Antibacterial Activities of Selected Vegetables (Ulam) Extracts

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ABSTRACT

This research was carried out to study the antibacterial activity of ten species of Malay traditional vegetables against some species of bacteria that were pathogenic to human such as Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium and Staphylococcus aureus. The ten species of table salads (ulam) used in this research were Averrhoa bilimbi L., Etlingera elatior, Vigna sinensis, Centella asiatica, Parkia speciosa, Diplazium esculentum, Manihot esculenta, Mentha arvensis L., Oenanthe javanica and Cosmos caudatus. The antibacterial activity was done by using disc diffusion method. The result showed that all the sample extracts except Parkia speciosa inhibited the growth of Staphylococcus aureus and Salmonella typhimurium. For the Escherichia coli, only Averrhoa bilimbi L., Vigna sinensis, Centella asiatica and Diplazium esculentum inhibit the growth of these bacteria. In addition, all samples inhibit the growth of Pseudomonas aeruginosa except Parkia speciosa and Mentha arvensis L. Extracts of Etlingers elatior showed the highest antibacterial activity against Pseudomonas aeruginosa with 10.1 mm diameter of inhibition zone while Centella asiatica show the lowest antibacterial activity against Pseudomonas aeruginosa with 2.2 mm diameter of inhibition zone. Besides, the Etlingera elatior, Parkia speciosa, Manihot esculenta, Mentha arvensis L., Oenanthe javanica and Cosmos caudatus showed a negative result for the antibacterial test onto the gram-negative bacteria Escherichia coli.

Key words: *antibacterial activity, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, inhibition zone*

Introduction

The increasing use of antibiotics for antibacterial therapy has initiated a rapid development and expansion of antibiotic resistance in human pathogens. An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity) or parasites (anti-parasitic activity). Antimicrobial drugs either kill microbes (microbidal) or prevent the growth of microbes (microbistatic) (Srimathi et.al, 2011).

Nowadays, many people are concern about their own health and complementing of their treatment with medicine from natural resources. The conscious of people towards medicine from natural resources is increasing due to the worry about the side effects of using synthetic chemical drugs. Natural resources provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to the increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action (Chanda et.al, 2010).

The explorations of the new sources of antimicrobial extracts from plant have become a great interest among researchers. It is mainly due to the current widespread belief that green medicine is safe and more affordable than the synthetic drug (Jigna and Sumitra, 2006). The screening of antibiotic resistance modifying compounds from plants sources are expected to provide the basis for identifying leads for the isolation of therapeutically useful compounds. The antimicrobial constituents are present in all parts of the plant viz. bark, stalks, leaves, fruits, roots, flowers, pods, seeds, stems, latex, hull and fruit rind (Rajaei et.al, 2010). Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and terenoides, amongst which can be cited xanthones, benzophenones, coumarins and flavonoids (Belguith et al., 2010). Fruit and vegetable can be utilized as a source of antimicrobials. It will be economic, eco friendly and reduce pollution (Chanda et.al, 2010).

Ulam is a Malaysian term for table salad which refers to any vegetable that is eaten raw. Malaysians usually eat *ulam* because of their therapeutic effect. Some Malaysian *ulam* are used to cure cough, fever, flu and headache. Numerous studies have been reported on the antimicrobial activities of plant extracts against different types of microbes, including food-borne pathogens (Hara-Kudo et.al, 2004). The ability of *ulam* to produce bioactive compounds and its nature resistance towards the microbial disease was made us conducted a study in order to explore the antibacterial potency of a common *ulam* against microbes such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhimurium.* Besides that, the purpose of the study also to produce scientific information of the antimicrobial extracted from *ulam* which is still lacking and limited. *Ulam* was identified as potential unlimited free resource for this study.

Materials and Methods

Plant Materials and Experimental Procedure

The ten species of Malay traditional vegetables used in this research were *Averrhoa bilimbi L., Etlingera elatior, Vigna sinensis, Centella asiatica, Parkia speciosa, Diplazium esculentum, Manihot esculenta, Mentha arvensis L., Oenanthe javanica* and *Cosmos caudatus.* The procedure for this study divided into three parts, the preparation of media, preparation of plant extracts and antibacterial studies using anti-bacterial tests. Two types of media consist of solid media (agar) and liquid media (broth) are prepared for the test according to the specification set by manufacturer.

Plants Extraction

Ulam samples were cut and dried in an oven at 50°C for two days. Then, the samples were grinded to get fine powder. About 4.0 g of the powder samples were dissolved with 25 ml of methanol to produce methanol extracts. The mixtures were stirred for three hour in the magnetic hot plate and then the suspension was filtered to get the methanol extracts.

Bacteria Culture

In order to study the effects of the antimicrobial activities of selected *ulam*, four different types of bacteria are chosen. These bacteria are chosen based on their impacts on human health and have been identified as pathogens to human health. For the bacteria, it can be classified as gram-positive and gram-negative bacteria. Bacterial colonies were inoculated into liquid media and were shake using incubator three hours at 37°C. The colonies were cultured to 0.2 concentrations at 625nm. About 1.5 ml of bacterial culture is then poured onto agar media. All of the bacteria's strain will be subculture every three weeks for its viability.

Four strains of bacteria used in this study are:

Gram-negative	Gram-positive
Escherichia coli *Pseudomonas aeruginosa* *Salmonella typhimurium*	*Staphylococcus aureus*

Disc Diffusion Method

Disc diffusion method used in this study to investigate the effect of antibacterial activity selected Malay traditional vegetables against four strains of bacteria. This method was performed in which the plant extracts (methanol extracts) pipette on 6 mm AA disc. Gentamicin was used as a positive control and empty AA disc with methanol used as negative control. The three discs are placed on the surface of the bacteria media. The Petri discs are kept in incubator at 37°C overnight. The inhibition zone appears if the samples have antibacterial activity.

Statistical analysis

Data will be analyzed using SPSS version 12 and analysis of variance (ANOVA). Mean comparison of the inhibition zone will be recorded as min ± standard deviation (SD).

Result

Table 1 shows the reluts of the study. The results revealed that the MeOH extracts of *Averrhoa bilimbi* was the most successful samples that can inhibit the growth of *Escherichia coli*. The average reading retardation zone diameter was 5.3 mm. Among these ten species, *Etlingera elatior, Parkia speciosa, Manihot esculenta, Mentha arvensis, Oenanthe javanica,* and *Cosmos caudatus* did not inhibit the *E. coli* at all. There is no inhibition zone diameter readings recorded for the antibacterial activity. There are only two species that cannot inhibit the growth of these bacteria. The species are *Parkia speciosa* and *Mentha arvensis*. The *Etlingera elatior* is the broad-spectrum antibacterial agent for P. aeruginosa. The inhibition zone is 10.1 mm. It can be concluded that nearly all selected species can inhibit the growth of *Pseudomonas aeruginosa.*

Based on Table 1, eight species give a positive result while the other two species give a negative test result. Species gives a positive result is the fruit of *Averrhoa bilimbi, Etlingera elatior, Vigna sinensis, Centella asiatica, Diplazium esculentum, Manihot esculenta, Mentha arvensis,* and *Oenanthe javanica.* The inhibition zone for these species is between 1.7 mm to 5.6 mm. Species that give negative result is *Parkia speciosa* and *Cosmos caudatus.* Besides that, *Etlingera elatior, Diplazium esculentum, Mentha arvensis* and *Oenanthe javanica* give almost the same inhibition zone diameter reading. In contrast, the *Parkia speciosa* can not inhibit the growth of *Staphylococcus aureus*. The others

samples give inhibition zone diameter reading between 2.7 mm to 7.5 mm. *Centella asiatica* give the biggest inhibition zone which is 7.5 mm.

Table 1: Antibacterial activity (mm inhibition zone diameter) of the methanol extracts of vegetables (ulam)

Sample	Inhibition one (mm) of bacteria			
	E. coli	***P. aeruginosa***	***S. aureus***	***S. typhimurium***
Averrhoa bilimbi L.	5.3 ± 0.47	4.2 ± 0.14	6.6 ± 1.14	5.6 ± 0.83
Etlingera elatior	-	10.1 ± 0.69	3.4 ± 1.79	1.7 ± 0.47
Vigna sinensis	2.6 ± 0.94	4.2 ± 1.48	3.2 ± 1.04	3.3 ± 1.47
Centella asiatica	2.2 ± 0.12	2.2 ± 1.48	7.5 ± 2.17	5.5 ± 1.36
Parkia speciosa	-	-	-	-
Diplazium esculentum	2.9 ± 0.59	7.5 ± 2.07	2.6 ± 1.51	1.9 ± 0.14
Manihot esculenta	-	2.5 ± 0.33	3.4 ± 0.19	3.0 ± 1.39
Mentha arvensis L.	-	-	3.6 ± 1.47	1.8 ± 0.41
Oenanthe javanica	-	6.2 ± 1.6	2.9 ± 1.13	1.7 ± 0.29
Cosmos caudatus	-	4.2 ± 0.66	6.9 ± 1.07	-

Mean (±SD) indicate statistically differences at $P \leq 0.05$.

Discussion

The different inhibition zones diameter caused by various factors. Among them are due to the differences of main compounds in the sample extract (Delamare et al., 2005). *Averrhoa bilimbi, Vigna sinensis, Centella asiatica, and Diplazium esculentum* showed antibacterial activity against all four strains of bacteria. According to the previous study, the samples contain the important compounds such as amino acids, lipids, proteins and nucleic acids (Ismail, 2000). In addition, it also contained the alkaloid compounds, flavonoids, glycosides, amino lysergic etc. (Nornisah et al., 2005). These compounds were important in determining whether a plant was potentially to be antibacterial agents (Fasihuddin et al., 1993). Fasihuddin (1993) also found that many plants used in medicine industry contained alkaloids. Alkaloids belong to the group of antibiotics and secondary metabolites.

Based on the results obtained, the growth of *Staphylococcus aureus* (gram-positive bacteria) can be inhibited by almost all plant MeOH extracts, except for *Parkia speciosa*. Growth of gram-negative (gram –ve) bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium* only inhibited by some samples extracts such as *Averrhoa bilimbi L., Vigna sinensis, Centella asiatica and Diplazium esculentum*. These showed that antibacterial activities against gram-positive (gram +ve) bacteria were higher than antibacterial activities against gram -ve bacteria. These results were expected because of the gram -ve bacteria generally more resistant to antibacterial agents compared to gram +ve bacteria (Romero et al., 2005). Gram -ve were resistant to antibacterial agents because of the structure of gram –ve bacteria have three layers of cell walls that consists of inner membrane, several layers of peptidoglycan and outer membrane compared to gram +ve bacteria which have only a two-layer cell wall (Tortora et al., 2004). Outer membrane contains thick and impermeable lipopolysaccharide (LPS, which consists of lipid A, core polysaccharide, and O antigen) outside the peptidoglycan layer (Delamare et al., 2005)

These layers serve as a barrier to antibiotics, digestive enzymes and so on. In this study, the antibacterial agent was unable to penetrate the membrane and inhibited the bacterial growth. However, gram +ve bacteria have thick peptidoglycan and teicoic acids. However, peptidoglycan can be easily digested by lisozim (Tortora et al., 2004). This simplifies the antibacterial agents to inhibit bacterial growth (Delamare et al., 2005).

The thickness of the media also influences the diameter of inhibition zone. If the diameter of inhibition zone is thick, the diameter of the inhibition zone is small and if the media is thin, the diameter of inhibition zone is large (Madigan et al., 2003). This is related to the content in the media. If the media is too thick, nutrient supplied to the bacteria is high. This allows the bacteria to growth to the maximum and difficult to control and retard. If the media is too thin, the nutrients can be supplied in small amounts. These can control the growth and easily to inhibit the bacteria growth.

Conclusion

In conclusion, it was found that all samples extracts except *Parkia speciosa* inhibited the growth of one or more of the bacteria used in this study. Overall, the *Averrhoa bilimbi* extracts is a broad-spectrum antibacterial agent found in this

study which can inhibit all the bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* and *Staphylococcus aureus*. The inhibition zone of all bacteria is more than 4 mm. The antibacterial activities of selected table salad against four strains bacteria was influenced by various factors such as the differences of major compounds in each extract (Delamare et al., 2005). In addition, the sensitivity of the bacteria tested, the thickness of the media and the substances of the antibacterial also was influenced the diameter of the inhibition zone. The future study will be focused on selection of the best concentration of sample extracts to inhibit bacterial growth using the MIC (Minimum Inhibitory Concentration) method. The MIC methods allow us to find the lowest concentration of the extract sample which can inhibits the growth of bacterial.

References

Belguith H., Kthiri F. (2010). Inhibitory effect of aqueous extract of Allium Sativum. African journal of Microbiology Research. Vol 4(5):328-338.

Chanda, S., Baravalia, Y., Kaneria, M. and Rakholiya K. (2010). Fruit and vegetable peels – strong natural source of antimicrobics. Current research, Technology and education topics in Applied Microbiology and Microbial Technology. 2010.

Delamare, A. P. L., Moschen-Pistorello, I. T., Artico, L., Atti-Serafini, L., Echeverrigaray, S., (2005). *Antibacterial activity of the essential oils of Salvia officinalis L. and Salvia triloba L. cultivated in South Brazil.* Food Chemistry, **100**, 603-608.

Fasihuddin, A., Hasmah, R., (1993). *Kimia Hasilan Semulajadi dan Tumbuhan Ubatan.* Dewan Bahasa dan Pustaka, Kuala Lumpur.

Hara-Kudo Y, Kobayashi A, Sugita-Konishi Y, Kondo K (2004). Antibacterial activity of plants used in cooking for aroma and taste. J. Food Prot. 67: 2820-2824.

Ismail, S., (2000). *Sayuran Tradisional Ulam dan Penyedap Rasa.* Penerbitan Universiti Kebangsaan Malaysia, Selangor.

Jigna, P. and Chanda, S. (2006). In vitro antimicrobial activities of extracts of *Launaea procumbeus* Roxb. *(Labiateae), Vitis vinifera al L.* (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr. J. Biomed. Res. 9(2): 89-93.

Madigan, M. T., Martinko, J. M., Parker, J., (2003). *Brock Biology of Microorganisms*, Pearson Education, United State of Amerika.

Nornisah, M., Shaida, F. S., Suriyati, M., Zuraini, Z., Habibah, A. W., (2005). *Khasiat Ulam-ulaman*, Penerbitan Universiti Sains Malaysia, Pulau Pinang.

Rajaei A, Barzegar M, Mobarez AM, Sahari MA, Esfahani ZH. Antioxidant, antimicrobial and antimutagenicity activities of pistachio (Pistachia vera) green hull extract. Food Chemistry and Toxicology. 2010;48:107-112.

Romero, C. R., Chopin, S. F., Buck, G., Martinez, E., Garcia, M., Bixby, L., (2005). *Antibacterial properties of common herbal remedies of the southwest.* Journal of Ethnopharmocology, **99**, 253-257.

Srimathi, S., Devi Narayani, K. S., Muthumary, J. (2011). Studies on Antimicrobial Activities of *Chaetomium atrobrunneum* Ames against Selected Microorganisms. *Journal of Experimental Sciences 2(5): 13-18.*

Tortora, G. J., Funke, B. R., Case, C. L., (2004). *Microbiology An Introduction*, Pearson Benjamin Cummings, United State of Amerika.

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