ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 2, 2014

ISSN - 0974-2441

Research Article

IN VITRO LIPASE INHIBITORY EFFECT OF THIRTY TWO SELECTED PLANTS IN MALAYSIA

SIEW-LING ONG[1], SUGUNA PANEERCHELVAN[1], HOW-YEE LAI[1]*, NALAMOLU KOTESWARA RAO[2]

[1]School of Biosciences, Taylor's University, No 1 Jalan Taylor's, 47500 Subang Jaya, Malaysia.[2]School of Medicine, Taylor's University, No 1 Jalan Taylor's, 47500 Subang Jaya, Malaysia. Email: howyee.lai@taylors.edu.my

Received: 22 March 2014, Revised and Accepted: 10 May 2014

ABSTRACT

Objective: The objective of the study was to assess the lipase inhibitory activities of methanolic extracts from thirty two selected medicinal plants in Malaysia, for potential use in the treatment of obesity.

Methods: Methanolic extracts of these plants were evaluated for lipase inhibitory activity using porcine pancreatic lipase (PPL: triacylglycerol lipase, EC 3.1.1.3) and *p*-nitrophenyl butyrate in an *in vitro* assay. Standard phytochemical tests for alkaloids, tannins, saponins, glycosides, flavonoids and terpenoids were conducted.

Results: Among the thirty two local plant species examined, four plants exhibited inhibition activities of more than 15%. *Eleusine indica* showed the highest pancreatic lipase inhibitory activity of 31.36%, followed by *Myristica fragrans* (20.23%), *Melastoma candidum* (19.67%)and *Phyla nodiflora* (18.26%), respectively. There was no significant difference between activity produced by *Eleusine indica* methanolic extract and the standard drug orlistat.

Conclusion: Crude methanolic extracts of *Eleusine indica, Myristica fragrans, Melastoma candidum* and *Phyla nodiflora* are potential candidates as pancreatic lipase inhibitory agents.

Keywords: *Eleusine indica,* pancreatic lipase inhibitory activity, *Melastoma candidum*, *Myristica fragrans*, *Phyla nodiflora*, *p*-nitrophenyl butyrate

INTRODUCTION

Obesity is the leading cause of major diseases such as hypertension, Type 2 diabetes, coronary heart disease and ischemic stroke [1]. It is also known to increase the risk of cancer of the breast, colon, prostate, endometrium, kidney and gall bladder. According to World Health Organization (WHO), approximately 2.8 million people die each year and 35.8 million global disability-adjusted life years (DALYs) are due to overweight and obesity [2].

Despite the significant advances in understanding obesity and the development of effective pharmacologic treatments, obesity is still quite a challenge for many health care systems especially in the developed countries [3]. Several approaches have been implied for the treatment of obesity targeting at specific mechanisms, which include lipase inhibition, suppressive effect on food intake, stimulatory effects on energy expenditure, inhibition of adipocyte differentiation and the regulatory effect on lipid metabolism [4]. Amongst the approaches available, inhibition of pancreatic lipase which will retard the absorption of fatty acid, is the most widely studied mechanisms for the evaluation of natural products as anti-obesity agents [4, 5].

The field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in treatment of diseases is increasing worldwide. Natural product (nutraceutical) interventions are currently being investigated on a large-scale basis as potential treatments for obesity and weight management [6]. At present, the potential use of natural products for the treatment of obesity is still largely unexplored and it might be an excellent alternative strategy for the development of safe and effective anti-obesity drugs [7].

Malaysia is home to a diverse range of natural herbs which are potentially rich in bioactive compounds that can be developed for pharmaceuticals and nutraceutical purposes. There are 12,000 flowering plant species in Malaysia of which 1,300 species were said to be medicinal but only about one hundred species have been systematically evaluated for medicinal properties [8]. Ethnobotanical studies on various communities in Malaysia have revealed the use of various plants as remedies to cure many ailmentsand complaints [9, 10]. The aim of this research study was to screen for potential lipase inhibitory properties from selected medicinal plants found in Malaysia.

MATERIALS AND METHODS

Plant Materials and Chemicals

The plant materials selected for the research study were based on ethnopharmacological uses of local edible plants. The list of plants selected for the study is given in Table 1. Orlistat, *p*-nitrophenyl butyrate (PNPB) and porcine pancreatic lipase (PPL Type II, 3.1.1.3.) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents are of analytical grade.

Plant samples were collected from a few locations: Putrajaya Botanical Garden, Perak Herb Garden and local plantations in Malaysia.

Extraction and Preparation of Crude Extracts

The plant material was cleaned from residual soil then freeze-dried. Methanol was added and the mixture was sonicated intermitently. The extracts were then filtered under reduced pressure. The residue was repeatedly extracted until the filtrate was light in colour. The filtrates were pooled and solvent was evaporated off. The extract was then again freeze-dried and kept at -20°C until further use. The percentage yield of the extract from fresh weight were recorded. The crude extract was dissolved in DMSO at a final concentration which will not affect enzyme activity within the total volume (2% v/v).

Phytochemical Analysis of Methanol Extract

The extracts obtained were subjected to standard tests for detection of alkaloids (Dragendorff's reagent) [43], tannins, saponins (frothing test) [44], glycosides (Molisch test) [45], flavonoids (Shinoda test) [44] and terpenoids (Salkowski test) [46].

Table 1: Ethnomedicinal uses and common names of the medicinal plants used in study

No.	Scientific name	Common Name	Ethnomedicinal Usage	Ref.
1	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	King of bitter; 'Hempedu bumi'	Relief heat, detoxification	[11]
2	*Azadirachta indica*A.Juss.	Neem	Hot water extract taken for fever, diabetes, tonic, refrigerant and anthelmintic	[12]
3	*Blechnum orientale* L.	Centipede fern, 'paku ikan'	Boils, blisters, abscesses and sores; diaphoretic for stomach and urinary bladder complains	[13]
4	*Cassia alata* L.	Candle brush	Skin infections, constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes	[14]
5	*Clinacanthus nutans* L.	Sabah snake grass, 'belalai gajah'	Inflammation, viral infection, diabetes, fever and diarrhoea	[15]
6	*Coffea spp.*	Coffee plant	Stimulant, nervine and diuretic	[16]
7	*Dicranopteris linearis* (Burm.f.) Underw.	Climbing fern, false staghorn	Indigestion, asthma and women sterility	[17]
8	*Elettariopsis triloba* (Gagnep.) Loes.	'Serai acheh'	Expel wind from body, facilitate digestion, reduce cholesterol, induce sweating, treatment for yellow fever and bone ache	[18]
9	*Eleusine indica* (L.) Gaertn.	Goosegrass	Urinary complaints and fracture of bones	[19]
10	*Hibiscus mutabilis* L.	Cotton rose	Menorrhagia, anodyne, antidote, demulcent, expectorant and refrigerant	[20]
11	*Hibiscus sabdariffa* L.	Roselle	Antiseptic, aphrodisiac, astringent, cholagogue, demulcent, diuretic, purgative, refrigerant, stomachic and tonic	[21]
12	*Melastoma candidum* D. Don. Varalbiflorum	Forest starr, Kim starr	Inflammation, toothache, wounds, diarrhoea, scar prevention and post-partum recovery	[22]
13	*Melilotus albus* Medik.	Honey clover, tree clover	Anaemia, sedative, constipantand anti-rheumatic	[23]
14	*Morus alba* L.	Mulberry	Inflammation, jaundice, hepatitis, diabetes, fever, headache, hypertension, dyslipidemia, anthelminthic and wound healing properties	[24]
15	*Murraya koenigii* (Linn.) Spreng	Curry plant	Antiemetic, anti-diarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic and flavouring agent in culinary cooking	[25]
16	*Myristica fragrans* Houtt.	Nutmeg	Diarrhoea, mouth sores, insomnia, stomachic, stimulant and flatulence; Essential oil used externally for rheumatism	[26]
17	*Pandanus amaryllifolius* Roxb.	Pandan leaves	Fever, headache, sore throat and toothache	[27]
18	*Passiflora edulis* Sims	Passion fruit	Anxiety, nervousness and regulation of cholesterol level	[28]
19	*Peperomia pellucida* Kunth	Soft bone leaf, shiny bush	Abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain	[29]
20	*Phyla nodiflora* (L.) Greene	Frog fruit, capeweed	Fever, pains, cough and joint	[30]

			pain	
21	*Phyllanthus watsonii* A. Shaw	Airy shaw	Diabetes, anaemia, bronchitis and hepatitis	[31]
22	*Piper sarmentosum* Roxb.	'Kadok'	Treat bone fracture; water decoction of the roots to relieve diabetes mellitus, dysmenorrhoea and urinary symptoms	[32]
23	*Plectranthus amboinicus* (Lour.) Spreng.	Mexican mint	Skin ulceration, scorpion bite, skin allergy, wounds, diarrhoea and fever	[33]
24	*Polygonum chinense* L.	Chinese knotweed	Gastritis, dysentery, promoting blood circulation, diuretic, dyspepsia and haemorrhage	[34]
25	*Rhinacanthus nasutus* (L.) Kurz.	Snake jasmine	Anti-pyretic and cough	[35]
26	*Sambucus javanica* Reinw. *ex*Blume	Chinese elder	Diuretic, purgative and depurative	[36]
27	*Selaginella involvens*(Sw.) Spring	Spikemoss	Infections and time immemorial	[37]
28	*Stachytarpheta australis* Moldenke.	White porterweed	Purgative, vermifuge, expectorant, diuretic, emmenagogue and general tonic	[38]
29	*Syzygium aromaticum* (L.) Merrill & Perry	Clove	Hypertension, nausea, diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment and improvement in digestion	[39]
30	*Vernonia amygdalina* Delile	Bitter leaf	High blood pressure, diabetes, malaria, fever and cough	[40]
31	*Vitex negundo* L.	Five-leafed chaste tree	Astringent, cephalic, stomachic, alterant, thermogenic, depurative, antipyretic and bronchitis	[41]
32	*Youngia japonica* L. (DC)	Oriental Hawksbeard	Painful urination, swelling, pain, heat, diarrhoea, detoxification	[42]

Pancreatic Lipase (PL) Inhibition Assay

Pancreatic lipase (PL) inhibitory activity was measured using the substrate *p*-nitrophenyl butyrate (PNPB), as described by Bustanji *et al.* (2011) with slight modification [47]. The enzyme solutions were prepared immediately before use by suspending crude porcine PL type II (Sigma, EC 3.1.1.3) in Tris-HCl buffer (50mMTris, 150 mM NaCl, 1 mM EDTA, 10 mM MOPS, pH 7.4) to give a concentration of 5 mg/mL (200 units/mL) and mixed using a stirrer for 15 min. The solution was then centrifuged at 1,500 rpm for 10 min and the clear supernatant was recovered. Plant extract with a concentration of 100 µg/mL were pre-incubated with 0.20 mL of PL solution for 5 min at 37°C before adding PNPB substrate (10 mM in acetonitrile). The volume was diluted to 1mL using the Tris-HCl buffer and absorbance was read at 410 nm against blank using denatured enzyme. The denatured enzyme was prepared by boiling the enzyme solution for 5 min. The PL activity was related to the rate of *p*-nitrophenol release, which can be estimated from the slope of the linear segment of absorbance versus time profiles. Extracts were dissolved in DMSO at the final concentration not exceeding 1%, so as not to affect the enzyme activity. The percentage of residual activity of PL was determined for each compound by comparing the lipase activity of PL with and without the compounds. Orlistat, a known inhibitor of PL, was used as a positive control in the assay mixture.

The activities of the negative control were checked with and without the inhibitor. The inhibitory activity (I) was calculated as accordance to the formula below:

$$I\% = \left(1 - \frac{B-b}{A-a}\right) \times 100 \quad [48]$$

Where A is the activity of the enzyme without inhibitor, a is the negative control without the inhibitor, B is the activity of the enzyme with inhibitor, b is the negative control with inhibitor.

Statistical Analysis

All results were expressed as mean ± standard deviation (n=3). Significance of difference from the control was determined by Post-hoc Tukey's test (one way ANOVA) and independent T-test with a p value < 0.05 using SPSS software (version 16.0).

RESULTS AND DISCUSSION

Methanolic extracts of selected plants were prepared and investigated at a concentration of 100 µg/mL using PL inhibition assay. The inhibitory activities are shown in Table 2 and extracts were sorted according to their percentage of inhibition. Among the forty four plant extracts examined, twenty seven crude extracts from different plant species showed inhibitory activity against porcine pancreatic lipase *in vitro*. Five of these extracts showed significant ($p<0.05$) pancreatic lipase inhibitory activity of more than 15%.

Highest inhibitory activity was shown by *Eleusine indica* i.e. 31.36 ± 0.58%, which was comparable ($p<0.05$) to that of the reference orlistat (34.49 ± 5.39%), against pancreatic lipase (PL). Orlistat was used as the positive control as it is the only pancreatic-lipase inhibitor approved by US Food and Drug Administration and is currently used clinically to treat obesity. As a lipase inhibitor, orlistat acts by reducing absorption of fat. Nevertheless, orlistat is associated with several serious side effects which include oily stool, flatulence and abdominal cramping [49], which has prompted the search for other lipase inhibitors. In this study, the percentage of inhibition shown by orlistat was found to be 34.5 ± 5.4% which was slightly lower than that reported by Roh & Jung (2012) i.e. 42% [48].

Other plants with moderate activity (10-20%) were *Myristica fragrans* (mace, 18-20%), *Melastoma candidum* 20%, *Phyla nodiflora* 18%, *Dicranopteris linearis* 14% and *Myristica fragrans* (pericarp, 11%). There were nineteen crude extracts that showed weak

inhibitory activity (<10%) against PL. On the other hand, seventeen extracts promoted the activity of PL, such as *Vernonia amygdalina*, had increased the enzyme activity by 32.3 ± 5.2 %.

A study by Roh and Jung (2012) [49] on forty four plant extracts at concentration of 0.1 mg/mL, reported a range of 1.8-38.0 % inhibition compared to the positive control, orlistat of 42.0% inhibition; which was similar to the range of inhibition in our study, 0.3-31.4%, and orlistat of 34.5%. Other studies on a few plant species have indicated inhibitory effects at various concentration of extracts (0.1-5.0 mg/mL) e.g. as those shown by *Ilex paraguariensis* at 3 mg/mL [50], and of thirty seven traditional Chinese medicinal herbs at 0.2 mg/mL [51]. There was also a wide variation in the lipase inhibition at various concentrations of the extracts tested, for example, at extract concentration of 200µg/mL, Zheng *et al.* (2010) reported the inhibition exhibited by 37 traditional Chinese herbs ranged from -11.0 to 74.7% compared to the positive control, orlistat of 93.5% inhibition [51].

Qualitative phytochemical analysis was done on the four extracts that showed highest PL inhibition. Table 3 shows the presence of alkaloids, flavonoids, tannins, saponins and glycosides in these active extracts. The phytoconstituents present in the plant extracts could be responsible for its PL inhibition activity although their exact mode of action is still unclear. Previously, several potential pancreatic lipase inhibitory extracts from plants have been reported to contain saponins, polyphenols and terpenes [52].

Table 3: Phytochemical evaluation of four selected plant extracts

Plant	Alkaloid	Flavonoid	Glycoside	Saponin	Tannin	Terpenoid
E. indica	-	-	+	-	-	-
M. fragrans (mace)	-	-	+	-	+	+
M. candidum	-	-	+	-	+	-
P. nodiflora	-	+	+	-	-	-

Note: (+) present; (-) absent

Table 2: Pancreatic lipase (PL) inhibitory activity of methanol extracts of sampled plants

No.	Scientific name	Plant Part	Family	Inhibition (%)
1	*Eleusine indica*	Aerial part	Poaceae	31.36 ± 0.58
2	*Myristica fragrans*	Ripe female mace	Myristicaceae	20.23 ± 0.25*
3	*Melastoma candidum*	Aerial part	Melatomataceae	19.67 ± 0.50*
4	*Phyla nodiflora*	Whole	Verbenaceae	18.26 ± 2.84*
5	*Myristica fragrans*	Ripe male mace	Myristicaceae	17.99 ± 0.74*
6	*Dicranopteris linearis*	Aerial part	Gleicheniaceae	14.34 ± 1.90*
7	*Myristica fragrans*	Ripe male pericarp	Myristicaceae	11.32 ± 0.30*
8	*Myristica fragrans*	Ripe female pericarp	Myristicaceae	10.33 ± 0.32*
9	*Peperomia pellucida*	Leaf	Piperaceae	8.62 ± 1.79*
10	*Coffea spp.*	Leaf	Rubiaceae	7.85 ± 1.39*
11	*Youngia japonica*	Whole	Asteraceae	6.90 ± 2.37*
12	*Myristica fragrans*	Green female mace	Myristicaceae	4.90 ± 0.34*
13	*Hibiscus sabdariffa*	Calyx	Malvaceae	4.23 ± 1.33*
14	*Polygala paniculata*	Whole	Polygalaceae	4.17 ± 1.37*
15	*Hibiscus sabdariffa*	Leaf	Malvaceae	3.71 ± 0.37*
16	*Hibiscus mutabilis*	Leaf	Malvaceae	2.85 ± 0.64*
17	*Myristica fragrans*	Female leaf	Myristicaceae	2.07 ± 1.95*
18	*Myristica fragrans*	Green female pericarp	Myristicaceae	2.02 ± 0.48*
19	*Sambucus javanica*	Leaf	Adoxaceae	1.89 ± 1.27*
20	*Elettariopsis triloba*	Leaf	Zingiberaceae	1.65 ± 0.77*
21	*Passiflora edulis*	Pulp & seed	Passifloraceae	1.63 ± 0.32*
23	*Blechnum orientale*	Leaf	Blechnaceae	1.26 ± 0.29*
24	*Morus alba*	Leaf	Moraceae	0.78 ± 1.38*
25	*Azadirachta indica*	Leaf	Meliaceae	0.51 ± 1.08*
26	*Myristica fragrans*	Mixed leaf	Myristicaceae	0.32 ± 0.50*
27	*Myristica fragrans*	Male leaf	Myristicaceae	0.26 ± 0.25*
28	*Passiflora edulis*	Leaf	Passifloraceae	-0.09 ± 0.44*
29	*Stachytarpheta australis*	Whole	Verbenaceae	-1.51 ± 0.36*
30	*Cassia alata*	Leaf	Fabaceae	-1.77 ± 0.39*
31	*Selaginella involvens*	Whole	Selaginellaceae	-1.97 ± 2.70*
32	*Murraya koenigii*	Leaf	Rutaceae	-4.58 ± 1.63*
33	*Rhinacanthus nasutus*	Leaf	Acanthaceae	-4.70 ± 1.09*
34	*Polygonum chinese*	Aerial part	Polygonaceae	-5.48 ± 0.68*
35	*Pandanus amaryllifolius*	Leaf	Pandanaceae	-5.70 ± 2.21*
36	*Phyllanthus watsonii*	Aerial part	Euphorbiaceae	-6.00 ± 0.99*
37	*Syzygium aromaticum*	Leaf	Myrtaceae	-6.25 ± 6.10*
38	*Plectranthus amboinicus*	Leaf	Lamiaceae	-6.30 ± 1.68*
39	*Piper sarmentosum*	Leaf	Piperaceae	-6.64 ± 4.41*
40	*Passiflora edulis*	Exocarp	Passifloraceae	-8.23 ± 0.30*
41	*Vitex negundo*	Leaf	Lamiaceae	-10.91 ± 1.78*
42	*Andrographis paniculata*	Leaf	Acanthaceae	-14.89 ± 5.01*
43	*Clinacanthus nutans*	Leaf	Acanthaceae	-22.56 ± 0.83*
44	*Vernonia amygdalina*	Leaf	Asteraceae	-32.27 ± 5.22*
45	Orlistat (positive control)			34.49 ± 5.39

*$p < 0.05$ compared with control orlistat, data presented as median ± standard deviation (n = 3), and "-" indicates a promotion of pancreatic lipase activity. The final concentration of the crude extracts was 100 µg/mL.

Obesity is the major cause of diseases like diabetes [53] and many others. Since orlistat is the only approved drug for as an effective pancreatic lipase inhibitor, extensive studies has been carried out to develop alternative drugs. Several studies done on different phytochemicals had given a brief insight of its mechanisms with its anti-obesity effect, such as extract of *Ziziphus mauritiana Lam* bark powder had shown significant inhibition of lipase activity *in vivo* in high fat diet induced obesity rats [54]. Another study conducted by Lee *et al*. (2013) [55] had proposed stilbene derivative from *Parthenocissus tricuspidata* showed strong inhibitory effect on adipocyte differentiation. Statistically significant results were obtained in this *in vitro* study which defines the possibility of potential pancreatic lipase inhibitors. Further studies are needed, via *in vivo* or cell culture model to further confirm the inhibitory activities of these plants and to elucidate the effective phytochemicals in these potential plants.

CONCLUSION

In this study, it was shown that the extracts of *Eleusine indica, Myristica fragrans, Melastoma candidum* and *Phyla nodiflora* were found to be possible candidates for further studies to isolate potential pancreatic lipase inhibitor among the thirty two different plants screened. According to our literature review, extracts of *Eleusine indica, Myristica fragrans, Melastoma candidum* and *Phyla nodiflora* have not been reported to have pancreatic lipase inhibition activities. Further studies are needed in order to isolate, identify and characterize phytoactive compounds to further define the nature of their lipid-lowering activity and verifying the potency of these via adipocytes or *in vivo* studies.

ACKNOWLEDGEMENT

We would like to acknowledge funding support from Taylor's University, Malaysia under the Taylor's Research Grant Scheme (No. TRGS/2/2011/SOBS/012).

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ABBREVIATIONS

PL- Pancreatic lipase; PNPB – p-nitrophenyl butyrate