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Antimicrobial Activities of Leaf Extracts from Algarrobo blanco (*Prosopis alba*), Tusca (*Acacia aroma*) y Mistol (*Ziziphus mistol*) to be Applied to Fresh Products

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Abstract

The aim of the present study was to analyze the *in vitro* antimicrobial activity against pathogens such as *Salmonella, Staphylococcus aureus, Staphylococcus spp., Bacillus cereus, Micrococcus, Escherichia coli* and *Listeria innocua*, using *Prosopis alba* (Algarrobo blanco), *Acacia aroma* (Tusca) and *Zizhiphus mistol* (Mistol) leaf extracts with 50: 50 v/v ethanol: water and 70:30 v/v acetone: water as antimicrobial preservatives. Determination by agar disk diffusion method was made. All extracts showed some antibacterial activity; however, *Bacillus cereus* was more resistant and *Staphylococcus aureus* was more sensitive to the extracts evaluated in comparison with the other bacteria strains used in the test. Algarrobo blanco extracts with 50:50 v/v ethanol: water inhibited the *Staphylococcus spp.* growth. Therefore, Algarrobo blanco, Tusca and Mistol leaf extracts could be used as potential natural preservatives for fresh products.

Keywords: Algarrobo blanco; Tusca; Mistol; Antibacterial Activity

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Introduction

Food spoilage is caused by various factors, one of them is the development of microorganisms, and one way to prevent it is through the use of preservative compounds [1]. These compounds can be synthetic or natural [1,2]. However, the use of synthetic preservatives can have adverse effects on the consumers' health, so natural compounds would be a good option [1]. These natural compounds can be obtained from different sources such as food [3], agri-food waste [4,5], flower petals [6] or stems, leaves, seeds or fruits [3,7-9], and they are rich in phenolic compounds [10] which may also contribute to replace common additives with antioxidant and antimicrobial properties that are currently the focus of consumers who demand the so-called "clean-label" food and minimally treated fresh products [11-13]. In this sense, these bioactive compounds in addition to their antioxidant or antimicrobial functions could provide safe products along with functional properties including better and healthier characteristics [6,13].

Antimicrobial preservatives that prevent bacterial growth, especially Gram positive, may be ineffective against resistant Gram negative bacteria due to the lipid bilayer with protective effect [2].

Santiago del Estero belongs to the Great American Chaco, rich in native vegetation such us *Prosopis alba,* commonly known as Algarrobo blanco, *Acacia aroma* or Tusca and *Ziziphus mistol* or Mistol. Algarrobo blanco and Tusca belong to the legume family and Mistol belongs to the rhamnaceae family [14]. According to the literature, the leaves of these native species are an important source of natural conservants [15-19].

The aim of the present study was to determine the in vitro antimicrobial activity of Algarrobo blanco, Tusca and Mistol leaf extracts by using 70: 30 v/v acetone: water and 50: 50 v/v ethanol: water as solvent mixtures to be used as conservants in food products.

Materials and Methods

Plant Material

Prosopis alba (Algarrobo blanco: AB), *Acacia aroma* (Tusca: T) and *Ziziphus mistol* (Mistol: M) leaves were collected from the Experimental Field Francisco Cantos of the National Institute of Agricultural Technology (Latitude: 28° 1'21.38"S; Length: 64°13'49.83"O) from Santiago del Estero, Argentina. Green leaves were harvested during November and December of 2015 and dried at room temperature (22°C). They were subsequently lyophilized and frozen at -20°C until processing.

Plant Extracts

Leaves were finely minced in a 1 mm mesh ml (CT 193 Cyclotec-

China). For the extraction of the bioactive compounds, two types of solvent mixtures, 50: 50 v/v ethanol: water and 70: 30 v/v acetone: water. 0,1 g of plant material and 10 mL of solvent mixtures were used. The extraction was performed by ultrasound for 1 hour with sonication periods of 15 minutes using an Ultrasound (Branson 2210 R- MT Ultrasonic Cleaner, USA). Subsequently, the mixture was centrifuged at 3000 x g for 15 minutes at room temperature. The liquid fraction was recovered in a 10 mL flask. The extract was obtained in triplicate and was stored in screw cap bottles at -25 °C until further analysis.

Evaluation of *in vitro* Antibacterial Activity

Bacterial strains and culture conditions: A total of seven bacterial strains were used to evaluate the antimicrobial effects of the leaf extracts. These include five strains of Gram positive bacteria (*Staphylococcus sp., Staphylococcus aureus, Listeria innocua, Bacillus cereus and Micrococcus luteus*) and two Gram-negative bacteria (*Salmonella and Escherichia coli*). The strains were isolated and identified in the Chair of Microbiology, Department of Food Sciences of the Faculty of Agronomy and Agro-industries of the National University of Santiago del Estero, Argentina.

Before they were used, the bacterial strains were activated by inoculation in Brain Heart Infusion (BHI broth, Britania, Argentina). 10µL of culture was transferred to a 10 mL liquid medium and the inoculated media were incubated at 37°C for 24 hours to obtain cells in the exponential phase.

Agar disk diffusion method: Antibacterial activity of ethanolic and acetonic extracts was evaluated by the agar disk diffusion method described by [10]. 100µL aliquot of active bacteria containing >106 cfu/mL (measured with the McFarland scale) was spread into the surface of BHI agar to create a microbial lawn and then left to dry. Sterile filter paper disks (6 mm diameter) were impregnated with 20µL of each extract and left to dry before being placed on each inoculated agar. A disk of ciprofloxacin with 5 µg was used as positive control and disks with 20µL of 50: 50 v/v ethanol: water and 70: 30 v/v acetone: water was employed as negative control to verify its antimicrobial action in the different strains studied. The assay was carried out in triplicate. The plates inoculated were incubated at 37°C for 24 hours. After incubation, the antimicrobial activity was determined by measuring the inhibition zone (clear zone) around each paper disk by means of a digital caliper. All measures included the disk diameter.

Results and Discussion

The antibacterial activity of leaf extracts was measured using agar disk diffusion assay. Various degrees of inhibition against bacterial strains were shown by leaf extracts and the results were given in Table 1. *B. cereus* was found to be very resistant because there was no inhibition in all the extracts studied. On the other hand, *S. aureus* was more sensitive to the extracts evaluated in comparison with the other strains of bacteria used in the test. Nevertheless, AB extracts with 50: 50 v/v ethanol: water significantly inhibited the growth of *S. sp*.

The differences in the antibacterial activity of these extracts could be due to the fact reported by several authors, who observed that Gram positive bacteria is more sensitive than Gram negative bacteria [20] because the latter has an extra protective outer membrane of peptidoglycan, therefore they are usually considered more resistant to antibacterial agents than their Gram positive counterparts [18,20, and 21]. In addition, the extracts of these leaves are rich in phenolic compounds [16,18,19, and 22]. In this sense, the phenolic compounds from different plant sources could inhibit various foodborne pathogens and there may be relationships between the antibacterial activity and the chemical structures of phenolic compounds. Also, the phenolic compounds have aromatic nuclei containing polar functional groups (e.g. hydroxyl groups -OH). The presence and position of the hydroxyl groups in phenolic compounds might influence their antimicrobial effectiveness. Hence, the higher the hydroxylation, the greater the toxicity [23]. The hydroxyl groups are highly reactive under aqueous conditions and react with several biomolecules, causing deformation of these molecules, which results in retardation of bacterial growth [24]. The potentially antimicrobial mechanisms of phenolic compounds include the interruption of the function of bacterial cell membranes [20] which could cause considerable morphological alteration and damage to the treated bacteria so as to exert their bacteriostatic or bactericidal effect [23]. In this manner, the native leaf extracts studied, rich in antioxidants compounds [19,22], have an inhibitory effect against most of the strain bacteria analyzed. It was shown that extract obtained from natural sources can prevent food decay and the growth of foodborne pathogens, and also can help to increase the storage shelf life of food products [25].

Conclusion

These preliminary results suggest that the native leaf extracts evaluated in the present study have antimicrobial potential, especially against *Staphilococcus* strains. In previous studies, it has been determined the antioxidant potential of these extracts, so they could be used as natural preservatives capable of extending shelf life and improving food safety by controlling foodborne pathogenic in fresh

Table 1: *In vitro* antimicrobial activity of Algarrobo blanco, Tusca and Mistol leaf extracts.

	***In vitro* antimicrobial activity**						
Microbial strain/ Antimicrobial	***Salmonella***	***Bacilus cereus***	***Staphylococcus aureus***	***Staphylococcus spp***	***Escherichia coli***	***Micrococcus luteus***	***Listeria innocua***
A	+	-	++	-	-	-	-
B	-	-	++	+++	-	-	-
C	+	-	++	-	-	-	-
D	-	-	++	-	-	++	+
E	+	-	++	-	-	-	-
F	-	-	++	-	+	-	++
C1	-	-	-	-	-	-	-
C2	-	-	-	-	-	-	-
CIP	++	+++	+++	+++	+++	+++	+++

References: -: no inhibition; +: low inhibition; ++: half inhibition; +++: high inhibition. A: Algarrobo blanco 70: 30 v/v acetone: water; B: Algarrobo blanco 50:50 v/v ethanol: water; C: Tusca 70: 30 v/v acetone: water; D: Tusca 50:50 v/v ethanol: water; E: Mistol 70: 30 v/v acetone: water; F: Mistol 50:50 v/v ethanol: water; C1: 50:50 v/v etano: water; C2: 70:30 v/v acetone: water; CIP: Ciprofloxacina.

products. There are plans to carry out this study with microbial reference cultures and also to determine the toxicity of these extracts so as to rule out possible harmful effects for the human organism.

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