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Antioxidant activity of fresh and dried extracted herbs mixture of *Psidium guajava*, *Pteridophytes*, *Cymbopogon*, and *Pandanus amaryllifolius*

Ummi Kalthum Ibrahim* and Amirah Adniruddin

Faculty of Chemical Engineering, Universiti Teknologi MARA, Selangor, Malaysia

Corresponding email: ummi985@salam.uitm.edu.my

Abstract

This study aims to investigate the effect of solvent concentration and extraction temperature on antioxidant activity of fresh and dried mixture of Psidium guajava, Pteridophytes, Cymbopogon, and Pandanus amaryllifolius. The samples were extracted using different concentration of methanol (0, 30, 50,and 70% (v/v)) in a shaking water bath at temperature of 60 °C and 90 °C for 30 minutes. From the results, the antioxidant activities were dependent on the solvent concentration and the temperature during extraction. The condition of both samples demonstrated different antioxidant activities. The highest antioxidant activities were found at 30% (v/v) methanol concentration and 60 °C indicating by the scavenging percentage of 53.85% and 65.93% for fresh and dried mixture, respectively. However, 70% (v/v) of methanol concentration exhibited the lowest antioxidant activities of fresh and dried mixture, showing 29.67% and 42.86%, respectively. Overall, the antioxidant activity in oven dried samples is higher than in the fresh samples. Other than that, GC-MS analysis presented the octen-1-ol,3,7-dimethyl (0.240%), cis-á terpineol (0.334%) and 6-octenal, 3,7-dimethyl (0.205%) as a major component in the extracts.

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1.0 Introduction

Traditional medicine deals with the knowledge, skills, and practices based on the theories, beliefs, and experiences that are used in the maintenance of health as well as in the inhibition, diagnosis, and treatment of physical and mental disease. Nowadays, herbs are used to treat chronic and acute conditions, and various ailments and complications such as cardiovascular disease, prostate problem, depression, inflammation, and to improve immune system (Watchel-Galor et al., 2011).

Previous study has been conducted to examine the antioxidant activity in guava (*Psidium guajava)*, fern (*Pteridophytes*), lemongrass (*Cymbopogon*), and pandan (*Pandanus amaryllifolius*). Guava is widely cultivated and consumed in Asia. The long history of guava's consumption has led many researchers to study guava extracts. Extracts and metabolites of this species, especially obtained from the leaves, possess useful pharmacological activities including antioxidant and antimicrobial properties, and have been widely used in the treatment of diarrhoea. Other reported uses include its function as a hypoglycaemic, antitussive, anti-inflammatory, and antioxidant thereby reinforcing its traditional consumption (Gutiérrez et al., 2008). With regard to *Pteridophytes*, which better known as fern, it belongs to a group of non-flowering plants. It is estimated that there are 1136 species of *Pteridophytes* in Malaysia (Lai and Lim, 2011). Bioactive components of fern mainly belong to the phenolic, flavonoid, alkaloid, and terpenoid families. Flavonoids and other phenolic compounds have been demonstrated to be potent antioxidants (Chai et al., 2012). Lemongrass (*Cymbopogon*) is a tropical perennial plant. It has been used by humans for thousands of years because of its characteristic and valuable flavour, and for the treatment or prevention of health disorders and diseases. Lemongrass has been classified as a safe herb for human consumption (Chukwuocha et al., 2016). Pandan (*Pandanus amaryllifolius*) is one of the terrestrial plant species found to adapt well to hydrophytic conditions and is commonly known as fragrant pandan. Ooi et al. (2006) isolated several interesting active proteins from this plant; and reported the purification and characterisation of a new mannose-binding protein. The oil from the leaf is described as stimulant, diuretic (Tan et al., 2010), antispasmodic, and is effective against headaches, rheumatism, and

epilepsy, and as a cure for sore throats (Nor et al., 2008). On top of that, guava leaves extract presented potential antioxidant and antimicrobial activities (Fernandes et al., 2014). Fern, lemongrass, and pandan also showed high antioxidants content (Ding et al., 2008; Ghasemzadeh & Jaafar, 2013; Balakrishnan et al., 2014).

Despite the improvement of new extraction techniques, conventional extraction technique dominates in many experimental works due to its simplicity and low economic outlay. The example of conventional extraction is Soxhlet, percolation, and maceration. Both the extraction yield and antioxidant capacity of extracts are strongly influenced by the solvent, due to the different polarity and different antioxidant potential of compounds extracted. For determination of antioxidant activity, the free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is commonly used.

Physiologically, antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative. Recently, according to Ministry of Health Malaysia, the most widely used additive in food are synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT). These additives are known to give bad effect to human health such as liver damage. Thus, natural antioxidant has potential to replace the synthetic ones. Further research on the feasibility of natural antioxidant is necessary. Nevertheless, the documented research on beneficial compound such as antioxidant of traditional medicinal plants in Malaysia is very limited to a few plant species and lack information are presented to prove its effectiveness. Therefore, it is of paramount importance to determine the beneficial compounds in traditional herbs mixture such as guava, fern, lemongrass, and pandan, due to its usage for refreshment and remedies after labour or during confinement period.

The objectives of this study are to investigate the effect of solvent concentration and temperature during extraction on chemical composition and antioxidant activity of fresh and dried mixture of *Psidium guajava*, *Pteridophytes*, *Cymbopogon*, and *Pandanus amaryllifolius*. This study is focusing on the effect of solvent concentration and temperature on the extraction a mixture containing guava, fern, lemongrass, and pandan.

2.0 Methodology

2.1 Sample preparation

Leaves of guava (*Psidium guajava*), fern (*Pteridophytes*), lemongrass (*Cymbopogon*), and pandan (*Pandanus amaryllifolius*) were freshly purchased from the market in Shah Alam, Malaysia. The twigs were removed from leaves and fresh leaves were washed with tap water. The clean leaves were air dried for 1–2 hours to remove surface moisture. The samples were separated into two groups, which were used as freshly minced and oven dried. The samples were oven dried at temperature of 50 °C for 24 hours. For the freshly minced sample, the leaves were cut into small pieces, kept in a plastic container and refrigerated at 4 °C for not more than one week. For the oven dried samples, the leaves were ground with an electric grinder and maintained in dark air-tight plastic containers. The samples were stored in a freezer at –20 °C before further analysis was carried out.

2.2 Sample extraction

For the extraction of mixture of *Psidium guajava*, *Pteridophytes*, *Cymbopogon*, and *Pandanus amaryllifolius*, a method of Deng et al. (2012) with slight modifications were adopted. Three different methanol concentrations (30, 50, and 70% (v/v)) and distilled water were used as solvent. Extraction was performed at two temperatures (60 °C and 90 °C) for 30 minutes. One gram of freshly minced and oven dried samples were extracted with 20 ml of organic solvent and distilled water at 60 °C and 90 °C for 30 minutes on a horizontal water bath shaker at 100 rpm. The extracted samples were then filtered through Whatman No. 1 filter paper using a Büchner funnel, and the filtrate were adjusted to 25 ml in volumetric flasks with appropriate organic solvent or distilled water. The extracted samples were stored at –18 °C for further analyses.

2.3 DPPH radical scavenging analysis

The antioxidant activity of the extracted samples was measured with the DPPH method with a slight modification (Do et al., 2014). A solution of DPPH was prepared by dissolving six milligram DPPH in 50 ml methanol (0.3 mM). The extracted samples (2.5 ml) and DPPH solution (2.5 ml) was mixed together in a test tube. The test tube was then incubated in the dark for 20 minutes at room temperature. The decreased in

absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of radicals was calculated using the following equation Eq. (1):

$$\% \text{ inhibition} = (Abs_{control} - A_{sample}) \times 100 / Abs_{control} \quad (1)$$

where $Abs_{control}$ is the absorbance of DPPH solution without extracted sample and Abs_{sample} is the absorbance of extracted sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. A lower IC_{50} indicates a higher antioxidant activity of a compound (Do et al., 2014). All tests were performed in triplicate, and graphs were plotted using the average of three determinations.

2.4 Gas chromatography-mass spectrometry(GC-MS) analysis

GC-MS were performed using Agilent Technologies 6890 Network GC System with Agilent Technologies 5973 inert Mass Selective Detector. The flow rates of gases were set to manufacturer's specifications. The column used was a HP-5MS fused silica capillary column, 30.0 m × 250 µm I.D and 0.25 µm capillary thickness. Injections were made in the split less mode. The temperature was programmed at an initial of 60 °C for two minutes, followed by an increase of 10 °C/min to temperature of 200 °C and held for 15 minutes. Both the injector temperature and the detector temperature were set at 250 °C. Compounds were then identified by matching their mass spectra with the National Institute Standard and Technology (NIST) spectral library with a resemblance percentage above 90% (Ajayi et al., 2016).

3.0 Results and discussion

3.1 DPPH radical scavenging activities

DPPH radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Hseu et al., 2008). Table 1 shows the DPPH scavenging activities of the extracts in terms of percent inhibition. The antioxidant activity of both fresh and dried samples extracted in 30% (v/v) methanol concentration at 60 °C yielded the highest DPPH radical scavenging activity at percent inhibition of 53.85% and 65.93%, respectively. However, at methanol concentration of 70% (v/v) and extraction temperature of 90 °C exhibited the lowest radical scavenging activity at percent inhibition of 29.67% and 42.86% for fresh and dried samples, respectively. Furthermore, all sample extracts from oven dried gave the stronger radical scavenging capacity as compared to the fresh sample. This is because of the drying process cause the brittleness of the tissue inside the sample which in turns result in rapid cell wall rupture during extraction using shaking water bath. The broken cell wall may release more antioxidant compound into the solvent when they are shaken during extraction. In addition, it could also be due to the enzymatic degradation which cause the sample to lose antioxidant compounds, as the enzyme were still active in the fresh samples (Hossain et al., 2010).

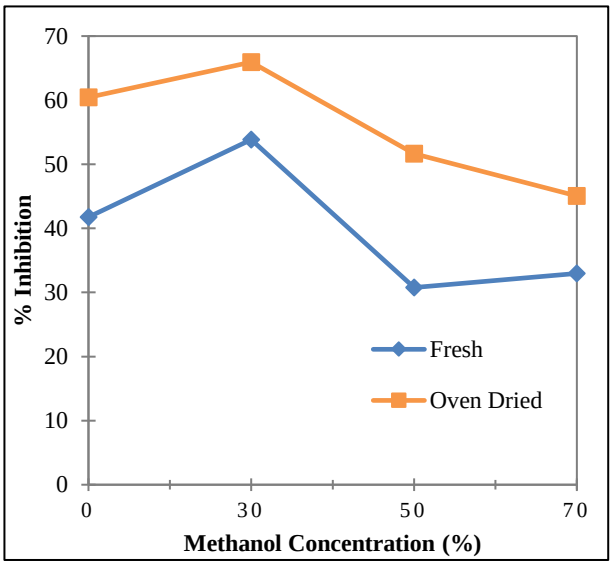
Table 1: DPPH scavenging activities.

Extraction solvent	Extraction Temperature (°C)	Percent Scavenging Activities (%)	
		Fresh	Oven Dried
30% Methanol	60	53.846	65.934
	90	41.758	54.945
50% Methanol	60	30.769	51.648
	90	43.956	50.549
70% Methanol	60	32.967	45.054
	90	29.670	42.857
Water	60	41.758	60.439
	90	34.065	54.945

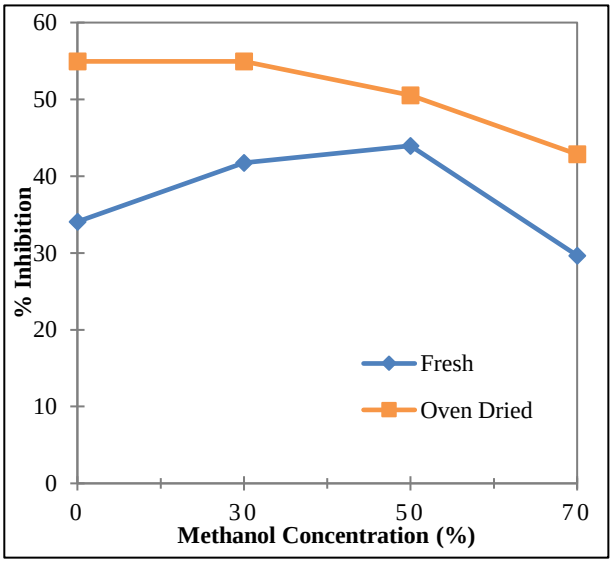
3.2 Effect of solvent concentration

From the results shown in Fig. 1(a) and (b), it is proven that the antioxidant activities were dependent on the solvent concentration. On the other hand, an increased in the percentage of methanol concentration in aqueous solutions had no positive influenced on the extraction efficiency of antioxidant, and the antioxidant activity was maximized at aqueous methanol extraction of 30% (v/v) then followed by distilled water extraction at 60 °C. The best conditions for extraction of antioxidants from the herb mixture appeared with methanol composition of about 30% (v/v) at 60 °C for 30 minutes for both fresh and oven dried sample. However, the differences in activity

among methanol extract and water extract were not significant, which is in agreement with the green chemistry principles whereby water could be considered as an efficient solvent for antioxidant extraction as well. The conventional extraction of antioxidant compounds in thyme and marjoram was conducted with 30, 50, and 70% aqueous methanol solutions, lasting 15–30 minutes, where better results were achieved with more water content in aqueous solutions of solvent (Fecka & Turek, 2008). The results were in agreement with previous studies which showed that solvent nature exert a great power in antioxidant extraction capacities in many species (Akowuah et al., 2005).



(a)



(b)

Fig. 1: Effect of solvent concentration on scavenging activities at (a) 60 °C; and (b) 90 °C.

3.3 Effect of extraction temperature

The radical scavenging activities on the extracts obtained at extraction temperature of 60 °C were ranged from 30.77% to 65.93%. The sample extracted by distilled water resulted in scavenging activities of 60.44% at 60 °C and 54.95% at 90 °C. The results showed that methanol extracts obtained at extraction temperature of 60 °C contained the higher content of antioxidant and they were selected for GC-MS analysis. Conversely, the antioxidant activity in water extracts slightly decreased with increasing extraction temperature. The extraction done at temperature of 60 °C gave higher yields of antioxidant due to increased solubility and diffusion coefficients, while extraction at 90 °C resulted in the decreased of antioxidant activities. Above a temperatures of 60 °C, the reduction in an extraction yield could possibly be due to degradation of phenolic compounds. According to Durling et al. (2007), an increased in temperature resulted to an increase of extracted yields, and the optimum extraction of polyphenols was observed at 40 °C. Furthermore, the recoveries for almost all phenolics were similar at both 40 °C and 60 °C, thus extractions at temperatures higher than 40 °C were extracting more non-active compounds from the sage. They also reported about increased solvent losses at high temperatures.

3.4 GC-MS analysis

The methanol concentration at 30%(v/v) has shown significantly higher antioxidant capacities when compared with the other concentrations. Hence, further analysis was carried out using GC-MS. GC-MS analysis was conducted with a total run time of 31 minutes and the comparison of mass fragmentation pattern of compounds with NIST library revealed the presence of 14 phytocomponents of different groups as shown in Table 2. Among the 14 phytocomponents, 7-octen-1-ol, 3,7-dimethyl (0.240%), cis-á-terpineol (0.334%),6-octenal, 3,7-dimethyl (0.205%) and 3-(Dimethylamino)-7-(methylamino) phenothiazin-5-ium (0.164%) were present in major amount. On the other hand, mequinol (0.0115%), 6-octen-1-ol,3,7-dimethyl-, propanoate (0.00268%), 2-propanone, 1-(4-methoxyphenyl) (0.00334%), piperidine, 1-methyl (0.0162%), limonene oxide, cis (0.00730%), anisaldehyde dimethyl acetal (0.00222%), 1-eicosene (0.00878%) and 1H-imidazole, 2-propyl (0.00550%) were minor components present in methanolic extracts of the herbs mixture.

These phytocomponents are well recognised for their antioxidative action (Dimitrios,2006; Deng et al.,2012) and these components were assumed could also be the contributing factor for antioxidant capacity of methanol extract of the herb mixture.

Table 2: GC-MS chemometric profile of methanolic extracts of *Psidiumguavajava*, *Pteridophytes*, *Cymbopogonn* and *Pandanus amaryfollius* mixture.

No	Peak RT (min)	Peak area	Peak area (%)	Compound detected
1	3.047	366658	0.240	7-Octen-1-ol,3,7-dimethyl
2	3.054	510335	0.334	cis-á-Terpineol
3	3.548	313638	0.205	6-Octenal, 3,7-dimethyl
4	4.737	17580	0.0115	Mequinol
5	6.022	4097	0.00268	6-Octen-1-ol,3,7-dimethyl-, propanoate
6	6.216	5102	0.00334	2-Propanone,1-(4-methoxyphenyl)
7	6.293	24777	0.0162	Piperidine, 1-methyl
8	7.140	11154	0.00730	Limonene oxide
9	8.403	3399	0.00222	Anisaldehyde dimethyl acetal
10	9.983	13418	0.00878	1-Eicosene
11	10.015	8411	0.00550	1H-Imidazole, 2-propyl
12	14.834	23698	0.0155	2,6-Dimethyl-6-nitro-2-hepten-4-one
13	15.238	6549	0.00428	Alloaromadendrene oxide-(1)
14	22.979	251123	0.164	3-(Dimethylamino)-7-(methylamino) phenothiazin-5-ium

4.0 Conclusions

In general, the antioxidant activity in oven dried samples was higher than that in the fresh samples. Hossain et al. (2010) reported that relatively low antioxidant estimation in fresh samples had a very strong correlation with high moisture content, which caused dilution effect toward the total antioxidant content in fresh samples. Fresh samples containing high moisture may also lose its antioxidant compounds through the enzymatic degradation process because the active enzymes in fresh samples are still high. The temperature and solvent concentration also affect the antioxidative activities of the herbs mixture. Investigating the effects of sample storage duration is recommended to maximize the determination of the antioxidant activity in the samples.

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