

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 faecalis* (clinical 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-Bauer 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.

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.

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

Tamizaje Antimicrobiano de una Planta Medicinal Barbadiana con Indicaciones para su Uso en el Tratamiento de Infecciones de Heridas en Diabéticos

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RESUMEN

Objetivo: La diabetes mellitus es una enfermedad crónica no transmisible, de alta prevalencia en la región de Norte América y el Caribe. El síndrome de pie diabético es una complicación asociada, que puede llevar al desarrollo de heridas y úlceras, con la consiguiente posibilidad de infección. *Justicia secunda* es una planta conocida localmente en Barbados como "bloodroot" (*sanguinaria canadensis*) y usada en la medicina folklórica para la curación de heridas. Esta planta fue seleccionada para analizar su capacidad de ayudar a curar las heridas de diabéticos por su actividad antimicrobiana. Por lo tanto, se la sometió a prueba frente a bacterias *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, y *Enterococcus faecalis* (cepa clínica) que normalmente se encuentran en las heridas del diabético.

Métodos: La planta fue proporcionada por usuarios locales. Usando un extractor Soxhlet, se prepararon extractos de metanol y acetona a partir de la planta. La actividad antimicrobiana se evaluó mediante el método de Kirby-Bauer modificado. Se usaron concentraciones de 200 mg/ml, 100 mg/ml,

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10 mg/ml, y 1 mg/ml del extracto, con un control positivo de 5 µg de ciprofloxacina estándar, y un control negativo de una solución de dimetil sulfóxido (DMSO) al 5%.

Resultados: Los extractos de metanol y acetona de *J secunda* con un rendimiento de extracción de 15.3% y 0.75% respectivamente, no arrojaron actividad alguna dentro del rango de la concentración contra las tres cepas de bacterias sujetas a prueba. Comparado con el control positivo, el diámetro de la zona de inhibición relativa (RIZD) arrojó valores de 0%, tanto para el control negativo como para los extractos, con un valor de 100% para el control positivo.

Conclusión: El tamizaje in vitro de los extractos preparados de *J secunda*, no arrojó actividad antimicrobiana alguna contra las tres cepas de bacterias analizadas, y por consiguiente no sustenta la afirmación de la medicina folklórica en relación con este mecanismo de acción.

Palabras claves: Antimicrobiano, barbadense, diabetes, extractos, *Justicia secunda*

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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



Figure: *Justicia secunda*, known as Bloodroot in Barbados.

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*, *Pseudomonas 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 *Pseudomonas aeruginosa* ATCC 27853 and also on a clinically acquired strain of *Enterococcus faecalis*.

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 they were 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, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis*, a clinically acquired strain, were obtained from the Microbiology Department of the University

Hospital of the West Indies. Muller Hinton agar media were 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 μ L of concentrations of 1 mg/ml to 200 mg/ml of the extracts were added. Also tested simultaneously were the negative control 5% DMSO solution, and the positive control 5 μ g 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:

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

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.

Table: 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 μ g/disc (0.005 mg)
<i>E. faecalis</i>	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
<i>S. aureus</i>	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
<i>P. aeruginosa</i>	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

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. aeruginosa* 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 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 to the bioactive constituent in the extract. Similarly, Rojas *et al* published data in 2006 outlining the activity of *J. secunda* against *S. aureus* with an ATCC 29737 strain being used (5). Similar to difference in activity of the plant's extract against the different strains of *S. aureus* mentioned earlier, the extract had no activity against *P. aeruginosa* ATCC 27653 used in this study while Rojas *et al* reported activity against *P. aeruginosa* ATCC 25619. However, limited publications are available which provide a direct comparison of the degree of resistance among different strains of the bacteria.

The absence of the antimicrobial activity of *J. secunda* does not eliminate its possible folklore claim of wound healing and therefore its ability to have a therapeutic 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 the phytochemical profile of the plant for future studies.

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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REFERENCES

1. World Health Organization. Health Topics: Diabetes. World Health Organization [cited May 13, 2011]. Available from: http://www.who.int/topics/diabetes_mellitus/en/
2. Hambleton IR, Jonnalagadda R, Davis CR, Fraser HS, Chaturvedi N, Davis CR et al. All-cause mortality after diabetes-related amputation in Barbados. *Diabetes Care* 2009; **32**: 306–7.
3. Porter RS. The Merck Manual – Home Health Handbook. New Jersey: Merck Sharpe & Dohme Corporation; 2010–2011 [cited October 6, 2010]. Available from: <http://www.merck.com/mmhe/sec06/ch095/ch095h.html>.
4. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW et al. Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2004; **39**: 885–910.
5. Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. *BMC Complement Altern Med* 2006; **6**: 2.
6. Herrera-Mata H, Rosas-Romero A, Crescente O. Biological activity of "Sanguinaria" (*Justicia secunda*) extracts. *Pharm Biol* 2002; **40**: 206–12.
7. Cantillio J, Guette J, Baldiris R, Jaramillio B, Olicero J. Evaluation of the acute toxicity (LC50) against *Artemia franciscana* and hemolytic activity of the aqueous extracts in dichloromethane and methanol in parts of *Justicia secunda* (Vahl.) [Serial on the internet]. *Scientia et Technica* 2007; **33**: 257–8.
8. Martin A. The use of antioxidants in healing. *Dermatol Surg* 1996; **22**: 156–60.
9. Alsaimary IEA. Bacterial wound infections in diabetic patients and their therapeutic indications. *Med Pract Rev* 2010; **1**: 12–15.
10. Sigma-Aldrich. Bloodroot (*Sanguinaria canadensis*). Plant Profiler. Sigma-Aldrich Co. LLW; 2012 [cited May 13, 2011]. Available from: <http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler/sanguinaria-canadensis.html>
11. Basch H, Gadebusch HH. In vitro activity of dimethylsulfoxide. *App Microbiol* 1968; **16**: 1953–54.
12. International Diabetes Federation. IDF Diabetes Atlas, North America and Caribbean. International Diabetes Federation; 2011 [2012; cited June 6, 2011] Available from: <http://www.diabetesatlas.org/content/north-america-and-caribbean>
13. Sharma VK, Khadha PB, Joshi A, Sharma R. Common pathogens isolated in diabetic foot infection in Bir Hospital. *Kathmandu University Med J* 2006; **4**: 295–301.