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Research Article

Antimicrobial Activities of Leaves Extracts of Mistol (*Ziziphus mistol*), Algarrobo blanco (*Prosopis alba*) and Tusca (*Acacia Aroma*) to be Applied to Meat Products Preservation

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Abstract

The aim of the present study is to analyze the in vitro antioxidant activity against pathogens such as *Salmonella*, *Staphylococcus aureus*, *Staphylococcus sp*, *Bacillus cereus*, *Micrococcus*, *Escherichia coli* and *Listeria innocua* using *Ziziphus mistol* (Mistol), *Prosopis alba* (Algarrobo blanco) and *Acacia aroma* (Tusca) extracts with 70% acetone and 50% ethanol as solvents extraction as antibacterial. Determination was made by agar disk diffusion method. All the 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 strains of bacteria used in the test. Nevertheless, Algarrobo blanco extracts in 50% ethanol significantly inhibited the growth of *Staphylococcus sp*. Therefore, extracts Mistol, Algarrobo blanco and Tusca could have potential as natural preservatives for fresh meat products.

INTRODUCTION

In the past numerous native populations distributed by different parts of the world had as a base of survival the diverse products that extracted from the native forests, which contributed to popular knowledge up to the present day [1].

Santiago del Estero belongs to the Great American Chaco, rich in vegetation which is characterized by having several natives spices such as Algarrobo blanco (*Prosopis alba*), Tusca (*Acacia aroma*) and Mistol (*Ziziphuz mistol*) [2] among others, that are attributed different properties such as medicinal, antioxidant and antimicrobial [1]. Algarrobo blanco develops naturally in argentinian Chaco and it is a spice with a lot of chemicals compounds in their different tissues, so it could be considered an interesting source of natural preservatives and additives with antioxidants capacity [3]. Mistol is a spinipherous fruit tree, sweet and edible, it is native from center- north of Argentina, characteristic of Dry Chaco, which has leaves rich in phenolic compounds [4]. On the other hand, Tusca is a native spice from Tucumán, Santiago del Estero, Salta, Catamarca, La Rioja, Formosa, Chaco, Córdoba, San Luis and Santa Fé, in Argentina [5] and like the previous ones, its leaves, stems and flowers are rich in phenolic compounds.

The use of plants is an economic and very important source of obtaining vegetal extracts with antimicrobial activity [6]. There have been numerous investigations in medicinal and edible plants regarding the obtaining of bioactive compounds [7-9]. The demand for fresh product minimally treated is increasing [10] and consumers prefer products with the fewest additives and preservatives [11-13] which in addition to their nutritional function provide other health characteristics such as functional foods. Due to this, it has been increasing the use of natural antimicrobial agents [10] derived from animal, plants and microbial sources [14]. In addition, many synthetic preservatives such as butylated hidroxylanisole (BHA), butylated hidroxytoluene (BHT) and tertiary butyl hidroquinone (THBQ) have been successfully used to prevent the spoilage in meat products [15]. However, the use of synthetic antimicrobial agents is currently questioned, which had led to the need to seek conservation alternatives [10] and the industry has been

replacing artificial by natural preservatives generating a trend in the food industry [16].

Antimicrobials are used in food for two main reasons: to control natural spoilage process (food preservation) and to prevent or control growth of microorganisms, including pathogenic microorganisms (food safety) [14]. Because of this, microbial growth during storage can be reduced by applying antimicrobial agents to be meat products, leading to a retardation of spoilage, extension of shelf life and maintenance of quality and safety [17].

As opposed to synthetic compounds, natural preservatives are generally obtained from plants [18] rich in phenolic compounds with antimicrobial and antioxidant properties [19,10] and can enhance overall quality through the decrease in microbial growth and also the lipid oxidation, thanks to the antioxidant activity of this phenolic compounds [18]. In addition to this, being obtained from natural sources would be considered safe for those who consume it [10].

The aim of the present study was to determine the in vitro antimicrobial activity of Mistol, Algarrobo blanco and Tusca leaves using 70% acetone and 50% ethanol as solvents to be used as additives in foods products, obtaining added value to these native and abundant species from Santiago del Estero, Argentina.

MATERIALS AND METHODS

Plant materials

Leaves of Mistol (*Zizhipus mistol*), Algarrobo blanco (*Prosopis alba*) and Tusca (*Acacia aroma*) were collected from the Experimental Annex Field "Francisco Cantos" of the Experimental Agricultural Station of Santiago del Estero of INTA.

The extracts were obtained at the Chemical Laboratories of the National University of Santiago del Estero (UNSE) and the antimicrobial activity was determined in the Pilot Plant of Food Processing of UNSE.

Leaves were collected during November and December, and a pool of this leaves (three plants of each spices) were analyzed in a sample composed by species. They were harvested green and dried at room temperature in closed rooms, then packed and frozen until use.

Preparation of extracts

0,1g of plant material was used and 10ml of 1% extract was prepared using the following solvents: 50% ethanol and 70% acetone. The extracted material was subjected to ultrasounds periods in a cooled water bath every 15 minutes for 1 hour using ultrasound equipment (Branson 2210 R- MT Ultrasonic Cleaner, USA). Subsequently, the residue was separated from supernatant and the latter was centrifuged at 3000 grams per minute for 15 minutes at room temperature. The supernatant obtained was transferred to a 10 ml flask and made up. The extracts were stored in a refrigerator in opaque bottles 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 leaves 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*). All strains used are well known food- borne bacterial pathogens in meat products. The strains were isolated and identified in the Chair of Microbiology, Department of Food Sciences of the Faculty of Agronomy and Agroindustries of the National University of Santiago del Estero.

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 ten ml liquid medium and the inoculated media were incubated at 37°C for 24 h to obtain cells in the exponential phase.

Agar disk diffusion method: Antibacterial activity of ethanolic and acetonic extracts was determined using Gram positive strains (*Staphylococcus sp., Staphylococcus aureus, Listeria innocua, Bacillus cereus and Micrococcus luteus*) and Gram negative strains (*Salmonella and Escherichia coli*). The antibacterial activity was evaluated by agar disk diffusion method described by [19] 100µl aliquot of active bacteria containing $>10^6$ cfu/ml (measured with the Mc Farland scale) was spread into the surface of BHI agar to create a microbial lawn and then left to dry. Sterile filter paper disks (6mm diameter) were impregnated with 20µl of each extracts and left to dry before being placed on each inoculated agar. A disk of ciprofloxacin with 5µg was used as positive control and disks with 20µl of 70% acetone and 50% ethanol were 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 h. 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 leaves extracts was measured using agar disk diffusion assay. Various degrees of inhibition against bacterial strains were shown by leaves extracts and the results were given in Table 1. From the results, it was observed that *Bacillus cereus* (Gram- positive bacteria) was found to be very resistant because there was no inhibition in all extracts studied. On the other hand, *Staphylococcus aureus* was more sensitive to the extracts evaluated in comparison with the other strains of bacteria used in the test. Nevertheless, Algarrobo blanco extracts in 50% ethanol significantly inhibited the growth of *Staphylococcus 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 [18], because the latter has an extra protective outer membrane of peptidoglycan, they are usually considerably more resistant to antibacterial agents than their Gram positive counterparts [20,19,5]. In addition, the extracts of these leaves are rich in phenolic compounds [3-5,13], also reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens and there may be relationships

Table 1: In vitro Antimicrobial Activity of leaves of Mistol, Tusca and Algarroboblanco extracts.

	In vitro antimicrobial activity						
Microbial strains/ 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: *Prosopis alba* 70% acetone; B: *Prosopis alba* 50% ethanol; C: *Zizhypuz mistol* 70% acetone D; *Zizhypuz mistol* 50% ethanol; E: *Acacia aroma* 70% acetone; F: *Acacia aroma* 50% ethanol; C1: 50% ethanol; C2: 70% acetone; CIP: Ciprofloxacin.

between the antibacterial activity and the chemical structures of de phenolic compounds. Also, the phenolic compounds have aromatic nucleus 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, in this way, the higher the hydroxylation, the greater the toxicity [13]. 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 [21]. Phenolic compounds are also involved in protein and cell wall binding inactivation of bacterial enzymes and intercalation into the bacterial DNA during replication (Brooks, Butel and Morse, 1998; Pelczar, Chan and Krieg, 1988 cited by [18]. In this way, the potentially antimicrobial mechanisms of phenolic compounds include the interruption of function of bacterial cell membranes [18], which could cause considerable morphological alteration and damage of the treated bacteria so as to exert their bacteriostatic or bactericidal effect [13] This explains why vegetal extracts studied to be rich in antioxidant compounds [4,5], have an inhibitory effect against most of the strain bacteria analyzed. [22], have shown that extract obtained from natural sources can prevent food decay and the growth of food borne pathogens, and also can help to increase the storage shelf lives of foods.

CONCLUSION

These preliminary results suggest that the extracts obtained under the conditions mentioned in the present study could have antimicrobial effects on certain bacteria, nevertheless it is necessary to deepen these studies in order to stablish the suitability of these extracts as possible preservative natural additives to improve food safety by control of food borne pathogenic bacteria, extending the shelf life of fresh meat products. In view of the use of these extracts in foods, it is planned to carry out this study with microbial reference cultures (MRC) and to determine the toxicity of these extracts to rule out possible harmful effects for the human organism.

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