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Nutritional Composition, Antioxidative and Inhibitory Effects Against Pancreatic Lipase, α-Amylase and α-Glucosidase of *Lasia spinosa*

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Abstract: The different parts (leaves, stems and roots) of *Lasia spinosa* in aqueous (hot and cold) and ethanol extracts were analyzed for nutrient composition, antioxidant capacity and enzyme inhibition of pancreatic lipase, α-amylase and α-glucosidase *in vitro*. Nutrient composition was determined using standard methods. Folin-Ciocalteu and aluminium chloride colorimetric were carried out to determine Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), respectively. Antioxidant activities were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and β-Carotene Bleaching (BCB) assay. Enzymes inhibition assays were determined using commercial enzyme kits. Proximate analysis of the samples showed content of moisture (78.20, 89.60, 79.09%), ash (0.97, 1.00, 0.97%), protein (1.24, 1.63, 5.33%), fat (0.13, 0.03, 0.07%), dietary fiber (42.40, 52.35, 45.34%), carbohydrate (19.45, 7.65, 14.33%) and energy value (168.77, 142.94, 171.88 kcal/100 g) of leaves, stems and roots, respectively. Results showed that stems contain higher mineral contents compared to leaves and roots. Hot aqueous leaves extracts contained the highest TPC and TFC. The cold aqueous leaves extract possess high antioxidant activities with EC_{50} value of 312 μg/mL. The highest pancreatic lipase inhibitory effect was the ethanol stems extract. For α-amylase, hot aqueous leaves extract showed the highest inhibition whereas ethanol leaf extracts showed the highest inhibitory effects of α-glucosidase. *Lasia spinosa* exhibits excellent nutrient composition, antioxidant contents and activities and inhibitory effects against pancreatic lipase, α-amylase and α-glucosidase which imply the inhibition of dietary fat and sugar absorption and thereby might have a great potential in chemoprevention of obesity and diabetes.

Key words: Nutrient composition, antioxidant content, antioxidant activity, enzyme inhibition, chemoprevention, obesity, diabetes

INTRODUCTION

Lasia spinosa (L.) Thwaites is belong to the family Araceae which is a perennial herb with watery, bitter or milky juice and usually an elongated or tuberous rhizome (Cook *et al*., 1974). *L. spinosa* is distributed in Bangladesh, Bhutan, India, Nepal, Sri Lanka, Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia, Malaysia, Papua New Guinea, China and Taiwan (Deb *et al*., 2010; Napiroon *et al*., 2014). This plant is known locally as Kantakachu (Bengali) (Goshwami *et al*., 2012) Phaknam (Thailand) (Nauheimer *et al*., 2012), Kohila (Sri Lanka) (Shefana and Ekanayake, 2009) Turang (India) (Kichu *et al*., 2015) and Geli-geli or Gegeli (Malaysia) (Bachok *et al*., 2014). Traditionally, *L. spinosa* is used as vegetables (Shefana and Ekanayake, 2009) added in curry to acidify the taste (Burkill, 1953) or eaten raw as "Ulam" (Bachok *et al*., 2014).

Nutritional composition of *L. spinosa* includes polyphenols, vitamin C and dietary fibres (Shefana and Ekanayake, 2009). Phytochemical consituents of this plant includes β-sitosterol acetate and stigmasterol from the rhizome (Dinda *et al*., 2004) and flavonol 3'-methyl quercetin-3-o-α-L- rhamnopyranosyl- (→6)-β- D-glucopyranoside (Van *et al*., 2006). The leaves and rhizomes are used in folk medicine to treat intestinal diseases such as worm's infections, colic, hemorrhoid and constipation (Shefana and Ekanayake, 2009). Scientific studies have demonstrated rhizome of *L. spinosa* was

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used for treatment of lung inflammation, bleeding cough and the whole plant in uterine cancer and high antimicrobial activities and significant toxicity to brine shrimp (Alam *et al*., 2011). Hydroalcoholic root extract of *L. spinosa* demonstrated antioxidant (Shefana and Ekanayake, 2009), antinociceptive, antinflammatory, antidiarrhoeal activity in a dose dependent manner (Deb *et al*., 2010) and antihyperglycemic properties (Hasan *et al*., 2014).

The use of plant-based antioxidant compounds in foods and preventive medicine are gaining a great deal of interest because of their potential health benefits (Parida *et al*., 2014). Epidemiological studies have suggested that relationships exist between the consumption of phenolic-rich foods or beverages and the prevention of chronic diseases such as cancer and cardiovascular diseases. Many plant phenolic compounds exhibiting antioxidant properties have been studied and proposed for protection against oxidation (Habauzit and Morand, 2012). An antioxidant is a stable molecule that can donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Antioxidants can delay or inhibit cellular damage through their free radical scavenging property. These low molecular weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Sharma *et al*., 2012). Hence, diseases associated with free radicals such as the risk for cancer, hypertension and heart disease can be avoided by antioxidant therapy which gained an immense importance.

There are also increasing research in exploring the therapeutic agents for obesity and diabetes from natural sources such as medicinal plants. This is because medicinal plants are affordable, easily accessible and excellent alternatives for safer and more effective therapeutic agents although further research is required to test on its efficacies (Prakash *et al*., 2015). Since, dietary triglyceride and carbohydrate are the main source of ingested lipid and glucose, respectively, controlling the absorption of these nutrients is the most effective approach to prevent excess calorie and sugar intake by inhibiting the main digesting enzymes of lipid (lipase) and carbohydrate (α-amylase and α-glucosidase) (Tucci *et al*., 2010). Broad studies have been demonstrated the phytochemicals in plants as the contributor to these inhibitory effects (Oboh *et al*., 2012). Furthermore, the excellent antioxidant properties could consequently improve the inhibitory activities, adding the potential to be used as natural therapeutic agents (Maiti and Majumdar, 2012).

This study has demonstrated the potential of *L. spinosa* as a functional food and natural therapeutic agent evidenced by its nutrient composition, antioxidant capacities and the inhibitory activities against lipase, α-amylase and α-glucosidase with optimal extraction conditions.

MATERIALS AND METHODS

Sample collection and extraction: The plant was collected from Kedah, Malaysia. Samples were separated into three different parts; Leaves, stems and roots. After that samples were cleaned and washed by tap water. For drying process, the samples were dried in the oven at 60 °C for 24 h. Next, samples were grinded and proceed with crude extraction. The samples were subjected to three types of extraction; hot (70 °C, 12 h) (Cheurfa and Allem, 2015) and cold (room temperature, 2 days) (Santos *et al*., 2014) aqueous extractions and 70% ethanol extraction (AOAC, 2005; Roh and Jung, 2012). The dried extracts were kept at -20°C until further analysis (Cheurfa and Allem, 2015; Nurdiana *et al*., 2013).

Proximate and mineral analysis: The proximate analysis of the samples for moisture, ash and carbohydrate contents were determined as described by AOAC (2005). The contents of Ca, Zn, Na, K, Mg and Fe in foods were measured by Atomic Absorption Spectrophotometer (AAS). Briefly, 5 g sample was placed in a previously weighed porcelain crucible and heated. The resulting white ash was weighed, dissolved in 3 mL of concentrated nitric acid and diluted with distilled water in a 25 mL calibrated flask. The solution was used to determine of Calcium (Ca), Zinc (Zn), sodium (Na), potassium (K), Magnesium (Mg) and iron (Fe). Standard stock solution of these minerals were prepared from AAS grade chemicals (Sigma, USA) by appropriate dilution (Santos *et al*., 2014).

Determination of Total Phenolic Content (TPC): The Folin-Ciocalteu (FC) reagent assay was diluted by added 1 mL of FC in 9 mL of distilled water. Then 0.6 g of sodium bicarbonate (Na_2CO_3) was prepared in 10 mL of distilled water. The serial dilution of gallic acid concentrations (1.95, 3.9, 7.8, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 μg/mL) also were prepared. After that 50 μL of sample, 50 μL of FC reagent and 100 μL of Na_2CO_3 were added in the 96 well plates. The absorbance was measured at 725 nm by using FLUOstar Omega microplate reader. The mean of three readings were used and the content of phenolic was expressed as Gallic Acid Equivalent (GAE) per 1 g of extract. The total phenolic content was calculated by September 29, 2018 using the following formula (Eq. 1):

$$\text{TPC for 1 g of extract} = \frac{\text{TPC per mL sample} \times \text{Dilution factor} \times \text{Total sample volume used}}{\text{Sample weight}} \quad (1)$$

Determination of Total Flavonoid Content (TFC): All reagents were prepared which are mixed 50 mL of distilled water with 2.5 g of 5% sodium nitrate ($NaNO_2$), 50 mL distilled water with 2.5 g of 10% aluminum chloride ($AlCl_3$) and 2.5 g sodium hydroxide (NaOH) with 62.5 mL distilled water. The serial dilution of catechin concentrations (1.95, 3.9, 7.8, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 μg/mL) also were prepared. After that 20 μL of each sample was added in 96 well plates. Then the samples were mixed with 6 μL of $NaNO_2$ solution and 48 μL of distilled water and incubated for 5 min. Six microliter of $AlCl_3$ solution was added to the mixture and allowed to stand for 6 min. Then 40 μL of NaOH solution was added with 80 μL of double distilled water and the mixture was allowed to stand for 15 min. The absorbance was measured at 510 nm. The mean of three readings were used and the content of total flavonoids was expressed as Catechin Equivalent (CE) per 1 g of extract. The total phenolic content was calculated by using the following formula (Eq. 2):

$$\text{TFC for 1 g of extract} = \frac{\text{TFC per mL sample} \times \text{Dilution factor} \times \text{Total sample volume used}}{\text{Sample weight}} \quad (2)$$

β-Carotene Bleaching (BCB) assay: The β-Carotene (βC) reagent was prepared by mixing 2 mg of βC in 10 mL of chloroform. A stock solution was prepared by mixed 1mL of βC reagent, 20 μL of linoleic acid and 200 μL of tween 40 into a round bottom flask. Then it was evaporated at 50°C using a rotary evaporator. Then, 50 mL of distilled water was added to form an emulsion. The 20 μL of sample and 200 μL of emulsion were added in the 96 well plates. Butylated Hydroxy Toluene (BHT) was used as standard. The absorbance was measured at 470 nm by using FLUOstar Omega microplate reader. The reading of absorbance was measured at time 20, 40, 60, 80, 100 and 120 min. Degradation Rate (DR) was calculated according to first order kinetics by using the following Eq. 3:

$$\text{Antioxidant activity}(\%) = \frac{A_t\text{-}C_t}{C_0\text{-}C_t} \times 100 \quad (3)$$

where, A_t and C_t referred as the absorbance values measured for the extraction sample and control, respectively, after incubation for t min and C_0 is the absorbance value for the control measured at zero time during the incubation.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay: The DPPH working solution was prepared by adding 2.4 mg of DPPH in 30 mL of methanol. Then the serial dilution of sample concentrations (15.63, 31.25, 62.5, 125, 250, 500, 1000 μg/mL) were prepared. The 100 μL of sample and 100 μL of DPPH were added in the 96 well plates. Butylated Hydroxy Toluene (BHT) was used as standard. The absorbance was measured at 517 nm by using FLUOstar Omega microplate reader. Radical scavenging capacity was expressed as percentage (%) and calculated using the following Eq. 4:

$$\text{DPPH radical scavenging activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (4)$$

Different sample concentrations were used in order to obtain antiradical curves for calculating the EC_{50} values.

Pancreatic lipase inhibition assay: The lipase inhibition assay was carried out using lipase activity assay kit (Sigma) with modifications (Roh and Jung, 2012). The reaction mixture (50 μL) were prepared by mixing lipase assay buffer (46.5 μL), peroxidase substrate (1.0 μL), enzyme mix (1.0 μL) and lipase substrate (1.5 μL). The reaction mixture was then pipetted into 96-well plate. The assay was initiated by transferring 25 μL of the sample extracts (0.1 mg/mL) into the wells. After that the plate was incubated at 37 °C and in every 5 min the absorbance were measured at 405 nm using FLUOstar Omega microplate reader.

α-amylase inhibition assay: The α-amylase inhibition assay was performed using amylase activity assay kit (Sigma) with modifications (Liu *et al*., 2013). The sample extracts (0.1 mg/mL) were mixed well with reaction mix (50 μL of amylase buffer and 50 μL of amylase substrate mix). After that the plate was incubated at 37°C and the absorbance were measured in every 5 min at 540 nm using FLUOstar Omega microplate reader.

α-glucosidase inhibition assay: The α-glucosidase inhibitory activity was performed using glucosidase activity assay kit (Sigma) with modifications. Briefly, an appropriate 20 μL of sample extracts (0.1 mg/mL) was added into 96 well plates. After that, 200 μL of master reaction mix (200 μL of assay buffer and 8 μL of α-NPG substrate) was transferred into the sample wells. Then the plate was incubated at 37°C and the absorbance were measured in every 5 min at 405 nm using FLUOstar Omega microplate reader (Liu *et al*., 2013). The percentage of inhibition of all enzymes activity is calculated by using following Eq. 5:

$$\text{Percentage of inhibition}(\%) = 100\text{-}\left[\frac{(\text{Absorbance of Sample})/}{(\text{Absorbance of Control})\times 100}\right] \quad (5)$$

Statistical analysis: Statistical analysis was performed by one-way Analysis of Variance (ANOVA) with post hoc test (Tukey HSD) and bivariate Pearson correlation test was used to determine the correlation between antioxidant content (TPC and TFC) and antioxidant activity (BCB and DPPH). All results were expressed in mean±standard deviation and were considered statistically significant when p<0.05.

RESULTS AND DISCUSSION

Proximate and mineral analysis: Results of proximate analysis of different parts of *L. spinosa* are shown in Table 1. The proximate analysis revealed that moisture content is within the range of 78-90%. Ash content was found ranging from 0.97-1.00% and not significant (p>0.05) between different parts. *L. spinosa* roots contained the highest and significant (p<0.05) protein (5.33%) compared to other parts. Proteins play important roles in human body such as act as enzyme, hormones and antibodies (Gropper and Smith, 2012). The fat composition in all parts of *L. spinosa* was very low. Thus, this may suggested that *L. spinosa* has low energy content which could benefits in prevention of obesity and other related diseases. Total dietary fibers were higher in all parts; Leaves, stems and roots (42.40, 53.35 and 45.34%, respectively). High-fiber intake is associated with the prevention of various chronic diseases such as cardiovascular diseases, diabetes and cancer (Kaczmarczyk *et al.,* 2012). Carbohydrate content was the highest in leaves (19%). *L. spinosa* leaves and stems contained high iron content (35.47 and 44.53 mg/100 g, respectively) whereas roots contained high zinc content (13.35 mg/100 g). Iron and zinc are essential micronutrients for human growth, development and maintenance of the immune system. Deficiencies of both nutrients remain a global health problems (Nguyen *et al*., 2012).

Results are expressed as mean±standard deviation (n = 3). Different letters in the same row indicate significant difference at p<0.05. Proximate is expressed by percentage and minerals by mg/100 g.

Total phenolic and flavonoid content: Table 2 shows the Total Phenolic Content (TPC) in hot aqueous extract, cold aqueous extract and ethanol extract of different parts (leaves, stems and roots) of *L. spinosa*. In hot aqueous extract, leaves had the highest TPC (42.36±0.42 mg GAE/g extract) followed by stems (27.42±2.33 mg GAE/g extract) and roots (13.68±1.29 mg GAE/g extract). In cold aqueous extract, leaves had the highest TPC (35.06±0.20 mg GAE/g extract) followed by stems and roots. For ethanol extract, leaves also showed the highest TPC (34.56±1.21mg GAE/g extract) followed by roots and stems. The optimum condition to obtain the TPC in *L. spinosa* was hot aqueous leaves extraction.

Table 1: Proximate and mineral composition of different parts of *L. spinosa*

	Plant parts		
Nutrients	Leaves	Stems	Roots
Energy (kcal/100 g)	168.77±2.13[a]	142.94±5.14[b]	171.88±2.38[a]
Moisture	078.20±0.60[b]	089.60±1.13[a]	079.09±0.66[b]
Ash	000.97±0.01[a]	001.00±0.02[a]	000.97±0.02[a]
Protein	001.24±0.09[c]	001.63±0.19[b]	005.33±0.08[a]
Fat	000.13±0.12[a]	000.03±0.00[a]	000.07±0.01[a]
Total dietary fiber	042.40±0.46[c]	052.35±0.43[a]	045.34±0.57[b]
Carbohydrate	019.45±0.63[a]	007.65±0.81[c]	014.33±1.04[b]
Potassium	003.09±0.01[b]	003.37±0.03[a]	002.71±0.13[c]
Sodium	000.20±0.00[a]	000.25±0.04[a]	000.12±0.01[b]
Calcium	003.71±0.66[a]	003.11±0.08[a]	003.51±0.04[a]
Manganese	000.04±0.02[c]	000.08±0.02[b]	000.23±0.01[a]
Magnesium	000.50±0.01[c]	000.91±0.01[a]	000.70±0.01[b]
Iron	035.47±0.92[b]	044.53±0.70[a]	001.82±0.18[c]
Zinc	003.77±0.20[b]	002.93±0.11[c]	013.35±0.33[a]

Table 2: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of *L. spinosa*

Types of extraction	Total Phenolic Content (TPC) (mg GAE/g extract)	Total Flavonoid Content (TFC) (mg CE/g extract)
Hot aqueous extract		
Leaves	42.63± .42[a]	15.62±1.23[a]
Stems	27.42±2.33[b]	10.34±3.75[a]
Roots	13.68±1.29[c]	06.21±1.34[b]
Cold aqueous extract		
Leaves	35.06±0.20[d]	11.57±1.02[c]
Stems	15.20±3.25[e]	08.51±0.85[c]
Roots	13.22 ± 0.57[e]	02.90±3.12[d]
Ethanol extract		
Leaves	34.56±1.21[f]	09.78±2.74[e]
Stems	05.00±0.20[g]	00.99±1.76[f]
Roots	09.85±1.40[g]	04.93±1.60[e]

a-d is significant values

Results are expressed as mean±standard deviation (n = 3). Different letters in the column within the same types of extraction indicate significant difference between parts at p<0.05.

Similar results in other studies revealed that sample in hot water extraction gives the higher value of total phenolic content (Nyirenda *et al*., 2012; Stankovic, 2010). It may due to some antioxidants such as polyphenolic compounds have greater solubility in polar solvents such as water and some others dissolve well in slightly-polar solvents such as ethanol (Boeing *et al*., 2014). It was also found that the phenolic content were lowest at room temperature compared with those obtained on heating. Increased temperature could promote the phenolic extraction by increasing both diffusion coefficient and solubility of phenolic compounds in

Table 3: Antioxidant activity of selected samples assayed by BCB

Types of extraction	Antioxidant activity (%)
Hot aqueous extract	
Leaves	85.27±1.97[a]
Stems	44.86±0.44[b]
Roots	ND
Cold aqueous extract	
Leaves	92.04±3.514[c]
Stems	3.07±9.96[d]
Roots	ND
Ethanol extract	
Leaves	9.71±6.68[e]
Stems	13.55±2.92[e]
Roots	16.68±9.49[e]

[a-d]Significant values

Table 4: The value of EC_{50} in different parts of *L. spinosa*

Types of extraction	EC_{50} (µg/mL)
Hot aqueous extract	
Leaves	455
Stems	970
Roots	825
Cold aqueous extract	
Leaves	312
Stems	912
Roots	ND
Ethanol extract	
Leaves	656
Stems	ND
Roots	ND
BHT	53

extraction solvent. Besides, intense heat from solvent was also able to release the cell wall phenolics and bounded phenolics by breaking down of cellular constituents and hence increases the phenolic yield in extract (La *et al*., 2013).

Table 2 also shows the mean of Total Flavonoid Content (TFC) in hot aqueous extract, cold aqueous extract and ethanol extract, of different parts (leaves, stems and roots) of *L. spinosa*. In hot aqueous extract, leaves had the highest TFC (15.62±1.23 mg GAE/g extract) followed by stems and roots. Similarly, in cold aqueous extract, leaves contained the highest TFC (11.57±1.02 mg GAE/g extract) followed by stems and roots. In ethanol extract, leaves had the highest TFC (9.78±2.74 mg GAE/g extract) followed by roots and stems. In similar to TPC, the optimum condition to obtain the TFC in *L. spinosa* was hot aqueous leaves extraction. Similar finding also indicate that the highest flavonoid content was measured in water extract. This is because the concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Wong *et al*., 2014) and may be useful as aqueous extract as it is safe and non-toxic either for direct consumption or application in foods or cosmetic products.

β-Carotene Bleaching (BCB) assay: Table 3 shows the of β-carotene bleaching activity of different parts (leaves, stems and roots) of *L. spinosa*. In hot aqueous extract, leaves had the highest antioxidant activity (85.26±1.97%) followed by stems (44.86±0.44%). In cold aqueous extract, leaves also showed the highest antioxidant activity (92.04±3.51%) followed by stems (3.07±9.96%). The antioxidant activity of roots in hot and cold aqueous root extract was not determined. In ethanol extract, root had the highest antioxidant activity (16.68±9.49%) followed by stems (13.55±2.92%) and leaves (9.71±6.68%). Overall results demonstrated the highest scavenging activity was cold aqueous leaves extract and to some extent possess higher antioxidant activity when compared to BHT (54.34±9.49%). The presence of antioxidant compounds can hinder the extent of β-carotene bleaching by neutralizing hydroperoxides (Othman *et al*., 2016). Thus, the low degradation rate has been increased in antioxidant activity. Results from previous research also showed the plant that homogenate extracted into water (polar solvent) shows higher antioxidant activity than that extracted into non-polar solvent (Boeing *et al*., 2014).

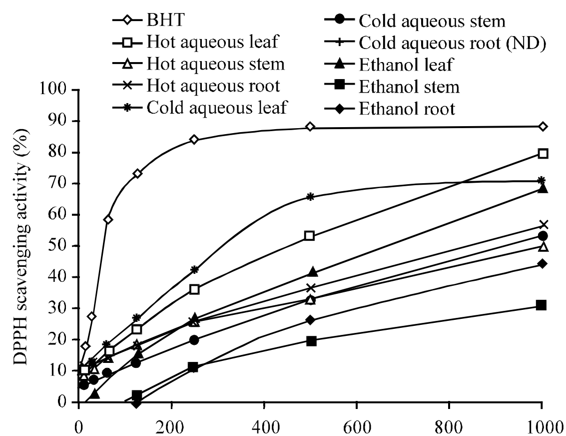

Fig. 1: Percentage of DPPH scavenging activity of *L. spinosa*

Values are expressed as mean±standard deviation (n = 3). Different letters in the same column within the same types of extraction indicate there are significant differences ($p<0.05$). ND indicates the antioxidant activity was not determined within the selected concentrations.

DPPH radical scavenging assay: Figure 1 and Table 4 show the percentage of DPPH radical scavenging activity and the EC_{50} value different parts (leaves, stems and roots) of *L. spinosa*, respectively. As shown in Fig. 1, cold aqueous leaves extract had the highest percentage of scavenging activity but lower when compare to BHT. In hot aqueous extract, the lowest EC_{50} value was in leaves (455 µg/mL) followed by roots (825 µg/mL) and stems (970 µg/mL). In cold aqueous extract, leaves also had the lowest EC_{50} value (312 µg/mL) followed by stems (912

μg/mL). The EC_{50} value of ethanol leaves extract was 656 μg/mL. The EC_{50} was not determined in the cold aqueous roots extract and both ethanol stems and roots extract. The highest antioxidant activity was cold aqueous leaves extract. The EC_{50} value for BHT was 53 μg/mL.

EC_{50} values represent 50% of the radicals scavenged by the samples. Lower EC_{50} value indicates higher antioxidant activity. ND indicates the EC_{50} value was not determined within the selected concentrations.

According to some study in the different extract, similar results reveal that hot water extract gave the higher value of DPPH radical scavenging activity (Nyienda *et al*., 2012; Wong *et al*., 2014). This is because aqueous extracts gave significantly higher radical scavenging rates compared to organic extract. Polar compounds are more soluble in aqueous solvents compared to organic solvents. This finding was similar to the previous study which stated that aqueous extract gave the lowest EC_{50} value compared to organic extract (Kaewpiboon *et al*., 2012). This may be due to phenolics compounds present in the samples are the main contributors to their antioxidant capacity (Khoo *et al*., 2012).

Correlation of antioxidant content and activity: Table 5 shows the correlation between Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant activities (BCB and DPPH). There were positive correlation between TPC and BCB ($p<0.05$) and TPC and DPPH ($p<0.05$). Similarly, there were positive correlation between TFC and BCB ($p<0.05$) and TFC and DPPH ($p<0.05$).

Inhibition effects of *L. spinosa* against pancreatic lipase, α-amylase and α-glucosidase: As shown in Table 6, inhibition of pancreatic lipase was the highest in ethanol stems extract (45.59±1.76%) and followed by cold aqueous roots extract (37.57±0.70%). The ethanol extract shows the highest and significant inhibitory effects ($p<0.05$) when compared with aqueous (hot and cold water) extracts. Previous studies also showed that ethanolic extract had higher result in the inhibition of pancreatic lipase when compared with hot and cold water extracts (Yang *et al*., 2014). The pancreatic lipase inhibitory phytochemicals includes polyphenols and flavonoids (Seyedan *et al*., 2015; Hassan, 2014).

Results are expressed as mean±standard deviation. Different letters in the same column indicate significant difference at $p<0.05$.

The inhibition of α-amylase in hot aqueous leaves extract showed the strongest inhibition effects (46.03±0.01%) followed by cold aqueous leaves extract (45.71±0.44%) and ethanol leaves extract (33.80±3.27%) (Table 6). Overall, the optimum conditions to obtain the α-amylase inhibitor in *L. spinosa* was hot aqueous leaves extract which is more polar extraction (water) compared to the less polar extraction (ethanol). Similarly, Park *et al*. (2013) also demonstrated that α-amylase inhibitory activity was at the highest in polar extracts of plant material.

Table 5: Correlation coefficient (r) between antioxidant contents (TPC and TFC) with antioxidant activities (BCB and DPPH)

Parameters	Values	BCB	DPPH
TPC	r value	0.699*	0.901*
TFC	r value	0.657*	0.837*

*Correlation is significant at $p<0.05$

Table 6: Enzymes inhibition of different parts of *L. spinosa*

Types of extraction	Pancreatic lipase	α-amylase	α-glucosidase
Hot aqueous extract			
Leaves	21.27±14.72^{a}	46.03±0.01^{a}	28.86±2.34^{a}
Stems	27.75±0.01^{a}	12.76±0.01^{b}	10.66±1.56^{b}
Roots	29.32±1.67^{a}	28.47±2.69^{c}	09.56 ± 1.56^{b}
Cold aqueous extract			
Leaves	17.74±2.5^{a}	45.71±0.44^{a}	22.24±5.46^{a}
Stems	21.66±1.94^{a}	ND	29.41±2.20^{ac}
Roots	37.57±0.70^{b}	22.82±1.81^{c}	17.83±0.78^{d}
Ethanol extract			
Leaves	18.07±0.01^{a}	33.80±3.27^{a}	36.24±0.20^{a}
Stems	45.59±1.76^{c}	ND	04.21±1.05^{a}
Roots	15.30±0.44^{a}	15.37 ± 0.53^{c}	27.73±4.00^{acd}

$^{a-d}$Significant values

Table 6 also shows the α-glucosidase activity of *L. spinosa*. The strongest inhibition of α-glucosidase activity was ethanol leaves extract (36.24±0.20%) followed by cold aqueous stems extract (29.41±2.20%) and ethanol roots extract (27.73±4.00%). Overall, the ethanol extraction demonstrated the strongest α-glucosidase inhibitory activity. Previous study by Lordan *et al*. (2013) also showed that ethanolic extract had higher inhibition of α-glucosidase when compared with water extract. Plant polyphenols such as flavonoids and tannins are known to have inhibitory effects on α-amylase and α-glucosidase (Bello *et al*., 2011).

CONCLUSION

Based on overall results, *L. spinosa* leaves demonstrated as a functional food with potent antioxidant content and activities and provide strongest inhibition effects against pancreatic lipase, α-amylase and α-glucosidase which strongly indicated their great potential as natural therapeutic agents.

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