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Research Article

In vitro Evaluation of Phytobiotics for the Inhibition of Common Enterobacteriaceae of the Gastrointestinal Tract

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Abstract

The extract mangosteen plant (Garcinia mangostana Lynn), with antibacterial capacity, was selected for its In vitro effect against frequent enterobacteria in the gastrointestinal tract (GIT) of living beings. In this work, the In vitro antibacterial activity was observed for enterobacteria and other selected ones (*Escherichea coli, Staphylococcos aureus, Streptococcus spp., and Klebsiella pneumoniae*). The value of the minimum inhibitory concentration (MIC) of the mangosteen extract, the supernatant, precipitate and dehydrated precipitate, commercial product XanGo®, xanthone 9-xanthene® and nanocellulose from Acacia farnesiana and the mixture between these phytobiotic compounds varied between 25 at 100% dilution and 5 to 10 mg/mL respectively. The results showed that there was a significant difference in the bacterial inhibition halos for enterobacteria ($P < 0.05$); The highest value (mm) was in XanGo® (20 ± 0.6), followed by mangosteen extract (16 ± 1.1), xanthone 9-xanthene® (7 ± 0.7) and nanocellulose (1 ± 0.1). For the Staphylococcus aureus group, the highest value recorded (mm) was XanGo® (20.13 ± 0.94), followed by mangosteen extract (16 ± 0.88), xanthone 9-xanthene® (7 ± 0.58) and nanocellulose (1 ± 0.33), so there are significant differences in the mixture between the phytobiotic compounds, where the highest achievement of inhibition was observed (11.3 ± 1.30), in the mixture of mangosteen extract and nanocellulose (1:1 dilution), with activity against *Staphylococcus aureus* strain 448. In the mixture of phytobiotic compounds, the highest achievement of inhibition for enterobacteria was observed (10.3 ± 0.2), with xanthone 9-xantene® and nanocellulose (1:1 dilution), for which is concluded in this study, the *In vitro* antibacterial activity of XanGo®, the mangosteen extract and xanthone 9-xanthene® is effective against enterobacteria, common pathogens of the digestive tract..

Keywords: Antibacterial; Enterobacteria; Mangosteen Extract, 9-Xanthene™

Introduction

The presence of multidrug-resistant bacteria has become an important cause of failure of antibacterial activity, it can be a major obstacle in the treatment of infectious diseases such as *Staphylococcos aureus* (MRSA), *Mycobacterium tuberculosis* [11]. As a result of antibiotic resistence in domestic animals and humans, currently facing one of the most serius public health and animal health dilemmas [12,28], plants are currently an alternative source to control bacterial growth. Given the appearance of new pathogenic and infectious bacterial strains of the gastrointestinal tract (GIT), since plants have secondary metabolites for their defense with the ability to inhibit and are commonly used in traditional medicine [1,7,14,17,42], the potencial of plants, extracts and phytobiotics are an alternative source of animal supplementation [2,4,5,8-10,34], strains of microbial pathogens have been evaluated, potencially using plants for the therapeutic treatment of plants, animal and human beings [32,35,41,46,48].

In the practice of medicine, neonatal individuals; calves, pigs, birds and others, they present a high degree of susceptibility to to infections caused by pathogenic microorganisms, particularly in the respiratory and digestive tracs, which causes diseases with different effects and clinical symptoms [18,19,21,23], antibacterial activity and modulation can be measured in the population. Of these, even recognizing that they are changing in susceptibility patterns [27]. The potential of some plant extracts against pathogens is considered; various phytobiotic compounds have antibacterial properties in experimental studies, such as mangosteen extract (Garcinia mangostana Lynn), xanthone 9-xanthene™, nanocellulose (from acacia farnesiana), which can have an inmunomodulatory effect at early ages; in both humans and animals, particularly diseases caused by *Enterobacteriaceae*, *Salmonella spp*., *Escherichia coli*, *Staphylococcos aureus*, *Streptococcos spp*., *Mycobacterium*, as well as parasites and viruses, its antiflammatory activity has also been described in these infection processes [14,15].

The objetive of this study was to record the *In vitro* antibacterial effect of mangosteen extract and some compounds of natural origin such as nanocellulose, for use in the natural therapeutics of digestive problems.

Material and Methods

Commercial acquisition of mangosteen extract™ (Xi'an Olin Biol. Tech. Co. Ltd), including xanthone α- mangosteen (10%) is obtained by the Soxhlet method in a semi-continuous extraction using ethanol as a solvent requiring 6-24 hours for extraction.

Acquisition of phytobiotic compounds

- The mangosteen extract was separated into fractions: 1) supernatant; 2) precipitated; and 3) dehydrated precipitate, which was obtained by centrifuging at 12,000 rpm for 10 min, respectively.
- The commercial product, XanGo™, which is processed using the described methodology [3]. It should be noted that the fruit extract is a natural fermentable liquid, to which citric acid, pectin, xanthene gum, and sodium benzoate are added and is distributed worldwide for use in the beverage and food industry.
- Xanthone 9-xanthene™ (Sigma Aldrich™), is a simple organic composition with a degree of 99% purity, whose preparation is derived from the xanthone α-mangostin which presents similar characteristics of this natural xanthone, whose ring Benzene has a modification of the carboxyl functional group and can have better availability and therefore achieves greater biological effects.
- Nanocellulose is a biotechnological organic polymer from Acacia farnesiana (huizache), being an aqueous suspension and microscopically with the formation of nanocrystals. Nanocellulose was obtained through acid hydrolysis of the formed lignocellulose fibers [8]. This nanocellulose corresponds to a defect-free rod form of nanoparticles that present notable properties, among which the following stand out: low molecular weight, low cost, viability of the raw material, nanoscale dimension and stable morphology, the crystalline domains of the raw being , which were designed at the Cold Plasma Center for Natural Polymers of the Department of Wood, Cellulose and Paper of the University Center for Exact Sciences and Engineering (CUCEI) of the Guadalajara University, cellulose microfibrils, which have excellent mechanical properties for use in pharmacokinetics in soluble form.

In vitro tests

The *In vitro* experimental phase was carried out in the Plant Ecophysiology Laboratory of the Department of Ecology and in the Mastitis and Molecular Diagnosis Laboratory of the Department of Veterinary Medicine of the University Center for Biological and Agricultural Sciences of the Guadalajara University (20º44´53.6"-103º30 ´52.2" W, altitude 1,659 meters above sea level).

- *Enterobacteriaceae* were initially isolated from bovine milk (Holstein-Friesian breed) from 2 stables located in the Tala area, Jalisco, Mexico. Milk samples (n = 336) were collected in test tubes (10 mL) directly from each udder quarter of the cows and were transported to the laboratory in a container at a temperature of 4 ± 2 °C. The test tubes with the milk were allowed to rest at room temperature for approximately 1 h, then 1 mL of milk was taken and placed in a Petri dish containing the general Bioxon™ lamb blood agar medium. This culture medium was prepared following the technique [27], which is described below: 40 g of the lyophilized medium were suspended in 1 L of distilled water, mixed thoroughly and heated with frequent stirring until boiling for 1 min with the in order for the dissolution to be complete. Subsequently, the medium was sterilized at 121ºC for 15 min and distributed in the Petri dishes, allowing it to solidify at room temperature. To observe bacterial growth, the Petri dishes were placed in an incubation oven (BINDER™) for 24 and 48 h at 37°C.

- The identification of the *Enterobacteriaceae* group was based on the methodology [39], which consists; 1) morphology; bacilli, cocci or spirillum, forming chains or groups; 2) colony shape; punctate less than 1 mm in diameter, round, irregular or filamentous; 3) margin of the colony: entire, curved, wavy, lobed or filamentous; 4) texture of the colony: smooth, concentric, wrinkled or with curves or contour; and 5) color and smell. Subsequently, to corroborate the identification of the enterobacteria, they were placed in the Mc Conkey agar differential culture medium, Bioxon™ Becton Dickinson), which is specific for enterobacteria and coliforms, the preparation of which is described below; 50g of the lyophilized medium were suspended in 1 L of distilled water and mixed until a uniform suspension was obtained. It was then allowed to stand for 10 to 15 min, stirred frequently and heated to boiling for 1 min. Finally, it was sterilized in an autoclave (FELISA™) at 121ºC for 15 min.
- The conservation of enterobacteria, either as reference material or for replication with the purpose of carrying out *In vitro* tests, began with the direct taking of bacteria samples in Petri dishes using a cotton swab, then the inoculum in a sterile test tube containing bovine brain-heart infusion liquid culture medium (Bioxon™), whose preparation is described below: 37g of the lyophilized medium were suspended in 1 L of distilled water, mixed thoroughly, then heated. with frequent stirring, it was distributed and sterilized at 121ºC for 15 min. 1 mL of this medium was placed in a test tube with the bacterial inoculum at a concentration of approximately 1.0 x 105 colony forming units (CFU), then the tubes were placed in an incubation oven for 24h at 37°C. It should be noted that another way to preserve the *Enterobacteriaceae* of the strain in the laboratory is based on the method [23], which consists of multiplying by inoculating the seed with a metal loop in a lamb blood agar medium (Bioxon™), whose preparation is described below: 40g of the lyophilized medium were suspended in 1 L of distilled water, mixed and heated with frequent stirring until it boiled for 1 min, achieving complete dissolution, then it was sterilized at 121°C for 15 min, they were placed in Petri dishes for 24-48h at 37°C in the incubation oven. Process carried out during the *In vitro* experimental phase, every 10-15 days.
- Measurements of the diameter of sensitivity of enterobacteria to the application of phytobiotic compounds (n = 27) were carried out in triplicate, as follows: 1) mangosteen extract; 2) supernatant; 3) precipitate; 4) dehydrated precipitate; 5) XanGo™; 6) xanthone 9-xanthene™; 7) nanocellulose; 8) mangosteen extract with nanocellulose (EM + NC, 1:1 ratio); and 9) the xanthone 9-xanthene with nanocellulose (9X + NC, 1:1 ratio). These phytobiotic compounds were added to the sterile filter paper discs and at 24h and 48h, the sensitivity diameter (mm) was measured with a digital Vernier ruler, with the reference site being the center of the radius of the halo.
- Determination of sensitivity in 3 groups of enterobacteria with the mangosteen extract at different dilutions (100%, 50% and 25%), according to the data generated in the *In vitro* sensitivity tests (previously described), and to corroborate the effect of incubation time, measurements of the sensitivity diameter were also carried out in triplicate (n = 27), which were added to the sterile filter paper discs and after 48h, the sensitivity diameter (mm) was measured with a ruler. Digital Vernier with the reference site being the center of the halo radius.
- The xanthone 9-xanthene™ was evaluated at different concentrations (5 mg and 10 mg), measurements of the sensitivity diameter (mm) were carried out in triplicate (n = 9) in the enterobacteria group. The xanthone 9 xanthene™ was added to the sterile filter paper discs and only after 24 hours, the sensitivity diameter (mm) was measured with a digital Vernier ruler, with the reference site being the center of the radius of the halo.
- *Escherichia coli* was identified, to corroborate (6), the growth sensitivity of this bacterial group and others of interest (*Streptococcos*, *spp*., *Staphylococcos aureus.*, and *Klebsiella pneumoniae*), to phytobiotic compounds, which could be isolated from milk samples, having importance in public and veterinary health, since they are latent pathogens and incidents of digestive diseases mainly in dairy cattle, and could potentially induce zoonosis in the general population, due to the consumption of contaminated food. by these pathogens. This bacterial group can also be present in different pathological conditions in livestock farms, and its importance of study lies in the characteristic of resistance to antibiotics, for this reason, in the microbiology laboratory of the State Research Institute of GieBen, Hessen, Germany, growth sensitivity tests were performed for this bacterial group.
- Identification of bacterial groups according to the methodology [27], which was described previously; and they consisted of; 1) morphology, 2) colony shape, 3) colony margin, 4) colony texture, and 5) color and odor. In the laboratory, the bac-

terial group *Streptococcus spp*., *Staphylococcos aureus*, *Escherichia coli* and *Klebsiella pneumoniae* were isolated from bovine milk obtained in stables with Holstein-Friesian cattle from the Hessen region, Germany. Milk samples (n = 1500) were collected (10 mL) directly from each udder quarter of the cows in test tubes and transported to the laboratory at a temperature of 4°C ± 2°C. From the test tubes, 1 mL of milk is taken and placed in a Petri dish with the lamb blood agar medium (whose preparation was described previously), for bacterial growth for 24-48 h at 37°C in the oven incubation. The specific differential media for the group of bacteria were as follows: for *Escherichia coli* it was methylene blue eosin agar which was prepared from a suspension of 36 g of the lyophilized medium in 1 L of distilled water, mixed perfectly and heated. With frequent stirring until it boiled for 1 min, achieving complete dissolution, it was then sterilized at 121ºC for 15 min, distributed in Petri dishes which were placed in the incubation oven for 24h and 48h at 37ºC. In the case of *Klebsiella pneumoniae*, Mc Conkey agar was used (preparation described above) and for *Staphylococcos aureus*, saline mannitol (also known as Chapman medium), which was prepared as follows: 111g of the lyophilized medium were suspended in 1 L of distilled water, it was mixed perfectly and heated with frequent stirring to boiling for 1 min until complete dissolution was achieved. It was then sterilized at 121°C for 15 min, distributed in Petri dishes, which were then placed with the bacteria in the incubation oven for 24 h at 37°C.

- Measurements of the growth sensitivity diameter, for each group of bacteria, (n = 396), with the phytobiotic compounds: 1) sterile water (control), 2) mangosteen extract (100%) and its fractions; a) supernatant, b) precipitate and c) dehydrated precipitate; which were obtained by centrifuging at 12,000 rpm for 10 min, respectively; 3) XanGo™; 4) xanthone 9-xanthene; 5) nanocellulose; 6) mangosteen extract with nanocellulose (EM + NC, 1:1 ratio); 7) mangosteen extract with xanthone 9-xanthene® (EM + 9X, 1:1 ratio); 8) 9-xanthene® xanthone with nanocellulose (9X + NC, 1:1 ratio); and 9) mangosteen extract with xanthone 9-xanthene® and nanocellulose (EM + 9X + NC, 1:1:1 ratio). These phytobiotics were added to the sterile filter paper discs and after 24 hours, the diameter of sensitivity (mm) was measured with a digital Vernier ruler with the reference site being the center of the halo radius

Statistic analysis

- The results were analyzed and it was considered to carry out the parametric test which consists of: independent data (t test of two or more groups), standard deviation with the Bonferroni test (as an indication of the dispersion of a set of data), normality (Anderson Darling test) and homogeneity of variances (Levene test).
- The results of the *In vitro* test were evaluated by analysis of variance (one-way ANOVA). The difference between means ($P < 0.05$) was evaluated according to the Tukey method [27], using the Minitab version 14® statistical package.

Results and Discussion

The results of the *In vitro* test are shown (Figure 1), with the different inhibition diameter values (mm) of the phytobiotic compounds evaluated after 24 h. The antibacterial effect revealed three disaggregated groups, the first with the largest inhibition diameters being XanGo™ (20 ± 0.6), followed by both the supernatant (17 ± 0.58) and the precipitate of the mangosteen extract (16 ± 0.29), and 100% mangosteen extract (16 ± 1.15). The second inhibition diameter group is made up of xanthone 9-xanthene™ with nanocellulose (10 ± 0.20), mangosteen extract with nanocellulose (7 ± 0.34) and xanthone 9-xanthene® (7 ± 0.73). The third group presented no bacterial inhibition and was the fraction of the dehydrated precipitate of the mangosteen extract and nanocellulose (0.3 ± 0.09 and 1 ± 0.12), respectively. *In vitro* evaluation of plants, their components and/or fractions of natural origin is promoted for the preparation of antibacterials [11,27,31], being important, since with this search for new bioactive molecules it can develop new phytobiotics for control of infectious diseases in humans and animals. For this reason [10,13,25,29,44,47], they have evaluated mangosteen for these applications, which is native to Southeast Asia and contains a large number of biologically active substances, including catechins, stilbenes, polysaccharides, flavonoids, vitamins and phenols, among which xanthones stand out, which may be the antioxidant and antibacterial factor of the fruit. The antibacterial activity of the extracts [16,24,25,40], confirms that it is due in part to the presence of chemical constituents and phenolic fractions. But [14,27,30,37,44], the fragmentation of the chemical components that constitute the extract such as the volatile matter of flavonoids, phenols, soluble or non-soluble substances during the extraction process, can reduce or eliminate the antibacterial action potential, indicating a synergistic effect of the active ingredients, compounds or equivalent.

In this study, the natural origin of the phytobiotic compounds was taken in to account and that they could have a bioactive action in their composition and mixture, being factors of the nutritional effect in the experimental animals. On the other hand, the total active substances of the extract and the observe in *In vitro* tests. From the results obtained in the In vitro tests after 24h of incubation (Figure 1), the phytobiotic compounds that presented the greatest action potential were XanGo™, mangosteen extract and xanthone 9-xanthene™, for the Enterobacteriaceae group. And, on the contrary at 48 h of incubation the diameters of inhibition of the phytobiotic compounds were minimal; this could have happened because they are organic products and may be subject to a fermentation process and tend to degrade due to the effect of time and exposure to temperature [27,36,38] also describe that these factors influence the chemical composition and pH, so they may present differences in biological and microbiological action.

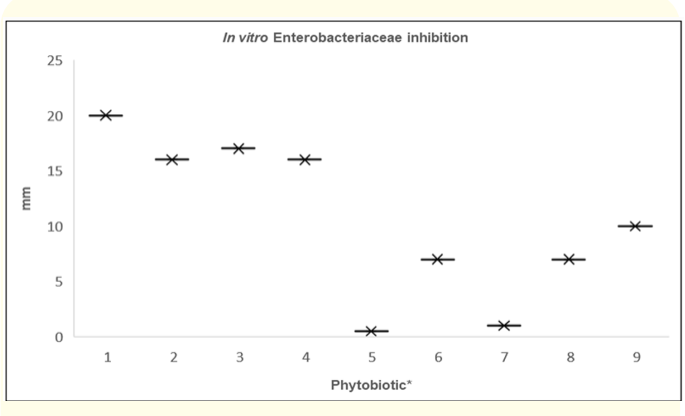

Figure 1: Inhibition of enterobacteria under *In vitro* conditions of some phytobiotics*: 1) XanGo™, 2) Mangosteen extract (100%), 3) Supernatant, 4) Precipitate, 5) Dehydrated precipitate, 6) Xanthone 9-xanthene™, 7) Nanocellulose, 8) Mangosteen extract (100%) and nanocellulose, 9) Xanthone 9-xanthene™ and nanocellulose. After 24 h of incubation, where the different values are the mean (n = 3) ± standard error (P < 0.05).

The sensitivity of the enterobacteria in the *In vitro* test with the phytobiotic compounds at 48 h of incubation (Table 1) was not significant (P < 0.05) and was determined with the inhibition diameter (mm) of a mean (n = 3) ± standard error with nanocellulose (0.5 ± 0.0) and the dehydrated precipitate (0.1 ± 0.1) being significant. Confirming that the *In vitro* bacterial sensitivity after 48 h of incubation of the mangosteen extract regardless of the dilution (100%, 50% and 25%) revealed minimal inhibition zones, which describe [31] that the concentration can influence directly on the effectiveness of the extracts. This situation also occurred in the concentration (mg) of the xanthone 9-xanthene™ determined in the *In vitro* test at 24h of incubation, where the sensitivity for this same bacterial group registered minimum inhibition diameters. In the case of nanocellulose, it is important to note that it did not have relevant effects as an inhibitor of enterobacterial growth, so the inhibition diameter was zero, even when it was mixed with other phytobiotic compounds; such as mangosteen extract and 9-xanthene™. For this reason [6,23], they demonstrate that their design is to be used as a vehicle for carrying bioactive particles, this influences the efficiency of nutrient performance. It also describes [20], that nanocellulose due to its natural characteristics has been proposed to be used with antioxidant interest and precursors for the dynamics of active ingredients [23], in nutritional principles which indicates that it can potentiate the transit of nutrients during the intestinal journey. The production of nanocellulose from natural fibers has become truly significant for its use in the bases of pharmacology applied in therapeutics and with the capacity for its use in phytobiotics [6,38].

Phytobiotic	Inhibition diameter*
Comercial product XanGo™	7.2 ± 0.3a
Supernant	6.4 ± 1.3a
Mangosteen extract 100%	6.3 ± 0.3a
Precipitate	5.1 ± 0.9a
Mangosteen extract + Nanocellulose	4.1 ± 0.3a
Xanthone 9-xanthene™ + Nanocellulose	3.4 ± 1.0a
Xanthone 9-xanthene™	3.3 ± 0.9a
Nanocellulose	0.5 ± 0.0b
Dehydratate precipitate	0.1 ± 0.1b

Table 1: Sensitivity of Enterobacteriaceae under *In vitro* conditions of phytobiotics, where the different values are the mean (n = 3) ± standard error (P < 0.05), the inhibition diameter (mm) was determined *In Vitro* after 48h.

*Means of different literals indicate statistical difference between groups.

The *In vitro* evaluation of the enterobacteria group with different concentrations of mangosteen extract (Table 2), the concentration at 100% (6.3 ± 0.88), 50% (5.3 ± 0.33) and 25% (5.0 ± 1.15) was not significant. For xanthone 9 xanthene™, it was determined that at concentrations 0, 5 and 10 mg, the inhibition diameter was minimally zero (3 ± 0.33 mm), between both, and was not

significant (P < 0.05). The effect of these phytobiotic compounds on bacterial inhibition in different groups of interest was evaluated; *Staphylococcos aureus*, *Streptococcos spp*., *Escherichia col*i, and *Klebsiella pneumoniae* and this action was recorded due to the effect mainly in the combination of mangosteen extract and xanthone α-mangostine. This characteristic of the mangosteen extract is the antibacterial action factor, which reinforces the bioactive potential and nutritional complement in organisms. It is important to note that mangosteen extract has xanthones and other biologically active substances, such as catechins, polyphenols and polysaccharides, which are apparently also responsible for some antibacterial and antifungal effects [10].

Mangosteen extract*			
Enterobacteriaceae	**100%**	**50%**	**25%**
1	6.6 ± 0.9a	5.6 ± 0.3a	4.8 ± 1.2a
2	6.3 ± 1.2a	6.3 ± 1.2a	4.7 ± 0.9a
3	6.1 ± 0.3a	5.8 ± 0.6a	5.0 ± 1.5a

Table 2: *In vitro* antibacterial activity of mangosteen extract at different concentrations determined after 48 h of incubation, using the inhibition diameter (mm), where the diferent values are the mean (n = 3) standard error (p < 0.05).

*Means of different literals indicate statistical difference between groups.

The *In vitro* experimental test (Table 3), the inhibition diameter was recorded for *Streptococcus spp*, *Staphylococcos aureus, Escherichia coli* and *Klebsiella pneumoniae* with the phytobiotics and their mixture (1:1 dilution). The greatest achievement of inhibition was observed in the mixture of mangosteen extract and cellulose nanocrystals with activity against *Staphylococcos aureus* strain 448 (11.3 ± 1.30), in the mixture of xanthone 9-xanthene® and nanocellulose with activity against enterobacteria it was (10.0 ± 0.33) and in the mixture of mangosteen extract, xanthone 9-xanthene™ and nanocellulose against *Staphylococcos aureus* strain 519/6 with (9.57 ± 0.10) and finally in the mixture of mangosteen extract and xanthone 9-xanthene® with activity against *Staphylococcos aureus* strain 447/6 with (8.94 ± 0.19). On the contrary, the inhibition zones were minimal (moderate), for some strains of *Escherichia coli* and *Klebsiella pneumoniae*, no significant differences were recorded (P < 0.05) between the phytobiotic compounds; mangosteen extract, xanthone 9-xanthene™; mangosteen extract plus cellulose nanocrystals; and mangosteen extract plus xanthone 9-xanthene™ and nanocrystals. The greatest inhibition effect observed was for *Klebsiella pneumoniae* 523/2 (3.41 ± 0.00) and the lowest inhibition zone recorded (1.33 ± 0.00) was for *Escherichia coli* strain 459/3 with the combination of mangosteen extract and cellulose nanocrystals. *Escherichia coli* was identified in milk samples, which has a worldwide presence, which is why the study was carried out in Hessen, Germany, with the purpose of recording the sensitivity effect of mangosteen extract, 9-xanthene™ and nanocellulose and its mixtures, which revealed zero inhibition diameters with mangosteen extract [30] and is important in public health and veterinary medicine, since these organisms coliforms are latent pathogens and incidents of digestive diseases mainly in dairy cattle, and can potentially induce a zoonosis in the general population, due to the consumption of food contaminated by these pathogens [5,22,45], they consider that this bacterial group may also be present in different conditions of pathological exposure in livestock farms, and its importance for study worldwide lies in its genomic characteristics that present resistance to antibiotics. . According to the phytobiotic compounds that presented the greatest action potential in the *In vitro* test, they can be evaluated in acceptance tests at an experimental level.

Finally, it was determined that the mangosteen extract has growth inhibitory capacity for the different groups of bacteria evaluated; significant for Enterobacteriaceae, moderate for *Staphylococcos spp*., *Streptococcos spp*., traditionally and historically being the factor for its use in the practice of traditional medicine [35]. Mangosteen extract has a low to no inhibition action on the bacterial growth of the *Escherichia coli* genus, a saprophytic bacteria that has a close relationship with the digestive tract of living beings [33], so it is of interest to apply a series of studies directed against common pathogenic bacteria in humans and domestic animals. The greatest halo recorded for bacterial inhibition in the study was for the Enterobacteriaceae group, therefore, phytobiotic compounds can exert a modulating action on the composition of coliform organisms and mesophilic bacteria, and can promote improvement in the performance of foods, forages and nutritional supplements without interfering with the administration of other types of therapeutic treatment. It is important to consider the incorporation of these phytobiotic compounds as nutritional supplements to strengthen the response capacity of the immune system in the presence of pathogens [23,39].

It is important to note that pharmacological foundations cannot be established because these phytobiotic compounds are not regulated by the Food and Drug Administration (FDA), as reported

[27], he used gentamicin and streptomycin as positive controls for bacterial inhibitors, which have a broad spectrum for Gram-positive and Gram-negative bacteria. Being relevant in this study, XanGo™ and mangosteen extract showed significant results in the inhibition diameter for enterobacteria [43], determining that xanthone 9-xanthene™ may have immunostimulatory activity. For this reason, the present study determined the *In vitro* effect directed for the genus of Enterobacteriaceae, for its use of phytobiotics in the treatment of digestive tract infections as alternatives to antibiotics.

Grupo Bacteriano	1	2	3	4	5	6	7	8	9	10	11
Estreptococcos spp.	16.0 ± 4.26a	17.0 ± 0.17a	16.0 ± 0.67a	0.2 ± 0.02c	17.0 ± 0.88a	6.0 ± 1.45b	0.3 ± 0.03c	8.0 ± 0.33b	*	4.0 ± 0.33b	*
Staphylococcus spp.	17.0 ± 0.88a	14.0 ± 0.33a	15.0 ± 0.58a	0.0 ± 0.00c	15.0 ± 1.15a	3.0 ± 0.58b	4.0 ± 0.58b	6.0 ± 0.33b	*	7.0 ± 0.88b	*
Staphylococcus aureus 43913	8.68 ± 0.0b	*	*	*	18.58 ± 0.0a	7.42 ± 0.00b	*	8.43 ± 0.0b	7.77 ± 0.0b	7.41 ± 0.0b	8.77 ± 0.0b
Staphylococcus aureus 445	8.67 ± 0.0a	*	*	*	6.91 ± 0.0b	6.11 ± 0.0b	*	9.04 ± 0.75a	8.46 ± 0.0a	8.54 ± 0.0a	8.58 ± 0.0a
Staphylococcus aureus 446	10.03 ± 0.0a	*	*	*	10.43 ± 0.0a	7.21 ± 0.0c	*	8.11 ± 0.0c	7.76 ± 0.0c	9.77 ± 0.36a	8.82 ± 0.0b
Staphylococcus aureus 447/6	11.21 ± 0.36a	*	*	*	8.03 ± 0.0c	7.95 ± 0.03c	*	8.81 ± 0.0b	8.94 ± 0.19b	7.45 ± 0.0c	9.23 ± 0.0b
Staphylococcus aureus 448	9.52 ± 0.0b	*	*	*	11.52 ± 0.0a	6.82 ± 0.0c	*	11.26 ± 1.30a	7.05 ± 0.0c	6.66 ± 0.0c	9.27 ± 0.0b
*Staphylococcus aureus 479/^*	9.14 ± 0.0a	*	*	*	7.05 ± 0.0c	7.88 ± 0.0b	*	8.69 ± 0.0a	7.06 ± 0.0c	6.94 ± 0.0c	8.71 ± 0.0a
Staphylococcus aureus 493	9.63 ± 0.0b	*	*	*	16.86 ± 0.0a	6.43 ± 0.0c	*	8.51 ± 0.0b	6.93 ± 0.0c	7.63 ± 0.0c	9.27 ± 0.0b
Staphylococcus aureus 493/2	9.51 ± 0.0b	*	*	*	12.63 ± 0.0a	6.58 ± 0.0c	*	8.51 ± 0.0b	7.18 ± 0.0b	7.91 ± 0.0b	8.54 ± 0.0b
Staphylococcus aureus 502	9.05 ± 0.0a	*	*	*	7.17 ± 0.0b	6.56 ± 0.0b	*	8.01 ± 0.0a	8.61 ± 0.0a	8.94 ± 0.0a	8.53 ± 0.0a
*Staphylococcus aureus 503/^*	10.29 ± 0.0a	*	*	*	10.55 ± 0.0a	6.45 ± 0.0c	*	8.96 ± 0.0b	7.13 ± 0.0c	6.29 ± 0.0c	8.45 ± 0.0b
Staphylococcus aureus 506/194	8.53 ± 0.0b	*	*	*	13.53 ± 0.0a	7.83 ± 0.0b	*	7.41 ± 0.0b	7.58 ± 0.0b	7.73 ± 0.0b	7.28 ± 0.0b
Staphylococcus aureus 519/6	8.06 ± 0.0b	*	*	*	20.13 ± 0.94a	6.43 ± 0.0c	*	8.44 ± 0.0b	8.27 ± 0.0b	6.51 ± 0.0c	.57 ± 0.1b
Staphylococcus aureus 508/44	9.48 ± 0.0b	*	*	*	11.77 ± 0.0a	7.86 ± 0.0c	*	8.63 ± 0.0c	7.81 ± 0.0c	8.11 ± 0.0c	8.57 ± 0.0c
Staphylococcus aureus 506/76	10.01 ± 0.0a	*	*	*	6.1 ± 0.0c	6.48 ± 0.0c	*	8.82 ± 0.0b	7.29 ± 0.0b	8.35 ± 0.0b	7.97 ± 0.0b
E. coli 525/20	3.14 ± 0.39a	*	*	*	*	3.24 ± 0.4a	*	1.87 ± 0.16a	*	*	2.35 ± 0.42a
E. coli 514/2	2.82 ± 0.0a	*	*	*	*	2.97 ± 0.0a	*	1.61 ± 0.0a	*	*	1.62 ± 0.0a
E. coli 459/3	1.83 ± 0.0a	*	*	*	*	1.92 ± 0.0a	*	1.33 ± 0.0a	*	*	3.09 ± 0.0a
Klebsiella pneumoniae 523/2	3.41 ± 0.0a	*	*	*		3.25 ± 0.0a	*	2.71 ± 0.0a	*	*	2.84 ± 0.0a

Table 3: Antibacterial activity of some phytobiotics* and their combinations in a 1:1 ratio where the different values are the mean (n=3) ± standard error. The inhibition diameter (mm) was determined *In vitro* after 24 h.

***** 1) 100% mangosteen extract, 2) Supernant, 3) Precipitate 4), Dehydratate precipitate, 5) XanGo™ 6) Xanthone 9-xanthene™, 7) Nanocellulose 8) Mangosteen extract and nanocellulose, 9) Mangosteen extract and xanthone 9-xanthene™, 10) Xanthone 9-xanthene™ and nanocellulose, 11) Mangosteen extract, xanthone 9-xanthene™ and nanocelullose

Conclusion

The *In vitro* antibacterial activity of XanGo™, mangosteen extract and xanthone 9-xanthene™ is effective against enterobacteria, common pathogens of the digestive tract.

In the present study, it is concluded that the *In vitro* antibacterial activity of the mangosteen extract; the supernatant, precipitate and dehydrated precipitate, nutritional benefits of natural food supplements will be able to expand the areas of biotechnological research.

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