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Antibacterial effect against foodborne bacteria of plants used in traditional medicine in central Mexico: Studies in vitro and in raw beef

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ABSTRACT

The antimicrobial effect of plant species used in traditional medicine in Hidalgo State, Mexico, against foodborne bacteria was evaluated in vitro and in raw beef. Plant extracts produced with water, ethanol, methanol, acetone, hexane and ethyl acetate were applied against fourteen foodborne bacteria by agar diffusion technique. For raw meat test, only the antibacterial effect of a mixture of water and methanol extracts of *Artemisia absinthium* was evaluated against *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* at 5 ± 2 °C. Only *Chelidonium majus*, *Dendropanax arboreus*, *Eucalyptus globulus* and *A. absinthium* exhibited an antimicrobial effect against at least two of the tested microorganisms. The *E. globulus* and *A. absinthium* extracts had an antimicrobial effect against eight of the tested bacteria. *L. monocytogenes*, *S. typhimurium* and *E. coli* O157:H7 multiplied on the pieces of meat untreated with a mixture of *A. absinthium* extracts. However, none of the tested microorganisms were capable of multiplying on the pieces of meat treated with the extract mixture; indeed, concentrations for all the pathogens decreased steadily over time. Treatments with *A. absinthium* extracts could be a way of controlling some foodborne microorganism pathogens in raw meat.

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1. Introduction

Foodborne disease is a worldwide public health challenge. Every country faces a different form of this challenge, but it is generally more accentuated in developing countries such as Mexico. A wide spectrum of pathogenic bacteria has been implicated in foodborne disease outbreaks around the world (CDC, 2009; Harris et al., 2003). In developing countries such as Mexico, the main pathogens reported are *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Listeria monocytogenes,* with the Diarrheagenic *Escherichia coli* pathotypes (DEP) coming into prominence recently (Aguilar, Giono, & Costarrica, 1983; Castillo, Villarruel-López, Navarro-Hidalgo, Martínez-González, & Torres-Vitela, 2006; Castro-Rosas et al., 2011, 2010; Estrada-Garcia et al., 2009; Moreno-Enriquez et al., 2007; Parrilla-Cerrillo, Vázquez-Castellanos, Saldate-Castañeda, & Nava-Fernández, 1993; Secretaria de Salud, 2012).

Refrigeration is often the principal, and sometimes the only, factor relied upon to control foodbome pathogens in different foods. Hence, temperature abuse of such foods could lead to foodbome illness. In addition, some psychrotrophic pathogens can grow in refrigerated foods with little or no obvious change in sensory quality (Berrang, Brackett, & Beuchat, 1989a,b). Using antimicrobial ingredients may provide an additional barrier protection beyond low temperature alone. However, consumers often oppose the inclusion of artificial or chemical additives (Ray, 1992). In addition, a number of studies have shown that chemical antimicrobials commonly used to inhibit or eliminate pathogenic microorganism development are limited or ineffective (Castro-Rosas & Escartín, 1999). They can also lead to negative effects in the consumer; for instance, the nitrites widely used in some meat products (Varnam & Sutherland, 1998). In response, various studies are now in progress searching for alternative antimicrobials from plants. Antimicrobial substances are commonly identified in different plant parts such as leaves, stems, seeds, fruit and roots. Some

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of these substances are elements in the fluid or tissues of plants whereas others are generated in response to infection and/or environmental stress. Over 1300 plant species are reported to produce chemical compounds with antimicrobial activity, many of which have been isolated and are used in many fields, including the food industry (Burt, 2004; Tajkarimi, Ibrahim, & Cliver, 2010).

A promising source of antimicrobials with potential uses in the pharmaceutical and food industries are the thousands of plant species used in traditional medicine (Grosvenor, Supriono, & Gray, 1995; Nimri, Meqdam, & Alkofahi, 1999; Saxena, 1997; Saxena & Sharma, 1999; Silva, Duarte, Cabrita, & Gomes, 1996). Of the approximately 3500 plants reportedly used in traditional medicine in Mexico, close to 800 are commonly used by the people of Hidalgo State, Mexico, to treat a range of health problems, as gastrointestinal diseases. However, most of the plant species used in traditional medicine in Mexico, including those used in Hidalgo, have not been evaluated to identify and confirm their possible antimicrobial effect. Some of the plants used in traditional medicine in Mexico may contain chemical compounds with antimicrobial activity against foodborne pathogens. If these compounds can be identified, the plants containing them could be used as a source of antimicrobials for use in food applications.

Identifying novel and acceptable means of ensuring the safety of refrigerated foods, as raw meat, is an important issue to the food industry. Using naturally occurring plant products to control foodborne pathogens may be one solution to this problem. Thus, the use of such naturally occurring plant products may provide an attractive potential barrier to the growth of foodborne pathogens in raw meat. However, most studies on antimicrobial activity of plant extracts have been conducted in vitro using microbiological media. Little information exists regarding the use of such antimicrobial extracts in foods. The effects of plant extracts in foods may not be the same as in test media since the microenvironments in foods are very different from microbiological media. Therefore, studies are needed to determine the efficacy of plant extracts in foods. The present study objective was to evaluate the antimicrobial effect of thirty plant species used in traditional medicine in Hidalgo State, Mexico, against pathogenic bacteria in vitro and in raw beef.

2. Materials and methods

2.1. Plants

Thirty plants used in traditional medicine in Hidalgo were analyzed (Table 1). Analysis was done only of those parts of each plant commonly used and to which medicinal effects have been attributed (Pérez, Villavicencio, & Ramírez, 2003). Fresh samples (500 g) of these parts were purchased at different public markets throughout Hidalgo. Samples were handled under aseptic conditions and transported to the laboratory on the day of purchase. They were dehydrated in an oven at 40 ± 2 °C for 7 days, placed in sterile plastic bags and stored at room temperature until use.

2.2. Extract production

2.2.1. Aqueous

Samples (25 g) of each dried plant part (Table 1) were aseptically weighed and placed in separate sterile glass flasks. Distilled water (225 mL) was added to each flask, the flasks heated to boiling for 10 min and allowed to cool to room temperature.

2.2.2. Methanol, ethanol, acetone, hexane and ethyl acetate

Samples (25 g) of each dried plant part (Table 1) were taken. These were aseptically weighed and placed in separate sterile glass flasks. Depending on the treatment, 225 mL 96% ethanol (Sigma–Aldrich, Mexico); 225 mL methanol (Sigma–Aldrich, Mexico); acetone (Sigma–Aldrich, Mexico); hexane (Sigma–Aldrich, Mexico); or ethyl acetate (Sigma–Aldrich, Mexico) were added to each flask. The flasks were hermetically sealed and stored at room temperature for three days with manual agitation once daily. After this extraction period, the liquid phase was filtered through Whatman No. 4 filter paper and the filtered extracts concentrated in a rotary evaporator (BÜCHI, Vacuum Controller, V-800). Solvents were completely eliminated from the concentrates by placing them in a recirculating air incubator (Lab-Line, Ambi-Hi-Low Chamber, USA) for 24 h at 50 ± 2 °C. A decimal dilution was prepared from these concentrates using water for the methanol and ethanol extracts, and a solution of tween 80 (Sigma–Aldrich, Mexico)-distilled water (1:10 v/v) for the acetone, hexane and ethyl acetate extracts (final extract concentration: 100 mg/mL).

2.3. In vitro antibacterial activity assays

2.3.1. Bacterial strains

Fourteen bacterial strains were used: *Salmonella typhimurium* (ATCC 14028); *Salmonella choleraesuis* (ATCC 10708), *L. monocytogenes* (ATCC 19115); *Staphylococcus aureus* (ATCC 25923); *Staphylococcus epidermidis* (ATCC 12228), *Shigella flexneri* (ATCC 12022); and *Shigella sonnei* (ATCC 25931), *Vibrio parahaemolyticus* (ATCC 17802), *Pseudomonas aeruginosa* (ATCC 27853). *E. coli* O157:H7 (E09; donated by E.F. Escartín of the Universidad Autónoma de Querétaro, México), three Diarrheagenic *E. coli* pathotypes (DEP): enterotoxigenic (1620 TL); enteropathogenic (52GM 291) and enteroinvasive (4VC81-5), (all these DEP were donated by Dr. F. Cerna-Cortes of the Instituto Politécnico Nacional, México); and *Vibrio cholerae* O1 (87151, serotipe Inaba; donated by E.F. Escartín). Rifampicin resistance (R+) was induced in all strains (Castro-Rosas et al., 2010). The R+ strains were kept in inclined trypticase soy agar (TSA; Bioxon, *Becton Dickinson,* México) tubes at 3–7 °C with monthly transfers over TSA. All strains maintained rifampicin resistance throughout the study.

2.3.2. Inoculum preparation

Tubes containing 3 ml trypticase soy broth (TSB, Bioxon, *Becton Dickinson,* Mexico) were inoculated with one of the tested bacterial strains and incubated at 35 °C for 18 h. The cultures were washed twice in sterile isotonic saline solution (ISS; 0.85% of NaCl) by centrifuging at 3500× *g* for 20 min, and resuspending the pellets in sterile peptone water at about 10^9 CFU/ml. A decimal dilution of these washed cultures was done with ISS to produce a final approximate concentration of 8 log CFU/ml.

2.3.3. Diffusion technique

A 100 μL sample was taken from the first dilution of each washed bacterial culture, inoculated onto TSA plates containing 100 mg/L rifampicin (Rif; Sigma–Aldrich St. Louis, MO, USA) and distributed over the agar using the surface extension technique. Filter paper (Whatman No. 5) discs (5 mm diameter) were placed on the surface of each agar plate. Aliquots (10 μL) of each extract were then placed on each disc (final doses per disc: 1 mg extract), including isotonic saline solution (ISS) as a negative control. Four replicates were done per extract. Discs containing chloramphenicol (25 μg) were used as positive controls. Once the extracts were absorbed by the agar, the plates were incubated for 24 h at 35 ± 1 °C. The diameter (mm) of any resulting inhibition zones was measured and average diameter values calculated for each extract.

2.3.4. Minimal inhibitory concentration (MIC)

Estimation of the MIC for each extract was done by the broth dilution method (Vanden & Vlietinck, 1991). Dilutions of plant extracts from 100 to 1 mg/ml were used. Tubes were inoculated with

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