Sumac Composition

Sumac possesses a diverse phytochemical profile within its chemical composition, as shown in **Tables 1, 2, 3,** and **4.** The chemical compositions of sumac fruit are comprised of hydrophilic and hydrophobic, polar, and volatile compounds that give the fruit its color, flavor, and aroma. Specifically, sumac reportedly contains volatile aldehydic compounds and essential oils that likely vary with environmental and growing regions or among sub-species [36-37]. Due to high water solubility as an important factor in food processing, sumac hydrophilic compounds may have greater potential to be studied in the food industry. In addition to its polyphenol content, sumac is reported to have 14.6% fiber, 9.6% moisture, 7.4% fat, 2.6% protein, and 1.8% ash [38]. Sumac is reported to have an abundance of organic acids, mostly malic and citric acids, and other complex chemical compositions, including protein, oils, and pigments [39-40]. Sumac has also been reported

as a rich source of vitamins, amino acids, and minerals, while polyphenols and their derivatives such as phenolic acids, flavonoids, hydrolysable tannins, anthocyanins, and terpenes are included within the fruit's chemistry profile.

The most abundant phenolic compound in sumac is gallic acid [41]. Gallic acid is an important constituent in the formulation of some medicinal drugs and food additives [42]. A stepwise process for the production of gallic acids from tannins is suggested through which consecutive hydrolysis reactions are hydrolyzed tannin to tannic acids in the presence of acids or enzymes. Tannic acids can then be broken down to gallic acids upon further hydrolysis [43]. Sumac is laden with galloglucosides, and gallotannins among its complex phytochemical composition. The palatability of sumac is attributed to its sour, acidic taste and bitter/astringency from gallotannins. Gallotannins are made from polyol-D-glucose esterified to hydroxyl groups of gallic acids. Fractionation and isolation of tannin derivatives such as β-pentagalloyl-D-glucose (5-GC) using Sephadex LH-20 column has shown the presence of gallic acid methyl ester, digallic acid methyl ester, and pentagalloyl glucose within sumac extract [17].

Table 1. Sumac chemical composition- Adopted from Rima Kossah et al., 2009

No.	Compound	Average	Unit	No.	Compound	Amount	Unit
1	Moisture	11.80	% dry weight	16	Riboflavin	24.68	mg/kg
2	Protein	2.47	% dry weight	17	Pyridoxine	69.83	mg/kg
3	Fat	7.51	% dry weight	18	Cyanocobalamin	10.08	mg/kg
4	Fiber	22.15	% dry weight	19	Nicotinamide	17.95	mg/kg
5	Ash	2.66	% dry weight	20	Biotin	4.32	mg/kg
6	Myristic acid	0.36	% total fatty acid	21	Ascorbic acid	38.91	mg/kg
7	Palmitic acid	27.41	% total fatty acid	22	K	7441.25	mg/kg
8	Palmitoleic acid	0.68	% total fatty acid	23	Na	101.04	mg/kg
9	Stearic acid	2.92	% total fatty acid	24	Mg	605.74	mg/kg
10	Oleic acid	36.95	% total fatty acid	25	Ca	3155.53	mg/kg
11	Linoleic acid	30.38	% total fatty acid	26	Fe	174.15	mg/kg
12	Linolenic acid	1.27	% total fatty acid	27	Cu	42.68	mg/kg
13	Total Unsaturated Fatty Acids (TUFA)	69.28	% total fatty acid	28	Zn	55.74	mg/kg
14	Total Saturated Fatty Acids (TSFA)	30.69	% total fatty acid	29	Mn	10.57	mg/kg
15	Thiamin	30.65	mg/kg	30	P	327.70	mg/kg

Table 2. Sumac polyphenolic content- Adopted from Hossein Fereidoonfar et al., 2019

No.	Content	Unit	Mean value
1	Phenolic acids	mg GAE/g dry weight	190.97
2	Tannin	mg GAE/g dry weight	108.15
3	Flavonoid	mg QE/g dry weight	4.64
4	Anthocyanin	mg CyE/g dry weight	18.49

Table 3. Sumac phytochemical composition- Adopted from Ibrahim M. Abu-Reidah et al., 2014

No.	Compound	No.	Compound
1	Gallic acid dihexose	67	Quercetin
2	Gallic acid hexose derivative	68	Quercetin dimer
3	Gallic acid O-malic acid	69	Quercetin glucoside
4	Digallic acid	70	Quercetin glucuronide
5	Trigallic acid	71	Quercetin arabinoside
6	Galloylhexose	72	Quercetin rhamnoside
7	Galloylhexose derivatives	73	Quercetin-hexose malic acid
8	Galloylhexose malic acid	74	Quercetin-rhamnose malic acid
9	Pentagalloyl-hexoside	75	Methyl-dihydroquercetin hexoside

No.	Compound	No.	Compound
10	Digalloyl-hexoside	76	Quercetin-3-O-(6″-3-hydroxy-3-methylglutaroyl)-a-galactoside
11	Tri-galloyl-hexoside	77	Isorhamnetin hexoside
12	Galloylpyrogallol	78	Hinokiflavone or Amenthoflavone or Agathisflavone
13	Hexagalloyl-hexoside	79	Rhamnetin
14	O-galloylnorbergenin	80	Linoleylhydroxamate
15	Trigalloyllevoglucosan	81	Betunolic acid
16	Galloylshikimic acid	82	Myricetin
17	Galloylquinic acid	83	Myricetin derivative
18	Trigalloyllevoglucosan	84	Myricetin-hexose malic acid
19	Tetra-O-galloylhexoside	85	Myricetin-3-O-glucuronide
20	Digalloyl-hexose malic acid	86	Myricetin-3-O-glucoside
21	Galloylshikimic acid	87	Myricetin-3-O-rhamnoside
22	Digalloyl-hexose-malic acid	88	Myricetin O-rhamnosylglucose
23	Eriodictyol hexoside or Dihydrokaempferol hexoside	89	Apigenin neohesperidoside
24	Kaempferol hexoside or Luteolin hexoside	90	Apigenin glucoside
25	Kaempferol rhamnoside	91	Apiin
26	Kaempferol rhamnose-malic acid	92	Ellagic acid
27	Kaempferol 3-glucuronide	93	Coumaric acid
28	O-galloylnorbergenin	94	Moroctic acid
29	Tetra-O-galloyl-scyllo-quercitol	95	Caftaric acid
30	Di-O-galloyl-3,4-(S)-hexahydroxydiphenoyl protoquercitol I	96	Protocatechoic acid
31	Di-O-galloyl-2,3-(S)-hexahydroxydiphenoyl-scyllo-quercitol II	97	Genistein-hexose malic acid
32	1,5-di-O-galloyl-3,4-(S)-hexahydroxydiphenoyl protoquercitol	98	Prorocatechuic acid hexoside
33	O-Galloyl arbutin	99	Coumaryl-hexose malic acid
34	Digalloyl-hexoyl-ellagic acid	100	Syringic acid hexoside
35	Galloyl-valoneic acid bilactone	101	Oxydisuccinic acid
36	Myricetin galloyl-hexoside	102	Isorhamentin hexose-malic acid
37	Cyanidin-3-O-(2″galloyl)-galactoside	103	Homoprotocatechuic acid
38	7-O-Methyl-cyanidin-3-O-(2″galloyl)-galactoside	104	Di-benzopyrano-furanacetic acid derivative
39	7-O-Methyl-delphinidin-3-O-(2″galloyl)-galactoside I	105	Dihydroxypalmitic acid
40	Apigenin-7-O-(6″-O-galloyl)-β-D-glucopyranoside	106	Hexadecadienoic acid
41	Hydroxy-methoxyphenyl-O-(O-galloyl)-hexose	107	Linoleic acid amide
42	Methyl gallate	108	Oxoglycyrrhetinic acid
43	Methyl digallate	109	Benzoic acid, 3,4,5-trihydroxy-2-oxo-1,3-propanediyl ester
44	Levoglucosan gallate	110	Penstemide
45	Dihydroxybenzoic acetate-digallate	111	Spinochrome A
46	Afzelin O-gallate	112	Vapiprost
47	Myricitrin O-gallate	113	Sespendole
48	Quercitrin 2″O-gallate	114	Chrysoriol
49	Malic acid	115	Vebonol
50	Malik acid hexoside	116	Horridin
51	Malic acid derivative	117	Isovitexin
52	Quinic acid	118	Rutin
53	Caffeoylquinic acid	119	Ampeloptin
54	O-Succinoyl-di-O-caffeoylquinic acid	120	Luteolin
55	Kaempferol	121	Butein
56	Kaempferol-hexose malic acid	123	Triterpenoid derivative
57	Kaempferol rutinoside	124	Ampelopsin glucoside
58	Eriodictyol hexoside or Dihydrokaempferol hexoside	125	Mingjinianuronide B
59	Coumaryl-hexoside	126	Spicoside E
60	Eriodictyol xyloyl-deoxyhexose	127	Petunidin-3-O-glucosdie pyruvate
61	Umbelliferone	128	Chrysoeriol-6-O-acetyl-4′-β-d-glucoside
62	Glycitein 7-O-glucoside	129	Chrysoriol derivative

No.	Compound	No.	Compound
63	Ascorbyl monomyristate	130	Mangiferitin
64	Deoxycorticosterone glucoside	131	Apigenin glucuronide
65	Dihydroisovaltrate	132	Camellianin A
66	Deacetylfoskolin	133	7-O-Methyl-cyanidin-3-O-galactoside

Table 4. Identified sumac fruit (*Rhus coriaria*) anthocyanin (A) and phenolic contents (B) from previously published study- Adopted from Flora V. Romeo et al., 2015

A)

No.	RT (min)	$[M]^+$ (*m*/*z*)	MS^n (*m*/*z*)	Anthocyanins	Relative Compositions (%)
1	10.2	465	303	delphinidin 3-glucoside	0.28
2	10.9	449	287	cyanidin 3-glucoside	7.84
3	12.7	601	287	cyanidin 3-(2″-galloyl) galactoside	3.83
4	13.4	463	301	7-methyl-cyanidin 3-galactoside	52.92
5	15.3	615	301	7-methyl-cyanidin 3-(2″-galloyl) galactoside	35.14

B)

No.	RT (min)	$[M-H]^-$ (*m*/*z*)	MS^n (*m*/*z*)	Phenolic Compounds	λ_{max}
1	2.6	169	125	gallic acid	269, 310
2	31.9	-	301	quercetin derivatives	255, 354
3	33.9	463	316	myricetin 3-rhamnoside	257, 366
4	35.3	463	301	quercetin 3-glucoside	255, 351
5	37.3	939	921/787/169	pentagalloyl-glucoside	280
6	39.9	1091	939/169	hexagalloyl-glucoside	282
7	41.9	1243	1091/169	heptagalloyl-glucoside	281
8	43.5	1395	1243/169	octagalloyl-glucoside	285
9	45.5	1547	1395/169	nonagalloyl-glucoside	283
10	50.5	1699	1547/169	decagalloyl-glucoside	278

Sumac Tannins

Tannins are high molecular weight polyphenolic structures that are considered soluble in water even though they create heterogeneous suspension when dissolved in water. The molecular weight

of tannins may differ based on the number of monomer units within their molecular structures, while the molecular weight of hydrolysable tannin is within the range of 500 to 3,000 Daltons (Da) [44], condense tannin usually have a higher molecular weight up to 1,000-20,000 Da [45]. However, the size and weight of these compounds can go beyond this range as the molecule may incorporate additional functional groups within its structure. The chemical structures of tannin molecules are shown in **Figure 2**. Tannins are divided into subgroups of condensed, complex, and hydrolysable tannins. Condensed tannins are also known as catechin tannins or proanthocyanidins and result from condensation of flavan-3-ols and/or flavan-4-ols and do not have a sugar moiety in their chemical structure. Complex tannins are tannins in which a catechin unit is bound glycosidically to a hydrolysable tannin [46].

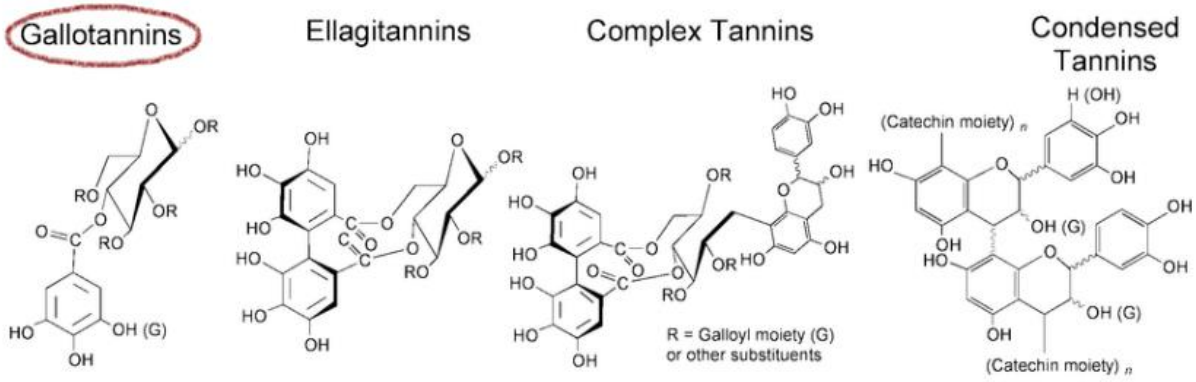


Figure 2. Chemical structures of tannin molecules- Adopted from Karamali Khanbabaee et al., 2001.

This type of tannin is composed of a flavagallonyl unit connected to a polyol derived from D-glycosidic bond in C-1 and three other ester bonds [47]. Complex tannins, as their name suggested,

are the most complexe type of tannin molecules. They are a type of tannins in which flavane-3-ol, the primary unit of condensed tannin, is attached to a hydrolysable tannin through carbon-carbon linkages [48]. Almost 500 glucogalloyl derivatives have been identified [49-50]. Hydrolysable tannins are synthesized in plants by esterifying a sugar molecule, most commonly glucose, by the hydroxyl groups on monomeric gallic acid, acid, or the ellagic acid precursor hexahydroxydiphenic acid to form the two primary subgroups of gallotannins and ellagitannins. Upon hydrolysis by either acid, bases, or enzymes gallotannins are broken down into their constituent free gallic acid and sugar, while ellagiannins produce free ellagic acid. Hydrolysable tannins account for almost 20% of *Rhus coriaria* fruit's mass, from which gallotannins are reported to be the abundant type of hydrolysable tannins present in sumac fruit extract [51].

Extraction of polyphenols from leaves and fruits of sumac has shown a similar composition of tannins ranging from penta to decagalloyl glucose. These galloylated compounds are separated by a constant 152 Da decrease in the mass values from deca to pentagalloyl glucose. The loss of galloyl moieties from pentagalloyl glucose (through the breaking of the m-depside bond) is more favorable than the loss of the core gallic acid attached to the sugar moiety. Due to the higher energy required to break down the direct bond between glucose core and gallic acid, the loss of gallic acid (170 Da) is observed only in pentgalloylglucose fragmentation pathway at *m/z* 921 [52]. Chromatography analysis of sumac fruit extract through HPLC-MS has identified 191 chemical components within the extract and classified them as 78 hydrolysable tannins and gallic acid, 59 flavonoids, 9 anthocyanins, 2 isoflavonoids, 2 terpenoids, 1 diterpene, 38 other unidentified compounds [53]. Gallotannins bear a basic chemical structure of polyol-D-glucose molecules esterified on gallic acid units at their hydroxyl groups. The product of the esterification reaction,

β-pentagalloyl-D-glucose, is susceptible to hydrolyzation with either weak acid, bases, or enzymes at defined temperatures. Upon hydrolysis, the compound is broken down into smaller molecules such as gallic acid, which may be further decarboxylated to even simpler molecular structures such as pyrogallol (also known as pyrogallic acid).

Interaction of Sumac Polyphenols with Protein

The presence of hydroxyl groups in the structure of tannins gives polyphenols the ability to physicochemical interactions with other macromolecule structures within a food matrix. Protein and polyphenolic interactions are among the most common interaction, developed through the formation of hydrogen bonds or hydrophobic interactions that cause precipitation haze and loss of functional properties such as their antioxidant capacity, among others [54]. The reaction also creates a unique sensation associated with increased friction between tannins and oral membranes [55]. When exposed to a commercial mixture of hydrolysable tannins, the lubricating quality of human saliva is decreased through lowering the viscosity associated with an increase in friction which further results in an increment in astringency characteristic of sumac [56-57]. The above-mentioned physicochemical interaction is highly pH dependent meaning that lowering the pH increases the protein binding property of tannins [58]. This property is suggested to help modulate the physiological properties of tannin as the tannin-protein complexation get stabilized through more acidic environment within digestive tract. The chemical binding between tannin and salivary protein is created upon the ability of prolyl residues to create multiple hydrophobic binding sites, which further connects the phenolic groups to the tertiary amide carbonyl group through hydrogen bonds [59]. Therefore, the magnitude of the interaction between saliva proline-rich protein and food

tannins is highly attributed to the size of tannins. That is, the more complex tannins have a higher affinity to the salivary protein as the number of the binding sites within their structures increase. However, the maximum interaction between tannin and protein is also attributed to the medium-size tannins. The Nuclear Magnetic Resonance (NMR) study on tannin binding dissociation constant has shown that the highest relative binding affinity to protein is limited to tetra and Penta galloyl glucose molecules. Further polygalloylation has not increased the protein binding property of tannins [60]. Despite the directly proportional relationship between the level of astringency and the number of hydroxyl groups attached to tannin, an inversely relationship is reported beyond seven hydroxyl groups within the structure of tannin. This is because the abundance of these functional groups may trigger the steric hindrance property to prevent a possible imbalance of the strength of hydrogen bonds [61]. The increase in molecular size and its binding sites changes the steric hindrance of the molecule, which defined as the available functional groups binding sites around the molecule by surrounding ligands. Very high molecular weight tannins have shown a greater steric hindrance due to less available binding sites to bond surrounding molecules [62-63].

Foodborne Pathogens

Foodborne disease burden epidemiology reference group report has indicated that almost 1 in 10 individuals fall ill after consuming contaminated food globally. The report has mentioned that 600 million foodborne illness causes 420,000 deaths annually around the world [64]. Also, according to a report published by the Centers for Disease Control (CDC), foodborne infections have caused 76 million illness, 325,000 hospitalizations, and 5,000 deaths in the USA every year. The economic toll is estimated at $10-83 billion due to medical expenses, lost productivity, legal fees, and other

monetary damages [65]. Foodborne illness occurs when a pathogen or a toxin produced by the pathogen is ingested with food and introduced into the human body. Based on the type of entering the human host, foodborne illnesses are classified into two major categories, including foodborne infection (bacterial pathogens) or toxins as foodborne intoxication [66]. Factors contributing to foodborne illness include, but are not limited to, large-scale food production, worldwide distribution, and overall globalization of the food supply. Other factors include eating outside the home, interindividual differences in genetics and resistance, the emergence of new pathogens, and an increasing population of at-risk consumers [67].

Antibacterial Activity of Polyphenols

Natural Replacement for Traditional Antibiotics

The emergence of antimicrobial drug resistance has increased the interest in the discovery and evaluating naturally-occurring antimicrobial agents to help the human body defend against foodborne pathogens. Medicinal plants are one such option and represent an alternative to synthetic drugs to treat pathogenic bacterial infections and serve as potential antibiotics that are not resistant to standard drug therapies [68]. In addition, there is a limitation on the use of synthetic antibacterial due to their potential for carcinogenic effects and potential environmental hazards [69]. When taken excessively, antibiotics may result in an uncontrollable outcome that challenges our future civilization's perspective. The development of antibiotic resistance is expanding among commonly used therapies and fighting microorganisms at earlier stages of exposure or contamination is likely a more sustainable approach [70].

The use of herbal remedies has increased worldwide, primarily since about 80 percent of the world's population relies on plant-based traditional medicine as their primary healthcare approach [71], but also due to excessive use of chemical and biological (pharmaceutical compounds extracted from biological sources) drugs. These synthetic drugs gradually elevate bacterial resistance and further suppress the human immune system. The mechanisms of action for immune system suppression by pathogenic bacteria are through the production of antigens and biomarkers that disturb the host immune responses, such as apoptosis induction, suppression of proinflammatory cytokines, and preventing neutrophil chemotaxis [72-73]. However, the occurrence of antibiotic resistance is not only attributed to direct consumption of antibiotics by humans, but also consumption of antibiotic residues from foods that may spread antibiotic resistance genes to human pathogens [74]. Although antibiotics are commonly used to defeat a targeted infectious microorganism, subsets of commensal microbes will also be eliminated by the action of antibiotics [75].

Plant extracts have also shown a potential role as a replacement for chemical preservatives in foods. For example, the use of vegetable extracts rich in natural nitrates/nitrites instead of synthetic or purified nitrate/nitrite is growing in popularity in the meat industry preventing products from oxidation and discoloration by inhibiting myoglobin conversion [76]. Compounds synthesized as part of the secondary metabolism of plants such as tannins, essential oils, and phenolic compounds, have also shown antimicrobial activity and other clinical benefits [77].

Antibacterial Activity of Sumac Polyphenols

There are various components in bacteria cell wall structures that provide the cell walls with an integrated and seamless biological structure that can define the cell susceptibility to its surrounding environment. That is, Gram-positive bacteria cell walls are composed of thick peptidoglycan layers (with covalently attached teichoic and teichuronic acids), and protein, while Gram-negative bacteria have protein embedded lipopolysaccharide outer membrane, thin peptidoglycan, and porin channels [78] (**Figure 3**).

The bacteria membrane structure comprises several constituents that are chemically bonded to one another. Although the bacterial cell wall primarily contains a peptidoglycan structure, it's not a seamless structure of this polysaccharide moiety. The cell wall also has other chemical, components including phospholipid bilayer, glycolipids, lipopolysaccharide, and proteins mainly lipoproteins [79]. These cell wall components are delicately embedded in one united structure by chemical binding. However, the surrounding molecules can approach, attach, and disrupt this balance.

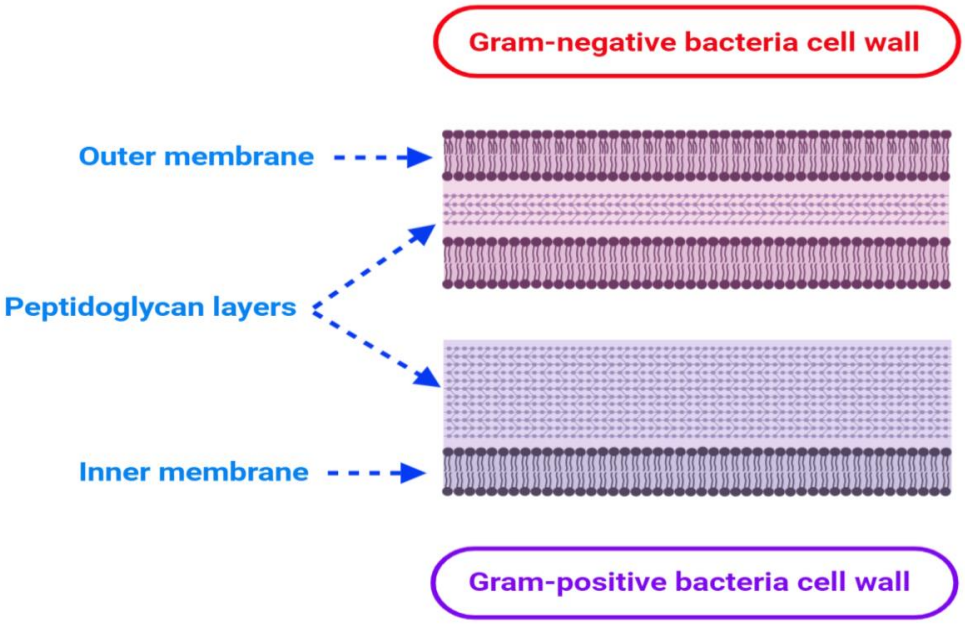


Figure 3. The difference in bacterial cell wall structures between Gram-negative and Gram-positive bacteria **(**created with BioRender.com).

Fruit polyphenolic extracts rich in tannin may play an antibacterial role through a mechanism involving a direct interaction of tannins with bacteria. The antibacterial mechanism of tannins is primarily associated with their protein binding property through which enzymatic activities or cell integrity of the targeted microorganism is affected. This interaction may be caused by tannin molecules that irreversibly change the structure of the cytoplasmic barrier, which further coagulates the enzymes necessary for cell metabolism and viability [80]. The bacterial growth inhibition associated with tannin activity has also suggested other mechanisms directly affecting microbial metabolism, inhibition of microbial extracellular enzymes, complexation with essential substrates such as metal ions necessary for activity and growth of bacteria. These mechanisms are

suggested based on the ability of tannin molecules to interact with different components of the bacterial cell wall structure. For instance, tannin may interact with lipoproteins and occupy all the protein binding sites (within the structure of lipoproteins) due to its concentration. The lipid moieties of the lipoproteins may also attach to an amino terminal cysteine reside to make this protein bonding even more complex [81].

Tannin also inhibits the extracellular microbial enzymes, deprives the substrates that microorganism needs for growth, and inhibits oxidative phosphorylation, which disrupts microbial metabolism [82]. The selective passage ability of the bacteria cells allows certain nutrients to pass through the cell membrane while other nutrients are prohibited from entering the cells. This is a natural mechanism to help bacteria overcome the harsh living conditions crucial to cell survival. The antimicrobial properties of fruit extracts can be determined by evaluating the magnitude of the disruption caused by the polyphenolic constituents of the extract against the bacteria cell wall structure and integrity.

The hydroxyl groups within the tannin structure donate hydrogens to the oxygen atoms within the phospholipid head group to form hydrogen bonds with the bacteria membrane lipid bilayers. The number of these hydrogen bonds defines the strength of the interaction between tannin molecules and the cell bilayer constituent. Hydrolysable tannins have shown the ability to penetrate the hydrophobic region of the bilayer and change the physiological properties of the membrane [83]. The ability of hydrolysable tannins to form ion channels in the lipid bilayer has resulted in a leakage current within the membrane. The intensity of this leakage has proportionally increased by the concentration of tannin molecules. The interaction between tannins and bacteria cell wall constituents depends on the concentration of the tannin and the type of tannin being

extracted from target fruit. Penta-galloyl-glucose, as the simplest and non-polar structure hydrolysable tannin, has disordered the acyl chains of the lipid bilayers while a highly polar condensed tannin catechin trimer exhibit lower disruption of the bilayer. As the polarity of tannin increases, the disruption of lipid bilayer by tannin molecule decreases [84].

Although all types of tannins have shown antibacterial activity against different types of bacteria, hydrolysable tannins have shown a high reactivity and bonding property with sulfhydryl functional groups mainly through C-S crosslink covalent bonds [85]. Tannins compromise the integrity of bacteria cell wall structure through detaching the ions from the outer membrane and further leak of cytoplasm but also interferes with polypeptides in the cell wall (reacting with sulfhydryl groups) that leads to membrane protein malfunction [86-87]. The sulfhydryl or thiol functional groups are essential components of the bacterial membrane by creating sulfide bound between amino acids and thus providing proteins with their configurations. The sulfhydryl groups are located on membrane proteins/exoproteins and exhibit different functionalities such as reducing oxidoreduction potential, which provides the cell with protection against oxidative stress. The redox sensing mechanisms in bacteria are related to the thiol-disulfide balance thereby, bacteria may sense the environmental redox state and adopt their cell activity [88].

As the hydrolysable tannins are broken down to their smaller metabolite, gallic acid, the amount of this phenolic structure is also increased following the hydrolysis. This may raise interest for its use as a functional food ingredient with applications in the food, pharmaceutical, and cosmetic industries [89]. The inhibition of intracellular constituents' functionality caused by tannins results from the cell membrane damage and further leakage of the essential internal components of the cell through increased membrane permeability. The antibacterial property is not attributed

to only tannin molecules. Gallic acid (3,4,5-trihydroxybenzoic acid) as a primary metabolite of tannins has also shown strong antibacterial activity when tested against pathogenic bacteria. Gallic acid has reportedly inhibited the growth of *L. monocytogenes*, *S. aureus*, *E. coli*, and *P. aeruginosa* at 2000, 1750, 1500, and 500 µg/mL, respectively.

The higher resistance among Gram-positive bacteria against samples being tested compared to that of Gram-negative bacteria was observed [80] indicating the role of peptidoglycan layer in cell membrane permeability. The lipopolysaccharide outer membrane in Gram-negative bacteria selectively diffuses chemical compounds (selecting passage of the surrounding substances). Gutiérrez et al., 2012 has suggested that the number and location of hydroxyl groups within phytochemicals structures (dihydroxylation in the 3´ and 4´ positions in flavones and flavonols, glycosylation on the 3 and 7 positions in flavonoids) maybe two key elements promoting toxicity to targeted *S. aureus* bacterium when investigating the antimicrobial effectiveness[90-91]. Antibacterial activity of sumac whole extract (100g of sumac powder was soaked in 1000 mL of distilled water for 5 days at 45 °C. The solution was then concentrated, dried, and rehydrated to desired concentration) against an isolated antibiotic-resistant (methicillin resistance) *S. aureus* was observed when the bacterium was treated at the concentration of 250 µg/mL of the fruit extract [92].

Encapsulation of Polyphenols

The food safety and health-promoting properties attributed to plant active compounds have provided the food with these compounds in their structure with good marketing opportunities. However, the plant bioactive compounds are susceptible to environmental conditions such as temperature, light, oxygen, pH, etc. in addition to these negatively impacting factors, the

gastrointestinal conditions (acidity, temperature, peristalsis, exposure to other food components), and enzymatic reactions (exposure to enzymes either produced by microorganisms or secreted from body cells). Encapsulation is a method for improving in vitro and in vivo stability of polyphenols is recommended [93].

The interaction between two different substances is developed through one of the following mechanisms: A hydrophobic interactions (the tendency of hydrophobic substances to cluster in an aqueous solution, host-guest interactions (a spherical non-covalent binding relationship between a host and its guest such as that in enzyme-inhibitor interactions), Π interactions (where the p orbitals in the atoms of two interacting substances overlap and share electrons), or hydrogen bonding (interaction between a hydrogen atom with a negatively charged particle) [94-95].

Tannic acid and PEG have previously exhibited a strong molecular network formation (polyethylene glycol (PEG) has a similar structure as PEO in Pluronic). Polymeric encapsulation with Pluronic copolymers has been used for hydrophobic and hydrophilic substances. The use of Pluronic F127 for encapsulation of curcumin has shown 4-6 log10 reduction when 270 μM of this polyphenolic compound was used to treat *S. mutans* and *C. albicans* bacteria [96]. Pluronic nanoparticles are also prepared from proanthocyanidin, epigallocatechin gallate, and quercetin (the latter being hydrophobic and the other two hydrophilic) at the ratio of 5:1 polymer to an individual substance (0.5, 2.5, and 0.1% *w/v* for Pluronic, DMSO, and Phenolics respectively). Interestingly, the results have indicated that encapsulation efficiency among the hydrophilic compounds (proanthocyanidin, epigallocatechin gallate) was significantly higher than that of the hydrophobic compound (quercetin). This may be due to the difference in water solubility for pluronic and quercetin (50 mg/mL vs. 2 mg/mL, respectively in water at room temperature). The highest loading

capacity in the core is occurred when water solubility values of the drug and hydrophobic core are equal [97]. Pluronics are known as poloxamers, long-chain triblock copolymers with a sandwich-like chemical structure made from two hydrophilic polyethylene oxide (PEO) blocks with hydrophobic propylene oxide (PPO) block in the middle [98]. The hydroxyl groups in tannin interact with an oxygen atom in PEO chains through hydrogen binding [99]. Within the micellar structure, while the hydrophobic substances are thought to be embedded in the inner core, the hydrophilic compounds are located within the corona with the relatively hydrophilic properties. In addition, hydrogen bonds or ionic interactions is formed between hydrophilic compounds and hydrophilic corona. However, the hydrophobic compounds are entrapped in the hydrophobic core due to the formation of Van der Waals forces [100].

By utilizing the encapsulation procedure, the plant phytochemicals and drug substances are protected against rapid degradation in human and/or animal cells, and within food matrices. In other word, encapsulation lowers the susceptibility of different polyphenolic compounds against degradation and physicochemical changes. At the same time, it enhances the stability and dispersibility of the polyphenolic compounds throughout the food systems [101]. Encapsulation can be used to suppress undesirable flavor and odors in the food and drug industries. The method can improve antimicrobial properties associated with the entrapped compounds. The role of encapsulation procedure in enhancing antibacterial activity attributed to plant polyphenolic compounds is suggested by other studies, although coating materials may exhibit different protection properties of the polyphenols against microorganisms [102].

Encapsulation of Hydrophilic Compounds

The most essential constituent within the structure of prokaryotic and eukaryotic cells is the membrane that separates the components of the cell from the extracellular environment. This separation in fact, protects the cells against their surroundings. The creation of bilayer structures such as liposomes was inspired by biological cell membranes' role and functional properties. Since then, scientists have been developing simpler, stronger, and more controllable analogs to better protect sensitive compounds, leading the creation of amphiphilic block copolymers called polymersomes [103]. **Figure 4** represents the structure of liposomes and polymersomes. Liposomes and polymersomes are both amphiphilic and self-assemble structures. However, liposomes are more sensitive and less stable compared to polymersomes despite the high biocompatible (not harmful to living tissues) property of lipid-based vesicles.

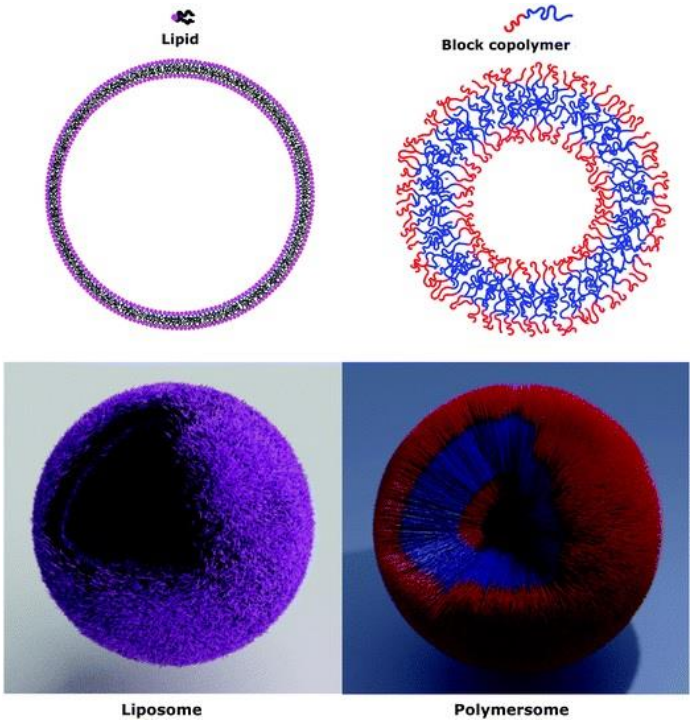


Figure 4. The structure of Liposome versus Polymersome. The thickness of the coating in liposomes may be in a 3-5 nm range while the same layer in polymersome has a thickness of 5-50 nm range- Adopted from Emeline Rideau et al. 2018.

The ability of polymeric vesicles to encapsulate a wide range of molecules (bearing various hydrophobic and hydrophilic properties) within their structures has provided them suitability with different applications and functionality. In general, polymer-based nanoparticles (molecules that are coated by polymers) are more stable compared to that of liposomes due to their rigid structure that causes a low permeability (the surrounding molecules are unable to get into the polymeric structure) to the particles [104]. The high permeability in liposomosomes increases the exchange and transportation of substances, which may cause a leakage of encapsulated compounds. However, this can also be considered as a downside for polymersomes when higher permeability in the

nanoparticles is intended, such as enzymatic reactions (or when higher exposure or penetration of the enzyme with encapsulated substances is needed) [105]. Polymersomes and liposomes trap the target hydrophilic compound into their centered aqueous core, while hydrophobic shell provides a barrier against the targeted compound to provide a controlled release of the compound and prevent rapid clearance at the site of action [106].

Nanoparticles Coating Materials

Different nanocarriers for encapsulation of polyphenolic compounds have been recognized as suitable delivery systems in foods. Numerous compounds, including polymers, polysaccharides, proteins, cyclodextrins, lipid, gelatin, or their combinations, are reportedly used for polyphenolic encapsulation [107]. The coating materials may be either cationic (such as chitosan, polydimethylaminoethyl methacrylate, polydiethylaminoethyl methacrylate) or ionic (such as alginate, polyacrylic acid, albumin, hyaluronic acid) [108]. However, some coating materials are non-ionic compounds with amphiphilic properties, which allows them the ability to bind a wide range of substances. Poloxamer 407 (Pluronic F127) is one of these compounds. Pluronic is a non-toxic, biocompatible triblock copolymer and proper coating material for the preparation of nanoparticles. FDA has also approved this compound for human drug nanopolymer applications. Although the individual monomers (PPO & PEO) in the structure of Pluronic polymers are already approved and used in foods, the use of the polymer as a whole is not yet approved by the agency to be used in food products. PF127 (EO_{98}-PO_{67}-EO_{98}) has a molar mass of 12600 g/mol (Da), from which 70% comes from 196 ethylene oxide units distributed in the two lateral blocks. The structure of Pluronic F127 is shown in **Figure 5**. There are 67 propylene oxide units in the central block which,

along with the other two polyethylene blocks, create a compatible amphiphilic coating material and makes the compound soluble (for water-insoluble phytochemicals such as carotenoids, tocopherol, alkaloids, etc.) in aqueous physiological fluids [109]. The PF127 can self-assemble itself into micellar structures resulting from dehydration of hydrophobic PPO core blocks and outer shell hydrated/swollen PEO chains. This physical development in the structure of PF127 leads the creation of well spherical-shaped systems that can incorporate a high loading of hydrophilic compounds into the structure [110].

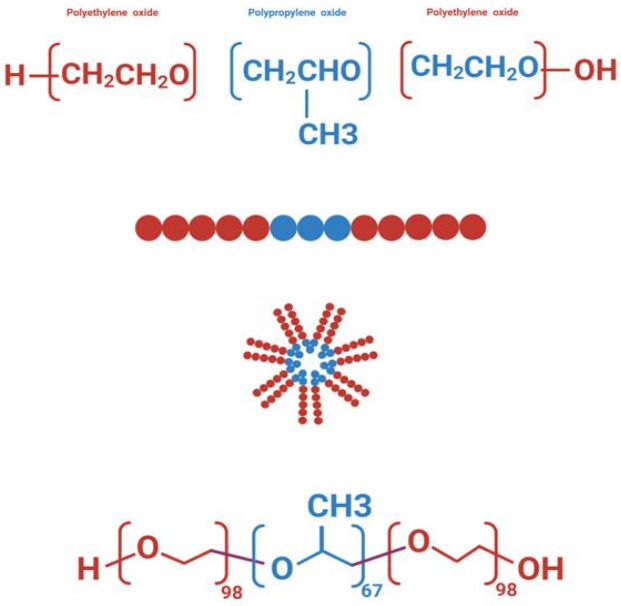


Figure 5. Structure of Pluronic F127 polymer (created with BioRender.com).

The difference in solubility between PPO and PEO is the crucial element to provide PF127 with an amphiphilic feature. The lateral PEO blocks are fully soluble in water, while the central PPO block is more hydrophobic and only partially soluble in water [111]. The application of

poloxamers in nanoparticles formulation may provide us a wide range of combinations by including additional copolymers to its primary structure and achieve the desired amphiphilicity. The base triblock copolymer system plays a role as a central building block that provides modification in the molecule by allowing various functional groups to attach to both ends of the chain [112]. The encapsulation process with Pluronic copolymer involves a lyophilization step which can potentially damage/degrade the sample in its original/free form. However, the long PEO chains within the structure of Pluronic F127 provide a cryoprotecting property to encapsulated particles [113]. The lyophilized sumac polyphenolic extracts may be stored for months before either being resuspended in an aqueous solution or being consumed directly.

The self-assembly or spontaneous formation of nanostructures provides PF127 the capability to act as a useful coating material in different applications, including administration of parent compounds in the pharmaceutical industry [114] or as a delivery vehicle (refers to the transportation of typical water insoluble compounds) [115-116]. This multipurpose ingredient has also been used in the formulation of drugs as emulsifying, coating, wetting, and dispersion agents. For research purposes, the application of polymeric nanoparticles of PF127 (EO_{98}-PO_{67}-EO_{98}) has also been used as a coating material for encapsulation of hydrophobic compounds such as curcumin, lutein, and quercetin [113] as well as polar phenolic compounds such as gallic acid, syringic acid, and sinapic acid [117-118].

Particle Size Distribution

Nanoencapsulation is a technology that brings principles from different science fields together to create modified particles with a size range of 1-100 nm in at least one dimension. However,

nanoparticles are commonly defined as those materials that are built up to several hundred nanometers in structure [119]. Based on their properties, shape, and size, nanoparticles are categorized in fullerenes, metal, ceramic, and polymeric nanoparticles [120].

Dynamic light scattering (DLS) has been used to measure the nanoparticle size distribution. DLS is an analysis technique used to study the physical properties of a molecule, polymer, or physicochemical complex. The name comes from exposing compounds to monochromatic light waves, following by detecting the scattered light reflected from them [121]. The monochromic incident light undergoes a successive destruction and construction phase so that the scattered light is constantly converted to a detectable signal [122]. It has been reported that increasing the number of nanoparticles per surface area has increased the internalization of the nanoparticles, which means that their passage through the biological membrane is increased [123].

The formation of nanoparticle clusters on the epithelial cell surface has promoted the permeability of nanoparticles compared to that of single particles. Although nanoparticles with 95-200 nm size are reported as the ideal size for the highest cellular uptake, experimental results have suggested higher bioavailability of the drug careers with the size range of 100-1000 nm [124]. The importance of the size of nanoparticles is not limited to their transportation across epithelial cells. The development of nanocarriers in anticancer therapy has suggested an association between the size of nanocarriers kinetic and tumor accumulation of the particles [125]. To design effective nanoparticles for diagnostic and therapeutic applications, the optimum size of nanoparticles to achieve the highest cellular uptake is suggested to be 50 nm [126]. Besides the particle size, other factors influencing the fate of compounds within nanoparticles can be identified as shape and surface charge of the nanoparticles [127]. Mechanisms of nanoparticle internalization have shown a

significant difference in the transportation of nanoparticles across the cell membrane between negatively and positively charged particles. The results have suggested up to a 5-fold increase in transport efficiency (percent transported versus internalized nanoparticle mass) of negatively charged nanoparticles compared to positively charged particles. However, the transportation of nanoparticles exhibited a directly proportional relationship with the dose of treatments regardless of the nanoparticles' surface charges [128].

Release Profile of Sumac Polyphenols

The released profile can be studied in two stages, including an initial burst release followed by a sustained and slow-release [129-130] has suggested a better release profile from the equal ratio of compound and coating material compared to the sample possessing a higher ratio of coating material to the compound. This resulted in a high burst effect followed by a shorter release period that caused a fast degradation and elimination of the target compound within the medium [131]. However, it is important to find a proper ratio of polymer to polyphenol to achieve the highest encapsulation efficiency; by either increasing the concentration of gallic acid or decreasing the concentration of polymer, the initial burst release may be controlled [129].

Antibacterial Activity of Encapsulated Polyphenols

Polymeric nanoparticles are known to have unique properties that distinguish them from other nanocarriers. These properties are easy to synthesize, cost-effective, biocompatible, biodegradable, and non-toxic [132]. The use of different coating materials in the formulation of emulsions and investigating their antibacterial activity has shown that surfactants have increased

the antibacterial effects of phytochemicals through the different possible mechanisms of action including an increase in passive transport across bacterial cell walls through an increase in surface area and nanoscale size of the particles [120, 133]. Engaging the emulsifier droplet and fusion with phospholipid bilayer may also help the compounds to reach the target site of action. Also, as the electrostatic between the negative charge on bacteria surface with the charge on the particles increases, the concentration of the particles at the site of action has increased [133].

Polyphenol Absorption, Distribution, Metabolism, and Elimination (ADME)

The fate of the administered polyphenols is studied through investigating the absorption, distribution, metabolism, and finally, excretion/elimination of the compound (ADME). The concentration of the compound, maximum time spent to achieve that concentration, the area under the curve in the time-concentration graph, the volume of distribution, half-life, and clearance of the compound from the body are the parameters that may affect ADME processes [134]. When food is digested, its chemical constituents are detached and available in various biocomponents including different vesicles, ionic particles such as salts, metal ions, or strongly polar components such as polyphenols or vitamins. While the hydrophobic micellar structures may cross the epithelial cell membrane through the phospholipid bilayers, the transportation of the hydrophilic compounds is taken place by an active transport called the endocytosis process. The process involves environing the particles with membrane and further taking in the particles inside the cells.

Although the common way to receive phenolic compounds is through consumption of food containing polyphenols, the instability, and degradation of polyphenols as well as the intestinal epithelial barrier have resulted in a very low absorption of such compounds into the systemic

circulation [135]. Research on food polyphenols and their possible interaction with human cells is through an oral administration of the compounds. The orally administrated polyphenol compounds undergo some chemical and physical changes caused by different digestive enzymes and pH conditions, leading to the degradation of these compounds in GIT.

Human Gut Microbiota

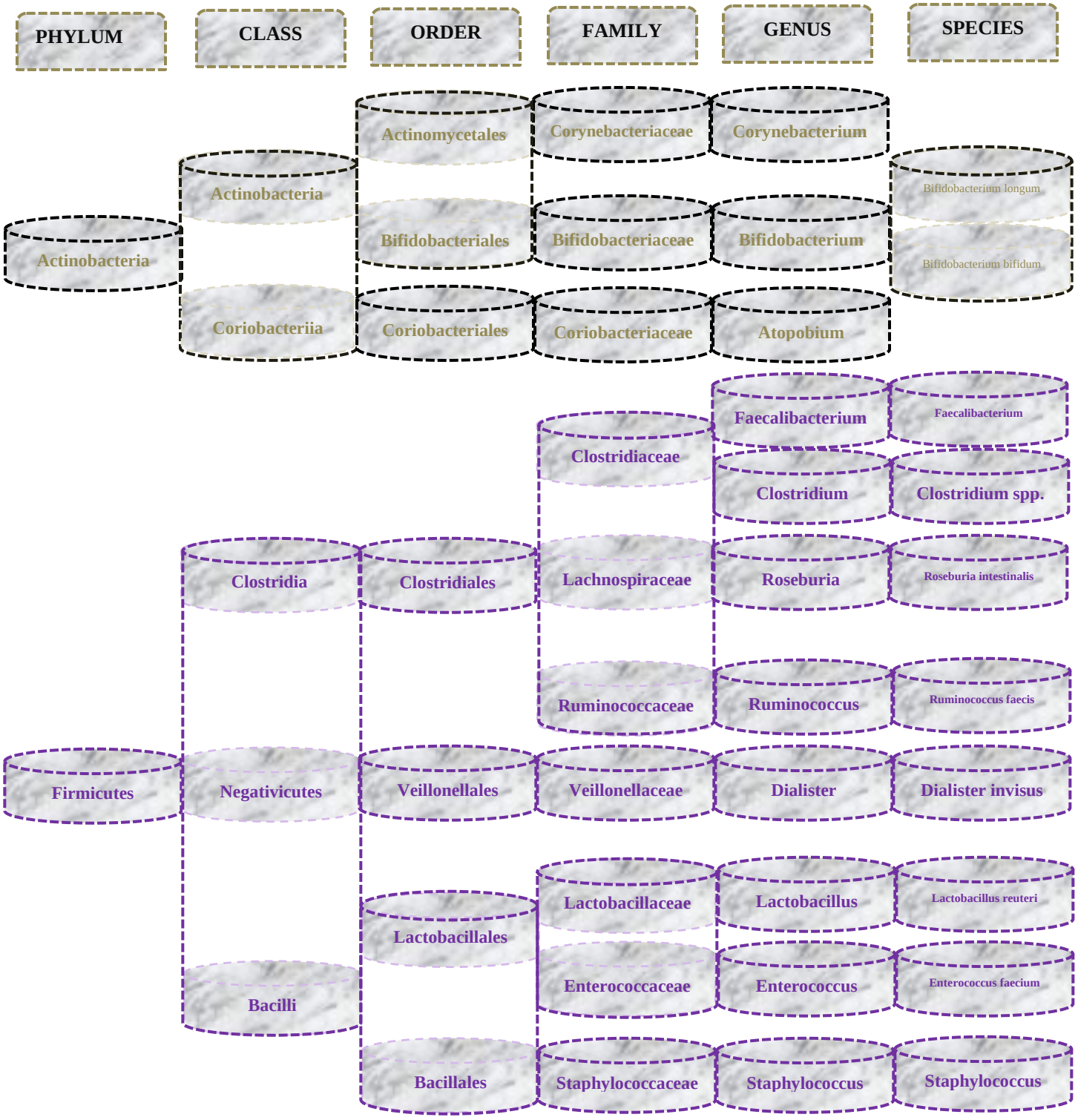
The human digestive system hosts about 100 trillion microorganisms which are almost 10 times more than entire somatic cells in the human body. While the human genome is only composed of 23 thousand genes, the total DNA of the human microbiome is more than 4 million genes. The number of bacterial cells in the human microbiome is also 100 times greater than that of the human body [136]. This interesting number and diversity of microorganisms within the human gastrointestinal tract have provided a unique ecology for probiotic bacteria responsible for the decomposition and digestion of undigested food products. The main portion of the gut microbiota is in large intestine. Most of the gut microbiota population consists of *Bifidobacterium, Lactobacillus, Bacteroides, Clostridium, Escherichia, Streptococcus,* and *Ruminococcus* bacteria [137].

Probiotic strains are usually harvested from either Lacto-fermented foods or the human gastrointestinal tract. Four common probiotic microorganisms are *Lactobacillus, Bifidobacterium,* certain *Bacillus* species, and *Saccharomyces boulardii.* However, *Akkermansia municiphila* and *Faecalibacterium prausnitzii* have also been found in the human microbiome but not yet commercialized [138]. In the large intestine, as the primary site for colonization of the human microbial ecosystem, harbors several hundred grams of microbes. **Figure 6** illustrates existing

bacteria phyla within the human gut microbiome. The main phyla identified as Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia which 90% of all the phylotypes belong to Firmicutes and Bacteroidetes phyla only [139]. The ratio of abundance of these two phyla is a key element in indicating the modulation of gut microbial composition. A lowered ratio of abundance of Firmicutes to Bacteroidetes indicates a healthy gut microbiome [140]. Given that the Firmicutes are the phyla inhabited within the intestinal environment and considered as gram-positive bacteria. While Bacteroidetes are gram-negative bacteria harbored in the same ecosystem, the key element in their susceptibility difference against polyphenol compounds may be associated with their different cell wall structure [141]. In other word, a healthy gut microbiome that prevents diseases such as obesity, psoriasis, autism, and mood disorders is identified as a community of mixed Gram stain bacteria in which Gram-negative bacteria have a higher ratio than Gram-positive bacteria [140].

Besides the impact of food composition on the quality and quantity of gut microbiota, other variables, including but not limited to age, gender, ethnicity, method of birth, and environmental factors, need to be considered while studying the gut microbiota profile [142]. The increased incidence of gut microbial imbalance among the western populations may be a result of the diet style in developed countries, yet the impact of other lifestyle variables cannot be ignored entirely [143]. However, modification of diet composition may promote human health through the modulation of gut microbiota. The positive effect of a diet rich in polyphenols on gut microbiome integrity and functionality has shown that the phenolic compounds may selectively suppress the growth of gut pathogenic bacteria while maintaining the growth of probiotic bacteria in the gut microbiome [144].

Despite the overall difference in gut microbiome compositions within different individuals, a comparison analysis between two different individuals has indicated that more than 65% of the resistance genes derived from cultured aerobes retrieved from their gut microbiome. An increasing number of currently becoming multidrug resistance species were once considered harmless residents of the human gut microbiota [145]. However, as individuals get older, the gut microbiome composition converges to a more similar microbiome. Human study has indicated that almost 40% of the genes from each individual were found, and similar in 50% of all participants [146].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Faecalibacterium
Clostridium
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Dialister
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Enterococcus
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Roseburia intestinalis
Ruminococcus faecis
Dialister invisus
Lactobacillus reuteri
Enterococcus faecium
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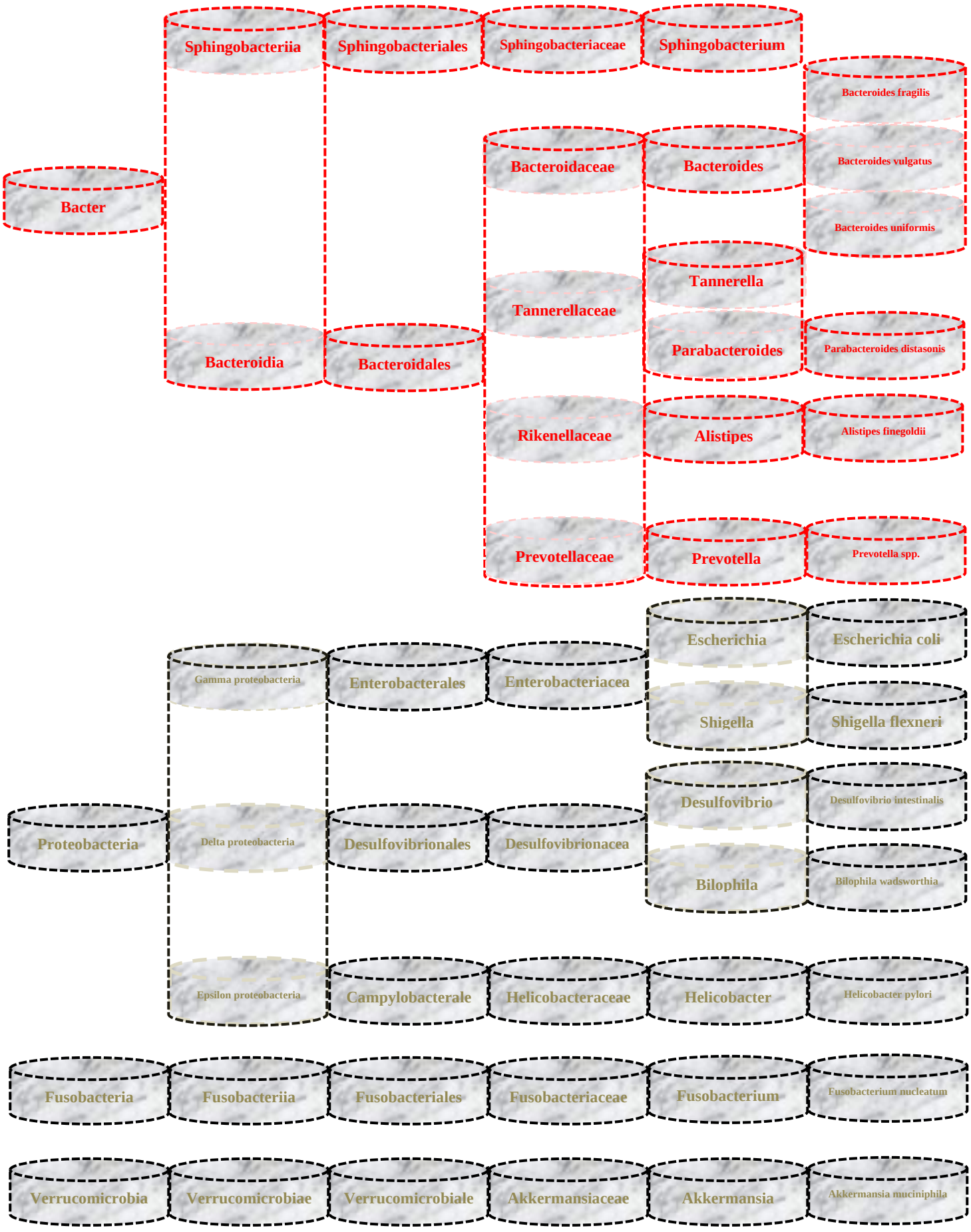
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Tannerellaceae
Tannerella
Parabacteroides
Parabacteroides distasonis
Rikenellaceae
Alistipes
Alistipes finegoldii
Prevotellaceae
Prevotella
Prevotella spp.
Proteobacteria
Gamma proteobacteria
Enterobacterales
Enterobacteriacea
Escherichia
Escherichia coli
Shigella
Shigella flexneri
Delta proteobacteria
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Fusobacteria
Fusobacteriia
Fusobacteriales
Fusobacteriaceae
Fusobacterium
Fusobacterium nucleatum
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Verrucomicrobiae
Verrucomicrobiale
Akkermansiaceae
Akkermansia
Akkermansia muciniphila

Figure 6. Schematic illustration of human gut microbiota composition- Adopted from Emanuele Rinninella et al., 2019.

The Synbiotic Effect of Polyphenol and Human Gut Microbiome

Among all strategies for modulating the metabolic activity and human gut composition, probiotics, prebiotics, and polyphenols (also known as the three Ps) are defined as the most well-stablished factors in promoting gut health [147]. Recently the new definition for prebiotics has introduced them as non-digestible compounds that are further metabolized by gut microorganisms and conferred beneficial physiological effects on the host. Polyphenols and particularly tannins are suggested to possess prebiotic effects and promote the growth of probiotic bacteria, specifically Lactobacilli and *Bifidobacteria* [148]. The ratio of predominant bacteria in human gut microflora is different from that of the dominant bacteria in animals' digestive tracts (including rats and guinea pigs). Regardless of this quantitative difference, a study on the effect of tannic acid on the population of rat microflora has shown that the numbers of all culturable microflora decrease significantly up to six days of treatment with tannic acid at the concentration of 45.0 mg per 100 g of rat body weight daily. However, a significant increase in the number of all tested microbial population including total bacterial flora, total coliforms, and a number of *E. coli* per mg feces by 10^7, 10^5, and 10^4 folds, respectively (compared to their respective normal levels) was observed after 21 days of the treatment [149]. Metabolism of gallic acid by human gut microbiota has shown that their gallic acid has reduced the growth of a group of harmful bacteria such as C. *histolyticum* at the concentration of 1000 mg/L while not having a negative effect on the bacteria being tested (*Atopobium* spp., *Bifidobacterium* spp., *Lactobacillus* spp., and *Bacteroides* spp.) [150].

One of the most common approaches in modulating the gut microbiota population and preventing the loss of probiotic bacteria in the gut environment is to take probiotic supplements, which can improve the quality and quantity of the gut microbiome. The tannic acid supplement is suggested to be beneficial in promoting intestine function (such as the beneficial effects on fermentation process in caecum) at the optimal concentration of 1-1.5%. The improvement in gut microbiota composition is attributed to the ability of a tannin-rich diet to increase the number of Bacteroides, *Bifidobacterium,* and *Lactobacillus* while decreasing the number of *Clostridium* species [151]. Total consumption of 30 grams of cranberry per day for 5 days has shown a decrease in the number of Firmicutes within the fecal samples while increasing that of Bacteroidetes. The decreased number of infection and antibiotic resistant bacteria such as *Clostridia* and *Oribacterium* within fecal samples has also suggested a positive association between consumption of cranberry powder and gut microbiota modulation [152]. An *in-vivo* study on pigs treated with Grape seeds extract (1% of the total diet) for six days has shown an increase in beneficial bacteria such as *Lacbnospiranceae, Ruminococcaceae,* and *Lactobacillus* [153]. After degradation of polyphenols by gut microbiota to their metabolites, parent compounds and their metabolites will then modulate gut microbiota, and this reciprocal benefit leads to a healthy gut microbiome [9].

The molar mass and size of tannins will influence their bioactivity. As the size of the molecule gets bigger, the antinutritional and biological activities such as cell permeability and absorption of the molecules are lowered [154]. The primary chemical structure of hydrolysable tannin can be cleaved into smaller fragment molecules by the action of enzymes. Tannase is naturally produced and available in plants, animals, and microorganisms, while the primary sources for the production of this enzyme are still microorganisms. The bacterial strains capable of the production of tannase

are reported as *Bacillus pumilus, Bacillus polymyxa, Corynebacterium sp., Klebsiella pneumoniae, Streptococcus bovis, Selenomonas ruminantium* as well as *Staphylococcus lugdunensis, S. gallolyticus, Lactobacillus plantarum,* and *Enterobacter* spp. that are genetically analyzed and identified as the only bacterial sources in production of tannases [49, 155]. The biochemical pathway involved in the degradation of tannins by gut microbiota is identified as bacterial secreted enzymes, including tannase and gallate decarboxylase [156]. For instance, *Lactobacillus plantarum,* as a member of lactic acid bacteria, is suggested to possess the ability to encode for tannin acyl hydrolase and gallic acid decarboxylase [157]. Tannase starts the tannin degradation process by catalyzing the hydrolysis of galloyl ester bonds to dissociate gallic acid from the central glucose moiety in the structure of hydrolysable tannin. The gallate decarboxylase enzyme then dissociates the carboxyl group from gallic acid to produce pyrogallol. The dissociation reaction may continue to produce resorcinol [158] or go through further methylation reaction to create methyl gallate [157]. As the ability of tannin to bind protein and iron becomes toxic to bacteria by inhibiting their growth and further killing them, some microorganisms are naturally equipped with specified genes in their genomes that encode degrading enzymes such as tannase [159]. The increased growth of bacteria strains after continued treatment with tannic acid is attributed to enterobacteria's ability to produce and secreting tannase enzyme. The beneficial effects associated with tannins in the digestion process may be affected by other food components such as iron and protein [160]. The enzyme not only dissociates the bonds between tannins and proteins, but also breaks down the internal chemical bond within the structure of tannins [161].

Sumac Polyphenols and Catabolism Pathway

Digestion is a complex phenomenon that involves several mechanical and chemical processes on food components upon their entries into GIT. The primary outcomes of the digestion process consist of enzymatic depolymerization of oligomers and polymers, destruction and decomposition of the compounds that are susceptible to acid and base exposure, and the formation of complexes with proteins, etc. These alterations in the chemical compositions of food components may result in significant changes in the concentration of the targeted compounds and their bioactivity power [162]. Although almost all plant bioactive compounds are affected by digestion, the change among tannin compounds is higher compared to that of the other phenolic compounds. Tannins and their metabolites may be resistant to changes in digestion pH; however, they may change the activity of proteins such as gastric enzymes. The interaction between tannins and enzymes is highly associated with the concentration of tannins. While low concentrations of tannins may boost the catalytic activity of enzymes, the high tannin concentration reduces the catalytic activity of the enzymes [163].

The lowest absorption of polyphenols occurs in the stomach, where despite the high acidic environment, the low molecular weight polyphenols are stable, and glucose bonded polyphenols are partially hydrolyzed. While low molecular weight polyphenols are partially absorbed either directly or after phase II metabolic conversion within the small intestine, the high molecular weight polyphenols such as tannins are transported into the colon intact. The transformation of polyphenols to their metabolites that increase their bioavailability and absorption occurs through a catabolism process of polyphenols. If not catabolized by the colon microbiome, they will then be excreted from the body without absorption. The catabolism pathways involved in catalyzing of

the polyphenols by microbial enzymes include hydrolysis (O-deglycosylations and ester hydrolysis), cleavage (C-ring cleavage, delactonization), and reduction (dihydroxylation and double-bond reduction) reactions [164-165]. Due to xenobiotic metabolism, more polar metabolites such as glucuronide sulfate and methyl conjugates will be excreted via kidneys or bile. These metabolites can be found in urine and plasma as the result of enterohepatic circulation thereby, the metabolites are efflux back to the intestinal lumen [166].

The large structure polyphenols such as tannins go through further intestinal enzymatic transformation steps before absorption in the systemic circulation. The final absorption of tannin molecules requires the completed transformation of these compounds to their smaller bioactive constituents by the action of a team of probiotic bacteria species [86]. A thorough metabolism and decomposition of hydrolysable tannins by gut microbiota includes an initial deglycosylation of the sugar moieties and carbon-carbon cleavage of heterocyclic and aromatic rings, dihydroxylation, decarboxylation, and hydrogenation of alkene constituents [167]. Deglycosylation of gallotannins through the action of tannase enzyme creates a so-called gallic acid pool which serves as gallic acid reservoir and may be either absorbed or undergone further decarboxylation to produce gallic acid metabolites upon microbial fermentation.

CHAPTER III

PHYTOCHEMICAL CHARACTERISTIC OF SUMAC (*RHUS CORIARIA*)

Introduction

Sumac has a broad range of culinary applications from use as the primary ingredient in za'atar (a common spice used for different Arab dishes) to marinating and tenderizing agent for meat products to directly dusting it on other foods, including cooked meat, salads, bread, and desserts has provided sumac the ability to fit into the Green Med Diet. Sumac is a ubiquitous and robust plant that can grow under various environmental conditions. The plant can grow on lowlands, soil eroded, or bare lands, even on calcareous substrates or steep rocky slopes where no competition exists among plants in the region. The fruit has reportedly been growing in different geographic altitudes (altitudinal range of 550-1354 m a.s.l.) and wide range of temperature and precipitation equal to or greater than 500 mm precipitation annually.

Sumac has been incorporated into food to prepare different middle eastern dishes. In Kurdish cuisine, finely powdered sumac on freshly cut onions are used as an appetizer. The use of sumac is not limited to its wide range of applications as a food ingredient. The fruit has been associated with treating many diseases, including upset stomach and bowel issues, fever, and gastrointestinal complications. The use of sumac as a remedy for internal sores, ulcers, and wounds on the mucous layer has also been reported [168].

Materials and Methods

Sumac Fruit

The dried sumac fruit was purchased from a retail grocery store in Houston, USA. The packages were shipped to the lab under ambient temperature at College Station, TX. Upon receipt, the sumac fruits were packaged into 50-gram using a lab vacuum sealer and stored at 4 °C for further analysis.

Chemicals and Reagents

Analytical-grade methanol, ethanol, and acetone were purchased from Sigma Aldrich Co. (St. Louis, MO, USA) and used for extraction purposes. References compounds of gallic acid, sodium carbonate (Na_2CO_3), formic acid, and Folin-Ciocalteu phenol reagent were also procured from Sigma Aldrich Co. and used as received. The bacteria strains *Listeria monocytogenes* (Scott A Ser 4b), *Enterococcus faecium* (NRRL B-2354), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* O157:H7 (43895™), *Salmonella enterica* Serovar Typhimurium (ATCC 700720), *Pseudomonas aeruginosa* (ATCC 10145) were obtained from the Food Microbiology lab (Department of Food Science, Texas A&M University, USA).

Preparation of Fruit Extract

Briefly, 10 g of the sumac dried fruits were ground into powder using a grinder (Miracle Mill MC-17B, 150 W, Danbury, CT, USA). The ground fruit was then subjected to 100 mL of targeted solvent (ratio 1:10 sample to solvent), either absolute ethanol, methanol, water, or acetone. Extraction was done by homogenizing the powder using a high-shear homogenizer (PowerGen

500S1, 500W, Schwerte, Germany) for 10 min at a speed level of 4 or 16000 rpm at ambient temperature. Further homogenization was achieved using a bath sonicator (Branson, 3510R-MT,130W, Danbury, CT) for 15 min. The sample was then centrifuged for 10 min at 3,000 × g (Eppendorf, 5810R,1300W, Hamburg, Germany). The supernatants were collected and passed through a filter paper (Whatman Grade-4 filter paper with particle retention of 25 µm at 98% efficiency, Maidstone, United Kingdom). The excess solvent was evaporated under reduced pressure using a rotary evaporator (Buchi, R-200, Essen, Germany) at 45°C for 1 hour. The extracts were further concentrated using vacuum evaporation equipment (Thermo-Fisher Scientific, Savant ISS 110, Waltham, MA, USA) to reach a final concentration of 5, 10, 15, 20, and 25 mg/mL. Finally, the samples were filtered through 0.22 µm PTFE members (Millipore, Burlington, MA, USA). The concentrated extracts were stored at 4°C for analysis of the total phenolic content and antibacterial assays.

Antioxidant Activity of Fruit Extracts (Folin-Ciocalteau Assay)

The total phenolic content was determined using the Folin-Ciocalteu method [169-170]. The extracts were diluted down and subjected to Folin's reagent, sodium carbonate, and water, respectively. The samples were then run in a spectrophotometer instrument (Thermo Electron Corp, Helios Gamma, UVG 1202E, Hemel Hempstead, UK). The optical density values were plotted against the Gallic acid standard curve to determine different polyphenol contents among the extracts. The total phenolic content of each extraction sample was calculated separately and reported as gallic acid equivalents (GAE) per g fruit dried weight (DW).

Determination of Phospholipid Content

Phosphate group quantification assay

The Thermo fisher scientific phosphate test kit was used to evaluate solvent extraction samples quantitatively. For each sumac sample, different concentrations of 15.62, 31.25, 62.50, 125, 250, 500, and 1000 ppm were prepared by mixing the sumac extract with adequate amounts of Milli-Q water. A volume of 50 µL of each sample was transferred to a 96-well polypropylene microplate, and 30 µL of the phosphate reagent was mixed with samples in each well. The final volume of each well was adjusted to 200 µL by adding 120 µL of Milli-Q water. The absorbances of the samples were measured at an Optical Density of 650 (OD560).

Standard Curve for Phosphate Content

Phosphate standard for colorimetric detection was prepared based on the method previously published [171]. Briefly, 10 mM Phosphate Standard stock solution (Sigma Aldrich, St. Louis, MO, USA) was purchased, and 10 µL of the stock was mixed with 990 µL of water to prepare a 0.1 mM phosphate standard solution. Then, a standard curve was calculated by generating 0 (blank), 1, 2, 3, 4, and 5 nmol/µL standard.

Phenolic Identification and Quantification

The phenolic compound in sumac fruits was analyzed using a Thermo-Finnigan Surveyor HPLC-PDA in tandem with an LCQ Deca XP Max ion trap spectrometer with an ESI source equipped with a C18 column (Sunfire C18 Reversed-phase, 4.6 × 250 mm, 18.5 µm particle size, Waters Company, Milford, Massachusetts, USA). The mobile phase consisted of water (mobile

phase A) and methanol (mobile phase B), both containing 1% formic acid at a flow rate of 0.45 mL/min. The gradient began with 100% mobile phase A and was maintained for 2 min, and then phase A was decreased to 90% over 10 min, 60% for 25 min, 35% at 35 min, and 15 at 41 min, and maintained for an additional 15 min. Analysis was in negative ion mode for gallic acid. ESI-MS parameters included sheath gas flow of 8, the potential of the ESI source voltage at 4.50 kV, the capillary temperature at 350 °C, the capillary voltage at 7 V and 80 μL, respectively (Martini et al., 2017). Semi-quantification was performed against mg/L gallic acid equivalents present in sumac extracts. After incubation, samples were deactivated using 6-fold acidified methanol. The samples were then purified through 0.22 μm PTFE members (Millipore, Burlington, MA, USA).

Statistical analyses

All results were expressed as mean ± standard error (SE) using GraphPad Prism version 5. Data of growth inhibition of the bacteria were evaluated in triplicate using the calculated means from ANOVA (analysis of variance) followed by comparing each pair with control.

Results and Discussion

Total Reducing Capacity in Sumac Extract

It is known that the antioxidant and radical scavenging activity of sumac is mostly proportional to polyphenolic content [172]. The antioxidant activity of the sumac extracts was evaluated by performing a Folin-Ciocalteau assay, which is commonly used to determine the total reducing capacity value of the samples. The extraction efficiency strongly depended on the nature of the solvent. With extraction by methanol, ethanol, water, and acetone, total reducing capacity

obtained ranged between 27.0 ± 0.4 g and 102.0 ± 0.6 g of gallic acid equivalent per g of dried weight (DW). The methanolic extraction yielded the highest total reducing capacity among all samples, followed by ethanolic, water, and acetone extraction. The total reducing capacity extracted by methanol was about twice of that by water and four times that by acetone. Ethanol solvent was selected for further analysis due to the possible risk of toxicity associated with other extraction solvents against human health. Ethanol has been reported as a high polar solvent that has been widely used for the extraction of more polar compounds. Another benefit associated with ethanolic extract is that ethanol has been known as a safe chemical compound for food safety while it is also categorized as a polar solvent in food industries [173]. That is, the solvent residual in food products does not convey any risk to consumers as ethanol has been categorized as a safe additive based on FDA's GRAS list [174]. This solvent has also shown higher extraction yield due to its high polarity and extraction temperature associated with this solvent [175]. Since the sample was prepared using the same extraction procedure, the total polyphenol content signifies different partition coefficients of sumac phenolic in various solvents. Such a variation can be attributed to the difference in the relative polarity of phenolic compounds and solvents. Likewise, the proticity of solvent (i.e. the ability to donate hydrogen bonding groups) may also play a role in the differences in the portioning of sumac phenolics in methanol, ethanol, and water.

The extraction of sumac (*Rhus coriaria*) dried fruit powder using different organic solvents including methanol, ethanol, acetone, and water exhibited various polyphenol contents within each extract (**Table 5).** The same extraction procedure was carried out using four different solvents to extract the highest polyphenol content. Unlike reported by [176], the ethanolic extraction did not show the highest polyphenol content. The lower polyphenol content in ethanolic extract compared

to that of the methanolic extracts may be due to decreased accessibility of the phenolic compounds to the Folin-Ciocalteu reagent. The emulsifiers present in sumac ethanolic extracts may create the biopolymer coats around the phenolic substances, which causes extra protection for polyphenols exposure to the reducing agent in the solution. A study on encapsulation of grape seed extracts with soy lecithin has shown a reduction in polyphenol content compared to intact extract indicating a minimized interaction between grape seed polyphenols and the reducing agent in Folin's assay [177].

Table 5. Total phenolic compounds of fruit sumac extracted using different solvents.

Type of solvents	Total phenolic content [a] (mg/g dried fruit)
Methanol	101.72±0.56
Ethanol	83.21±0.34
Water	51.41±0.51
Acetone	27.24±0.41

[a] Values represent the means and standard errors from triplicate of total phenolic contents of fruit samples.

The solvent type plays an essential role in optimizing the phytochemical content of the extraction [178]. The use of water along with an organic solvent increased the extraction yield of the polar compounds [179], while the use of non-polar solvents for the extraction of hydrophobic compounds was suggested [180]. However, the highest yield has been reported from a mixture of four solvents, including methanol, ethanol, water, and acetone. Applying the same ratios of the solvents has resulted in a higher extraction yield compared to that of individual solvents [181]. Although the addition of water in the solvent is recommended to increase the solubility of the

compounds and maceration of the fruit [182], use of water in the solvent solution repel the hydrophobic compounds. While using methanol as an organic solvent with the highest extraction yield was recommended [183], other studies have reported the highest extraction yield using ethanol in their extraction procedure [184]. Moreover, the difference in polyphenol contents may be associated with the chemical structure of the solvent that would interact with the chemical components of the sample. This interaction may also depend on the presence of unknown and interfering substances impacting the extraction rate of the phenolic compounds.

Sumac phospholipid

The concentration of phosphate groups within sumac extracts (as an indicator for phospholipid content) was tested, as shown in **Figure 7**. It has been recommended to choose a proper solvent for extraction based on the chemical nature and particle size of the compound of interest. However, the presence of interferences can affect on the overall behavior of the compound. Generally, some organic solvents are more polar than the others are. That is, methanol, ethanol, acetone, diethyl ether, and ethyl acetate have been used for extraction of polyphenolic compounds; however, due to the hydrophilic nature of some of the phenolic groups such as benzoic and cinnamic acid, a mixture of water in different ratios to the organic solvents has been recommended. On the other hand, the less polar solvents are used for extracting hydrophobic compounds such as waxes, oils, sterols, chlorophyll [185].

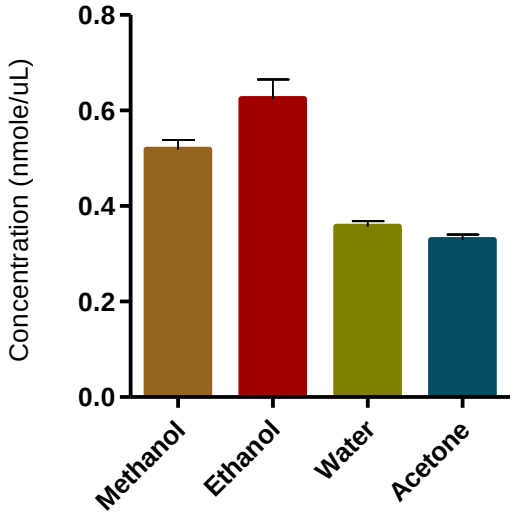
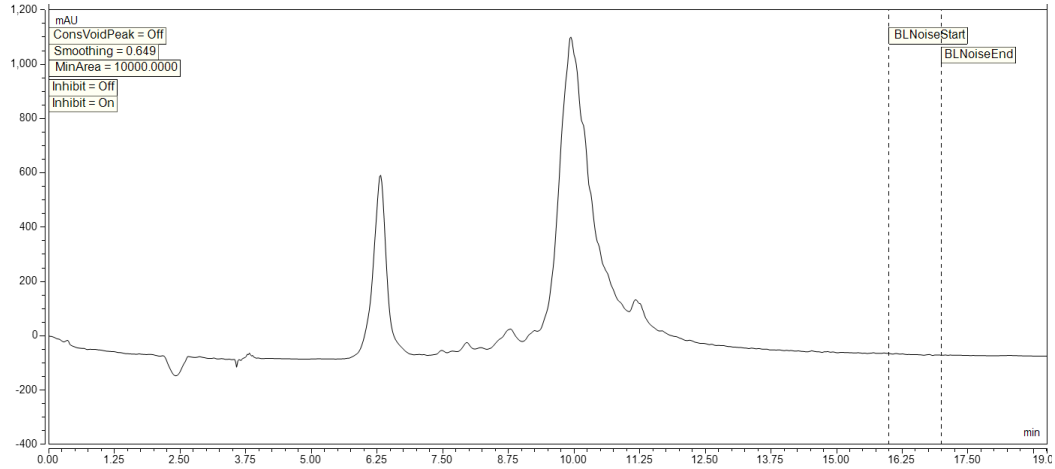


Figure 7. Sumac phospholipid content.

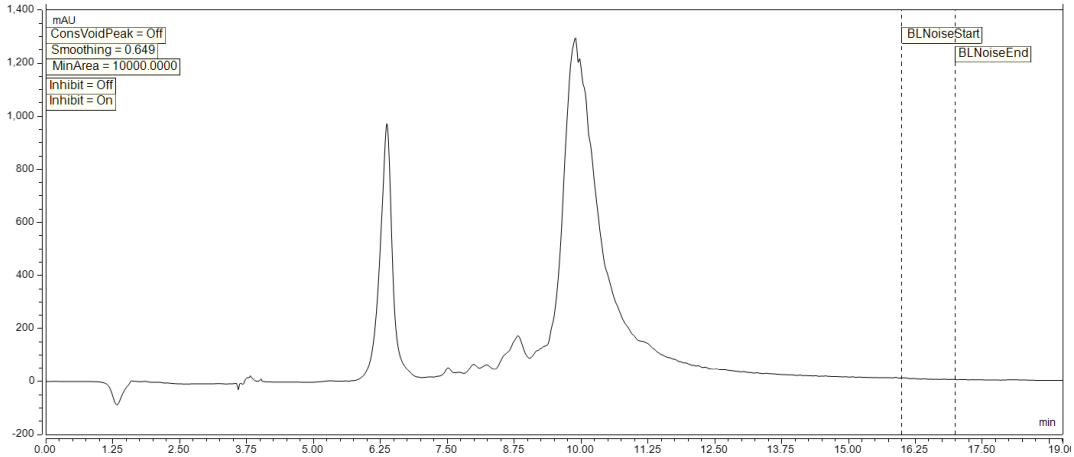
UPLC/ Mass Spectrometric Analyses

Further details of polydispersity characteristics of polyphenols can be seen from LC-MS spectra. Regardless of the solvent being used, gallic acid was the predominant phenolic compound within all sumac extracts, while the concentration of gallic acid showed a weak variation from solvent to solvent. Moreover, galloyl derivatives metabolism was assessed through the chromatography analyses on the samples (**Figure 8**).

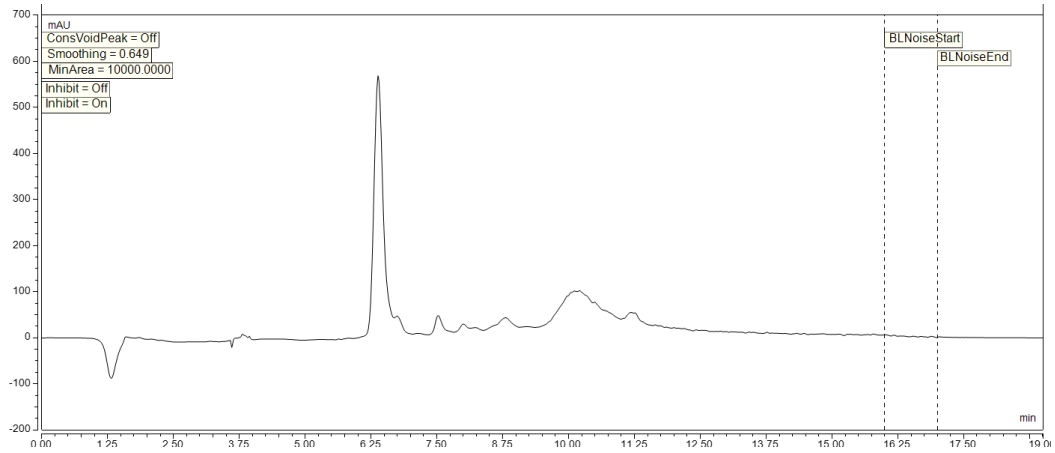
A) Ethanol extract



B) Methanol extract



C) Water extract



D) Acetone extract

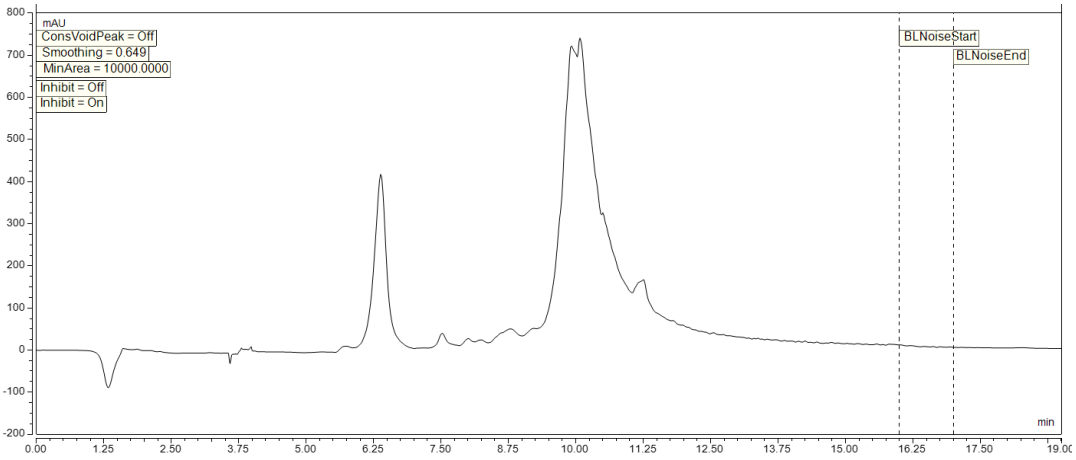
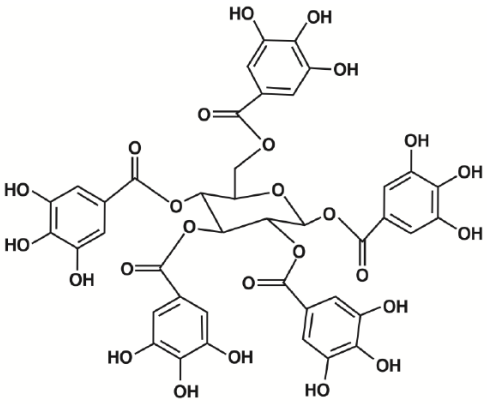


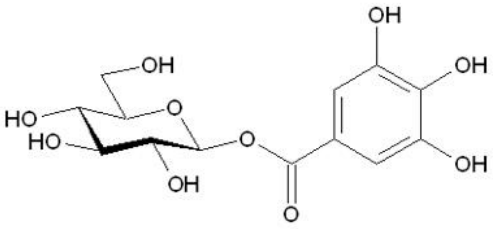
Figure 8. Representative ion monitoring chromatogram of UPLC showing different elution of sumac ethanolic (A), methanolic (B), aqueous (C), and acetone (D) extracts.

Additionally, the initial concentration of high molecular galloyl glucosides and their galloyl derivatives was investigated (**Figure 9**). The standard solutions for penta-galloyl-

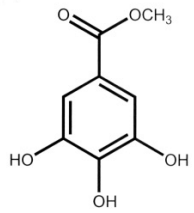
glucoside, mono-galloyl-glucoside, methyl gallate, ethyl gallate, gallic acid, protocatechuic acid, and pyrogallol were referenced, and the samples were analyzed against the standards.



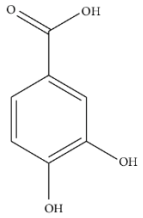
Penta-Galloyl-Glucose



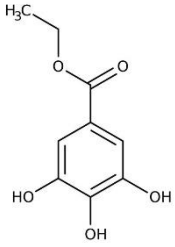
Mono-Galloyl-Glucose



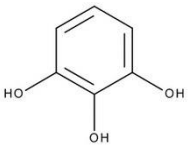
Methyl Gallate



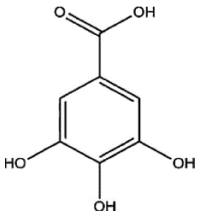
Protocatechuic acid



Ethyl Gallate



Pyrogallol



Gallic acid

Figure 9. Galloyl derivatives (created with BioRender.com).

The amount of methyl gallate and ethyl gallate in the samples was highly impacted by the organic solvent being used to extract of the fruit polyphenols. Samples extracted with ethanol exhibited a significantly higher concentration of ethyl gallate within their polyphenols profile. Samples prepared using methanol for their extraction showed substantially higher concentrations of methyl gallate in their profile, indicating the role of ethyl and methyl groups in the production of ethyl and methyl gallates in the samples. In addition, the concentration of protocatechuic acid was greater in water and acetone extracts. Acetone and Ethanol samples exhibited the highest and second-highest gallic acid content, respectively (**Figure 10**).

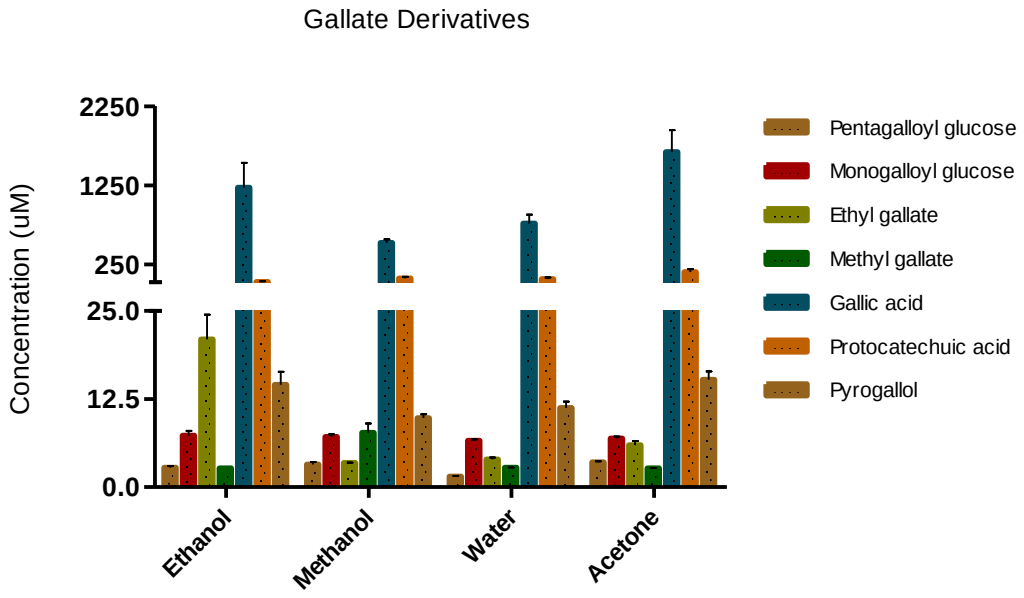


Figure 10. Concentration (µM) of gallate derivatives.

Figures 11 and **12** shows the conversion of free gallic acid from free and nanoparticle complexed tannins that occurred through the activity of the tannin acyl hydrolase enzyme, commonly known as tannase. The enzyme can cleave the ester bond between galloyl and glycosyl moieties to produce gallic acid molecule, which undergoes further reactions [186].

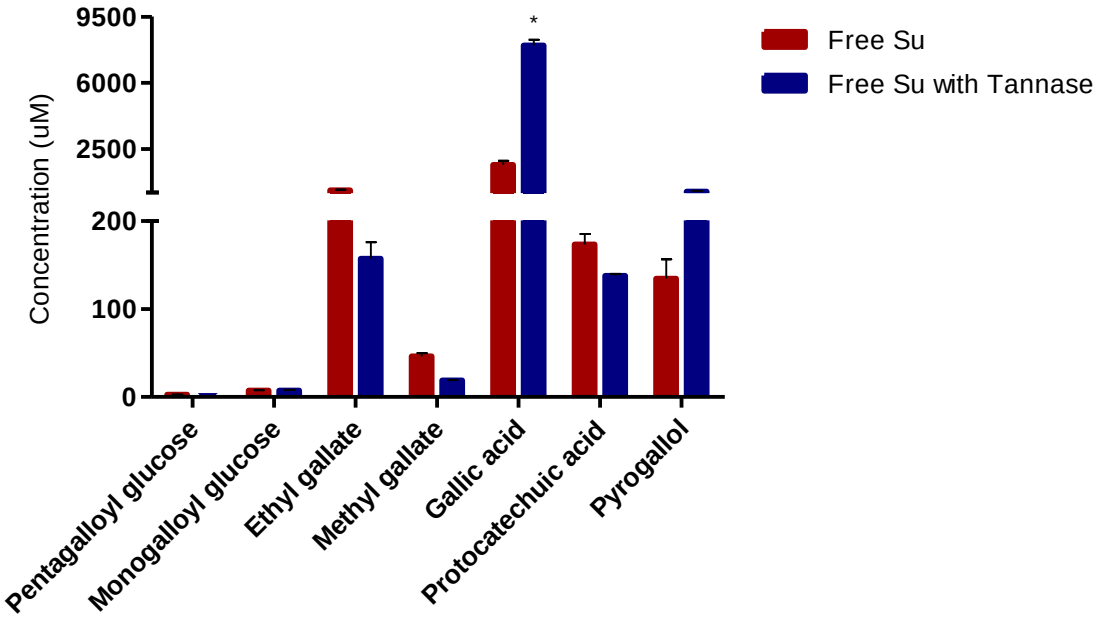


Figure 11. Sumac ethanolic extract-Gallate derivatives (penta-galloyl-β-D-glucose, mono galloyl-β-D-glucose, ethyl gallate, methyl gallate, gallic acid, protocatechuic acid, and pyrogallol) treated with and without tannase at a concentration of 0.4 mg/mL at 37 ºC.

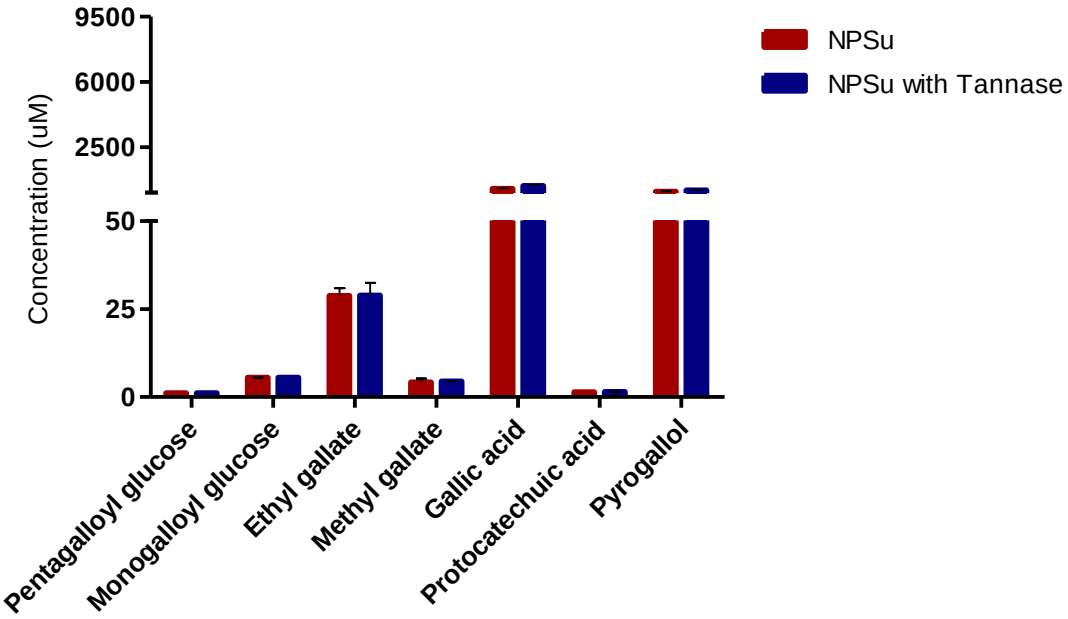


Figure 12. Nanoparticles of sumac ethanolic extract-Gallate derivatives (penta-galloyl-β-D-glucose, mono galloyl-β-D-glucose, ethyl gallate, methyl gallate, gallic acid, protocatechuic acid, and pyrogallol) treated with and without tannase at a concentration of 0.4 mg/mL 37 ºC.

The degradation of gallic acid may undergo either dihydroxylation or decarboxylation reactions on its hydroxyl or carboxyl groups respectively. The results of gallic acid dihydroxylation may result in smaller molecular compounds such as protocatechuic acid (3,4-dihydroxybenzoic acid), 2,5-dihydroxybenzoic acid, also known as gentisic acid, 3 and/or 4-hydroxybenzoic acid, etc. metabolites. The decarboxylation reaction of gallic acid may result in the production of pyrogallol metabolite [187]. The concentration of pentagalloyl glucoside as the initial product of tannin hydrolysis (becomes available from simplest tannin called 2-*O*-digalloyl-1,3,4,6-tetra-*O*-galloyl-β-D-glucopyranose) and Pyrogallol (the most broken-down structure in the list) were picked from the list of compounds being tested in the samples.

Conclusion

Based on the solvent polarity and its potential to interact with the target compound, each organic solvent has been recommended for extracting specific chemical compounds. The higher concentration of smaller compounds, including gallic acid, protocatechuic acid, and pyrogallol in acetone and water samples, may be attributed to the high number of free gallic acid molecules in the extracts. The production of methyl and ethyl gallates in methanol and ethanol extracts requires the usage of free gallic acid in the solution. This may be explained by which the amount of the same molecules within ethanolic extract have increased after treating with tannase. Finally, the addition of the tannase enzyme catalyzed the dihydroxylation and decarboxylation of the larger galloyl esters to their simple phenolic constituents.

CHAPTER IV

ANTIBACTERIAL ACTIVITY OF FREE SUMAC EXTRACTS AGAINST PATHOGENIC BACTERIA

Introduction

Food spoilage and food poisoning are a concern the food manufacturing. Most of the food poisonings are associated with bacteria contamination, especially Gram-negative bacteria (*Escherichia coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*), and the bacteria associated with foodborne diseases are known to be *Staphylococcus aureus* and *Bacillus cereus*. The chemical preservatives are usually applied to prevent those issues among food industries which may have a negative impact on human health due to chemical residue present in farm to fork supply chain and microbial resistance to the used chemicals [188]. Therefore, some friendly antibacterial agents and naturally occurring plant compounds are required to be used that are considered safe if they are applied in an effective concentration, due to being non-toxic, effective on pathogenic and spoilage bacteria, being economical, and feasible [189].

Materials and Methods

Microorganisms Preparation and Inoculation

The antibacterial activities of the sumac extracts were studied on Gram-positive bacteria *L. monocytogenes* (Scott A Ser 4b), *E. faecium* (NRRL B-2354), *S. aureus* (ATCC 6538) and Gram-negative bacteria *E-coli* O157:H7 (43895™), *S. enterica* Serovar Typhimurium (ATCC 700720), *P. aeruginosa* (ATCC 10145). Isolation of the microorganisms was performed based on previously

reported method [190-191]. Briefly, the target cultured microorganisms were aseptically harvested from tryptic soy agar (TSA; Becton, Dickinson, and Co., Sparks, MD, USA) slants using a sterile inoculating loop. One loop-full (10 µL) of each target cultured bacteria was transferred to 9.0 mL of brain heart infusion broth (BHI; Becton, Dickinson, and Co.) and incubated at 37°C for 24 h. The second consecutive transfer from BHI tubes post-incubation was prepared by transferring a loop of culture (10 µL) to a sterile BHI test tube. The inoculated BHI tubes, each inoculated with a single bacterial isolate, were then incubated for 24 h at 37°C. Serial dilutions of inoculated BHI tubes were prepared using 0.1% (w/v) peptone water (Becton, Dickinson, and Co.). Tryptic soy agar plates were then prepared by inoculating serial dilutions onto their surfaces to enumerate numbers following revival. The plates were then incubated at 37°C for 24 h. The numbers of bacteria were enumerated to allow for the preparation of microbes for later study to a standard 6.0 to 7.0 $\log_{10}$ CFU/mL inoculum. A daily culture was also prepared by transferring a loop of culture (10 µL) from a culture tube to a 10 mL sterile BHI tube and incubation for 24 h at 37°C.

In Vitro Screening Using Disc Diffusion Method

The antimicrobial activity of treatment samples of sumac extracts was determined by the diffusion technique on TSA growth medium according to previously reported method with a slight modification [192-194]. The bacteria suspension was standardized to 10^6-10^7 CFU/mL in Brain Heart Infusion broth (BHI). 120 µL of the suspension containing bacteria were then mixed with 120 mL of Tryptic Soy Agar (TSA). The prepared bacteria suspension in medium (20 mL) was then transferred into the Petri dishes and placed for 10 min at ambient temperature. In the meantime, the discs (6mm Whatman grade AA discs, GE Healthcare Life Science, Buckinghamshire, UK)

were immersed and impregnated with 50 µL of corresponding concentrations of prepared extract samples. The filter discs were then dried and placed onto the surface of agar. Ethanol, methanol, distilled water, and gentamicin were used as experimental controls for each set of runs. A clear zone around each disc was observed after incubation time indicating the absence of bacteria in that area. The assessment was conducted by measuring the diameters of all bacteria strains' growth inhibition zones at 37 °C after 24 h exposure to the treatments. The diameters of inhibition zones were used as the measurement of antimicrobial activities. To ensure the precision and accuracy of the experiments, each assay was carried out in a triplicate fashion.

In vitro Screening Using Broth Microdilution Method

This assay was conducted based on broth microdilution assay previously reported with a slight modification [195]. The growth curves of bacteria strains treated with sumac extracts were measured using 96 well plate, two-fold Brain Heart Infusion (BHI) medium, and 6-7 $\log_{10}$ inoculum bacteria. Sumac extracts were also diluted with two-fold BHI (2X BHI) to obtain final concentrations of 5, 10, 15, 20, and 25 mg/mL. In the 96 microwell plates, 100 µL of samples containing BHI were transferred to the wells. To obtain a total volume of 200 µL, 100 µL of each microorganism was then transferred to each well. The BHI medium was considered as a negative control while positive control was a mixture of inoculum bacteria suspension and BHI medium. The microplate was loaded into an Epoch UV/Visible scanning spectrophotometer (Bio-Tek® Cytation Hybrid Multi-Mode Reader, Cytation 5 imaging, USA) at 37 °C for 24 h and readings were conducted every 10 min. Changes in optical density of bacteria suspensions were observed as the measurements for determination of pathogens' growth at 630 nm (OD_{630}).

Scanning Electron Microscopy (SEM)

The cells preparation was followed by the previously reported method [196]. Briefly, the treated bacteria cells were centrifuged at 12000 rpm for 5 min and washed with Milli-Q water 3 times to remove the media residuals. The cells samples were then placed on dry poly-lysine coated glass coverslips and fixed using glutaraldehyde (2.5 w/v%) for 2 hours under the fume hood. The cells were then washed using 0.1 molar cacodylate buffer gently. The Post fixed-step was followed by applying 1 wt% osmium tetroxide and leaving the poly-lysine coated glass-loaded samples at ambient temperature for 1 hour. The cells were then washed with cacodylate buffer and rinsed with 50% ethanol solvent for 30 seconds, moving up to absolute ethanol. Finally, samples were coated with ~10 nm carbon in an evaporator and imaged using scanning electron microscope SEM at Material Characteristic Facility (MCF) in Texas A&M University.

Statistical Analyses

All results were expressed as mean ± standard error (SE) using GraphPad Prism version 5. Data of growth inhibition of the bacteria were evaluated in triplicate using the calculated means from ANOVA (analysis of variance) followed by comparing each pair with control.

Results and Discussion

The Bacterial Inhibition Zones from Sumac Polyphenols

The disc inhibition zones of sumac extract against six strains of Gram-positive and Gram-negative bacteria are shown in **Table 7**. Based on the data, the following conclusions can be drawn: First, sumac extracts demonstrated a larger inhibition growth zone for Gram-positive than that for

Gram-negative bacteria. Second, sumac phenolic extracted by ethanol and methanol resulted in a much higher growth inhibition zone against Gram-positive and Gram-negative bacteria compared to sumac phenolic extracts by acetone (**Figure 13**). Extracts obtained by acetone were mostly ineffective in inhibiting growth. Third, while polyphenolics obtained by ethanol and methanol had statistically similar efficacy against Gram-positive bacteria, the growth inhibition for Gram-negative bacteria was higher with extracts obtained by ethanol. This is surprising given that the total polyphenol content was higher for methanolic extraction. Fourth, increasing extract concentration increased bacterial growth inhibition zone, but gradually less and less. Fifth, *S. aureus* had the largest inhibition zones among all bacterial tested while *E. coli* O157:H7 and *S.* Typhimurium had the smallest inhibition zones (**Figure 14**).

Table 6. The growth inhibition zone (mm) of sumac free extracts from disc diffusion against Gram-positive and four Gram-negative pathogens.

Type of Bacteria							
		Gram-positive			Gram-negative		
Concentration (mg/mL)	Type of solvent	*L. monocytogenes*	*E. faecium*	*S. aureus*	*E. coli* O157:H7	*S.* Typhimurium	*P. aeruginosa*
5	Methanol	6.7±0.1A	6.9±0.2A	6.6±0.1B	0.0±0.0A	0.0±0.0A	0.0±0.0A
	Ethanol	6.9±0.1A	6.8±0.1A	7.9±0.07A	0.0±0.0A	0.0±0.0A	0.0±0.0A
	Water	0.0±0.0B	0.0±0.0B	0.0±0.0C	0.0±0.0A	0.0±0.0A	0.0±0.0A
	Acetone	0.0±0.0B	0.0±0.0B	0.0±0.0C	0.0±0.0A	0.0±0.0A	0.0±0.0A
10	Methanol	10±0.1A	9.2±0.2A	11.5±0.3A	1.5±0.9B	0.0±0.00	0.0±0.0A
	Ethanol	10.1±0.2A	9.5±0.2A	11.5±0.2A	7.3±0.3A	6.6±0.1A	0.0±0.0A
	Water	2.2±1.1B	0.0±0.0B	3.1±1.2B	0.0±0.0C	0.0±0.0B	0.0±0.0A
	Acetone	0.0±0.0C	0.0±0.0B	0.0±0.0C	0.0±0.0C	0.0±0.0B	0.0±0.0A
15	Methanol	12.3±0.3B	11.9±0.1A	13.1±0.4B	6.9±0.2B	3±1.2B	0.0±0.0A
	Ethanol	13.9±0.1A	11.2±0.2A	15.0±0.2A	8.3±0.3A	9.0±0.2A	0.0±0.0A
	Water	7.4±0.3C	2.2±1.1B	7.8±0.3	3.1±1.2C	0.0±0.0C	0.0±0.0A
	Acetone	0.0±0.0D	0.0±0.0C	0.0±0.0B	0.0±0.0D	0.0±0.0C	0.0±0.0A
20	Methanol	14.9±0.2A	12.5±0.2B	13.5±0.2B	7.8±0.3B	7.3±0.3B	7.0±0.1A
	Ethanol	14.9±0.1A	13.9±0.1A	15.7±0.2A	9.8±0.5A	11.4±0.4A	8.0±0.2A
	Water	8.4±0.3B	7.9±0.3C	9.6±0.3C	6.9±0.17C	6.8±0.1B	1.6±1.1B
	Acetone	6.6±0.1C	0.0±0.0D	6.5±0.1D	0.0±0.0D	0.0±0.0C	0.0±0.0C
25	Methanol	15.6±0.2A	14.6±0.2A	14.1±0.2B	9.2±0.5B	8.7±0.3B	8.4±0.2B
	Ethanol	16.1±0.1A	14.6±0.2A	16.8±0.2A	11.2±0.5A	12.1±0.4A	11.2±0.2A
	Water	9.6±0.3B	9.0±0.3B	10.3±0.3C	8.7±0.3B	7.1±0.1C	7.7±0.1B
	Acetone	6.8±0.1C	6.3±0.04C	6.7±0.1D	0.0±0.0C	0.0±0.0D	0.0±0.0C

Results are presented as means of the three observations.
[A]Mean values± standard error sharing the capital letters in the columns are significant at $P<0.05$.

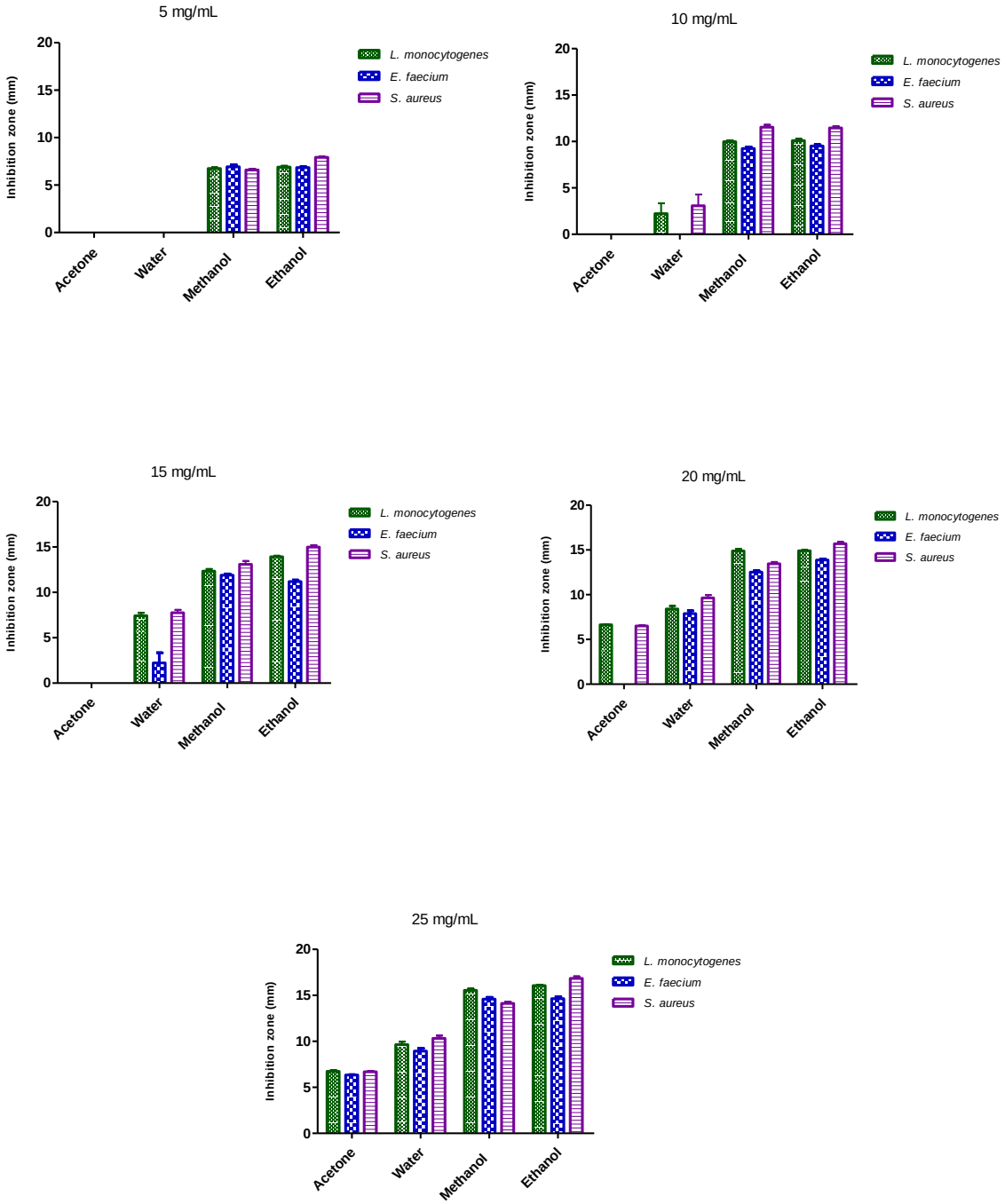
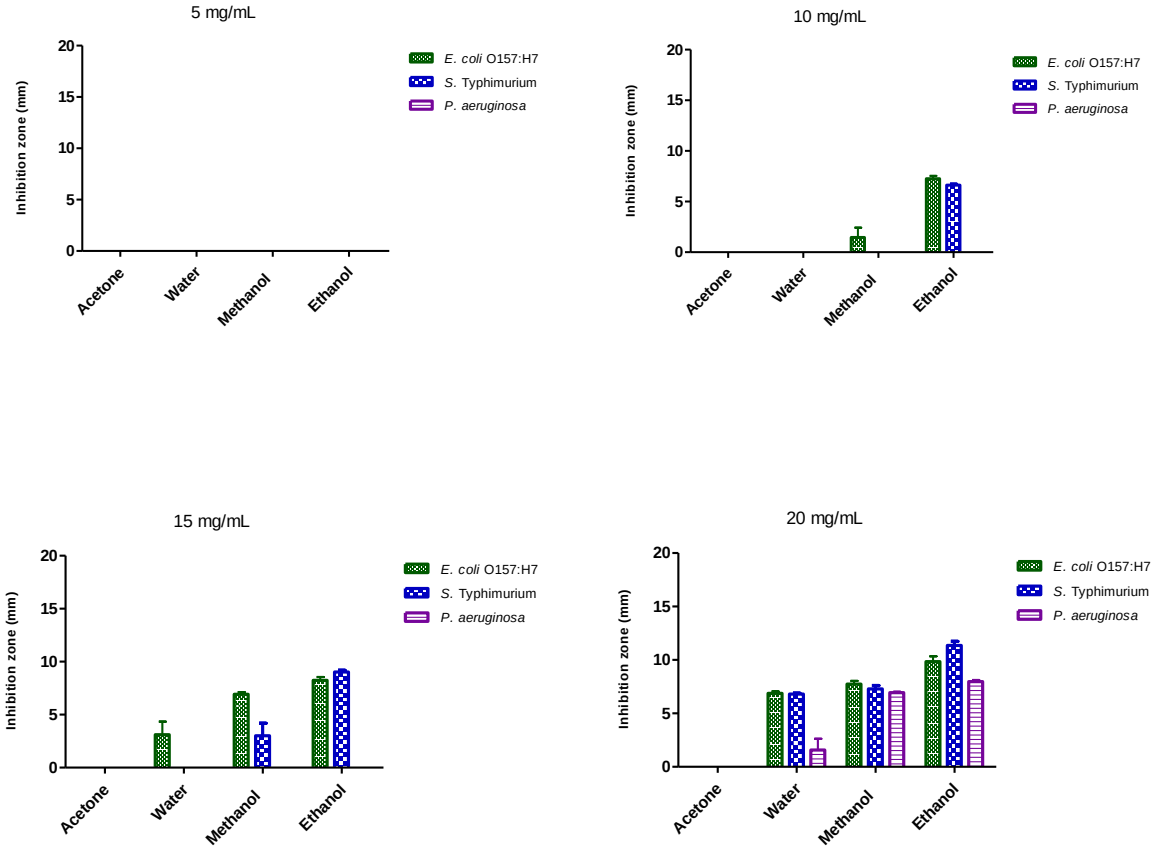


Figure 13. The growth inhibition zone against Gram-positive bacteria.

Permeability of smaller molecules across bacterial wall is higher in Gram-positive bacteria due to the absence of an outer membrane, which can cause more susceptibility to the treatments compared to Gram-negative bacteria [173]. Knowing that the cell wall of Gram-positive bacteria involves layers of peptidoglycans rich in teichoic acid groups, the cell of Gram-negative bacteria usually contains lipopolysaccharides with phosphate groups in the inner core polar hydroxyl groups in sugar repeating units [197].



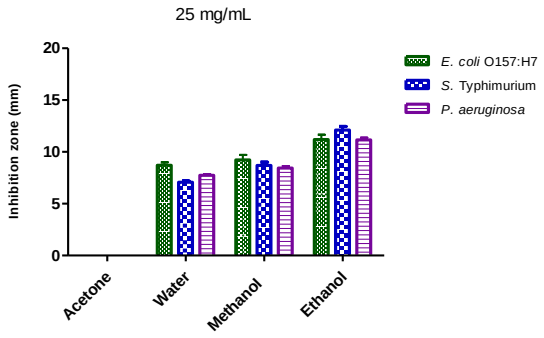


Figure 14. The growth inhibition zone against Gram-negative bacteria.

It is important to underline that disc diffusion assay is not optimal for hydrophobic compounds as the solubilization and diffusion of nan-polar compounds is relatively limited in agar media [198]. Hence, complementary assays are needed to properly evaluate the antibacterial characteristics of sumac extracts, as it is shown in the next sections.

The Growth Inhibitory of Bacteria Strains

The proliferation and growth of bacteria in a liquid phase medium have been studied and well-established for a long time. As a bacterium is introduced to a new nutritional environment, they start to increase their population, followed by infection. This process follows a standard trend called the growth curve [199]. Four main stages in all growth curves have been distinguished and identified as being: the non-replicative stage or lag phase, the exponential proliferation stage or log phase, the stationary stage during which the nutrient exhaustion followed by suspension on replication occurs, and the final death stage at which the starvation of bacteria results in a declined

number of viable cells. The bacteria growth curve provides the opportunity to monitor and interpret the bacteria susceptibility to the environment they have been introduced to.

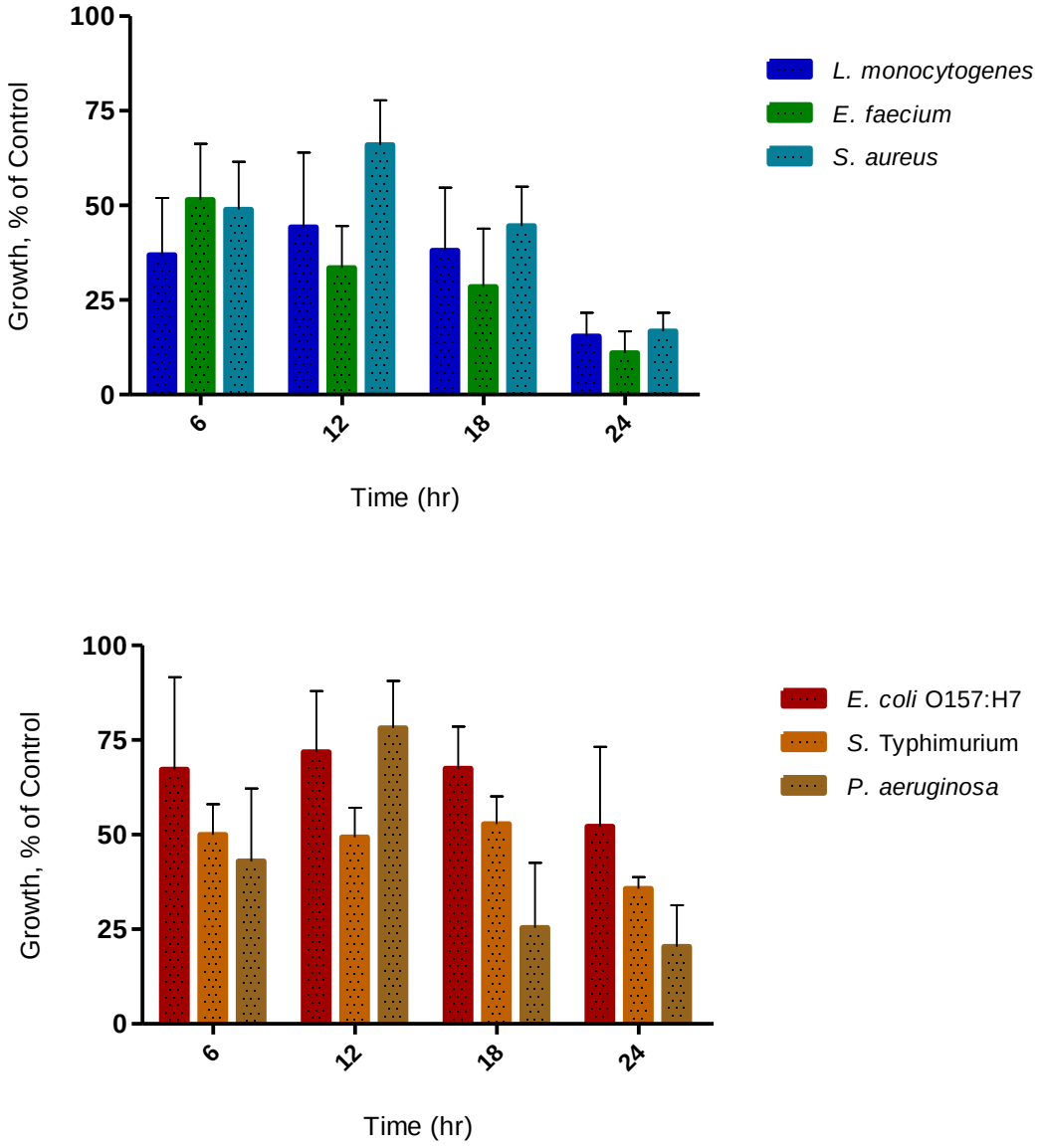


Figure 15. The growth of bacteria strains treated with free sumac polyphenolic extracts.

The inhibitory effects of different concentrations of ethanolic sumac extract on the growth of various tested bacteria strains are reported in **Table 8**. The extract was found to be effective against all bacteria tested. However, increasing the concentration of polyphenols in ethanolic extract, has increased the inhibitory effect. Statistical analysis showed a significant difference *(P<0.01)* between the various concentrations of treatments against Gram-positive bacteria at the exposure time of 24 h. The *Staphylococcus aureus* showed to be more sensitive to the treatments among Gram-positive bacteria, followed by *L. monocytogenes* and *E. faecium.* Statistical analysis showed a significant difference (*P<0.01*) within the Gram-negative bacteria. *P. aeruginosa* was found to be more sensitive among Gram-negative strains. Gram-negative bacteria were more resistant to the treatments than Gram positives. This is consistent with the results reported in a previous study [200]. The difference in bacteria susceptibility to the treatments may be due to structural variabilities in their cell wall compositions. The most important component within cell structure is the peptidoglycan layer which its presence or absence may differ one bacterium from another. This layer stabilizes the inner cell layer and controls osmotic pressure by balancing the internal and external pressures [81, 173]. The difference in cell wall structures may also result in variabilities in penetration potential among the targeted bacteria.

Table 7. Growth of pathogen strains treated by different concentration of free sumac (Su) extract at 24 hrs exposure time.

Type of Bacteria	Concentrations of Free Samples [a] (mg/mL)										
	0	0.3125	0.625	1.25	2.5	5	6	7	8	9	10
L. monocytogenes	0.8±0.09	0.8±0.08	0.7±0.08	0.7±0.07	0.6±0.06	0.5±0.05	0.4±0.04	0.3±0.02	0.3±0.04	0.2±0.03	0.2±0.03
E. faecium	1.0±0.1	0.9±0.1	0.8±0.1	0.8±0.10	0.8±0.09	0.4±0.04	0.3±0.03	0.2±0.01	0.2±0.02	0.2±0.02	0.2±0.01
S. aureus	0.8±0.1	0.7±0.09	0.7±0.09	0.6±0.10	0.5±0.08	0.3±0.09	0.3±0.08	0.3±0.08	0.3±0.04	0.2±0.06	0.2±0.05
E. coli O157:H7	0.6±0.06	0.6±0.05	0.5±0.05	0.5±0.05	0.4±0.03	0.3±0.03	0.2±0.02	0.2±0.01	0.1±0.01	0.1±0.01	0.05±0.01
S. Typhimurium	1.0±0.1	0.5±0.05	0.5±0.05	0.4±0.04	0.4±0.03	0.3±0.03	0.3±0.03	0.2±0.02	0.2±0.01	0.1±0.01	0.1±0.01
P. aeruginosa	0.8±0.1	0.7±0.09	0.7±0.10	0.7±0.10	0.5±0.08	0.3±0.08	0.3±0.05	0.3±0.05	0.2±0.05	0.2±0.03	0.2±0.04

Results are presented as means of the three observations.
[a] Values represent the means and standard errors from triplicate of growth curve of bacteria strains at 24 hrs exposure time to free (unencapsulated) samples.

In this experiment, the highest antibacterial property exhibited in sumac ethanolic extract against the tested pathogens was consistent with other studies in the literature investigating the antimicrobial activities in spices. It has been shown in several reports that the bioactive components present in spices, such as the naturally occurring compounds in essential oils, may attach to the surface of the cells and penetrate the biolayers of the cell membrane. As the result of the accumulation and penetration of bioactive compounds into the cell membrane, the integrity of the cell structure and metabolism is disturbed, which may further result in cell death [201].

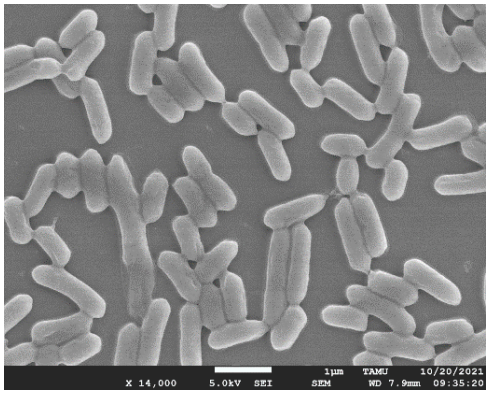
Gallic acid is the main polyphenol compound in all sumacs extracts regardless of the solvent being used for extraction. The possible mechanisms of action of gallic acid against different bacteria strains include the change in the hydrophobicity of bacteria cell membrane via gallic acid deposition, the reduction of the surface potential of bacteria cell wall via electrostatic interaction, and the accumulation of gallic acid in the membrane, thereby causing the membrane damage and sequential leakage of intracellular components [92]. However, according to the extraction obtained by LC-MS, gallic acid has been equally well-captured by all solvents. Since the antibacterial activity of extracts obtained by different solvents varied, it can be concluded that other polyphenolic compounds play an important role in the context of antibacterial characteristics of sumac extracts.

SEM Micrographs of The Treated Cells of Bacteria Samples

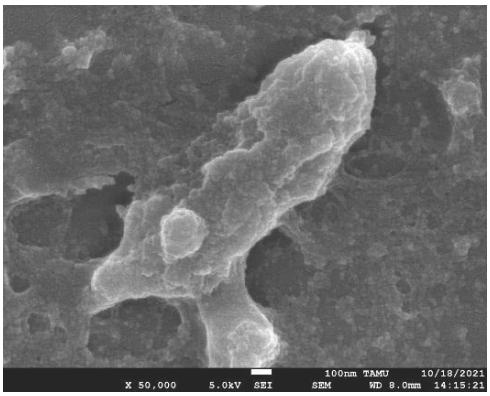
The antibacterial activity of free Su extracts was done on the same microbial cultures of *L. monocytogenes* (Scott A Ser 4b), *E. faecium* (NRRL B-2354), *S. aureus* (ATCC 6538), *E. coli* O157:H7 (43895™), *S.* Typhimurium (ATCC 700720), and *P. aeruginosa* (ATCC 10145). The

purpose was to observe morphological alteration of tested bacteria strains after exposure to free Su extract at a concentration of 10 mg/mL for 24 hr. The bacteria cells treated with sumac extract were compared with untreated cells (**Figure 16**).

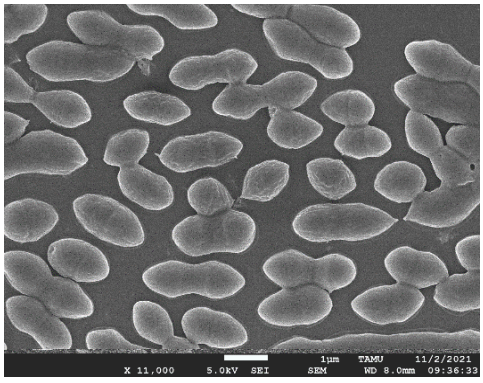
A)



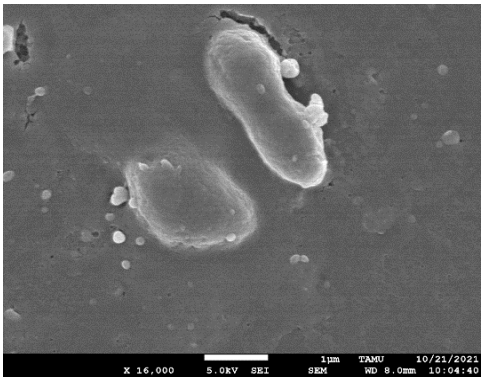
B)



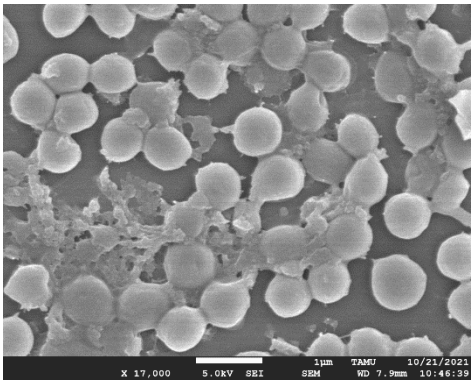
C)

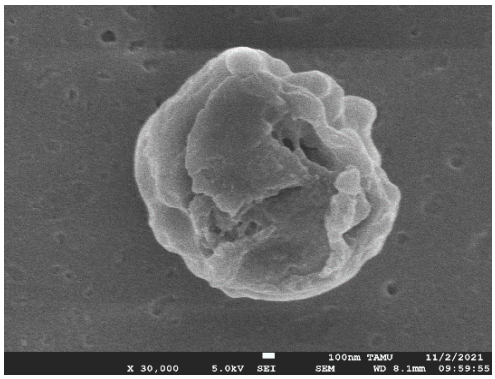


D)



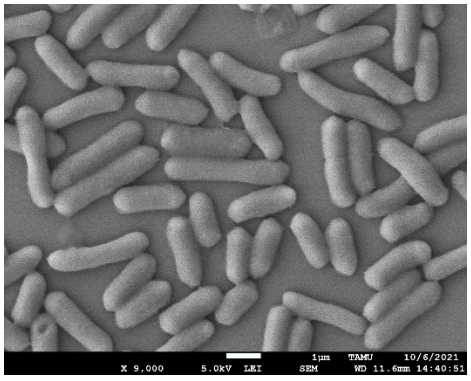
E) **F)**

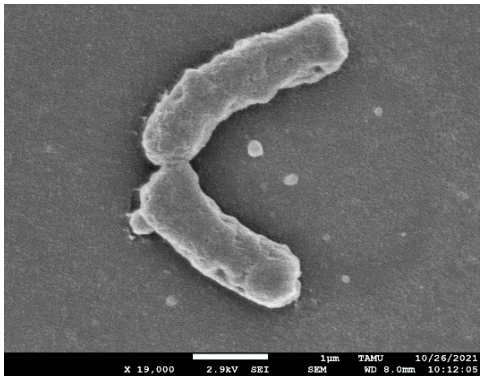




As a result, the treated cells appeared to be degraded and damaged. The mechanisms of action of the compounds within sumac extract may be due to their effect on the bacterial cell wall components resulting in degradation and gradually disrupting of the cell wall. This result was in agreement with the study that investigated the effect of *O. basilicum* extract against *L. monocytogenes.* The mechanism of action was proposed to be the degradation of cell wall following damage to a cytoplasmic membrane protein, the binding proteins, and finally leakage of cell contents [202].

G) **H)**





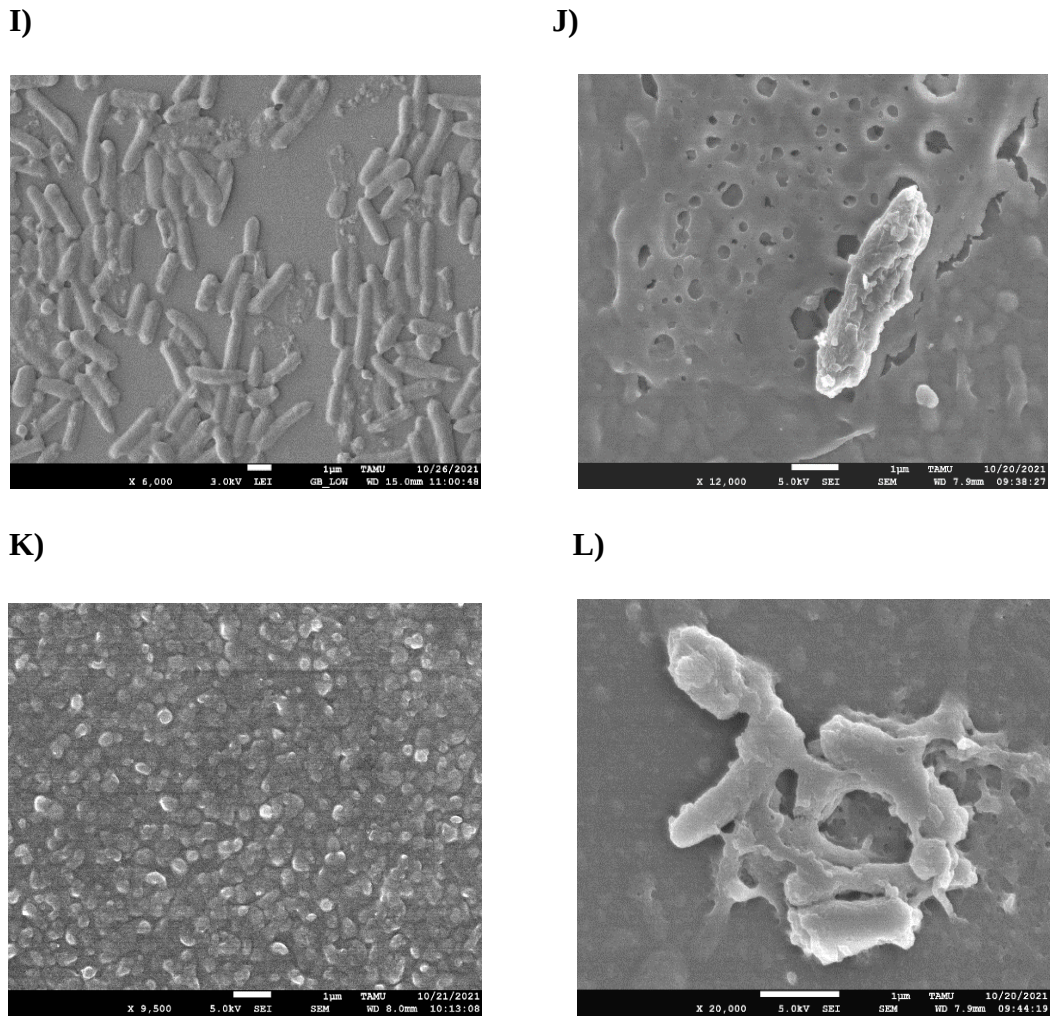


Figure 16. Scanning electron micrographs of untreated *L. monocytogenes* (A), *E. faecium* (C), *S. aureus* (E), *E. coli* O157:H7 (G), *S.* Typhimurium (I), and *P. aeruginosa* (K), whereby represents *L. monocytogenes* (B), *E. faecium* (D), *S. aureus* (F), *E. coli* O157:H7 (H), *S.* Typhimurium (J), and *P. aeruginosa* (L) treated with free sumac extract at a concentration of 10 mg/mL for 24 hrs incubation time at 37 °C.

Non-treated *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 cells showed intact cell morphology with smooth surfaces. However, the bacterial cell wall morphology of treated bacteria with f extract was changed, showing a disrupted structure and wrinkled cells. The results were

similar to other studies that reported fruit extracts caused deformation of the cell surface, increased cell membrane permeability, and effect cell morphology [203-204].

Conclusion

The sumac fruit extract has a high amount of polyphenolic extract that can be utilized as antimicrobial agents in food. The impact of sumac polyphenolic extract on the structure of pathogenic bacteria has exhibited the ability of the fruit extract to disrupt the cell wall integrity and finally destruction of microorganisms. Sumac ethanolic extract had the highest bacterial growth inhibition zone among all tested samples. This may be partly due to the unique complexation of hydrophobic and hydrophilic constituents in one extract. This structure has provided the polyphenols compounds in an ethanolic extract with an improved physicochemical property for easy transportation through biological membranes.

CHAPTER V

IN VITRO ANTIBACTERIAL ASSESSMNET OF ENCAPSULATED SUMAC POLYPHENOLS AGAINST TESTED BACTERIA

Introduction

The galloyl derivatives have been studied for their antioxidant, anti-inflammatory, antibacterial, antiviral properties. Encapsulation is a method of protection for these compounds against degradation is introduced. The method may minimize the negative effects of environmental factors, including light in moisture, and oxygen [205]. There are many different coating materials to create nanoparticles among which Pluronic® F127 has been frequently used in drug delivery systems development. The application of this copolymer is due to its non-toxic, inexpensive, and high biocompatibility characteristics. PF127 has also been approved by FDA for biomedical applications [191]. A comparison study on the antibacterial activity of free versus encapsulated plant extract has suggested a higher antibacterial activity associated with samples loaded with Pluronic copolymers compared to that of free samples. This antibacterial improvement is attributed enhancing the extract's physicochemical properties [206].

Materials and Methods

Preparation of Sumac Nanoparticle

The preparation of the encapsulated sumac extracts was carried out according to the method previously described [207]. Sumac extracts and Pluronic® polymer were weighted in a 0.4:0.64 ratio, then dissolved in tetrahydrofuran (THF) solvent. The sample was then impinged rapidly into a beaker containing 200 mL of Milli-Q water. The process was carried out under continuous

sonication for 10 minutes to produce encapsulated sumac. The flow rate of water and THF in the procedure stayed the same as 50.0 and 5.0 mL/min for all the samples tested. The nanoencapsulated dispersion was dialyzed to remove the THF from the samples under a fume hood for 5 hrs following the encapsulation process. Particle size, zeta potential values, encapsulation efficiency, and drug release assays were then evaluated.

Size Distribution and Nanoparticle Zeta-Potential

The procedure was followed from previously described by [208]. The size of nanoparticles was determined by the Dynamic Light Scattering (DLS) technique using Malvern Zetasizer ZS90 nano series instrument (Malvern Instrument, Ltd., Westborough, MA). To run this experiment, plastic cuvettes were then used by adjusting with a pinhole set to 20 μm, 165° scattering angle at 1 cm path length.

Encapsulation Efficiency (EE%) Assessment

The nanoparticles' encapsulation efficiency (EE%) was determined by running the samples through a UV-1800 UV/Visible scanning spectrophotometer (Shimadzu Corp., Columbia, MD) at 280 nm. A percentage of entrapped sumac polyphenols was determined using a standard curve method for encapsulation efficiency (EE%). To evaluate the %EE of encapsulated sumac polyphenols rich in gallic acid, a calibration curve was first calculated in a concentration gradient $Y=4.5356x+0.0267$ with an $R^2=0.9980$. The corresponding results obtained at 280 nm were plotted in the standard curve equation to determine the percentage of encapsulation efficiency for encapsulated sumac polyphenols.

$$\text{EE}\ (\%) = \text{loaded sumac polyphenols/initial concentration} \times 100$$

Sumac Nanoparticle Release Kinetic in Vitro

The *In vitro* release kinetic study of sumac polyphenolic nanoparticles was conducted using membrane equilibrium procedure in Milli-Q water (pH 7.4) according to the method previously described by [209]. Briefly, a 10 mg/mL nanoparticle suspension was prepared, and a 30 mL of already prepared nanoparticle suspension was placed into a pouch with a molecular weight cut-off of 12,000-14,000 Da (MWCO, Thermo Scientific Inc., Rockford, IL, USA) and a mesh size of 2.0 nm. The pouches were submerged in 2000 ml beakers containing Milli-Q water for 5 hrs at ambient temperature to release the remainder of THF solvent in the solution prior to drug release assay. A 10 mL of the contents of the pouches were then transferred to new membrane pouches, immersed in 200 mL Milli-Q water, and stirred under 90 rpm at 25 ºC to mimic the physicochemical condition. The beakers were sealed using Parafilm to minimize the evaporation of the sample during the time of the experiment. At each time interval, 3 mL of released samples were taken after 0, 1, 2, 4, 8, 16, 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hrs in an exponential timely repetition. To quantitively analyze free polyphenolics, a UV-1800 UV/Visible scanning spectrophotometer (Shimadzu Corp., Columbia, MD) was used, and the absorbance was read at 280 nm. Three samples (n=3) were taken for measurement for each group. The highest correlation coefficient (R^2) was referenced for plotting the concentration of the compounds.

Scanning Electron Microscopy

The shape and size of the encapsulate sumac polyphenols were characterized and tested based on the method previously described [196] by scanning electron microscopy instrument in the Material Characterization Facility (MCF) at Texas A&M University.

Statistical Analysis

The results from triplicate trials were analyzed using Prism and the Origin v.5 software (OriginLab Corp) and reported as mean ± standard deviation. The two ways ANOVA followed by Bonferroni post-test tests were run to compare replicates means of each concentration in different time points.

Results and Discussion

Size Distribution and Zeta-Potential values of Nanoparticles

The size of four different sumac extracts was determined, and results were obtained using the Dynamic Light Scattering technique. **Figure 17** represents the SEM images of free Su and Pluronic® F127. The dynamic light scattering for nanoencapsulated sumac ethanolic extract was significantly more promising than the one of methanolic, water and acetone extracts, suggesting a better emulsifying property associated with the ethanolic extracts compared to the other samples. The average size of 100 nm for particles resulted from sumac whole extract nanoparticles of methanolic, aqueous, and acetone, while the ethanolic extract had a range size of larger than 100 nm with a higher intensity value.

A) **B)**

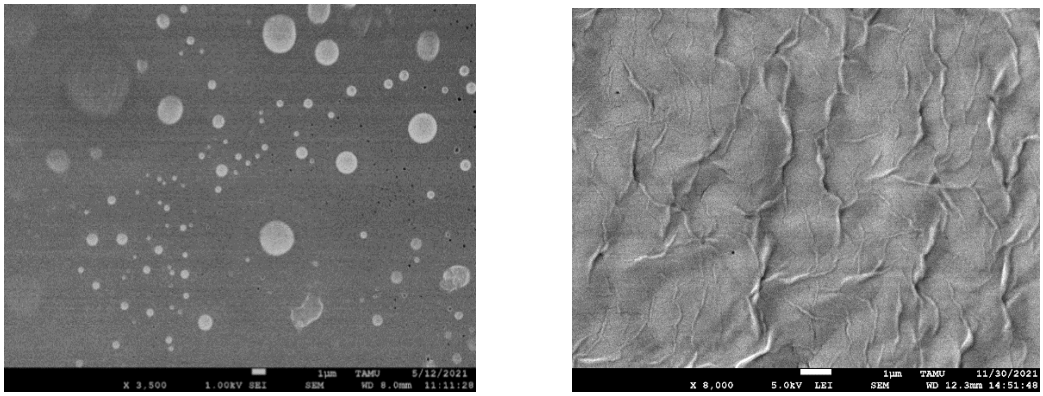
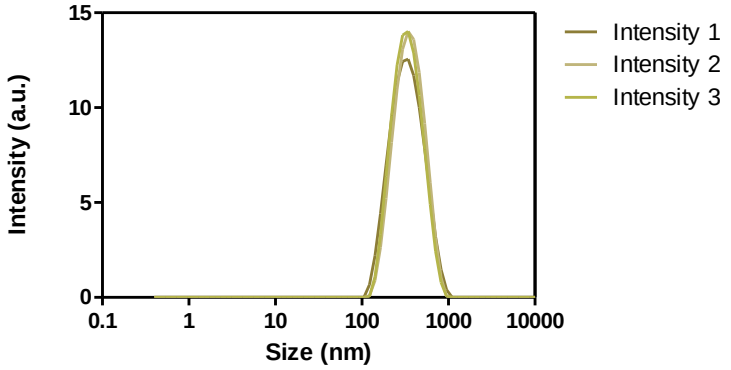


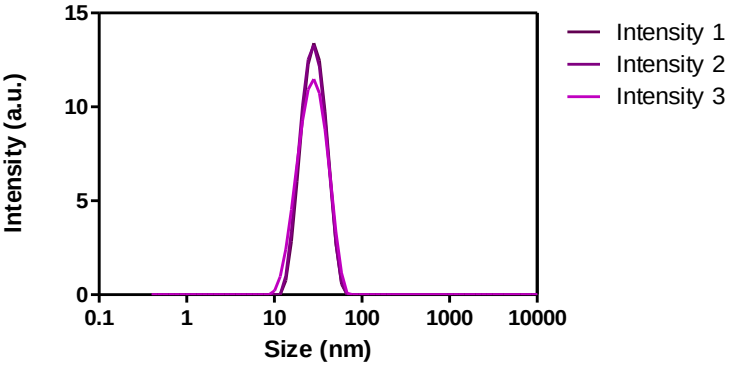
Figure 17. SEM image of free Su extract (A) whereby (B) represents Pluronic® F127 as a wall material.

The results of this experiment were supported by other studies reporting slightly below 100 nm or an average of 100 nm particle size when using Pluronic® F127 for making nanoparticles [210-211]. The conjugation of Pluronic® F127 with lutein-loaded zein nanoparticles demonstrated an improvement in polydispersity index (PDI) value after 24 hours dialysis in phosphate-buffered saline (PBS). A study on coating the grape seed extracts with lecithin has shown a reduction in polyphenol content, suggesting the association between polyphenol content values and their exposure surface size with reducing Folin reagent [177].

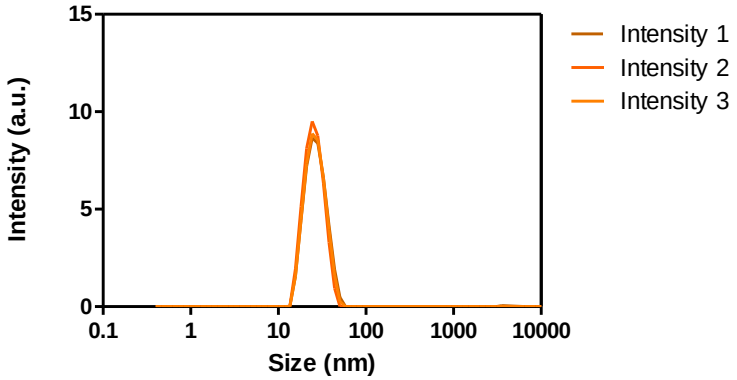
A) EtOH Ext



B) MeOH Ext



C) W Ext



D) Ac Ext

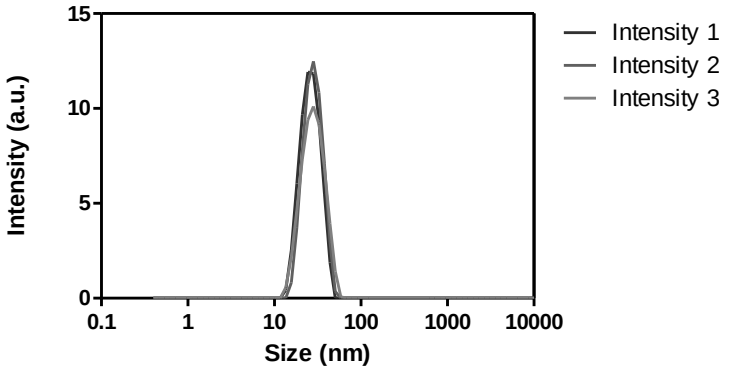
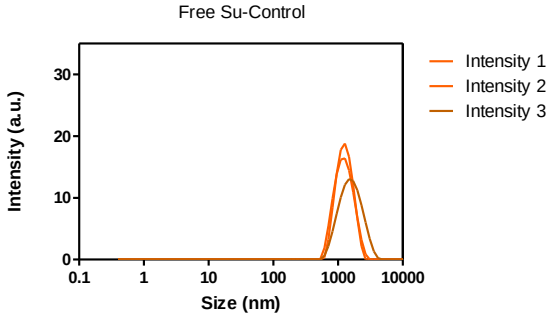
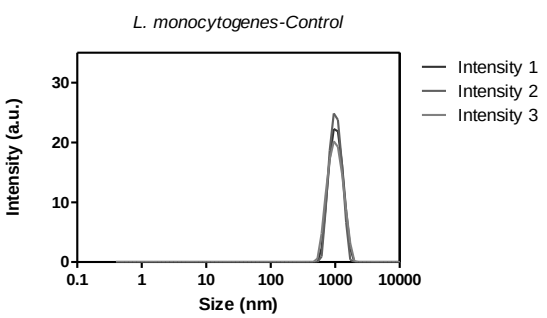
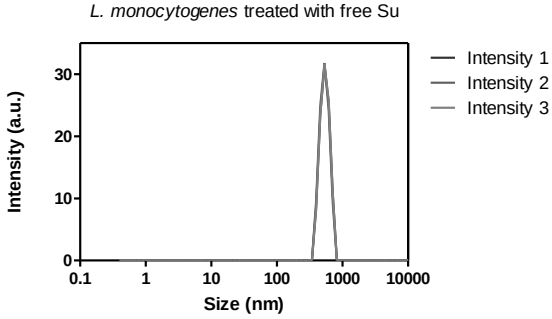


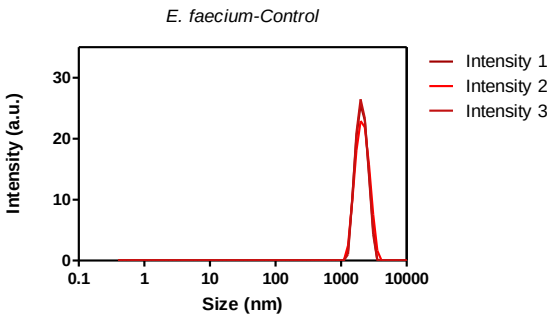
Figure 18. Particle size distribution of nanoparticles made of ethanol (A), methanol (B), water (C), and acetone (D) sumac extracts.

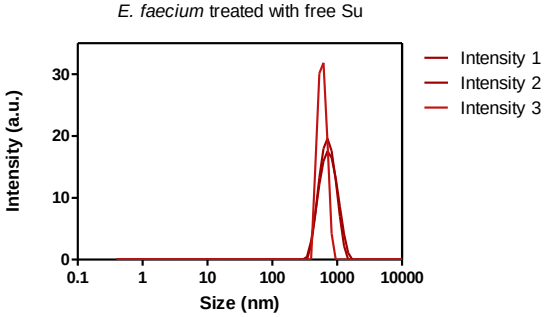
Despite an up to 40% increase in size, nanoparticles made with poloxamer 407 exhibited less than 0.3 PDI value compared to the 0.33-0.48 range for nanoparticles made without polymer. The data is consistent with the results of sumac encapsulation study, where the PDI for sumac encapsulated polyphenols exhibited a promising value of 0.2 and an average size distribution of 100 nm [212].

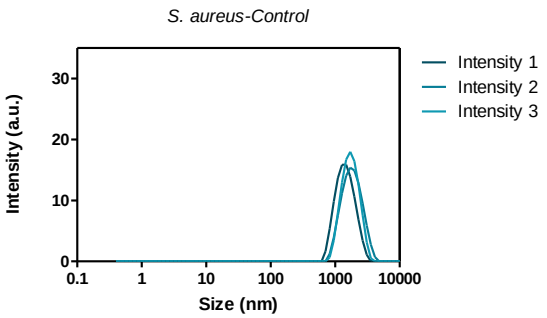

Free Su-Control
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)

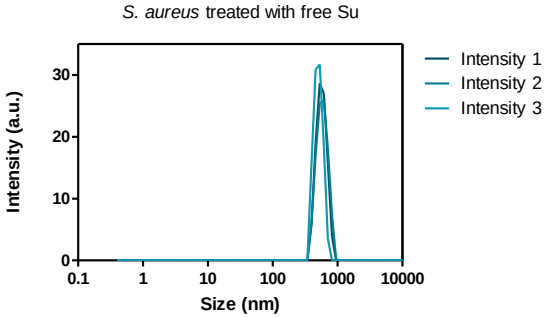

L. monocytogenes-Control
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)


L. monocytogenes treated with free Su
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)


E. faecium-Control
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)


E. faecium treated with free Su
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)


S. aureus-Control
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)


S. aureus treated with free Su
Intensity 1
Intensity 2
Intensity 3
Intensity (a.u.)
Size (nm)

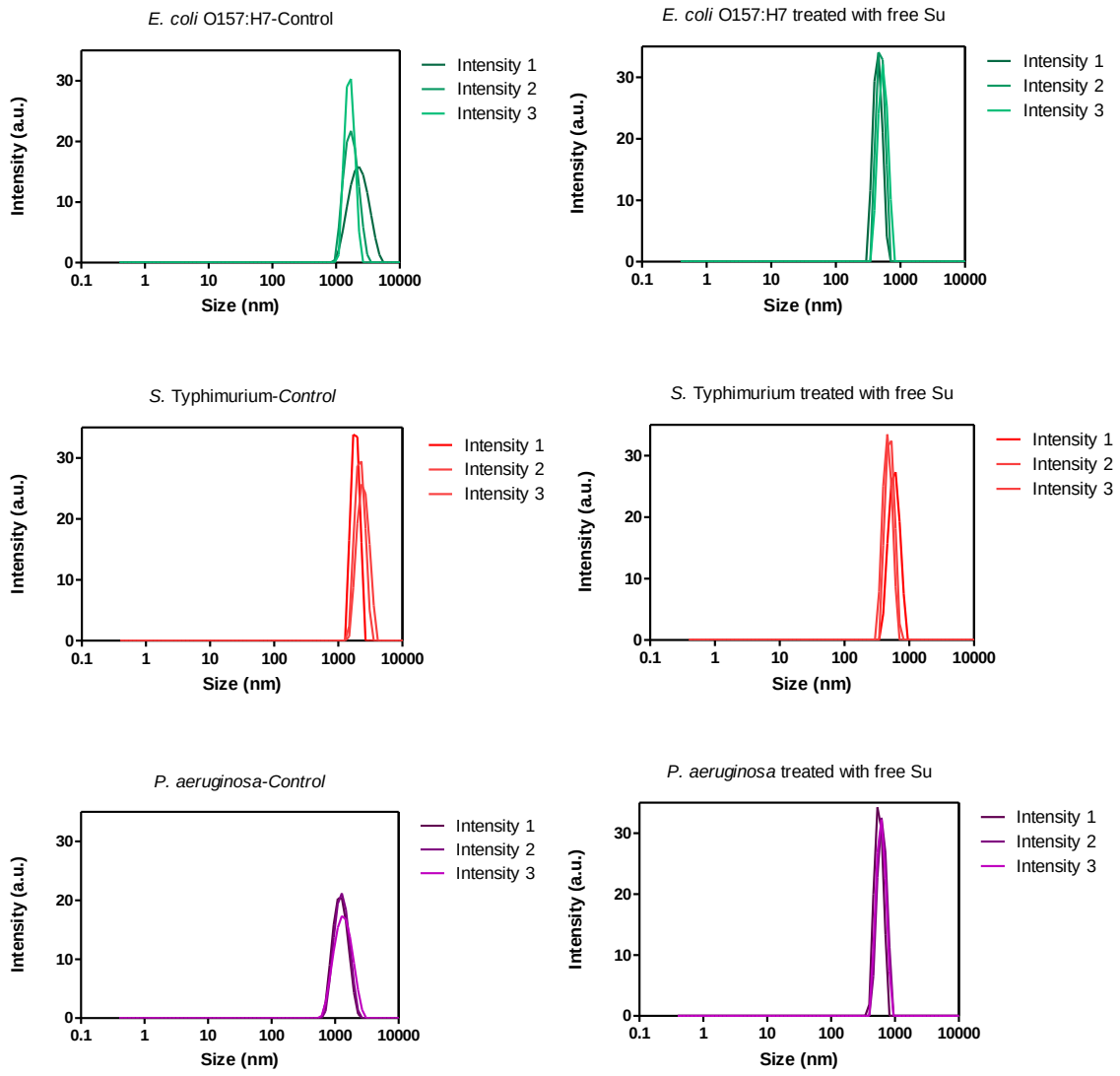
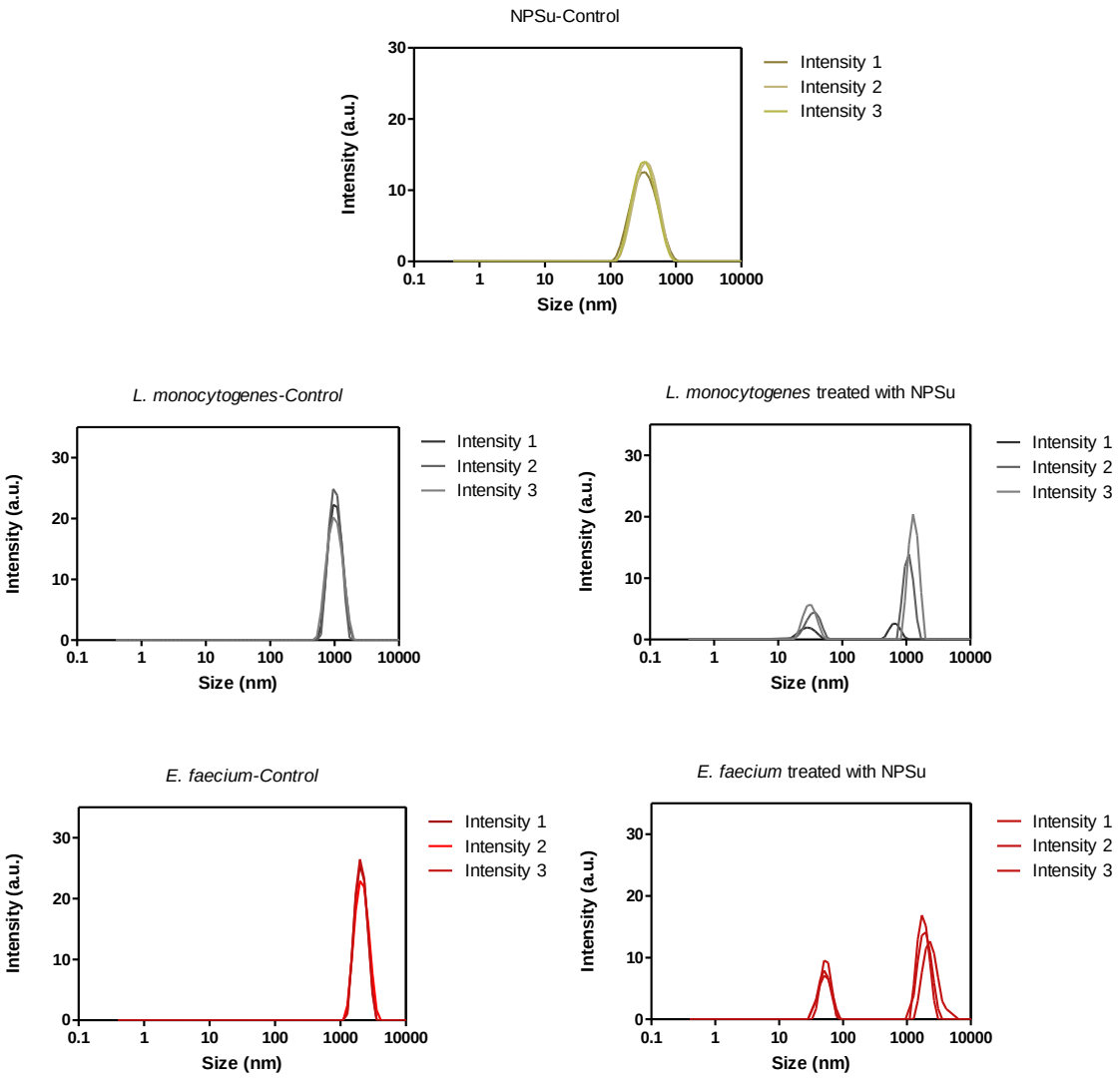


Figure 19. A comparison analysis of particle size distribution between free Su and bacteria controls versus treated bacteria strains.

The data showed an expansion in the range of particle sizes in all NP samples treated with tested bacteria (**Figure 20**). The interaction of bacteria with sumac extract nanoparticles caused a reduction in the size of polyphenols and fragmentation of bacteria after exposure to the treatments.

Preliminary data on the effect of the tannin molecular size on their protein bonding capacity demonstrated that as the molecular size of hydrolysable tannin gets bigger, the percentage of these molecules retained within the protein content samples goes higher. That is, the higher molecular weight tannins may have a higher binding affinity to the surrounding protein molecules [213].



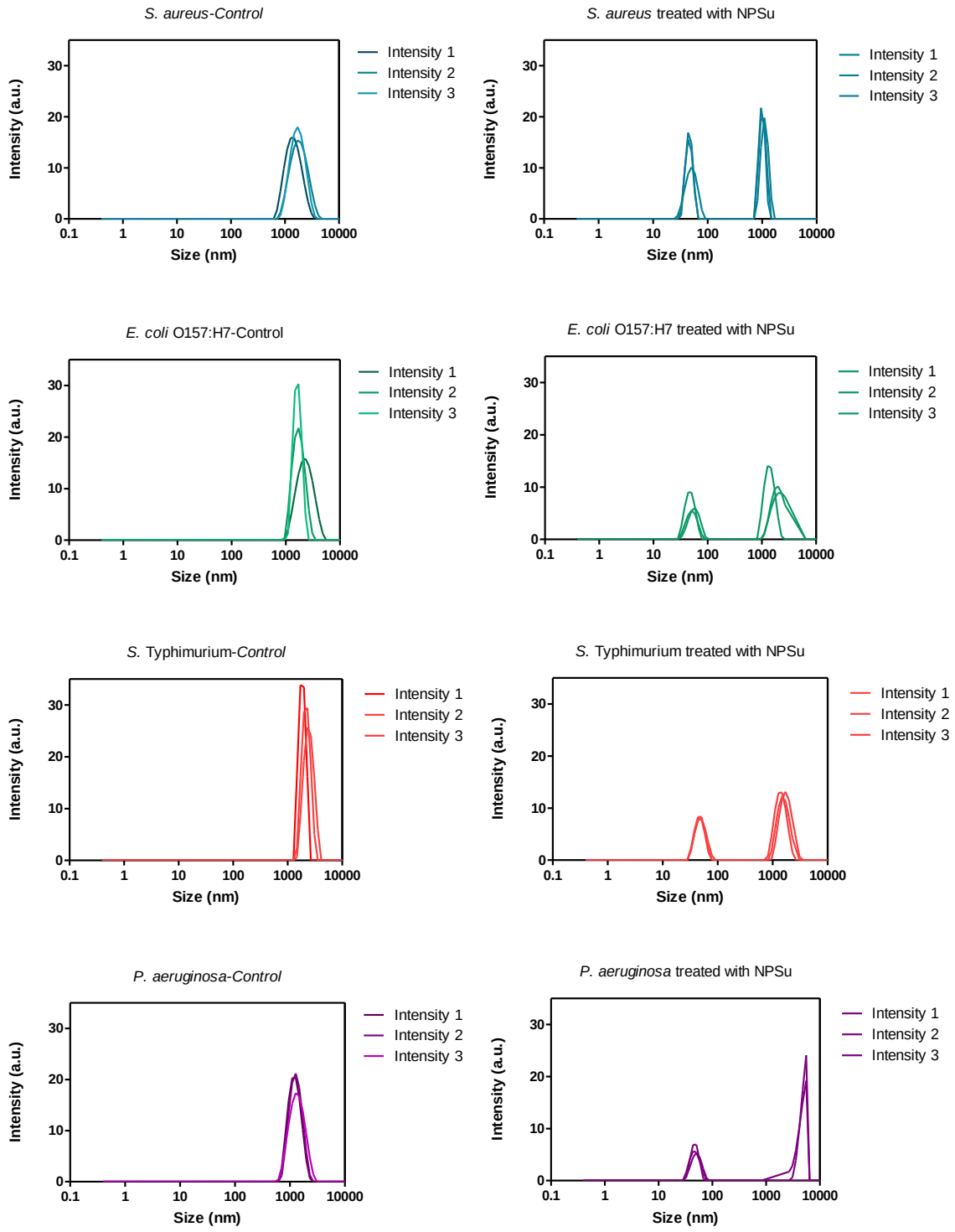


Figure 20. A comparison analysis of particle size distribution between nanoparticle sumac and bacteria controls versus treated bacteria strains.

Treatment of bacteria with nanoparticles caused a wider range of particle size distribution values as the larger molecules are broken down into smaller size particles. The smaller particles may be originated from either bacterial fragmentation of destruction of bacterial structure or product of hydrolyzation of bigger polyphenolic molecules to their smaller metabolites.

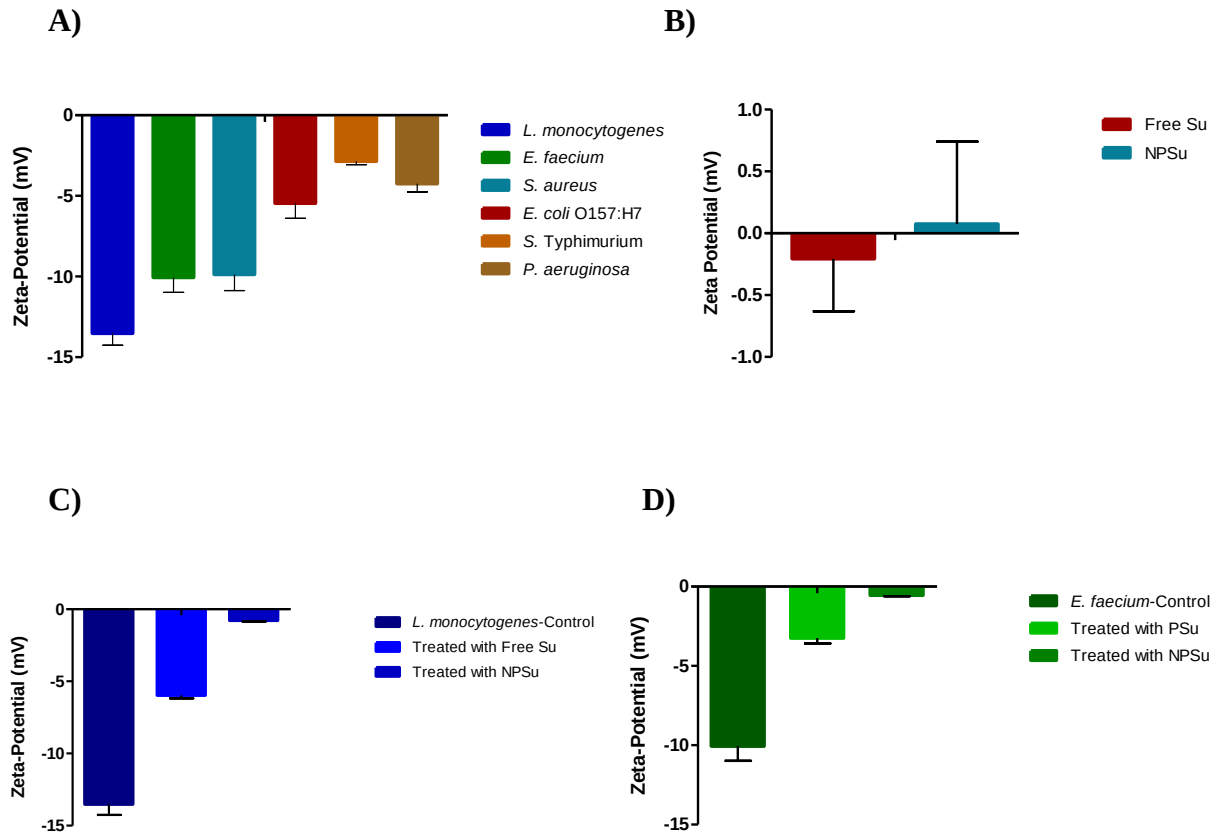
Table 8. Characteristics of control bacteria, treated with free Su, and NPSu.

Type of Bacteria	Average Particle Size[a] (nm)			Polydispersity Index[b] (PDI)		
	Bacteria Control	Treated with free Su	Treated with NPSu	Bacteria Control	Treated with free Su	Treated with NPSu
L. monocytogenes	987.4±19.8	568.0±11	842.7±131.7	0.03±0.03	0.5±0.2	0.7±0.2
E. faecium	2010±28.8	666.8±33.5	812.4±82.4	0.03±0.02	0.2±0.3	0.3±0.3
S. aureus	1465±49.0	575.2±18.6	11.4±216.5	0.2±0.01	0.4±0.3	0.5±0.3
E. coli O157:H7	1723±151.6	536.7±50.0	475.7±104.5	0.2±0.07	0.8±0.2	0.9±0.1
S. Typhimurium	2182±305.1	577.9±18.8	481.4±33.3	0.2±0.2	0.7±0.4	0.7±0.4
P. aeruginosa	1176±31.7	680.6±13	1017±71.3	0.1±0.01	0.9±0.1	1.0±0.1

[a,b]Values represent the means and standard deviation from triplicate of average particle size and polydispersity index.

The zeta-potential values of tested bacteria, free Su and NPSu, as well as treated bacteria with the treatment are shown (**Figure 21**). Results indicated that despite the negative values for both free Su and NPSu polyphenolic extract, the absolute values for nanoparticles were higher than free extract. Although adhesion of less negative surfaces to the outer membrane of microorganisms may be higher at first, the growth inhibition property may not necessarily be higher. Pluronic copolymers are inherently neutral since they are considered non-ionic coating materials. The slight

negative value for Pluronic-based sumac polyphenol nanoparticles is attributed to the compounds already attached to the surface of already created nanoparticles [214]. As the particles get close to the surface of bacteria, chemical bonding occurs between components of negatively charged molecules and bacteria outer membrane constituents. This phenomenon disrupts cell metabolism and, finally death of the bacteria of interest as exhibited by Scanning Electron Microscopy (SEM) method.



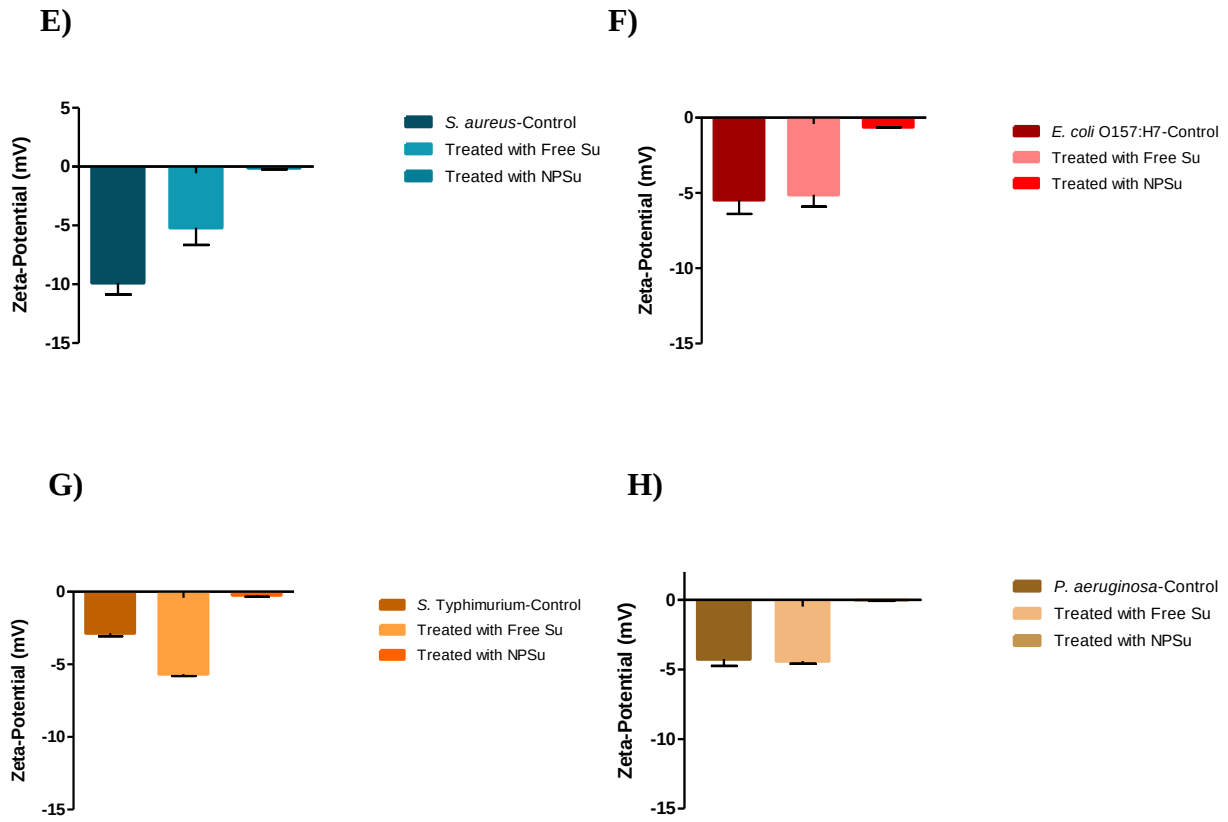


Figure 21. Zeta-potential distribution of tested bacteria (A), free Su, and NPSu treatments (B), *L. monocytogenes* (C), *E. faecium* (D), *S. aureus* (E), *E. coli* O157:H7 (F), *S.* Typhimurium (G), and *P. aeruginosa* (H) treated with free Su and NPSu after 24 hrs incubation time at 37 °C.

Entrapment Efficiency of Encapsulated particles

The encapsulation efficiency (EE) of sumac ethanolic extract was determined using the following equation Y=4.5356x+0.0267 with R^2=0.99 (**Figure 22**). The EE value for sumac nanoparticles was determined and reported as 75.99 ± 0.27.

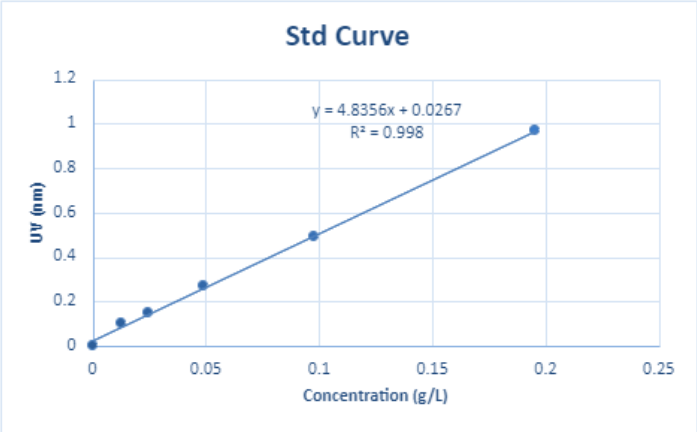


Figure 22. Standard curve of encapsulated sumac for Encapsulation Efficiency (EE).

Pluronic® F127 has been utilized as a coating material in different studies with promising encapsulation efficiency values. Study on zein protein-based nanoparticles stabilized/coated by PF127 as surfactant has significantly improved entrapment efficiency. The study indicated that 20% increase in encapsulation efficiency of nanoparticles made with PF127 compared to samples made without the polymer. The 83% entrapment efficiency [212] is consistent with 76% encapsulation efficiency for sumac ethanolic extract. The study has suggested that the use of PF127 in lutein-loaded nanoparticles have improved the conductivity of the particles by decreasing the zeta potential value of nanoparticles conjugated with the polymer compared to that of without polymer.

Release Kinetic of Sumac Nanoparticle

The sumac nanoparticle solution was transferred into Spectrum™ dialysis cellulose membrane with a molecular weight cut-off of 12,000-14,000 Da and 2 nm diameter to test the durability of

coating material in a hydrophilic environment. The release kinetic study of the integrity of nanoparticles exhibited a controlled gradual release of sumac polyphenolics rich in gallic acid by measuring the cumulative concentration of the targeted compound at periodic intervals at 280 nm using a spectrophotometer instrument equipped with a UV-1800 detector (Shimadzu Corp., Columbia, MD). The scanning range was set from 200 to 800 nm. The UV spectra for tannin have been reported to be within the range of 250 and 310 based on the number of gallic acids and tannic acid moieties in their structure [215].

Chemical properties of the loaded compound, the material of polymer carrier, and environmental factors can affect the release of encapsulated molecules from a polymeric nanocarrier [216]. **Figure 23** demonstrated the release kinetics of sumac polyphenols loaded into nanocarrier PF127 involving PEO and PPO blocks. Based on the data, the release profile was involved an initial release in first 4-8 hrs that exponentially increased over time. Secondly, the controlled release behavior of NPSu polyphenols showed a sustained exponential release trend for 24-240 hrs at 25 ºC.

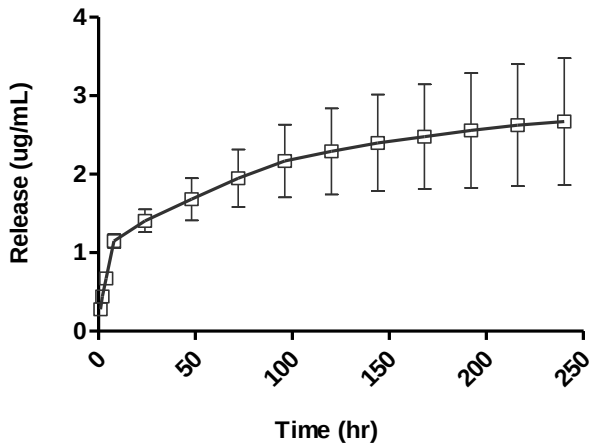


Figure 23. The release kinetic of sumac polyphenolic loaded in PF127.

In other word, the controlled release model exhibited a high rate in the first 8 hours followed by an exponential rate in the 10 following days. Due to the size differences between free Su extract and NPSu, a gradual increase in the concentration of the substances of interest was expected, whereas the bigger encapsulated particles were not able to pass through the membrane. This can be explained by gallic acid affinity to the hydrophilic medium, which causes the loose or weakly attached compounds to the surface of nanoparticles to release into the medium [130].

In vitro Antibacterial effect on the Tested Bacteria

The growth of pathogen strains treated by different encapsulated sumac extract concentrations at 24 hours exposure time is shown in **Figure 24**. The growth inhibition zone values for all tested bacteria were increased in the higher concentration of treatments. However, the difference between inhibitions zones is small in higher concentrations. For *L. monocytogenes*, concentration of 0.625 mg/mL to 10 mg/mL was statistically significant with a p value of less than 0.001 ($p < 0.001$).

However, the inhibition was not significant at the concentration of 0.312 mg/mL. For *E. faecium* all concentrations showed significant inhibition at the time points of 4-24 hrs; however, inhibition was not significant at the first two hours of the experiment. For *S. aureus*, significant growth inhibition was observed from the concentration of 1.25-10 mg/mL at 6 to 24 hours. The results were not significant at the first four hours of incubation time.

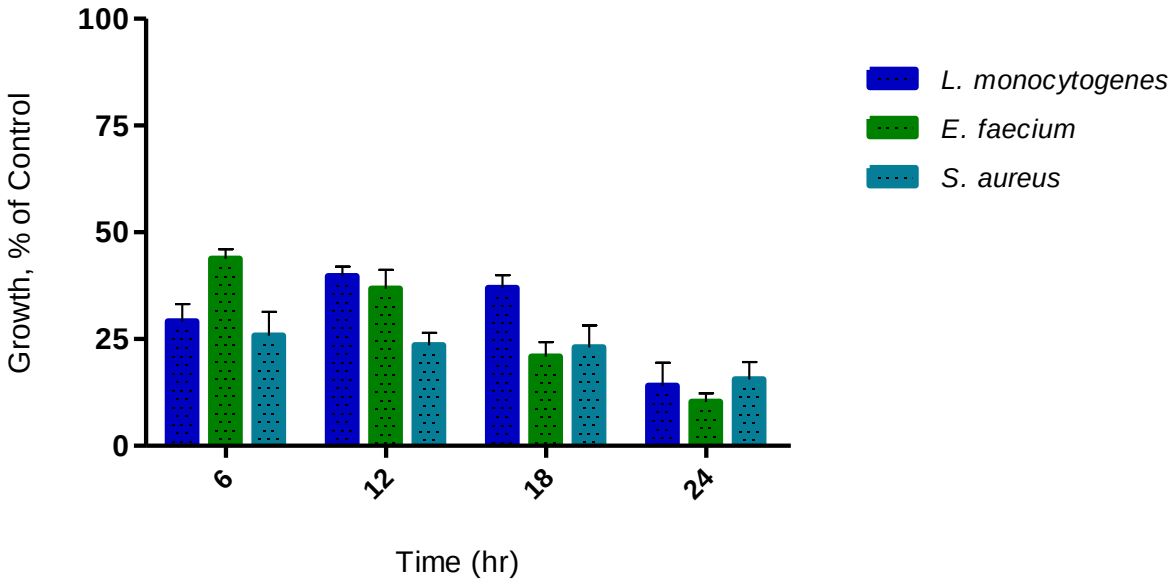
Table 9. Growth of pathogen strains treated by different concentration of encapsulated sumac extract at 24 hrs exposure time.

Type of Bacteria	Concentrations of Encapsulated Samples [a] (mg/mL)										
	0	0.3125	0.625	1.25	2.5	5	6	7	8	9	10
L.monocytogenes	0.9±0.08	0.6±0.06	0.4±0.04	0.4±0.04	0.2±0.02	0.2±0.03	0.2±0.02	0.2±0.01	0.2±0.02	0.2±0.02	0.1±0.03
E. faecium	1.2±0.1	0.8±0.1	0.7±0.1	0.5±0.08	0.4±0.03	0.3±0.02	0.3±0.03	0.2±0.02	0.1±0.02	0.1±0.02	0.1±0.01
S. aureus	0.8±0.1	0.7±0.1	0.5±0.08	0.4±0.03	0.2±0.02	0.2±0.02	0.2±0.02	0.1±0.02	0.1±0.02	0.1±0.01	0.1±0.01
E. coli O157:H7	1.0±0.09	0.5±0.05	0.4±0.03	0.3±0.04	0.2±0.03	0.2±0.02	0.2±0.02	0.3±0.02	0.1±0.01	0.1±0.01	0.1±0.01
*S.*Typhimurium	0.7±0.1	0.4±0.04	0.4±0.05	0.3±0.04	0.3±0.04	0.3±0.03	0.2±0.03	0.2±0.02	0.2±0.02	0.1±0.01	0.1±0.01
P. aeruginosa	0.90±0.1	0.7±0.1	0.5±0.07	0.4±0.03	0.3±0.04	0.3±0.03	0.1±0.01	0.1±0.02	0.1±0.01	0.1±0.01	0.1±0.01

Results are presented as means of the three observations.

[a] Values represent the means and standard errors from triplicate of growth curve of bacteria strains at 24 hrs exposure time to encapsulated samples.

Among the Gram-negative bacteria, *E. coli* O157:H7 demonstrated an inhibition zone from the lowest concentration at 0.3125 mg/mL to 10 mg/mL. Interestingly, the nanoparticle treatments inhibited the tested bacterium from the first two hours except for a concentration of 0.325 mg/mL, that the treatment was not effective for the first six hours. *S.* Typhimurium exhibited more resistance against the treatment, which means the treatment was not effective enough to inhibit the bacterium in the first ten hours during the incubation time. For *P. aeruginosa*, the concentration of 0.3125 mg/mL was not statistically significant (with a $p > 0.05$) to inhibit the growth of the bacterium.



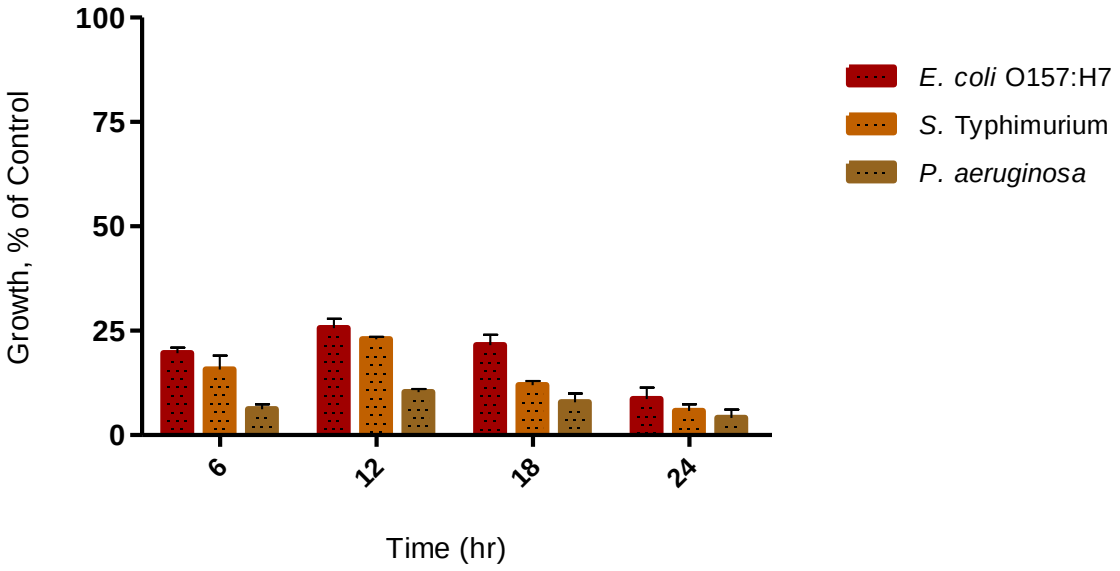


Figure 24. The growth of bacteria strains treated with nanoparticle sumac polyphenolic extracts.

An antibacterial comparison study of neutral, negatively, and positively charged particles against Gram-negative and Gram-positive bacteria suggested higher bacterial growth inhibition in positively charged particles among all samples being tested [217]. However, a different study has shown an otherwise growth kinetic by time. The study has concluded that although the electrostatic attraction was relatively higher in positively charged surfaces, only the growth of Gram-negative bacteria was inhibited. The positively charged biomaterial surfaces exhibited antibacterial properties against Gram-negative bacteria but not against Gram-positive bacteria [218]. This is in agreement with the results from a study investigating the potential of negatively charged particles to interact with bacterial cell wall constituents. The study has indicated that no electrostatic interaction was observed between tested bacteria and negatively charged particles. However, the prolonged antibacterial activity against Gram-positive bacteria strains has been attributed to the interaction between negatively charged nanoparticles and teichoic acid components within Gram-

positive cell wall structure. Also, chemical bonding between teichoic acid of bacteria and nanoparticles [219].

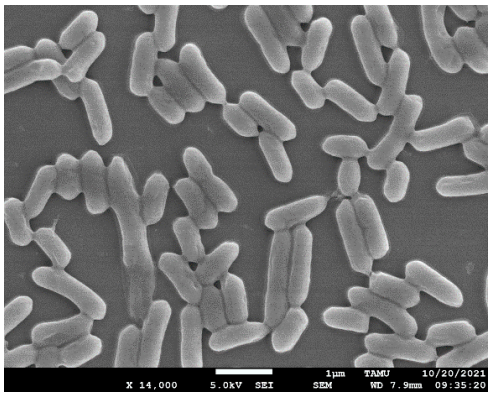
SEM Micrographs of The Treated Bacteria with Encapsulated Sumac

The antibacterial effect of the sumac extracts loaded polymer PF127 was investigated to better understanding its roles towards the antibacterial of the coated nanoparticle. **Figure 25** represents SEM micrographs of six types of tested microbial cells treated with NPSu. There was an accumulation of nanoparticles on the surface of *L. monocytogenes* and *S. aureus* cell walls (**Figures 25B**, and **F**), resulting the changes in the bacteria cell wall morphology. On the other hand, inhibition of Gram-negative has not involved an accumulation of the bacteria. However, these types of bacteria were inhibited individually due to an improvement of nanoparticle adhesion to the negatively charged cell membrane (**Figures 25H**, **J**, and **L**). For *S.* Typhimurium, a length alteration was also observed, and the bacterium shortened to as little as 1 µm.

As obtained from surface charge values of encapsulated sumac sample, a cationic tendency of particles has a potential penetration ability to have an electrostatic interaction with negatively charged bacteria. The mechanism of action may be due to the presence of outer membrane (OM) with negatively charged molecules such as Lipopolysaccharide (LPS) and Lipoprotein (LP) and their interaction with the bacterial cell wall structure. It has been reported that the higher affinity

to the ion molecules among negatively-charged Gram-negative bacteria leads to increase uptake of ion and eventually damages the cell wall [78].

A)



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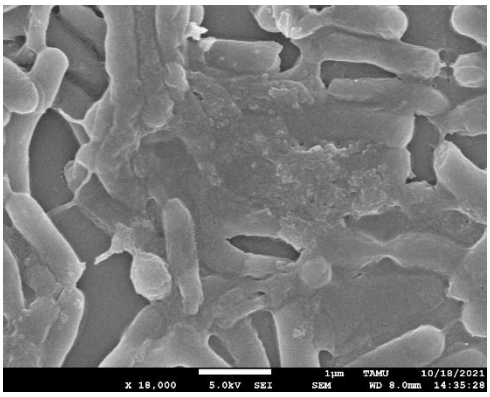

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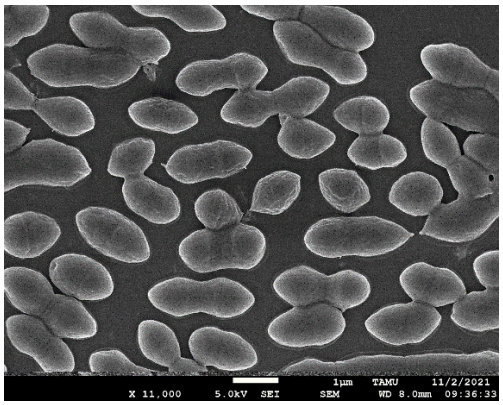
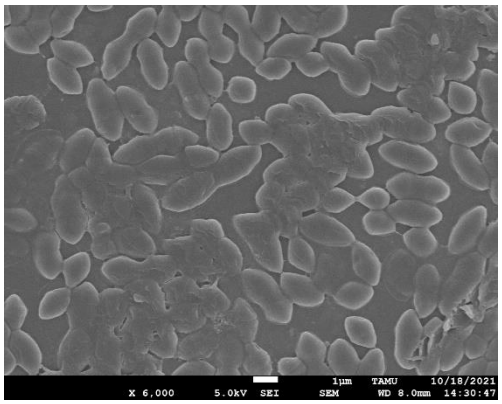


D)



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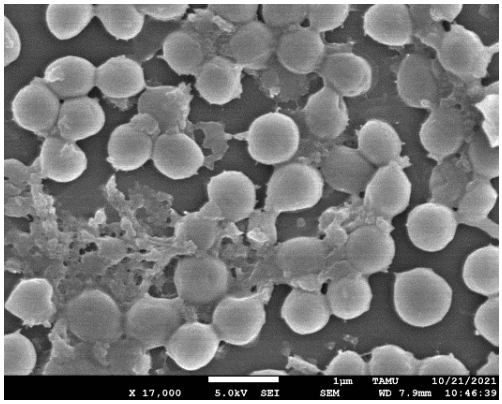
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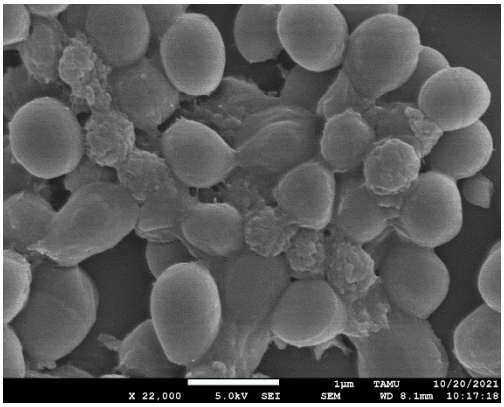
F)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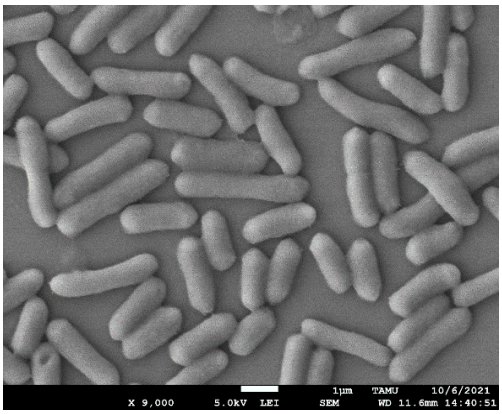
G)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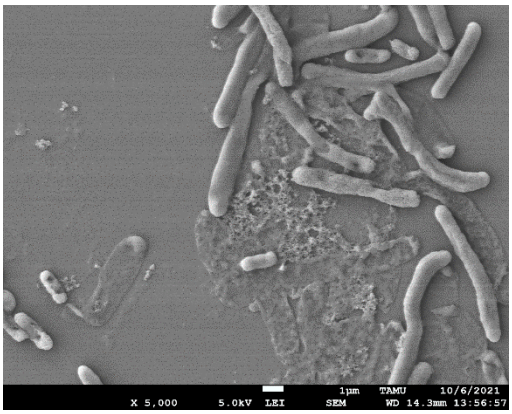
H)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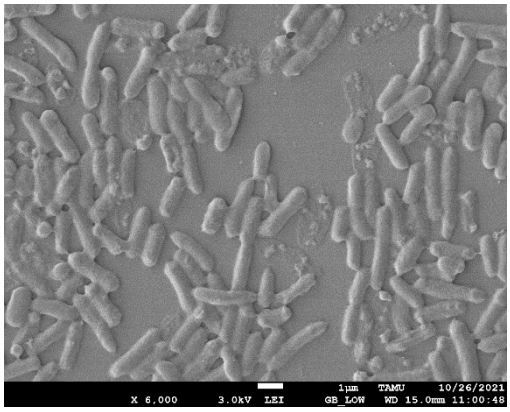
I)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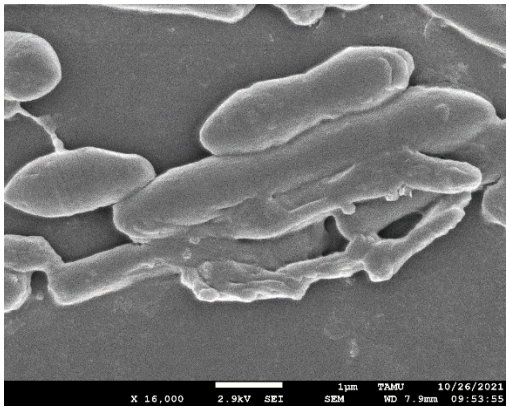
J)


1µm TAMU 10/26/2021
X 16,000 2.9kV SEI SEM WD 7.9mm 09:53:55

K) **L)**

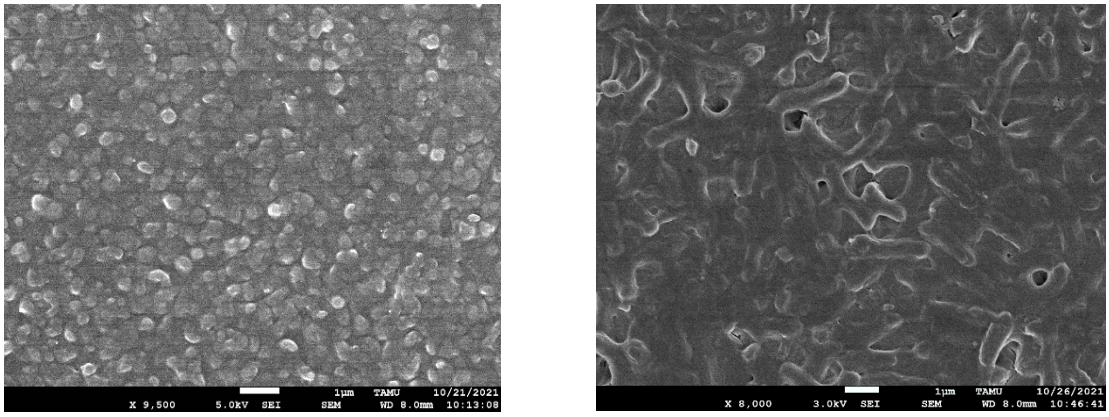


Figure 25. Scanning electron micrographs of untreated *L. monocytogenes* (A), *E. faecium* (C), *S. aureus* (E), *E. coli* O157:H7 (G), *S.* Typhimurium (I), and *P. aeruginosa* (K), whereby represents *L. monocytogenes* (B), *E. faecium* (D), *S. aureus* (F), *E. coli* O157:H7 (H), *S.* Typhimurium (J), and *P. aeruginosa* (L) treated with encapsulated extract at a concentration of 10 mg/mL for 24 hrs incubation time.

Conclusion

The higher antibacterial activity in encapsulated sumac polyphenols compared to that of sumac free extract is attributed to different factors, including particle size distribution, surface charge, encapsulation efficiency, nanoparticle stability, etc. The initial burst in our drug release experiment may be attributed to the release of randomly attached sumac polyphenolic compounds on the outer layer of nanoparticles that have been released in the first few hours of the dialysis experiment as they have come in contact with media. The same effect may be responsible for the higher initial negative surface charge of sumac nanoparticles as the hydroxyl groups on galloyl derivatives have provided the compounds with a negative surface charge.

CHAPTER VI

CHEMICAL STABILITY OF SUMAC POLYPHENOLS IN AN IN VITRO SIMULATED GASTEROINTESTINAL DIGESTION SYSTEM

Introduction

The outcome of a digestion process can is to transport the food constituents into the body cells to provide them with the nutrients they need for their existence. The gastrointestinal tract in human is designed and responsible for the process of extraction, breaking down, and absorption the food constituents into the bloodstream. When studying the structure and function of food components and their effects on human health, one approach may be conducting a human clinical trial. However, human studies are time consuming, costly, and restricted by ethical concerns [220]. The composition of gut microbiota strains may differ from one body site to another making it hard to attribute a particular action to each strain. The use of *in vitro* and *ex vivo* studies are an alternative approach in effort to isolate critical steps in digestion and evaluate specific physicochemical changes under controlled experimental conditions. For example, quantitative analysis may include identification of strains, species abundance, functionality of biomolecular compounds, and bioinformatics profiling of the resulting data [221]. The above-mentioned evolution of methodologies for studying the human microbiome has been continued by introducing *in vitro* models. The primary advantages associated with the *in vitro* gastrointestinal model used in our study is its cost effectiveness and flexibility to manipulate the conditions prevailing the human gastrointestinal tract. Such *in vitro* studies are also more rapid and involve less ethical restrictions than in vivo and human clinical trials [222]. However, using isolated bacteria in this study may allow us to investigate

their direct interactions with plant polyphenolic compounds individually. The role of gut microbiota such as commensal *E. coli* and *Lactobacillus* bacteria in breaking down the larger polyphenolic compounds and their impact on the overall permeability of intestinal cells to the compounds through altering the tight junction protein distribution have been already evaluated [223]. This chapter, however, concentrates on the impact of the isolated probiotic bacteria on the metabolite production from sumac extract.

The parameters affecting the overall structure and function of plant polyphenolic compounds can be divided into internal and external categories. The internal parameters refer to the chemical structure of polyphenols and external factors include temperature, pH, oxygen, light, interaction with other polyphenols and/or food constituents [224]. One of the approaches to assess the chemical and physical stability of plant polyphenols during digestion is to simulate the approximate conditions prevailing in the human gastrointestinal tract. To evaluate the effect of the prevailing physicochemical conditions in the human gastrointestinal tract on the structure and function of sumac tannins, a simulated digestion model was designed to represent a healthy human's upper gastrointestinal digestion tract. The physiological conditions such as the dynamics of peristaltic mixing, oral saliva interactions, gastric digestion, and emptying into the small intestine based on transit times and the acidity of the oral, gastric, and intestine along with chemical composition and interaction of the secretory products were stimulated. There may be more conditions than the factors mentioned above that affect the final structure of sumac hydrolysable tannins. The aim of this experiment was to investigate the impact of endogenous bacterial secreted enzymes (esterase and decarboxylase) on the production of sumac metabolites in a simulated gastrointestinal digestion.

Another factor impacting the bioavailability of polyphenolic compounds is the action of gut microbiota through which these colon habitants secrete various enzymes and hydrolyze the polyphenols to low molecular weight metabolites as their defense mechanism. Most of the parent polyphenolics found in foods, especially high molecular weight tannins, will escape absorption in small intestine and enter to the colon. These compounds then undergo a biotransformation through enzymatic action expressed by gut microbiota to convert compounds into more absorbable forms for the human body. Some probiotic bacteria such as *Lactobacillus* spp. (i.e. *L. plantarum*) and *Bifidobacterium* spp. (i.e. *B. breve*) can produce tannase enzymes (i.e. tannin acyl esterase) to target the gallotannins found in sumac and other fruits. Tannase catalyzes the hydrolysis of galloyl ester bonds from its glucose core to produce free gallic acid with remenants of lower molecular weight galloglucoses. The biochemical pathway for the metabolite production of tannins by probiotic bacteria first involves this (gallotannase) while the same bacteria may also express gallate decarboxylase in a second reaction to form pyrogallol [49].

As an indicator of probiotic bacteria' metabolizing activity, the concentration of targeted low and high molecular weight tannin metabolites was evaluated. It was hypothesized that the production of low molecular weight metabolites increases as the high molecular weight compounds undergo bacterial hydrolysis activities. Despite the presence of more complex galloylated structures in sumac, the highest molecular weight hydrolysable tannin in this experiment was determined to be penta-galloyl glucose (PGG) molecule. PGG is the smallest sugar conjugated tannin that can be metabolized to its simplest aglycones upon bacteria's' secretion of endogenous enzymes by bacteria. To assess the concentration of the metabolites, quantitative and qualitative analyses were carried out.

Materials and Methods

Simulating Gastrointestinal Model

The physicochemical conditions prevailing human gastrointestinal digestion tract was simulated in a model as previously described [225-226]. The primary digestion phases consisted of physicochemical simulation of oral, gastric, and small intestinal compartments. The model was designed to evaluate the impact of digestive chemicals and physical processes on an extract of sumac polyphenolics. To assess the physicochemical impact of digestion process, the free Su and NPSu extracts (as previously described in chapters IV and V) were applied to a simulated gastrointestinal model consisting of the first three phases of oral, gastric, and intestinal. Comparison analyses were based on the physicochemical alteration of polyphenols were conducted. The qualitative and quantitative analyses were made after collecting aliquots from the digesta at the defined time intervals followed by characterizing the polyphenols present in each sample. These isolates were assessed for biotransformation of the parent compounds after each stage of the digestive model.

Oral Phase Simulation

A saliva fluid was simulated by preparing a mixture of chemicals as previously described (Mao & McClements, 2012). Briefly, sodium chloride (1.6 g/L NaCl), ammonium nitrate (NH_4NO_3), potassium phosphate (KH_2PO_4), potassium chloride (KCl), potassium citrate ($K_3C_6H_5O_7H_2O$), uric acid sodium ($C_5H_3N_4O_3Na$), lactic acid sodium ($C_3H_5O_3Na$), urea (H_2NCONH_2), porcine gastric mucin (Type II) were added to water to prepare the oral phase compartment of the digestion model. 40 mL of the solution was then mixed with 1 g of free Su

and 2.5 g of NPSu extracts. The amount was adjusted for the difference based on the total polyphenolic content values (the ratio of polymer to compound was 1.5:1 in NPSu). The pH of the oral solution was adjusted to 6.8 using 1N NaOH solution. The mixture was placed in a temperature-controlled incubator that maintained continuous shaking at 90 rpm at 37 °C for 3 minutes. Aliquots (2 mL) were removed from the digesta and the oral digestion reaction immediately stopped by adding 6-fold volume of methanol (acidified with 0.1% formic acid). The samples were then centrifuged at 12,000 x g at 4º C for 20 minutes. The supernatants were filtered through 0.22 µm PTFE filters and stabilized by storing at -80º C until LC-MS analysis.

Gastric Phase Simulation

Immediately following the oral digestion phase, the simulated gastric fluid (SGF) was added according to a previously described method [225]. A mixture of 2 mg/mL sodium chloride (NaCl), 5 mg/mL hydrochloric acid solution (0.05 M, pH 1.2), and 5 mg/mL pepsin was mixed with distilled water and prepared in a 1L flask. A volume of 200 mL of the SGF was mixed with the oral digesta at a ratio of 1:5 (v/v). The pH of the mixture was adjusted to 2.5 with 6 M HCl and incubated in a temperature-controlled water bath to maintain 90 rpm continuous shaking at 37 °C for 2 hours. To prevent sample degradation by external factors such as air and light, the sampling procedure was carried out in an anaerobic chamber while the sample containers were wrapped in aluminum foil. The experiment was run in three independent experiments without replication (a total of 3 values). The aliquots of 12 mL were removed and mixed with 6-fold acidified methanol to stop the digestion reaction. The samples were then centrifuged at 12,000x g at 4º C for 20

minutes followed by filtration of the supernatants through 0.22 µm PTFE filters. The samples were stored at -80º C until LC-MS analysis.

Small Intestine Compartment Simulation

A simulated intestinal fluid (SIF) was prepared according to a method previously described [225]. Briefly, 24 mg/mL porcine pancreatin, 50 mg/mL bile salts, and saline solution (0.5 M $CaCl_2$ and 7.5 NaCl) were mixed with distilled water to prepare a volume of 1L. Before using the digesta, the pH of digesta was adjusted to 5.8 by using 1M $NaHCO_3$ solution. After 10 minutes of incubation at 37 °C, the pH was readjusted to 6.8 using 0.25 M NaOH solution. Then, 10 mL of the SIF solution was added to the digesta (recovered from the gastric phase) and pH was readjusted to 6.8 using the same sodium hydroxide solution and incubated at 37 °C for 2 hours while shaking at 90 rpm in the absence of light and oxygen. Aliquots of 13 mL were removed, and samples were mixed with 6-fold acidified methanol (0.1% formic acid content) to stop the enzymatical reactions. The samples were then cooled down to the refrigerator temperature by placing them in an icebox for 10 min. Then, the digesta were centrifuged at the speed of 12,000x g at 4 °C for 20 minutes. The supernatants were separated and filtered through 0.22 µm PTFE filters and kept at -80 ºC until further analysis.

Physicochemical Stability Analyses of Free and Encapsulated Sumac polyphenols

Size Alteration as a Function of pH Changes

The role of pH conditions in physicochemical alterations of free Su and NPSu polyphenols was assessed using the same simulated digestion fluids (including the enzymes as described in the

previous section). For sample preparation, a 1.5:1 polymer to compound ratio was used. The formula adjusted for the difference so that the concentration of polyphenols in both free (0.650 g) and encapsulated (1.625 g) sumac extracts was the same. The polyphenolic extracts were then individually mixed with 150 mL of Simulated Gastric Fluid (SGF; pH 2.5) for 2 hrs at 37ºC. To simulate the upper intestinal conditions, 12.5 mL of Simulated Intestinal Fluid (SIF; pH 5.8) was transferred to the chamber containing the mixture of sumac extract and SGF and kept for 10 min. The pH of the mixture was then adjusted to 6.8. The SIF mixture was held for 32 hrs on a shaking incubator at 90 rpm at 37 ºC. Aliquots (1 mL) of digestions were removed at time intervals of 0, 2, 4, 8, 16, and 32 hrs. To test for particle size at the target pH values, the samples were immediately cooled down to 4 ºC using an icebox (no methanol was added or pH modification conducted). To determine the effect of pH changes and gastrointestinal fluids compositions on sumac polyphenols particle size diameter and distribution, the Dynamic Light Scattering (DLS) technique was utilized to measure particle size at a scattering angle of 90º at 25 ºC using a Malvern Zetasizer ZS90 nano series instrument (Malvern Instrument, Ltd., Westborough, MA) was used.

Impact of pH on the transportation rate of sumac extract polyphenols through a dialysis membrane (Release Kinetic)

To assess the effect of tannase enzyme on NPSu polyphenols, the enzyme was exogenously (at the concentration of 20 U or 4 mg/mL) added to both free Su and NPSu extracts. Four samples of treated and untreated with tannase were then prepared and labeled as free Su without tannase, free Su with tannase, NPSu without tannase, and NPSu with tannase. Aliquots of 1 g of free Su and 2.5 g of NPSu polyphenols were directly (no preconditioning such as hydration or dilution

involved) loaded in Spectrum™ dialysis cellulose membrane with 2 nm diameter and a molecular weight cut-off of 12,000-14,000 Da. The tubes were sealed and immersed in 1x PBS (diluted down from a stock solution of 10x) containing jars (150 mL) at variable pH values of 2.5, 5.8, 6.8, and 7.4 to simulate acidity levels throughout human digestion of those present in food systems. The optimal pH and temperature at which most tannases have shown the highest enzymatic activity are 6-8 and 30-40°C respectively [159]. This overlaps the conditions prevailing oral cavity and intestinal environments. However, the pH condition in stomach may not be in favor of tannase functionality and greatly inhibit its activity in such environment.

The containers were sealed and placed on magnetic stirrers at 90 rpm at ambient temperature over 7 days. Aliquots were pulled from the jars and analyzed using a UV-Visible spectrometer (UV-1800 Shimadzu Corp., Columbia, MD). The concentrations of tannin derivatives were assessed at different time intervals of 0, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 hrs at 280 nm wavelength according to a method previously described [227-228]. As the smaller tannin derivatives are identified at the above-mentioned wavelength, the 280 nm was picked for this experiment as an indicator of whether tannase was able to reach the encapsulated compounds within the membrane. The smaller compounds as the products of tannase activity may release and enter the media environment. The impact of the lowest pH value of 2.5 on inhibiting the activity of exogenously added tannase may be determined when comparing the results from other pH values (5.8, 6.8, and 7.4) being tested. The ability of the tested polyphenols to leave the nanoparticle structure or polymeric micells (autohydrolysis) and freely pass right through the membrane into the permeate (PBS) was defined as its release kinetic. Based on the concentration of targeted polyphenolic compounds (280 nm) in the permeate at the defined time intervals release profile was

plotted. The pH of the permeate was constantly controlled to ensure that the designed pH conditions are maintained throughout the experiment. In addition, the stability of free Su and NPSu polyphenols treated with tannase enzyme were evaluated and compared to those of the free Su and NPSu samples without tannase treatment.

Promoting the Growth of Probiotic Bacteria

Culture Media Preparation

The preparation of the media to culture probiotic bacteria from a commercially available probiotic mix supplement *Lactobacillus* (*L. casei, L. rhamnosus, L. reuteri, L. acidophilus, L. paracasei, L. bulgaricus, L. salivarius,* and *L. plantarum*) and *Bifidobacterium* (*B. bifidum, B. longum, B. breve,* and *B. lactis*) from Renew Life dietary supplements company. *E. coli* HS was donated by *E. coli* Reference Center from Department of Food Science at Penn State University) followed the method of [229]. The free Su and NPSu polyphenolic extracts were evaluated with and without 20 U/mL of tannase enzyme in the presence and absence of the probiotic supplement (anaerobically). The bacteria were inoculated in Man-Rogosa Sharpe broth (MRS purchased from Anaerobe Systems Co.) in an anaerobic chamber at 37 °C for 24 hrs. The already lyophilized bacteria recovered from the probiotic capsules were first preconditioned (rehydrated in 2x media at 37 °C anaerobically for 48 hours) in their selective media. The bacteria were then inoculated in fresh 1x media suspensions followed by incubation for 48 hours under the same anaerobic condition. The suspensions were centrifuged at speed of 4,000x g at 4 °C for 15 minutes. After discarding the supernatant, the pellets were recovered and resuspended in phosphate-buffered saline (PBS) and standardized according to the McFarland protocol. The standard bacterial

suspensions were then treated with sumac polyphenolic extracts and the growth kinetic of the bacteria was evaluated using a plate reader instrument (CLARIOstar® Plus Multi-mode Microplate Reader-BMG LABTECH).

Assessment of possible symbiotic effect between probiotic bacteria and sumac extracts by evaluating metabolite production

The production of microbial metabolites was assessed as previously described methods [230]. The metabolite production from sumac gallotannins including gallic acid and pyrogallol were identified and quantified by UHPLC-MS.

For microbial sample preparation, previously preconditioned probiotic bacteria suspensions including *Lactobacillus, Bifidobacterium,* co-culture, and *E. coli* HS were individually treated with sumac polyphenolic extracts anaerobically. The samples were then incubated at 37 °C for 48 hrs. During the incubation, the optical density of the sample was read at 0, 2, 4, 8, 16, 32, and 48 hrs. At the end of the experiment, the wells contents were deactivated with acidified methanol (0.1% formic acid) at the ratio of 1:6 (v/v). The suspensions were then centrifuged at 4000 x g for 20 minutes at 4 ºC. The supernatants were removed and filtered using 0.22 µm PTFE filters and stored at -80 ºC until UHPLC-MS analysis.

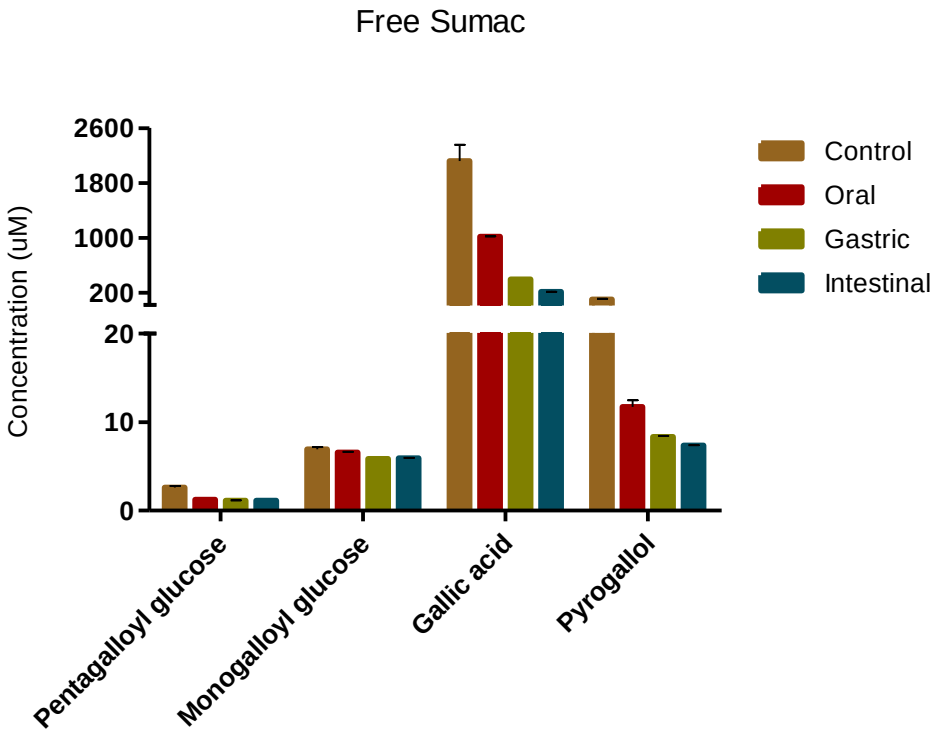
Results and Discussion

The Effect of Simulated Gastrointestinal on Polyphenolic Sumac Extract

Free Su and NPSu polyphenolic extracts treated with and without tannase enzyme were exposed to simulated digestive conditions in the absence of light at 37 ºC. Aliquots were taken at

the end of each digestive stage based on timing of each transition from oral to colon and changes in polyphenolics evaluated by LC-MS analyses. The compounds of interest were identified as penta-galloyl glucose (5GG), monogalloyl glucose (1GG), gallic acid (GA), and pyrogallol (**Figure 27A**). The result of free Su treatment indicated the concentration values of pentagalloylglucose (5-GG) and monogalloylglucose (1-GG) were 1.2 µM and 6.6 µM after oral phase at pH value of 6.8 whereas losses of 52.4% and 5.3% for 5-GG and 1-GG were observed compared to the controls, respectively. A loss of 7.6% in gastric phase and an additional of 1.9% in intestinal digestive phase were also observed for 5-GG. The concentrations of gallic acid and pyrogallol were also decreased to 1017 µM and 1.25 µM compared to that of the initial concentrations by 51% and 88% in free sample in oral phase, respectively. Moreover, the gallic acid was decreased 61% in gastric phase and an additional 46% in intestinal phase. The concentration of pyrogallol was indicated a reduction from 8.33 µM in gastric condition to 7.38 µM in intestinal phase that showed a decrease of 29% to 11% from transition to intestinal digestive phase.

A)



The **Figure 27B**. shows the concentrations of targeted compounds in free Su treated with tannase treatment. The 5-GG exhibited a decrease by 63%, whereas the concentration of this compound was increased by 28% in gastric phase compared to the oral digestive phase. However, a decrease by 45% was observed when pH raised to 6.8 in intestinal phase. The 1-GG also exhibited a similar trend of reduction compared to 5-GG, whereas the concentration of this compound was decreased by 22% in the oral phase and reached a value of 5.20 µM. However, 1-GG concentration was enhanced by 1.6% at pH value of 2.5 in gastric condition compared to that of oral digestive phase.

B)

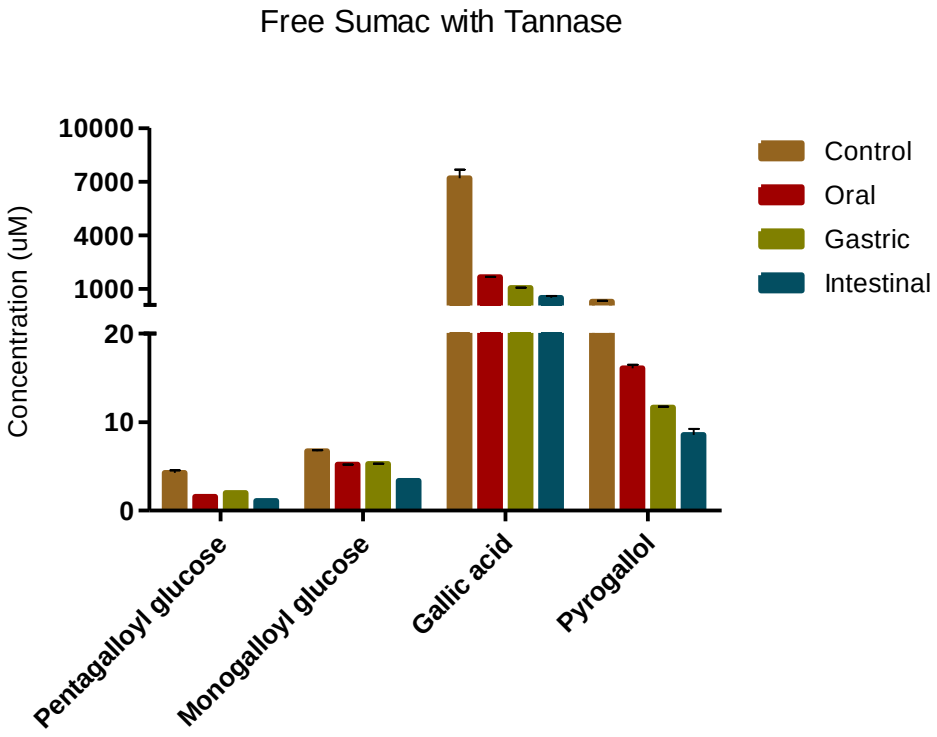


Figure 26. The presence of tannin and its metabolites in free Su (A) and free Su treated with Tannase enzyme (B) (5-GG, 1-GG, Gallic acid, and Pyrogallol) in a simulated oral, gastric, and intestinal conditions at pH values of 6.8, 2.5, 5.8, and 6.8 after 15 min, 2 hrs, and 2 hrs digestion at 37 ºC.

The concentrations of gallic acid and pyrogallol were also decrease by 77% and 94% in oral phase in the free Su treated with Tannase enzyme with a concentrations of 1661 µM and 16.1 µM. However, this reduction was observed for GA and pyrogallol when a transition occurred from a higher pH of oral phase to lower pH of gastric digestive phase, so that, the concentration values of 1055 µM and 11.7 µM were exhibited losses by 36% and 27% in gastric phase and an additional 53% and 26% in intestinal digestive phase. Results of the current study showed that the amount of high molecular weight galloylated compounds (pentagalloylglucose and monogalloylglucose)

were lost during the initial oral phase. Pentagalloyl glucose and monogalloyl glucose molecules were shown more concentration reduction after the oral phase (pH of 6.8) in free Su treated with Tannase compared to that of free Su in oral digestive phase. A possible explanation can be due to the higher number of galloyl group in their structure which provides them with higher hydroxyl group sites to interact with the enzyme in oral phase.

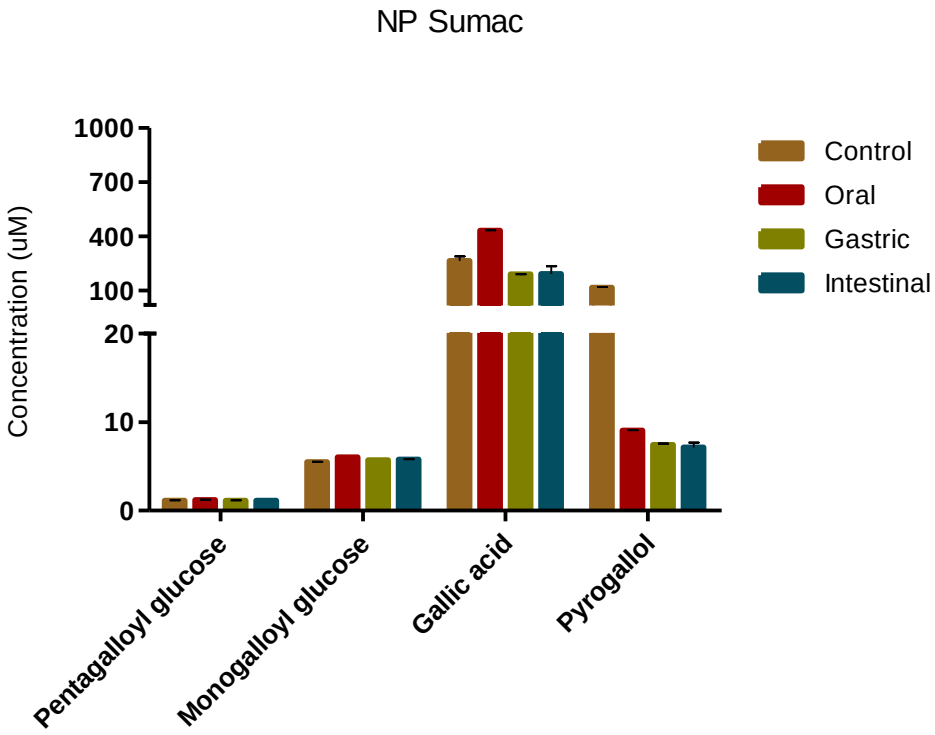
Although the oral cavity as the first part of the digestion system has only few minutes possession of food to impact its polyphenolic content. In this work, it was however observed that the initial concentration of galloyl derivatives were lost during the oral phase of the simulated digestive model. The chewing process was not simulated in this model; however, chewing may increase the enzyme exposure of tannins which can result in a reduction in high molecular weight tannins in oral phase In addition to tannin-mucin complexation, the higher pH value in the mouth (6-7) may negatively affect the stability of galloyl derivatives.

The presence of galloyl derivatives in NPSu and NPSu with Tannase is shown in **Figure 28**. The result suggested that tannase enzyme may hydrolyze tannains structure in different pH conditions of simulated gastrointestinal conditions. The result of NPSu extract treated with exogenous tannase enzyme treatment indicated that the concentration values of high molecular weight galloyl derivatives (5-GG and 1-GG) slightly decreased from 1.22 µM to 1.17 µM and from 6.04 µM to 5.55 µM compared to that of the samples without tannase treatment after the oral phase. After oral phase at pH value of 5.8 of gastric condition, the concentration of 5-GG and 1-GG molecules were lost by 3.5% and 8.8%, respectively. When comparing the results in gastric phase, the concentration of tested polyphenolic compounds showed different trend than oral, so

that, a lower loss of the high molecular weight compounds 5-GG was decrease from 6.6% to 3.2% after treated with exogenously added tannase.

In NPSu treated with tannase enzyme, the concentration of lower molecular weight tannin derivatives gallic acid increased by 11% with the value from 432 µM to 780 µM in oral phase. Moreover, the concentration of pyrogallol was also showed an increase by 1.5% with a value from 9.06 µM to 11.1 µM. The loss of pyrogallol increased from 22% (NPSu treated with tannase) to 18% (NPSu) compared to the oral phase. However, addition of tannase enzyme exogenously did not change the loss percentage of the gallic acid in NPSu which counts for 56% for both samples in gastric digestive phase. Nanoparticle sumac extract when treated with tannase after intestinal phase at pH value of 6.8 exhibited losses of 0.91%, whereas the loss of 1.1% was observed in NPSu. Sumac tannins were protected from tannase activity during digestion; however, the degradation of polymeric coating material during the process could provide a controlled release of the compounds to be available for bacteria. After treatment of sumac NPSu extract with tannase, the concentration of 5-GG in intestinal phase was slightly decreased from 1.13 µM to 1.12 µM while the concentration of 1-GG decreased from 5.80 µM to 5.51 µM under the same conditions in intestinal phase. In contrary, the concentration of gallic acid and pyrogallol (possibly due to auto degradation of gallic acid in an alkaline condition) were slightly increased in intestinal digestive phase. The results indicated an increase from 192 µM to 287 µM for gallic acid and from 7.2 µM to 7.6 µM for pyrogallol concentration after treatment of the sumac nanoparticle extract with tannase.

A)


NP Sumac
Concentration (uM)
1000
700
400
100
20
10
0
Control
Oral
Gastric
Intestinal
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol

B)

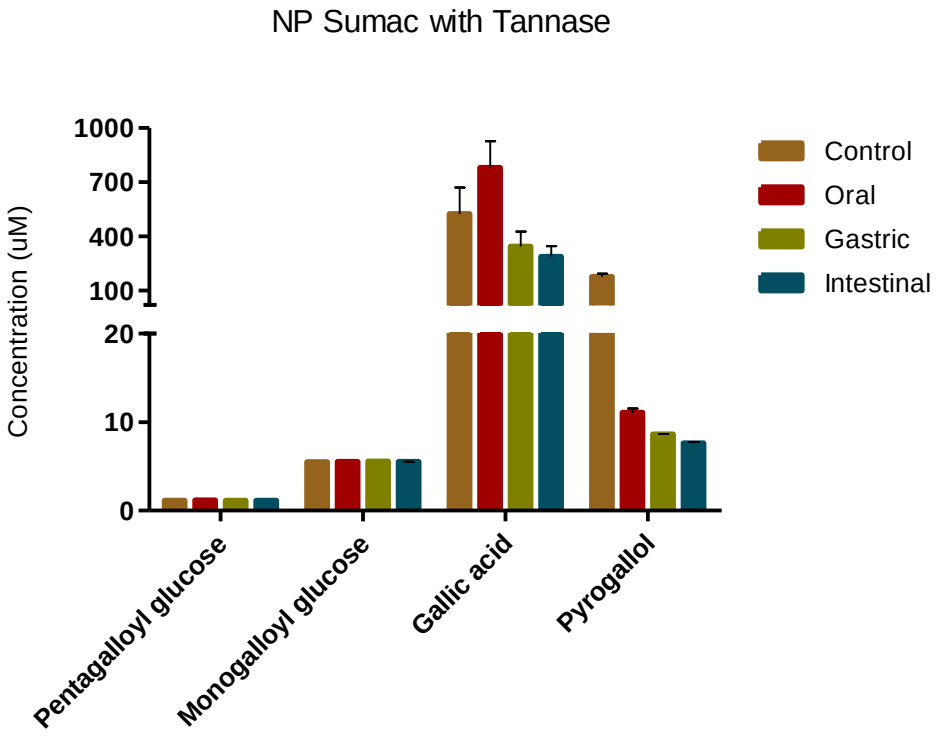


Figure 27. The presence of tannin and its metabolites in NPSu (A) and NPSu treated with Tannase enzyme (B) (5-GG, 1-GG, Gallic acid, and Pyrogallol) in a simulated oral, gastric, and intestinal conditions at pH values of 6.8, 2.5, 5.8, and 6.8 after 15 min, 2 hrs, and 2 hrs digestion at 37 ºC.

The oral phase of the gastrointestinal tract is a sophisticated part of the system with many components contributing the pre-digestion of food. The complexation of the system has made it hard to simulate the oral phase for research purposes. Although the real saliva was not used in this model; however, it is worth mentioning that the interindividual and intraindividual differences in saliva compositions is still a challenge toward using the real saliva for research. The composition of saliva varies from one individual to another. Even the composition of saliva within one subject may vary due to diet, age, gender, time of day, interaction with other food ingredients, etc. The

primary difference between actual and simulated saliva is the unique lubrication property of human saliva [231-232]. This property is attributed to a type of anionic glycosylated protein called mucin apparently responsible for all the rheological behaviors of saliva. The mucin provides this unique lubrication property by interaction with and incorporating other compounds in a specific structure called saliva pellicle. The pellicle is formed as a result of multilayer network between mucins and other salivary proteins. This structure provides the saliva with a visosity near to ($\rho \sim 1000 kgm^{-3}$) and surface tension lower than water ($\lambda \sim 50 mNm^{-1}$) [233]. The exposure of four common epicatechin compounds (epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate) has shown a significant loss of all compounds (20%-40%) with epicatechin showing the highest stability and epigallocatechin gallate the lowest. The results were on par with the loss of gallic acid in this model, although gallocatechin has a different structure than gallic acid, which may not justify this comparison analysis. The depletion of the above-mentioned tannins in part is attributed to the number of galloyl groups within their structures. That is, the catechins with more galloyl group were lost more than those with lower degree of galloylation [234].

The active site of an enzyme may also bind to a PEO (polyethylene oxide) monomer within the structure of pluronic copolymer. Such conjugation may result in a cross-linked enzyme-polymer conjugate that temporarily immobilizes and protects the enzyme against denaturalization. The driving force for the modification in the enzyme-substrate interaction may be through protein conformation change, high substrate concentration, or changes in the hydration site of the enzyme [235]. Chemical substances may act as substrates for tannase and immobilize/deactivate the enzyme. Different polysaccharides (alginate, chitin, agarose, methacrylamide), co-polymers, ion-exchange resins, metal ions (Cu, Zn, Fe, etc.), and proteins/peptides are reportedly covalently interacted with

tannase and occupied its binding sites [236]. The covalent conjugation between pluronic-based particles and tannase may be formed through the interaction between an active group (thiol, carboxyl, etc.) of polyethylene oxide molecule (the hydrophilic chains on the structure of pluronic copolymer) and a functional group on the enzyme [237].

Regardless of the source of tannase (bacteria, yeast, or fungi), they all share a common pentapeptide active site pattern of Glycine-X-Serine-X-Glycine structure. This typical motif for serine hydrolases has provided tannase with the ability to hydrolyze either ester or depside bond (galloyl ester of alcohol or gallic acid moieties, respectively) [238]. When binding to gallic acid, the carboxylic acid group on gallic acid bonds to a catalytic triad (consisting of Ser163, His451, and Asp419) through hydrogen bonding. The three hydroxyl groups are also bonded to Asp421, Lys343, and Glu357 to create additional hydrogen bindings making the complex even stronger [239]. Tannase may also be trapped within the blank/void spots between multiple pluronic nanoparticle structures. As shown in **Figure 29**, the pluronic-based micelles created various locations in their surroundings with different viscosities. The void regions contain high polar and low viscose media, which is attractive for a protein or enzyme. Unlike the void areas, the PPO cores are nonpolar and highly viscous, while PEO areas are also hydrophilic with moderate viscosity and water penetration property [240].

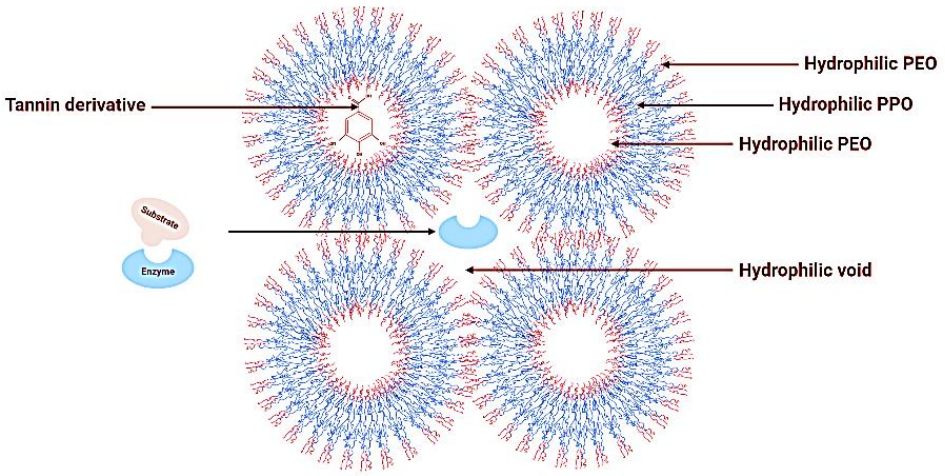


Figure 28. The porous steric structure of polymeric micelles. The self-assemble property of pluronic results the surrounding molecules to get trapped inside the above structures. Tannin and tannin acyl hydrolase (tannase) are both entrapped within different regions of this schematic structure (created with BioRender.com).

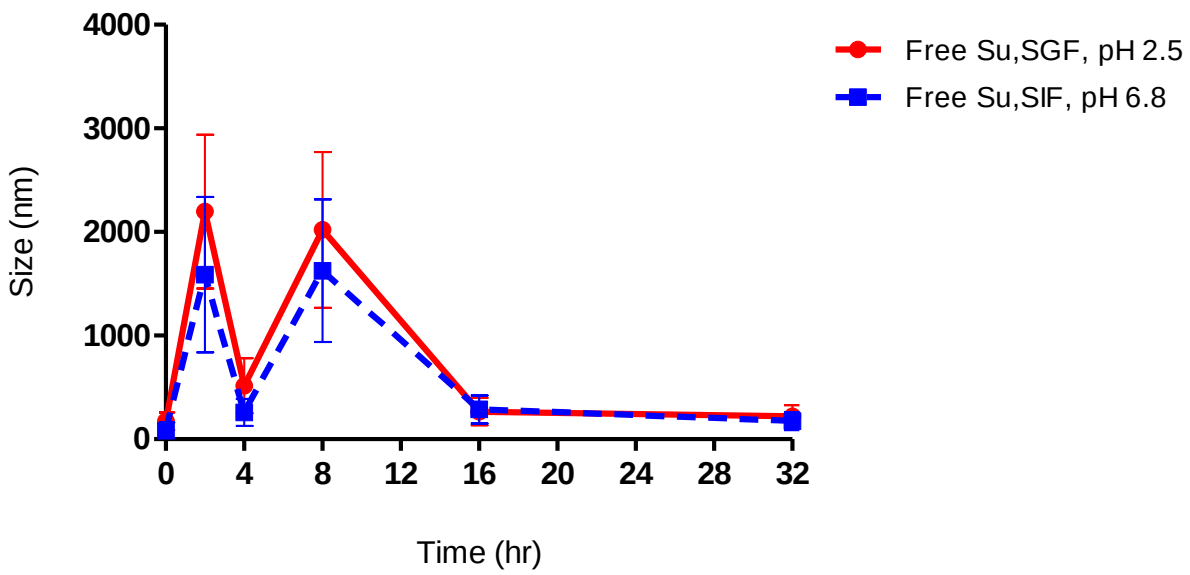
Size Alteration as a Function of pH Changes

The size fluctuation over time exhibited a lower rate in NPSu samples compared to that of free Su samples in different pH conditions (**Figure 30**). The free Su sample in SGF at a pH value of 2.5 showed a mean size of 899.6 nm, while a correspondent size became smaller at the pH of 6.8 with a size mean of 669.5 nm. Moreover, free Su with Tannase exhibited a mean size of 1422 nm at a pH of 2.5. However, this value was changed to 505.1 nm when the sample was exposed to intestinal conditions at the pH value of 6.8. In the gastric state with a pH of 2.5, the size mean of

NPSu was found to be 1664 nm, while the same sample got smaller to 596.5 nm when exposed to SIF with a pH value of 6.8. On the other hand, the NPSu with Tannase sample exhibited a larger size mean value of 1475 nm than the NPSu sample with a size mean value of 702.1 nm.

In addition to the impact of pH on particle size in all sumac samples, the particles in NPSu samples showed higher resistance against the changes in pH compared to that of free Su samples. The possible explanation can be associated with the stronger structures of NPSu samples that may protect the particles against pH changes compared to the free Su samples. However, the size change rate was different throughout 32 hrs of the experiment. There was an observed difference in the particle size growth rate. The rapid kinetic as a function of pH suggested that the size growth rate decreased as pH increased.

A)



The kinetic shifted from second order to first order in the higher pH value (pH=6.8). In an acidic environment, the generation of more ionic particles may cause aggregation and precipitation of the molecules, casing a rapid increase in particle size. On the other hand, the lower concentration of H^+ in the solution at higher pH resulted in lower aggregation and higher dissociation of particles. The result was in agreement with a study that investigated the size distribution of silver nanoparticles in the simulated gastrointestinal fluids. It was suggested that under highly acidic conditions (pH 2), there were rapid changes in particle size while the size fluctuation decreased in higher pH of 5 [241].

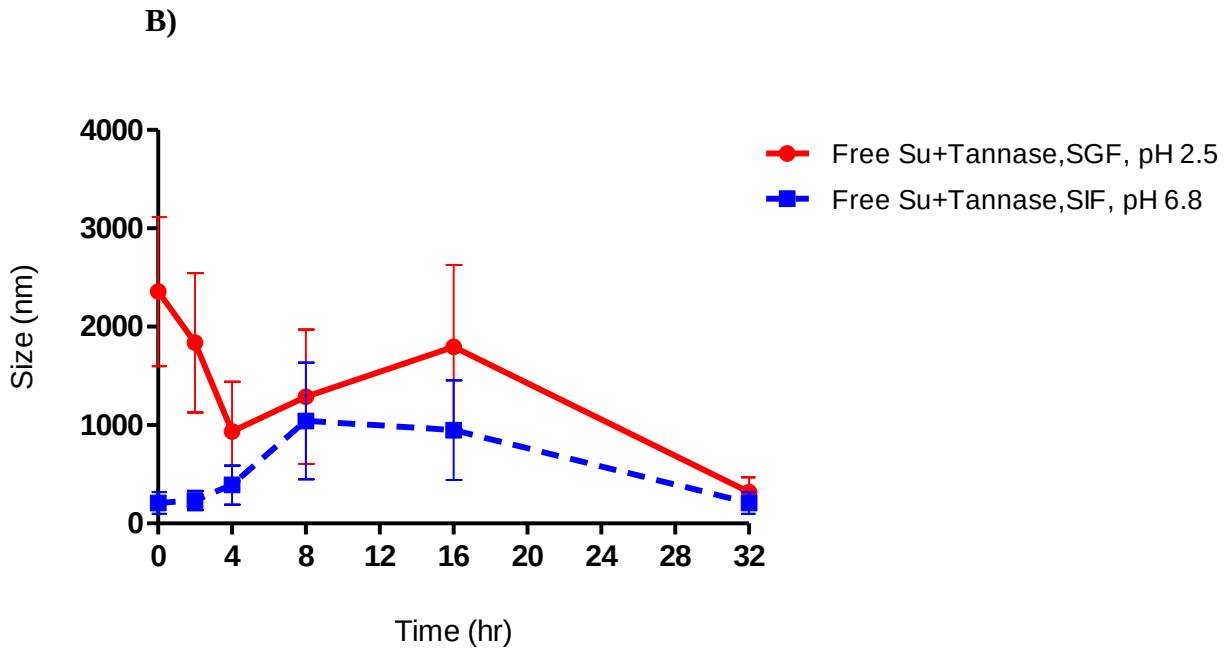
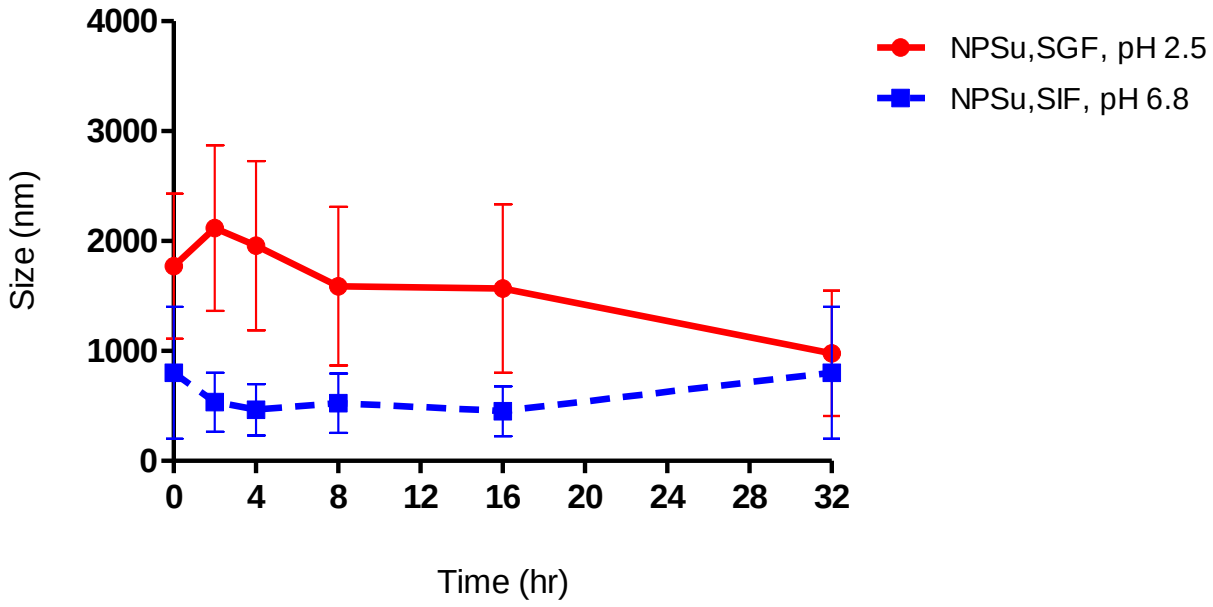


Figure 29. Size stability of free Su (A) and free Su with Tannase enzyme (B) when exposed to SGF and SIF at pH values of 2.5 and 6.8 for 32 hrs at 37 ºC.

The particle size changes due to the instability of particles were greater in free Su samples in both pH conditions compared to that of the NPSu samples. However, the size stability over the time of 32 hrs experiment was higher at less acidic pH (pH=6.8) compared to that of more acidic pH conditions (pH=2.5). It can be concluded that encapsulation of tannin and other galloyl derivatives extracted from sumac may reduce the chemical interaction between hydroxyl groups on galloyl groups with hydrogen or oxygen from other surrounding molecules possessing hydroxyl groups (such as proteins, polysaccharides, alkaloids, etc.) to create hydrogen bonding. This is especially important when studying the chemical stability of tannin derivatives in different pH ranges such as the gastrointestinal tract. Tannin molecule does not exist in its molecular form at pH above six, where it is fully broken down and ionized [242]. The results were in agreement with

the previous study on tannic acid encapsulated particles showing an increase of 93% (106 to 198 nm) in the particle diameter mean when the value of pH lowered from 8 to 2 [243].

A)



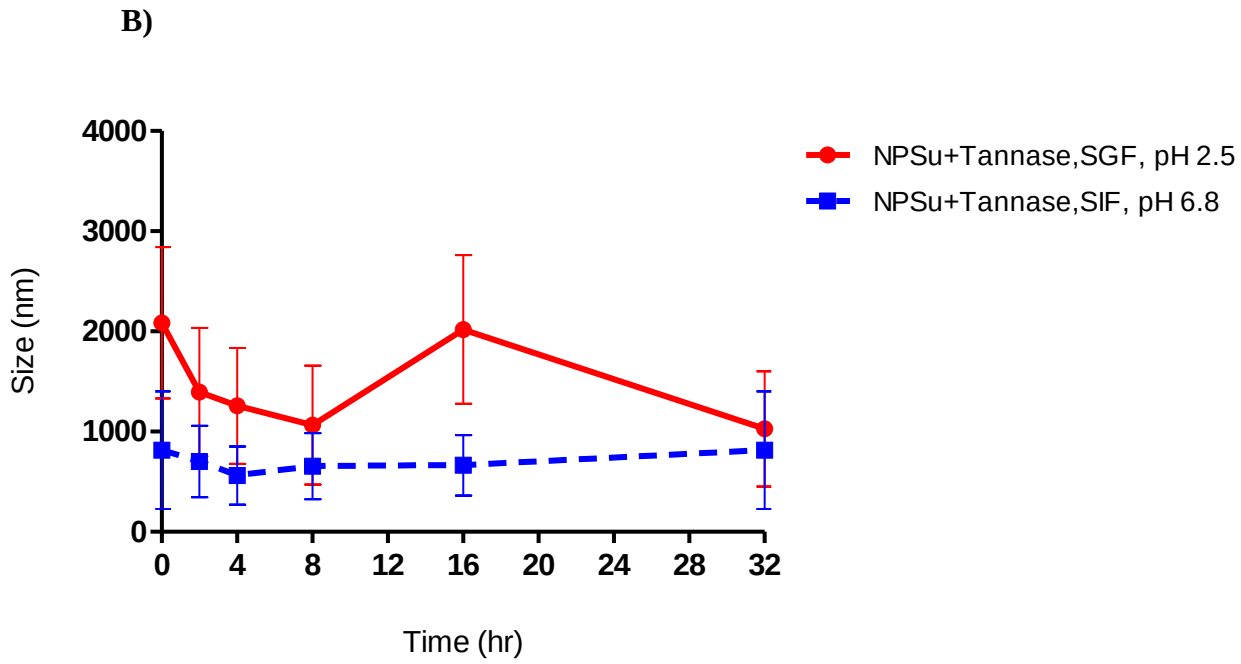


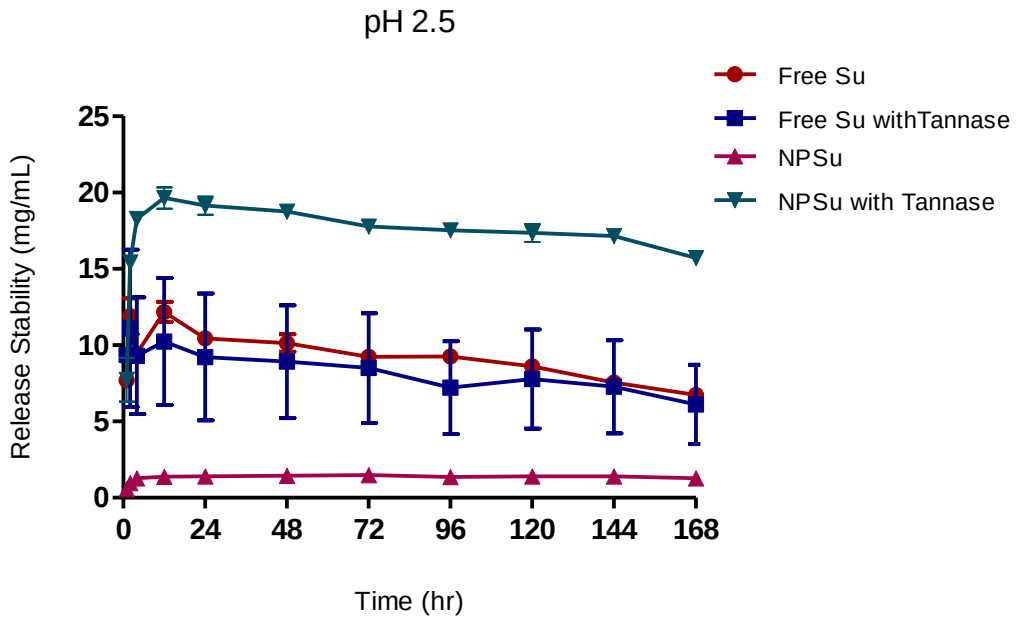
Figure 30. Size stability of NPSu (A) and NPSu with Tannase enzyme (B) when exposed to SGF and SIF at pH values of 2.5 and 6.8 for 32 hrs at 37 ºC.

Release Kinetic Fluctuation as a Function of pH After GI Digestion

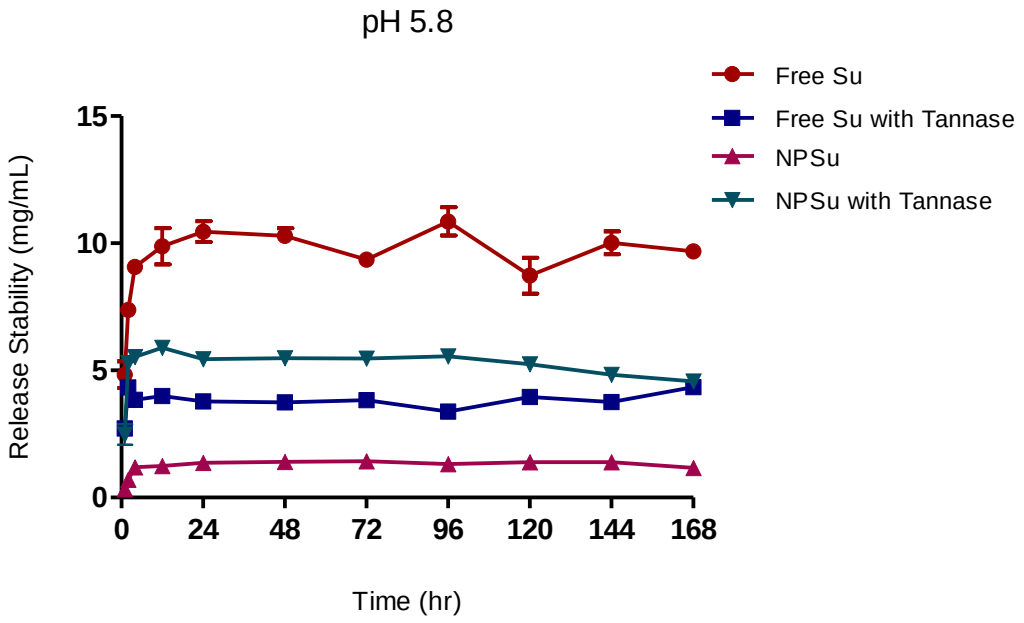
The physicochemical alteration in nanoparticle structure as a function of the particles' environmental conditions was assessed. Free and Nanoparticle Sumac polyphenolic extracts were exposed to exogenously added tannase while exposed to Phosphate Buffer Saline (PBS) medium for seven days. The effect of pH on the stability of sumac polyphenols is shown in **Figure 32**. The free Su treated with tannase showed a higher release of phenolic compounds at 280 nm while NPSu treated with tannase sample exhibited an inverse trend for the release experiment compared to that of the free sample treated with tannase. the encapsulation procedure allows a continuous release of the target compound through the gastrointestinal tract [244]. The lower but constant release of the encapsulated samples with and without the intervention of tannin may be associated with the

ability of coating materials to retain and gradually release the targeted polyphenols from the dialysis membrane into the media solution surrounding them.

A)



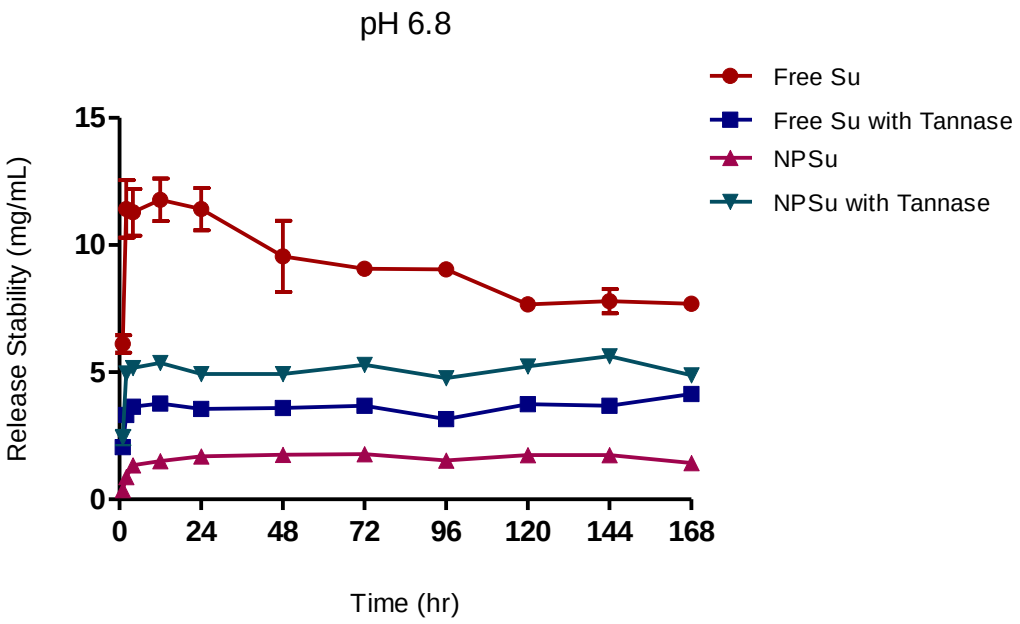
B)



The samples of free Su and NPSu polyphenolic extracts exhibited different susceptibility when exposed to tannase enzyme at different pH values. The free Su treatment showed more susceptibility and degradation under all tested pH conditions compared to that of NPSu extract. The greater stability of polyphenolic compounds in a higher pH value of 5.8 compared to that of lower pH (pH=2.5) was observed. The NPSu samples showed higher stability in both neutral and slightly acidic pH conditions (5.8, 6.8, and 7.4) compared to that of high acidic conditions (pH=2.5). It can be explained that the encapsulation of sumac extract not only provided the samples with stability against pH changes, but also protected the samples against the activity of tannase enzyme. Free Su samples treated with tannase showed a constant but higher release of polyphenolic compounds over 168 hrs of the membrane dialysis experiment. In contrast, NPSu treated with tannase exhibited a lower concentration of polyphenolic compounds indicating a gradual and slow release of the compounds from encapsulated samples. Tannase functionality at

pH 2.5 was higher in the first 48 hours of the experiment compared to that of the other pH conditions being tested.

C)



D)

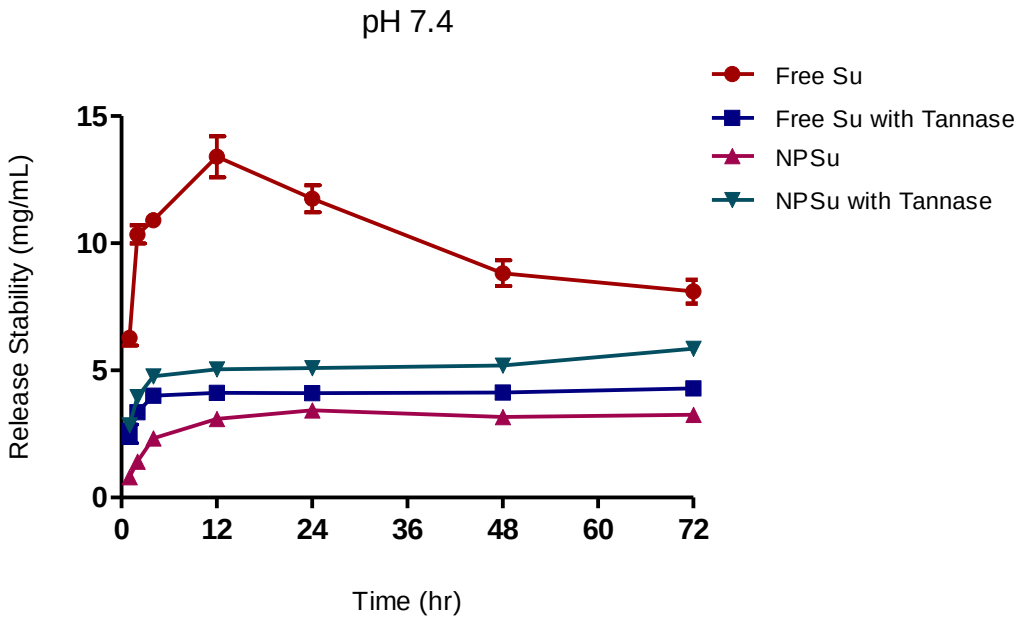


Figure 31. The effect of tannase enzyme on the stability of free Su and NPSu extract in Phosphate-buffered Saline (PBS) at a function of pH of 2.5, 5.8, 6.8, and 7.4 over 168 hrs.

The release stability study on free and encapsulated sumac extract showed a higher concentration of tannins with a constant release at all tested time points. The controlled release of encapsulated particles allows the targeted compounds to gradually release and maintain a constant concentration at the time during their passage through the digestive system [245]. The gradually decreasing concentration in free Su sample after 168 hrs of dialysis indicated the susceptibility to degradation among sumac polyphenolic compounds compared to that of NPSu sample. In addition, the result indicated that regardless of the concentration of sumac extract, there was a constant release profile characteristic associated with the NP samples in the solution.

Sumac Tannins Metabolism by Probiotic Bacteria

After exposing sumac extracts to isolated probiotic bacteria and as a result of their biodegradation reactions, the concentration of hydrolysable tannins in the samples was decreased while the amount of subsequently produced metabolites increased. High molecular weight tannins are reported to be transported to and excreted from the large intestine in their original form. The polyphenols transported into the large intestine may also be subjected to enterobacteria catabolism if not excreted. Although the stomach and small intestine are reportedly the least potential site of absorption for polyphenols among all gastrointestinal tract components, hydrolysis of hydrolysable tannins may take place in the stomach or small intestine before compounds reach the colon [167].

In this study, the stability and hydrolysis of tannin molecules in sumac fruit extract have been studied both with and without exposure to probiotic bacteria to investigate the impact of gastrointestinal fluids and environment on the production of smaller molecule gallates. After treatment of probiotic bacteria with sumac samples, the concentration of higher molecular weight compounds such as 5-GG and 1-GG decreased while low molecular weight compounds exhibited an increase in the concentration, especially in gallic acid and pyrogallol. The ability of probiotic bacteria to secrete dehydroxylase, decarboxylase, etc, enzymes provide them with the ability to break down higher molecular weight tannin structures to smaller and lower molecular weight gallic acid, pyrogallol, etc. Although there is no evidence suggesting a constant for a conversion rate of pyrogallol from galloyl glucosides; however, a study on mango tannin metabolism has previously shown that almost 68% of the total hydrolysable tannin consumed were detected as urinary gallic

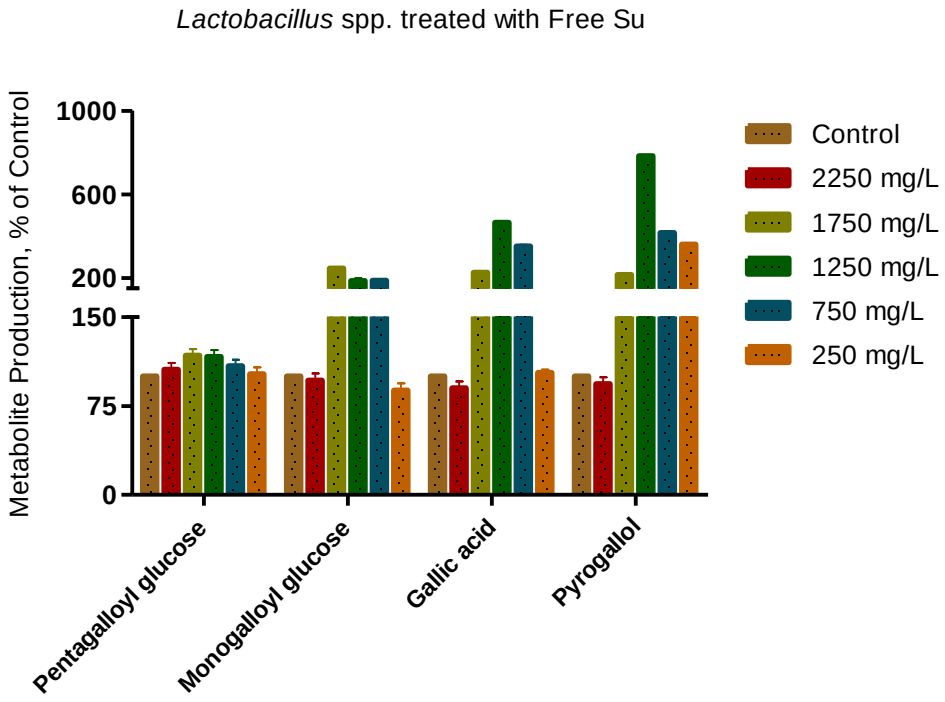
acid metabolites mainly pyrogallol glucuronide, pyrogallol sulfate, and methyl-pyrogallol sulfate [246].

The results of this experiment indicated that *Lactobacillus* spp. were able to increase the concentration of all four tannin metabolites being tested (5-GG, 1-GG, gallic acid, pyrogallol). As shown in **Figure 33**, the concentration of all four compounds is increased even in lower concentrations of the treatments. The concentrations of pentagalloyl glucose and monogalloyl glucose were increased at all the concentrations being tested (2250 mg/L, 1750 mg/L, 1250 mg/L, 750 mg/L, 250 mg/L). The concentration of 5-GG constantly increased even by reducing the total polyphenolic content (TPC) of the specimens. The results showed 5%, 18%, 17%, 9%, and 2% increase in the concentration of 5-GG at 2250 mg/L, 1750 mg/L, 1250 mg/L, 750 mg/L, 250 mg/L, respectively. The lowest galloylated compound within our sumac extract was 1-GG and the results exhibited almost the trend as that of 5-GG. However, the amount of monogalloyl glucose at the highest and lowest concentrations of 2250 mg/L and 250 mg/L showed decreased by 3% and 12% in the concentration compared to that of control. At the other tested concentrations however, the amount of 1-GG was constantly increasing by 146% at 1750 mg/L, 86% at 1250 mg/L, and 87% at 750 mg/L.

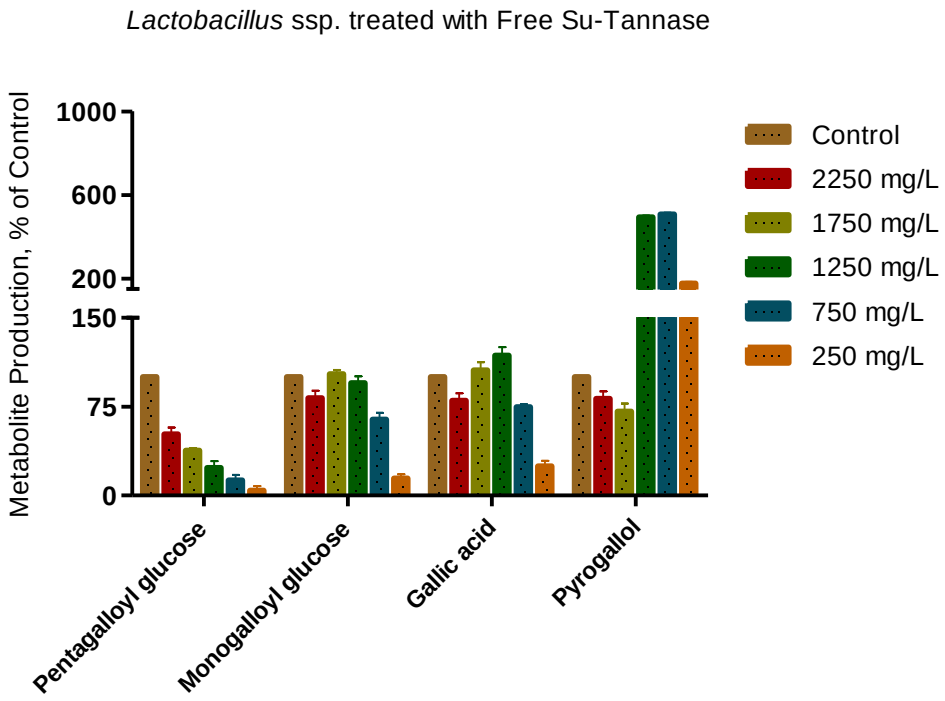
The concentration of the low molecular weight tannin metabolite gallic acid exhibited a reduction in 2250 ppm by 10% compared to the control. After this point, the concentration of gallic acid was constantly increasing by 125%, 365%, 251%, and 3% at the concentration levels of 1750 mg/L, 1250 mg/L, 750 mg/L, and 250 mg/L, respectively. Moreover, among the four tested compounds, pyrogallol exhibited a constant increase in its concentration even at the lowest concentration of 250 mg/L. Except for the highest concentration of 2250 mg/L at which a decrease

by 6% was observed. However, the concentration of pyrogallol was increased by 114%, 683%, 316%, and 261% at the concentration of 1750 mg/L, 1250 mg/L, 750 mg/L, and 250 mg/L, respectively.

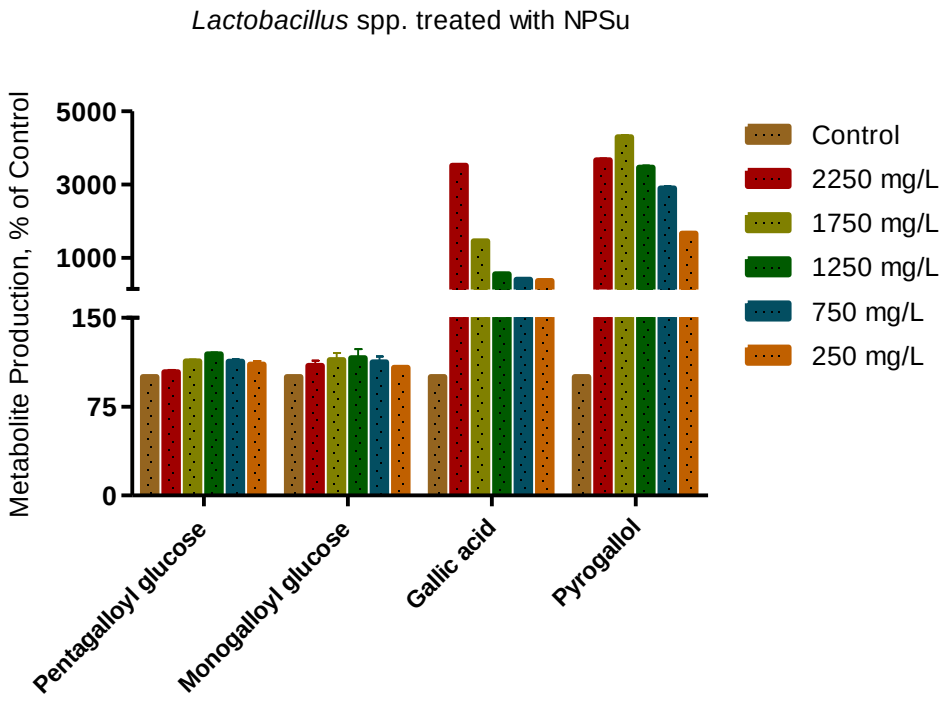
A)



B)


Lactobacillus ssp. treated with Free Su-Tannase
Metabolite Production, % of Control
1000
600
200
150
75
0
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L

C)



The results were in an agreement with a previously reported study suggesting the effect of *Lactobacillus* spp. to synthesize tannase enzyme as their defense mechanism against tannin derivatives. Due to the ability of *Lactobacillus* spp. such as *L. plantarum* to produce tannin acyl hydrolase, these bacteria can hydrolyze ester bonds in tannins and gallic acid esters to produce gallic acid and glucose. In the further subsequent stage, gallic acid can be converted to pyrogallol by *Lactobacillus* spp. [247]. Therefore, the ability of *Lactobacillus* spp. (*L. plantarum*) to excrete decarboxylase and hydrolase enzymes which can break down tannins and gallic acid is attributed to the production of 5-GG, 1-GG, gallic acid, pyrogallol phenolic compounds in this work.

D)

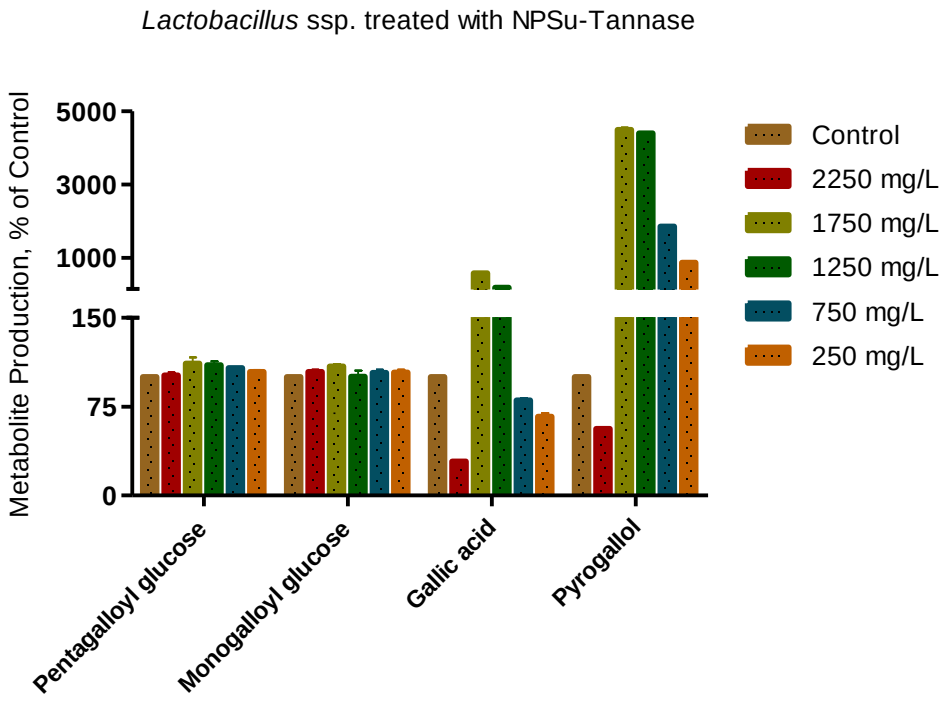
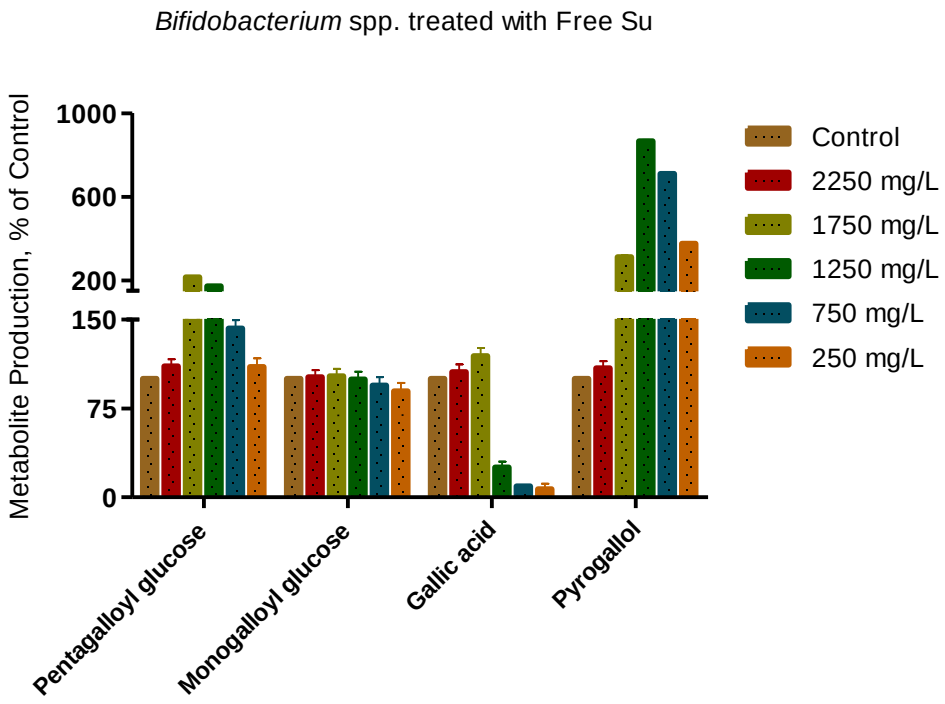


Figure 32. *Lactobacillus* ssp. bacteria fermentation metabolites after treating with free Su, free Su with Tannase, NPSu, and NPSu with Tannase treatments in anaerobic condition at 37 ºC ($p < 0.05$).

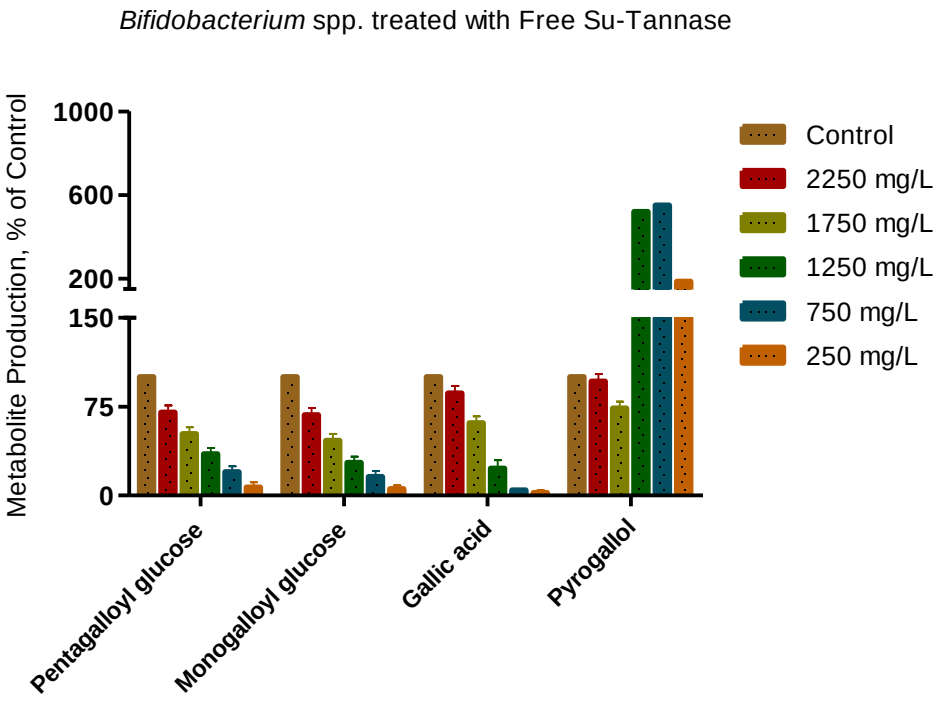
As seen in **Figure 34**, the amount of pentagalloyl glucose and pyrogallol increased at all the tested concentrations. 5-GG was increased by 11%, 115%, 71%, 43%, and 10% at 2250 mg/L, 1750 mg/L, 1250 mg/L, 750 mg/L, 250 mg/L, respectively. The amount of pyrogallol in the treatments was also increased by 9% at 2250 mg/L, 112% at 1750 mg/L, 766% at 1250 mg/L, 610% at, 750 mg/L, and 277% at 250 mg/L. The concentration of 1-GG and gallic acid, however, decreased at and below 1250 mg/L. The amount of monogalloyl glucose increased by 2% at both concentrations of 2250 mg/L and 1750 mg/L. However, the amount of 1-GG decreased by 1%, 6%, and 12% at 1250 mg/L, 750 mg/L, and 250 mg/L, respectively. Similar to this trend, the

amount of gallic acid increased by 6%, and 19% at 2250 mg/L and 1750 mg/L, while its concentration decreased by 75% at 1250 mg/L, 91% at 750 mg/L, and 93% at 250 mg/L.

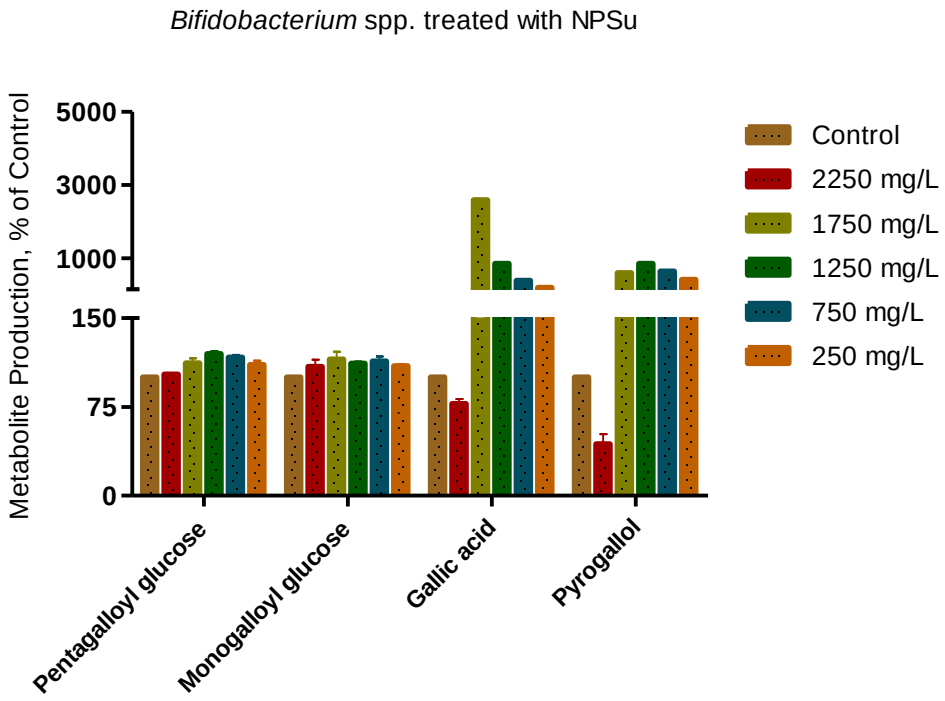
A)



B)


Bifidobacterium spp. treated with Free Su-Tannase
Metabolite Production, % of Control
1000
600
200
150
75
0
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L

C)


Bifidobacterium spp. treated with NPSu
Metabolite Production, % of Control
5000
3000
1000
150
75
0
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L

D)

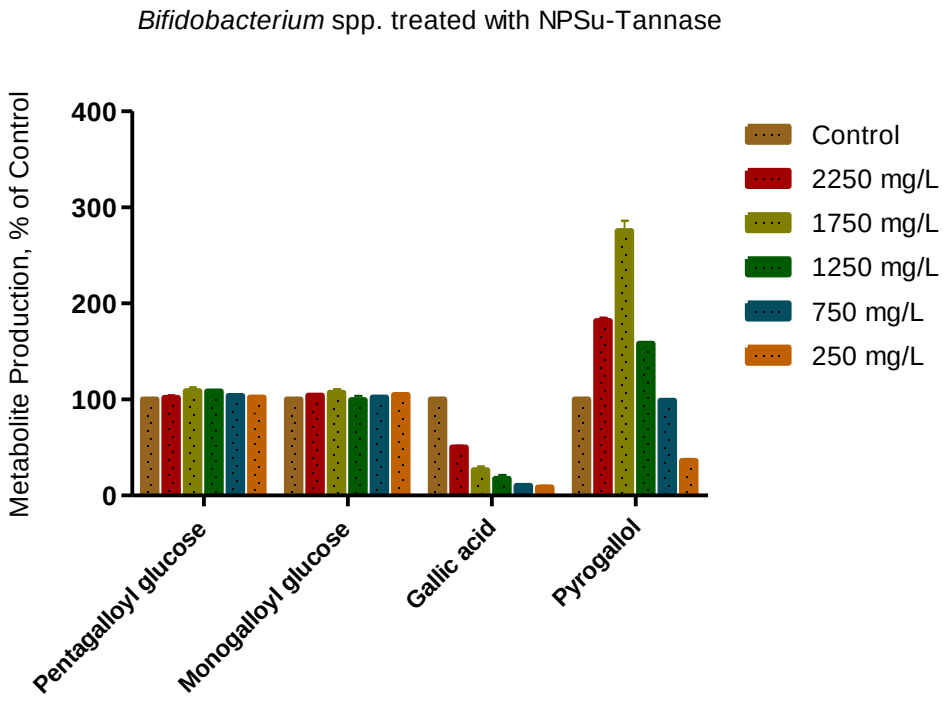
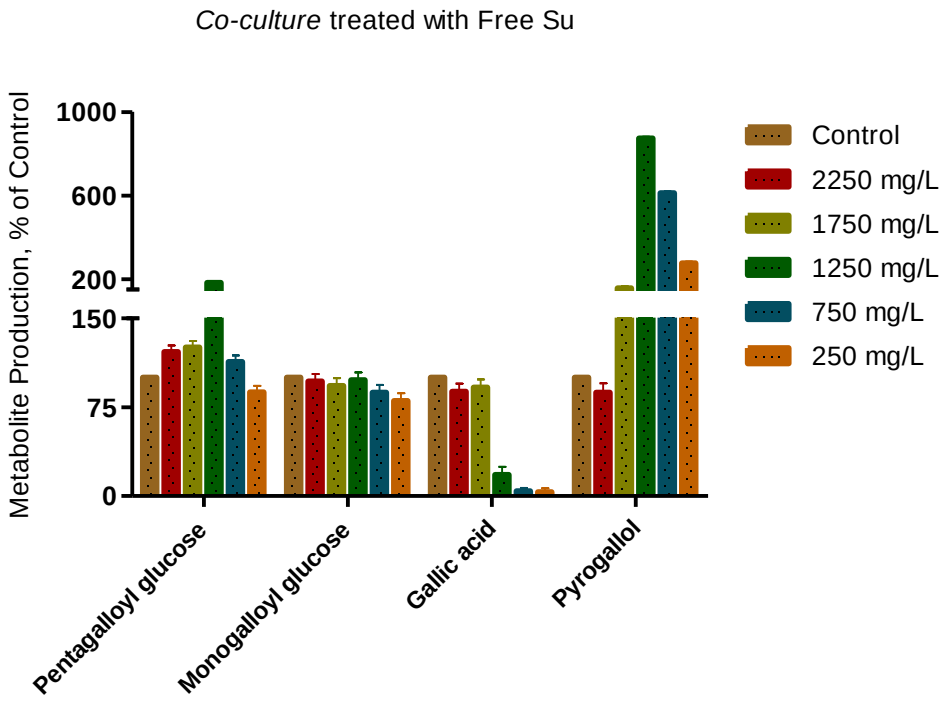


Figure 33. *Bifidobacterium* spp. fermentation metabolites after treating with free Su, free Su with Tannase, NPSu, and NPSu with Tannase treatments in anaerobic condition at 37 ºC.

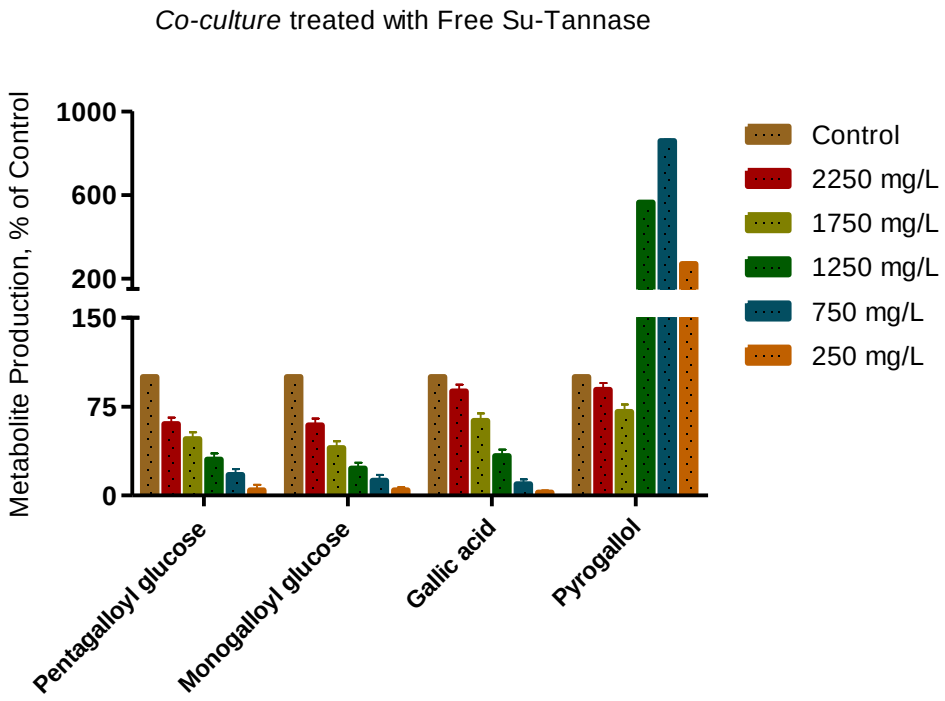
Figure 35 shows the metabolite production of free Su and NPSu polyphenolic extract when treated with co-culture bacteria. The results indicated that the amount of 5-GG exhibited a constant increase up to 250 mg/L at which 5-GG was decreased by 12% compared to the control. The amount of 5-GG increased by 22%, 26%, 83%, and 13% at 2250 mg/L, 1750 mg/L, 1250 mg/L, and 750 mg/L, respectively. An opposite trend was observed in pyrogallol at which the amount of this compound was decreased by 13% only at the highest concentration of 2250 mg/L. However, the concentration of pyrogallol constantly increased by 58%, 773%, 612%, and 176% at 1750

mg/L, 1250 mg/L, 750 mg/L, and 250 mg/L, respectively. The results also showed a reduction in the concentration of monogalloyl glucose and gallic acid in all the treatments. The amount of 1-GG was decreased by 3% at 2250 mg/L, 7% at 1750 mg/L, 2% at 1250 mg/L, 12% at 750 mg/L, and 19% at 250 mg/L. The amount of gallic acid was also decreased compared to the control by 12%, 8%, 82%, 96%, and 97% at 2250 mg/L, 1750 mg/L, 1250 mg/L, 750 mg/L, and 250 mg/L, respectively.

A)

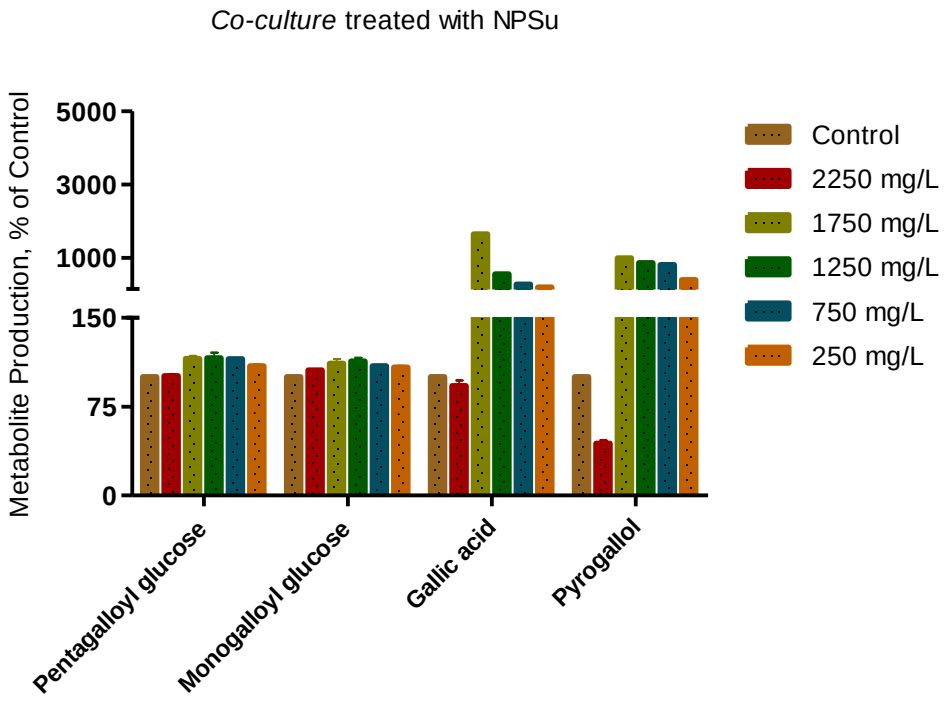


B)



The presence of *Lactobacillus* spp. in combination with *Bifidobacterium* spp. in the co-culture bacteria exhibited the effect of *Lactobacillus* spp. to boost the mixture culture against tannin derivatives. This was an agreement to the previously reported results indicating the higher resistance to tannins among *Lactobacillus* spp. bacteria [248].

C)


Co-culture treated with NPSu
Metabolite Production, % of Control
5000
3000
1000
150
75
0
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L

D)

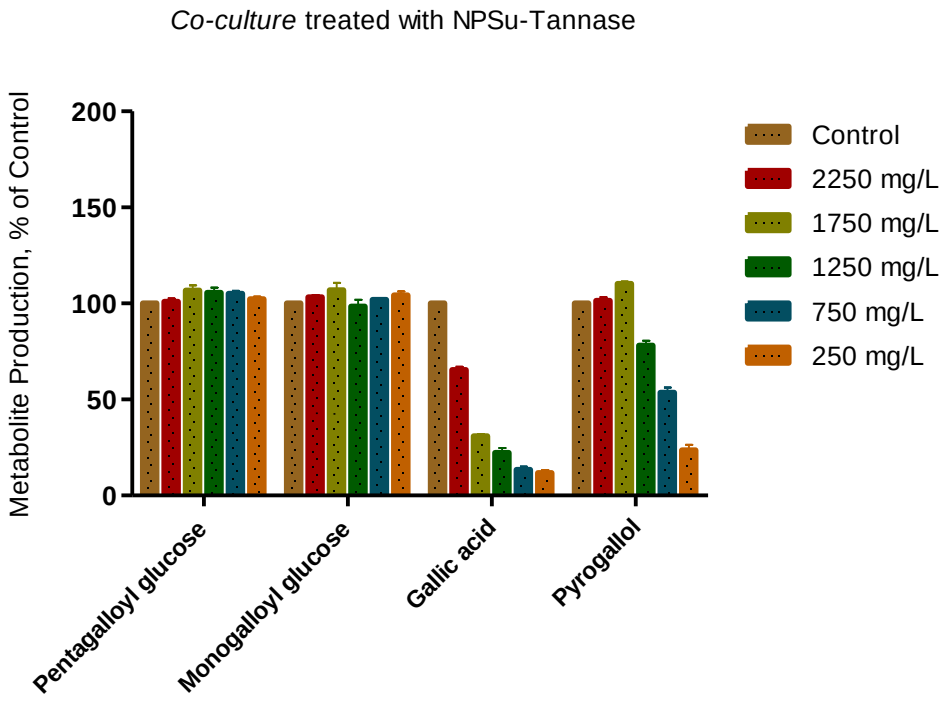


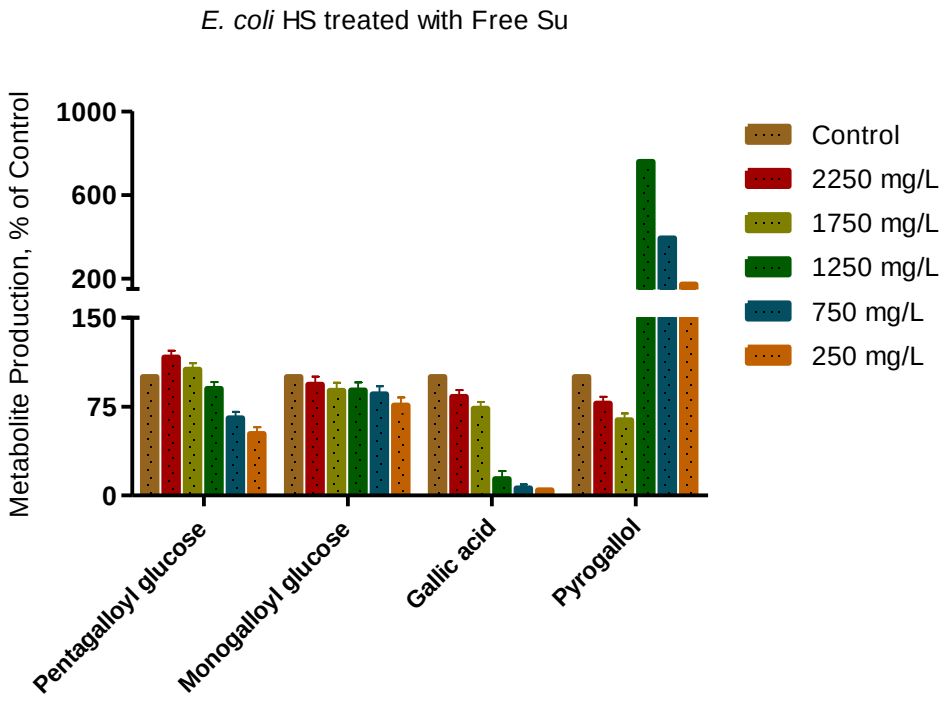
Figure 34. Co-culture bacteria fermentation metabolites after treating with free Su, free Su with Tannase, NPSu, and NPSu with Tannase treatments in anaerobic condition at 37 ºC.

The treatment of *E. coli* HS by sumac extract showed a decrease in the concentration of all the tested compounds (except for pyrogallol) directly proportional to the reduction in TPC in the specimens (**Figure 36**). While pyrogallol concentration showed reduction by 12% and 36% at 2250 mg/mL and 1750 mg/mL, respectively, there was an increase in the other tested concentration even by diluting the treatments (1 to 10-fold). That is, the amount of pyrogallol increased by 658%, 292%, and 72% at the concentration of 1250 mg/L, 750 mg/L, and 250 mg/L, respectively. The concentration of 5-GG was also increased by 17% at 2250 mg/L and 6% at the concentration level of 1750 mg/L. However, the amount of this compound decreased as the treatments were diluted.

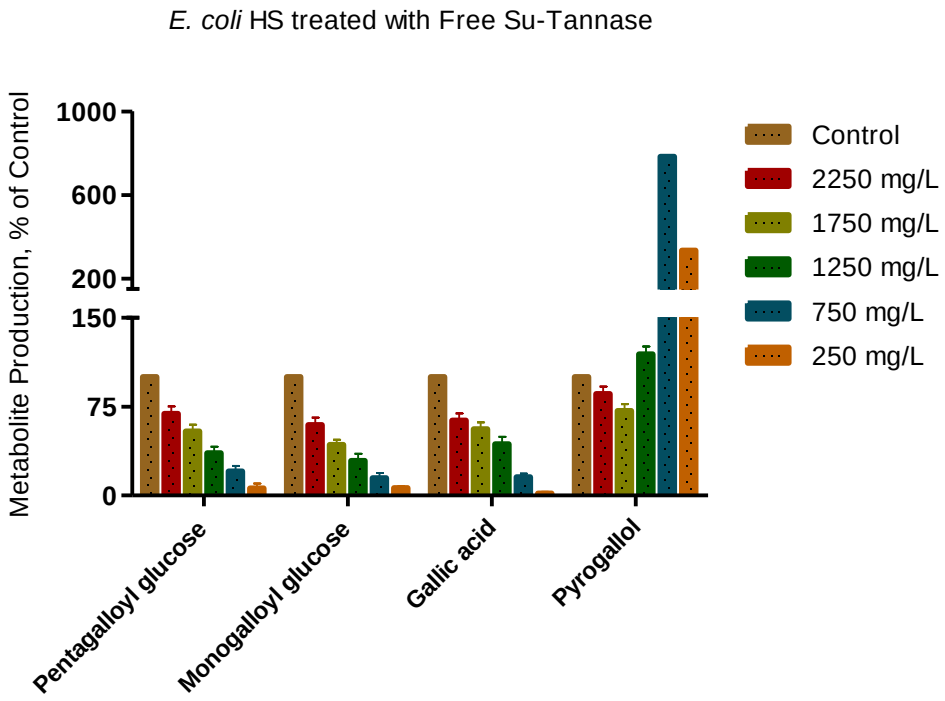
The amount of 5-GG decreased by 10%, 35%, and 48% at 1250 mg/L, 750 mg/L, and 250 mg/L respectively. The amount of 1-GG and gallic acid were constantly changed at all concentrations compared to their controls. The amount of 1-GG decreased by 6%, 12%, 11%, 14%, and 24% at 2250 mg/L, 1750 mg/L, 1250 mg/L, 750 mg/L, and 250 mg/L, respectively. The amount of gallic acid was also decreased at all the concentration by 17% at 2250 mg/L, 27% at 1750 mg/L, 86% at 1250 mg/L, 94% at 750 mg/L, and 96% at 250 mg/L.

These results may be attributed to the difference between metabolism in facultatively anaerobe versus anaerobe bacteria which allow the *Lactobacillus* spp. and *Bifidobacterium* spp. to grow faster with better competition compared to commensal *E. coli* HS. The effect of tannins on *E. coil* has shown a concentration-dependent trend as the effect of tannic acid on the *E. coli* growth stood out at the concentration of 40 µg/mL among 20 µg/mL, 30 µg/mL, and 50 µg/mL, so that, a decreasing trend was observed on the bacteria growth. However, some factors play an important role in the growth of bacteria including interactions of tannins with bacterial and growth medium protein, the interaction of tannins with bacterial cell wall plasma membrane, and chelation of metal ions which all result in producing a less favorable conditions in growth media for bacteria growth even by addition tannins compounds [249].

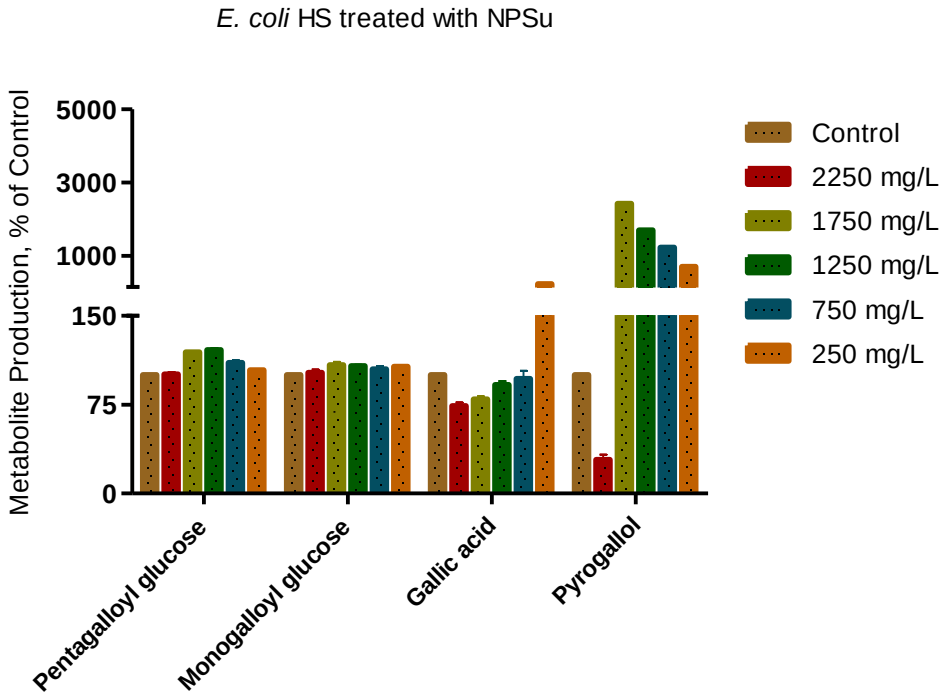
A)


E. coli HS treated with Free Su
Metabolite Production, % of Control
1000
600
200
150
75
0
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L

B)


E. coli HS treated with Free Su-Tannase
Metabolite Production, % of Control
1000
600
200
150
75
0
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol

C)


E. coli HS treated with NPSu
Metabolite Production, % of Control
5000
3000
1000
150
75
0
Pentagalloyl glucose
Monogalloyl glucose
Gallic acid
Pyrogallol
Control
2250 mg/L
1750 mg/L
1250 mg/L
750 mg/L
250 mg/L

D)

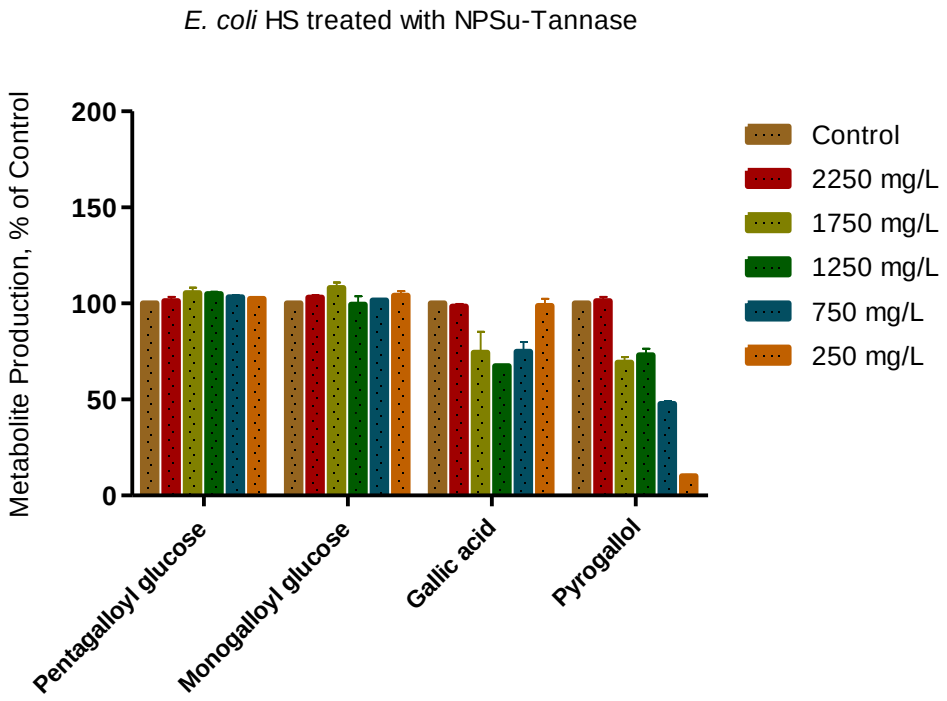


Figure 35. *E. coli* HS fermentation metabolites after treating with free Su, free Su with Tannase, NPSu, and NPSu with Tannase treatments in anaerobic condition at 37 ºC.

Conclusion

Sumac is traditionally consumed in combinations with other food ingredients. The bioavailability of sumac polyphenolic compounds may be affected by physicochemical properties of the human gastrointestinal tract and the interactions with the other food constituents (fibers, lipids, proteins, and polysaccharides) consumed in combination with the polyphenols. In this study, the whole sumac fruit extract was used without further isolation, purification, or filtration process. By adapting nanoparticle formation as a method of protection for these bioactive compounds, their stability during gastrointestinal digestion may be increased. The accessibility of

these compounds for gut microbiota and bioavailability for human body cells (as their ultimate destination) is highly impacted by their interaction with the human gut microbiota [245]. Encapsulation is an approach to increase the production of sumac tannin metabolites by minimizing the interactions between their parent compounds and gastrointestinal fluids, enzymes, and other food macromolecules. Encapsulation of tannic acid as a complex hydrolysable tannin molecule has been previously evaluated [243]. By protecting the high molecular weight tannins during the early stages of the digestion process (where the maximum elimination of polyphenols occurred), the higher amount of intact higher molecular weight tannins may be available for gut microbiota. The availability of these compounds for the bacteria is also dependent on the release of the compounds from the polymeric-based micelles. The process of hydration and collapse of the polymer structure starts with water penetration into the most easily accessible PEO region, where water molecules can be bound to the PEO segment. This results in a conformational change that forms a disordered structure [250]. The release kinetic shows the hydration of the Pluronic polymers in an aqueous solution that results in hydrogen bond formation between polymer and water molecules. The hydration process eventually loosens the existing bonds between the two and breaks down the micelles' structure.

The effect of simulated GI digestion was studied on free Su, free Su with Tannase, NPSu, and NPSu with Tannase samples. The results indicated that the concentration of the targeted high molecular weight tannin was lower in all three experimental compartments (oral, gastric, and intestinal). The amount of these compounds was higher at the beginning of the digestion process (oral phase) indicating their higher release rate at the beginning. However, the presence of these compounds later in the digestion process was lower than that of encapsulated particles indicating

the ability of encapsulation to retain these compounds from degradation thus their higher availability in the intestinal compartment. It was suggested that encapsulation of sumac phenolic extract provided the targeted phenolic compounds with extra protection against the physicochemical factors prevailing gastrointestinal tract. The size alteration as a function of pH change and release stability of tested samples were studied in this work. It was observed that the size fluctuation over time exhibited a lower rate in NPSu samples compared to that of free Su samples in different pH conditions. On the other hand, the release date for free Su and NPSu was found to exhibit different susceptibility when exposed to tannase at different pH values. The susceptibility and degradation under all tested pH conditions were observed in free Su sample compared to that of NPSu extract. Also, the effect of probiotic bacteria on all tested samples was studied in a fermentation process. The result demonstrated that the concentration of gallic acid was decreased as the concentration of sumac extract was lowered in the samples. However, the amount of pyrogallol was increased as the gallic acid concentration lowered. The ability of probiotic bacteria to decarboxylase gallic acid and produce pyrogallol subsequently may be attributed to the above-mentioned trend. Lower molecular weight tannin metabolites such as pyrogallol are beneficial but very unstable to resist in gastrointestinal conditions. The production of endogenous tannase produced by probiotic bacteria especially those from *Lactobacillus* spp. indicated the ability of these microorganisms to tolerate the antibacterial properties attributed to sumac polyphenolic extract. The result of this experiment also indicated that regardless of the type of bacteria being treated, the nanoparticle sumac extract did not allow either tannase (endogenous or exogenous) enzyme to clearly interact with tannin derivatives to produce the target metabolites. The effect of tannase on dissociating the galloyl ester bonds of the high molecular weight

hydrolysable tannins was obvious where the production of low molecular weight tannin metabolites was significantly higher than that of their controls.

CHAPTER VII

CONCLUSIONS

Rhus coriaria, also known as sumac, is a plant from the Anacardiaceae family with resistance to harsh environmental conditions. The plant has small berry liked fruits that form clusters on sumac shrubs. After harvesting, the fruits are dried and ground into fine or coarse powder to be used as a spice in different Mediterranean recipes. Due to the high amount of phytochemical compounds in sumac, extraction and characterization of the polyphenolic sumac extract has been attractive for industries and academia. The polyphenol profile of sumac is mainly constated of hydrolysable tannins that have been associated with many foods safety as well as human health benefits. Antibacterial activity of sumac extract has not only positively impacted food safety by inhibiting the growth of pathogenic bacteria but also contributed to modulating the gut microbiota population. For bacteria to have the best efficient exposure to sumac polyphenols, specifically galloyl derivatives, the compounds need to be protected throughout their way to the human gut. There are many hurdle conditions such as pH change, mechanical move, interaction with other food constituents, etc. across the gastrointestinal tract, which may negatively impact the effectiveness of sumac polyphenolic extract against gut microorganisms. Polymeric nanoencapsulation as a safe and effective method was utilized to protect and sustain the polyphenols of interest from early degradation. To investigate the potential interaction between sumac extract and gut microbiota, a simulated gastrointestinal tract was developed, and different conditions were tested against some most commonly known and ubiquitous probiotic bacteria. The bacteria were picked in a way that presented the overall Gram-negative to Gram-positive ratio in

an actual gut microbial ecosystem. The results indicated that encapsulation of sumac polyphenolic extract improved physicochemical properties of the extract by bringing uniformity to particles shape and distribution within the extract. Sumac polyphenolic extract may be encapsulated with FDA-approved Pluronic F127 copolymers and sold as prebiotic supplements. The direct consumption of sumac may not have the same health benefits as encapsulated samples due to its lower impact on gut microbiota. The results indicate that sumac fruit extract the way it was encapsulated in Pluronic F127 copolymer in the present study, did not seem to be a proper method for protecting the hydrolysable tannin against chemical degradation during gastrointestinal digestion. The results of the study however, indicated that a major percentile of the initial polyphenolic concentration was lost in the early stage of digestion suggesting the high interaction of the compound with gastrointestinal enzyme in the oral phase.

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