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PHYTOCHEMICALS SCREENING, ANTIOXIDANT ACTIVITY AND FRYING QUALITY AS AFFECTED BY AQUEOUS EXTRACT OF MALAYSIANSERAIKAYU (*EUGENIA POLYANTHA)*

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ABSTRACT

SeraiKayuor its scientific name is *Eugenia polyantha* is widely found in the Western part of the South East Asian peninsular and in Western Indonesia. The leaves are commonly taken as "ulam" in Malaysian communities. This paper reports the investigation of the phytochemical constituents and antioxidant potential of aqueous extract from leaves of *E.polyantha* and the frying quality of coconut oil were evaluated during deep frying of French fries. The amounts of total phenolic and flavonoids content were determined spectrometrically. There were three types of parameter used for frying quality of coconut oil; coconut oil without BHT as a negative control, coconut oil with BHT as a positive control and coconut oil with *E.polyantha* as a natural antioxidant. The oil quality was assessed by measuring the peroxide value, free fatty acid, iodin value and viscosity. Phytochemical screening of the crude extracts revealed the presence of different kind of chemical groups such as flavonoids, phenol, tannins, saponins, steroids and terpenoids. The result shows the extract had high phenol(213.15 ±1.1 mg GAE/g plant extract) and flavonoid content (2.47 ± 0.1 mg QE/g plant extract) with high DPPH scavenging ability (IC_{50} value: 0.15 ± 0.01) compared to BHT (IC_{50} value: 0.19 ± 0.01). The result for frying quality of coconut oil indicated that the extract delayed the oil deterioration. The *E.polyantha* extract significantly ($p< 0.05$) lowered the rate of oxidation in crude coconut oil, compared to negative and positive control. In general, the present findings suggest that the crude aqueous extract of *E.polyantha* leaves is a potential source of natural antioxidants and theextract was capable of extending the stability and quality of crude coconut oil and therefore has potential as new source of natural antioxidant for use in deep frying.

KEYWORDS: Eugenia Polyantha, Aqueous Extract, Phytochemicals, Antioxidant Activity, Frying

INTRODUCTION

In Malaysia, traditional medicines are widely used and practiced in the community for maintaining health and also for treatment of many diseases. Plants and herbal extracts are considered as important materials in modern medicine, due to their phytochemical and medicinal contents in their natural form. Ulam or Malaysian herbs can be defined as the vegetation consists of leaves, fruit and rhizome. Ulam is usually used for medical uses either for preventative or even curative process.

In Malaysian culture ulams eaten raw and used in many dishes especially among the Malay communities. Most of these herbs are believed to be associated with antioxidant activities and have many beneficial effects. However the benefits

of their active component are not well documented Many species have been recognised to have medicinal properties and beneficial effect on health and food such as antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, and anticarcinogenic potential (Aneta et al, 2007). They are consumed because of their taste, which adds variety and flavour to the diet, as well as for health benefit. Nutritional studies have indicated that many of these vegetables are rich in carbohydrate, proteins, minerals and vitamins (Faridah et al, 2006).

Eugenia polyantha is known as seraikayu among the locals comes from family of Mythaceae. It distribute in a lowland forest like in Burma and Malaysia (PulauLangkawi) and Singapore. *E. polyantha*leaves grows wildly in the western part of Peninsular Malaysia and in western Indonesia. The leaves are slightly astringent or sour and the flavor develops more after frying. It has long been used as spices in cooking (Azizah et.al, 2014). It also has medicinal uses. The roots and fruits extract have ability to neutralize overdoses of alcohol consumption. Extract of the leafis used to stop diarrhea, gastritis, diabetes mellitus,itchy, astringent and scabies. It side effects are lowercompared to synthetic drugs. The main chemical constituents of this plant are euginol, citral, tannins, flavonoids and metachavicol.

Deep fat frying remains as one of the major problems in food industry preparation that may contribute to the effect of pleasant taste, aroma and health problems. Lipid oxidation and deterioration of the fats and oils can lower the nutritional value by the formation of oxidation products. Thus the use of synthetic antioxidants such as butylated hydroxyltolune (BHT) used to protect the quality of oil. However it believed to have the carcinogenic effect (Nor et al, 2008) and the use of natural antioxidantis an optional due to its safety.

The aims of this study are to determine the phytochemical screening,total phenolic content, flavonoid content and antioxidant activity from aqueous extract in *Eugenia polyantha* leaves and to investigate the stability and quality of their antioxidant properties during frying.

MATERIAL AND METHODS

Plant Material Preparation

The leaves of SeraiKayu *(Eugenia polyantha)*were obtainedfresh from KampungPandan 2 and Kampung Padang, Kuantan Pahang. These specimens were then sent to the botanist for confirmatory identification. All samples were washed using tap water and the damaged and disease portions were removed.All chemicals and solvents used were of analytical grade unless otherwise specified.

Sample Collection and Extraction

The plant sample was cut into small pieces and the leaves were dried in a dryer (Protech) at 40°C for two weeks, grind (Qingdao Dahua Double Circle), labeled and stored in airtight container for further used. An amount of 50g of ground samples was extracted using 250 mL deionized water. The mixture was allowed to stand for 24 hour at 60°C. The aqueous extract was obtained by filtering the mixture through Whatman No. 1 filter paper and used for analysis without further treatment. The extract was kept in Biomedical refrigerator(CHRIST, BETA 1-8 LD plus) (at -20°) C and freeze dried (Fiocchetti, Sientific Refrigerator) for 8 days.

Preliminary Phytochemical Analysis

The qualitative screening of leaves were performed in order to verify the presence of alkaloid, tannins, saponins,

flavonoids, (FarazMojab, 2003) steroids, terpenoids, phenolic, (C. Chitravadivu, 2009) of this plant.

Determination of Total Phenol Contents

Total phenolic contents of methanol extracts were measured using the modified Folin- Ciocalteu method (Amin et al., 2004) with slight modification. Aliquot (1 ml) of each extract was mixed with Folin-Ciocalteu reagent, shaken using a vortex mixer and added 4 ml of 20% aqueous sodium carbonate solution. The mixture was shaken once again and left to stand for 60 minutes to obtain maximum color development. The absorbance was measured at 760 nm using UV-Vis spectrophotometer (Shimadzu UV-1650PC spectrophotometer with 10mm path length cell) with distilled water as a blank. The total phenolic contents were expressed as gallic acid equivalents (GAE) in milligrams per gram of extract, using a standard curve generated with 10 – 100 µg of gallic acid. All determinations were performed in triplicate.

Determination of Flavanoid

Total flavonoid content was evaluated using aluminium nitrate nonhydrate according to the procedure reported by Woisky and Salatino (1998) with some modification. 0.01g of extract was diluted with distilled water to make the concentration of the sample 0.01g/mL. 1.0 mL sample was mixed with 4 mL of distilled water and subsequently with 0.3 mL of a $NaNO_3$ solution (10%). After 5 minutes, 0.3 mL $AlCl_3$ solution (10%) was added followed by 2.0 mL of NaOH solution (0.1%) to the mixture. The sample were measured the absorbance at 510 nm with UV-Visible spectrophotometer (Shidmadzu, UV1800) with distilled water as a blank. The total flavonoid concentration in water extract was calculated from quarcetin hydrate (Qu) calibration curve (0.1-0.5 mg/mL) and expressed as quarcetinequavalents (Qu)/g of dry extract averaged of 3 measurement.

Determination of Antioxidant Activity (DPPH Scavenging Assay)

Radical scavenging activity of extracts was measured method previously reported method previously reported (Blois, 1958). The DPPH solution with a concentration of a 0.007% (w/v) was prepared. Three ml of this solution was mixed with one ml extract solution in a test tube. Each mixture was then shaken vigorously and held for 30 min in the dark at room temperature. Butylated hydroxyl toluene (BHT) was used as a positive control. Discolorization was measured at 517 nm after incubation for 30 min by using a spectrophotometer. The actual decrease in absorption induced by the test compounds was compared with the positive controls. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

Inhibition % = [(Abst0 min – Abst30min) / Abst0] x 100

Where:

Abst0min: the absorbance of DPPH at time zero

Abst30min the absorbance of DPPH after 30 minutes of incubation.

Antioxidant capacity was expressed as IC_{50}; extract concentration (mg/ml) that required for scavenging 50% of DPPH. All measurements were carried out in triplicate.

Frying Experiment

Oil samples were obtained from coconut milk purchased from Kuantan market. The coconut milk was cooked at

low temperature using cooled extraction process (80˚C) for 5h. In order to assess the oxidative stability, oil samples were stored in a dark bottle at -5˚C before used.

French fries (LDPE) were purchased in a local market at Kuantan.

Frying experiments were carried out according to the method of Koh and Long (2012) using four various treatments that contained: Coconut oil without antioxidant or control (Treatment I); Coconut oil with xanthan gum (Treatment II)Coconut oil with 200 ppm BHT (Treatment III) and Coconut oil with 200 ppm *E.polyantha*crude extract (Treatment IV); and. Frying experiments were conducted in two replicates on each treatment. Thus, treatment IV was used as control for the procedure used.

A total of 4.5 kg oil was used in each experiment and put in the fryer (Anvil). In the treatment IV xanthan gum was used to emulsify the *E.polyantha*crude extract in the oil by dispersing the xanthan gum (0.35% w/v) in distilled water and mixing for 1.5h at 60˚C using a magnetic stirring bar and magnetic stirrer hotplate (IKA ®, C-MAG HS7) (Traynor et al., 2013). The coconut oil was then heated to 60˚C and slowly added to the aqueous phase while stirring. The pre-emulsion was allowed to mix for a further 5 minutes before being homogenized with a food homogenizer for 3 minutes at speed 5. In the case for treatment II, the oil also mixed with xanthan gum with the same procedure. In the treatment III, the temperature was raised to 60˚C, and then 200 ppm of BHT was added in the oil. The oil was stirred for 10 min at 60˚C to ensure dissolution of antioxidant. In the case of treatment I (control), the oil also was held for 10 min at 60˚C, but no antioxidant was added. The temperature was then raised to 180˚C during 20 min. Frying started 20 min after the temperature had reached 180˚C. A batch of 100 g potato fries was fried for 2.5 min at 17.5 min intervals for a period of 3.5 h per day for 5 consecutive days. During the frying period, the fryer was left uncovered. At the end of 10^{th} frying, the dryer was switched off and temperature was allowed to drop to 60˚C. The oil (300 g) was collected in amber bottles and stored at -4˚C for further analysis. The lid of the fryer was then put on the remaining oil was allowed to cool overnight. No fresh oil was added to the frying vessel on the subsequent frying days.

Analyses of Oil

Free fatty acid value, peroxide value and iodine value of fried oil was determined by Ranganna (2001). The oil viscosity was measured according to the method of Koh& Long (2012) using a Brookfield viscometer, (DV-I prime)with spindle 3 and the shear rate was set a 20 s^{-1}.

Statistical Analysis

Data were statistically analyzed by one-way analysis of variance procedure usingStatistical Package for Social Science (SPSS version 20). Significant differences ($p < 0.05$) between means were determined by Duncan multiple range test.

RESULT AND DISCUSSIONS

Phytochemical Screening

The phytochemical screening of the *Eugenia polyantha* studied showed the presence of flavonoids, phenol, tannin,, saponins, steroids and terpenoids (Table 1). Previous study revealed that *Eugenia polyantha*consist of tanines, flavonoid and essential oils (0.05%), including citric acid and eugenol (Sumonoet,al., 2008).

Table 1: Phytochemical Screening of *Eugenia Polyantha* (Leaves)

	Flavanoid	Phenol	Tannin	Saponin	Steroids &Terpenoids
Eugenia polyantha (leaves)	+++	+++	+++	+++	++

*+++ Copiously present, +++ Moderately present

Total Phenolics (TP) and Total Flavonoids (TF)

The level of phenolic compounds in aquoes extracts of the leaves *E.polyantha*are presented in Table 2. Wong et al. (2006) reported that 11 mg gallic acidequivalent (GAE)/ g extract in the water extract of *E. Polyantha* leaves from Singapore (MansorHakimanet,al. 2009) while the result from this study demonstrated higher TPC value at 213.15±1.10 mg GAE/g extract. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds.This activity is believed to be mainly due to their redox properties, which plays animportant role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Polyphenolic flavonoids are occurring ubiquitously in foodand medicinal plants. They occur as glycosides and containseveral phenolics hydroxyl groups. Many flavonoids arefound to be strong antioxidants effectively scavenging thereactive oxygen species because of their phenolics hydroxylgroups. Table 2 showedthe total flavonoid content (TFC) of *E.polyantha*is 2.47 ± 0.06 mg QE/g extract which might play an important role in improving of oxidative stress.

Antioxidant Activity

DPPH test was performing in order to determine antioxidant activity of the *Eugenia polyantha* and BHT as a positive control. The capacity to neutralize DPPH radicals was found for *Eugenia polyantha*which inhibited 50% of free radicals (IC_{50}) at the 0.15±0.01mg/mL as par as BHT 0.19±0.01 mg/mLrepresented in Table 2.

Table 2: Total Flavanoid, Total Phenolic and Antioxidant Activity of *Eugenia Polyantha* (Leaves)

	Total Flavanoid (Mg Qe/G Extract)	Total Phenol (Mg Gae/G Extract)	Antioxidant Activity Ic_{50} (Mg/Ml)
Eugenia polyantha (leaves)	2.47 ± 0.06	213.15±1.10	0.15±0.01[a]
BHT	-	-	0.19±0.01[a]

Viscosity, Iodine Value, FFA and PV

Table 3 shows the changes in viscosity, IV, FFA and PV value and of the coconut oil during frying. There was noteworthy significantly ($p<0.05$) increase in viscosity with the increase in days of frying. The observed increases in viscosity were due to polymerization resulted in formation of higher molecular weight compound (Gray, 1978). It resulted in the formation of higher molecular weight compounds. The xanthan gum had a consistently higher level of viscosity among the 4 treatments during frying and other treatments in order of the viscosity increase were *E.polyantha*> BHT > control > xanthan gum.

Table 3: Changes in Viscosity, Iodine Value, FFA Content and Peroxide of the Coconut Oil during Frying

Characteristics	Day	Treatment I (Control)	Treatment II (Xanthan Gum)	Treatment IV (BHT)	Treatment III (Seraikayu)
Viscosity (centipose)	0	65.85 ± 0.29^{c}	61.90 ± 0.24^{a}	66.45 ± 0.66^{b}	66.95 ± 0.34^{d}
	1	71.90 ± 0.39^{d}	61.90 ± 0.24^{a}	63.78 ± 0.22^{b}	67.85 ± 0.13^{c}
	2	74.93 ± 0.25^{c}	69.56 ± 0.01^{a}	75.48 ± 0.13^{d}	72.75 ± 0.13^{b}
	3	75.40 ± 0.34^{b}	71.90 ± 0.18^{a}	79.05 ± 0.13^{d}	75.98 ± 0.26^{c}
	4	76.40 ± 0.22^{a}	76.33 ± 0.45^{a}	79.05 ± 0.13^{b}	79.78 ± 0.13^{c}
	5	82.28 ± 0.15^{a}	84.35 ± 0.17^{d}	82.80 ± 0.33^{b}	83.23 ± 0.05^{c}
Iodine Value (g of I_2/100 g oil)	0	7.78 ± 0.51^{b}	7.49 ± 0.55^{a}	7.48 ± 1.09^{a}	7.72 ± 1.39b
	1	7.77 ± 0.36^{c}	6.35 ± 0.56^{a}	7.23 ± 0.30^{b}	7.72 ± 1.39^{c}
	2	7.21 ± 0.54^{b}	6.77 ± 0.39^{a}	7.03 ± 0.68^{b}	7.00 ± 2.69^{b}
	3	6.73 ± 0.73^{b}	5.02 ± 0.74^{a}	6.95 ± 0.49c	6.96 ± 1.18^{c}
	4	6.55 ± 0.61^{c}	5.50 ± 0.49^{a}	6.20 ± 1.32^{b}	6.56 ± 0.69^{c}
	5	5.59 ± 0.83^{b}	5.05 ± 0.74^{a}	6.08 ± 0.66^{b}	6.45 ± 0.80^{c}
FFA Content (%)	0	0.18 ± 0.01^{a}	0.28 ± 0.05^{b}	0.20 ± 0.02^{a}	0.23 ± 0.04^{ab}
	1	0.22 ± 0.02^{a}	0.35 ± 0.04^{b}	0.26 ± 0.07^{ab}	0.30 ± 0.01^{ab}
	2	0.35 ± 0.05^{a}	0.44 ± 0.03^{b}	0.32 ± 0.03^{a}	0.34 ± 0.02^{a}
	3	0.47 ± 0.12^{a}	0.51 ± 0.05^{a}	0.40 ± 0.04^{a}	0.40 ± 0.05^{a}
	4	0.51 ± 0.02^{a}	0.54 ± 0.04^{a}	0.47 ± 0.05^{a}	0.50 ± 0.03^{a}
	5	0.59 ± 0.01^{a}	0.70 ± 0.02^{b}	0.60 ± 0.07^{a}	0.54 ± 0.05^{a}
Peroxide Value (meqhydroperoxide /kg Oil	0	0.00 ± 0.00^{a}	3.81 ± 0.08^{c}	0.00 ± 0.00^{a}	1.87 ± 0.10^{b}
	1	2.43 ± 1.02a	9.25 ± 0.38b	2.29 ± 0.91^{a}	3.72 ± 0.24^{a}
	2	3.62 ± 0.23a	10.19 ± 0.40^{c}	5.76 ± 0.21^{b}	5.56 ± 0.25^{b}
	3	6.63 ± 1.07a	14.07 ± 1.52^{b}	5.91 ± 1.14^{a}	5.43 ± 0.11^{a}
	4	8.76 ± 0.42^{b}	16.58 ± 0.90^{c}	5.97 ± 1.14^{a}	5.69 ± 0.44^{a}
	5	11.08 ± 0.72^{b}	18.28 ± 0.52^{c}	5.46 ± 0.25^{a}	5.41 ± 0.13^{a}

[a]Each value in the table represents the mean ± standard deviation of 4 analyses of from 3 replications. Means within row with different superscripts are significantly ($P < 0.05$) different.

Iodine value indicates the overall unsaturation and liquidity of the oil at a specific temperature. IV for coconut oil ranges from 7.0-9.5 is highly stable towards atmospheric oxidation. (Diana et al, 2012;Ankrah, 1998). The changes in IV over 5 days of frying oil were 2.19, 2.47, 1.4 and 1.27 g I_2/100 g oil for all treatment respectively. A significantly ($P<0.05$) larger change in iodine value in control and xanthan gum (treatment I and II) compared to the BHT and *E.polyantha*indicated that the rate of oxidation of unsaturated fatty acids was affected by the antioxidant content. The oil degradation can be caused by oxidation, hydrolysis and polymerization which can influenced by several factors such as moisture, enzymatic catalyst, heat, and chemical reaction (McGill, 2009). The smaller changes in iodine value in treatment III and IV showed less oxidation occurred in both treatments. Therefore, the changes in IV showed that extract from *E. polyantha*were comparatively more effective in protecting oxidation of unsaturated fatty acid than BHT. FFA content is common indicator for the oil quality in foods. It also showed the occurrence of hydrolytic rancidity in the oil that may contribute to breakdown of fatty acid in the triglycerides chain. From the results obtained, the FFA content in treatment I, II and III were 0.40, 0.42 and 0.40 % respectively. However smaller change in FFA content for treatment IV as compared to other treatments.

Although the FFA content is not a reliable measurement of oil deterioration, thus result showed that there are slightly higher value of FFA content in treatment II and IV at day 0 compared to other treatments. These condition may be due to the present of water in the preparation of oil that cause the breakdown more fatty acids that affect the FFA content in both treatments. These mentioned by Tyagi and Vasishtha (1996), the formation of FFA can be attributed by oil

hydrolysis and the presence of carboxylic groups of the oil. However, the oil treated with crude *E.polyantha*gives lowest FFA content (0.31%) compared to other 3 treatments (≥ 0.4%). In summary, the FFA content in oil for all treatments after 5 days of frying were less than 2%, below the discard points of 2.0-5.0% (Koh& Long, 2012).

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils (Marina et al. 2009).Peroxide value represents primary reaction products of lipid oxidation, which can be measured by their ability to liberate iodine from potassium iodide (Nor et al. 2008). The present study shows that after 5 days of frying, the PV rose for treatment I and II. However in treatment III and IV the PV was slightly increased and fell on the last day of frying, which the same pattern is observed for peroxides in most deep-fat frying studies (Che Man & Tan, 1999; Rady&Madkour, 1995). These mainly due to the decomposition of the peroxide products to produce carbonyl and aldehydric compounds under deep frying operation (Makhoul et al, 2006).

Coconut oil with the addition of antioxidant (treatment III and IV) had PV not significantly different ($p < 0.05$) lower than control and xanthan gum (treatment I and II) throughout the duration of the study. Although there was no significant different between treatment III and IV, the PV for oil with*E.polyantha*extract, rose slightly lower (3.54 meqhydroperoxide /kg Oil) than coconut oil treated with BHT (5.41meqhydroperoxide/kg oil). Thus,the *E.polyantha*extract should be considered as a potential natural source of antioxidant in frying oils that can prevent oil rancidity.

CONCLUSIONS

The present studies denote that the present of phytochemicals in *E.polyantha*and contain high antioxidant activity comparable with BHT that maybe donated from phenol or flavonoid. The high antioxidant activity of extract from *E.polyantha*extract greatly improved the peroxide value and free fatty acid of the frying coconut oil. These finding indicate that the *E. polyantha extract* can as the natural antioxidant in frying coconut oil.

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