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PREFACE

This book constitutes the refereed proceedings of the International Conference of Natural Products 2014, held in conjunction with INCP2014, in Putrajaya, Malaysia, in March 2014. ICNP2014 received 258 participants from 7 countries in all continents. From these, after a blind review process, 60 were presented orally, and 190 were presented as poster. The 36 full papers presented here were carefully reviewed and published based on the classifications provided by the Program Committee. This conference provides a platform for natural products researchers to interact and form collaborations with researchers in other fields of drug discovery and herbal medicine development. The theme of this conference `Moving Translational Research in Natural Products Forwards' signifies the importance of effective collaboration between researchers of various disciplines to bring knowledge developed and insight gained in the laboratories to the clinics and to the market.

Head of Editor,
Jamia Azdina Jamal
Proceedings of the 30th Annual Seminar of the Malaysian Natural Products Soceity
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Pancreatic Lipase Inhibitory Potential of Selected Malaysian Plants

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ABSTRACT
Pancreatic lipase inhibitors from natural sources are considered as potential tool for the treatment of obesity. The inhibitory activity of 24 crude extracts of selected Malaysian plants against porcine pancreatic lipase (PPL) was successfully screened and tested by using in vitro approach. Orthosiphon stamineus, Phyllanthus niruri, Murraya paniculata and Averrhoa bilimbi leaves extracts showed more than 70% inhibitions when incubated with PPL at a final concentration of 500 µg/mL for 15 minutes at 37°C. P. niruri crude extract exhibited strongest lipase inhibitory activity, with an IC_{50} value of 27. 65 µg/mL followed by O. stamineus, M. paniculata and A. bilimbi with IC_{50} values of 34.74, 41.45 and 55.18 µg/mL, respectively. Inhibition mode study disclosed that O. stamineus and A. bilimbi could act as uncompetitive inhibitor while P. niruri and M. paniculata could act as noncompetitive inhibitor. The results suggested that these four plant extracts may be useful for obesity treatment.

Keywords: Pancreatic lipase inhibitor, obesity.

INTRODUCTION

Natural products provide a vast collection of pancreatic lipase inhibitors with potential for being developed into clinical products to treat obesity. Birari and Bhutani (2007), reviewed a variety of plant extracts and secondary metabolites that have inhibitory activity against pancreatic lipase. The main phytochemicals contributing towards pancreatic lipase inhibition consist of polyphenols, flavonoids, saponins and caffeine (Kim and Kang, 2005; Han *et al*., 2006; Moreno *et al*., 2006; Shimoda *et al*., 2006). Presently, studying on lipase inhibitors is extensively conducted worldwide particularly in Asia. Lipase inhibitors have been detected in different plant species, including *Nageia wallichiana* (Sirinamarattana *et al*., 2010), *Eisenia bicyclis* (Eom *et al*., 2013) and *Ligustrum purpurascens* (Wu *et al*., 2014) and shown their inhibitory ability without having detailed mechanistic details. Therefore, the aim of this study is to screen a group of plants grown in Malaysia for pancreatic lipase inhibition activity.

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACTS

Twenty-four selected plant materials were collected from University Agriculture Park, Universiti Putra Malaysia, Serdang. The whole plant materials were air-dried and ground into fine powder. Hundred g of the powdered materials was extracted by maceration in 1 L of 80 % methanol for 3 days at ambient temperature (25 – 30 °C). The extracts were concentrated in a rotary vacuum evaporator.

LIPASE INHIBITION ASSAY

The inhibition towards porcine pancreatic lipase was performed according to the modified method by Lewis and Liu (2012). The lipase activity was quantified by calculating the conversion of *p*-nitrophenyl butyrate to *p*-nitrophenol at 405 nm using UV-transparent 96-well plates on a microplate reader (BIO-TEK, Synergy HT, U.S.A.). Lipase assays were performed by incubating the plant extracts (final concentration 500 µg/ml) with PPL and pNPB in reaction buffer (50 mM potassium phosphate buffer, pH 7.2, 0.5 % Triton X-100) for 15 min. pNPB was first solubilised with dimethylsulfoxide (DMSO), then diluted with the reaction buffer to a final concentration of 2.5 mM in a 100 µl reaction. All assays were run at 37 °C and the results are the average of three replicates that were blank substracted. Orlistat was used as a positive control. DMSO was used as a negative control and the activity was also examined with and without the inhibitor. Inhibition of the lipase activity is defined as the decrease in percentage

of activity when PPL is incubated with the crude extracts. Lipase inhibition (%) is determined by using the formula:

$$\text{Inhibitory activity (I \%)} = 100 - [(B - b)/(A - a) \times 100]$$

where, A = the activity without inhibitor, a = the negative control without inhibitor, B = the activity with inhibitor and b = the negative control with inhibitor. The concentrations of extracts giving 50 % lipase inhibition (IC_{50}) were calculated from the least squares regression line of the semi-logarithmic plot against percentage inhibition curves using the GraphPad Prism software (GraphPad Software Inc., San Diego, USA).

MODE OF INHIBITION TEST

Crude extracts exhibiting more than 70 % inhibition towards pancreatic lipase were subjected to kinetic study in order to determine the inhibition mode. The inhibition mode was determined by Hanes-Woolf plot analysis resulted from the enzyme assay data containing increasing concentrations of pNPB (0.25, 0.5, 1.0, 2.0, 4.0 and 6.0 mM) with the absence and presence of difference concentration of extracts (10 and 50 µg/ml). All samples were analyzed in triplicate. Hanes-Woolf plots were constructed by plotting the ratio of the substrate concentration to the reaction velocity ([S]/v) against the substrate concentration [S]. Hanes-Woolf plots and nonlinear regression analysis for determination of Michaelis–Menten constant, K_m and maximal velocity, V_{max} were done using GraphPad Prism 4.0 software.

RESULTS AND DISCUSSION

LIPASE INHIBITION

Preliminary lipase inhibitory assay screening detected 4 extracts exhibiting high (> 70 %) inhibition while 9 extracts with medium (30 – 70 %) inhibition and the remaining 11 plant extracts showed either low (< 30 %) or no inhibition when incubated with PPL at a final concentration of 500 µg/ml for 15 min at 37 ^{0}C (Table 1). The findings from IC_{50} values showed that *P. niruri* extract was the most potent pancreatic lipase inhibitor followed by *O. stamineus*, *M. paniculata* and *A. bilimbi* with the IC_{50} value of 27.7 < 34.7 < 41.5 < 55.2 µg/ml, respectively. However, all extracts were less effective than orlistat (control) in inhibiting pancreatic lipase.

INHIBITION MODE

The mode of inhibition of four most active plant extracts was visualized using Hanes-Woolf plot; [S]/v versus [S] as shown in Figure 1. In Hanes-Woolf plot, the intercept on the [S]/v axis (*y*-axis) gives K_m/V_{max}. Thus, when [S]/v = 0, the intercept on the [S] axis (*x*-axis) gives $-K_m$. The result disclosed that *P. niruri* and *M. paniculata* extracts showed a noncompetitive inhibitory effect on pancreatic lipase. *O. stamineus* and *A. bilimbi* showed uncompetitive inhibition towards pancreatic lipase activity. Theoretically, noncompetitive inhibitors bind to a site other than the active site. The binding of the extracts has no effect on the substrate binding, and vice versa, thus did not change the K_m. Uncompetitive inhibitors only bind to the enzyme-substrate complex, but not to free enzyme. As a result, uncompetitive inhibitors decrease both V_{max} and K_m to the same extent.

TABLE 1: PPL inhibitory activity of 24 metabolic plant extracts (80%).

Scientific name	Family	Common name	Part used	Inhibition (%)[a]	IC_{50} (µg/ml)[b]
Andrographis paniculata	Acanthaceae	King of bitter/ creat	Leaf	n	nd
Gynura procumbens	Asteraceae	Mollucan spinach	Leaf	32.5 + 3.2	nd
Gynura bicolour	Asteraceae	Mollucan spinach (purple)	Leaf	14.6 + 1.9	nd
Carica papaya	Caricaceae	Papaya	Leaf	20.5 + 5.1	nd
Garcinia atroviridis	Clusiaceae	Asam gelugor / asam keping	Fruit	32.1 + 2.7	nd
Garcinia atroviridis	Clusiaceae	Asam gelugor	Leaf	34.6 + 1.0	nd
Momordica charantia	Cucurbitaceae	Bitter gourd	Fruit	41.2 + 4.4	nd
Tamarindus indica	Fabaceae	Tamarind	Leaf	28.1 + 2.5	nd
Orthosiphon stamineus Benth	Lamiaceae	Cat whisker	Leaf	85.3 + 3.1	34.7
Hibiscus sabdariffa	Malvaceae	Roselle	Fruit	29.6 + 0.2	nd
Syzygium polyanthum	Myrtaceae	Bay	Leaf	38.2 + 6.5	nd
Averrhoa bilimbi	Oxalidaceae	Cucumber tree/ tree sorrel	Leaf	73.9 + 2.0	55.2
Phyllanthus acidus	Phyllanthaceae	Malay gooseberry	Leaf	33.9 + 4.4	nd
Phyllanthus niruri	Phyllanthaceae	Dukung anak	Whole plant	76.7 + 0.4	27. 7
Piper betle L.	Piperaceae	Betel	Leaf	50.6 + 1.8	nd
Cymbopogon nardus	Poaceae	Lemongrass	Leaf	28.3 + 2.2	nd
Cymbopogon nardus	Poaceae	Lemongrass	Stem	16.6 + 0.8	nd
Morinda citrifolia	Rubiaceae	Noni	Leaf	37.0 + 1.4	nd
Murraya paniculata	Rutaceae	Orange Jessamine	Leaf	75.6 + 5.4	41.5
Phaleria macrocarpa	Thymelaeaceae	Mahkota dewa	Leaf	45.2 + 1.4	nd
Kaempferia galangal	Zingiberaceae	Fingeroot	Leaf	26.9 + 2.7	nd
Zingiber cassumunar	Zingiberaceae	Cassumunar ginger	Leaf	11.7 + 3.3	nd
Alpinia galanga	Zingiberaceae	Galanga root	Rhizome	11.4 + 0.7	nd
Curcuma aeruginosa Roxb.	Zingiberaceae	Temu itam/ireng	Rhizome	29.6 + 0.2	nd
Orlistat (Control)				99.6 + 0.3[c]	1.7

[a] Percentage of inhibition represents the ability to extract to inhibit pancreatic lipase activity in the medium; concentration tested was 500 µg/ml in the assay except for [c]. Determined based on the average of three independent replications.

[b] IC_{50}, concentration causing 50% inhibition; concentration tested were varied from 10^{-2} to 10^{3} µg/ml in the assay.

[c] Concentration tested was 10 µg/ml in the assay.

n = no inhibition

nd = not determined

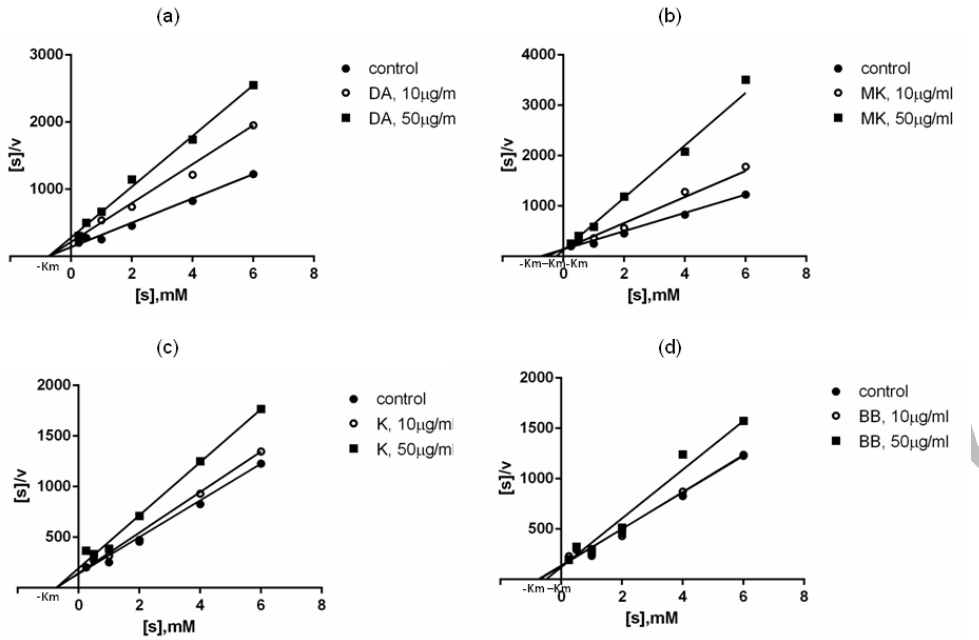

FIGURE 1: Hanes-Woolf plot; [S]/v versus [S] of kinetic analysis for PPL at 2 different concentrations of (a) *P. niruri* extract (Abbreviated as DA = dukung anak), (b) *O. stamineus* extract (Abbreviated as MK = misai kucing), (c) *M. paniculata* extract (Abbreviated as K = kemuning and (d) *A. bilimbi* extract (Abbreviated as BB = belimbing buluh).

CONCLUSIONS

The results of this study outlined a good preliminary basis for the screening of local plant to find new lipase inhibitory compound. Further study on the isolation and identification of these potential plants (*P. niruri*, *O. stamineus*, *M. paniculata* and *A. bilimbi*) bioactive constituents warranted and is currently underway.

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