

Red Cabbage Methanol Extract inhibits the growth of Vancomycin-resistant *Enterococcus faecalis* during Kirby-Bauer Disk Diffusion Susceptibility Test

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This study was designed to establish whether red cabbage methanol extract can inhibit the growth of various strains of bacteria which include, *Micrococcus luteus*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, Vancomycin-resistant *Enterococcus faecalis* (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. While most of the bacterial strains showed resistance to the red cabbage extract, Vancomycin-resistant *Enterococcus faecalis* became susceptible to it. The susceptibility of VRE to the red cabbage methanol extract is notable since VRE is resistant to streptomycin.

Keywords: Red cabbage methanol extract; Vancomycin-resistant *Enterococcus faecalis*

Introduction

The indiscriminate use of antimicrobial drugs (specifically antibiotics) has led to the development of multidrug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Pseudomonas aeruginosa*. [2] Antibiotics are drugs that are produced naturally by microorganisms. Certain individual cells of bacteria develop resistance to the activity of drugs. Such resistance occurs through mutations of chromosomal genes or by acquiring resistance genes on R-plasmids. [1] When antimicrobial drugs act on bacterial cells, majority of them die or become inactive. The bacterial cells that resist the antibiotic gain dominance. For instance, penicillin, discovered by Alexander Fleming in 1929, was hailed as a wonder drug because of its action against numerous species of bacteria. At the time, penicillin was a common drug prescribed for almost every bacterial infection. However, the overuse of penicillin has rendered it ineffective against bacteria such as *Staphylococcus aureus*. The ineffectiveness of penicillin led to the development of the methicillin which is now ineffective against MRSA. [1] In this day and age where the market is filled with antimicrobial products including hand soaps, bar soaps and even dishwashing agents, multidrug resistant pathogens are developing and creating headaches for pharmaceutical companies and the general public. This has created the need to frequently develop new antimicrobial

drugs or other alternative forms of treatments. Cruciferous vegetables, which belong to the *Brassica* group, include some of the most commonly grown crops such as cabbage, broccoli, cauliflower, Brussels sprouts, kale and turnip which are consumed all over the world. Almost all clinical trials and studies have established that the increase in consumption of fruits and vegetables leads to the decrease in occurrence of cardiovascular and metastatic diseases. [6] Among the wide variety of vegetables, cruciferous vegetables have been found to be good sources of nutrients and phytochemicals that reduce the risk of age-related chronic illnesses such as cardiovascular health and other degenerative diseases. [5] Among the numerous phytochemicals in cruciferous vegetables, flavonoids have been shown to be associated with many biological effects such as antibacterial, antiviral, anti-inflammatory, antiplatelet, antioxidant, free radical scavenging and vasodilatory actions. [2] Flavonoids are phenolic substances linked to aromatic rings. [2] They are known to be synthesized by plants in response to an antimicrobial infection. Therefore, it is not a surprise that flavonoids are effective against a wide array of microorganisms. [2] The presence of hydroxyl groups enable flavonoids to react effectively against proteins (importantly bacterial proteins). [2] Thus, more hydroxyl groups potentially mean greater antimicrobial activity.

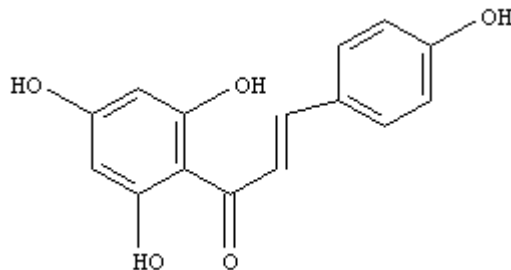


Figure 1: Chemical structure of flavonoids. Red cabbage is known to be a main source of these phenolic substances with antimicrobial activities

Red cabbage which belongs to the family Brassicaceae, is an important vegetable with anticancer, anti-inflammatory and anti-diabetic effects [2]. High activity of flavonoids was found in intensely colored vegetables such as red cabbage rendering them likely sources of novel antimicrobial activities that might lead to a breakthrough discovery of new antibiotic drugs [1]. This research tests whether crude methanol extract of red cabbage can significantly inhibit several species of bacteria. This could lead to the development of new and inexpensive antibacterial drugs as a substitute for drugs to which bacteria has developed resistance.

Materials and Methods

The red cabbages were bought from three local markets. The fresh cabbages were stored in the refrigerator for 48 hours before using for research. The cabbage leaves were peeled and manually shredded in to small pieces in order to facilitate blending. Then, 200 grams of red cabbage leaves were blended with 100mL of methanol. Following blending, the cabbage-methanol solution was filtered and 2.5 mm filter paper discs were placed into a volumetric flask that contained

10 mL of the filtrate. The filter paper discs in the volumetric flask were allowed to evaporate for three weeks before transferring them to the agar plates. The methanol used was Sigma-Aldrich ChromasolV for HPLC, 99.9%. Mueller-Hinton agar (BBL) was used for testing antimicrobial activity. All the eight strains of bacteria were from Presque Isle Cultures, Presque Isle, PA. The materials and facilities used for the research such as the Mueller-Hinton agar, nutrient agar plates, autoclave and incubators were provided by Felician College, NJ.

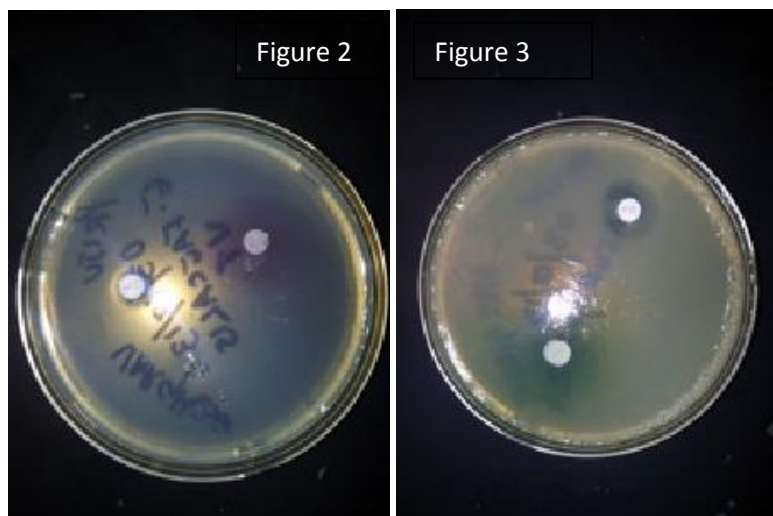
The Kirby-Bauer Disk Diffusion Susceptibility Test is a common method used by microbiologists to test the antimicrobial sensitivity of bacteria [1]. 1 mL of each of the bacterial cultures were inoculated to a set of 3 Mueller Hinton agar plates. Then, a 10 µg streptomycin disc and a red cabbage methanol extract disc were placed on to each of those plates. These plates were incubated at 25°C for a period of 48 hours. After 48 hours, the plates were examined for areas of clearing around the discs, which are known as zones of inhibition (ZOI). In general, the higher the diameter of the zone, the more susceptible are the bacteria to the antibiotic.

Results

Table 1: Comparison of the average zones of inhibition (ZOI) for streptomycin and the red cabbage methanol extract.

Bacteria	Average ZOI for 10 µg streptomycin discs (mm)	Average ZOI for Red Cabbage Methanol Extract Discs (mm)
<i>Micrococcus luteus</i>	33±2.5 (S)	-
<i>Escherichia coli</i>	13±0.6 (I)	-
<i>Staphylococcus aureus</i>	15±0.6 (I)	-
<i>Bacillus subtilis</i>	9±0.6 (R)	-
<i>Enterococcus faecalis</i>	>30 (S)	>30.00
<i>Enterococcus faecalis</i> VRE	8±0.6 (R)	30±0.6
MRSA	0 (R)	-
<i>Pseudomonas aeruginosa</i>	14±0.6 (I)	-

*S = susceptible (15 mm or more), I = intermediate (12-14 mm) and R = resistant (less than 11 mm). "-" indicates the absence of antimicrobial activity. The readings are based on the standard zone of inhibition chart provided by Microbiology Labs.



Figures 2 and 3: The plate on the left contains the vancomycin-resistant strain of *Enterococcus faecalis*. The 30 mm zone of inhibition on this plate is due to the red cabbage methanol extract. The plate on the right contains *Pseudomonas aeruginosa*. The 14 mm zone of inhibition on the right plate is due to streptomycin.

Discussion

The objective of this study was to test whether red cabbage methanol extract inhibited the growth of various species of bacteria. Even though majority of the bacterial strains resisted the cabbage extract, it is noticeable that the vancomycin resistant strain of *Enterococcus faecalis* was susceptible to it. It is especially remarkable that the zone size of 30 mm was obtained due to the extract's inhibition of the vancomycin resistant strain.

Among the eight bacteria that were tested using Kirby-Bauer, only *Micrococcus luteus*, and *Enterococcus faecalis* were completely susceptible to streptomycin. The antimicrobial effect of streptomycin on *Escherichia coli* and *Pseudomonas aeruginosa* is considered intermediate. *Bacillus subtilis*, VRE and MRSA were resistant to streptomycin

Hafidh et al (2011) tested 12 strains of bacteria including *E.coli* O157:H7 and MRSA. They were able to obtain significant zones of inhibition with the red cabbage methanol extract. These zones of inhibition were only obtained with an extract which was prepared through strenuous and intricate procedures. However, there seems to be no preceding studies regarding the antimicrobial effect of the red cabbage methanol extract on VRE. Since VRE is responsible for nosocomial infections, the

forementioned result should be investigated in order to curb such outbreaks.

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