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ANTIOXIDANT PROPERTIES OF RAMBUTAN PEEL EXTRACT AND SUBFRACTIONS AND THEIR POTENTIAL AS VEGETABLE OIL PRESERVATIVE

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By

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Chair: Professor Amin Ismail, PhD
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A number of tropical fruit peel has been known to possess high antioxidant activity, however, antioxidant data on the Malaysian tropical fruit peel is still lacking. In this study, red rambutan (*Nephelium lappaceum)* peel were reported to possess high antioxidant activities in comparison with peels obtained from tropical fruits, namely mangosteen (*Garcinia mangostana*), "langsat" *(Lansium domesticum*), Sapodilla (*Manilkara zapota*), passion fruit (*Passiflora edulis*), soursop (*Annona muricata*), and mango (*Mangifera indica*). The extraction method of rambutan peel was determined by using single factor experiments. Results showed that rambutan peel had the lowest EC_{50} (9.30±0.35 mg/g) and the highest TPC (359.60±20.85 GAE mg/g). Thus, rambutan peel was selected for further investigation on different extraction time (1 to 5 hours) and extraction temperature (25-60ºC). The extraction conditions of the red rambutan were determined to be 80% ethanol for 2 hours at 50ºC. The crude extract of the rambutan's peel showed EC_{50} of 6.39±0.32 mg/g (DPPH), 333.70±13.35 mg/g (ABTS), 676.96±12.93 trolox equivalents mg/g (FRAP), 269.70±15.90 GAE mg/g (TPC) and 88.67±0.14% antioxidant activity (as measured by BCB). The crude extract of rambutan peel was then fractionated into three sub-fractions by using silica-packed column chromatography. Results reported the presence of phenolic compounds such as geraniin, ellagic acid and gallic acid. In the last part of this study, the crude extracts and the sub-fraction (SF II) reported with the highest antioxidant activity were supplemented into sunflower oil to study their effects in delaying lipid oxidation under accelerated conditions. Rambutan extract is a potential source of antioxidant. The oxidative activities of the crude extracts and sub-fraction (SF II) at all concentrations were significantly ($p < 0.05$) higher than the control. Results reported that with two years storage period at ambient temperature, the sub-fraction, (SF II) at 300ppm, was observed to work more effectively than the synthetic antioxidant, *t*-tocopherol. SF II possessed a protective effect comparable with Butylated Hydroxyanisole (BHA). Therefore, rambutan extract could be used as a potential alternative source of antioxidant in the oil industry or other fat-based products to delay lipid oxidation.

Abstrak tesis yang dikemukakan kepadaSenatUniversiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah master sains

SIFAT ANTIOKSIDAN EKSTRAK DAN FRAKSI KULIT BUAH RAMBUTAN DAN POTENSINYA SEBAGAI PENGAWET MINYAK SAYURAN

Oleh

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Oktober 2015

Pengerusi: Professor Amin Ismail, PhD
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Kulit buah-buahan tropika dibukti mempunyai aktiviti antioksidan yang tinggi.Walaubagaimanapun, data antioksidan kulit buah-buahan tropika Malaysia masih lagi tidak mencukupi. Dalam kajian ini, kulit rambutan (*Nephelium lappaceum*) didapati memiliki aktiviti antioksidan yang tinggi berbanding dengan kulit diperolehi daripada buah-buahan tropika lain, iaitu manggis (*Garcinia mangostana*), langsat (*Lansium domesticum*), ciku (*Manilkara zapota*), buah Markisa (*Passiflora edulis*), durian belanda (*Annona muricata*), dan mangga (*Mangifera indica*). Kaedah pengekstrakan kulit rambutan telah dipilih dengan menggunakan eksperimen faktor tunggal. Hasil kajian menunjukkan bahawa kulit rambutan itu mempunyai EC_{50} yang paling rendah (9.30 ± 0.35 mg / g) dan TPC yang tertinggi (359,60 ± 20.85 GAE mg / g). Oleh itu, kulit rambutan telah dipilih untuk kajian lanjut dengan masa pengekstrakan yang berbeza (1-5 jam) dan suhu pengekstrakan yang berbeza (25-60ºC). Keadah pengekstrakan telah dipilih pada 80% etanol selama 2 jam pada 50ºC. Ekstrak mentah dari kulit rambutan menunjukkan EC_{50}, 6.39 ± 0.32 mg / g (DPPH), 333,70 ± 13.35 mg / g (ABTS), 676.96 ± 12,93 trolox setara mg / g (FRAP), 269.70 ± 15.90 GAE mg / g (TPC) dan 88.67 ± 0.14% aktiviti antioksidan (seperti yang diukur oleh BCB).Ekstrak mentah daripada kulit rambutan kemudian dipecah kepada tiga sub-pecahan dengan menggunakan silika kromatografi. Keputusan kajian melaporkan kehadiran sebatian fenolik seperti geraniin, asid ellagik dan asid gallic. Dalam bahagian akhir kajian ini, ekstrak mentah dan pecahan yang mempunyai aktiviti antioksidan yang paling tinggi ditambah ke dalam minyak bunga matahari untuk mengkaji kesan mereka dalam melambatkan pengoksidaan minyak. Ekstrak rambutan adalah sumber potensi antioksidan yang kuat. Aktiviti pengoksidaan ekstrak mentah dan fraksi (SF II) pada setiap kepekatan ketara ($p <0.05$) didapati lebih tinggi daripada kawalan. Keputusan melaporkan bahawa dalam tempoh penyimpanan 2 tahun pada suhu bilik, sub-pecahan, SF II pada 300 ppm, didapati lebih berkesan daripada antioksidan sintetik, t-tokoferol, dan ia mempunyai kesan perlindungan yang setanding dengan butylatedhydrioxynanisole (BHA). Kesimpulannya, ekstrak rambutan boleh digunakan sebagai sumber alternatif potensi antioksidan dalam industri minyak atau produk berasaskan lemak untuk melambatkan pengoksidaan lipid.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
AOM	Active oxygen method
AP	Andascorbyl palmitate
BCB	β-carotene bleaching
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FFA	Free fatty acid
FDA	Food and Drug Administration
GAE	Gallic acid equivalent
GC	Gas chromatography
GRAS	Generally recognized as safe
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
HPTLC	High performance think layer chromatography
IV	Iodine value
LC-MS	Liquid chromatography mass spectrometry
LDL	Low density lipoprotein
MDA	Malondialdehyde
MGC	Monoacylglycerol citrate
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
OSI	Oil stability index
PG	Propyl gallate
PUFA	Polyunsaturated fatty acids
RBD	Refined, bleached and deodorized
R·	alky radical
ROO·	peroxyl radical
ROOH	hydroperoxides
Rpm	round per minute
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	*tert*-butylhydroquinone
TEAC	Trolox equivalent antioxidant capacity
TLC	Thin layer chromatography
TOTOX	Total oxidation
TPC	Total phenolic content

TPTZ	2,4,6-Tripyridyl-s-Triazine
UV	Ultra-violet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Antioxidants offer resistance against the oxidative stress by maintaining the oxidant-antioxidant equilibrium. It inhibits the formation of the reactive oxygen species that initiate lipid peroxidation (Alessio and Hagerman 2006). They are able to neutralize free radicals by donating one of their own electrons, ending the electron-"stealing" reaction. There are two types of antioxidant, namely endogenous which produced by body and exogenous which acquired from the diet. The exogenous antioxidant can be easily found from fruits, vegetables and legumes. They are found to contain high amount of potent antioxidant compounds such as phenolic compounds, vitamin A and vitamin C. According to Naczk and Shahidi (2006), fruits such as apples, cranberries, blueberries, and citrus are high in phenolic acids (Naczk and Shahidi 2006). Acerola, strawberry, guava are rich in vitamin C. Avocado was reported to contain high amount of beta carotene (Leong and Shui 2002). Studies also shown that high intake of fruits and vegetables rich in antioxidant compounds are less likely to get chronic diseases such as cancer, heart disease and stroke (Ames et al., 1993; Liu et al., 2000; Hung et al., 2004).

The health benefits of consuming fruits are mainly contributed by the presence of antioxidant compounds in the fruits. Many studies have been done to screen on the antioxidant potential of tropical fruits. Most of the studies reported tropical fruits are good source of antioxidant compounds such as vitamin C and phenolic compounds (Leong and Shui 2002; Lim et al., 2007; Alothman et al., 2009; Ashraf et al., 2011). In a study done by Lim et al. (2007), the phenolic content of seedless guava (179±44 mg/100g), star fruit (131±54 mg/100g), and langsat (100±29mg/100 g) are higher than that of orange (75±10 mg/100g). The vitamin C content of guava (131±18.2 mg/100g) and solo papaya (67.8±12.6 mg/100g) were also reported to be higher than the orange (36.1±15.9 mg/100g) (Leong and Shui, 2002). Solo papaya, star fruit, seeded guava, seedless guava were reported to have IC_{50} lower than 5 mg/ml which indicating that these fruits are potential source of antioxidants (Lim et al., 2007).

In fruits and vegetables industries, they usually produce a large amount of by-products which might be a rich source of bioactive compounds (Balasundram et al., 2006). According to Lim et al. (2007), the edible portion of the sapodilla was found to possess weak antioxidative capacity; whereas Leong and Shui (2002) reported that the whole fruits of sapodilla (including the edible portion and peels) contain a high amount of ascorbic acid and was high in antioxidants activities. This shows that the antioxidant capacity of the fruits are mainly comes from the peels. Guo et al. (2003) also found that peels of the pomegranate, grape seed, hawthorn, longan and lychee possessed relatively high antioxidant capacity as compared to the pulp.

The antioxidant potential of the peels of tropical fruits such as banana, papaya, kaffir lime ("limau purut"), pomelo, and watermelon have been reported (Yong 2009; Chua

2010; Lam 2010; Leong 2010; Ng 2010). The crude extracts of the peel demonstrated promising antioxidative potential. The extracts contain a relatively high amount of total phenolic content, which have been shown to possess strong positive correlations with their antioxidant capactiy. The peel of kaffir lime was found to exhibit promising antioxidant activity and the phenolic compounds such as catechin, tannic acid, apigenin and ascorbic acid were identified (Chua 2010). According to the study of Amin and Mukhrizah (2006), they suggested the peels of pink guava, roselle seed and cocoa shell as the potential source of antioxidant and can be exploited as food preservatives or nutraceuticals. With all the research done, it increased the interest of most researchers to conduct studies on the peels of fruits for their antioxidative potential. It is considered economically beneficial to use the fruits' peels, turning them from waste into a valuable source of antioxidants derived from natural resources.

Vegetable oils such as palm oil, corn oil, canola oil, sunflower oil and olive oil are recommended as ideal cooking media. However, all kinds of oils contain certain amount of unsaturated fatty acids which are susceptible to lipid oxidation. Lipid oxidation is the principal cause of food deterioration. It is the major problem in oil industry. It reduces nutritional quality of oils by producing off flavour and toxic compound. As a result, it leads to unpleasant tastes and shorten the shelf life of the oils (Rubalaya and Neelamegam 2012; Alfonso et al., 2003). Among the commonly used vegetable oils, palm oil is the most stable and is least susceptible to lipid oxidation with its high saturated fatty acids content. In early years, saturated fatty acids were found to bring adverse effect on human health. In turn, PUFA was evidenced to possess many beneficial effects on the human body especially in cholesterol lowering effect (Ruxton et al. 2004). Therefore, it is highly recommended to consume vegetable oils contain high amount of PUFA. However, polyunsaturated fatty acids (PUFA) are relatively unstable and susceptible to lipid oxidation (Alfonso et al., 2003). When lipids are exposed to environmental factors such as air, light or high temperature the oxidation process will be induced. There is a need to source for antioxidant to be incorporate in high PUFA vegetable oils to prevent lipid oxidation and extend their shelf-life.

In order to prevent or retard the lipid oxidation, synthetic antioxidants have been widely used to maintain the quality and extend the shelf life of the oil. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (THBQ) are examples of synthetic antioxidant commonly added to cooking oil (Rubalya and Neelamegam 2012). The effectiveness on the use of natural extract in retarding the lipid oxidation has been well studied. Rosemary, garlic, kenaf seed, roselle seed, coffee bean, catnip, sage, thyme, potato peel and sugar beet pulp are among the examples of natural extracts that found to work effectively in preventing oil rancidity (Ahmed and Jacques 1999; Anwar et al., 2006; Iqbal et al., 2007; Mohdaly, et al., 2010; Nyam et al., 2013). The antioxidant activities are mainly due to the presence of phenolic acid found in the extract. Fruits are one of the main sources of phenolic compounds and they were found to possess high antioxidant activity. There are few studies described the efficiency of the fruit's extract in stabilizing vegetable oil. Iqbal et al. (2008) revealed that promeganate peel to be a potent source of antioxidant in stabilization of sunflower oil.

From all the literature search, the by-product (peel) of tropical fruits possesses promising antioxidant potential and can be a good source of antioxidants. Hence, further evaluation on the antioxidant properties of peels of locally available tropical fruits would provide a fundamental knowledge for their potent antioxidative components. The fractionation and identification of the potent antioxidant compounds would then lay a platform for the development of useful natural antioxidants and its application as natural preservatives in cooking oil. For fruits' farmers and those small business owner selling fruits, the positive results from this study would definitely provide an additional stream of income by selling the peels that are often discarded to those industrial players for their antioxidant extraction and application. Hence, the general objective of the present study was to investigate the antioxidant potential of the peel of tropical fruits. The specific objectives of the study were:

1. To evaluate the effect of extraction parameter (ethanol concentrations, extraction time and extraction temperature) on the antioxidant activity of the peels of tropical fruits.
2. To fractionate and identify the potent antioxidant compounds from rambutan's peel.
3. To evaluate the effectiveness of the fractionated fraction(s) from rambutan's peel in stabilizing cooking oil during accelerated conditions.

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