ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF PLANTAIN PEELS EXTRACTS.

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE DEGREE BACHELOR OF FOOD SCIENCE WITH HONOURS IN THE FIELD OF FOOD SCIENCE AND NUTRITION

SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2007
PENGESAHAN STATUS TESIS

JUL: ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF PLANTAIN PEELS EXTRACTS

ZAH: SARJANA MUDA SAHIS MACULANAN CMACULANAN DAN PENDAHULUAN

SESU PENGAJIAN: 2004 / 2005

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The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

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ACKNOWLEDGEMENT

First and foremost, I would like to express my greatest appreciation to Dr. Chye Fook Yee, my supervisor for giving me guidance, advices and being patient and helpful in conducting this study.

Grateful appreciation is dedicated to Mr. Tin Hoe Seng and Ms. Tham Kar Yee for their guidance, encouragement, advices, suggestions, kindness and helpfulness. Special thanks are also dedicated to the laboratory assistants of SSMP especially Mr. Taipin and Mr. Othman, for their kindly assistances and for giving me a helping hand when I faced difficulties during conducting this study.

My sincere thanks are also extended to Ms. Lim Sze Hui, Ms. Lorraine Lau Pei Jin, Ms. Chin Mee Yee and Ms. Wong Mum Yee for giving support and advices to me to deal with the difficulties during conducting this study.

Nevertheless, grateful and appreciation also go to my family especially my parents, Mr. Chong Lai Ming and Mrs. Choong Lai Kuei for their understanding and giving me courage, mentally and financially support in order to complete this study.

Once again, I would like expressed my deepest appreciation to everyone who had been giving me a helping hand in conducting this study including those who are not being mentioned.
ABSTRACT

Methanolic extract and fractions of “Pisang Saba” and “Pisang Raja” peels were assayed for their “antimicrobial” and “antioxidant” activity. The methanolic extract and dichloromethane fraction of Pisang Raja peels exhibited the strongest antimicrobial activity among all the extract and fractions by showing bigger inhibition zone in disc diffusion screening, whereas no antimicrobial potency was observed when the hexane fraction was tested against four pathogenic bacteria strains. A few extract and fractions were found to have comparable antimicrobial activity as the antibiotics. Minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC) of extract or fractions which showed inhibitory activity in previous screening were determined by time-kill assay. Total phenolics contents of extract and fractions were evaluated using Folin-Ciocalteu method while the antioxidant activities were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Results obtained showed that no antioxidant activity was found in the hexane fraction of both samples. Correlation of method for determination of total phenolic contents and antioxidant activity was analyzed by simple bivariate and partial correlation. Significant correlation was found between the antioxidant activities assayed by two different methods.
ABSTRAK

AKTIVITI ANTIMIKROBIAL DAN ANTIOXIDAN DALAM EKSTRAK KULIT PISANG.

Ekstrak metanol dan bahagian-bahagian daripada kulit "Pisang Saba" dan "Pisang Raja" telah dikaji berasaskan aktiviti "antimikrobial" dan "antioxidan". Ekstrak metanol dan bahagian diklorometana daripada kulit Pisang Raja mempamerkan aktiviti antimikrobial yang paling kuat berbanding dengan ekstrak dan bahagian ekstrak yang lain dengan menunjukkan zon rencatan yang lebih besar apabila diuji dengan kaedah disc diffusion. Manakala, bahagian hexana daripada kedua-dua sampel tidak menunjukkan aktiviti antimikrobial sewaktu diuji dengan empat jenis bakteria patogenik. Beberapa ekstrak dan bahagian ekstrak yang dikaji didapati mempunyai aktiviti antimikrobial yang setara dengan antibiotik. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) ekstrak dan bahagian ekstrak yang mampu merencatkan pertumbuhan bakteria yang dikaji dalam ujian sebelumnya ditentukan dengan ujian "time-kill". Jumlah kandungan phenolic and aktiviti antioxidan juga dikaji dengan menggunakan ujian 2, 2-diphenyl-1-picrylhydrazyl (DPPH) dan "ferric reducing antioxidant power" (FRAP). Keputusan yang didapati menunjukkan bahawa aktiviti antioxidan tidak wujud dalam bahagian hexana daripada kedua-dua sampel. Perkaitan antara kaedah penentuan jumlah kandungan phenolic dan aktiviti antioxidan juga dikaji dengan "simple bivariate" dan "partial correlation". Perkaitan yang nyata di antara jumlah kandungan phenolic dan aktiviti antioxidan yang dikaji dengan menggunakan dua kaedah yang berbeza.
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CHAPTER 1

INTRODUCTION

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety (Jayaprakasha et al., 2003). The discovery of medicinal plants in different parts of the world is important both to the agricultural and medicine sectors, helping in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits (Barbour et al., 2004). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase in the incidence of new, re-emerging infectious diseases and concern on the development of resistance microorganism towards current clinical use of antibiotics (Rojas et al., 2003). Although most antibiotics in clinical use have been obtained from microorganisms, a renewed interest in plant antimicrobials in the last 20 years has emerged but only a very small fraction of the known plant species has been evaluated for the presence of antifungal compounds (Stein et al., 2005). Natural products like fruit phenolic acids possess antimicrobial properties in particular as potent fungal growth inhibitors which could be very useful in controlling plant disease, and their content may increase after infection (Naidu, 2000).

The plant kingdom is recognized as an enormous source of antimicrobial compounds with applications in the food, cosmetic and drug industries (Ippolito &
Nigro, 2003). They have an almost limitless ability to synthesize aromatic substances, mainly phenols or their oxygen-substituted derivatives which in many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores such as the terpenoid capsaicin from chili peppers that is responsible for plant flavor, and some of the same herbs and spices used by humans as seasoning yield useful medicinal compounds (Cowan, 1999). According to Tegos et al. (2002), plant compounds are routinely classified as “antimicrobial” on the basis of susceptibility tests that produce Minimum Inhibitory Concentrations (MICs) in the range of 100 to 1,000μg/ml, orders of magnitude weaker than those of typical antibiotics produced by bacteria and fungi (MICs, 0.01 to 10μg/ml).

The prominent phytochemicals in fruits and vegetables are flavonoids. According to Naidu (2000), flavonoids play different roles in the ecology of plants due to their attractive colors, flavonols, flavones, and anthocyanidins are likely to be a visual signal for pollinating insects. Phenolic compounds are secondary metabolites of plants and one of the most widely occurring groups of phytochemicals, which has physiological (such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects) and morphological importance in plants (Balasundram et al., 2006). Major component of naturally occurring antimicrobials in plants can include those present in the intact plant and those released due to infection or injury such as alkaloids, dienes, flavonols, flavones, glycosides, lactones, organic acids, protein-like compounds (Davidson & Zivanovic, 2003) and phenolics compounds such as phytoalexins can be biosynthesized de novo after infection and elicit antimicrobial activity as a defense mechanism in plants towards microbial infection (Cowan, 1999; Naidu, 2000; Davidson & Zivanovic, 2003). As a result, antimicrobials in plants can be effective in
preserving a food by either controlling the overall growth of the microorganisms or directly destroying the microorganisms in foods (Davidson & Branen, 1993).

Some of these antimicrobial compounds are also known to have antioxidative properties due to their phenolic compounds (e.g. flavonoids) with known free radical scavenging properties, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Pourmorad et al., 2006). Oxidation of lipids which occurs during raw material storage, processing, heat treatment and further storage of final products is one of the basic processes causing rancidity of food products leading to deterioration. Therefore, in order to prolong the stability of foods during storage, synthetic antioxidants are used for industrial processing (Tepe et al., 2005).

Tropical fruits grow under hot and intense sunlight that facilitates lipid peroxidation and Kanazawa & Sakakibara (2000) considered that banana (a tropical plant) should contain antioxidants in pulp and peel to shield against peroxidizing factors which may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant. Bananas are one of the most popular fruits in the world and the Musa Cavendish has been proved to contain various antioxidants compounds such as gallocatechin (Someya et al., 2002) and dopamine (Kanazawa & Sakakibara, 2000) both in pulp and peel to shield against peroxidizing factors. The antibacterial and antioxidant activities of Cavendish fruit peel had also being studied by Mokbel & Hashinaga (2005). Pulp of plantain which was studied by Lewis et al. (1999) suggested that they contained active protective agents such as polyphenolic, flavonoid and leucocyanidin.
According to Schieber et al. (2001), the peels of several other fruits have also been found to contain higher amounts of phenolics than the edible fleshy parts. For instance, peels from apples, peaches and pears were found to contain twice the amount of total phenolics as found in the pulps. While the edible pulp of banana (Musa cavendish) contains 232 mg/100 g dry weight phenolics, this amount is about 25% of that present in the peel. The study of Manthey & Grohmann (2001) had shown that citrus processing byproducts potentially represent a rich source of natural flavonoids, owing to the large amount of peel produced, and that citrus peels contain a high concentration of phenolic compounds. On the other hand, antimicrobial effect of apple skin (Alberto et al., 2006) and phenolic compounds in apple peels (Wolfe & Liu, 2003) had also being studied.

Worldwide production of cooking bananas (plantains) amounts to nearly 30 million tons per year and the peels constitute up to 30% of the ripe fruit (Schieber et al., 2001). Banana cultivation is one of the most important fruit growing activities in Malaysia after durian and it covers about 15% of the area cultivated with fruit (39,000 ha) with about US$7.5 million in export revenue (Anon, 1999). Report on Crops Hectareage and Production in Sabah in year 2004 showed that production of banana in 2,546 hectareage (2,207.3 producing area) was 37,349.6 mt. As referred the production of banana by districts in Sabah, Tawau was the district providing the most banana production (5,571.1 mt) in 2004 with 265.3 hectareage, following by Kota Belud, Tuaran, Sipitang, Lahad Datu with the production ranged from 3,600 mt (Lahad Datu) to 5,571.1 mt (Tawau) (Department of Agriculture Sabah, 2004).

The processing of plant foods results in the production of by-products that are rich in bioactive compounds which can be classified into insoluble (e.g. fibers), water-
soluble (e.g. phenolics) and lipid-soluble (e.g. carotenoids) compounds (Schieber et al., 2001). The availability of phenolic compounds from agricultural and industrial residues, their extraction and antioxidant activity have been reviewed by Balasundram et al. (2006). Banana and plantain peels are currently treated as waste of processing and this practice is not only a waste of resource, but also causes environmental pollution (Schieber et al., 2001). Banana peel, which is usually discarded, should also be considered to be a functional food source against cancer and heart disease, since the banana peel is rich in gallicatechin (Someya et al., 2002). Thus, utilization of the by-product which used to be treated as waste is an issue that are greatly concerned by all manufacturers especially in the food industry since it would be an advance waste treatment program that benefits the industry economically.

Food poisoning is still a threat for both consumers and the food industry despite the use of preserving processes although the safety of foods containing preservatives has remained a concern by the consumers. Therefore, there has been a growing interest in new and effective techniques to reduce cases of food-borne illnesses using antimicrobial substances from natural sources like plants to achieve higher levels of food safety (Alzoreky & Nakahara, 2003). Utilizing the peels of plantain serves as a type of waste treatment in plantain processing and provides an alternative for valued added ingredients such as sources of natural antimicrobial and antioxidant agents in food industry. As the peels bananas and plantain are normally treated as waste in food processing, the cost of raw material can be eliminated. However, studies and researches need to be carried out in order to find the most suitable way to cover the cost of producing value added ingredients by utilizing the
peels for bananas and plantains. Thus, the aim of the study was to determine the antimicrobial activity of plantain peels.

**The Specific Objectives of this Study are:-**

1. To evaluate antimicrobial activity of extracts from *Pisang Saba* and *Pisang Raja* peels in full green stage.

2. To determine Minimum Inhibitory Concentration (MIC) and Minimum Bacteriacidal Concentration (MBC) of the peel extracts against foodborne bacterial pathogens.

3. To study the total phenolic content and antioxidant activity of *Pisang Saba* and *Pisang Raja* peel extract and fractions.
CHAPTER 2

LITERATURE REVIEW

2.1. Overview of Natural Antimicrobial and Consumer Trend

According to Naidu (2000), naturally occurring antimicrobials are abundant in the environment which exists in animals and plants where they evolved as host defense mechanisms. They can be categorized into six different classes of natural systems which included lacto-, ovo-, phyto-, bacto-, acid- and milieu- antimicrobials. The spoilage and poisoning of foods by microorganisms is a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available (Rauhaa et al., 2000). Poisoning outbreaks caused by foodborne pathogenic bacteria have consequently increased the interest in preventing food contamination by food-borne pathogens where the addition of antimicrobial agents has been particularly effective in controlling microbial growth (Kabuki et al., 2000). Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve sufficiently long shelf life of foods and a high degree of safety with respect to foodborne pathogenic microorganisms (Rauhaa et al., 2000).

Before the discovery of the existence of microbes, the idea that certain plants contained antimicrobial which had healing potential was well accepted since plants have been used to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies
(Rios & Recio, 2005). This included the phytochemical studies of medicinal plants from Africa (Tshibangu et al., 2002), Siberian (Kokoska et al., 2002), Lebanon (Barbour et al., 2004), Argentina (Salvat et al., 2004) where their antimicrobial activity were being screened by a few groups of researchers.

In nature, there are a large number of different types of antimicrobial compounds (phytoalexins) that play an important role in the natural defence of all kinds of living organisms (Rauhaa et al., 2000). Major classes of antimicrobial compounds from plants can be divided into phenolics, essential oils, alkaloids, lectins and polypeptides, and polyacetylenes by Cowan (1999). According to Skocibusic, Bezic & Dunkic (2006), a novel way to reduce the proliferation of microorganisms is by using essential oils that are extracted from plant materials because of their antibacterial, antifungal, antioxidant and anticarcinogenic properties. Research have been carried out to study the antimicrobial activity of essential oils of plants (Baydar et al., 2004; Mourey & Canillac, 2002; Skocibusic, Bezic & Dunkic, 2006; Tepe, 2005), polyphenols from apple skins (Alberto, Canavosio & Manca de Nadra, 2006), phenolics compounds in fruits and plants (Robards et al., 1999; Rauhaa et al., 2000) and flavonoids in plants (Rauhaa et al., 2000; Cushnie & Lamb, 2005).

2.2. Overview of Banana and Plantain Production

According to Morton (1987), bananas and plantains are grown in every humid tropical region and constitute the 4th largest fruit crop of the world, following the grape, citrus fruits and the apples where India is the leading banana producer in Asia.

International commodity prices are used by FAOSTAT (2006) to calculate the total value of commodity produced by each country and subsequently used in the
ranking of commodities and countries. They are applied in order to avoid the use of exchange rates for obtaining continental and world aggregates, and also to improve and facilitate international comparative analysis of productivity at the national level. Data which displayed as Appendix I showed that India, Brazil and China are the top three banana producers in the world which provide 16,820,000 mt, 6,702,760 mt and 6,390,000 mt of bananas in year 2005 respectively. On the other hand, as focusing on the production of plantains, the top three plantains producer countries are Uganda (9,900,000 mt), Colombia (3,400,000 mt) and Rwanda (2,593,080 mt) in the year 2005 as referred to Appendix II. Data in Appendix III displays the 20 most important food and agricultural commodities (ranked by value) in Malaysia for year 2005 in which banana is ranked as the eighth out of twenty commodities but it is the most type of fruits that being produced followed by pineapples (FAOSTAT, 2006).

According to the Department of Agriculture (2005), bananas production in year 2000, 2002, 2003, 2004 and 2005 was found to be 178,958 tonnes, 290,763 tonnes, 274,426 tonnes, 328,099 tonnes and 381,771 tonnes, respectively. Meanwhile hectareage of fruit crops by districts, Sabah for year 2000-2005 were showed in Appendix IV to IX but crops production in Sabah only started to be recorded in year 2004 where the production of bananas being compared between 2004 and 2005, higher production was observed in the year 2004 with 37,349.60 mt of banana being produced. When the production of banana was being reviewed according districts in Sabah in 2004, it was found that Tawau, Kota Belud and Tuaran were the three districts providing the highest banana production sequentially throughout year 2004 (Department of Agriculture, 2005).
2.3. Banana and Plantain

According to Norgrove & Hauser (2002), Musa species, which include sweet bananas, plantains and cooking bananas, are referred as groups which indicate their ploidy and genomic composition with respect to the parent species (A, Musa acuminata; B, Musa balbisiana). Most cultivars of edible bananas and plantains are derived from Musa acuminata and Musa balbisiana, which are the two members of the family Musaceae. All varieties derived only from Musa acuminata are more sugary and are mainly eaten as a dessert fruit but on the other hand, varieties which contain Musa balbisiana characteristics shown a slower process of ripening when starch is converted to sugar (Robinson, 1996).

Species and genome of bananas can be divided into Musa acuminata Diploid (AA) and Triploid (AAA), Musa paradisiaca Triploid (AAB), Musa paradisiaca Triploid (ABB), Musa paradisiaca Tetraploid (ABBB) and Triploid (BBB) (Anon, 1999). There was no single scientific name can be given to all the edible bananas. Musa acuminata could be applied to pure, seedless diploid (AA) and triploid (AAA) forms of dessert bananas such as 'Pisang Mas' and 'Grand Nain' respectively while Musa balbisiana could be applied to pure, seedless diploid (BB) and triploid (BBB) forms of cooking bananas such as 'Abuhon' and 'Saba' respectively, but many hybrids cannot carry a specific name due to their mixed composition and differences in ploidy (Robinson, 1996).

In West Malaysia, bananas are produced by farms, estates and household plantations (Rukayah, 2000). There are about 70 banana cultivars in Malaysia with different nutritional value and can be classified into bananas that being eaten raw and cooking or processed bananas which known as plantain. The two obvious tissues
that constitute both the bananas and plantains are the pulp and the peel. The peel is the ovary wall while the pulp originates from cell division of the innermost layers of the pericarp. The growth of the peel begins to slow down as the fruit matures but the growth of the pulp continues, consequently peel splitting often occurs in very mature green fruit (Turner, 1997).

Significant difference between bananas and plantains would be the degree of ripening stage that being consumed and the ways of consumption. Bananas are mainly eaten raw as a dessert fruit because in the ripe stage. They are sweet and easily digested while plantains are generally much starchier and can be eaten either ripe or unripe in which they are usually either boiled, fried or roasted (Robinson, 1996). Traditionally, the stage of ripening of banana, cooking banana and plantain have been closely linked with the changes in peel colour and the matching of the peel colour against a set of standard colour plates is a common method used to assess the ripeness of the fruit. The disappearance or loss of peel green colour and the corresponding increase in yellowing of the peel during ripening are the obvious manifestations in banana, cooking banana and plantain (Dadzie et Orchard, 1997).

Completely green plantains are 50% flesh and 50% peel and the ripening stages of banana are scored on the colour of the peel, from 1 for all green to 8 for duffel (Shahidi & Naczk, 2004).

Bananas that are being eaten raw in Malaysia are pisang mas, pisang rastali, pisang embun, pisang berangan and pisang lemak manis while the types that are subjected to processing are pisang nangka, pisang tanduk, pisang awak, pisang abu and pisang raja (Zainun, 1992). ‘Horn’ plantain is one of the two main types of plantain subgroup of AAB triploid hybrids while the other type is the ‘French’ plantain.
(Robinson, 1996) which is also known as *pisang tanduk* in Malaysia (Anon, 1999). *Pisang raja* is a well-known dessert clone of AAB triploid hybrids in Malaysia and Indonesia while ‘Saba’ is the BBB pure *balbasiana* clones which known as *pisang nipah* in Malaysia (Robinson, 1996).

There are many different products which can be processed from banana and plantain, with different methods of processing such as canning, drying, freezing, frying or fermentation (Robinson, 1996). Products that can be processed from bananas are snacks, jam, flour, banana powder, puree, drinks and so on (Rukayah, 2000; Zainun, 1992). However, according to Robinson (1996), processed banana products represent a very minor proportion of around 0.003% if compared with the volume of fresh fruit exports. Banana is also a health-promoting fruits since it contained nutrients such as vitamin A, B, C and fiber which are essential for maintaining good health, improve digestion and prevent constipation (Ismail, 2000; Rukayah, 2000). As referred to the nutrient content, there are some differences in term of concentration of nutritional value between banana and plantain (Table 2.1).
REFERENCES


