

Formulation and conservation of a pharmaceutical form with leaf extracts from *Acacia aroma* Gill. ex Hook et Arn.

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Abstract

Leaf fluid extracts of *Acacia aroma* GILL. ex Hook et Arn showed antibacterial activity against antibiotic multi-resistant bacteria isolated from clinical samples, antioxidant and anti-inflammatory activities. Toxicological studies carried out on *Artemia salina* and *Allium cepa* attested none toxicity potential. The aim of this work was to elaborate a formulation of topical antibacterial hydrogel with Carbopol acrylic acid polymer containing an *A. aroma* fluid extract in order to compare with a hydrogel containing commercial antibiotic. The optimal extract concentration in this formulation was determined according to the values of minimal inhibitory concentration and minimal bactericidal concentration for *Staphylococcus aureus*, methicillin-resistant (F7) and *Pseudomonas aeruginosa* (F352). Physical, chemical, rheological and microbiological stability was observed at least during one year. The hydrogel containing *Acacia* leaves fluid extract shows remarkable antibacterial effect with a broad-spectrum efficacy against Gram positive and Gram negative bacteria at low concentration.

Acacia aroma Gill. ex Hook et Arn, common name tusca (Burkat, 1952), a native species of Argentina, is member of genus *Acacia* subgenus *Acacia*, widely distributed in the provinces of Tucumán, Salta, Santiago del Estero, Catamarca, La Rioja, Formosa, Chaco, Córdoba, San Luis and Santa Fe. This plant is used in Argentine folkloric medicine as wound healing, antiseptic and for the treatment of gastrointestinal disorders. Leaves and bark infusions have diuretic, anti-inflammatory and cicatrizant uses. Its fruits are important components in the diet of the animals living in this area. In a previous work (Arias et al, 2004), we described antibacterial activity of *Acacia aroma* extracts against antibiotic resistant Gram positive cocci species and Gram negative bacteria. Otherwise, the extracts were not found to be cytotoxic or genotoxic (Arias et al, 2002). The acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants.

The purpose of the present study was to prepare a

pharmaceutical formulation containing *Acacia* extracts as antibacterial and antioxidant agent. For the present investigation, different hydrogel types were prepared using an optimal stabilizer combination of hydrogel-forming poly (acrylic acid) polymer (Carbopol® 974) and antibiotic agent (fluid extract or gentamicin sulphate) without preservatives. Macroscopical and microscopical examination supported that the hydrogel formed was homogeneous in aspect and particle size. Figure 1 shows the changes in viscosity with increasing shear rate for hydrogel containing leaves fluid extract. The rheological properties of hydrogel was not affected during one year (Fig 2). Otherwise, a pseudoplastic behavior was observed.

Control of microbial contamination in each pharmaceutical formulation was done. The preparation containing leaves fluid extract were microbiologically stable during one year at room temperature as well as hydrogel with commercial antibiotic. Susceptibility test using agar well diffusion method indicate that preparations were active against antibiotic-multi-resistant Gram negative and Gram positive bacteria as well as the fluid extract of *Acacia* leaves.

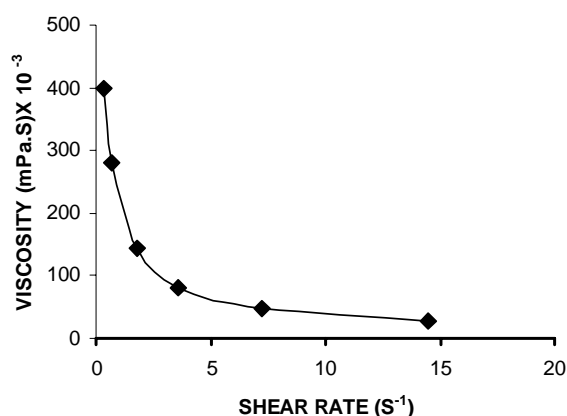


Figure 1. Apparent viscosity values of hydrogel containing *Acacia* fluid extract as a function of shear rate.

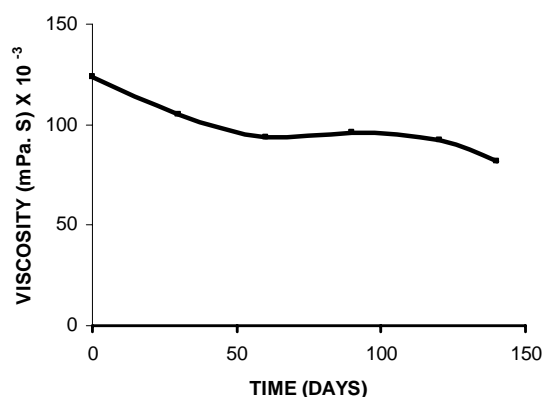


Figure 2. Apparent viscosity values of hydrogel containing *Acacia* fluid extract as a function of stored time.

The hydrogel containing *Acacia* leaves fluid extract shows antibacterial effect against Gram positive and Gram negative bacteria isolated from clinical sample of a Hospital of Tucumán, Argentina and microbiological, chemical, physical and rheological stability, compatibility with the formulation components, heat stability. Furthermore, the addition of antioxidant as preservatives in order to store the pharmaceutical formulation was not necessary. This formulation is a promising topical antibacterial pharmaceutical form against *S. aureus* and *P. aeruginosa*.

Material and Methodos

Plant material: The plants used for the present study were collected from September to March in Trancas, Tucumán, Argentina. For future reference, voucher specimens were deposited in the Herbarium of the Instituto de Estudios Vegetales (IEV), Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina). The parts used were leaves.

Preparation of *Acacia aroma* extracts: Lixiviation (fluid extract): ground air-dried plant material (leaves) was extracted with 60% ethanol (1g dry tissue / 1 ml 60% ethanol), using a percolator apparatus at room temperature until total extraction (Farmacopea Argentina, 1978).

Gel preparation: Aqueous dispersions containing only 1.0% (W/W) Carbopol® 974 acrylic acid polymer (BF Goodrich) or Carbopol containing 0,1% gentamicin sulphate or Carbopol containing 10% fluid extract were prepared by dispersing the required amounts of each component in 100 ml of water under stirring (2000 rpm). Polymer dispersions after 1 h were neutralized with triethanolamine until the desired apparent pH (pH= 7) measured in situ with a digital Orion pHmeter. The systems were left to stand for 24h at room temperature prior to the evaluation of their rheological properties. The hydrogels were stored at room temperature and analyzed each month during one year. The fluid extract concentration used in these preparation was determined according to the minimal inhibitory concentration (MIC) determined by agar macrodilution method and the minimal bactericidal concentration (MBC) determined by broth microdilution method (Arias et al, 2004). The strains assayed were *Staphylococcus aureus*, methicillin - resistant (F7) and *Pseudomonas aeruginosa* (F352). The strains were recovered from clinical samples obtained from the Hospital Nicolás Avellaneda, San Miguel de Tucumán, Tucumán, Argentina. The MIC values were 125 to 250 µg.ml⁻¹. The following parameters were determined: homogeneity (Lupa), particle size (optical microscope), pH (pHmeter) and extensibility

Rheograms were determined with a cone and plate Brookfield Digital Viscometer, equipped with a recirculating water-bath Brookfield for control of sample-container temperature. The viscosity determination were done in 0.5 ml of sample, sequentially increasing and decreasing values

of shear rate in the range 0.05-20 r.p.m. The temperature was kept constant at 26±0.5°C and all samples were equilibrated at run temperature on the plate for 5 min prior to viscosity measurement. The material was monitored as function of stored time.

Microbiological assays:

- The number of UFC (colony forming units) were determined in each preparation maintained at room temperature during one year (Farmacopea Española, 1999). The samples were analyzed monthly.
- Freshly samples of hydrogel preparations (1g) were inoculated with 10⁶ UFC of *Staphylococcus aureus*, methicillin - resistant (F7) or *Pseudomonas aeruginosa* (F352). Aliquots of the formulations were taken at different times from inoculation. Then, the number of UFC was determined each 15 days during one year (Farmacopea Española, 1999).
- Agar-well diffusion method**
Petri dishes (9 cm in diameter) were prepared with 10 ml of a base layer of Müller-Hinton agar medium (MHA, Laboratorio Britania, Argentina) and a top layer (3ml) of 0.2% BHI agar medium inoculated with 30 µl of each bacterial suspension (10⁵ bacteria x ml⁻¹). After drying, 0.1 g of each hydrogel preparation, 10 µl of fluid extract or 40 µg of gentamicin sulphate were placed in each well. The dishes were incubated at 35°C for 16-20 hours. The antibacterial activity was expressed as the mean of inhibition diameters (mm) produced. All tests were performed by duplicate.
- The minimal inhibitory concentration (MIC) was determined by agar macrodilution method and broth microdilution method (NCCLS, 2002).

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