ISOLATION AND CHARACTERIZATION OF ANTIMICROBIAL PROTEINS FROM FRUITS
(KIWI, BANANA AND COCONUT)

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THIS DISSERTATION IS SUBMITTED AS A PARTIAL REQUIREMENT FOR OBTAINING
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Antimicrobial proteins can be found in organisms ranging from bacteria to plants and mammals. Researches have been focusing on studying the antimicrobial proteins as infections caused by pathogenic bacteria and fungi can cause an expressive negative impact on both human and plants. Based on this study, antimicrobial proteins were extracted from the kiwi fruit, banana and coconut via three different methods of extraction of sodium chloride extraction, Tris-HCl buffer extraction and acetic acid extraction. Bradford assay was then carried followed by screening for antimicrobial activity and concentration dependency test. Lastly, the SDS-PAGE was carried out and the crude protein samples were purified via gel filtration chromatography. The highest crude protein recovery was achieved through the extraction of the antimicrobial proteins via Tris-HCl buffer extraction. The growth of both gram-positive bacteria and gram-negative bacteria of B. cereus, S. aureus, S. thyphimurium and E. Coli were able to be inhibited by antimicrobial proteins extracted from kiwi via Tris-HCl buffer and acetic acid, and also banana and coconut via sodium chloride. However, the antimicrobial proteins extracted from the fruits samples do not show any inhibition activity towards B. cinerea. Protein bands for M.a.1, C.n.1, A.d.2, M.a.2, C.n.2 and A.d.3 extracted via sodium chloride, Tris-HCl buffer and acetic could clearly be observed and has the size within the range of 14.4 kDa- 20 kDa, 24 kDa-30 kDa and 30- 43 kDa. The protein bands for the other fruits samples were not clearly visualized on the SDS-PAGE gel. The crude antimicrobial proteins were then purified using gel filtration chromatography which resulted in high protein recovery of the purified proteins for the fruit samples of kiwi, banana and coconut which corresponds to the high absorbance peaks which were obtained at 280 nm. Further studies on antimicrobial proteins extracted from fruits should be carried out to help in the treatment of several pathogen-infected diseases.
ABSTRAK

Protein yang mempunyai ciri-ciri antimikrobial dapat dijumpai dalam kebanyakan organism yang merangkumi bakteria, tumbuhan dan haiwan. Para pengkajian telah giat menjalankan kajian berdasarkan protein yang mempunyai ciri-ciri antimikrobial kerana jangkitan yang disebabkan oleh bakteria merbahaya dan fungi bukan sahaja dapat membahayakan manusia bahkan juga terhadap tumbuhan. Berdasarkan kajian yang dijalankan, protein yang mempunyai ciri-ciri antimikrobial dapat diekstrak daripada buah kiwi, 'Pisang Mas' dan kelapa melalui tiga kaedah pengekstrakan yang berbeza iaitu melalui penggunaan larutan sodium klorid, penimbal Tris-HCl dan asid asetik. Selepas kaedah pengekstrakan, ujian Bradford dijalankan ke atas sampel protein dan diikuti oleh penyaringan terhadap aktiviti antimikrobial. SDS-PAGE dijalankan ke atas sampel protein dan diakhiri dengan penulenan sampel protein tersebut menggunakan kromatografi penyaringan gel. Antimikrobial protein daripada sampel bagi buah kiwi, 'Pisang Mas' dan kelapa yang diekstrak menggunakan larutan sodium klorid, penimbal Tris-HCl dan asid asetik adalah berkesan dalam menyebabkan perencetan terhadap pertumbuhan kedua-dua bakteria gram-negatif dan bakteria gram-positif. Berdasarkan SDS-PAGE, hanya jalur protein bagi sampel M.a.1, C.n.1, Ad.2, M.a.2, C.n.2 dan A.d.3 dapat diperhatikan secara jelas di atas gel dan mempunyai berat molekul di dalam lingkungan 14.4 kDa - 20 kDa, 24 kDa-30 kDa and 30 kDa- 43kDa. Jalur protein bagi sampel selebihnya tidak dapat diperhatikan di atas gel SDS-PAGE. Proses penulenan menggunakan kromatografi penyaringan gel sampel dapat memberikan nilai pemulihan antimikrobial protein yang tinggi dimana selari dengan puncak graf resapan yang tinggi. Oleh itu, kajian yang lebih mendalam perlu diadakan terhadap antimikrobial protein daripada buah-buahan untuk membantu dalam merawat penyakit-penyakit yang disebabkan oleh fungi dan bakteria yang merbahaya.
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1.1 Background

Recently, infections that have been caused by pathogenic bacteria have increased. Infections caused by pathogenic bacteria such as tuberculosis, pneumonia, tetanus, typhoid fever and syphilis, are feared by the public as they could lead to a detrimental impact on human health. Moreover, researchers have discovered that there is a significant increase in the bacterial resistance to commercial antibiotics. Pharmaceuticals such as antibiotics, analgesics and hormones used to treat these infections are produced based on the production of relatively small organic molecules synthesized by microbes or by organic chemistry. However, recently much attention has been given to the use of larger and more complex protein molecules as therapeutic agents. Proteins are large organic molecules that consist of long chains of amino acids. The function of a given protein is determined by the amino acids sequence of the protein itself. Therefore, it also determines the three-dimensional conformation or structure of the protein. Proteins that function to fight against infections are known as therapeutic proteins. These types of protein have many potential therapeutic uses in preventing and curing diseases and disorders. Insulin was the first protein used to treat disease. It is used for the treatment of diabetes. Since 1982, many therapeutic proteins or peptides have been licensed for production using bacterial, fungal and mammalian cells grown in sterile cultures and there are still additional therapeutic proteins are being developed and tested until recently (Thomas et al., 2002).
Infections are normally caused by pathogenic bacteria and fungi. Therapeutic proteins that have the ability to inhibit the growth of bacteria are known as antibacterial proteins. Antibacterial proteins are thus, able to protect organisms from the pathogenic effects caused by the bacteria. On the other hand, antifungal proteins are proteins that inhibit the growth of fungi and thus, able to protect organisms from the pathogenic effects caused by the fungal pathogens (Xia & Ng, 2004). However, both of the antibacterial and antifungal protein can generally be known as antimicrobial proteins. Most of researchers focused only on isolation and production of antimicrobial proteins normally from microbes and plants. Apart from plants and animals, antimicrobial proteins can also be successfully isolated from fruits. Until recently, many antimicrobial proteins isolated from fruits are increasingly and commercially used to produce pharmaceuticals products.

Generally, it is known that fruits are naturally and famously consumed due to their nutritional benefits. Nowadays, people are getting more conscious about what they eat due to the increase in their interest in nutrition. Thus, making their interest in fruits and its contents also grows. However, our knowledge about fruits is still limited and there is still much to discover about fruits and the substances that the fruits contain. Protein is one of the substances that can be found in fruits. Raw fruits and vegetables are also the best source of protein apart from meat. Eating fruits is known to allow us to live a healthier life and also help to prevent or cure diseases. Three types of fruits that will be used in this study are green kiwi fruit, banana and coconut. There have been many studies carried out to study the therapeutic properties of antimicrobial proteins isolated from fruits. However, knowledge about the fruits therapeutic proteins is limited in each type of fruits as different types of fruits has different sets of defence proteins which are effectively used to fight and protect invading against pathogens (Wang & Ng, 2002).

Thus, the purpose of this study is to investigate the antimicrobial activity of fruit protein. The antimicrobial activity of the fruit protein isolated from three types of fruit which are green kiwi fruit, banana and coconut will be tested by using the antibacterial assay and antifungal assay. Apart from that, this study will also be specifically carried out to learn the difference of the antimicrobial activity between crude extract protein and also protein that will be purified. From the study, it is
being hoped that the examination on the antimicrobial activity of the protein isolated from the three fruits will able to provide further information and knowledge on the types and activities of the antimicrobial proteins for the purpose of future research or treatment of variety of diseases caused by microbes.

1.2 Objectives of Research

There are four main objectives of this study:

i) To extract antimicrobial protein based on sodium chloride extraction, Tris-HCl buffer extraction and acetic acid extraction from kiwi, banana and coconut.

ii) To conduct Bradford, antibacterial and antifungal assay on the crude protein extract of kiwi fruits, banana and coconut.

iii) To purify the crude antimicrobial protein extract from kiwi fruits, banana and coconut.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Fruits

Fruit is the ripened seed-bearing part of a plant when the part become fleshy and edible or can generally be defined as any fleshy material covering a seed. The three main parts involves in the growth of the fruit from the seeds are fertilization, embryology, and the development of fruits and seeds. Fruits are a well known food to be consumed for their fresh and nutritional value. Formerly, fruits are readily consumed in their raw form. However, as time passes by, the serving style of fruit also changes. Nowadays, fruits are served and consumed as food by million of people in the form of salads, soups, juices, jams and pickles. Apart from being consumed as nutritional and dietary food, fruits have also been exploited for the production of commercial products such as health and beauty products. The reason for the fruits' fame and popularity is due to the fact that researchers have managed to discover that fruits are actually low in calories and packed with vitamins, minerals, and antioxidants and photochemical.

Fruits are available in three basic types which are simple fruit, aggregate fruit and multiple fruit. Simple fruit can be found either in the form of dry or fleshy. The simple fruit is a result from the ripening of a simple or compound ovary with only one pistil. Examples of simple fruits are coconut, walnut, tomato, banana and cranberry. On the other hand, aggregate fruit develop from a flower with numerous simple pistils and such fruits are raspberry, blackberry and strawberry. Lastly, multiple fruit is formed from a cluster of flowers in which each flower produces a fruit and mature into a single mass. Pineapple, mulberry and figs are the examples of multiple fruit.
2.1.1 Types of Fruits and Taxonomy

Fruits can be found in various types and species within different parts of the world. Fruits are usually being classified based on their different characteristics such as the climate of the place where they can be found, shape, taste and nutritional value. However, only three types of fruits are investigated in this study which is kiwi fruit, banana, and coconut. Detail description and information about the three types of fruits will be emphasized in this study.

a. Kiwi Fruit

Figure 2.1: *Actinidia deliciosa*

Kiwi fruit is the fruit of the large woody vine of the genus *Actinidia* from the order of *Ericales*. The large woody vine of the genus *Actinidia* is normally found growing in temperate climates. Kiwi fruit is also known as "Chinese gooseberry" as the fruit originates from China and it tastes similar to a gooseberry fruit. The kiwi fruit has a sweet and mildly tangy taste. Kiwi fruit have been discovered since several centuries back and found mostly growing wild in China. Unfortunately, New Zealand only started to discover and began cultivating the kiwi fruit on a large commercial scale in the early 20th century onwards. Generally, the kiwi fruit has a hairy, dull-brown skin
with edible black seeds in either green or yellow flesh (Wang & Ng, 2002). The kiwi fruit that is used in this study is a kiwi fruit with green edible flesh which is scientifically known as *Actinidia deliciosa*.

b. Banana

Figure 2.2: *Musa acuminata*

Banana has a smooth, thick skin and has a few vertical ridges that run the length of the fruit. The flesh of the banana is creamy and soft. Bananas are usually yellow in colour but green when unripe. Besides that, there are also red and brown cultivars of bananas. The bananas grow in large bunches near the top of banana plants. The banana plants are originated from the genus *Musa* of the family *Musaceae*. Bananas are commonly found in the tropical region of Southeast Asia. *Musa paradisiaca* is the first scientific term given to banana by Linnaeus in his book *Species Plantarum*, the origin of modern botanical which was published in 1753. He described that the plantain cultivar produce long and slender fruits that remain starchy even when fully ripe. The fruits are cooked before they become palatable and consumed. Apart from that, he also described that the male flowers and bracts of plantains are usually
persistent and remain as relics on the male bud rachis. Limeans then introduced another species of banana, *Musa sapientum*, published in Systema Nature in 1759 which. He described a desert banana that produces sweet fruits which are eaten upon ripening. However, the *Musa sapientum* have dehiscent male flowers and bracts, exposing a clean rachis. The two scientific names remained in wide usage for almost ten centuries. Unfortunately, the bananas’ adoption in Southeast Asia generated confusions from early on as the bananas started to produce varieties of hybrids in order for them to adapt to different parts of the environment in various places in the Southeast Asia (Valmayor *et al.*, 2000). However, the banana that is used in this study is one of the various hybrids of bananas that can be found in Southeast Asia which comes from the species of *Musa acuminata* or commonly known as ‘Pisang Mas’ in Malaysia and Indonesia.

c. Coconut

![Diagram of Coconut seed](image)

**Figure 2.3: Cocos nucifera**

Coconut or scientifically known as *Cocos nucifera* is a member of the family *Arecaceae* and is also the only species of the genus *Cocos*. A coconut palm can grow to 30 m tall. Coconut palms are normally distributed in all tropical and subtropical regions. Coconut is believed to be originated from the Indo-Malayan region in which it spread throughout the tropics. Coconut palm is generally known as the “world’s
most useful plants" as each part of the coconut palms has its own usage and benefit. The coconut palm has also been called as the "tree of heaven" and "tree of life". The main use of a coconut palm is as staple food, wood, handicrafts, drink, fiber, match, oil, medicine and other uses. Apart from that, coconut palms also play an important role in coastal stabilization, windbreak and overstory (Chan & Elevitch, 2006). The coconut is called coco, which means "monkey face", by early Spanish explorers as the three indentations on the hairy nut resembles the head and face of a monkey. On the other hand, nucifera means "nut-bearing". The coconut's wall is the most important feature for the coconut. The coconut fruit is a fibrous drupe which consists of a thin hard skin (exocarp), the hard endocarp, a thicker layer of fibrous mesocarp, the hard endocarp (shell), the white endosperm (kernel) and a large cavity filled with water (Chakraborty & Mitra, 2008). The exocarp of the coconut fruit is usually green and sometimes bronze when it is immature. Fresh coconut flesh or the jelly-like young kernel of the immature nut is the solid white eatable lining of the coconut fruit. Mature kernel is either eaten fresh or dried and grated to make dishes, cakes and confections. Shredded kernel is often and widely used in a variety of local deserts in Malaysia. On the other hand, the sweet water from the coconut is a popular and refreshing drink. However, only the kernel of the coconut is used in this study to isolate antimicrobial protein.

2.2 Therapeutics Properties of Fruits

Fruits are famously known for their therapeutic values and also for their freshness. Scientists carried out many researches to study the therapeutic values of fruits and they managed to discover that fruits are low in calories and packed with vitamins, minerals, antioxidants and phytochemicals. Regular consumption of fruits are believed to be able to reduce risks of cancer, cardiovascular disease, stroke, Alzheimer disease, cataract and some of the functional declines associated with aging.


REFERENCES


