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Antibacterial activity of ethanolic and aqueous extracts of *Punica granatum* L. bark

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ABSTRACT

For thousands of years, *Punica granatum* L. has been used in traditional medicine all over the world and predate the introduction of antibacterial drugs. The aim of the present study was to investigate the antibacterial activity of aqueous and ethanolic extracts of *Punica granatum* L. bark obtained by decoction and maceration. The different extracts of *Punica granatum* L. (Lythraceae) bark have been tested for antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus stearothermophilus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) by disc diffusion method. The ethanolic macerate extract showed the strong *in vitro* antibacterial activity against *Pseudomonas aeruginosa* with zone inhibition of 24.4 mm. However, the results tests by disc diffusion method revealed the effectiveness of ethanolic decoctate against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus stearothermophilus*) with diameter zone of inhibition varying with 21.1mm and 23.75 mm respectively.

Keywords: *Punica granatum* L. bark, antibacterial activity, maceration, decoction.

INTRODUCTION

The steadily increasing bacterial resistance to existing drugs is a serious problem in antibacterial therapy. *Staphylococcus aureus* is an example of bacterial resistance serial and is considered as the principal contaminant of clinical infections. Recently, the acceptance of traditional medicine as an alternative form for health care and the development of bacterial resistance to the available antibiotics has led authors to investigate the antibacterial activity of medicinal plants (Scrivanasan, 2001; Kumarasamy, 2002; Ali, 2001; Masika and Afolayan, 2002; Hamill, 2003; Shah, 2005; Lahlou, 2009). Plants and plant derived agents have long history to clinical relevance as source of potential chemotherapeutic agents (Cushnie *et al.*; 2005). Thousands of plant species have been screened for their antimicrobial activity, but relatively few were found to be sufficiently active (Poyart-Salmeron, 1990; Meng, 2000) and non toxic to humans (Izzo, 2004). The tree of *Punica granatum* L. (Lythraceae) is extensively abundant in South-West of Algerian Sahara. The different parts of this plant such as flowers, seeds and bark have been employed against inflammatory and infectious pathologies. The purpose of the present study was to investigate the antibacterial activity of bark extracts of *Punica granatum* against Gram-positive and Gram-negative bacteria. The selected bacteria were antibiotic resistant or multi-resistant human pathogens. The extracts with the highest antibacterial effectiveness were chosen for subsequent use in pharmaceutical formulations.

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MATERIALS AND METHODS

Plant material

The plant used for the present study was collected in September 2010 in Timimoun area, Adrar Department, Algeria. The bark was separated from fruits and dried at room temperature for 12 days. The dried bark was milled to a fine powder in an electrical mill and stored in the dark at room temperature in closed containers until required.

Preparation of *Punica granatum* extracts

Maceration: About 5 g of powdered material was macerated with 20 ml of ethanol for 30min. The ethanol extract was filtered using whattman filter paper and then concentrated under vacuum at 65°C using a Rotary Evaporator. The residue obtained was stored at 4°C prior to analysis.

Decoction: Dried and powdered plant material 5 g was boiled with 50 ml of water for 30 min. After cooling, the mixture was filtered and stored to 4°C prior to analysis.

Similar protocols were used for preparation of ethanol decoctate and aqueous macerate.

Microorganisms and media

The antibacterial activity of ethanol and aqueous extracts of *Punica granatum* bark were evaluated using the following strains of bacteria, Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25922), *Bacillus stearothermophilus* (ATCC 11778), Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853). These bacterial strains were obtained from the Pasteur Institute, Algiers, Algeria.

The strains were identified by the use of Biochemical profiles according to the recommendations of the Manual of Clinical Microbiology (Murray *et al.*; 1999). All organisms were maintained in brain-heart infusion (BHI medium) containing 30% (v/v) glycerol at -20°C. Before testing, the suspensions were transferred to trypticase soy agar supplemented with 5% of sheep blood (Difco) and aerobically grown overnight at 35°C. Individual colonies were isolated and suspended in 5 ml of 0.9% NaCl solution. The inocula were prepared by adjusting the turbidity of the suspension to match the 0.5 McFarland standard and diluted in CAMHB (cation - adjusted Mueller–Hinton broth) in order to achieve the adequate inoculum in each case.

The cell number in CAMHB was estimated using a serial dilution technique (NCCLS, 2002) for each assay.

Disk diffusion method

Petri dishes (90 cm in diameter) were prepared with 10 ml of a base layer of Mueller–Hinton gelose medium (MHG) and inoculated with 100 µl of each bacterial suspension (velickovic *et al.*; 2003). After drying in a sterile hood, 6 mm diameter disks soaked with aqueous decoctate 5.5 µl; ethanolic decoctate 5.8 µl; aqueous macerate 5.4 µl and ethanolic macerate 5.7µl. The different extract dilutions were placed on the gelose. The dishes were incubated at 35°C for 24hours. All tests were performed in

duplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced.

RESULTS AND DISCUSSION

The results of the disk diffusion test indicated that crude ethanol and aqueous extracts of *Punica granatum* bark showed different degrees of growth inhibition, depending on the bacterial strains (Tables 1 and 2). The aqueous and ethanol decoctate extracts showed the broadest antibacterial activity by inhibiting growth of all bacterial strains tested (the diameter of inhibition zone, 15.85-22.85 mm and 21.30-23.75 mm respectively). Of all ethanol and aqueous macerate extracts tested, the aqueous macerate extract showed the highest antibacterial activity against some strains, such as *Pseudomonas aeruginosa* (24.4mm), and *Escherichia coli* (23.3mm; 23.85 mm) respectively for aqueous and ethanol macerates.

Table 1: Antibacterial activity of the ethanolic and aqueous decoctates of *Punica granatum* bark by disc diffusion method.

Microorganisms	Aqueous decoctate	Ethanolic decoctate
	Inhibition zone (mm)	Inhibition zone (mm)
Gram-positive bacteria		
<i>Staphylococcus aureus</i>	19.00	21.30
<i>Bacillus stearothermophilus</i>	15.85	23.75
Gram-negative bacteria		
<i>Escherichia coli</i>	20.40	22.10
<i>Pseudomonas aeruginosa</i>	22.85	22.75

Table 2: Antibacterial activity of the ethanolic and aqueous macerates of *Punica granatum* bark by disc diffusion method.

Microorganisms	Aqueous macerate	Ethanolic macerate
	Inhibition zone (mm)	Inhibition zone (mm)
Gram-positive bacteria		
<i>Staphylococcus aureus</i>	23.05	20.90
<i>Bacillus stearothermophilus</i>	21.20	21.05
Gram-negative bacteria		
<i>Escherichia coli</i>	23.30	23.85
<i>Pseudomonas aeruginosa</i>	24.40	19.45

The disc diffusion bioassay showed that bark decoctate extracts have the highest activity against all Gram-positive bacteria and they also showed good activity against Gram-negative bacteria. The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971).

Based on inhibition zone values, bark aqueous macerate showed to be potent inhibitors against all bacterial strains.

Same results were obtained via the bark ethanolic macerate with different zone diameter of inhibition. These results are in agreement with those reported by Curtay and Jung (2010). They found that *Punica granatum* bark have to be *in vitro* antibacterial activity.

These results suggest that the inhibitory effect exhibited by the macerate and decoctate crude extracts of *Punica granatum* bark may be attributable to the tannins that represent 28% of bark constituents (Blansky and Newman, 2007). This family of compounds has been found *in vitro* to have various pharmacological properties such as antioxidant, antimicrobial and anti-inflammatory (Curtay and Jung, 2010). Furthermore, the observed antibacterial activity may be due also to other secondary metabolites like phenolic compounds and saponins. In general, we observed that the ethanol decoctate extract was more efficacy than aqueous extract because ethanol allowed to extract well the less polar compounds such as terpenic derives (Emmanuel *et al*; 2002).

The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to the evaluation of possible synergism among extract components for their antimicrobial activity. Investigations are in progress to determine the degree of toxicity of these extracts.

CONCLUSION

Extracts of *Punica granatum* L. bark in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria with different diameter zone of inhibition. The broad-spectrum antibacterial activities of the plant extract, possibly due to the secondary metabolites such as tannins, phenolic compounds or saponins that were abundant in this plant. This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antibacterial effect.

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