Abstract

The purpose of this study was to test for antimicrobial activity in leaf and stem extracts of Jacquemontia ovalifolia sandwichensis, an endemic Hawaiian plant which is used traditionally for the treatment of thrush. A hexane, methanol, and water plant extraction method was used followed by a Kirby-Bauer disc diffusion susceptibility test on Candida albicans, Escherichia coli, and Staphylococcus aureus. Zones of inhibition were observed only in S. aureus and E. coli. A one-way ANOVA and post-hoc Tukey pairwise comparisons showed a significant difference compared with positive controls (p<0.001).

Keywords: Jacquemontia ovalifolia sandwichensis, Pa’uohi’iaka, anti-microbial, thrush, plant extracts, Kirby-Bauer disc diffusion

Introduction

Various species of plants from the morning glory family are used therapeutically in traditional medicines around the world. The aborigines of Australia used Ipomoea pes-caprae to treat wounds and skin infections (Pereda-Miranda 2005). In Mexico, the Aztecs used Ipomoea murcicocida to treat tinnitus and rashes (Cherigo 2009). Plants from the morning glory family also have been used in many countries to treat colic, and gonorrhea (Souza 2000). Jacquemontia ovalifolia sandwichensis, known as Pa’uohi’iaka or Kakanihi’iaka in Hawaii (Nagata 1971), is a member of the morning glory family (Walther 2004), and can be found on all of the Hawaiian isles except for NC’s Maui (Robertson 1974, Nagata 2009). It grows from sea level to about 100 feet in elevation (Walther 2004) primarily in coastal habitats (Namoff 2009) and is most common on the leeward side of the island (Chock 1968)(Fig. 1). In ancient Hawaii, J. ovalifolia was used to treat thrush and infected wounds using the stems and leaves which were typically pounded and the resulting poultice was applied to sores or wounds or by mixing with water to create an ingestible solution (Chock 1968, Ka’aiakanamanu 2003, Walther 2004). No literature to date has had any results confirming whether or not this plant is effective in treating thrush. The purpose of this study was to screen leaf and stem extracts of J. ovalifolia for antimicrobial activity.

Materials & Methods

The microorganisms tested were Escherichia coli B (#155070A Carolina Biological), Staphylococcus aureus (coagulase positive) (#155554 Carolina Biological), and Candida albicans (#155965 Carolina Biological). The organisms E. coli and S. aureus were grown on LB agar (Sigma-Aldrich) or Mueller Hinton agar (Fluka), and C. albicans was grown on a YEPAD agar that was prepared in the lab (0.9% yeast extract, 1.8% peptone, 1.8% glucose, supplemented with 1.8% agar 7.5% adenine stock solution, and 91% distilled water). All of the organisms were incubated for about 16 to 18 hours at 37°C with a non-shaking environment. Each organism needed to be placed on new media every three weeks and refrigerated.

The leaves and stems of J. ovalifolia were collected at Moku‘aua Island (23° 39’ 49” N 157° 55’ 18” W) on Oahu in May 2014. Fresh leaves and stems were frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. Solvent extractions were performed by suspending five grams of the powdered sample in 25 ml of water, methanol, or hexane and stirred at room temperature for 24 hours. Each extract was vacuum filtered through a Whatman GF C filter paper. The solvents were evaporated using a Buchi Rotovap, employing a pressure of 70 mbar for hexane, 46 mbar for methanol, and 8 mbar for water, and using a temperature of 60°C. The dried extracts were weighed and re-suspended in dimethyl sulfoxide to achieve concentrations of 0.05, 5, 50, and 100 mg/mL.

A Kirby-Bauer disc diffusion susceptibility test was used to assay for microbial inhibition (Jorgenson 2009). For the positive controls, antibiotic discs of Kanamycin were used for E. coli, Ampicillin was used for S. aureus, and Nystatin for C. albicans. Negative controls used dimethyl sulfoxide. All of the plates were inoculated with freshly-cultured 1.5 x 10⁸ CFU/mL, which is equivalent to a 0.5 McFarland Standard. Each 6 mm sterile disc (Microexpress) was impregnated with 20 µg of each extract and the differing concentrations. The same was done for the positive and negative controls. All of the discs were allowed to dry, and deposited on the surface of 15 Mueller-Hinton plates (Fluka) which were filled to a height of 4 mm. Only after about 5 minutes from inoculation were the discs placed on the media or until the media was dry, but no longer than 15 minutes. For every test run five plates were used for each organism. All organisms were incubated for 12 to 16 hours at 37°C. Each assay was performed three times for each organism. Additionally, each test run, the zones of inhibition were measured using a metric ruler. A one-way ANOVA and post-hoc Tukey pairwise comparison test was used to analyze the data.

Results

The greatest zone of inhibition was observed in Staphylococcus aureus with the most clearance observed in the hexane extracts of Jacquemontia ovalifolia sandwichensis extracts. Hexane extracts yielded zones of inhibition with an average diameter of 19 ± 1 mm at concentrations of 100 and 19 ± 0.3 mm at concentrations of 50 mg/ml (Fig. 1, 2, 3). Escherichia coli was inhibited by the water of Jacquemontia ovalifolia sandwichensis extracts at 100 mg/ml, with an average of 5 ± 4.62 mm (Fig. 4). Zones of inhibition for S. aureus and E. coli were significantly different (p<0.001) from the positive controls.

Conclusions

Jacquemontia ovalifolia sandwichensis extracts, specifically the hexane and methanol extracts, did show consistent antimicrobial effects against Staphylococcus aureus. There could be some unknown factor that affects the potency of J. ovalifolia in relation to Candida albicans and Escherichia coli, such as the freshness of the extract. The plant material should be extracted and used as soon as possible since that may allow degeneration of the potency of any compounds in the plant. Even though the variable of the time of year was not considered in the study nor proven, it could have possibly played a role in the potency as well as the effectiveness of the plant extract since plants can change morphologically and physiologically throughout the year in respect to seasons, and possibly even more so in Hawaii since there is a relative rainy and dry season. For future research, the roots and seeds should be examined for any antimicrobial properties in accordance with this research since only the leaves and stems were used. Since this study was focused primarily on whether or not J. ovalifolia had any antimicrobial activity whatsoever, studies of how the ancient Hawaiians used and applied J. ovalifolia should be thoroughly examined since there is evidence of inhibition of S. aureus and E. coli.

References


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