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Full Length Research Paper

Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens

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Three strains of pathogenic bacteria were treated with condensed tannins (CT) purified from eight different woody plant species to investigate their inhibition effect on the growth of these bacteria *in vitro*. *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were tested against low (0, 2, 4 and 8 mg CT/ml) and high dose levels (0, 50, and 100 mg CT /ml) of CT extracted from different plant species. When exposed to purified tannin extracts at 4 mg extract/ml dosage, growth inhibition of *S. aureus* was dose dependent manner and observed in the following order: Shinnery oak > Post oak > Locust > Blackjack oak ≥ Skunk bush > Sericea lespedeza > commercial Quebracho ≥ Sumac > Plum. The extracts from Shinnery and Post oaks were particularly inhibitory against *S. aureus*, having growth inhibition zones exceeding 23 mm at 8 mg tannin extract/ml. *S. aureus* and *E. coli* exhibited dose dependent and susceptibility ($P < 0.01$) when exposed to 4 mg/ml of the following tannin monomers which exhibited differential inhibitory activity: catechin > ellagetannin ≥ tannic acid ≥ epi-catechin ≥ gallotannin. In the presence of high dose levels at 0, 50, and 100 mg tannin extract/ml, inhibition zones of growth were varied among plant species. The findings indicated that source and concentration are important factors that influence antimicrobial activity of tannins. Because some of the plant tannin extracts are highly inhibitory to selected pathogens, they may provide alternatives and supplements to conventional antimicrobial feed additives.

Key words: Pathogenic bacteria, tannins, tree leaves, zone of inhibition.

INTRODUCTION

The leaves of trees and shrubs are a component of most natural pastures for ruminant diets (Kumar and Vaithiyanathan, 1990). Many tree leaves have anti-nutritional or anti-microbial factors, like tannins, essential oils, or other aromatic compounds (Kumar and Singh, 1984a,b). Nutritional and toxic effects of tannins present in various foodstuffs, feed and fodder have been reviewed (Kumar and Singh, 1984a, b; Mehanso et al., 1987). In addition, many biological activities and antibacterial-promoting effects have been reported for plant tannins and flavornoids, and their investigation is now increasingly relevant (Haslam, 1989; Scalbert, 1991; Chung et al., 1998).

There remains a pronounced lack of detailed knowledge; however, concerning the antimicrobial activity of tannins in tree leaves that are consumed by ruminants against bacterial pathogens. Organisms commonly isolated from dairy cow quarters are *Staphylococcus aureus* (44.3%), *Streptococcus* sp. (18%), coagulase-negative staphy-lococci (12.8%) and others (Waage et al., 1999). Within gram-negative rods, *Klebsiella* sp., *Escherichia coli* and *Enterobacter* sp. have been reported as important mastitis-causing bacteria (Guifry, 1985). The aim of this study was to screen and evaluate the antimicrobial activity of tannin extracts from eight plant species common to the Southern Great Plains of the USA and compare these to the activity of a range of technical grade hydrolysable and condensed tannin preparations to estimate the relative anti-microbial contribution of hydro

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lysable and condensed tannin fractions of the novel plant tannin extracts. An *in vitro* disc diffusion methodology was employed for these comparisons.

MATERIALS AND METHODS

Experimental design

In experiment 1, an initial *in vitro* study was conducted to compare potential antimicrobial activity of purified tannin extracts from eight different woody plant species obtained from the Western Oklahoma area and to assess the efficacy of high level of tannins (0, 50, and 100 mg/ml) against three mastitis-causing pathogens. A second *in vitro* study was designed to determine efficacy of low dose level of tannin extracts (0, 2, 4, and 8 mg/ml) on *S. aureus* only. A comercially available Quebracho (Chemtan Co., Exeter, NH 03833, USA) tannin extract was also tested because this is known to contain high level of condensed tannins. In experiment 2, a series of *in vitro* experiments were conducted to evaluate the antimicrobial activity exerted by purified condensed (catechin and epicatechin) and hydrolysable tannin monomers (ellagetannins, gallotannins and tannic acid) against *S. aureus* (gram-positive) and *E. coli* (gram-negative).

Plant sampling procedures

The specimens used for the present study were collected in Black Kettle National Grasslands, Reydon, Western Oklahoma, U.S.A. Trees and brush forage species selected for evaluation were Blackjack oak (*Quercus marilandica*), Post oak (*Quercus stellata*), Sand shinnery oak (*Quercus havardii*), Sumac (*Rhus copallina*), Locust (*Gleditsia triacanthos*), Sand plum (*Prunus angustifolia*), and Skunk bush (*Rhus aromatica*). Sericea lespedeza (*Lespedeza cuneata*) was collected separately at the E (kika) de la Garza Institute for Goat Research farm, Langston University, Langston, Oklahoma. Each sampling site was split into three areas. Within each area, three plants of each species were sampled by stripping all available leaves, which were placed into plastic bags and immediately placed on ice to slow respiration. Samples were taken from May to September, 1999 and transported to the laboratory and stored at -20°C until analysis of tannin content. Plant samples were freeze-dried, and ground to pass 1 mm mesh sieve for tannins extraction.

Preparation of condensed tannin extracts

Condensed tannins were purified from the leaves of each plant by the methods of Terrill et al. (1992) and Min et al. (2005) with the following procedures. Leaves were homogenized in 70% aqueous acetone (Hagerman, 1988) containing 0.1% ascorbic acid, and three rounds of diethyl ether extraction removed plant pigments. Remaining tannin fractions was freeze-dried, redissolved in 50% methanol (v/v) and purified by gel filtration using a Sephadex LH-20 column (Pharmacia, Uppsala, Sweden) washing with 50% methanol (v/v) and eluting with 70% acetone (v/v). The tannin extracts were freeze-dried and stored in the dark at 4°C. Total condensed tannins in browse and tree leaves was determined using a butanol-HCL colorimetric procedure (Terrill et al., 1992). Proportion (%) of condensed (CT) and hydrolysable tannins (HT) in purified (LH-20 extraction) tannin compounds were analyzed with radial diffusion method (Hagerman, 2002).

Monomers of CT are either catechin or epicathechin and their galloyl derivatives, whereas constitutes of HT are ellagetannins, galotannins and galloyl derivatives. Monomers in catechin, epica tachin ellagetannins, gallotannins, and tannic acid (Sigma; St. Louis, MO, USA) were used in this study to compare the inhibitory effects of different types of tannin monomer on the mastitis-causing pathogens. The site (s) and number of hydroxyl groups on these tannin monomers are thought to be related to their relative toxicity to microorganisms (Geissman, 1963). Each tannin monomer was dissolved in methanol (5% v/v) and then diluted with sterile water.

Bacterial pathogens

The *Klebsiella pneumoniae*, *S. aureus* and *E. coli* strains used were obtained from G. Tomita (University of Montreal, Quebec, Canada). All the organisms were preserved during storage at –20°C in Muller-Hinton Broth (MHB) medium containing 17% (v/v) glycerol. Before testing, each inoculum was prepared and cultivated in tryptic soy broth (TSB; BD, Sparks, MD) for 24 h at 37°C. Microbial cultures were serially diluted (10-fold increments) in sterile phosphate-buffered saline (pH 7.0) to obtain the cell suspension of 10^5 CFU/ml. A 100 µl inoculum of the diluted (10^5 CFU/ml) test organism was spread on agar plates and then incubated 24 h.

Antibacterial activity of plant tannins

Susceptibilities were determined using the disc diffusion method (National Mastitis Council, 1999). Discs (BD, Sparks, MD) of 6 mm in diameter saturated with each plant extract solution were aseptically placed on agar media that had been spread with test bacteria. Tests were conducted aerobically using AC agar medium (all culture agar media; Sigma; P.O. Box 14508, St., Louis, MO 63178 USA), except for *E. coli*, which was tested on Mac Conkey's agar plates (BD, Sparks, MD). Negative control discs were separately prepared by saturating with sterile water. For comparison, sensitivity of each bacterium was also tested against a commercial Penicillin-G disc (10 IU/IE/UI; BD, Sparks, MD).

Statistical analysis

Data from each experiment were analyzed using the MIXED model procedure (SAS Institute, Cary, NC), with the factors examined being dose levels and types of tannins, and dose levels x types of tannins interactions included in the model. Variables included the proportion tannins content, bacterial strains and the zone of inhibition. The tannins dose level effects were tested by an orthogonal contrast for equally spaced treatments estimated by the MIXIED model procedure of SAS. The *F*-test-protected least squares means procedure of SAS was used to separate treatment means.

RESULTS AND DISCUSSION

Tannins content and composition of forage and browse species

Condensed tannins concentration in tree and browse leaves varied among plant species (Figure 1) and descending order of tannin concentration were: Sericea lespedeza ≥ Plum > Blackjack oak ≥ Locust ≥ Post oak ≥ Shinnery oak > Sumac ≥ Skunk bush (Figure 1). The proportions of purified CT and HT are presented in Table 1. The proportion of CT was greater ($P < 0.001$) for Sericea lespedeza, quebracho and Plum tannins than for other plant tannins. In contrast, HT content was greater ($P < 0.001$) for Sumac, Post oak, Shinnery oak and Locust tannin extracts than for other plant extracts. Plant tannins composition varied among tree and browse leave

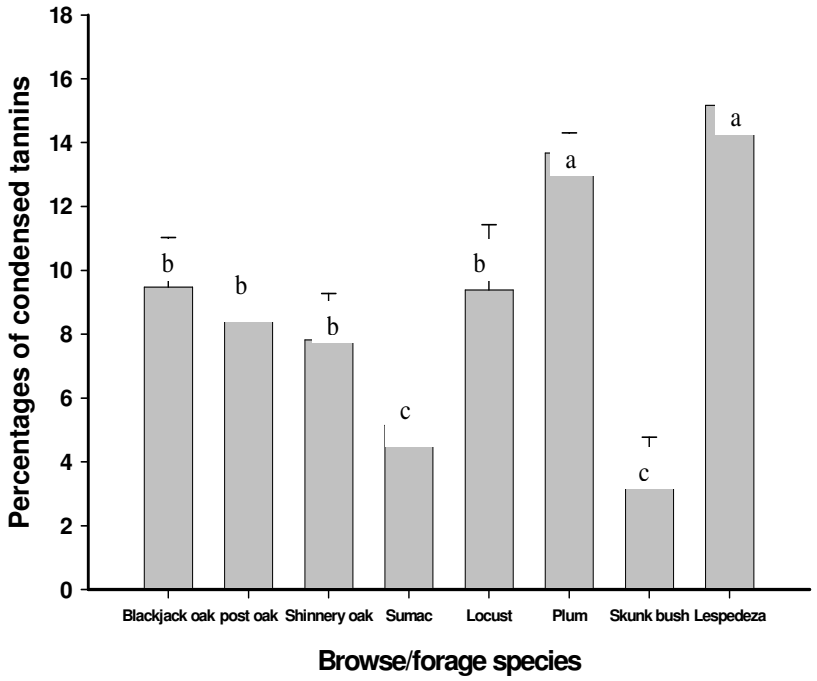

Figure 1. Percentages of condensed tannins in browse and forage species in Western Oklahoma. [a,b,c] Numbers in a row with superscript letters are significantly different ($P < 0.05$).

Table 1. Proportion (%) of purified condensed (CT) and hydrolysable tannin (HT) contents extracted from browse and forage species in Western Oklahoma.

Proportion of tannins content (%) item	CT	HT	SEM
Black jack	73.2[b]	26.8[d]	2.71
Sericea lespedeza	98.6[a]	1.4[e]	0.76
Sumac	16.7[e]	83.3[a]	5.56
Plum	95.9[a]	4.1[e]	3.29
Post oak	31.5[d]	68.5[b]	0.23
Locust	30.9[d]	69.1[b]	1.40
Shinnery oak	29.2[d]	70.8[b]	1.53
Skunk bush	46.2[c]	53.8[c]	0.12
Quebracho	98.5[a]	1.50[e]	0.20

[a,b,c,d,e] Numbers in a column with superscript letters are significantly different ($P < 0.05$).
Plant tannins were purified by gel filtration using a Sephadex LH-20 column from various plant sources. Proportion of condensed (CT) and hydrolysable tannins (HT) concentration was conducted by radial diffusion method.

consistent with previous reports (Feeny and Bostock, 1968; Kumar and Vaithiyanathan, 1990; Villena and Pfister, 1990; Broderick and Albrecht, 1997; Pasch and Pizzi, 2002; Muetzel and Becker, 2006). Leaves of oak, maple, and other fodder trees contained both CT and HT depending on seasons and different plant species (Bate-Smith, 1977; Feeny and Bostock, 1968; Hagerman, 1988; Kumar and Vaithiyanathan, 1990) and this data supports our study (Table 1). There was a greater ($P < 0.01$) proportion of CT in Blackjack oak leaves (73.2%) than in either Post oak (31.5%) or Shinnery oak (29.2%), illustrating tremendous variation among species of the same genus *Quercus*. The high proportion of CT in Plum extracts was similar to that found in Sericea lespedeza and quebracho extracts. The overall tannin content in plum leaf extracts was similar to that found in Sericea lespedeza extracts. In the present study, Post oak, Shinnery oak, and Black jack leaves contained less CT concentrations than Sericea lespedeza, quebracho and Plum tannins (Figure 1), but having a higher level of antimicrobial activity than other purified tannin extracts at the equivalent concentration of tannins (Table 2). This suggests that sources of tannins may play a role in antimicrobial activity.

Purified tannin extracts antimicrobial activity against food pathogens

Several possible explanations of the effects of plant tannins on food-borne pathogens have been proposed (Haslam, 1989; Scalbert, 1991; Chung et al., 1998). Susceptibilities of three major mastitis-causing bacteria exposed to 0 to 100 mg/ml of 9 different sources of puri-

Table 2. Relative susceptibilities of representative mastitis-causing bacterial strains to various plant tannin extracts.

Item	Diameter of inhibition (mm)[1]		*P*-value		
	50	100 mg/ml	SEM	Linear	Qudratic
Black jack					
K. pneumoniae	13[a]	16[a]	0.54	0.01	0.04
S. aureus	18[b]	25[b]	0.71	0.01	0.03
E. coli	17[a]	17[a]	0.26	NS	NS
Sericea lespedeza					
K. pneumoniae	10	12[a]	0.62	0.01	NS
S. aureus	17[b]	20[b]	0.56	0.01	0.01
E. coli	11	10	1.65	NS	NS
Sumac					
K. pneumoniae	7	9	0.18	0.01	NS
S. aureus	16[b]	19[b]	0.43	0.02	0.04
E. coli	12	18[a]	1.00	0.01	NS
Plum					
K. pneumoniae	9	11[a]	0.49	0.01	NS
S. aureus	16[b]	15[b]	0.72	0.01	NS
E. coli	13	13	0.41	NS	NS
Post oak					
K. pneumoniae	13[a]	16[a]	0.39	0.01	NS
S. aureus	27[b]	35[b]	2.96	0.01	NS
E. coli	18[a]	23[a]	1.71	0.01	NS
Locust					
K. pneumoniae	12[a]	12[a]	0.12	NS	NS
S. aureus	17[b]	20[b]	0.69	0.01	0.02
E. coli	12	14	0.68	0.01	0.06
Shinnery oak					
K. pneumoniae	12[a]	14[a]	0.27	0.01	0.01
S. aureus	26[b]	32[b]	0.67	0.001	0.01
E. coli	19[a]	24[a]	2.34	0.01	NS
Skunk bush					
K. pneumoniae	*9*	10[a]	0.62	0.01	NS
S. aureus	17[b]	21[b]	0.55	0.01	0.01
E. coli	10	16[a]	0.85	0.01	NS
Quebracho					
K. pneumoniae	9[a]	12[a]	0.19	0.01	NS
S. aureus	11[b]	14[b]	0.35	0.01	NS
E. coli	12	14[a]	0.91	0.01	NS
Control (sterile water)	-	-	-	-	-
Penicillin-G	10 IU				
K. pneumoniae	6.1[b]		0.12		
S. aureus	44[a]		1.29		
E. coli	12[b]		0.23		
ANOVA					
Sources of tannins (ST)			0.001		
Bacterial strain (BS)			0.001		
ST x BS			0.001		
Dose (D)			0.001		
ST x D			0.001		
BS x D			0.001		
ST x BS x D			0.001		

NS = not significant ($P > 0.05$). Inactive (-); Moderately active (7-14); highly active (> 15).

[1]Included diameter of disc (6 mm).

[a,b,c]Numbers in column (Penicillin-G vs. types of plant CT) and with different superscripts are significantly different ($P < 0.05$).

Table 3. Relative susceptibilities of mastitis-causing *S. aureus* to various plant tannin extracts.

Item	Diameter of inhibition (mm)[1]			*P*-value		
	2	4	8	mg/ml SEM	Linear	Qudratic
Black jack	12	14	17	0.30	0.001	9,91
Sericea lespedeza	9	10	12	0.56	0.01	NS
Sumac	8	9	10	0.36	0.02	NS
Plum	7	7	7	0.01	NS	NS
Post oak	15	19	23	1.09	0.001	NS
Locust	15	15	19	1.44	0.01	0.05
Shinnery oak	18	22	23	0.75	0.01	0.01
Skunk bush	10	14	13	0.98	0.01	NS
Quebracho	7	9	11	0.15	0.01	0.05
Control (sterile water)	-	-	-			
Penicillin-G		10 IU				
ANOVA		51		0.98		
Sources of tannins (ST)				0.001		
Dose (D)				0.001		
ST x D				0.001		

NS = not significant ($P > 0.05$). Inactive (-); Moderately active (7 - 14); highly active (> 15).
[1]Included diameter of disc (6 mm).

purified tannin extracts are shown in Table 2. Antimicrobial activity of plant tannins varied depending on dose levels and bacteria and ranged from no inhibition to an inhibition zone diameter of over 35 mm. The *in vitro* study reported here is the first to document that tannin extracts from plants common to the Southern Great Plains of the United States have the ability to inhibit selected mastitis-causing pathogens.

Generally, inhibition of *K. pneumoniae* and *S. aureus* strains increased in a dose dependent manner in the presence of all tannins, except locust plant tannin extracts. In the presence of plant extracts, antimicrobial effect was quantitatively lower in *K. pneumoniae* (zone, ≥ 9 to 16 mm) than other bacteria (zone, ≥ 10 to 35 mm). *K. pneumoniae* also was the most penicillin-G (10 IU) resistant strain evaluated. Sumac tannins extract was the only tannin extract that failed to exhibit greater inhibitory effect on *K. pneumoniae* greater than penicillin-G at 100 mg/ml tannin dose levels. Sumac, Sericea lespedeza, Plum and Skunk bush tannin extracts were ineffective against *K. pneumoniae* at 50 mg/ml dosages.

Inhibition of *E. coli* by tannins extracts dosed at 50 mg/ml varied among plant species and was equal to or better than that of penicillin-G (Table 2). Only the three oak species exhibited an inhibitory effect greater ($P < 0.05$) than penicillin-G at the 50 mg/ml dose. Inhibition of *E. coli* increased at 100 mg/ml dosage Sumac, Post oak, Shinnery oak, Skunk bush and Quebracho. The variable dosage levels led to significant ($P < 0.001$) interaction.

Response in *E. coli* inhibition among species and between dosage levels led to significant ($P < 0.001$) interaction.

The source of tannins x dose levels of tannins interactions and bacteria x dose levels of tannins interactions ($P < 0.001$) generally indicate that antibacterial activity increasing with increase dose levels of tannins through at different rates, and this treatment effect was associated with type of tannins and bacteria, with *S. aureus* (gram-positive) being more susceptible to plant tannin extracts than *K. pneumoniae* and *E. coli* (gram-negative). It has been reported that complex phenolic polymers in berries of the genus *Rubus* (cloudberry, raspberry, and artic bramble) and *Vaccinium* (cranberry) exhibit significant antimicrobial effects against food-borne pathogens, e.g. *S. aureus, E. coli* and *Salmonella* sp. (Foo et al., 2000; Pupponen-Pimia et al., 2004). Several mechanisms of action in the growth inhibition of bacteria are involved, such as destabilization of cytoplasmic and plasma membranes, inhibition of extracellular microbial enzymes and metabolisms, and deprivation of the substrate required for microbial growth (Bell et al., 1965; Ikigai et al., 1993; Puupponen-Pimia et al., 2004).

The overall linear inhibitory effect ($P < 0.001$) of plant tannin extracts observed in experiment one (Table 2) may have been resulted from high dose levels employed. Therefore, a second *in vitro* study (Table 3) was designed to determine efficacy of low dose level of tannin extracts (0, 2, 4, and 8 mg/ml) against *S. aureus.* Inhibition of *S. aureus* by tannin extracts (0 to 8 mg/ml) was similar trend in Table 2. When exposed to lower concentrations of tannin extracts at 4 mg extract/ml dosage, growth inhibition of *S. aureus* was dose dependent manner and observed in the following order: Shinnery oak > Post oak > Locust > Blackjack oak ≥ Skunk bush > Sericea lespe-

Table 4. Relative susceptibilities of mastitis-causing *S. aureus* and *E. coli* strains to various tannin fractions.

Item	Diameter of inhibition (mm)[1]		Contrast		
	2	4 mg/mL	SEM	Linear	Qudratic
E. coli					
Condensed tannins					
Catechin	16[a]	18[a]	0.73	0.01	0.02
Epi-catechin	13[b]	14[b]	1.62	0.03	0.28
Hydrolysable tannins					
Ellagetannin	10[ab]	17[a]	2.56	0.07	0.58
Gallotannin	8[b]	8[b]	1.88	0.14	0.58
Tannic acid	10[ab]	13[b]	0.88	0.01	0.51
Mean	10	14			
Control (sterile water)	-	-	-		
Penicillin-G	10 IU				
E.coli	15[b]		1.29		
S. aureus	25[a]		2.19		
ANOVA					
Sources of tannins (ST)			0.07		
Dose (D)			0.001		
Strains			0.002		
ST x D			0.03		
ST x strain			0.48		
Strain x D			0.03		
ST x D x strain			0.71		

NS = not significant ($P > 0.05$). Inactive (-); Moderately active (7-14); highly active (> 15).
[1]Included diameter of disc (6 mm).
[a,b,c] Numbers in column with different superscripts are significantly different ($P < 0.05$).

deza > commercial Quebracho ≥ Sumac > Plum. The extracts from Shinnery and Post oaks were particularly inhibitory against *S. aureus*, having growth inhibition zones exceeding 23 mm at 8 mg tannin extract/ml (Table 3). There was a source of tannins x dose level interactions ($P < 0.001$), indicating that antibacterial activity increased with increasing dose levels and different types of tannins. Chung et al. (1993) observed that the minimum inhibitory concentration of tannins for *Pseudomonas fluorescens, S. aureus*, *E. coli*, *K. pneumoniae*, and other pathogenic bacteria was 5 mg and the proportional range was between 5 and 25 mg/ml. These findings agree with our data in the presence of plant tannins, which showed that susceptibility of *S. aureus* linearly increased when exposed to plant tannins except Plum tannins. One mode of action of plant tannins is to complex with dietary nutrients through hydrogen and hydrophobic effects, as well as by covalent bond formation (Haslam, 1989; Scalbert, 1991). Thus, their mode of antimicrobial effect may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and mineral uptake (Bell et al., 1965; Scalbert, 1991; Min et al., 2003).

The chemical composition of the tannins extracts from the 9 plant species are not known and we utilized previous published reports and third in vitro trial using purified technical grade tannins and tannic acids to attempt to describe the nature of the observed responses in food pathogens. Compared to oak tree leaves (mainly contain ellagitannins), Sumac leaves contained high level of HT (poly-gallic acids; Table 1; Pasch and Pizzi, 2002), but sumac tannins have less significant in antimicrobial activity in the present study. Muetzel and Becker (2006) reported that tannins from Sumac had the highest tannin contents and consequently also the highest total protein precipitation capacity with bovine serum albumin protein, but its specific protein precipitation activity was lowest among tested tannins (walnut, chestnut, and willow tannin extracts). The same authors also observed that ruminal gas production (mixed rumen microorganisms was collected from a dry non-lactating Holstein cow) and ammonia concentration were higher for Sumac than for other tannin sources (chestnut, walnut and willow tannins), implicating that source of tannins are important factors to inhibiting microorganisms (Scalbert, 1991).

In vitro mean diameters of zones of inhibition caused by commercially available CT (catechin and epi-catechin) and HT monomers (ellagetannins, gallotannins and tannic acid) for two test bacteria are shown in Table 4. Susceptibility of *S. aureus* exhibited dose dependent and susceptibility ($P < 0.01$) when exposed to 4 mg/ml of the following tannin monomers which exhibited differential inhibitory activity: catechin ≥ ellagetannin > tannic acid ≥ epi-catechin ≥ gallo tannin at 4 mg/ml dosage. Inhibition of *E. coli* by tannin monomers was similar trend in *S. aureus* and observed in the order: catechin ≥ ellagetannin > tannic acid ≥ gallo tannin ≥ epi-catechin at 4 mg/ml dosage. There was a source of tannin monomers x dose levels of tannin fractions and bacterial strain lx dose level

Table 5. Representative examples of tannin types and their molecular weight (MW).

Plant source	Tannin identified	Type of tannins	MW (Da)
Oak (*Quercus* spp.)	Gallotannin	HT	170
	Ellagitannin		302
	Catechin	CT	
Sumac (*Rhus* spp.)	Gallotannin	HT	152
	Ellagitannin		
Serecea lespedeza	Prodelphinidin	CT	14-20 kDa
(*Lespedeza cuneata*)			
Quebracho	Catechin	CT	1978
(*Schinopsis* spp.)			

HT = hydrolysable tannins; CT = condensed tannins; MW = molecular weight
Sources: Bell et al. (1965); Mcleod (1974); Tang et al. (1992); Streit and Fengel (1994); Bhat et al. (1998); Mueller-Harvey (2001); Pasch and Pizzi (2002).

of tannin interactions ($P < 0.03$), indicating that antibacterial activity increased with increasing dose levels and different types of tannin monomers and bacteria. Chung et al. (1993) demonstrated that tannic acid, but not gallic acid, inhibited the growth of 15 bacterial species at a concentration of 5 mg/ml including *E. coli*, *K. pneumoniae*, and *S. aureus*. However, Toda et al. (1990) reported that pyrogallol tannins, extracted from green tea, showed a strong antibacterial activity against *S. aureus* and *Vibrio cholerae* 01. A higher antimicrobial activity in catechin compared to epi-catechin also has been reported and can possibly be an explained by an increase (30%) in the precipitation rate that occurs when mixed with salivary protein (Kallithraka et al., 2000). Furthermore, Puupponen-Pimia et al. (2004; 2005) reported that red raspberry and cloudberry contained high levels of ellagitannin (HT), but cranberry contained CT (catechin), which had strongly inhibited the food-borne pathogens, such as *S. auresus* and *Salmonella*. This data supports our study.

Oak tree leaves (Table 5) are reported to contain high levels of ellagitannin and gallotannin (HT) as well as some of CT (catechin; Tang et al., 1992; Haslam and Cai, 1994; Bhat et al., 1998). It is worth noting that some plant species produce either gallotannin or ellagitannin, whilst others produce complex mixtures containing HT (gallo- and ellagi-tannins) and CT (Mueller-Harvey, 2001; Mingshu et al., 2006). The site (s) and number of hydroxyl (-OH) groups on the tannins are also thought to be related to their relative toxicity to microorganisms, with increased hydroxylation resulting in increased toxicity (Geissman, 1963; Mangan, 1988; Tang et al., 1992; Bhat et al., 1998). However, there was a considerable variation in response to the types of purified tannin monomers, ranging from the higher antimicrobial activity of catechin and ellagetannin to less activity displayed by epicatechin, gallotannin and tannic acid in a dose dependent manner (Table 4). Results from this study showed that the effects of the tannin monomers on the test pathogens were concentration dependent and in some cases linked to the chemical structure of tannin fractions (Sivakumaran et al., 2004).

Compared to gallotannin, ellagitannin are much more difficult to be degraded by microbes because of their complex structure (Scalbert, 1991; Mingshu et al., 2006). Deschamps et al. (1980) reported a detailed study on the degradation of gallotannin by aerobic bacteria, and isolated fifteen bacterial isolates belonging to the genera *Klebsiella*, *Bacillus*, and *Staphylococcus* using gallo-tannin as a sole carbon source. However, the specific effects of purified tannins fractions on mastitis-causing bacteria *in vivo* are not known and need further study.

Antibiotic resistance in common food-borne pathogens such as *S. aureus*, coagulase-negative *Staphylococci*, *E. coli*, *Salmonella*, *Campylobacter* sp., *Listeria mono-cytogens*, and *Clostridium perfringens* have been reported (Teuber, 1999). The high incidence of food-bone pathogens in raw meat together with a therapeutic and nutritional application of antibiotics in agriculture reveals antibiotic resistance problems of global dimensions. Addition of purified tannin extracts significantly increased zone of inhibition in the presence of most tannin extracts compared to Penicillin-G (10 IU) when incubated with *K. pneumoniae* strain, indicating that *K. pneumoniae* has Penicillin resistance strain among tested bacteria. In the presence of plant extracts, antimicrobial effect was quantitatively lower in *K. pneumoniae* than other bacteria. *E. coli* were less susceptible to Penicillin-G. In contrast, *S. aureus* was highly susceptible to Penicillin-G when compared to the other tested bacteria.

In summary, our results showed that purified tannin extracts from tannin-containing plants exhibited a range of antimicrobial activity. The tannins obtained from oak tree leaves had greater antimicrobial activity than other type of tannins, which suggest the source of tannins and therefore the chemical composition influences antimicrobial activity. The mechanisms for increasing antimicrobial activity in oak tree species on the test bacteria are not clear at this time. The potential value of plant tannin

extracts needs to be further tested to investigate the mode of action of tannins against microorganisms, particularly the structure-reactivity relationship of each tannin component. Further research is required to determine the tannin compounds responsible for antimicrobial activity associated with extracts from these 9 plant species.

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