Food and Nutrition Sciences, 2013, 4, 554-558
http://dx.doi.org/10.4236/fns.2013.45071 Published Online May 2013 (http://www.scirp.org/journal/fns)

Lipase Inhibitor from Fruits of *Solanum stramonifolium* Jacq.

Watinee Chanmee[1], Chanya Chaicharoenpong[2*], Amorn Petsom[3]

[1]Program of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand; [2]Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok, Thailand; [3]Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.
Email: [*]chanya.c@chula.ac.th

Received March 8th, 2013; revised April 8th, 2013; accepted April 15th, 2013

ABSTRACT

Obesity is usually considered as an overweight or excess body fat, leading to increased health problems. Obesity is a major risk factor for a number of serious diseases. Decreasing dietary fat absorption, through inhibition of pancreatic lipase activity, has been reported to be one of the most effective ways for managing obesity. The present study was aimed at investigating lipase inhibitors from edible plants. A lipase inhibitor was isolated from *n*-hexane and ethyl acetate extracts of the ripe fruits of *Solanum stramonifolium* Jacq. by column chromatography and identified by spectral analysis. Its structure was elucidated as (22*R*)-3β-benzoyloxy-22-hydroxy-4α-methyl-5α-stigmast-7-en-6-one or carpesterol (**1**). Carpesterol exhibited moderate lipase inhibition activity with IC_{50} value of 56.0 μg/mL while orlistat, a well-know pancreatic lipase inhibitor, had IC_{50} value of 3.5 ng/mL. Moreover, the kinetic properties of carpesterol on pancreatic lipase were evaluated. Carpesterol is a competitive inhibitor and exhibited antagonistic interaction when combined with orlistat on lipase inhibition activity.

Keywords: *Solanum stramonifolium* Jacq.; Lipase Inhibitor; Carpesterol; Orlistat; Obesity

1. Introduction

Obesity or hyperlipidemia is usually considered as an overweight or excess body fat, leading to increased health problems [1]. Obesity is a major risk factor for a number of serious diseases, including cardiovascular diseases, heart disease, hypertension, atherosclerosis, diabetes, certain types of cancer, and osteoarthritis [2,3]. Decreasing dietary fat absorption, through inhibition of pancreatic lipase activity, has been reported to be one of the most effective ways for managing obesity. Orlistat is a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini* [4] and has been proved to be effective for the treatment of human obesity by the Food and Drug Administration. However, consumption of orlistat caused unpleasant side effects such as hyperuricemia, diarrhea, nausea, nyositis, gastric irritation, steatorrhes, oily spotting, flatulence, flatus with discharge, fecal incontinence and dry skin [5]. To find less toxic side effects than the synthetic drug, the lipase inhibitors from various plant species have been investigated and reported, including *Cassia nomame* [6], soybean seed [7], tea saponins [8], tea polyphenols [9], grape seed [10], *Eriochloa villosa* [11], *Orixa japonica* [11], *Setaria italica* [11] and *Platycodon grandiflorum* [12]. In our preliminary screening of lipase inhibition activity of edible plants, fruits of *Garcinia schomburgkiana* Pierre., *Phyllanthus acidus* Linn. Skeels, *Phyllanthus emblica* Linn., *Solanum stramonifolium* Jacq. and *Solanum trilobatum* Linn., the methanol extract of the ripe fruit of *S. stramonifolium* exhibited the strongest activity with an IC_{50} value of 0.36 mg/mL at a concentration of 1.25 mg/mL.

Solanum stramonifolium or coconilla is a hairy fruited pea-eggplant of the Solanaceae family that grows on hill fields and agroforest orchards of Thailand. In Thailand, this plant has been traditionally used to stimulate urine discharge and ripe fruits are normally eaten raw at mealtimes. In the present study we report on the isolation of a lipase inhibitor from the ripe fruit of *S. stramonifolium*. Furthermore, the kinetic properties of isolated compounds on pancreatic lipase were evaluated.

[*]Corresponding author.

2. Materials and Methods

2.1. General

Optical rotation was measured with a Perkin-Elmer 341 polarimeter (CH_2Cl_2; c 0.2). IR spectra were performed on a Perkin-Elmer (Spectrum One). TOF-MS was recorded on a Micromass Platform II mass spectrometer (Micromass). The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer (Bruker) in deuterated chloroform. Synergy HT Multi-mode microplate spectrophotometer (Bio-Tek) was used as a microplate reader. The PS microtiter plates with 96 flat bottom wells were purchased from Corning Coster. Thin layer chromatography and column chromatography was performed on silica gel 60 F_{254} precoated plates (Merck) and 70 - 230 mesh silica gel 60 (Merck), respectively. Lipase from porcine pancreas type II and *p*-nitrophenylpalmitate were purchased from Sigma Chemical Co. Tris [hydroxymethyl]aminomethane (MB Grade) was purchased from USB Corp. Orlistat was obtained commercially with the trade name Xenical (Hoffmann-La Roche).

2.2. Plant Materials

Ripe fruits of *S. stramonifolium* were purchased from the Thonburi Railway market Bangkok, which had been collected from Amphoe Nakhon-chai-si, Nakhon-pathom province, Thailand. The voucher specimens (BKF No. 183757) were authenticated by a botanist of the Bangkok Forest Herbarium and have been deposited at the Bangkok Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

2.3. Extraction and Isolation

The fresh ripe fruits of *S. stramonifolium* (26.0 kg) were ground and dried (1.3 kg), then macerated for three days with *n*-hexane (10 L × 2) at room temperature. The extract was filtrated with Whatman no. 1 filter paper and then was evaporated *in vacuo* to give the *n*-hexane extract (5.0 g) as green-yellow oil. After that the residue was macerated for three days with ethyl acetate (10 L × 2) at room temperature. The extract was filtrated with Whatman no. 1 filter paper and then evaporated *in vacuo* to obtain the ethyl acetate extract (10.8 g) as dark brown oil. The residue was extracted with water (10 L × 2) and then filtrated with Whatman no. 1 filter paper and evaporated *in vacuo* to give a water extract (772.0 g) as dark brown gum. The *n*-hexane extract was re-crystallized with a mixture of *n*-hexane and ethyl acetate (7:4 v/v) to yield white crystals of compound **1** (978.0 mg, 0.08% w/w of dried fruits). The ethyl acetate extract was separated by silica gel column chromatography with *n*-hexane-ethyl acetate gradient elution in a stepwise fashion (100:0, 5:1, 5:2, 1:1, 6:7, 5:6, 1:2 and 0:100 v/v) to obtain 8 fractions (frs. 1 - 8). The obtained fractions were tested *in vitro* for their lipase inhibition activities. The active fractions were further separated. Only fraction 4 (554.2 mg) showed anti-pancreatic lipase activity and was further purified by preparative TLC on silica gel plates using *n*-hexane/ethyl acetate (8:2 v/v) as developing solvent to give compound **1** (173.6 mg, 0.01% w/w of dried fruits).

2.4. Chemical Characterization of Compound 1 ((22*R*)-3β-Benzoyloxy-22-Hydroxy-4α-Methyl-5α-Stigmast-7-en-6-One or Carpesterol)

White crystals; $[\alpha]_D^{25}$ + 41.6 (c 0.20, CH_2Cl_2); IR (KBr) ν_{max}: 3453, 2960, 1705, 1680, 1630, 1605, 1580, 1450, 1380, 1273, 1110 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (^{2}H, d, J = 8.5 Hz, H-2' and H-6'), 7.56 (^{1}H, t, J = 7.5 Hz, H-4'), 7.45 (^{2}H, t, J = 7.6 Hz, H-3' and H-5'), 5.70 (^{1}H, s, H-7), 4.7 (^{1}H, m, J = 10.6, 4.5 Hz, H-3), 3.73 (^{1}H, d, J = 10.7 Hz, H-22), 1.10 (^{3}H, d, J = 5.5 Hz, H3-30), 0.96 (^{3}H, d, J = 6.7 Hz, H3-21), 0.92 (^{3}H, s, H3-19), 0.90 (^{3}H, d, J = 6.8 Hz, H3-27), 0.88 (^{3}H, t, J = 7.3 Hz, Ha-29), 0.80 (^{3}H, d, J = 6.8 Hz, H3-26), 0.62 (^{3}H, s, H3-18); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.3 (C-6), 166.5 (C-31), 161.1 (C-8), 133.0 (C-4'), 130.6 (C-1'), 129.7 (C-2' and C-6'), 128.4 (C-3' and C-5'), 123.7 (C-7), 79.1 (C-3), 71.2 (C-22), 60.1 (C-5), 55.0 (C-14), 53.2 (C-17), 51.1 (C-9), 45.2 (C-13), 42.7 (C-20), 41.5 (C-24), 39.4 (C-10), 38.8 (C-12), 36.3 (C-1), 31.9 (C-4), 30.1 (C-23), 28.8 (C-25), 27.1 (C-16), 26.3 (C-2), 23.7 (C-28), 22.7 (C-15), 21.8 (C-11), 20.6 (C-27), 17.7 (C-26), 17.6 (C-30), 14.8 (C-19), 12.6 (C-21), 12.4 (C-18), 11.9 (C-29); EI-MS: m/z $[M]^+$ 562; HR-EI-MS; m/z $[M]^+$ 562.3998 (calcd for $C_{37}H_{54}O_4$, 562.4052).

2.5. Lipase Inhibitory Activity *in Vitro*

The method of Slanc *et al.* [13] was employed with some modifications. *p*-nitrophenylpalmitate (PNP) was dissolved in acetonitrile to give a stock solution with a concentration of 20 mM. Ethanol was then added to a final concentration of 1:2 (v/v) acetonitrile:ethanol, resulting in 6.66 mM PNP. The solution was stored at −20˚C. The test sample was dissolved in DMSO (50 mg/mL). Porcine pancreatic lipase (type II, crude, Promega) was dissolved in 0.061 M Tris-HCl buffer (pH 8.5) to a final concentration of 5 mg/mL. Reaction mixtures containing 0.60 mg/mL of enzyme, 2.5 mg/mL of crude extracts, 0.333 mM of PNP and 0.061 M of Tris-HCl buffer, were incubated at 37˚C for 25 min. Then ethanol was added to stop the reaction. The absorbance of released *p*-nitrophenol was measured at 405 nm using a microplate reader. The activity assay was performed in triplicate for

each treatment, and the results were averaged and expressed with standard deviations. A solution of orlistat was prepared in DMSO as a positive control with a concentration of 0.01 μg/mL.

2.6. Investigation of Inhibitory Properties of Isolated Compound

Inhibition mode and K_i value were determined by Lineweaver-Burk plot analysis of the data resulted from enzyme assays containing various concentrations of PNP as substrate (3.996, 3.33, 1.665, 1.11 and 0.8325 mM) in the absence and presence of four different concentrations of compound **1** (2.5, 12.5, 25 and 125 μg/mL) [14].

2.7. Determination of Efficacy of the Compound 1 in Combination with Orlistat

Varying amounts of orlistat including 0.00125, 0.0025, 0.0100, 0.0250, and 0.0500 μg/mL were combined with 6.25 μg/mL of compound **1** and subjected to enzyme assay.

3. Results and Discussion

The dried ripe fruits of *S. stramonifolium* were successively extracted with *n*-hexane, ethyl acetate and water. The resulting three crude extracts were evaluated for anti-lipase activity using *p*-nitrophenylpalmitate (PNP) as substrate. The results are summarized in **Table 1**. The ethyl acetate extract exhibited strong activity with % inhibition values of 94.6% ± 8.3% and the *n*-hexane extract showed moderate activity with % inhibition values of 33.4% ± 2.7%. However, water extract showed no activity with % inhibition value of −1.1% ± 1.5%. Based on their inhibition values, the hexane and ethyl acetate extracts were further purified and evaluated for lipase inhibition activity *in vitro*. The *n*-hexane extract was purified by crystallization with a mixture of *n*-hexane and ethyl acetate (7:4 v/v) to obtain compound **1** as white crystals. The ethyl acetate extract was separated by silica gel column chromatography with *n*-hexane-ethyl acetate gradient elution to obtain 8 fractions (frs. 1 - 8). Only fraction 4 showed anti-pancreatic lipase activity and then was further purified by preparative TLC on silica gel plates using *n*-hexane/ethyl acetate (8:2 v/v) as developing solvent to give compound **1**. The ^1H, ^{13}C NMR and MS analyses of compound **1** indicated it was (22*R*)-3β-benzoyloxy-22-hydroxy-4α-methyl-5α-stigmast-7-en-6-one or carpesterol (**Figure 1**) [15-18]. Compound **1** showed lipase inhibition activity of 62.3% ± 6.2% at concentration of 0.125 mg/mL. It exhibited moderate inhibition activity with IC_{50} value of 56.00 μg/mL, while orlistat, a well-know pancreatic lipase inhibitor, showed lipase inhibition activity of 102.5% ± 5.1% at concentration of 1×10^{-5} mg/mL and IC_{50} value of 0.0035 μg/mL.

Table 1. Lipase inhibition activity of crude extracts of fruits of *S. stramonifolium* at concentration of 1.25 mg/mL.

Sample	% inhibition
n-hexane extract	33.4 ± 2.7
ethyl acetate extract	94.6 ± 8.3
water extract	−1.1 ± 1.5

The Lineweaver-Burk plot was determined for enzyme kinetic study of compound **1** using PNP as the variable substrate. The results shown in **Figure 2** demonstrated that compound **1** acted as a competitive inhibitor of pancreatic lipase having PNP as a substrate, while orlistat is a potent and irreversible pancreatic lipase inhibitor [19]. The V_{max} value was found to be constant, 2.05 min^{-1}. Summary of kinetic parameters of the inhibition is shown in **Table 2**, K_m and K_i values were increased with increasing concentrations of compound **1**.

In addition, the efficacy of compound **1** in combination with orlistat was determined on lipase inhibitory activity. Varying amounts of orlistat including 0.00125, 0.0025, 0.0100, 0.0250, and 0.0500 μg/mL were combined with 6.25 μg/mL of compound **1** and subjected to enzyme assay. The results showed that the %inhibition of

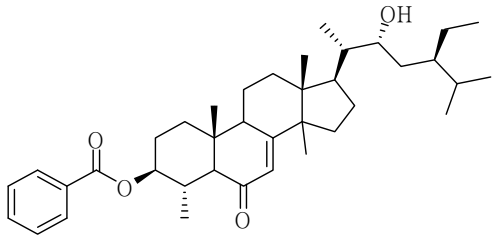

Figure 1. The structure of compound 1.

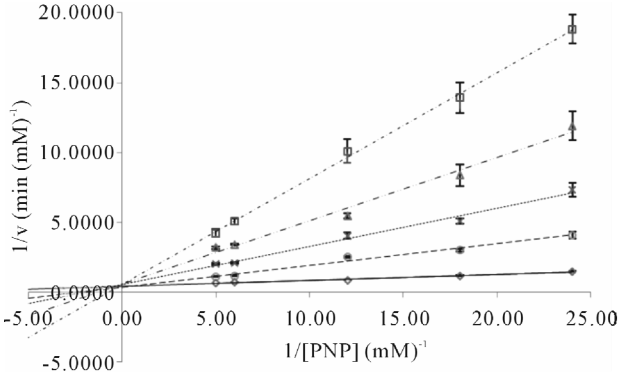

Figure 2. The Lineweaver-burk plots of kinetic analysis for inhibition of pancreatic lipase by compound 1 at five different PNP concentrations. (◊) Compound 1 = 0 μg/ml; (○) Compound 1 = 2.5 μg/ml; (×) Compound 1 = 12.5 μg/ml; (Δ) Compound 1 = 25 μg/ml; (□) Compound 1 = 125 μg/ml. The values are expressed as means ± S.D. (n = 3 for each activity assay).

the mixture was lower than for orlistat (**Figure 3**), indicating that the interaction between orlistat with compound **1** on lipase inhibitory activity was antagonistic.

In the literatures, methanol extract of fruit of *S. stramonifolium* was reported to exhibit low antioxidant activity because of low total phenolics contents [20-22]. Protein in aqueous extract of seed of *S. stramonifolium* was reported to show antibacterial activities with good inhibition against pathogenic bacteria both Gram positive and Gram negative according to the disc diffusion method [23]. Moreover, ethyl acetate and methanol extracts of *S. stramonifolium* inhibited *Escherichia coli* with diameter of inhibition zone 10.3 and 11.5 mm, respectively at the concentration of 10 mg/disc [24]. However, no chemical constituents have been reported. This is the first report of the isolation of chemical constituents of *S. stramonifolium* with lipase inhibition activity. Compound **1** or carpesterol was first extracted from *S. xanthocarpum* [25]. Carpesterol was also found in *S. sisymbrifolium* [18], *S. xanthocarpum* [17,26] and *S. indicum* [16]. It showed anti-inflammatory activity and hypoglycaemic activity in rats and mice [14]. The analysis of the structure-function of derivatives of carpesterol is in progress.

4. Conclusion

This study indicated that carpesterol displayed moderate lipase inhibition with IC_{50} value of 56.00 µg/mL. Moreover, it is a reversible competitive inhibitor while orlistat is a potent and irreversible pancreatic lipase inhibitor and it exhibited antagonistic interaction when administered with orlistat. On the basis of the results it is suggested that the ripe fruits of *S. stramonifolium* could be of used for obesity treatment but people under treatment with orlistat should avoid consumption of this fruit. Although, the potency of carpesterol on lipase inhibitory activity is not as strong as orlistat, its activity is in the same level as compounds extracted from natural sources [8].

Table 2. Summary of kinetic parameters for the inhibition of pancreatic lipase under different concentrations of PNP and compound 1.

Concentration of compound **1** (µM)	K_m $(mM)^{-1}$	K_i $(mM)^{-1}$
0	0.0951	-
2.5	0.4283	0.9213
12.5	0.4691	2.2785
25	0.7843	2.5542
125	1.3455	7.3289

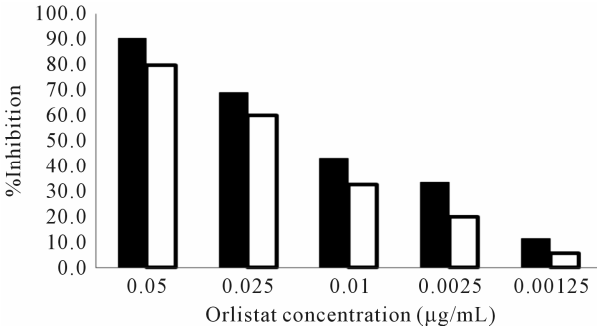

Figure 3. The efficacy of combination of varying amounts of orlistat with compound 1 (■ = orlistat; □ = orlistat + compound 1).

5. Acknowledgements

We are grateful to Associate Professor Dr. Chaiyo Chaichantipyuth and Associate Professor Dr. Pairoh Pinphanichakarn of Chulalongkorn University for suggestions. This work was financially supported by the grant from Graduate School, Chulalongkorn University and the grant from National Research University Project of Thailand, Office of the Higher Education Commission (FW1021A).

REFERENCES

[1] E. Cairns, "Obesity: The Fat Lady Sings?" *Drug Discovery Today*, Vol. 10, No. 5, 2005, pp. 305-307. doi:10.1016/S1359-6446(05)03375-1

[2] P. G. Kopelman, "Obesity as a Medical Problem," *Nature*, Vol. 404, No. 6778, 2000, pp. 635-643.

[3] G. A. Bray, "The Underlying Basis for Obesity: Relationship to Cancer," *The Journal of Nutrition*, Vol. 132, No. 11, 2002, pp. 3451S-3455S.

[4] P. Hadváy, H. Lengsfeld and H. Wolfer, "Human Digestive and Metabolic Lipases," *Biochemical Journal*, Vol. 256, Pt. 2, 1988, pp. 357-361.

[5] T. D. Filippatos, C. S. Derdemezis, I. F. Gazi, E. S. Nakou, D. P. Mikhalidis and M. S. Elisaf, "Orlistat-Associated Adverse Effects and Drug Interactions: A Critical Review," *Drug Safety*, Vol. 31, No. 1, 2008, pp. 53-65. doi:10.2165/00002018-200831010-00005

[6] M. Yamamoto, S. Shimura, Y. Itoh, T. Ohsaka, M. Egawa and S. Inoue, "Anti-Obesity Effects of Lipase Inhibitor CT-II, an Extract from Edible Herbs, Nomame Herba, on Rats Fed a High-Fat Diet," *International Journal of Obesity and Related Metabolic Disorders*, Vol. 24, No. 6, 2000, pp. 758-764. doi:10.1038/sj.ijo.0801222

[7] K. Satouchi, K. Hirano, O. Fujino, M. Ikoma, T. Tanaka and K. Kitamura, "Lipoxygenase-1 from Soybean Seed Inhibiting the Activity of Pancreatic Lipase," *Bioscience Biotechnology and Biochemistry*, Vol. 62, No. 8, 1998, pp. 1498-1503.

[8] R. B. Birari and K. K. Bhutani, "Pancreatic Lipase Inhibitors from Natural Sources: Unexplored Potential," *Drug Discovery Today*, Vol. 12, No. 19, 2007, pp. 879-889. doi:10.1016/j.drudis.2007.07.024

[9] M. Nakai, Y. Fukui, S. Asami, Y. Toyoda-Ono, T. Iwa-

shita, H. Shibata, T. Mitsunaga, F. Hashimoto and Y. Kiso, "Inhibitory Effects of Oolong Tea Polyphenols on Pancreatic Lipase *in Vitro*," *Journal of Agricultural and Food Chemistry*, Vol. 53, No. 11, 2005, pp. 4593-4598. doi:10.1021/jf047814+

[10] D. A. Moreno, N. Ilic, A. Poulev, D. L. Brasaemle, S. K. Fried and I. Raskin, "Inhibitory Effects of Grape Seed Extract on Lipases," *Nutrition*, Vol. 19, No. 10, 2003, pp. 876-879.

[11] N. Sharma, V. K., Sharma and S. Y. Seo, "Screening of Some Medical Plants for Anti-Lipase Activity," *Journal of Ethnopharmacology*, Vol. 97, No. 3, 2005, pp. 453-456. doi:10.1016/S0899-9007(03)00167-9

[12] L. K. Han, B. J. Xu, Y. Kimura, Y. N. Zheng and H. Okuda, "Platycodi Radix Affects Lipid Metabolism in Mice with High Fat Diet-Induced Obesity," *The Journal of Nutrition*, Vol. 130, No. 11, 2000, pp. 2760-2764.

[13] P. Slanc, B. Doljak, A. Mlinaric and B. Strukelj, "Screening of Wood Damaging Fungi and Macrofungi for Inhibitors of Pancreatic Lipase," *Phytotherapy Research*, Vol. 18, No. 9, 2004, pp. 758-762. doi:10.1002/ptr.1548

[14] T. K. Bhattacharya, M. N. Ghosh and S. S. Subramanian, "A Note on Anti-Inflammatory Activity of Carpesterol," *Fitoterapia*, Vol. 51, No. 5, 1980, pp. 265-268.

[15] Y. H. Tsay, J. V. Silverton, J. A. Beisler and Y. Sato, "The Structure of Carpesterol," *Journal of the American Chemical Society*, Vol. 92, No. 23, 1970, pp. 7005-7006. doi:10.1021/ja00726a068

[16] J. A. Beisler and Y. Sato, "The Chemistry of Carpesterol, a Novel Sterol from *Solanum xanthocarpum*," *The Journal of Organic Chemistry*, Vol. 36, No. 25, 1971, pp. 3946-3950. doi:10.1021/jo00824a022

[17] G. Kusano, T. Takemoto, J. A. Beisler and Y. Sato, "Steroidal Constituents of *Solanum xanthocarpum*," *Phytochemistry*, Vol. 14, No. 2, 1975, pp. 529-523. doi:10.1016/0031-9422(75)85123-5

[18] A. K. Chakravarty, S. Mukhopadhyay, S. Saha and S. C. Pakrashi, "A Neolignan and Sterols in Fruits of *Solanum sisymbrifolium*," *Phytochemistry*, Vol. 41, No. 3, 1996, pp. 935-939. doi:10.1016/0031-9422(95)00671-0

[19] S. Hogan, A. Fleury, P. Hadvary, H. Lengsfeld, M. K. Meier, J. Triscari and A. C. Sullivan, "Studies on the Antiobesity Activity of Tetrahydrolipstatin, a Potent and Selective Inhibitor of Pancreatic Lipase," *International Journal of Obesity*, Vol. 11, No. 3, 1987, pp. 35-42.

[20] W. Samee, M. Engkalohakul, N. Nebbua, P. Direkrojanavuti, C. Sornchaithawatwong and N. Kamkaen, "Correlation Analysis between Total Acid, Total Phenolic and Ascorbic Acid Contents in Fruit Extracts and Their Antioxidant Activities," *Thai Pharmaceutical and Health Science Journal*, Vol. 1, No. 3, 2006, pp. 196-203.

[21] C. Junmatong, J. Uthaibutra and K. Saengnil, "Antioxidant Activity and Total Phenolic Content in Skin and Pulp of Eggplant Fruits," *Agricultural Science Journal*, Vol. 39, No. 3, 2008, pp. 384-387.

[22] P. Wetwitayaklung and T. Phaechamud, "Antioxidant Activities and Phenolic Content of Solamun and Capsicum sp.," *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, Vol. 2, No. 2, 2011, pp. 146-154.

[23] R. Sarnthima and S. Khammuang, "Antibacterial Activities of *Solanum stramonifolium* Seed Extract," *International Journal of Agriculture and Biology*, Vol. 14, No. 1, 2012, pp. 111-115.

[24] A. Sakunpak and P. Panichayupakaranant, "Antibacterial Activity of Thai Edible Plants against Gastrointestinal Pathogenic Bacteria and Isolation of a New Broad Spectrum Antibacterial Polyisoprenylated Benzophenone, Chamuangone," *Food Chemistry*, Vol. 130, No. 4, 2012, pp. 826-831. doi:10.1016/j.foodchem.2011.07.088

[25] I. Z. Saiyed and D. D. Kanga, "Fruits of *Solanum xanthocarpum*," *Proceedings of the Indian Academy of Sciences*, Vol. 4, No. 3, 1936, pp. 255-260.

[26] O. M. Singh, K. Subharani, N. I. Singh, N. B. Devi and L. Nevidita, "Isolation of Steroidal Glycosides from *Solanum xanthocarpum* and Studies on Their Antifungal Activities," *Natural Product Research*, Vol. 21, No. 7, 2007, pp. 585-590. doi:10.1080/14786410701369458

