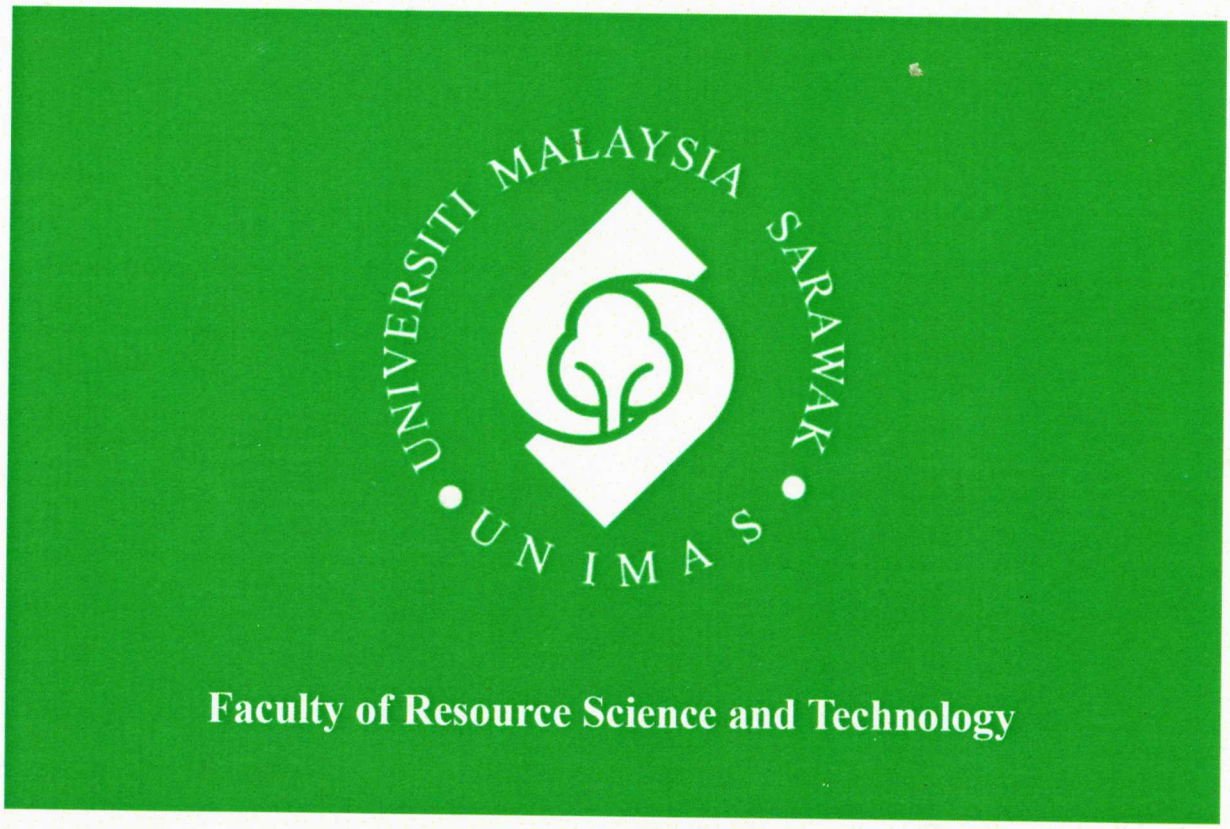

Effect of Botanical Drink against Food Related Pathogenic Bacteria

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Bachelor of Science with Honours
(Resource Biotechnology)
2018

Effect of Botanical Drink against Food Related Pathogenic Bacteria

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A project final report submitted in partial fulfilment of the
Final Year Project 2 (STF 3015) Course

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Resource Biotechnology Programmme

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2018

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Effect of Botanical Drink against Food Related Pathogenic Bacteria

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Abstract

The demand of healthy products that are free from chemicals and preservatives has led to the production of botanical drinks. The botanical drinks or natural products have been claimed to give many health benefits to the consumers. However, the evidence of botanical drinks that give an effect to the growth of the bacteria has yet to be ascertained. Botanical drinks that been use for this study consist of lemon juice, garlic, ginger, apple cider vinegar and honey. Methods that had been used for this study are the antibacterial activity assay and the minimum inhibitory concentration (MIC) by using the disc diffusion method on the Brain Heart Infusion (BHI) agar. The bacteria that been tested were gram positive bacterium, *Staphylococcus aureus*, and gram negative bacteria, *Escherichia coli* and *Salmonella typhii*. Apple cider vinegar shows the largest inhibition zone for the materials when tested on the gram positive and gram negative bacteria. The commercial botanical drink that has the largest inhibition zone is Ihsan. The botanical drinks are 75% effective against the food-related pathogenic bacteria. However, the individual materials showed bigger inhibition zone compared to the botanical drinks. This study gives confidence to the consumer regarding the benefits of the botanical drinks. Consumer can rely on botanical drinks and the individual materials compared to the medicine.

Key words: *Botanical drink, antimicrobial activity, Minimum inhibition zone, gram positive bacteria, gram negative bacteria*

Abstrak

Permintaan produk yang bebas daripada bahan kimia dan bahan pengawet telah mendorong kepada penghasilan minuman botani. Minuman botani atau produk semula jadi didakwa memberi banyak manfaat kesihatan kepada pengguna. Walaubagaimanapun, fakta mengenai minuman botani dapat memberi kesan kepada pertumbuhan bakteria belum dapat dikenalpasti. Minuman botani yang telah digunakan untuk kajian ini diperbuat daripada jus lemon, bawang putih, halia, cuka epal dan madu. Kaedah yang telah digunakan untuk kajian ini ialah antibakteria aktiviti dan kepekatan pembantut minimum (MIC) dengan menggunakan kaedah penyebaran cakera pada agar Brain Heart Infusion (BHI). Bakteria yang telah diuji adalah bakteria gram-positif, Staphylococcus aureus, dan bakteria gram-negatif, Escherichia coli dan Salmonella typhii. Cuka epal mempunyai zon perencatan terbesar untuk bahan-bahan apabila diuji dengan bakteria gram-positif dan bakteria gram-negatif. Minuman botani memberi 75% kesan yang efektif kepada bakteria yang menyebabkan keracunan makanan. Walaubagaimanapun, bahan-bahan individu menunjukkan zon perencatan lebih besar berbanding kepada minuman botani. Melalui kajian ini pengguna akan lebih yakin terhadap kelebihan minuman botani. Selain itu, pengguna juga akan lebih bergantung kepada minuman botani atau bahan-bahan tersebut berbanding dengan ubat-ubatan.

Kata kunci: *minuman botani, aktiviti antimikrobial, zon perencatan, bakteria gram-positif, bakteria gram-negaif*

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List of abbreviations

%	Percent
°C	Degree Celcius
µl	Microliter
BHI	Brain Heart Infusion
E.coli	*Escherichia coli*
EMB	Eosin Methylene Blue agar
MIC	Minimum Inhibitory Concentration
ml	Milliliter
mm	Millimeter
MSA	Mannitol Salt Agar
PBS	Phosphate Buffer Saline
S.aureus	*Staphylococcus aureus*
S.typhii	*Salmonella typhii*
v/v	Volume per volume
XLD	Xylose Lysine Deoxycholate agar

1.0 Introduction

Nowadays, most drug stores and pharmaceuticals have natural and botanical products. The consumption of healthy foods, drinks and medicines becomes a concerned meanwhile any consumption of products that contained chemicals and preservatives will be avoided. Thus, more products are being formulated from natural ingredients such as fruits and herbs to give more benefits to the human body. One of the health products is botanical drink. Botanical drink is a drink that is made from plants or plant parts for its functional health benefits, medicine, flavouring and scent (Food Processing, 2012). Materials in botanical drink are includes of lemon juice, garlic, ginger, apple cider vinegar and honey. This botanical drink has been claimed to benefit one's health. The main use of the botanical drink is to reduce the cholesterol level in the body. However, observation on the materials in this botanical drink shows most of them have antibacterial activity but the effect of this botanical drink on the pathogens has yet to be studied.

Food poisoning occurred because of the ingestion of food contaminated by pathogenic bacteria, toxins of bacteria, viruses or parasites. Food borne pathogens include *Escherichia coli, Salmonella, Listeria monocytogenes* and *Staphylococcus aureus*. There are more than 1.3 billion cases of human salmonellosis annually and the enterohemorrhagic *E.coli* O157:H7 is the most *E.coli* serotypes that cause food borne outbreaks in the worlds (Indu *et al.*, 2006). Food poisoning is a serious problem that turns up every year even though many techniques regarding the preparation of food had been improved. This food poisoning outbreak can be controlled by using the antimicrobial agents to inhibit the growth of the bacteria or to kill the bacteria (Dabbah *et al.*, 1969). Therefore, the used of the botanical drink to inhibit the growth of the food poisoning bacteria should be studied.

Problem statements for this study are:

1. Botanical drinks have been claimed to give many health benefits to the consumers but there is no scientific evidence to the fact especially on the effect of botanical drink against food-related pathogens bacteria.
2. There is no *in vitro* study to evaluate of the effectiveness of the components in botanical drinks against the food-related pathogens.

The hypothesis for this experiment is the botanical drink has the antibacterial activity that can affect the growth of the bacteria.

The objectives of this study are:

1. To determine the effect of the botanical drink against food-related pathogenic bacteria.
2. To evaluate the effectiveness of the materials in the botanical drink on the inhibition of the bacteria growth.
3. To compare which of these ingredients will play most important role to inhibit the growth of the bacteria.

2.0 Literature Review

2.1 Botanical Drink

Botanical drink is one of the products that have been produced by the entrepreneurs as a supplement for the consumers. Botanical is a plant or parts of plant that been used for therapeutic or medicinal purposes, fragrance and flavouring (Food Processing, 2012). Parts of plant that may be used are seeds, roots, stalk, flowers, barks and extracted chemicals from the plants (Synovitz & Larson, 2013). Synovitz and Larson also stated that botanical may be formed as constituent chemicals or in a crude form. Botanical drink that will be used in this experiment consists of lemon (*Citrus limon*) juice, garlic (*Allium sativum*), ginger (*Zingiber officinale*), honey and apple cider vinegar.

2.1.1 Lemon juice

Lemon, *Citrus Limon,* is from the family *Rutaceae* that acts as an essential nutrients and source of vitamins (Jumare *et al.*, 2015). Lemon juices have flavonoids that function as antioxidant and free radical scavengers. It also inhibits cell proliferation and exhibiting the capacity to modulate enzymatic activities. Lemon fruit is a citrus fruit or acidic fruit that is used as medicine, especially during flu or cold. In the past, lemon was used as an ornament and medicine. Lemon fruit consists of 5% of citric acid on its juice and it has many biological activities include antibacterial, anticancer, antiviral and antifungal (Bata, 2014). Lemon was able to inactivate the *Escherichia coli* and *Salmonella* at -11 °C (Nogueira *et al.*, 2003).

2.1.2 Ginger

Zingiber officinale or also known as ginger is the perennial herb that from Zingiberaeae family. In Malaysia, the traditional medicine by using ginger is by extract it in the hot water and will be consume orally by the women after giving birth or by the women that had went through abortion. The antibacterial activity of the ginger is including the weak production of *Bacillus subtilis, Escherichia coli* and *Streptococcus aureus* after the insertion of ethanol extract of dried rhizome (Ross, 2005). Indu *et al.* (2006) has stated ginger extract had a moderate antibacterial property against *E.coli* serogroups O8 and O88. However, their experiment on antibacterial activity of spice extracts showed a negative result as ginger extracts did not had any antibacterial activity against the serogroups of the *E.coli*.

2.1.3 Garlic

Garlic has been used for medicinal and dietary for several centuries. It is used to treat diarrhea, respiratory tract infections and discomfort (Jumare, Yakubu, & Ameh, 2015). According to Daka (2011), in 1150 BC, during the ancient Egyptians, garlic was used as medicine especially for the people that doing the labor worked. The properties of garlic as the antibacterial, antifungal, antiseptic and anti-inflammatory had been documented. Garlic extracts also can exhibit the activity against the gram positive bacteria and the gram negative bacteria. Garlic oil was able to inhibit the production of toxin by *Clostridium botulinum* type A but unable to inhibit toxin production by the *C. botulinum* type B and type E in meat slurry (De Wit *et al.*, 1978).

2.1.4 Honey

Honey is a sweet food product that produced by bees. It has distinct flavour and taste, high viscosity, sweetness and has a range of colours (Verma, 2009). Medicinally honey promotes rapid growth of healthy tissues, it is non- irritant and is useful in pruritus value, bed sores, skin and intestinal disorders. It is very commonly used internally in treatments of cough, cold, fever and gastrointestinal disorders. It helps to replenish the energy lost in many kinds of physical activities. Divers, athletes, mountaineers, workers and patients in hospitals are some of the society who requires the honey the most (Verma, 2009). Honey has the antibacterial activity that produces hydrogen peroxide and it contained high osmolarity (El-Sukhon *et al.*, 1994). Honey is operated as a wound treatment as it has the antibacterial activity and the anti-inflammatory properties (Bata, 2014). Honey inhibited more than 20% of bacterial growth at the lowest concentration except for *Bacillus* cereus (Zainol *et al.*, 2013).

2.1.5 Vinegar

Vinegar is usually used for cooking, preparing salad and pickling of vegetables and fruits. Vinegar has the bioactive components that can cause antimicrobial, antidiabetic, anti-oxidative and cholesterol-lowering responses (Budak *et al.*, 2014). Examples of bioactive components are acetic acid, ferulic acid and catechin. Vinegar can cause the inhibition of enterohemorrhagic *E. coli* strain because vinegar has 0.1% concentration of acetic acid (Entani *et al.*, 1998). Apple, grape and other fruit juices are the starting materials for the vinegar production (Budak *et al.*, 2014). Vinegar also has the strongest antibacterial activity against *Salmonella enterica, Listeria monocytogenes* and *E. coli* (Medina *et al.*, 2007). Apple cider

vinegar is one of the traditional medicines that already been used in many years ago (The Alternative Daily, 2014).

2.2 Food poisoning

Food poisoning or foodborne disease is a disease that caused by bacteria, viruses, protozoa, fungi and toxins. The major symptom of food poisoning is involving gastrointestinal symptoms such as diarrhea and vomiting (Jones, 2007). Some of food poisoning disease can cause neurological disease that can cause mortality or morbidity. In Malaysia, the food poisoning bacteria has the highest incidence rate in food and vector borne disease in 2016 (Department of Statistic Malaysia, 2016). Food poisoning outbreaks are involving more than two person that have a similar illness because they consume a food that already been infected by the bacteria or viruses (Jones, 2007).

To reduce the risk of foodborne disease, medical care, diagnostic capabilities and improved the basic sanitation can be applied. The pathogens' growth also needs to be controlled to reduce the food poisoning disease outbreak (Sethi *et al.*, 2013). The increasing of foodborne disease throughout the year can cause the increasing use of artificial antimicrobials or preservatives that made from chemicals to inhibit the growth of the pathogenic bacteria that can cause food poisoning disease (Tajkarimi *et al.*, 2010).

2.3 Food-related Pathogens

Food-related pathogens are including bacteria, viruses and protozoa. Most of the food poisoning disease is caused norovirus, *Salmonella*, *Clostridium perfringens* and *Campylobacter* spp. (Scallan *et al.*, 2011). The nontyphoidal *Salmonella* spp. is the bacteria that mostly can causes hospitalization and causes death. According to Tajkarimi *et al.* (2010),

Bacillus sp. can be inhibited by clove, mustard, cinnamon, garlic, ginger and mint. Mustard, clove, cinnamon, garlic, ginger and mint can inhibit the *E.coli*.

3.0 Materials and methods

3.1 Bacterial strains

Salmonella, *Staphylococcus aureus* and *Escherichia coli* strains are the food poisoning bacteria that were used from Microbiology Laboratory Stock Culture Collection. These bacteria were reconstituted into Luria bertani (LB) broth (BD Difco, United States) and were incubated at 37 °C for 24 hours (Zainol *et al.*, 2013). Then, these bacteria were sub-cultured on their selective agar. *E.coli* was sub-cultured on Eosin Methylene Blue (EMB) agar (Oxoid, United Kingdom), *S.typhii* was sub-cultured on Xylose Lysine Deoxycholate (XLD) agar (BD Difco, United States) and *S.aureus* on Mannitol Salt (MS) agar (Merck, United States). The primary culture of the bacteria will be inoculated into a universal bottle that contained 10 mL of LB broth and the inoculum will be incubated for 24 hours at 37 °C before proceed to the next steps.

3.2 Botanical drink

All the individual materials that involve in making botanical drink were bought at local store or market such as Survey Hypermarket, H&L and Mydin. Firstly, the experiment was conducted by testing lemon juice, garlic, ginger, honey (Hexa, Taiwan) and apple cider vinegar (Halawatussunah, Malaysia) on the effect of the bacteria growth. Honey and apple cider vinegar were prepared by dissolving it in the distilled water so that it was reached 50% concentration and the pH value for both will be taken by using the pH meter.

Lemon fruits (*Citrus limon*) were being squeezed in a juicer so that the lemon juice can be obtained. The pH of the lemon juice was taken so that the range of the pH is around 1.8 to 2.2 (Tomotake *et al.*, 2006).

Garlic and ginger were cleaned, descaled and washed by using sterile distilled water. 100 g of the garlic were sterilized by using ethanol. The ethanol was allowed to evaporate in sterile laminar flow chamber. Then, the garlic were homogenized aseptically by using a sterilize mortar and pestle. The extract was squeezed out by using sterile muslin cloth (Indu *et al.*, 2006). For ginger, 100 g of the ginger was crushed with mortar and pestle. The extracts of the ginger were sieved by using a muslin cloth and the ginger's extract was sterilized by using membrane filter that has 0.45-micron filter.

Three different commercial botanical drinks were bought at the local store such as Sharifah Trading. Examples of the botanical drinks are Jus Selesa (Al Ejib, Malaysia), Jus Pembersih Kolesterol (Ihsan Al Barakah, Malaysia) and Jus Lega (Lega Health Sdn Bhd, Malaysia). Then, it was prepared 50% (v/v) of the concentration by mix it with distilled water as recommended by Zainol *et al.* (2013).

For the preparing formulated botanical drink, 250 ml of the honey, 125 ml of lemon juice, 125 ml of ginger extract, 250 ml of garlic and 250 ml of apple cider vinegar were mixed together. After the ingredients were being mix, the juice was put in the refrigerator for at least five days before being used for the study.

Table 3.1 shows the summary of the preparation that was involved in the methodology of botanical drink. The concentration of all the materials and the botanical drink was summarized in the Table 3.2.

Table 3.1. Summary of botanical drink preparation

Activity	Explanation
Botanical drinks	Preparing honey (Hexa, Taiwan), apple cider vinegar (Halawatussunnah, Malaysia), fresh lemon juice, garlic and ginger extract,
	Preparing the commercial botanical drink which are Jus Selesa (Al Ejib, Malaysia), Jus Pembersih Kolesterol (Ihsan Al Barakah, Malaysia) and Jus Lega (Lega Health Sdn Bhd, Malaysia)
	Preparing the own formulation for the botanical drinks by using the materials

Table 3.2. Summary of the concentration of the materials and botanical drinks

Materials	Concentration
Honey	50% (v/v) = 5 ml of honey with 5 ml of distilled water
Garlic and ginger extract	50% (v/v) = 2.5 ml of garlic or ginger extracts with 2.5 ml of distilled water
Lemon juice	50% (v/v) = 2.5 ml of lemon juice with 2.5 ml of distilled water
Apple cider vinegar	50% (v/v) = 2.5 ml of apple cider vinegar with 2.5 ml of distilled water
Botanical drinks	50% (v/v) = 5 ml of botanical drinks with 5 ml of distilled water

3.3 Antibacterial Activity Assays

Antibacterial activity assay was done on Brain Heart Infusion (BHI) agar (Oxoid, United Kingdom) medium by using the disk diffusion method as recommended by Silva *et al.* (2016). The bacterial cultures were adjusted to 0.5 McFarland standards (1×10^8 cfu/ml) with sterile saline solution that is Phosphate Buffer Saline (PBS) solution. Then, by using sterile cotton swab, the bacterial cultures were spread over the control and test plates that contained BHI agar so that a uniform microbial growth result will be obtained. Six mm diameter of sterile paper discs were filled with 20 µl of the ingredients and the botanical drink. After that,

the sterile paper discs were placed on the surface of the inoculated BHI agar plates. The plates containing the BHI agar with the materials and botanical drinks were incubated at 37 °C for 24 hours and the zones of inhibition were measured. The antibacterial activity was assessed by the formation of inhibition zone that are "there is inhibition zone" and "there is no inhibition zone". For the control, a disc that containing the antibiotic, ciprofloxacin, was used as reference and the negative control was the distilled water. Ciprofloxacin was used as positive control because ciprofloxacin is a commonly used antibiotic and it is active against wide range of gram negative and gram positive bacteria. The antibacterial assay was repeated three times for each of the ingredients and the botanical drink.

3.4 Minimum Inhibitory Concentration Assay

Minimum Inhibitory Concentration (MIC) assay was done on the BHI agar plate by using the disc diffusion method as recommended by Silva *et al.* (2016). Each of the ingredients and the botanical drink were dissolved in an distilled water solution so that it achieve 50%, 25%,15%, 5% and 1% (v/v) of the final concentration. The sterilized disc was filled with 20 µL of mixtures (materials or botanical drinks with distilled water). Then, it was put on the BHI agar surface. The ciprofloxacin and distilled water were the positive and the negative control respectively. The plates were incubated at 37 °C for 18-24 hours and the zones of inhibition were measured. The MIC was repeated three times for each of the materials and botanical drinks.

4.0 Result

4.1. Antibacterial Activity Assay

The antibacterial activity of five materials (honey, apple cider vinegar, lemon juice, ginger and garlic) was shown in the Table 4.1. All of the materials were diluted with distilled water. Hence, the concentrations of each of the materials, botanical drinks and formulation botanical drink were 50%. Table 4.1 represents the average results of the inhibition zone of the triplicate assay. As shown in Figure 4.2 and Figure 4.3, apple cider vinegar has the largest inhibition zone when tested on gram negative (*Salmonella typhii*) and gram positive bacteria (*Staphylococcus aureus*). The inhibition zone for the apple cider vinegar against *S. typhii* and *S.aureus* are 11 mm and 10.67 mm respectively. However, garlic has the largest inhibition zone, which is 9.33 mm, referred to Figure 4.1, when tested on *Escherichia coli*. Materials that have the lowest inhibition zone for gram positive bacteria and gram negative bacteria are honey and ginger respectively.