

Full Length Research Paper

Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts

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Accepted 13 October, 2010

The extractive capability of phenolic components from herb material is considerably depended on the type of solvent. In our research three kinds of solvents (methanol, acetone and chloroform) extracts from different parts (leaves, stems and rhizomes) of two Malaysian young ginger varieties (Halia Bara and Halia Bentong) were used to examine the effects of extraction solvent on total phenolics (TP), total flavonoids (TF), quercetin, catechin and rutin content and antioxidant activity [1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay]. Results showed that extraction solvent had significant effects on TP, TF, quercetin, catechin and rutin content and antioxidant activity. The highest content of TP, TF and DPPH scavenging activities were found in methanol extracts. Additionally, High performance liquid chromatography results shown that methanol had the highest extraction capacity for quercetin, rutin and catechin. Between varieties Halia Bara had high content of TP, TF and antioxidant activities to compare with Halia Bentong. Accumulation and partitioning of TP and TF in both varieties were: leaves > rhizomes > stems in all the three solvent extracts. However, according to the results extraction yield of phenolic compounds is greatly depending on the solvent polarity. With increased in solvent polarity from chloroform to methanol, amount of phenolic compounds and antioxidant activities increased in both varieties. Thus, for routine screening of young ginger varieties with higher antioxidant activity, methanol was recommended to extract phenolic compounds from young ginger.

Key words: Solvent, TP, TF, DPPH, *Zingiber officinale*.

INTRODUCTION

Natural bioactive compounds especially from plant sources, including spices have been investigated for their characteristics and health effects. Ginger has been used as a medicine traditional, recorded in Sanskrit and Chinese texts and ancient Greek, Roman and Arabic medical published works (Bone et al., 1997). *Zingiber officinale* Roscoe is a member of the family Zingiberaceae

is well known in Asia. The plant is widely cultivated in village gardens in the tropics for its medicinal properties and as a marketable spice (Saadiah et al., 1995). In Asia and especially in Malaysia rhizomes of young ginger (family Zingiberaceae) have been widely used as spices or condiments. Ginger is the third most important spice originated in South Asia. The components in ginger include: extractable oleoresins, many fats, carbohydrates, vitamins, minerals, medicine compounds such as: antioxidant, flavonoids and anticancer (Shukla et al., 2007). Flavonoids are large family of polyphenolic components synthesized by plants. It was found that flavonoids functioned to reduce blood-lipid and glucose and to enhance human immunity (Atoui et al., 2005). Flavonoids were also a kind of natural antioxidant

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Abbreviations: TP, Total phenolics; TF, total flavonoids; DPPH, 1,1-diphenyl-2-picryl-hydrazyl

capable of scavenging free superoxide radical, anti-aging and reducing the risk of cancer. At present, flavonoids are extracted from ginkgo leaves (Feng et al., 2002), kudzu root (Liao et al., 2003), lotus leaves (Chen et al., 2002) and ginger rhizomes (Yang et al., 2002). It also had a benign effect on alleviating malaise such as hyperlipidemia (heart disease) and hypertension. However, flavonoids contents of some materials are limited and other materials are mainly of dietary or medicinal use, none of these materials can be produced on a large scale and meet the demand for flavonoids. Ginger is known as a resource with higher phenolic components, wide source and low price (Rozanida et al., 2005; Tang et al., 2001) and therefore it can serve as a cheap and important material in food.

Today's plant secondary metabolites and especially herbs are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process. Usually, complete extraction begins with careful preparation and selection of plant parts, and thorough review of the suitable literature for indications of which protocols are most suitable for a specific group of compounds or plant species. During the extraction of herb material, it is important to minimize interference from compounds that may co-extract with the chemicals, and to avoid contamination of the extract, as well as to prevent decomposition of metabolites or artifact formation as a result of extraction conditions or solvent impurities. The technique of phenolic components (flavonoids) isolation from a plant material, including the methods and type of extracting solvent, depends generally on the type of phenolic compound and the solvents (Golo et al., 2004). Results of previous studies showed that the extraction yield of phenolic and flavonoid content is greatly depending on the solvent polarity (Turkmen et al., 2006; Lapornik et al., 2005).

Maximum total phenolic content were obtained from barley flour with mixtures of ethanol and acetone (Bonoli et al., 2004). Similarly, Chatha et al. (2006); Siddhuraju et al. (2003) reported highest content of phenolic compounds from rice bran and *Moringa oleifera* leaves when extracted by aqueous methanol. Anwar et al. (2006) reported high content of antioxidant compounds from various plant materials including rice bran, wheat bran, oat groats and hull, coffee beans, citrus peel and guava leaves extracted by aqueous 80% methanol (methanol: water, 80:20 v/v). It can be concluded that it is not clear which type of solvent is more effective for extracting total phenolics and flavonoids of ginger organs and evaluating the antioxidant activity of them. On the other hand, little is known about phenolic and flavonoid contents and antioxidant activity of ginger extracted by different solvents with different polarity. Therefore, the objectives of this research were to investigate the effect of different extracting solvents with different polarity (from low to

high) on total phenolics (TP), total flavonoids (TF), quercetin, catechin and rutin content and antioxidant activity [1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay] of two varieties of Malaysian young ginger (*Zingiber officinale*) organs. The organic solvent systems with different polarities included methanol, acetone and chloroform.

MATERIALS AND METHODS

Plant material and maintenance

Two varieties of *Z. officinale* Roscoe (Halia Bentong and Halia Bara) seed rhizomes were germinated for two weeks in small pots and then transferred to polyethylene bags containing soilless mixture of burnt rice husk and coco peat at a ratio of 1:1. The plants were grown under glasshouse conditions at the glasshouse complex of University Putra Malaysia (UPM) where daily irradiance was approximately $790 \mu\text{molm}^{-2}\text{s}^{-1}$. The plants were harvested after 16 weeks, with the leaves, stems, and rhizomes separated. Once dried, they were all kept at -80°C for future analysis.

Extract preparation

Leaves, stems and rhizomes were freeze dried to constant weights prior to being used in the extraction. For TF and TP extraction, the leaves, stems, and rhizomes were powdered and 1 g of the powder was extracted continuously with three different aquatic solvents with different polarity (polar protic solvent: methanol, CH_3OH , 80%, polar aprotic solvent: acetone, CH_3COCH_3 , 80%, non polar: chloroform, CHCl_3 , 80%), (50 mL). The solution was then swirled for 1 h at room temperature using an orbital shaker. Extracts were then filtered under suction and stored at -20°C for further use.

Determination of total phenolic content

The total phenolic content was determined using Folin-Ciocalteu reagents with analytical grade gallic acid as the standard. 1 mL of extract or standard solution (0 to 500 mg/L) was added to deionized water (10 mL) and Folin-Ciocalteu phenol reagents (1.0 mL). After 5 min, 20% sodium carbonate (2.0 mL) was added to the mixture. After being kept in total darkness for 1 h, the absorbance was measured at 750 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Amounts of TP were calculated using gallic acid calibration curve. The results were expressed as mg gallic acid /g of dry plant matter (Kim et al., 2003).

Determination of total flavonoid

Spectrophotometric method described by Bushra et al. (2009) was used for TF measurement. Extracts of each plant parts material (1 mL containing 0.1 mg/mL) were diluted with distilled water (4 mL) in a 10 mL volumetric flask. Initially, 0.3 mL NaNO_2 solution (5%) was added to each volumetric flask, at 5 min, 0.3 mL AlCl_3 (10%) was added; and at 6 min, 2 mL NaOH (1.0 M) was added. Water (2.4 mL) was then added to the reaction flask and mixed well. Absorbance was read at 430 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). For each sample, three readings were taken to get the averaged results. The results were expressed in mg quercetin/g dry weight by comparison with the quercetin standard curve, which was made under the same condition.

Determination of antioxidant activities

DPPH radical scavenging assay

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Sigma–Aldrich (USA). Butylated hydroxytoluene (BHT) and α -tocopherol were purchased from Merck (India). In order to determine the radical scavenging ability, the method reported by Mensor et al. (2001), was used. Briefly, 0.3 mM alcohol solution of DPPH (1 mL) was added to samples (2.5 mL) containing different concentrations originating from different parts of ginger varieties' extracts. The samples were first kept in a dark place at room temperature and their absorbance was read at 518 nm after 30 min. The antiradical activity (AA) was determined using the following formula:

$$AA\% = 100 - \frac{(\text{Abs: sample} - \text{Abs: empty sample})}{\text{Abs: control}} \times 100$$

Blank samples contained 1 mL solvent + 2.5 mL from various concentrations of ginger extract; control sample containing 1 mL of 0.3 mM DPPH + 2.5 mL solvent. One synthetic antioxidant, BHT (butylhydroxytoluene) and α -tocopherol were used as positive controls.

High performance liquid chromatography (HPLC) apparatus

Extract preparation

0.25 g aliquots of leaves and rhizomes were extracted with 20 ml of aqueous methanol, acetone and chloroform. The 5 mL of 6 M HCl was added to each extract to obtain a 25 mL solution of 1.2 M HCl in solvents. Extracts were refluxed at 90°C for 2 h. Extract aliquots of 500 μ L, taken both before and after hydrolysis, were filtered through a 0.45 μ m filter (Crozier et al., 1997).

Analysis of flavonoid composition by HPLC

Reversed-phase HPLC was used to assay compositions of flavonoids. Agilent HPLC system (Tokyo, Japan) consisted of a Model 1100 pump equipped with a multi-solvent delivery system and L-7400 ultraviolet (UV) detector. The column type was Agilent C18, 5 μ m, 4.0 mm internal diameter 250 mm. The mobile phase composed of (A) 2% acetic acid (CH₃COOH) and (B) 0.5% acetic acid -acetonitrile (CH₃CN), (50:50 v/v), and gradient elution was performed as follows: 0 min, 95:5; 10 min, 90:10; 40 min, 60:40, 55 min, 45:55; 60 min, 20:80; and 65 min, 0:100. The mobile phase was filtered under vacuum through a 0.45 μ m membrane filter before use. The flow rate was 1 mL/min. UV absorbance was measured at 280 to 365 nm. The operating temperature was maintained at room temperature (Wng et al., 2007). Identification of the flavonoids was achieved by comparison with retention times of standards, UV spectra and calculation of UV absorbance ratios after co-injection of samples and standards. Commercial standards were purchased from Sigma–Aldrich (USA).

Statistical analysis

The results were expressed as mean \pm standard deviation of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's Multiple Range test using the Statistical Analysis System (SAS, 1999) programme. P-value of

< 0.05 was regarded as significant.

RESULTS AND DISCUSSION

Total phenolics (TP) and total flavonoids (TF)

The level of phenolic compounds in different solvent extracts (methanol, acetone and chloroform) of the leaves, rhizomes and stems in the two varieties of *Z. officinale* are shown in Table 1. With increased in solvent polarity, TF and TP content increased in extract. High content of TF (7.05 mg/g DW) and TP (39.06 mg/g DW) obtained from methanolic extract in Halia Bara leaves. After methanol, acetone had high content of phenolic compounds in extract. In both varieties, the total flavonoid and phenolic contents in the leaves were more than the rhizomes, followed by contents in the stems. Significant differences between solvents at $p \leq 0.05$ were observed for TP and TF extraction. Our results is similar to that reported by Sun et al. (2005), where methanol solvent was most effective in extracting phenolic components from oat bran. In that study, the content of phenolic components extracted by methanol was about 3 times higher than that extracted by acetone and 4 times higher than that extracted by hexane. Thurkmen et al. (2006) reported that solvent with different polarity had significant effect on polyphenol content and antioxidant activity in in higher content in more polar solvents (Siddhuraju et al., 2003; Sultana et al., 2007). The phenolics compounds often associated with other biomolecules (poly-saccharides, proteins, terpenes, chlorophyll, inorganic compounds etc) and a solvent must be found that it is suitable for extracting them. Research conducted by Jayaprakash et al. (2001) confirmed the ineffectiveness of acetone, methanol and water for the extraction of total phenols of grapes seeds (*Vitis vinifera*). However, ethanol / water or acetone / water were better solvents compared to ethanol or acetone (Kallithraka et al., 1995; Yilmaz et al., 2006). These authors also showed that the methanolic extract was better for flavonoid extraction such as catechin, epicatechin and epigallocatechin.

Radical scavenging activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

Solvents used for phenolic compounds extraction had significant effects on DPPH scavenging capacity determination for two varieties of ginger extracts (Table 2). Recently, DPPH scavenging method has been widely used in antioxidant activity studies of herb extracts (Chatha et al., 2006; Canadanovic-Brunet et al., 2005; Pinelo et al., 2004). In fact, free radical scavenging method (DPPH) show the reduction of alcoholic DPPH solutions in the presence of an hydrogen donating antioxidant (Koleva et al., 2002) and phenolic compound

Table 1. Total flavonoids and phenolic contents in different parts of ginger (*Z. officinale*) varieties extracted by different solvent.

Variety	Extraction source	TF (mg quercetin/g dry weight)			TP (mg gallic acid/g dry weight)		
		solvent			solvent		
		methanol	acetone	chloroform	methanol	acetone	chloroform
Halia Bentong	Leaves	5.5±0.54bc	4.7±0.55cd	4.54±0.64cd	33.1±1.21b	30.1±0.22c	28.8±0.21c
	Stems	1.3±0.12g	0.83±0.14g	0.74±0.102g	7.8±0.89ijk	7.2±1.05jk	7.07±0.99k
	Rhizomes	3.6±0.12def	3.4±0.13ef	3.23±0.12f	10.1±0.21efg	9.8±0.22efgh	9.2±0.66fghi
Halia Bara	Leaves	7.05±1.67a	6.2±1.71ab	6.01±1.65ab	39.06±1.62a	34.6±1.88b	33.6±1.99b
	Stems	1.7±0.49g	0.95±0.2g	0.9±0.16g	8.5±1.02hijk	8.06±0.92ijk	8.8±0.82ghij
	Rhizomes	4.4±0.57de	3.8±0.12def	3.7±0.15def	13.4±0.34d	11.1±0.87e	10.8±0.75ef

All analyses are the mean of triplicate measurements ± standard deviation; Means not sharing a common letter were significantly different at $p < 0.05$.

Table 2. Capabilities of scavenging DPPH free radicals from young ginger (*Z. officinale*) parts extracted by different solvent.

Variety	Extraction source	solvents		
		Methanol	Acetone	Chloroform
Halia Bentong	Leaves	51.12±0.36d	48.99±1.07e	47.12±0.97f
	Stems	32.83±1.02g	30.76±2.39h	28.95±0.75i
	Rhizomes	51.48±0.72d	49.22±0.46e	47.47±0.64f
Halia Bara	Leaves	56.38±0.23b	54.16±0.91c	52.53±0.33d
	Stems	31.33±0.55h	29.11±1.01i	27.46±0.62j
	Rhizomes	58.21±0.39a	56.18±0.51b	54.36±1.12c

All analyses are the mean of triplicate measurements ± standard deviation; Results expressed in percent of free radical inhibition; Means not sharing a common letter were significantly different at $P < 0.05$.

have been reported and provided to be potent hydrogen donors to the DPPH radical (Von Gadow et al., 1997) because of their excellence structural chemistry (Rice-Evans et al., 1997). The results of the effects of extracting solvent on the free radical scavenging of black tea- Results of the present study showed that among three solvent extracts, the aqueous methanol extracts had the highest TP and TF. This may be due to the fact that phenolics and flavonoids are often extracted extracts of different parts of young ginger (*Z. officinale*) materials at concentration of 50 µg/mL are shown in Table 2. The DPPH activities of the two varieties of young ginger extracts increased in a concentration dependent manner (Figure 1). The values of absorbance for the different part of ginger extract solutions at concentration of 50 µg/mL determined in this assay, ranged from 27.46 to 58.21 and followed the order of effectiveness as: aq. methanolic extract of Halia Bara rhizomes (58.21%) > aq. acetone extract of Halia Bara leaves (56.18%) > aq. chloroform of Halia Bara rhizomes (54.36%). In general, the aqueous organic solvent extracts of the ginger varieties materials, exhibiting greater TP and TF content, also depicted good reducing power in the present analysis. The free radical

scavenging power of antioxidant components is very much associated with their TP and TF content (Ghasemzadeh et al., 2010). The plant extracts with higher levels of total phenolics and flavonoids also exhibit greater free radical scavenging (Ghasemzadeh et al., 2010; Yingming et al., 2004).

According to the previous studies on ginger DPPH activities varieties with high level of TP and TF had high activity of free radical scavenging (Hasna et al., 2009; Praven et al., 2007). However, the inhibitory action of herb extracts could be enhanced by more recovery of phenolic compounds using suitable solvents because the connection of phenolics complex is not the same for all types of solvents used (Silva et al., 2007). It can be concluded that the extracts obtained using high polarity solvents (methanol) were considerably more effective radical scavengers than those using less polarity solvents (acetone and chloroform), indicating that antioxidant or active compounds of different polarity could be present in ginger varieties parts. With change in solvent polarity its ability to dissolve especial group of antioxidant compounds alters and influences the antioxidant activity estimation (Zhou et al., 2004).

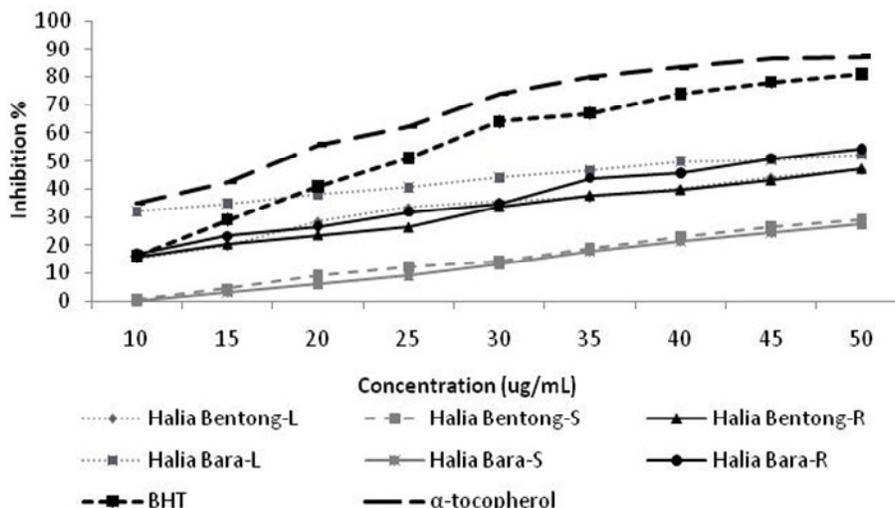


Figure 1. DPPH radical scavenging activity of different parts of two varieties of *Z. officinale* extracted by methanol compared with positive controls, BHT and α -tocopherol. L, S and R, respectively are: Leaves, Stems and Rhizomes of ginger.

High performance liquid chromatography

Table 3 shows the results of HPLC analysis in two varieties (Halia Bentong and Halia Bara) of young ginger (*Z. officinale*) in different parts of plant. According to the results, different solvents had significant effects on flavonoids extraction. High content of quercetin, rutin and catechin obtain from methanol extract and after that acetone and chloroform. This finding is in agreement with Kallithraka et al. (1995) who indicated that the methanol extraction of grape seed had high content of catechin, epicatechin and epigallocatechin. Rodtjer et al. (2006) reported high content of phenolic compounds in the studies showed that the extraction yield of phenolic compounds is greatly depending on the solvent polarity (Turkmen et al., 2006; Lapornik et al., 2005). According to the Yilmaz et al. (2006) research aqueous solutions of ethanol, methanol or acetone were better than a pure compound solvent system for the extraction of the phenolics compound from *Muscadine* seed. Also, other studies have established that the phenolics and flavonoids content of extracts are strongly dependent on the type of the solvent as well as on the different concentrations of solvent (Turkmen et al., 2006; Lapornik et al., 2005). As shown from the Table 3, quercetin extracts of 70% solvent-water mixtures. Results of some content was high (0.97 mg/g DW) in methanol extract of Halia Bara leaves. High content of rutin (0.324 mg/g DW) obtained from methanol extract of Halia Bara rhizomes and the content of catechin (0.56 mg/g DW) was high in methanol extract of Halia Bara leaves. Comparing the varieties, it was found that Halia Bara had higher contents of phenolic compounds (flavonoids) than Halia Bentong. Also, partitioning of quercetin and catechin was high to leaves and rutin was high to rhizomes.

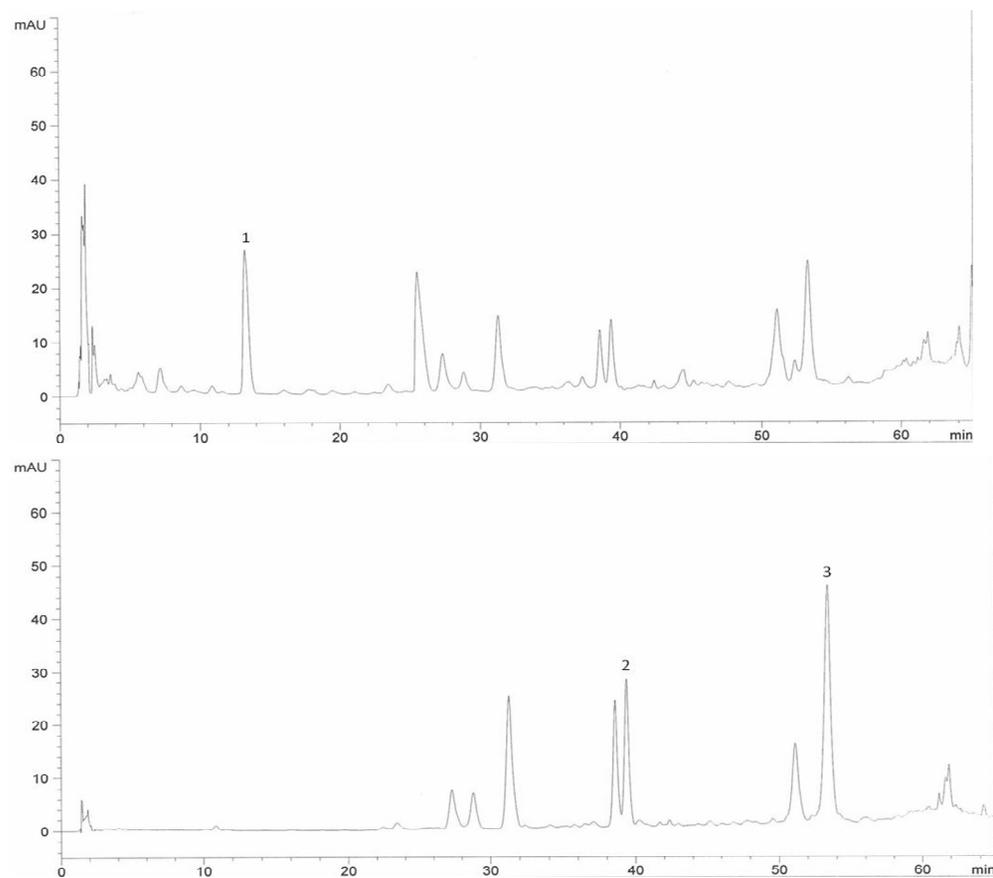
The HPLC chromatograms from the extracts of the leaves (Figure 2) show some of the flavonoid compounds found in Halia Bara. The extraction efficiency and yield of caffeine and catechins of green tea were higher with pure methanol comparing to pure ethanol (Perva-Uzunalic et al., 2006). Therefore, it seems that methanol proved to have better characteristics as a solvent for phenolics and flavonoids than ethanol (Lapornik et al., 2005). Contrary to our results and previous studies results, Wang et al. (2001) reported that aqueous ethanol was better than aqueous methanol and acetone for extraction of the flavonoids from tea. In extracting phenolic compounds from peanut skin, ethanol and methanol were more effective than water, with ethanol being the most efficient extraction solvent (Yu et al., 2005). Meanwhile, the methanol was the solvent with best results for phenols from pine sawdust, while in almond hulls ethanol was the best extraction solvent (Pinelo et al., 2004). Jung et al. (2006) also compared the influence of different solvents and they found out that the ethanol extracts contained higher amounts of total phenolics and flavonoids than water and methanol extracts from wild ginseng leaves.

Conclusions

According to the our results, it seems that the yield and efficiency of the phenolics extraction depends on the type and kind of the solvent as well on the flavonoids, which is being isolated. For total phenolics and flavonoids extraction from ginger parts methanol was more efficient compare to acetone and chloroform. Similar was for some individual flavonoids, such as quercetin, catechin and rutin. Extracting solvent significantly affected antioxidant activity of young ginger extracts. In the conventional

Table 3. HPLC analysis of flavonoids extracted by different solvents from different parts of young ginger (*Z. officinale*) varieties.

Variety	Parts	Quercetin			Rutin			Catechin		
		Methanol	Acetone	Chloroform	Methanol	Acetone	Chloroform	Methanol	Acetone	Chloroform
Halia Bentong	Leaves	0.836±0.001bcd	0.773±0.075def	0.69±0.069gh	0.053±0.005f	0.043±0.009f	0.043±0.005f	0.31±0.045de	0.28±0.037ef	0.211±0.022g
	Rhizomes	0.803±0.028bcde	0.78±0.002cdef	0.505±0.034i	0.31±0.022a	0.271±0.021b	0.226±0.016c	0.36±0.055cd	0.25±0.04fg	0.244±0.016fg
Halia Bara	Leaves	0.978±0.092a	0.846±0.006bc	0.75±0.082efg	0.205±0.02cd	0.16±0.017de	0.143±0.013e	0.56±0.07a	0.537±0.03a	0.436±0.047b
	Rhizomes	0.865±0.044b	0.733±0.035fg	0.666±0.026h	0.324±0.038a	0.269±0.038b	0.221±0.034c	0.45±0.03b	0.398±0.02bc	0.324±0.02de

**Figure 2.** HPLC chromatogram of methanolic extract from ginger (*Z. officinale*) leaves. Identification of compounds: Catechin (1), Rutin (2), Quercetin (3).

solvent extractions, methanol showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced by DPPH among the three solvents (methanol, acetone and chloroform). This study produced results which corroborate with the findings of a great deal of the previous work in this field.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Higher Learning Malaysia for financing this work under the Fundamental Research Grant Scheme FRGS/PHASE1-2009/FUNDAMENTAL SCIENCE/UPM/ (01-11-08-646FR).

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