**Abstract**

Traditional healers have long used plant-derived products to prevent or cure infectious diseases. The main reason is that these natural products may contain antimicrobial agents that are effective against drug-resistant bacteria. The Bizzy nut is a “cure-all” herbal medicine that is useful for many medical purposes, such as removal of poisons from the body; birth control; control of diabetes; weight loss; and contains antimicrobial activity. Previously, we reported on the antimicrobial property of the natural product, Bizzy nut, in gram-positive bacteria. In this study, we extended this analysis to gram-negative organisms. Solid-liquid extraction was used to divide the analytes present in *Cola acuminata* into five unique bioactive fractions. To determine the antimicrobial activity, five mm depth wells were created on a confluent lawn of gram-negative bacteria. Different concentrations of the acetone extract (0-50µL) was added to individual wells and the zone of inhibition was allowed to form at 30°C overnight. We observed that a concentration between 30µg/mL to 50µg/mL of Bizzy nut was effective in killing gram-negative organisms. The order of potency of the organisms examined is *Klebsiella pneumoniae* > *Escherichia coli* > *Enterobacter aerogenes*. This study suggests that Bizzy nut contains selective antimicrobial activity.

**Introduction**

The misuse of antibiotics and the counter pharmaceuticals have led to the evolution of drug-resistant bacteria. A World Health Organization report in April 2014 states that antibiotic resistance is a global health risk. This has led to a search for an alternative treatment for diseases caused by certain human pathogens. In the past few years, several studies have been conducted on the antimicrobial properties of natural medicinal plants. Many of these natural plants have been used because their antimicrobial compounds differ in their mechanism of action in comparison to current antimicrobial agents. These antimicrobial compounds present in plants are secondary metabolites of the plant, which makes them potentially more effective against resistant microbial strains.

*Cola acuminata*, commonly referred to Bizzy Nut, is a natural product produced from the Cola nut plant. The Ettu people of Jamaica consider *Cola acuminata* to be a “cure-all” herbal medicine.

**Hypothesis**

Previously we have demonstrated that an acetone extract of the natural product, *Cola acuminata*, exhibits important biological activities. Since *Cola acuminata* is likely to be rich in a wide variety of secondary metabolites, we hypothesized that these metabolites contain antimicrobial properties that inhibit gram-negative bacteria.

**Materials and Methods**

**Preparation of Cola acuminata Extracts**

Finely ground Bizzy nut samples (100g) were sequentially extracted in a Soxhlet apparatus (120cm x 500cm) using 100% hexane, ether, acetone, methanol, and water to produce five independent extracts with compounds of unique polarity.

**SPE Purification of Antimicrobial Activity from Biz-3W**

![Image of SPE purification process](image)

**Results**

The antimicrobial activity of the acetone extract of *Cola acuminata* (Biz-3) was assayed using the agar-diffusion assay, turbidity assay, and the bacterial viability assay using *Staphylococcus aureus* as the model organism. A standard curve of *Staphylococcus aureus* inhibition was generated using the turbidity assay and vancomycin as the standard antibiotic. One unit of inhibition was determined by linear regression and is defined as the amount of antibiotic or Biz-3 required to produce an OD 0.75 @650nm. The acetone extract (Biz-3) was acidified then purified through a DEC-SCX ion exchange SPE column. The bound sample was eluted using a different concentration of methanol and each sample assayed for antimicrobial activity.

- A. Agar-Diffusion Assay using *Staphylococcus aureus*.
- B. Quantification of the Antimicrobial Activity in Biz-3 samples using the turbidity assay. The amount of inhibitory activity in each sample was determined using a vancomycin standard curve. One unit of activity is defined as the amount of antibiotic or Biz-3 required to produce an OD 1.0 @650nm.

**Conclusions**

Our results suggest that gram-negative bacteria are not as sensitive towards the acetone extract of *Cola acuminata* as compared to gram-positive bacteria.

**References**
