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Full Length Research Paper

Effects of shading on synthesis and accumulation of polyphenolic compounds in ginger (*Zingiber officinale* Roscoe) varieties

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Extracts of leaves and rhizomes of two varieties (Halia Bara and Halia Bentong) of Malaysian young ginger (*Zingiber officinale*) grown under different shade net (0 and 60%) were examined as potential sources of phenolics and flavonoids compounds for antioxidant activities. High performance liquid chromatography (HPLC) with UV detection was employed for the identification and quantification of the polyphenolic compounds (flavonoids and phenolic acids). Flavonoid compounds (quercetin, apigenin, luteolin, and myricetin) and phenolic acids (gallic acid, vanillic acid, ferulic acid, tannic acid and caffeic acid) were identified with different concentration in leaves and rhizomes of ginger varieties. The most abundant phenolic acid in ginger was gallic acid, and flavonoids were quercetin and apigenin. Accordingly, accumulations of flavonoids in the leaves were high under 60% shade, while most phenolic acids were observed in the rhizomes under 0% shade. Furthermore, caffeic acid was only detected from ginger grown under 0% shade, while tannic acid only accumulated in the leaves of ginger grown under 60% shade level. The results indicated phenolic acids and flavonoids absolutely light dependent and them biosynthetic rate is related to light intensity. Additionally, this study also validated Halia Bara and Halia Bentong medicinal potential based on polyphenolics compound.

Key words: High performance liquid chromatography, shade, flavonoids, phenolic acids, Halia Bentong, Halia Bara.

INTRODUCTION

Zingiber officinale is one of the traditional folk medicinal plants that have been used for over 2000 years by polynesians for treating diabetes, high blood pressure, cancer, fitness and many other illnesses (Tepe et al., 2006). Ginger used for food and cooking has old history in Asia. *Z. officinale* contains a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, rutin, etc (Aruoma et al., 1997). Easily cultivable, *Z. officinale* with its wide range of antioxidants can be a major source of natural or phytochemical antioxidants (Kikuzaki et al., 1993; Chan et al., 2008). Many plants and herbs used to flavor dishes are an excellent source of phenolics and flavonoids compounds which have been reported to show good antioxidant activity (Ghasemzadeh et al., 2010). Some flavonoids compounds found in galagal root, lemon grass and kaffir lime leaves, which are major herbal ingredients of the soup in Thailand, are effective in inhibiting tumors in the digestive tract (Murakami et al., 1995).

Flavonoids are a large family of polyphenolic compounds synthesized by plants. Currently, there are more than 6000 flavonoids already being identified (Harborne and Williams, 2000). Flavonoids have important role in human life and health because of their high pharmacological activities as free radical scavenging agents (Cook et al., 1996; Heijnen et al., 2001; Chun et al., 2003; Byers et al., 2005). The functionality in human health is supported by the ability of flavonoids to induce human protective enzyme system (Lotito and Frei, 2006).

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Flavonoids have been labeled as high level natural antioxidants on the basis of their abilities to scavenge free radical and to quench active oxygen with the inhibition of enzyme activity (Clifford and Cuppett, 2000). High content of natural phenolics compounds and flavonoids are found in green tea, fruits and vegetables, while some amount of phenolics exist in red wine and coffee (Ho et al., 1992). The influences of ecological environment on flavonoids content are synthetically effective. The same cultivar can be tamed into different ecological types and flavonoids content of the same cultivar also could be different under different ecological environment. Light is the most imperative factor among all the ecological factors. Flavonoids and phenolic biosynthesis requires light or is enhanced by light, and flavonoid formation is absolutely light-dependent, and its biosynthetic rate is related to light intensity and density (Xie et al., 1996; Graham, 2006). Different shade levels with changes in plant morphology and physiological characteristics affected the secondary metabolites like as phenolic compounds in plants (Kurata et al., 1991; Briskin and Gawienowski, 2001). However, the different plants had a diverse reaction to shade levels, which alters the production of total phenolics (TP) and total flavonoids (TF). Previous studies showed that change in light intensity was able to modify the production and accumulation of total flavonoids and total phenolics in herbs. According to previous studies varied light intensities with changing in plant morphology and physiology characteristics exerted substantial impact of the medicinal compounds in herbs (Hemm et al., 1997; Briskin and Gawienowski, 2001; Kurata et al., 2004). Changes in light intensity with shade net were able to change synthesis of phenolic compounds in plants (Graham et al., 1998). Whether similar environmental conditions when exerted upon ginger was able to modify the production and profiling of its bioactive compounds in different plant parts, or totally alter the plant bioactive constituents, or could there be a species-related response to the impact of environmental factors on accumulation and distribution of the secondary metabolites, was still not fully elabo-rated and documented. The main objective of the current study was to consider effect of shading on biosynthesis and partitioning of various flavonoids and phenolics constituents in two varieties of ginger (*Z. officinale*).

MATERIALS AND METHODS

Plant material and maintenance

Rhizomes of Halia Bentong and Halia Bara (*Z. officinale)* was germinated for 2 weeks in small pots, and then transferred to polyethylene bags which were to be filled with soilless mixture of burnt rice husk and coco peat (ratio1:1) under 2 levels of greenhouse shade (0 and 60% shade). The experiment was factorial based on randomized complete block design (RCBD) with 3 replications. Plants were harvested 16 weeks after exposure to different light intensities. At the end of 16 weeks, the flavonoids and phenolics compounds and antioxidant activities in different parts of plants were measured. The experiment was carried out at the Faculty of Agriculture greenhouse complex, University Putra Malaysia (UPM).

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH) together with flavonoids (quercetin, apigenin, luteolin and myricetin) and phenolic acids standards (gallic acid, ferulic acid, vanillic acid, tannic acid and caffeic acid) were purchased from Sigma–Aldrich (USA). Methanol HPLC grade and phosphoric acid were obtained from Fisher Chemical (UK).

High performance liquid chromatography (HPLC) apparatus

Extract preparation of flavonoids

Flavonoids extraction from ginger parts was carried out according to method by Crozier et al. (1997). Samples of 0.25 g aliquots of leaves and rhizomes were extracted with 20 ml of 60% aqueous methanol. 5 ml of 6 M HC1 was added to each extract to make a 25 ml solution of 1.2 M HC1 in 60% aqueous methanol. Extracts were refluxed at 90°C for 2 h and solution was filtered through a 0.45 µm filter.

Analysis of flavonoids composition by HPLC

The HPLC analyses of flavonoids were done using the procedure established by Wang et al. (2007). 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 a L-7400 ultraviolet (UV) detector. The column type was Agilent C18, 5 µm, 4.0 mm internal diameter 150 mm. The mobile phase was composed of acetic acid (aqueous) and acetic acid (aqueous) and acetonitrile (50:50 v/v). The mobile phase was filtered under vacuum through a 0.45 µm membrane filter before use. The flow rate was 1 ml/min. The UV absorbance was measured at 280 to 365 nm using a spectrophotometer. The operating temperature was maintained at room temperature. Identification of the flavonoids was achieved by comparison with retention times of the standards, UV spectra and calculation of UV absorbance ratios after co-injection of samples and standards.

Extract preparation of phenolics

Extraction of phenolic acids was done according to the standard operating protocol (SOP, 2001). Phosphoric acid 0.1% (H_3PO_4) at 1.2 ml was carefully pipetted into about 950 ml water in a 1 L volumetric flask. Solution was missed well before brought to volume with distilled water; and 0.25 g of leaves and rhizomes were extracted with 20 ml of phosphoric acid. A 5 ml of 6 M HC1 was added to each extract to give a 25 ml solution of 1.2 M HC1 in 50% aqueous methanol. Extracts were refluxed at 90°C for 2 h and solution was filtered through a 0.45 µm filter.

Analysis of phenolics acids composition by HPLC

The standard operating protocol was used for HPLC analysis of phenolic acids. Agilent HPLC system (Tokyo, Japan) consisted of

Table 1. Concentration of some flavonoids in two varieties of *Z. officinale viz.* Halia Bentong and Halia Bara grown under different light intensity.

Flavonoids	Halia Bentong				Halia Bara			
	Shade 0%		Shade 60%		Shade 0%		Shade 60%	
	Leaf	Rhizome	Leaf	Rhizome	Leaf	Rhizome	Leaf	Rhizome
Quercetin	0.871 ± 0.031cd	0.803 ± 0.028d	0.985 ± 0.015b	0.9 ± 0.042bc	0.978 ± 0.024b	0.865 ± 0.027cd	1.123 ± 0.11a	0.986 ± 0.032b
Apigenin	0.227±0.043d	0.205±0.027d	0.372±0.032c	0.26±0.018d	0.38±0.038c	0.34±0.019c	0.51±0.46a	0.427±0.041b
Luteolin	0.072±0.04c	0.028±0.033d	0.126±0.019b	0.067±0.026c	0.118±0.01b	0.072±0.016c	0.177±0.04a	0.116±0.011b
Myricetin	n.d	n.d	0.077±0.042b	n.d	n.d	n.d	0.152±0.037a	0.014±0.018c

All analyses are mean of triplicate measurements ± standard deviation. Means not sharing a common letter in each row were significantly different at $P \leq 0.05$. Results expressed in mg /g of dry plant material; nd, non detected.

a Model 1100 pump equipped with a multi-solvent delivery system and ultraviolet (UV) detector. The column type was C18, 5 µm, 4.6 mm. The mobile phase was phosphoric acid 0.1% (aqueous) and acetonitrile (HPLC grade). The mobile phase was filtered under vacuum through a 0.45 µm membrane filter before use. The flow rate and injection volume was 1 ml/min and 20 µl. UV absorbance was measured at 260 to 280 nm. The operating temperature was maintained at room temperature (SOP, 2001). 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.

Statistical analysis

The experimental design was factorial based on RCBD with three replicates and the results were expressed as mean ± standard deviation. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and the differences among samples were determined by Duncan's Multiple Range Test using the Statistical Analysis System (SAS, 1999) and MSTATC programme. P value of ≤ 0.05 was regarded as significant.

RESULTS

The results obtained from the analysis of flavonoids compounds (quercetin, apigenin, luteolin, and myricetin) by HPLC are shown in (Table 1). It is apparent that flavonoids accumulation and partitioning in the ginger varieties was considerably affected by the shade. The different shade level had a significant (P ≤ 0.01) effect on the flavonoids synthesis (Table 1). The results showed that in both varieties of ginger, the leaves had a higher flavonoids content under 60% shade level compared to 0% shade level. The partitioning of flavonoids at 16 weeks after planting was: leaves > rhizomes. According to the results, factors (shade, varieties and plant parts) had a significant effect (P ≤ 0.01) on flavonoids accumulation and partitioning in both varieties. Between varieties Halia Bara exhibited high concentration of studied flavonoid compounds compared to Halia Bentong. The HPLC chromatograms from the extracts of the leaves (Figures 1 and 2) show some of the flavonoids compounds found in Halia Bara.

Basically, Malaysian ginger especially that of variety Halia Bara contained considerably high amount of quercetin (1.23 mg/g dry weight). The amount of quercetin recorded here (0.852 to 1.23 mg/g dry weight) was also higher than or comparable to those found in other plant species for example red chilli (0.799 mg/g dry weight), black tea (1.107 mg/g dry weight) and onion (1.49 mg/g dry weight) (Miean et al., 2001) suggesting that Halia Bara, particularly the leaves, is rich in bioactive compounds, when plants grown under low irradiance. Increasing the light intensity reduced quercetin production in Halia Bara leaves (17.0%) and rhizomes (15.7%). Halia Bentong, on the other hand, registered lower quercetin concentrations than Halia Bara in both the leaves and rhizomes Apigenin is rare and famous isoflavonoids component in plants. Previous studies showed that apigenin had anti-inflammatory (Lee et al., 2007), anti-carcinogenic and strong antioxidant (Patel et al., 2007) effects. According to Table 1, ginger leaves and rhizomes exhibited good potential of this flavonoid. Concentration of this flavonoid in leaves was higher than rhizome in both varieties. Halia Bara leaves grown under 60% shade exhibited high content (0.51 mg/g dry weight) of apigenin. It seemed that apigenin content could also be improved by decreasing light intensity in both of varieties especially in Halia Bara. Ginger varieties showed good potential of apigenin compared to some other plants like as Chinese cabbage (0.187 mg/g dry weight), bell pepper, garlic (0.217 mg/g dry weight), French peas (0.176 mg/g dry weight),

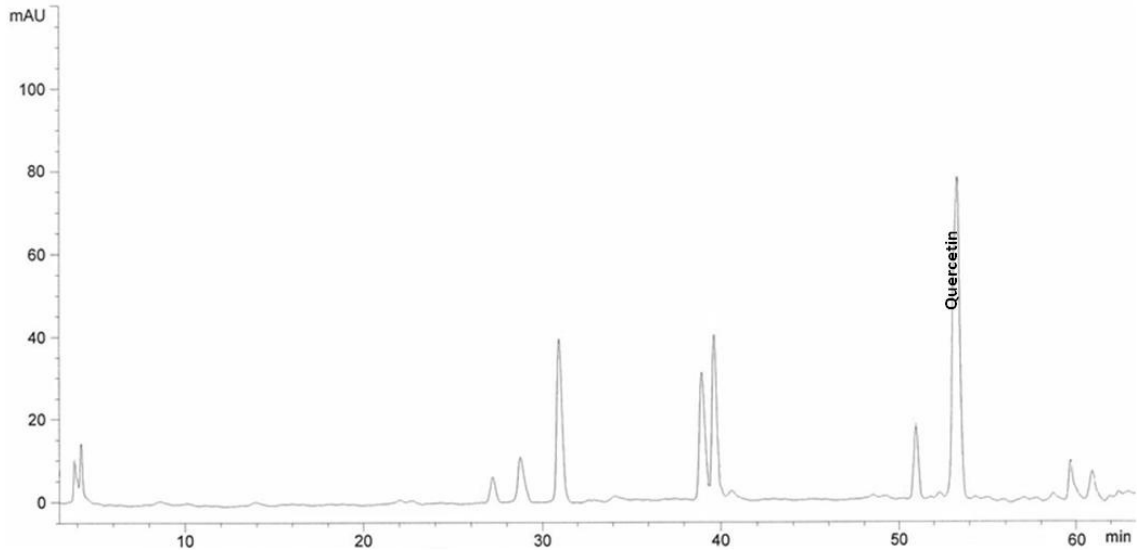

Figure 1. HPLC chromatogram of flavonoid compounds detected from Halia Bara (*Z. officinale*) leaves extracts (shade 60%).

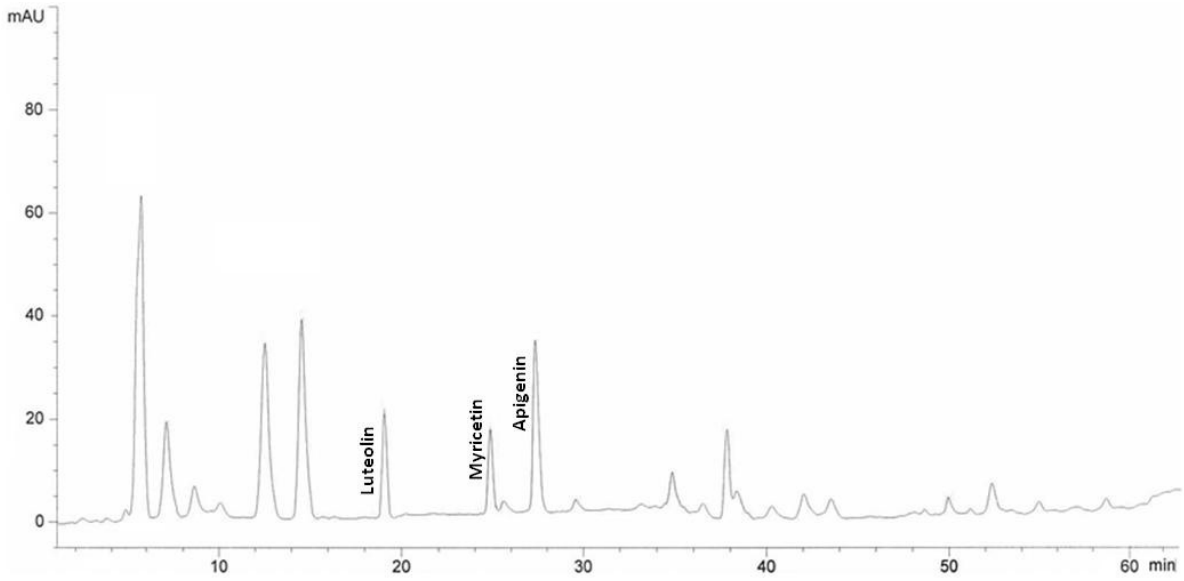

Figure 2. HPLC chromatogram of isoflavonoid compounds detected from Halia Bara (*Z. officinale*) leaves extracts (shade 60%).

snake gourd (0.0424 mg/g dry weight), local celery (0.338 mg/g dry weight), daun turi (0.0395 mg/g dry weight), and kadok (0.0345 mg/g dry weight) but concentration of Apigenin was lower than some plants like as wolfberry leaves (0.547 mg/g dry weight) and guava (0.579 mg/g dry weight), (Mean et al., 2001). Luteolin is another flavonoids belonging to isoflavonols group. The importance of luteolin as anticance agent has also been widely recognized (Lin et al., 2008; Shi et al., 2007, Bagli et al., 2004). Increasing shade level had increased accumulation of luteolin in the leaves of Halia Bentong from 0.028 to 0.177 mg/g DW implying a variety-specific response in accumulation of luteolin with regard to light intensity. Lowest luteolin concentration, however, was observed in rhizome raised from the latter light condition. Generally, Halia Bara had higher concentration than Halia Bentong with more accumulation found in the leaves than in the rhizomes, especially under low light condition. Halia Bara leaves grown under 60% shade showed high content of luteolin (0.177 mg/g dry weight) compared to broccoli (0.074 mg/g dry weight), green chili (0.033 mg/g dry weight), French bean (0.011 mg/kg),

Table 2. Concentration of some phenolic acids in two varieties of *Z. officinale viz.* Halia Bentong and Halia Bara grown under different shade level.

	Halia Bentong				Halia Bara			
Phenolic acids	**Shade 0%**		**Shade 60%**		**Shade 0%**		**Shade 60%**	
	Leaf	**Rhizome**	**Leaf**	**Rhizome**	**Leaf**	**Rhizome**	**Leaf**	**Rhizome**
Gallic acid	1.087±0.11b	1.086±0.03b	0.161±0.0095d	0.233±0.023d	1.762±0.048a	1.7639±0.074a	0.183±0.008d	0.437±0.038c
Vanillic acid	0.349±0.038d	0.514±0.027c	n.d	n.d	0.652±0.03b	0.859±0.025a	0.076±0.012e	n.d
Ferulic acid	0.495±0.053c	0.723±0.034b	0.073±0.018d	0.102±0.016d	0.640±0.047b	1.240±0.166a	0.062±0.011d	0.143±0.015d
Tannic acid	n.d	n.d	0.0375±0.066a	n.d	n.d	n.d	0.0207±0.035b	n.d
Caffeic acid	0.144±0.061c	0.113±0.02c	n.d	n.d	0.227±0.031a	0.185±0.037b	n.d	n.d

All analyses are mean of triplicate measurements ± standard deviation. Means not sharing a common letter in each row were significantly different at $P \leq 0.05$. Results expressed in mg /g of dry plant material; n.d, non detected.

carrot (0.0375 mg/g dry weight), white radish (0.009 mg/g dry weight), local celery (0.08 mg/g dry weight) and limau purut leaves (0.03 mg/g dry weight), (Mean et al., 2001).

Myricetin is belonging to flavonol group (sub group of flavonoids). It is found in several foods such as red grapes, berries, tea, onions, and herbs. Myricetin exerts extensive biological effects, including anticancer and antioxidant activities. High content (0.152 mg/ g dry weight) of this compound was detected from Halia Bara leaves grown under 60% shade level. The results indicated myricetin absolutely light dependent and its biosynthetic rate is related to light intensity. High light intensity inhibited synthesis of myricetin in ginger varieties.

The results obtained from the analysis of phenolic acids (gallic acid, vanillic acid, ferulic acid, tannic acid and caffeic acid) by HPLC are shown in (Table 2). Shade levels had a significant ($P \leq 0.05$) impact on the production of phenolic acids production. As the shade level increased from 0 to 60%, the concentration of phenolic acids decreased significantly in both varieties. These results suggest the ability of shade to alter or modify the concentration and profiling of phenolic acids in ginger varieties; although accumulation of phenolic acids favored high light intensity (low shade), in contrast, low light intensity (heavy shade) generally promoted the accumulation of flavonoids. The results were consistent with Caldwell (2003) and Xiangfei et al. (2007), which reported that plants grown under grater light intensity showed higher phenolic acids content and antioxidant capacity. The HPLC chromatograms from the extracts of the leaves (Figure 3) show some of the phenolic acids compounds found in Halia Bara variety.

Among phenolics acid compounds studied, gallic acid had more content in both varieties followed by ferulic and vanilic acids. Similar to pattern of flavonoids partitioning, phenolic acids in leaves were found to be higher than in rhizomes (Table 2). What is interesting with these data is that vanillic acid, tannic acid and caffeic acid, regardless the varieties, were not detected in ginger grown under 60% shade, which was more severely observed with rhizomes. Conversely, tannic acid was not detected from gingers grown under 0% shade. The increase in phenolic acids such as intermediates in lignin biosynthesis can indicate the typical anatomical change induced by stressors: An increase in cell wall endurance and the creation of physical barriers prevent walls (Diaz et al., 2001).

DISCUSSION

The synthesis of flavonoids and phenolic acids depends on ecological and physiological factors, but also on ginger. Light has been shown to be the imperative environmental factor influencing phenolic acids and flavonoids synthesis in most plants (Grisebach, 1982). The results of current study suggest the ability of different shade level in altering or modifying both the concentration and profiling of phenolic compounds in ginger plants; although accumulation of phenolics compounds favoured high light intensity, the opposite was found for flavonoids where low light intensity generally promoted the accumulation of flavonoids compounds into plant parts. On the other hand, different shade level had a significant effect on the synthesis, accumulation and partitioning in different part of gingers and also different varieties had a different concentration of flavonoids and phenolic acids in different part of plants. Ginger

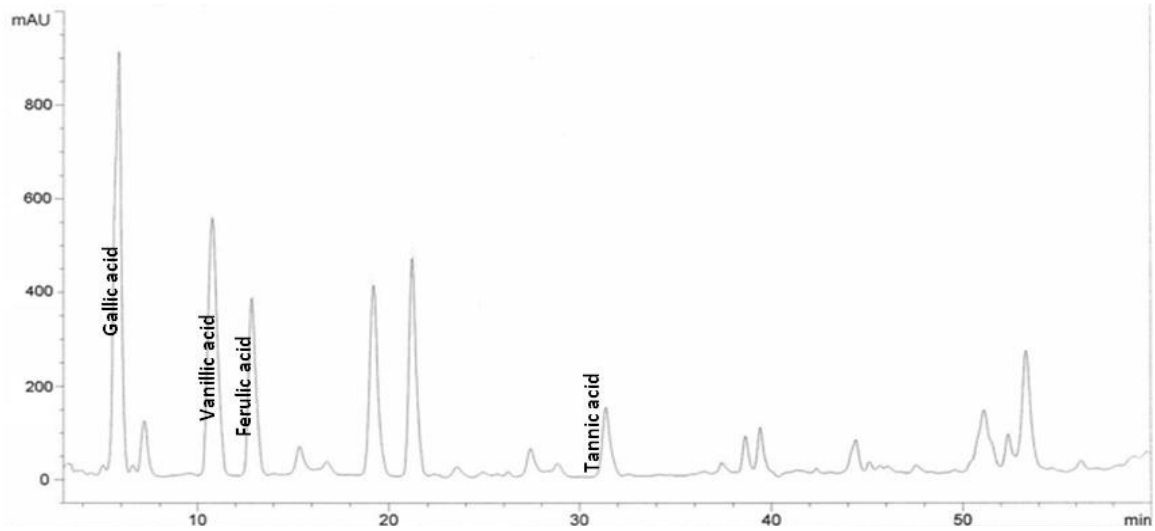

Figure 3. HPLC chromatogram of phenolic acids detected from Halia Bara (Zingiber officinale) leaves extracts (shade 60%).

reported as a semi shade plant, and the results of this study showed that low light intensity is suitable for maximum flavonoids production while High light intensity was suitable for phenolic acid production. Shiow et al. (2009) reported significant effect of light on flavonoid synthesis in red raspberries. A similar trend of increasing flavonoids with decreasing light intensity were reported in *Tanacetum parthenium* and strawberry (Fonseca et al., 2006; Mosaleeyanon et al., 2005) and in some medicinal plants illustrating a considerable influence of low irradiance on enhancement of plant flavonoids (Hall et al., 1972). Zengqiang et al. (2010) also reported high light intensity induced stress on Anoectochilus growth and reduced photosynthetic capability and the flavonoid accumulation. The use of shade netting for production of baby spinach is acceptable as it is related to increase both flavonoids concentration and composition (Bergquist et al., 2007). Xiao-feng et al. (2009) showed the content of total flavonoids of *Dichondra repens* were enhanced under 30% shade. Concurrently, it is necessary to consider whether the enhanced of secondary metabolites production under different light intensity is due to the increased amount of carbon production through photosynthesis or the stress induced by different light intensities, which stimulates secondary metabolites production. Wen-hua1 et al. (2006) showed light intensity and light quality significantly affect the growth and total flavonoid accummulation of *Erigeron breviscapus*. However, information on flavonoid and phenolic compounds in Malaysian young ginger is still limited. The results of our work were consistent with those of other studies to suggest that high content of some phenolic compounds found in ginger such as cinnamic acid could inhibit flavonoids synthesis by inhibiting of phenylalanine ammonia lyase (PAL) enzyme activity (Shui-Yuan et al., 2009). The key enzyme of the flavonoid pathway is the chalcone synthase (Chs) and this enzyme is extremely sensitive to UV and blue light (Logemann et al., 2000; Loyall et al., 2000). In addition, decreasing of flavonoids synthesis in ginger varieties grown under 0% shade level could be related to inhibition of chalcone synthase enzyme by high light intensity. Phenylalanine ammonia-lyase (PAL), an important enzyme in the biosynthesis of phenolic acids, and previous studies showed activity of this enzyme induced by high light intensity and UV (Kumari et al., 2009). Therefore increasing of phenolic acids production under 0% shade could be related to increasing of PAL enzyme activity. However, many other compounds such as anthocyanin, cutin and lignin are also synthesized during the course of phenolic compounds being transformed into flavonoids. In this study, caffeic acid was not detected in plants grown under 60% shade where instead high content of flavonoids was registered.

The most abundant phenolic acid in ginger was gallic acid, and flavonoids were quercetin and apigenin. In recent years, research about carcinogenic potential of quercetin has ranged to examination of its promise as an anti-cancer agent. In this study Halia Bara and Halia Bentong exhibited good potential of quercetin content in leaves and rhizomes. According to the obtained results synthesis of flavonoids in Malaysian ginger varieties will be enhanced with low light intensity and following that medicinal power of ginger could be improved.

Conclusion

This study demonstrated that shade is able to enhance synthesis of flavonoid compounds in ginger. HPLC analysis revealed that leaves of young ginger had high concentration in almost all flavonoids compounds tested,

whilst most phenolic acids seemed to favour the rhizomes. Among phenolics acid compounds studied, gallic acid had more content in both varieties followed by ferulic and vanilic acids. Accordingly, Halia Bara, particularly the leaves, is rich in bioactive compounds, especially in apigenin, when plants grown under low irradiance. Our results in this study indicate that some compounds in Malaysian young ginger varieties like as quercetin, apigenin, luteolin and myricetin posses anticancer activities and may contribute to the therapeutic effect of this medicinal herb. Further work is required to establish this.

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REFERENCES

Aruoma OI, Spencer JP, Warren D, Jenner P, Butler J, Halliwell B (1997). Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. Food Chem., 60: 49–156.

Bagli E, Stefaniotou M, Morbidelli L, Ziche M, Psillas K, Murphy C, Fotsis T (2004). Luteolin inhibits vascular endothelial growth factor-induced angiogenesis; inhibition of endothelial cell survival and proliferation by targeting phosphatidylinositol 3′-kinase activity. Cancer Res., 64: 7936-7946.

Bergquist S, Gertsson U, Nordmark LY, Olsson ME (2007). Effects of shade nettings, sowing time and storage on baby spinach flavonoids. J. Sci. Food Agric., 87: 2464-2471.

Briskin DP, Gawienowski MC (2001). Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. Plant Physiol., 39: 1075–1081.

Byers T, Guerrero N (1995). Epidemilogic evidence for vitamin C and vitamin E in cancer prevention. Am. J. Clin. Nutr., 62: 1385-1392.

Caldwell CR (2003). Alkylperoxyl radical scavenging activity of red leaf lettuce (*Lactuca sativa* L.) phenolics. J. Agric. Food Chem., 51: 4589–4595.

Chan EWC, Lim YY, Wong FL, Lianto FS (2008). Antioxidant and tyrosinase inhibition properties of leaves and rhizomes in ginger species. Food Chem., 109: 477–483.

Chun OK, Kim DO, Lee CY (2003). Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem., 51: 8067-8072.

Clifford AH, Cuppett SL (2000). Review: anthocyanins-nature, occurrence and dietary burden. J. Sci. Food. Agric., 80: 1063-1072.

Cook NC, Samman S (1996). Review: flavonoids chemistry, metabolism, cardioprotective effects and dietary sources. J. Nutri. Biochem., 7: 66-76.

Crozier A, Jensen E, Lean MEJ, Mc DMS (1997). Quantitative analysis of flavonoids by reversed-phase high performance liquid chromatography. J. Chrom., 761: 315-321.

Diaz J, Bernal A, Pomar F, Merino F (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicumannum* L.) seedlings in response to copper stress and its relation to lignification. J. Plant Res., 161: 179–188.

Fonseca JM, Rushing JW, Rajapakse NC (2006). Potential implications of medicinal plants production in controlled environments: the case of feverfew (*Tanacetum parthenium*). Horticult. Sci., 41: 531–535.

Ghasemzadeh A, Jaafar HZE, Rahmat A (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of malaysia young ginger (*Zingiber officinale* Roscoe). Mol., 15: 4324-4333.

Graham TL (1998). Flavonoid and flavonol glycoside metabolism in Arabidopsis. Plant Physiol. Biochem., 36: 135-144.

Grisebach H (1982). Biosynthesis of anthocyanins. In P. Markakis (Ed.), Anthocyanins as food colors. New York, NY: Academic Press. Pp. 47–67.

Hall IV, Stark R (1972). Anthocyanin production in cranberry leaves and fruit, related to cool temperatures at a low light intensity. Hort. Res., 12: 183–186.

Harborne JB, Williams CA (2000). Advances in flavonoid research science. Phytochemistry, 55: 481-504.

Heijnen CG, Haenen GR, Vanacker FA, Vijgh WJ, Bast A (2001). Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups. Toxi. In Vitro, 15: 3-6.

Hemm MR, Rider SD, Ogas J, Murry DJ, Chapple C (2004). Light induces phenylpropanoid metabolism in Arabidopsis roots. Plant J., 38: 765–778.

Ho CT, Lee CY, Hungan MT (1992). Phenolic compounds in food and their effects on health (analysis, occurrence, and chemistry). Am. Chem. Soc., Washington, USA. P. 338.

Kikuzaki H, Nakatani N (1993). Antioxidant effect of some ginger constituents. J. Food Sci., 578: 1407–1410.

Kumari R, Singh S, Agrawal SB (2009). Effects of supplemental ultraviolet-b radiation on growth and physiology of *Acorus calamus* l. (sweet flag). Act. Bio. Cra. Ser. Bot., 51: 19–27.

Kurata H, Matsumura S, Furusaki S (1997). Light irradiation causes physiological andmetabolic changes for purine alkaloid production by a *Coffea arabica* cell suspension culture. Plant Sci., 123: 197–203.

Lee JH, Zhou HY, Cho SY, Kim YS, Lee YS, Jeong CS (2007) Anti-inflammatory mechanisms of apigenin: inhibition of cyclooxygenase-2 expression, adhesion of monocytes to human umbilical vein endothelial cells, and expression of cellular adhesion molecules. Arch. Pharm. Res., 10: 1318-1327.

Lin Y, Shi R, Wang X, Shen HM (2008). Luteolin, a flavonoid with potential for cancer prevention and therapy. Curr. Cancer Drug Targets, 8: 634-646.

Logemann E, Tavernaro A, Shulz W, Somssish IE, Hahlbrock K (2000). UV light selectively co induces supply pathways from primary metabolism and flavonoid secondary product formation in parsley. Proc. Nat. Acad. Sci., 97: 1903-1907.

Lotito S, Frei B (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Rad. Biol. Med., 41: 1727–1746.

Loyall L, Uchida K, Braun S, Furuya M, Frohnmey H (2000). Glutathione and a UV light-induced glutathione S-transferase are involved in signaling to chalcone Ssnthase in cell cultures. Plant Cell, 12: 1939–1950.

Miean KH, Mohamed S (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J. Agric. Food Chem., 49: 3106-3112.

Mosaleeyanon K, Zobayed SMA, Afreen F (2005). Relationship between net photosynthesis rate and secondary metabolite content in Strawberry. Plant Sci., 169: 523–553.

Murakami A, Kondo A, Nakamura Y, Ohigashi H, Koshimizu K (1995). Glyceroglycolipdis from *Citrus hystrix*, a traditional herb in Thailand, potenly inhibit the tumor promoting activity of 12-O-tetradecanoylphorbol 13-acetate in mouse skin. J. Agric. Food Chem., 43: 2779-2783.

Patel D, Shukla S, Gupta S (2007). Apigenin and cancer chemoprevention: progress, potential and promise. Int. J. Oncol., 30: 233-245.

Shi R, Huang Q, Zhu X, Ong YB, Zhao B, Lu J, Ong C, Shen HM (2007). Luteolin sensitizes the anticancer effect of cisplatin via c-Jun NH_2-terminal kinase–mediated p53 phosphorylation and stabilization. Mol. Cancer Ther., 6: 1338–1347.

Shiow YW, Chi TCh, Chien YW (2009). The influence of light and maturity on fruit quality and flavonoid content of red raspberries. Food Chem. 112: 676–684.

Shui YC, Feng X, Yan W (2009). Advances in the study of flavonoids in *Ginkgo biloba* leaves. J. Med. Plants Res., 3: 1248–1252.

Standard operating protocol for phenolics (SOP), SOP No.: CB0103, Botan. Cent. Age-renal. Disea. 2001, 9 p.

Tepe B, Sokmen M, Akpulat HA, Sokmen A (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chem., 95: 200–204.

Wang TC, Chuang YC, Ku YH (2007). Quantification of bioactive compounds in citrus fruits cultivated in Taiwan. Food Chem., 102: 1163–1171.

Wen HSU, Guang FZH, Xiu HLI, Fa XGU, Bing LSHI (2006). Effect of light intensity and light quality on growth and total flavonoid accumulation of *Erigeron breviscapus.* Chin. Trad. Herb Drug, 17: 73-79.

Xiangfei L, Shane A, Marisa B, John P, Kequan ZH, Cecil S, Frank S, Liangli Y, Patricia K (2007). Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. LWT, 40: 552–557.

Xiao FZ, Shou BZH, Ji HY, He QZH, Shou FL (2010). Effects on leaf characteristics and total flavone content of *dichondra repens* under different light intensity. Act. Las. Biol. Sin., 22: 45-51.

Xie BD, Wang HT (2006). Effects of light spectrum and photoperiod on contents of flavonoid and terpene in leaves of *Ginkgo biloba* L. J. Nanji. Fores. Uni., 30: 51-54.

Zengqiang MA, shishang LI, Meijun ZH, Shihao JI, Yulan XI (2010). Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *anoectochilus* plants. Hort Sci., 45: 863-867.