The Antimicrobial Screening of a Barbadian Medicinal Plant with Indications for Use in the Treatment of Diabetic Wound Infections

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ABSTRACT

Objective: Diabetes mellitus is a chronic non-communicable disease with high prevalence in the North American and Caribbean region. Diabetic Foot Syndrome which is an associated complication can lead to the development of wounds and ulcers which can become infected. *Justicia secunda*, a plant known locally in Barbados as Bloodroot used in folklore for wound healing, was selected to test its ability to aid diabetic wound healing by antimicrobial activity. It was therefore tested against the bacteria *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus feacalis* (clincal strain) which are commonly found in diabetic wounds.

Methods: The plant was collected by local users. Methanol and acetone extracts of the plant were prepared with use of soxhlet extraction. The antimicrobial activity was assessed with the use of a modified Kirby-baurer method. Concentrations of 200 mg/ml, 100 mg/ml, 10 mg/ml, and 1 mg/ml of the extract were used, with a standard ciprofloxacin 5 µg positive control, and a 5% dimethyl sulfoxide (DMSO) solution negative control.

Keywords: Antimicrobial, Barbadian, diabetes, extracts, *Justicia secunda*

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Results: The *J secunda* methanol and acetone extracts with an extraction yield of 15.3% and 0.75%, respectively yielded no activity within the concentration range against the three strains of bacteria tested. In comparison with the positive control, relative inhibition zone diameter (RIZD) values of 0% resulted for both the negative control and the extracts, with the positive control having a value of 100%.

Conclusion: The *in vitro* screen of the extracts prepared from *J secunda*, yielded no antimicrobial activity against the three strains of bacteria tested and therefore does not support the folklore claims by this mechanism of action.

INTRODUCTION

Diabetes mellitus is a chronic non-communicable disease which has a high degree of prevalence within the Caribbean. This disease is characterized by the inability of the pancreas to secrete sufficient insulin to lower blood sugar levels, as well as the body's inability to adequately utilize insulin (1). One major complication associated with this illness is the development of foot ulcers (2, 3). These injuries can become infected with different bacterial organisms such as *Staphylococcus aureus* and also gram negative organisms such as *Pseudomonas aeruginosa* (4). If these infections go untreated, the area may become gangrenous and amputation of the limb may be required.

The plant *Justicia secunda* of the Acanthaceae family is known in Barbados as "Bloodroot" and in other countries such as Venezuela as "Sanguinaria" which means blood in English (Figure). The term bloodroot in Barbados was coined due to the red colour that the plant imparted to water when any part of the plant was boiled. This plant is used by Barbadian locals to treat wound infections by taking it as a decoction and also in teas.

Most recent literature reviews revealed two articles which screened aqueous extracts of *J secunda* against a range of gram positive and gram negative micro-organisms. Antimicrobial studies by Rojas *et al* showed no activity against *S aureus* ATCC 29737 bacterium, but activity against *Escherichia coli* (*E coli*)*, Bacillus cereus, Pseudomanas aeruginosa* ATCC 25919 and *Candida albicans* (5). Conversely, studies conducted by Herrera-Mata *et al* produced evidence of activity against *S aureus* ATCC 6538P, but no activity against *E coli* ATCC 0389 (6). This research paper evaluates extracts from this plant against different strains of previously tested bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonaus aeruginosa* ATCC 27853 and also on a *c*linically acquired strain of *Enterococcus feacalis*.

MATERIALS AND METHODS

The plant was harvested by a local who was well versed in its use, and identified using the University of the West Indies Cave Hill Campus, Plants of the Eastern Caribbean Online Database.

The plant material was dried for two days at room temperature and drying was completed in a 50 ˚C oven for 12 hours. Fifteen grams of the dried plant material was then ground into a fine powder and analytically weighed. The ground material was then extracted with the use of a soxhlet apparatus at 70 ˚C. Extraction was carried out first with methanol then successively with acetone. Both extracts were then rotary evaporated to dryness, then re-dissolved in a small volume of their respective extraction solvents in which it was stored at 15 ˚C.

The extracts were rotary evaporated to dryness and final weights of the extracts obtained were then used to create standard solutions of concentrations 200 mg/ml, 100 mg/ml, 10 mg/ml and 1 mg/ml for the methanol and acetone extracts. The dilutions were performed with solutions of 5% dimethyl sulfoxide (DMSO) solution.

The test organisms *Staphylococcus aureus* ATCC 25923, *Pseudomonaus aeruginosa* ATCC 27853 and *Enterococcus feacalis,* a *c*linically acquired strain, were obtained from the Microbiology Department of the University Hospital of the West Indies. Muller Hinton agar media was used for all screening performed. Overnight cultures of each of the organism were used to isolate four to five colonies which were then suspended in a tube of sterile Muller Hinton broth. This was then adjusted to 0.5 McFarland Standard by visual comparison. The adjustment ensured a colony suspension of 10^8 CFU/ml was used for plating in the disk diffusion test. The agar plates were loaded with blank filter paper disk to which 50 µL of concentrations of 1 mg/ml to 200 mg/ml of the extracts were added. Also tested simultaneously were the negative controls

5% DMSO solution, and the positive control 5 µg standard ciprofloxacin disk. The plates were then covered, incubated inverted for 24 hours at 35 ˚C until a confluent lawn of bacteria was observed on the surface of the plate. All tests were performed in duplicate.

Zones of inhibition around the disk were measured and zone sizes were recorded in millimeters. The antimicrobial activity was expressed as relative inhibition zone diameter, using the calculation:

$$\%RIZD = \frac{\text{(inhibition zone diameter – inhibition zone diameter for negative control)}}{\text{(inhibition zone diameter for antibiotic standard)}} \times 100 \quad [5].$$

RESULTS

The yields of the chemical extraction from the original 15 g of ground dried plant material were 15.3% and 0.75% for methanol and acetone, respectively. The Table shows the antimicrobial activity of each concentration of extract tested.

DISCUSSION

The strains selected to assess the antibacterial activity of the extract were utilized because of their documented prevalence in diabetic wound infections, with *S aureus* and *P aeurginosa* being the two most prevalent bacterial isolates from diabetic wound cultures (7). These provided a basis for testing the ability of the extract for efficacy in the treatment of diabetic wound infections. Results of the experiment indicated that the prepared extracts of *J secunda* had no activity against the bacterial strains tested.

Studies by Herrera-Mata *et al* outlined conflicting results documenting activity of *J secunda* against *S aureus* and also used the disk diffusion assay to test its activity (6). However, the main difference noted for differences between that study and this research paper is with the strain of *Staphylococcus aureus* tested. This study used a standard American Type Culture Collection (ATCC) strain of 25923 while their study utilized ATCC 6538P. The possibility therefore exists that the strain utilized in this experiment is more resistant. Similarly, Rojas *et al* published data in 2006 outlining the negative activity of *J secunda* against *S aureus* with an ATCC 29737 strain being used (5). This was also the case with the difference between activity in the studies by Rojas *et al* and this study, with *P aeruginosa* ATCC 27653

used in this study and ATCC 25619 used by Rojas' team. However, limited publications are available which provide a direct comparison of the degree of resistance among different strains of the same bacterium.

The absence of the antimicrobial activity of *J secunda* does not eliminate its possible folklore claim of positive effect of wound healing in general, and therefore its ability to have an effect on diabetic wounds. Other possible mechanism by which it may exhibit this effect may be by an antioxidant mechanism such as scavenging free radicals in the wound (8) or providing anti-inflammatory activity.

Limitations encountered in this study relate to access to adequate equipment to further characterize the plant extracts with chromatographic techniques. This could have provided insight into some of the possible prospective activity of the plant, based on its chemical profile.

CONCLUSION

The results of this experiment showed that *J secunda* did not exhibit effects on wound healing by an antimicrobial mechanism against the bacteria tested in this study, and is therefore not likely to treat diabetic wound infections *via* this mechanism. This, however, does not nullify the folklore claims of its activity. Future research should be conducted to investigate other possible mechanisms by which this plant can affect wound healing and diabetic wound infections. Stability studies are also required on the various extracts of this plant.

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Figure: Bloodroot

Table 1: The antimicrobial activity of each extract by concentration expressed as percentage inhibition

Bacterium	**% Relative Zone of Inhibition Diameter**									
	Methanol				**Acetone**					**Control (Ciprofloxacin)**
	200 mg/ml	**100 mg/ml**	**10 mg/ml**	**1 mg/ml**	**200 mg/ml**	**100 mg/ml**	**10 mg/ml**	**1 mg/ml**	**0 mg/ml**	**5 ug/disc (0.005 mg)**
E feacalis	0	0	0	0	0	0	0	0	0	98%
	0	0	0	0	0	0	0	0	0	101%
Average	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**99.5%**
SD	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**± 2.12**
S aureus	0	0	0	0	0	0	0	0	0	101%
	0	0	0	0	0	0	0	0	0	98%
Average	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**99.5%**
SD	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**± 2.12**
P aeruginosa	0	0	0	0	0	0	0	0	0	99%
	0	0	0	0	0	0	0	0	0	101%
Average	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**99.5%**
SD	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**± 2.12**