

Antimicrobial Activity of *Spondias dulcis* Parkinson Extract Leaves Using Microdilution and Agar Diffusion: A Comparative Study

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Abstract

The present study investigates the antimicrobial activity of the hydroalcoholic extract of *Spondias dulcis* Parkinson. Leaves of *S. dulcis* were dried in oven (40°C) and pulverized in grinder. The hydroalcoholic extract was produced using turboextraction, 1:50% (ethanol:water) and concentrated in vacuum using a rotary evaporator. The microorganisms used to microbiology activity were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* sp. using Agar diffusion and microdilution method. The results suggest low activity of extract against Gram-negative microorganism. The extract can use to obtained subfractions and isolated compounds with antimicrobial potential.

Keywords: Antimicrobial Effect; Medicinal Plants; Cajarana; *Spondias dulcis*

Introduction

The natural products are an important strategy to development new potential antimicrobial drugs. Extracts and fractions can use to study antimicrobial activity against different microorganism [1].

Spondias dulcis Parkinson (syn *Spondias cythera* Sonn) belongs to Anacardiaceae family, known as “cajarana”, is a widespread plant in the Northeast of Brazil, plant from Polynesia and is widespread in Latin America and the Caribbean [2]. This specie was used in traditional medicine in the treatment of many diseases, for example, anti-inflammatory and anti-thrombolytic [3].

The phytochemical study suggest presents of flavonoids and tannins [4]. Gunguly, *et al.* [5] isolated three compounds, in special triterpene.

Although there are many studies on antimicrobial activity of medicinal plants, there are few studies that compare the same extract against two *in vitro* techniques. The aim of the present work is to evaluate the antimicrobial activity of 50% ethanolic leaves extract of *S. dulcis* using two different antimicrobial methods.

Material and Methods

Plant extract

Leaves of *Spondias dulcis* Parkinson were collected from the Uiraúna-PB, Brazil (6°30' 55,8" S; 38°24' 48,9" W). The tree was identified from the plant label, the identity was confirmed and voucher specimen (ACAM-999, SisGen A9BC7AC) was deposited at the “Herbário Ar-ruda Camara” - Paraíba State University, Campina Grande-PB, Brazil. The leaf material was air-dried at 40°C in over and milled into a fine powder. Ground material (20% w/v) was extracted with ethanol:water (1:1) using UltraTurrax Ika T-20 model for 20 minutes. The extract was concentrated under vacuum using a rotary evaporator at 40°C and stored under refrigeration at -4°C for later use.

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Antimicrobial activity

The microorganisms used in this study were: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella* sp. (ATCC 19196). All micro-organisms were cultured in Tryptone Soy Broth (TSB) and incubated at 37°C for 24h.

The conventional antimicrobial used in the study: Ampicillin to *S. aureus*, cefalotin to *S. epidermidis* and meropenem to gram negative microorganisms. All antimicrobial was prepared in sterile distilled water, to a concentration of 200 µg/ml.

Two methods were used to study the antimicrobial activity: microdilution [6] and agar diffusion [7].

Microdilution Method

The microbial suspension of the microorganisms were prepared separately from 24h broth cultures (TSB) and incubated at 35°C. These microbial suspensions were adjusted using spectrophotometer in 580 nm to 25% transmittance. This value corresponds to 1×10^8 CFU/mL. The inoculum was prepared with dilution (1:100 ratio) of the microbial suspension yielding an approximate inoculum size 1×10^6 CFU/mL.

The 96-well plates were prepared by adding 100 µL distilled sterile water into each well. Then, 100 µL of the plant extracts (starting stock 64 mg/mL) and antimicrobial control were transferred into the microtitre plate. Serial dilutions were performed, leading to a final volume of 100 µL per well. An amount of 100 µL of the inoculum was added to each well. The plates were incubated at 35°C for 24 hours. After 24 hours, 20 µL of 100 µg/mL of resazurin was added into all wells of the microtitre plates. The color change from blue to pink by visual examination and MIC value was defined as the lowest concentration of drug that prevented a color change (Figure 1A).

Agar Diffusion Method

The microbial suspension of the microorganisms were prepared separately from 24h broth cultures (TSB) and incubated at 35°C. These microbial suspensions were adjusted using spectrophotometer in 580 nm to 25% transmittance. This value corresponds to 1×10^8 CFU/mL.

The tryptone soya agar (TSA) medium was introduced in plastic Petri dishes (90 mm in diameter). The Petri dishes were all oved on the flat slab top for the medium to solidify within 30 minutes. An aliquot of 1% inoculum was spread (5 mL/Petri dishes) on the surface of agar plates through of the TSA and solidify within 5 minutes. The extract (16 mg/mL) and antimicrobial control (100 µg/mL) were additions using templates (100 µL/sample). The plates were incubated at 35°C for 24 hours. The results were recorded as the mean diameter of the zones of growth inhibition surrounding the templates (Figure 1B).

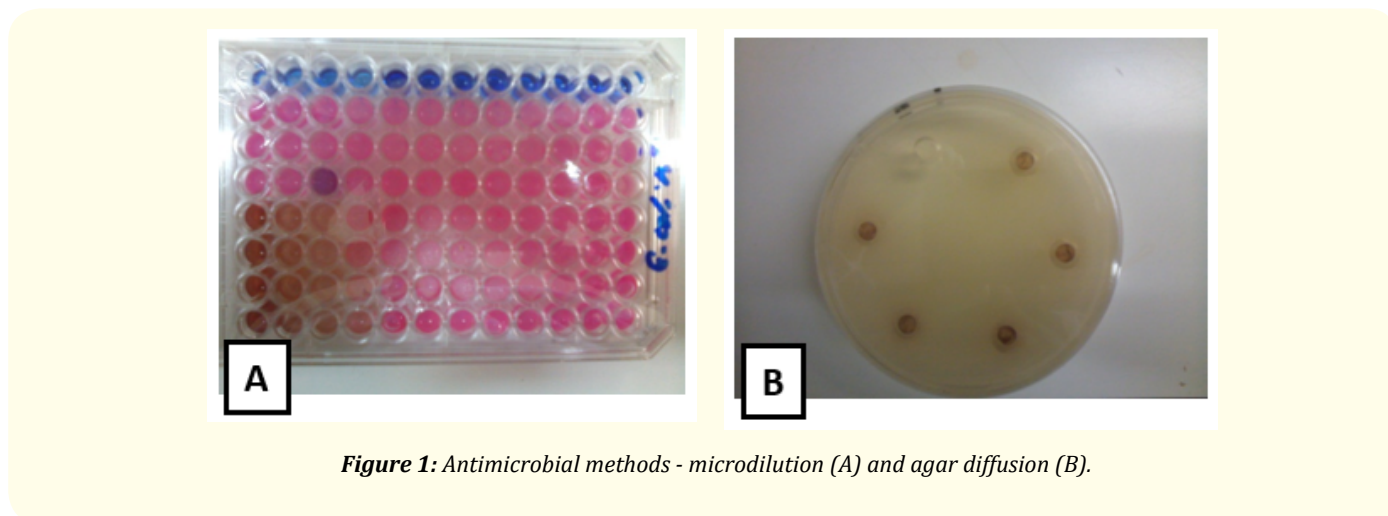


Figure 1: Antimicrobial methods - microdilution (A) and agar diffusion (B).

Statistical analysis

The analyses were carried out in triplicate. The results are expressed as means \pm SD. Microsoft® Excel 2013 software was used to calculate averages and standard deviations. The Nonparametric statistic (Kruskal Wallis test) was analyzed in Past 3.0 software.

Results and Discussion

The present study was conducted to investigate the antimicrobial activity of hydroalcoholic extract of leaves of *S. dulcis* Parkinson using two microbial methods: agar diffusion and microdilution assay.

Agar diffusion was described by Kirby-Bauer in 1940 and based of diffusion of solution in agar by Fick's diffusion theory [6]. In this condition, the microorganism growth in solid medium and the antimicrobial activity were measured by inhibition zone [10].

Microdilution assay was described by Eloff in 1998 [7]. This method used micro plate (for example 96 well microplate) and test solutions were diluted. In this condition, the microorganism was planktonic mode of growth. The inhibition was detected by visual examination, colorimetric methods (for example resazurin) or spectrophotometric analysis [6].

Table 1 provides the antimicrobial results obtained from the two methods. The results suggest low activity of hydroalcoholic extract of leaves *S. dulcis* Parkinson against all microorganisms. The statistical analysis of Agar diffusion suggest difference with Gram-positive and Gram-negative microorganism ($p < 0.05$), except to *S. aureus*/*P. aeruginosa* and *E. coli*/*P. aeruginosa*. The Agar diffusion results suggest other studies with fractions of extracts. Islam., *et al.* [4] studied *S. dulcis* extract (chloroform, methanol and dichloromethane) obtained by leaves and fruits using agar diffusion. The results suggest good antimicrobial activity against *Pseudomonas aeruginosa*. Other *Spondias* (*S. mombin*, *S. tuberosa* and *S. pinnata*) showed antimicrobial activity, in special to Gram-negative microorganism [2].

| | Microdilution assay | Agar diffusion |
|-------------------------|-----------------------|--|
| | MIC mg/mL (\pm SD) | Inhibition zone (mm \pm SD) |
| <i>S. aureus</i> | > 16 | 9.28 \pm 1.05 ^a |
| <i>S. epidermidis</i> | > 16 | 8.09 \pm 0.52 |
| <i>E. coli</i> | > 16 | 9.64 \pm 0.90 ^b |
| <i>P. aeruginosa</i> | > 16 | 10.32 \pm 0.52 ^a |
| <i>Salmonella</i> spp. | > 16 | 10.67 \pm 0.96 ^b |
| Ampicillin ¹ | < 0.1 | 26.82 \pm 1.96 |
| Cefalotin ² | < 0.1 | 22.84 \pm 0.97 |
| Meropenem ³ | < 0.1 | 23.35 \pm 1.46 / 34.75 \pm 0.43 / 32.55 \pm 1.05 |

Table 1: Antimicrobial activity of hydroalcoholic extract of leaves of *S. dulcis* Parkinson using two antimicrobial methods.
^{1,2,3}: Antimicrobial control to *S. aureus*, *S. epidermidis* and Gram-negative microorganism (*E. coli*, *P. aeruginosa* and *Salmonella*), respectively.

^{a,b}: Similar letters indicate not difference between the means (Kruskal-Wallis test with Dunn Post-hoc test).

Some authors classify extracts of plant material on the basis of MIC is as follows: A) High inhibition: MIC < 500 μ g/ml; B) Medium inhibition: MIC from 500 μ g/ml to 1500 μ g/ml; C) Low inhibition: MIC > 1500 μ g/ml [11]. To agar diffusion assay, the classification was bases in zone diameter inhibition (Resistant, Intermediate or Susceptible) [12]. However, this classification may chew the potential biological potentials of the extract. The lowest concentration showing some microbial growth inhibition, crucial information for the research and production of new antimicrobial drugs [13].

Conclusion

The present work demonstrates the possible antimicrobial potential of *Spondias dulcis* Parkinson leaves extract by using different antimicrobial methods. The results could indicate that hydroalcoholic extract can use to obtained fractions and potential antimicrobial substances from Gram-negative microorganism.

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