Comparison on Antioxidant Activity of Fresh and Oven Dried Extracted Mixture of Five Different Types of Traditional Herbs (*Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel*)

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Abstract— **This study aimed to assess the effect of solvent concentration and temperature during extraction on chemical composition and antioxidant activity of fresh mixture and dried mixture of Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel using DPPH radical scavenging analysis and gas chromatography-mass spectrometry (GC-MS) analysis. The herbs were divided into two which are fresh sample and oven dried sample. The sample were then extracted using different concentration of methanol (0,30,50,70 %) in a shaking water bath at temperature 60°C and 90°C for 30 min at 100 rpm. The antioxidant activities were dependent on the solvent concentration used and the temperature during extraction. The condition of the sample whether dried or fresh also shows different antioxidative activities. The optimum conditions for extraction of antioxidant from the herb mixture appeared with methanol composition of about 30% at 60 °C for 30 min with oven dried sample followed by distilled water extraction at 60°C with oven dried samples. The antioxidant activity in oven dried samples was higher than that in the fresh samples due to the high moisture content of the fresh sample. The GC-MS analysis characterized 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.**

Keywords—Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel; effect of solvent concentration and temperature; DPPH radical scavenging analysis; GCMS analysis;

INTRODUCTION

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs and experiences of our ancestors, whether reasonable or not, 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 the treatment of chronic and acute conditions and variety of ailments and complications such as cardiovascular disease, prostate problem, depression, inflammation, and to improve immune system and many more (Watchel-Galor, 2011).

Previous study has been conducted to examine the antioxidant activity of various solvent extracts using different in vitro models and to determine the most antioxidant content of each Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel. According to Fernandes et al (2014), Psidiumguavajava leaves extract showed present potential antioxidant and antimicrobial activities. Fern, Cymbopogonn and Pandanus amaryfollius also studied for its antioxidant activities and showed high antioxidants content.(Ding et al., 2008; Ghasemzadeh & Jaafar, 2013 ; Balakrishnan, Paramasivam, & Arulkumar, 2014). In spite of the improvement of new extraction techniques, classic extraction monopolize in many laboratories usually due to its simplicity and low economic outlay such as 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 (Oreopoulou, 2003). For determination of antioxidant activity, the free radical scavenging activity using DPPH assay is used.

Physiologically, antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes. Lately, according to The Ministry of Health (Malaysia) the most widely used antioxidants are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) which can affect human health such as liver damage. The documented research on traditional remedies or traditional medicinal plants in Malaysia is very limited to a few plant species and very little information are present to prove its effectiveness. In Malaysia, for decades our ancestors have been bath with a mixture of boiled Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel 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 mixture of Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius, Betel and to investigate the effect of solvent concentration and temperature during extraction on chemical composition and antioxidant activity of dried mixture of Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel.

This study will focus on extracting mixture of Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel using different solvent concentration and temperature to study its chemical composition and antioxidant activity. Raw material for this experiment, Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel were collected at local market in Shah Alam. The solvents used for extracting samples are methanol and water. The types of leaves used for extraction are oven dried leaves and

fresh leaves. Gas chromatography–mass spectrometry (GC-MS) is used to analyze the composition of the mixture and the free radical scavenging activity is determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay as described by Lee et al. (2013) with minor modification.

METHODOLOGY

A. Materials

Leaves of Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel were freshly collected 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 dried using oven at temperature of -50°C for 24 hours. For the freshly minced sample, the leaves were cut into small pieces, maintained in a plastic container and refrigerated at 4°C for not more than 1 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.

B. Sample Extraction

For the extraction of mixture of Psidiumguavajava, Fern, Cymbopogonn, Pandanus amaryfollius and Betel, a method of Dent et al, 2012 with slight modifications are used. Two different aqueous solutions (30, 50 or 70 %) of methanol and distilled water are used as solvent. Extraction was performed at two temperatures (60°C or 90 °C) for 30 minutes. Approximately 1 g of freshly minced and oven dried sample was 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 with at 100 rpm. The extracts were then filtered through Whatman no. 1 filter paper using a Büchner funnel, and the filtrates were adjusted to 25 mL in volumetric flasks with appropriate organic solvent or distilled water. The extracts were stored at –18 °C until analyses.

C. DPPH Radical Scavenging Analysis

The antioxidant activity of the extract was measured with the DPPH method with a slight modifications (Do et al, 2014). A solution of DPPH was prepared by dissolving 6 mg DPPH in 50 mL ethanol (about 0.3 mM). The extract (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 decrease in absorbance was measured at 517 nm using a UV VIS spectrophotometer. The percentage inhibition of radicals was calculated using the following formula:

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

where Acontrol is the absorbance of DPPH solution without extract and Asample is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC50) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. A lower IC50 indicates a higher antioxidant activity of a compound (Do et al, 2014). All tests were performed at least in triplicate, and graphs were plotted using the average of three determinations.

D. Gas Chromatography-Mass Spectrometry Analysis

GC-MS were performed on 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 x 250 µm I.D and 0.25 µm capillary thickness. Injections were made in the splitless mode. The temperature programmed was set at an initial 60 °C for 2 min, followed by an increase of 10 °C min-1 to 200 °C and held for 15 min. 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, Sadimenko & Afolayan, 2016).

RESULTS AND DISCUSSION

A. 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). Fig. 1 shows the DPPH scavenging activities of the extracts in terms of percent inhibition. The extract obtained by 30% methanol at 60°C by oven dried sample yielded the highest DPPH radical scavenging activity at percent inhibition of 65.93 %. However, at solvent concentrations of 30%, 50% and 70%, for oven dried sample, its DPPH radical scavenging activity is not significantly different from those of the other extracts. All extracts obtained by the oven dried sample gave the stronger radical scavenging capacity than that of the fresh sample. This may be because the drying process caused the tissue inside the sample to be brittle, thus result in rapid cell wall breakdown during extraction using shaking water bath. The broken cell wall may release more antioxidant compound into the solvent when they are shaken during extraction. Other reasons may be because of 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).

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

Table 1: DPPH scavenging activities

B. The effects solvent concentration

In this research distilled water and aqueous solution of methanol (30, 50 and 70 %) for extraction of a mixture of 5 different herbs were used. From the results shown in Fig. 1 and Fig. 2, it is evident that the antioxidant activities were dependent on the solvent concentration used. On the other hand, an increase in the percentage

of methanol concentration in aqueous solutions had no positive influence on the extraction efficiency of antioxidant, and the antioxidant activity was maximized at aquoues methanol extraction of 30% then followed by distilled water extraction at 60°C. The optimum conditions for extraction of antioxidants from the herb mixture appeared with methanol composition of about 30% at 60 °C for 30 min with oven dried sample. Using 30 % methanol solvents comparing with aqueous extracts, antioxidant activities is higher. However, the differences in activity among methanol extract 30% and water extracts are not significant, so in agreement with green chemistry principles water could be considered as an efficient solvent for antioxidant extraction, too. 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*)*.

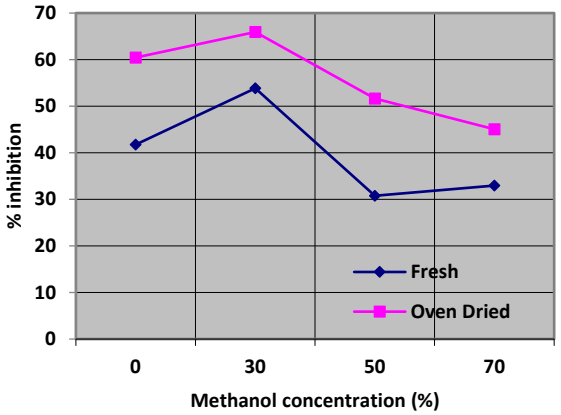

Fig. 1: Effect of solvent concentration on scavenging activities at 60°C

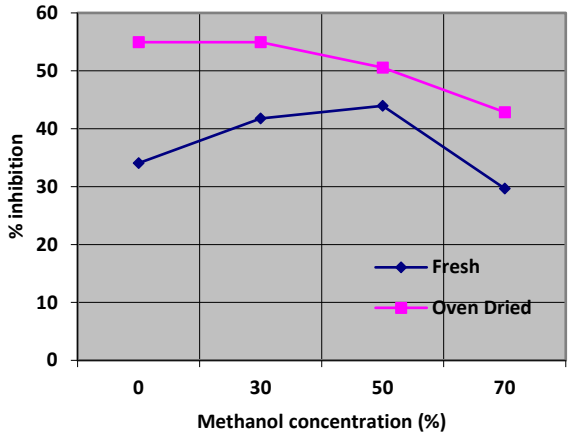

Fig. 2: Effect of solvent concentration on scavenging activities at 90°C

C. The Effects of Temperature

The scavenging activities on the extracts obtained by using methanol solutions at 60 °C ranged from 30.769% to 65.934%. The antioxidant extraction by the distilled water resulted in scavenging activities of 60.439% at 60°C and 54.945% 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 decrease of antioxidant activities. Temperatures above 60°C probably produced an extraction yield decrease due to a possible degradation of phenolic compounds. According to Durling et al, 2007, an increase in temperature resulted in increased extract yields, and the optimal extraction of polyphenols was observed at 40°C. Furthermore, the recoveries for almost all phenolics were similar at both 40 and 63°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.

D. GC-MS Analysis

In the present investigation, the methanol extract at 30% has shown significantly higher antioxidant capacities when compared with the aqueous extract. Hence, we decided for further analysis of this extract by GC-MS. The GC-MS analysis was done with a total run time of 31 min and the comparison of mass fragmentation pattern of compounds to that of in NIST library revealed the presence of 14 phytocomponents of different groups (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 found to be 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.

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

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

These phytocomponents are well recognized 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.

CONCLUSION

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 and high moisture contents of samples 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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