Pancreatic Lipase Inhibitory Activity of Selected Herbal infusions

Nuanla-ong KAEOTHAISONG[1], Rachanee NAM-MATRA[1], and
Saranyu KHAMMUANG[2,*]

[1]*Biodiversity and Conservation Research Unit Group, Biodiversity Program, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Kantarawichai District, Maha Sarakham, 44150, Thailand*
[2]*Protein and Enzyme Technology Research Unit, and Center of Excellence for Innovation in Chemistry Department of Chemistry, Faculty of Science, Mahasarakham University, Kantharawichai, Maha Sarakham, 44150, Thailand*

([*]Corresponding author: skhammuang@yahoo.com)

Abstract

Obesity is now an important public health issue throughout the world. Many methods have attempted to resolve this problem including specific pharmaceutical or herbal medicines as well as exercise regimes. Orlistat is one drug designed to treat obesity. It prevents the absorption of fats from the human diet by acting as a lipase inhibitor, thereby reducing caloric intake. However, orlistat has some side effects. Thus, the use of plant extracts as treatment programs is more suitable than synthetic drugs. This study screened the potential anti-obesity effects of plant extracts and their ability to inhibit the pancreatic lipase enzyme. Pancreatic lipase inhibitory activity was tested with five Thai medicinal plants namely *Bauhinia strychnifolia* Craib, *Cassia fistula* L., *Morus alba* L., *Moringa oleifera* Lam, and *Nelumbo nucifera* Gaertn. The effect of herbal infusions on lipase inhibitory activity *in vitro* was examined by the colorimetric method, using *p*-nitrophenyl dodecanoate as a substrate. *C. fistula* leaf extract exhibited the best activity against pancreatic lipase enzyme with an IC_{50} of 38.372±1.509 µg/mL. Orlistat was used as the positive control for pancreatic lipase inhibition. *C. fistula* leaf extract showed high inhibitory activity toward pancreatic lipase (0.353±0.055 mmol Orlistat/g dry weight). The results provide a basis for further investigations to identity active compounds from potential herb species. Further research is necessary for future development of effective and economical herbal anti-obesity products.

Keywords: Pancreatic lipase, anti-obesity, plant extract, herbal product

Introduction

Obesity is a major global health problem and the major cause of chronic illnesses including diabetes, cardiovascular diseases, hypertension, strokes, mental health disorders and increased risk of cancer of the breast, colon, prostate, endometrium, kidney, and gall bladder [1] Overweight and obesity usually result from an energy imbalance due to excess caloric intake relative to energy expenditure; the excess energy is stored as adipose mass and adipose tissue. Many methods have attempted to resolve this problem such as suppression of food intake and stimulation of energy expenditure. Lipase inhibition is another way to regulate lipid metabolism and inhibition of adipocyte which results in weight control [2].

Pancreatic lipase (EC 3.1.1.3) catalyzes the hydrolysis of ester bonds in triacylglycerols to produce free fatty acids, diacylglycerols, monoacylglycerols, and glycerol. This enzyme serves to digest fats and oils resulting in smaller molecules which can be absorbed easily into the body. For obese people who want to stop or minimize fats from foods and drinks, lipase inhibition is one way to help reduce obesity. Therefore an inhibitor of digestive lipase could become a useful anti-obesity agent [2].

Orlistat is one drug used to treat obesity. It functions by preventing the absorption of fats from human diet and acts as a lipase inhibitor. However, orlistat has some side effects [3]. Therefore, attempts to find natural products with lipase inhibitory activity have increased. Polyphenols can affect the functions of the enzymes that regulate glucose, protein, and lipid metabolism. Many studies have shown that extracts of lotus [4], berries [5], grape seed [6], and green, white, and black tea [7] inhibit pancreatic lipase in vitro.

This study tested the pancreatic lipase inhibitory activity of five plant extracts. Results were presented for phenolic content, total flavonoids content, and concentration of extract to inhibit 50% of lipase activity (IC_{50}).

Materials and methods

Plant materials and extract preparation

Plant materials were collected in 2015 from Maha Sarakham Province, Thailand. Five leaves of plants were tested namely *B. strychnifolia*, *C. fistula*, *M. alba*, *M. oleifera*, and *N. nucifera*. Fresh plant leaves were washed with water. Airing in the shade and air-dried at 50°C for 8 h. The dried leaf was cut into smaller pieces. The dried plant materials were weighed at 2 g, put into tea bags and moved to flasks. The samples were extracted with 200 ml of boiled water at 70°C, infused for 15 min and stirred with a rotary shaker at 100 rpm. After cooling, the infused herbals were filtered through Whatman No.1 filter paper [7].

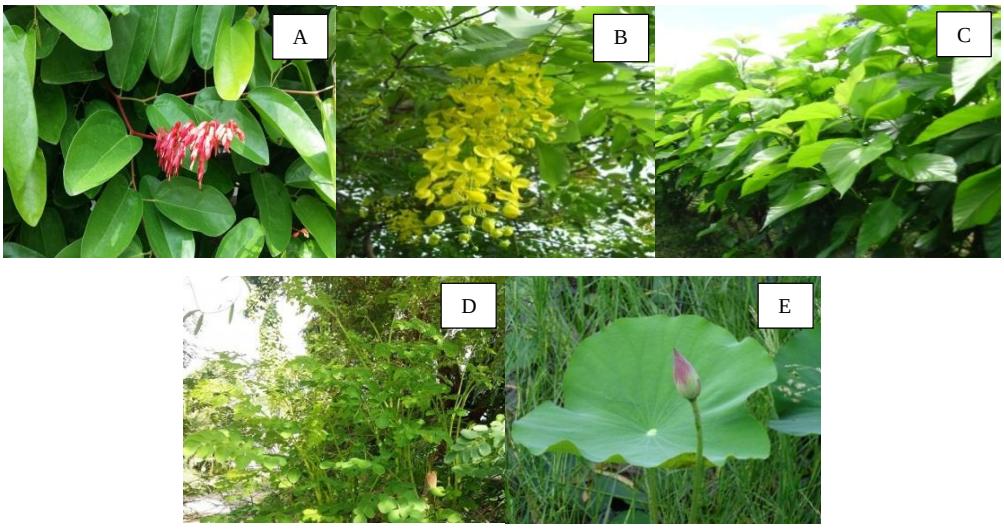

Figure 1. Plant meterials; A *(B. strychnifolia* Craib.), B *(C. fistula* L.), C (*M. alba* L.), D *(M. oleifera* Lam.), and E (*N. nucifera* Gaertn.).

Total phenolic content determination

Total phenolic content of the plant extracts was determined according to the Folin-Ciocalteu method of 10% Folin-Ciocalteu reagent was added to 0.5 ml of the plant extracts) [8]. After 5 min, 0.5 ml of 35% sodium carbonate and 3.5 ml of distilled water were added and the mixture was stored at room temperature for 90 min. The absorbance of the mixture was measured at 725 nm on a UV-vis spectrophotometer. Total phenolic content was expressed in terms of gallic

acid equivalent (mg gallic acid/g dry weight). The calibration equation for gallic acid was *y=106.14x* (R^2*=0.9955)*, when x is the gallic acid concentration in mg/mL and y was the absorbance reading at 725 nm.

Total flavonoid content determination

Total flavonoid content was determined by aluminium chloride colorimetric assay [9]. 0.5 ml of plant extract was placed into test tubes and 0.15 ml of 5% sodium nitrite solution was added into each. After 5 min, 0.15 ml of 10% aluminum chloride was added followed by adding 1 ml of 1 M sodium hydroxide. Finally, the volume was made up to 5 ml with distilled water and mixed well. The absorbance of the mixture was measured at 510 nm. Results of triplicate analyses were expressed as mg catechin /g dry weight. The calibration equation for catechin was *y=3.2463x* (R^2 *= 0.9989)*, where x is the catechin concentration in mg/mL, and y is the absorbance reading at 515 nm.

Free radical-scavenging ability using a stable ABTS radical cation

The $ABTS^{\bullet+}$ radical was used for the antioxidant activity experiment [10]. The $ABTS^{\bullet+}$ solution was diluted with distilled water to give initial optical density at 734 nm at around 0.7. For the 100 µl of samples (various concentrations) was added to 900 µl of $ABTS^{\bullet+}$ radical solution. The antioxidant activity was assayed by measuring the decreasing absorbance at 734 nm after incubation in the dark at room temperature for 5 min. The percentage inhibition of antioxidant was calculated using the equation:

$$\%\mathbf{Inhibition} = \frac{\mathbf{absorbance\ of\ control} - \mathbf{absorbance\ of\ test}}{\mathbf{absorbance\ of\ control}} \times \mathbf{100}$$

The concentration of plant extracts required to scavenge 50% of $ABTS^{\bullet+}$ radicals (IC_{50}) was obtained from graph between the percentage of inhibition and plant extract concentration. The antioxidant activity was reported as Trolox equivalent antioxidant capacity (TEAC) using the regressin equation *y = 4631.7x, R^2 = 0.9965* which expressed as µmol Trolox/g dry weight and also reported as Vitamin C equivalent antioxidant capacity (VCEAC) using the regressin equation *y = 7880.7x, R^2 = 0.999* which expressed as µmol Vitamin C/g dry weight.

Free radical-scavenging ability using a stable DPPH radical

DPPH scavenging activity was determined by an assay modified previously [11]. The DPPH• solution was diluted with distilled water to give initial optical density at 515 nm at around 0.7. For the samples (various concentrations), 100 µl was added to 900 µl of DPPH• solution. Each mixture was kept in the dark at room temperature for 20 min. The antioxidant activity was reported as the inhibition concentration of plant extracts required to scavenge 50% of DPPH• radicals (IC_{50}). TEAC values were derived from the regressin equation $y = 4110.1x$, $R^2 = 0.9998$ which expressed as µmol Trolox/g dry weight and VCEAC values were derived from the regressin equation $y = 5440.8x$, $R^2 = 0.9979$ which expressed as µmol Vitamin C/g dry weight. The percentage inhibition of antioxidant was calculated similar to the ABTS radical scavenging activity.

Pancreatic lipase inhibition assay in vitro

This assay used lipase from porcine pancreas Type II (Sigma, L3126) dissolved in ultra-pure water at 10 mg/ml. The supernatant was used after centrifugation at 12,000 rpm for 5 min. The assay buffer was 100 mM Tris buffer (pH 8.2) and 1.5 mM *p*-Nitrophenyl dodecanoate (Sigma, 61716) were used as the substrate. In total volume 1 ml of reaction, consisted of 150 µl enzyme, 80 µl of plant extracts, 250 µl of substrate and appropriated volume of buffer. The mixture were incubated at 37°C for 30 min and read at 410 nm on a UV-Vis spectrophotometer [7]. The results were expressed as IC_{50} and Orlistat equivalent (Sigma, O4139). The percentage inhibition of Pancreatic lipase enzyme was calculated by the following equation percentage inhibition of lipase, when E was absorbance of enzyme mixed with substrate, SB was absorbance of substrate, EB was absorbance of enzyme, I was absorbance of enzyme mixed with substrate and add plant extract (inhibitor), ISB was absorbance of substrate mixed with plant extract (inhibitor), IEB was absorbance of enzyme mixed with plant extract (inhibitor).

$$\%\ \mathbf{Inhibition\ of\ lipase} = \frac{(\mathbf{E} - (\mathbf{SB} + \mathbf{EB})) - (\mathbf{I} - (\mathbf{ISB} + \mathbf{IEB}))}{(\mathbf{E} - (\mathbf{SB} + \mathbf{EB}))} \mathbf{x\ 100}$$

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p < 0.05$) using the Statistical Package for the Social Sciences (SPSS). Data were expressed as mean±standard deviation of three replicates.

Results and discussion

Total phenolic content

Dried plant material (2 g) in tea bag was infused in 200 mL of hot water as description in materials and methods. After dried out it was found that each extracts concentrations were shown in Table 1. In addition, percentages of moisture for each plant materials were also shown.

Table 1. The percentage of moisture in plants materials and the amount of dried weight extract derived from herbal infusions

Plant extract	% Moisture in plants materials	Amount of extract (mg/mL)
B. strychnifolia	7.896±0.203	0.784±0.003
C. Fistula	5.472±0.118	1.611±0.005
M. alba	9.223±0.132	2.313±0.008
M. oleifera	7.518±0.129	1.973±0.040
N.nucifera	8.102±0.152	1.597±0.010

Total phenolic content in five plant extracts showed significantly difference ($p < 0.05$) (Table 2.). The extract of *B. strychnifolia* showed highest levels of total phenolic content with the value of 144.854±3.005 mg gallic acid/g dry weight. Leave extract of *B. strychnifolia* has been reported to be rich in phenolic content at 149.32±2.89 mg gallic acid/g [12], which closely related with our finding. There has been reports that phenolic componds likely involved in antioxidants ability [8, 9]. In addition, phenolic compounds probably involved in the inhibition

of the enzyme lipase, a previous report showed that phenolic compound could inhibition lipase and reduced fat absorption [6].

Table 2. Total phenolic contents and total flavonoid contents of plant extracts

Plant extract	Total phenolic content (mg gallic acid/g dry weight)	Total flavonoid content (mg catechin/g dry weight)
B. strychnifolia	144.854±3.005^{e}	1221.036±11.346^{c}
C. Fistula	56.719±0.253^{c}	628.354±3.980^{a}
M. alba	46.739±1.469^{b}	999.243±34.531^{b}
M. oleifera	38.435±0.316^{a}	651.480±9.147^{a}
N.nucifera	91.450±2.763^{d}	1710.412±42.101^{d}

All data are means ± S.D. of triplicate measurements. Different superscript letters indicate significant difference ($P < 0.05$) within the same column.

Total flavonoid content

The total flavonoid content in plant extracts showed content in order of *N. nucifera* > *B. strychnifolia* >*M. alba* > *M.olefera*> *C. fistula* (Table 2.). Extract of *N. nucifera* had the highest total flavonoids content with the values of 1710.412 ± 42.101 mg catechin/g dried weight extract. Flavonoids and other phenolic compound are potent water-soluble antioxidant and free radical scavenger that prevent oxidative cell damage. Flavonoids has beneficial effects on human health, a previous report showed that flavonoid probably involved in the inhibition lipase and reduced fat absorption such as Epigallocatechin-3-gallate (EGCG), Kaempferol and Quercetin [6]. Flavonoids in leave extract of *N.nucifera* showed high inhibitory activity against pancreatic lipase and significantly lowered the lipid component [4]. Flavonoids in lotus leaf that have been reported to have the ability to inhibit lipase is Quercetin-3-O-β-D-glucuronide and Rutin [13].

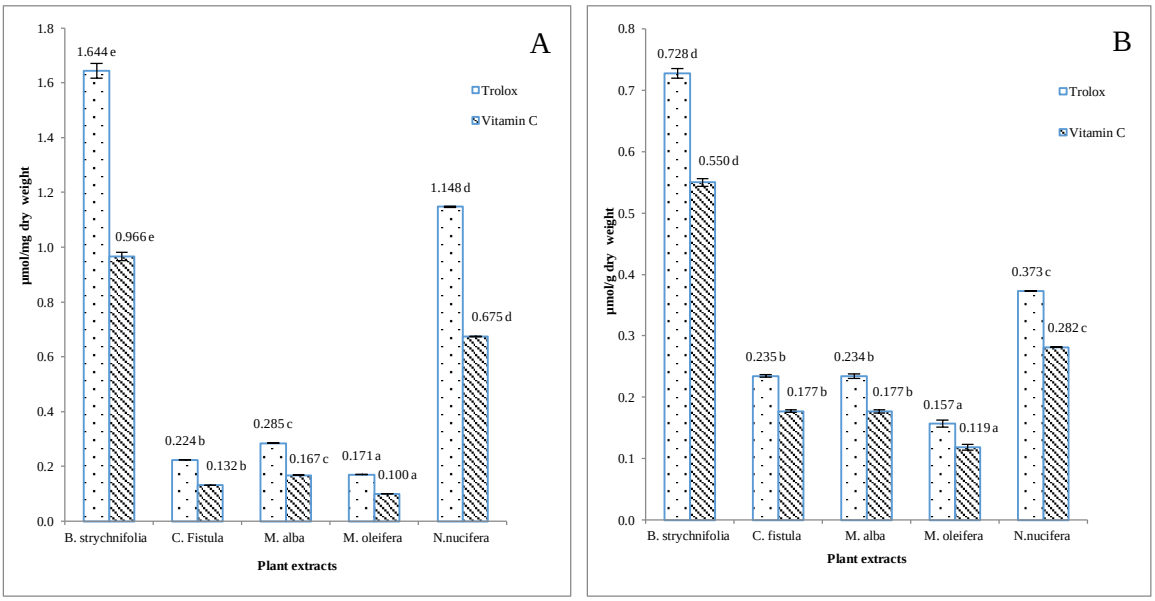

Figure 2. Amount of free radical-scavenging ability were express as µmol/mg dried weight extract with TEAC and VCEAC values of ABTS assay (A) and DPPH assay (B). Different superscript letters indicate significant difference ($P < 0.05$) within the same column.

Free radical-scavenging ability using a stable ABTS radical

The ABTS radical scavenging ability from high to low of plant extracts tested could be shown as following: *B. strychnifolia> N. nucifera> M. alba > C. fistula >M. olefera* (Figure 2A.). IC_{50} values for ABTS radical scavenging ability were shown in Table 3. It was found that extracts from *B. strychnifolia* and *N. nucifera* showed interested results for ABTS radical scavenging ability. This ability related with amount of total phenolic content and total flavonoid content which reported in Table 2. Therefore, phenolic compounds and flavonoids should be involving in ABTS radical scavenging ability.

Free radical-scavenging ability using a stable DPPH radical

The DPPH radical scavenging activity from high to low of plant extracts tested could be shown as following: *B. strychnifolia > N. nucifera > C. fistula >M. alba > M. olefera* (Figure 2B.). IC_{50} values for DPPH radical scavenging ability were shown in Table 3. It was found that extracts from *B. strychnifolia* and *N. nucifera* showed interested results for DPPH radical scavenging ability similar found in the ability to scavenging of ABTS radicals. Therefore,

phenolic compounds and flavonoids should be major type molecules response for DPPH radical scavenging ability. Leave extract of *B. strychnifolia* has been reported to be an effective antioxidant [12] which agree with our result.

Table 3. The inhibition concentation of plant extracts required to 50% radical scavenging ability and the inhibition concentation of plant extracts required to 50% pancreatic lipase

Plant extract	Inhibition of ABTS IC_{50}(µg/mL)	Inhibition of DPPH IC_{50}(µg/mL)	Inhibition of Pancreatic lipase IC_{50}(µg/mL)
B. strychnifolia	6.493±0.024 [a]	15.995±0.134 [a]	121.859±5.738 [d]
C. fistula	46.855±0.088 [d]	48.602±0.883 [c]	38.372±1.509 [a]
M. alba	36.616±0.090 [c]	51.095±1.020 [c]	111.164±6.868 [c]
M. oleifera	60.964±0.238 [e]	75.475±4.380 [d]	105.593±0.939 [c]
N. nucifera	8.926±0.011 [b]	32.215±0.437 [b]	77.266±0.865 [b]

Pancreatic lipase inhibition

The inhibitions of plant extracts against pancreatic lipase were expressed as Orlistat equivalent (mmol Orlistat/g dry weight) (Figure 3A). It was found that *C. fistula* and *N. nucifera* leave extracts showed interesting lipase inhibitory ability whilst other leave extracts also showed minor lipase inhibitory activity. Since total phenolic content and flavonoid content of *C. fistula* were not so high. However, it is possible that type of phenolic compounds or flavonoids that exist in *C. fistula,* but could not found in other tested leave extracts, might play a role in inhibition of lipase. In addition, other type of molecule which we are not test in this time may play a role in lipase inhibition. Previous reports showed that the structure of substances affecting the inhibition of enzyme lipase such as flavan-3-ols and strictinin [7]. Different phenolic compounds showed different ability to inhibit lipase such as EGCG, curcumin and flavonols had a stronger inhibitory effect than ellagic acid, resveratrol and luteolin [6]. Previous report showed that *C. fistula* extract could inhibit lipase because tannins [14]. Therefore, it is possible that tanins might response for inhibit lipase of *C. fistula* extract.

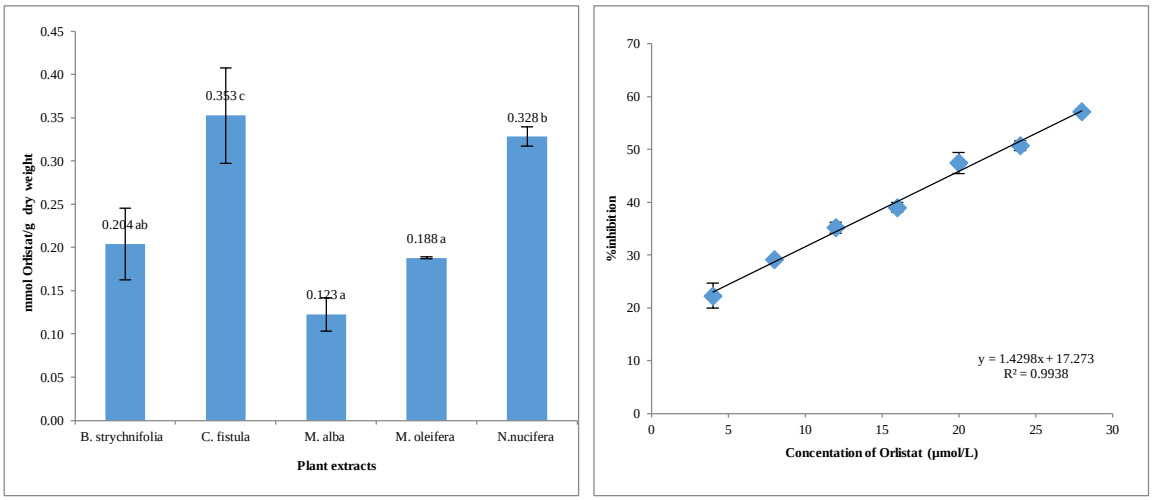

Figure3. (A) Inhibitory activity of pancreatic lipase expressed as Orlistat equivalent (mmol Orlistat/g dry weight). (B) Showed standard calibration curve of Orlistat and equation for pancreatic lipase.

Conclusions

In conclusion, *B. strychnifolia* extract contained highest amount of total phenolic content (144.854±3.005 mg gallic acid/ g dry weight) and *N. nucifera* extract contained highest amount of total flavonoids content (1710.412±42.101 mg catechin / g dry weight). *B. strychnifolia* extract showed the best antioxidant activity for scavenging of both ABTS and DPPH radicals. *C. fistula* leave extract showed the best activity for inhibition of the pancreatic lipase enzyme with IC_{50} of 38.372±1.509 µg/mL.

Acknowledgements

The research was financially supported by Research and Researchers for Industrie (RRI). The authors thank The Giving Tea. Program in Biodiversity, Walai Rukhavej Botanical Research Institute, Mahasarakham University and Faculty of Science, Mahasarakham University are acknowledged for facilities supports.

References

[1] L. Zhang, and Y. Lu. Inhibitory activities of extracts from *Cleistocalyx operculatus* flower buds on pancreatic lipase and α-amylase. *Eur Food Res Technol*. 235 (2012), 1133-1139.

[2] M. Ado, F. Abas, A.S. Mohammed and H.M. Ghazali. Anti-and pro-lipase activity of selected medicinal, herbal and aquatic plants, and structure elucidation of an anti-lipase compound. *Molecules.* 18(2013) 14651-14669.

[3] G.A. Mohamed, S.R. Ibrahim, E.S. Elkhayat, R.S. El Dine. Natural anti-obesity agents. *Bulletin of faculty of pharmacy.* 52 (2014) 269-284.

[4] S. Liu, D. Li, B. Huang, Y. Chen, X. Lu and Y. Wang. Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves. *J Ethnopharmacol.* 149 (2013) 263-269.

[5] G.J. McDougall, N.N. Kulkarni, and D. Stewart. Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chem.* 115 (2009) (1) 193-199.

[6] T. Sergent, J. Vanderstraeten, J. Winand, P. Beguin, Y.J. Schneider. Phenolic compounds and plant extracts as potential natural anti-obesity substances. *Food Chem.* 135 (2012) 68-73.

[7] A. Gondoin, D. Grussu, D. Stewart and G.J. McDougall. White and green tea polyphenols inhibit pancreatic lipase in vitro. *Food Res Int.* 43 (2010) 1537-1544.

[8] H. Cheung, Q. Zhang and Z. Zhang. Antioxidant activity of Rhizoma smilacis Glbrae extracts and its key constituent-astilbin. *Food Chem.* 115 (2009) 297-303.

[9] S. Kamkekar, V. keer and V. Patil. Estimimation of phenolic content, Flavonoid content, abtioxidant and alpha amylase inhibitory activity of marketed polyherbal. *J Applied pharmaceutical science*. 4 (2014) 61-65.

[10] K. Lo and K. Cheung. Antioxidant activity of extract from fruiting bodies of Agrobeaegerita var. alba. *Food Chem.* 89 (2005) 533-539.

[11] K. Thaipong and U. Boonprakop. Comparison of ABTS, DPPH, FRAP and ORAC assay for estimation antioxidant activity from guava fruit extracts. *J. Food Comp. Anal.* 19 (2006) 669-675.

[12] S. Sayompark, A. Itharat and P. Hansakul. Comparation study of antioxidant activities and total phenolic content of *Bauhinia strychifolia* leaves extracts. *Sci Health*, 2012.

[13] K. Shukla and N. Chaturvedi. *In vitro* antioxidant properties of different parts of *Nelumbo nucifera* Gaertn. IJAPBC, 5 (2016) 196-201.

[14] S. Niumsakul, D. Pattamadilok, N. Limpeanchob, K. Ingkaninan, P. Wongsinkongman and S. Srisop. Chemical constituents of *Nelumbo nucifera* Gaertn leaf extract exhibiting hypolipidemia Activity. *Journal of Thai Traditional & Alternative Medicine*, 8 (2010) 2-3.

[15] P. George and P. Paduchuri. A review on antiobesity properties of some medicinal plants. *An International Journal of Pharmaceutical Sciences*, 3 (2012) 1863-1874.