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Antioxidant and antimicrobial capacity of three agroindustrial residues as animal feeds

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Abstract Currently, different countries' policies prohibit the use of synthetic antibiotics in animal production. As a consequence, researchers have been looking for sources of these molecules in plants, vegetables, and agro-industrial waste in order to inhibit pathogenic microorganisms, such as *Escherichia coli*, *Salmonella* and *Listeria* and control livestock health. Hydro-alcoholic extracts of the leaves of three different plants- Avocado (*Persea americana* Mill) Hass variety, guava (*Psidium guajava* L.) Calvillo variety, and cherry plum (*Prunus cerasifera* Ehrh) Pissardii variety, at three different ethanol:water ratios (20:80, 50:50, and 80:20 volume/volume) were analyzed. Total phenols in the extracts were quantified by the Folin-Ciocalteu Method and the inhibitory spectrum test against Gram+: *Listeria monocytogenes* ATCC 19115, *Bacillus subtilis* ATCC 662, *Enterococcus* sp., *Staphylococcus* sp. and Gram−: *Escherichia coli* ATCC 25922, *Salmonella enterica serotipo* Enteriditis ATCC 13076, *Klebsiella* sp. and *Pseudomonas* sp. using the agar well-diffusion method. The highest phenol content and antioxidant capacity were found in the guava leaf extract at 50:50 (111.7 ± 8.8 EAG mg/mL dry matter, 450 ± 3 μM TE/g dry matter), and this was the only extract that showed total inhibitory spectrum activity for all the microorganisms evaluated among the extracts tested, with a range of 0.62–1.25 mg/mL minimal inhibitory capacity (MIC). A hydroalcoholic extract of guava leaves had strong antimicrobial activity against different pathogenic microorganisms and could be considered as a potential alternative to synthetic antibiotics for use in animal production.

Keywords Hydroalcoholic extract · Agroindustrial waste · Total phenols · Saponin · Antioxidant capacity · Antimicrobial capacity

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Introduction

It has been recognized since the 1940's that animal health and welfare are essential aspects of livestock production, which prevent metabolic disease and improve meat quality. This has led to the excessive use of growth promoters and antibiotics (Akyildiz and Denli 2016). However, increased concern among consumers, coupled with the January 2006 European prohibition of synthetic antibiotics use as growth promoters or animal treatment pathologies in animals intended for human consumption (CE 1831/2003). This has imposed an important challenge on animal producers to find natural sources of substances that have antimicrobial properties and stimulate animal welfare (Pereira et al. 2017). Currently the use of natural alternatives include prebiotics, probiotics, organic acids, essential oils and plant extracts (Akyildiz and Denli 2016).

Plant extracts and essential oils have shown antimicrobial, antioxidant, antiparasitic, anti-inflammatory, antidiarrheal and antifungal properties, improve food conversion and stimulate digestive enzymes when they are used in animal diets (Frankic et al. 2009; Martínez et al. 2015). Cinnamon, oregano, cumin, garlic, clove, anise, mint, coriander, and ginger are the most frequently used spices in animal production (Akyildiz and Denli 2016). These spices' antimicrobial and antioxidant activities depend on their secondary metabolites concentrations such as phenols, saponins, flavonoids, alkaloids, terpenoids and tannins (Díaz-de-Cerio et al. 2016; Li et al. 2016).

While some natural additives have shown favorable results when used in vitro, the use of these additives in vivo as dietary components presents high implementation costs because of the high doses needed (Pereira et al. 2017). However, if similar properties could be found in materials usually considered agroindustrial waste, these products could be used with a lower cost. High antioxidant activity and total phenol concentration have been suggested as an initial screening criterion to find natural sources of dietary additives from agroindustrial waste (Oboh et al. 2016; Yamassaki et al. 2017). Avocado, cherry plum, and guava leaves have been reported to have antioxidant and antimicrobial properties (Rasool et al. 2017; Camarena-Tello et al. 2018; Chen et al. 2018). However, these effects vary widely depending on the growing conditions, extraction method, and a host of aspects of the chemical composition of the active compounds, such as the quantity, hydroxyl position, molecular weight, particle size, solvent concentration, temperature, contact time and mass-solvent ratio, among others factors (Soto-García and Rosales-Castro 2016; Robles-García et al. 2016), in addition to the family, genus, an even species of plant. As such, the main objective of this study was to determine the antioxidant and antimicrobial activities of Mexican agroindustrial waste from avocado, cherry plum, and guava leaves for potential use as feed additives to improve animal health and welfare.

Materials and methods

Plant material

Arboreal leaves were randomly taken from the central branches of young and mature trees from a rural production area during the summer. Leaves with mechanical or biological damage were avoided. Guava (*Psidium guajava* L.) variety Calvillo and avocado (*Persea americana* Mill) Hass variety leaves were collected from Uruapan, Michoacan, Mexico. 19°25′16″N, 102°3′47″W. Cherry plum (*Prunus cerasifera* Ehrh) variety Pissardii leaves were taken from Toluca, Mexico State, Mexico (19°17′32″N, 99°39′14″W). Leaves were dried at 50 °C, 48 h and ground in an electric mill until a 1 mm particle size was achieved.

Microorganisms used and growth conditions

The microbiological strains included Gram-positive bacteria *Listeria monocytogenes* ATCC 19115 (M1), *Bacillus subtilis* ATCC 662 (M2), *Enterococcus* sp. (M3), *Staphylococcus* sp. (M4), and Gram-negative *Escherichia coli* ATCC 25922 (M5), *Salmonella enterica serotipo* Enteriditis ATCC 13076 (M6), *Klebsiella* sp. (M7) and *Pseudomonas* sp. (M8). These organisms were obtained from the collections of the Laboratory of Microbiology of the Technological Institute of Veracruz (Laboratorio de Microbiologia, UNIDA, Instituto Tecnologico de Veracruz) Mexico, where they are stored at − 40 °C in Luria–Bertani (LB) broth with 40% glycerol as a cryoprotectant. Microorganisms stains used were reactivated in LB broth for 24 h, 37 °C, followed of a second grown in

LB broth for 18 h, 37 °C before beginning each experiment.

Preparations of plant extracts

Plant extracts were obtained according to Salem et al. (2011). Hydroalcoholic extractions were made using 1 g dry cherry plum, guava, or avocado leaves per 8 mL of ethanol:water solvent mixture. The stock solvent mixture was made using 20, 50 y 80% ethanol (99/100, analytical grade, Fermont®, Monterrey, Mexico). Leaves were macerated at room temperature for 72 h in amber flasks (50 mL screw-capped glass flasks), then placed in a water bath at 39 °C for 30 min and filtered with Whatman N45 filter paper in order to be stored in amber flasks at 4 °C until use.

Experimental design

A 3 × 3 completely randomized factorial design using leaves (agroindustrial waste) of *Persea americana* Mill, *Prunus cerasifera* Ehrh and *Psidium guajava* L., in three ethanol:water ratios- 20:80 v/v, 50:50 v/v, 80:20 v/v was used. Total phenol Folin-Ciocalteu determination (TP), saponin quantification (SP), and antioxidant capacity (AC) analyses were carried out by triplicate (details follow).

Total phenol Folin-Ciocalteu determination

Total phenol equivalents were estimated by the Folin-Ciocalteu method (Spizzirri 2009; Arizmendi-Cotero et al. 2016). 120 µL plant extract were mixed with 10 mL distilled water. A 120 µL aliquot was mixed with 47.5 µL of Folin-Ciocalteu reagent and 300 µL of Na_2CO_3 15% in a 2.5 mL cuvette, and incubated for 15 min. 1080 µL distilled water was added, mixed and allowed to rest for 2 h. Absorbance was measured at 760 nm and total phenolic equivalents were expressed as gallic acid equivalents per g dry matter (DM).

Saponins quantification

Secondary metabolites were separated in a separation funnel. 10 mL of hydroalcoholic extract from each plant were mixed with 20 mL of ethyl acetate (99.7/100, analytical grade, Fermont®, Monterrey, Mexico) to eliminate phenolic compounds. 20 ml of *n*-butanol (99.9/100, analytical grade, Fermont®, Monterrey, Mexico) was added to the resulting phenol-free mixture to obtain saponins (SP), which were quantified by evaporating the solvent and weighing (Makkar et al. 1998; Salem et al. 2011).

Antioxidant capacity

Antioxidant capacity was quantified using ABTS [2.2′-azinobis-(3-ethylbenzothiazoline-6-acid)] reagent by the Mehta et al. (2014) method. Radical formation was by a reaction of 7 mM ABTS solution and 140 mM potassium persulfate, which was incubated in the dark at 25 °C for 16 h. The fresh radical solution was diluted in analytic grade ethanol to achieve a 0.7 ± 0.02 absorbance reading at 734 nm. 10 mL plant extract was diluted in 100 mL ethanol and 30 mL of this plant extract solution was mixed with 3 mL ABTS radical solution. Absorbance was measured at 734 nm after 6 min reaction, and a Trolox curve pattern was generated to express TEAC mmol/g dry matter.

Antimicrobial activity and minimal inhibitory capacity (MIC)

Antimicrobial activity

Antimicrobial activity was tested only for the 50:50 ethanol:water extraction for each plant species, since this was the extraction ratio that had the highest concentration of the secondary metabolites in the analyses described above. Antimicrobial activity was tested using the agar diffusion disc method following the Clinical and Laboratory Standards Institute (2012) for eight pathogenic microbial strains described above (M1, M2, M3, M4, M5, M6, M7 and M8; see "Microorganisms used and growth conditions" section). Microorganisms stains used were inoculated in LB broth for 18 h, 37 °C. Then a 1 mL was taken and used to inoculate a second LB broth for 2 h, 37 °C to reach a microbial concentration of 1×10^5 CFU/mL (verified with plate counts). Microbes were inoculated into Petri dishes and LB agar was poured later. 6 mm diameter filter paper discs with 10 µL of leaf extract were plated on the solidified agar and incubated overnight at 4 °C, then incubated again at 37 °C for 24 h. All samples were analyzed by triplicate. Antimicrobial activity was estimated by measuring the

transparent inhibition zone around each filter paper disc.

Minimal inhibitory capacity

The MIC was only quantified for the 50:50 v/v guava leaf extract because it was the only extract that showed antimicrobial capacity in the test described in the previous section. The MIC was quantified with a Microplate Reader (Microplate 5.0, BIORAD) using the kinetic growth protocol of each microorganism. The extract was analyzed at 0.31, 0.62, 1.25, 1.87 and 2.60 mg DM/mL concentrations as the antimicrobial solution. Sterile 96-well microplates were used. Negative control (50 µL antimicrobial solution + 150 µL LB broth), test assay (50 µL inoculum + 50 µL antimicrobial solution + 150 µL LB broth), positive control (50 µL inoculum + 150 µL LB broth) and positive blank (150 µL LB broth) were run for each sample determination. Absorbance was measured at 655 nm at 37 °C every 30 min until the sensitive microorganisms tested reached its stationary phase. Results were expressed in mg/g dry mass and calculated based on microorganism adaptation phase increase (Andrews 2001).

Results

Quantification of secondary metabolites and antioxidant capacity

Table 1 shows the results of the multifactorial ANOVA ($P \geq 0.05$) including as factors the concentration, plant species, and their interaction. There were significant effects over the concentration and the concentration × species interaction ($P \geq 0.05$) for response variables TP, SP, and AC studied. Plant species had a significant effect over TP and AC, but not for SP ($P \geq 0.05$) variable. Upon detecting significant effects in the overall test of means using a Tukey's test (95%) (Table 2). Concentration factors TP, SP, and AC were higher when using 50:50 ethanol:water ratio (TP = 89.9 mg GAE/g DM, SP = 105.1 mg/g DM and AC = 421.9 mM TEAC/g D) than for the 20:80 (TP = 52.9 mg GAE/g DM, SP = 39.4 mg/g DM and AC = 204.7 mM TEAC/g DM) or 80:20 extractions (TP = 56.0 mg GAE/g DM, SP = 48.02 mg/g DM and AC = 271.8 mM TEAC/g DM; Table 2). In Specie factor, guava leaves had the highest values (TP = 86.88 mg GAE/g DM, SP = 69.1 mg/g DM and AC = 332.0 mM TEAC/g DM), followed by avocado (TP = 63.77 mg GAE/g DM, SP = 67.47 mg/g DM, and AC = 299.7 mM TEAC/g DM), then cherry plum (TP = 47.49 mg GAE/g DM, SP = 63.1 mg/g DM and AC = 266.8 mM TEAC/g DM).

Concentration and species interaction results are shown in Table 3. Concentration-species combination with the highest values was guava in 50:50 ethanol:water ratio (TP = 111.7 ± 8.8 mg GAE/g DM, SP = 88.5 ± 6.9 mg/g DM and AC = 450.3 ± 18 µM TEAC/g DM). The remaining interactions were as follows, in decreasing order: avocado leaf at 50:50 (TP = 82.47 ± 6.2 mg GAE/g DM, SP = 113.15 ± 20 mg/g DM, AC = 446.7 ± 18 µM TEAC/g DM), guava leaf at 80:20 (TP = 76.40 ± 3.7 mg GAE/g DM, SP = 52.55 ± 5.8 mg/g DM, AC = 407.8 ± 7 µM TEAC/g DM), plum leaf at 50:50 (TP = 73.29 ± 4.8 mg GAE/g DM, SP = 113.15 ±

Table 1 Multifactorial analysis of the effects of extract concentration (20:80, 50:50, and 80:20 ethanol:water ratio), species (avocado, guava, and plum), and their interaction on total phenols, saponins, and antioxidant capacity among hydroalcoholic leaf extracts

Factor	DF Sum of squares	Total phenol	Saponins	Antioxidant capacity
Concentration	2	7053*	13,194*	445,410*
Plant species	2	7,2655*	503	38,283*
Interaction	4	136*	3,51*	205,710*
Total	26	14,8435	22,367	766,531

DF Degrees of freedom

*Significant effect of the factor

Table 2 Total phenol, saponins, and antioxidant capacity by species and ethanol:water ratio in leaf extracts, analyzed using a multifactorial analysis

Factor	Total phenol (mean mg GAE/g DM)	Total saponins (mean mg/g DM)	Antioxidant capacity (mean μM TEAC/g DM)
Concentration (ethanol:water ratio of the extract)			
20:80	52.9[a]	39.4[a]	204.7[a]
50:50	89.2[b]	105.1[c]	421.9[c]
80:20	56.0[a]	48.0[b]	271.8[b]
Plant species			
Avocado	63.7[b]	67.4[a]	299.7[ab]
Cherry plum	47.4[a]	63.1[a]	266.8[a]
Guava	86.8[c]	69.1[a]	332.0[b]

DM Dry matter

[a,b,c]Within columns, cells that do not share letters are significantly different

Table 3 Interaction of concentration and species in total phenols, total saponins and antioxidant capacity of hydroalcoholic leaf extracts at three different ethanol:water ratios

Specie	Concentration (ethanol/water) v/v	Total phenol (mg GAE/g DM)	Saponins (mg/g DM)	ABTS antioxidant capacity (μM TEAC/g DM)
Avocado leaves	20–80	52.7 ± 0.1[b]	37.6 ± 5.3[a]	265.4 ± 57[c]
	50–50	82.47 ± 6.2[d]	113.15 ± 20[b]	446.7 ± 18[de]
	80–20	56.1 ± 1.6[b]	51.23 ± 6.7[ab]	187.1 ± 38[ab]
Cherry plum leaves	20–80	33.61 ± 0.1[a]	35.85 ± 1.2[a]	210.8 ± 33[abc]
	50–50	73.29 ± 4.8[c]	113.15 ± 2.5[b]	368.9 ± 59[d]
	80–20	35.5 ± 3.6[a]	40.33 ± 5.8[a]	220.6 ± 51[bc]
Guava leaves	20–80	72.51 ± 5[c]	44.65 ± 2.7[a]	138.0 ± 51[a]
	50–50	111.7 ± 8.8[e]	88.5 ± 6.9[b]	450.3 ± 18[e]
	80–20	76.40 ± 3.7[cd]	52.55 ± 5.8[a]	407.8 ± 7[de]

DM Dry matter

[a,b,c,d,e]Within columns, cells that do not share letters are significantly different from each other

2.5 mg/g DM, AC = 368.9 ± 59 μM TEAC/g DM), guava leaf at 20:80 (TP = 72.51 ± 5 mg GAE/g DM, SP = 44.65 ± 2.7 mg/g DM, AC = 138.0 ± 51 μM TEAC/g DM), avocado leaf at 80:20 (TP = 56.1 ± 1.6 mg GAE/g DM, SP = 51.23 ± 6.7 mg/g DM, AC = 187.1 ± 38 μM TEAC/g DM), avocado leaf at 20:80 (TP = 52.7 ± 0.1 mg GAE/g DM, SP = 37.6 ± 5.3 mg/g DM, AC = 265.4 ± 57 μM TEAC/g DM), plum leaf at 80:20 (TP = 35.5 ± 3.6 mg GAE/g DM, SP = 40.33 ± 5.8 mg/g DM, AC = 220.6 ± 33 μM TEAC/g DM), and plum leaf at 20:80 (TP = 33.61 ± 0.1 mg GAE/g DM, SP = 35.85 ± 1.2 mg/g DM, AC = 210.8.3 ± 33 μM TEAC/g DM).

Antimicrobial activity

Table 4 shows the inhibition halos sizes due to the antimicrobial activity using agar disc diffusion method with the three plant species proved in eight microorganism's strains. Note that only the 50:50 concentration was used since this was the concentration with the highest TP, SP, and AC in the previous tests. Guava leaf extract had the strongest antimicrobial activity against all of the microbial strains evaluated since the inhibition halos were larger than 6 mm, the diameter of the sensidisc used. While the guava leaf extract had good antimicrobial capacity against all of the Gram+ and Gram− bacteria tested, it was particularly effective against *Klebsiella* sp. (17 ± 0.71 mm) compared to the remaining strains (16 ± 0.71 mm for *B. subtilis* and *E. coli*,

Table 4 Antimicrobial activity based on diffusion disc test from hydroalcoholic leaf extracts

Microorganisms	Guava leaves $\bar{X} \pm SD$ Halo in (mm)	Avocado leaves	Cherry plum leaves
Gram positive			
Listeria monocytogenes ATCC 19115	15.2 ± 0.45	–	–
Bacillus subtilis ATCC 662	16 ± 0.71	–	–
Enterococcus sp.	15.6 ± 0.55	–	–
Staphylococcus sp.	15.6 ± 0.55	–	–
Gram negative			
Escherichia coli ATCC 25922	16 ± 0.71	–	–
Salmonella enterica serotipo Enteriditis ATCC 13076	14.6 ± 0.55		
Klebsiella sp.	17 ± 0.71	–	–
Pseudomonas sp.	14.8 ± 0.84	–	–

Inhibition areas, $\bar{X} \pm SD$ = average of three replicates ± standard deviation. Disc diameter was 6 mm

"–" No inhibitory activity

15.6 ± 0.55 for *Enterococcus* sp and *Staphylococcus* sp, 15.2 ± 0.45 for *Listeria Monocytogenes*, 14.8 ± 0.84 for *Pseudomonas* sp., and 14.6 ± 0.55 for *Salmonella enterica serotipo* Enteriditis *ATCC 13076*). Avocado and plum extracts showed no antimicrobial activity.

Minimal inhibitory capacity (MIC)

The MIC results test are shown in Table 5. Analyses were only carried out for guava leaf extract (50:50 concentration) since this was the only species that showed antimicrobial activity. Six of the microbial strains were inhibited by guava extract at 0.62 mg/mL, *Staphylococcus* sp. and *Salmonella enterica serotipo* Enteriditis *ATCC 13076* were inhibited at 1.25 mg/mL.

Discussion

Total phenols, saponins, and antioxidant capacity were all affected by the interaction of concentration and plant species. With respect to the concentration factor is shown that the highest concentrations of TP, SP, and AC were obtained with a 50:50 ethanol:water ratio. This may be due to the different polarities of the solvents used; ethanol is a medium-polarity solvent

Table 5 MIC and from guava leaf extract

Microorganisms	Guava leaves (mg/mL)
Gram positive	
Listeria monocytogenes ATCC 19115	0.62
Bacillus subtilis ATCC 662	0.62
Enterococcus sp.	0.62
Staphylococcus sp.	1.25
Gram negative	
Escherichia coli ATCC 25922	0.62
Salmonella enterica serotipo Enteriditis ATCC 13076	1.25
Klebsiella sp.	0.62
Pseudomonas sp.	0.62

Minimal inhibitory capacity (MIC) of guava leaf extract

and water is a high-polarity solvent. The different secondary metabolites of all three species are attracted to solvents of a more similar polarity to a 50:50 mixture of ethanol and water, compared to when a higher concentration of either solvent is used (Masschelein-Kleiner 2004). These results are consistent with those from Kiassos et al. (2009) who, in a similar study in onion also found that TP and AC values were highest when a 50:50 ratio of ethanol:water was used. In addition, because ethanol:water provides efficient extraction of secondary metabolites at a lower cost, it is considered a relatively low-toxicity, sustainable, and cost-effective solvent compared to other non-polar solvents such as methanol and acetone that are often used for this type of extraction (Amyrgialaki et al. 2014).

There were no significant differences for plant species and SP among the species ($P \geq 0.05$), but guava leaves had higher TP and AC values than avocado and cherry plum leaves. These suggested results are proposed due to the diversity of secondary metabolites reported in leaves. In a guava leaves secondary metabolite research up to 72 secondary metabolites were detected; the main metabolites related to TP and AC were Gallic acid, Catechin, Gallocatechin, Procyanidin B Isomer, Morin, Ellagic acid, Quercetin, glucuronide, Reynoutrin, Guajaverin and Avicularin (Qian and Nihorimbere 2004; Díaz-de-Cerio et al. 2016). Other factors that may also play a role are agro-climatic conditions since the presence of secondary metabolites is related to stress or conditions which threaten the plant's homeostasis, as well as the plant genus, family, and even species (Robles-García et al. 2016; Domingo and López-Brea 2003).

With respect to the concentration-by-species interaction, the highest levels of TP and AC were found in guava leaf extract in 50:50 ethanol:water, followed by guava leaf extract at 80:20 ethanol:water, avocado leaves at 50:50 and cherry plum at 50:50. All of these values are higher than values reported for other plants in similar studies (e.g. Lu et al. 2011 and Gorinstein et al. 2008): *garlic*: TP = 19.40 ± 1.2 mg GAE/g DM, AC = 43.73 ± 1.7 µM TE/g DM, *ginger*: TP = 9.20 ± 0.5 mg GAE/g DM, AC = 75.66 ± 1.1 µM TEAC/g DM., *cumin*: TP = 9.00 ± 0.1 mg GAE/g DM, AC = 57.41 ± 2.7 µM TEAC/g DM, *cinnamon*: TP = 45.24 ± 2.4 mg GAE/g DM, AC = 525.85 ± 27.6 µM TEAC/g DM and *anise*: TP = 14.94 ± 0.1 mg GAE/g DM, AC = 188.13 ± 6.2 µM TEAC/g DM, with the exception of AC in *cinnamon*. Spices including *cinnamon, oregano*, *cumin, garlic, cloves, anise, mint, coriander,* and *ginger* are the most widely used as feed additives for animal production in order to manipulate rumen function and improve feed conversion (Akyildiz and Denli 2016). On the other hand, the oxidative stability of animal feeds depend on the antioxidant capacity ingredients, present either as natural components of the feed or added specifically, to avoid the oxidation of compounds like lipids and vitamins (Frankič et al. 2009). A hydroalcoholic extract of guava leaf could, therefore, be considered as a natural additive with high antioxidant capacity, which could improve the oxidative stability of feeds and therefore improve weight gain efficiency.

With respect to the variable SP, the highest concentrations were in the avocado and plum 50:50 extracts, followed by the guava leaf 50:50 extract, showing that all three of these agro-industrial waste products are important sources of saponins, compounds which at appropriate concentrations can help inhibit protozoans in the rumen, reducing methanogenesis. This is beneficial both because it increases the energy efficiency of ruminal metabolism and because it reduces methane gas emissions into the environment (Holtshausen et al. 2009).

Antimicrobial capacity

Only guava leaf extract had antimicrobial activity in this study. Inhibition diameters were between 14 and 17 mm in eight Gram+ and Gram− organisms. These results are similar to those of other extracts used in animal feed, such as *cinnamon, clove, garlic, ginger, mint,* and *mustard*, whose ranges were from 9 to 18 mm (Tajkarimi et al. 2010). With respect to the results of this study, it should be noted that Gram- microorganisms (*E. coli, Salmonella*) are related to high neonatal mortality indices due to diarrhea in ruminants (Barrington et al. 2002), piglets (Mantecón and Ahumada 2000), and chickens (Geetha and Palanivel 2018). Among Gram+ microorganisms, the most important are *L. monocytogenes*, which leads to encephalitis, and uterine infections that can lead to abortion in ruminants (Nightingale et al. 2004) and *Staphylococcus* sp., which leads to several different animal pathologies in ruminants, swine, and chickens (Smith 2015). As such, based on our in vitro results, a 50:50 hydroalcoholic extract of guava leaf could be

used to decrease the prevalence of these microorganisms in live animals in order to improve their homeostasis and weight gain, and could be considered as a possible broad-spectrum antibiotic.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of guava leaf extract for Gram negative (*E. coli, Salmonella enterica serotipo* Enteriditis *ATCC 13076, Klebsiella* sp., *Pseudomonas* sp.) and Gram+ (*L. monocytogenes, B. subtilis, Enterococcus* sp., *Staphylococcus* sp.) strains testes was between 0.62 and 1.25 mg/mL, demonstrating wide spectrum inhibition at low doses. Compared to other natural antibiotics studied by Kittisakulnam et al. (2017) and Baljett et al. (2015) such as garlic (8.8 mg mL^{-1}), pepper (560 mg/mL), ginger and cumin (both 12.5 mg/mL), guava is effective at lower doses. This effect may be explained by the secondary metabolites antimicrobial capacity to rupture the cell wall or disturb the intracellular matrix, leading to microorganism's death (Biswas et al. 2013).

Conclusions

The results of this study show that a 50:50 ethanol:water extract of guava leaf is the most effective species-concentration combination among those tested, showing the highest total phenol concentration, antioxidant capacity, and inhibition of eight microorganisms. As such, we suggest that it may be a viable substitute for synthetic antibiotics and could be used in different animal species to decrease pathologies related to Gram− and Gram+ microorganisms. In addition, this extract presented excellent antioxidant capacity, which could be used to increase the oxidative stability of animal feeds, and therefore the efficiency of weight gain. It is also a source of saponins, which could help decrease methanogenesis and increase the energy efficiency of digestion in livestock, decreasing the emission of methane gas into the environment. Further tests in vitro and tests in vivo are required to test the effects of this extract in different animal species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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