

Antimicrobial Activity of an Iridoid Rich Extract from *Morinda citrifolia* Fruit

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Abstract: The aim of the current study was to evaluate the antibacterial activity of iridoids in *Morinda citrifolia* (noni) fruits. An iridoid rich extract from noni fruits was prepared and incubated with aliquots of twenty-four hour cultures of *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* at total iridoid concentrations of 0.096, 0.19, 0.45, 0.82 and 1.41 mg/mL. was monitored by Optical Density (OD) at 600 nm. Iridoid concentration dependent decreases in cell growth were observed in all organisms. The results suggest that deacetylasperulosidic acid and asperulosidic acid, the major phytochemical constituents of noni fruit, possess antibacterial activity.

Key words: Antimicrobial, iridoids, *Morinda citrifolia*, noni

INTRODUCTION

Morinda citrifolia is a tree that grows throughout the tropics. It is a small to medium sized fruit bearing tree, Fig. 1. The common names for this plant are Indian mulberry and noni, with the latter being more frequently used since the global market introduction of noni fruit juice products in the mid 1990's. Noni fruits have long been consumed as a traditional food (Morton, 1992). But the fruits have also been used in Polynesian folk medicine for a variety of health related conditions, including the treatment of infections (Whistler, 1992). For example, Tongans place noni fruit juice directly into infected wounds, cuts, or bruises (referred to as "palangia") to clear the infection and promote healing (Palu, 2004). Antimicrobial experiments with ripe noni fruits have provided some substantiation of the efficacy of this traditional use (Bushnell *et al.*, 1950; Leach *et al.*, 1988). Additionally, many commercial noni juice products do not contain any chemical preservatives, further indicating the presence of phytochemicals with potential antimicrobial activity.

A large number of nutrients and phytochemicals have been identified in noni fruit. Among these, iridoids have recently been found to be the most abundant, especially deacetylasperulosidic acid and asperulosidic acid, Fig. 2 (Deng *et al.*, 2010; West *et al.*, 2011). Interestingly, the presence of iridoids in noni fruit and their possible antimicrobial activities had been investigated decades ago. Levan (1963) isolated a tetraacetate derivative of an iridoid from noni fruit in an effort to identify the compounds responsible for the antimicrobial activity observed in previous experiments. Although the activity



Fig. 1: Noni tree growing on the island of Tahiti, French Polynesia

of the tetraacetate derivative of asperuloside was not active, asperuloside itself had significant antimicrobial activity, leading Levand to conclude that other active iridoids are likely present in the fruit (Levand and Larson, 1979).

The current study was conducted by the research department of Tahitian Noni International, Inc. during 2010 to evaluate the antimicrobial activity of the major iridoids in noni fruit in Gram positive and Gram negative bacteria, as well as in yeast. As iridoids are the major phytochemical constituents of noni fruit, this investigation provides further insight into their biological activities, as well as provides an identification of the compounds likely responsible for at least some of the antimicrobial properties observed in previous studies of noni fruit.

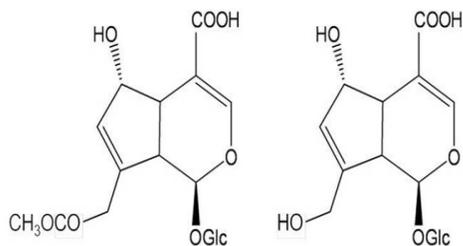


Fig. 2: Chemical structures of asperulosidic acid (left) and deacetylasperulosidic acid (right)

MATERIALS AND METHODS

Sample preparation: This study was conducted by the research department of Tahitian Noni International, Inc., American Fork, Utah, USA, during 2010. Noni fruits were harvested in French Polynesia and allowed to fully ripen. The fruit was then processed into a puree by mechanical removal of the seeds and skin, followed by pasteurization at a good manufacturing certified fruit processing facility in Mataiea, Tahiti, and shipped to the United States. Noni fruit puree was freeze-dried and extracted with methanol by percolation to produce a methanol extract. Following the addition of water, the methanol extract was partitioned with ethyl acetate (three times) to remove non-polar impurities. The aqueous extract was further partitioned with n-butanol (three times) to yield an n-butanol extract which contained 10% total iridoids. Prior to the antimicrobial assays, the n-butanol extract was prepared at 5 mg/mL in DMSO.

Chemical analysis: The iridoid content of the n-butanol extract, inclusive of Deacetyl Asperulosidic Acid (DAA) and asperulosidic acid (AA), was determined by HPLC, according to a previously reported method (Deng *et al.*, 2010). Briefly, the extract was diluted with water:methanol (1:1), mixed thoroughly, and filtered. Chromatographic separation was performed on a Waters 2690 separations module coupled with a 996 PDA detector, equipped with a C18 column (4.6 mm x 250 mm; 5 µm). The pump was connected to two mobile phases: A; acetonitrile, and B; 0.1% formic acid in water (v:v), and eluted at a flow rate of 0.8 mL/min. The mobile phase was programmed consecutively in linear gradients as follows: 0-5 min, 0% A; and 40 min, 30% A. The PDA detector was monitored in the range of 210-400 nm. The injection volume was 10 µL for each of the sample solutions. The column temperature was maintained at 25 °C.

Antimicrobial assay: Twenty-four hour broth cultures of *E. coli* (ATCC 8739), *C. albicans* (ATCC 10231), and *S.*

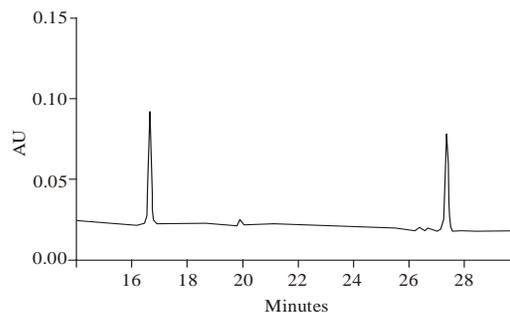


Fig. 3: HPLC chromatogram of the n-butanol extract of noni fruit. The retention times of deacetylasperulosidic acid and asperulosidic acid are approximately 17 and 27 min, respectively

aureus (ATCC 6538) were prepared using KwikStik™ lyophilized microorganism pellets (MicroBioLogics, St. Cloud, Minnesota, USA). Samples of broth (250 mL) were inoculated with the respective microorganisms. *E. coli* and *S. aureus* cultures were prepared with tryptic soy broth, while *C. albicans* was prepared with Sabouraud dextrose broth. Next, 0.1 mL of each inoculated broth was transferred into 5 mL aliquots of fresh broth. The n-butanol extract was then added to this diluted inoculated broth to produce total iridoid concentrations of 0.096, 0.19, 0.45 and 1.41 mg/mL. All culture samples were incubated for 24 h at 35°C. Microbial growth of the samples was measured by optical density at 600 nm, by spectrophotometer. Samples were tested in triplicate.

RESULTS AND DISCUSSION

HPLC analysis of the n-butanol extract revealed the presence of two prominent compounds (Fig. 3). These were asperulosidic acid and deacetylasperulosidic acid, which comprised 5.13 and 5.24% of the extract, respectively. The total iridoid content of the extract was approximately 10.4%. This information was used to determine the appropriate volumes of dissolved n-butanol extract to be added to cell cultures to achieve the various iridoid concentrations reported in the experiment. It should be noted that preparation of the extract enriched the iridoid concentration, as well as changed the deacetylasperulosidic to asperulosidic acid ratio. In processed noni fruit, the total iridoid concentration is approximately 0.18% with a deacetylasperulosidic to asperulosidic acid ratio of 3.55:1 (West *et al.*, 2011). This ratio is essentially 1:1 in the n-butanol extract, but provides a better material for testing the antimicrobial efficacy of iridoids found in noni fruit.

The effect of iridoid concentration on the growth of *E. coli*, *S. aureus*, and *C. albicans* cultures is demonstrated in Fig. 4. All three organisms experienced

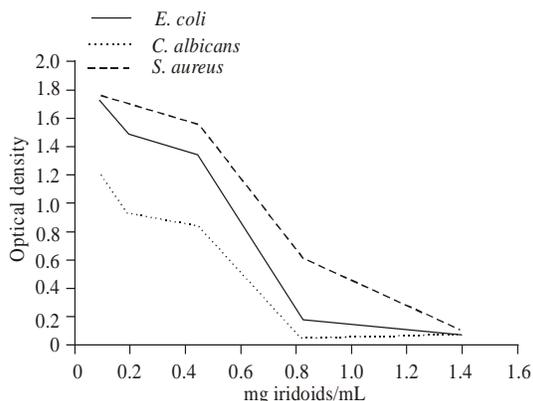


Fig. 4: Decreases in cell growth, as measured by optical density at 600 nm, during 24 h incubation with increasing iridoid concentrations

concentration dependent decreases in cell culture growth. Of the three organisms, *C. albicans* appears to the most sensitive to the antimicrobial activity of the iridoids. At 0.8 mg iridoids/ml, all *C. albicans* growth is arrested.

At the same concentration, most of the *E. coli* growth is suppressed, but complete cessation of growth does not appear until 1.4 mg iridoids/mL. *S. aureus* sensitivity to iridoid concentration is less than the other two organisms, with a more linear response throughout the concentrations evaluated. Even so, the results of the experiment demonstrate antimicrobial activity of the iridoid rich n-butanol extract against Gram negative and positive bacteria, as well as against yeast.

CONCLUSION

The results of our investigation lend support to the suggestion of Oscar Levand that the antimicrobial activity of noni fruit is due to the presence of iridoids, as iridoid concentration dependent decreases in cell culture growth were evident. Further, iridoids from noni fruit appear to be active against yeasts, Gram negative, and Gram positive bacteria. The current study also provides additional corroboration to the traditional use of noni fruit as an anti-infective agent. It is of interest to note, however, that the anti-microbial properties do not appear to pose any safety risks to humans or animals. Multiple toxicity tests and human trials have revealed that noni

fruit juice is safe to consume, even in large quantities and over extended periods of time (West *et al.*, 2009; West *et al.*, 2006).

ACKNOWLEDGMENT

This research was supported by Morinda Holdings, Inc.

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