Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v44i1.56879

CC BY

Medicinal plants in Sabah (North Borneo) exhibit anti-pancreatic lipase, anti-amylase, and antioxidant properties

See Choon How[1], Lucky Poh Wah Goh[1], Norhaniza Johansah[1], Azlinah Matawali[2], Jualang Azlan Gansau[1] and Siew-Eng How[1*]

[1]Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia. [2]Preparatory Centre for Science and Technology, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia. *Author for correspondence. E-mail: sehow@ums.edu.my

ABSTRACT. Medicinal plants have been widely used for their notable health benefits and help in disease prevention for generations. In recent years, obesity has become among the risk factors of hyperglycemia and oxidation stress. This study aims to investigate the potential of plants in Sabah, North Borneo to inhibit the key enzymes involved in obesity, hyperglycemia and oxidative stress. A total of 46 plant extracts were subjected to anti-pancreatic lipase, α-amylase inhibition and antioxidant assays. It was observed that S43 (*Lantana camara*) exhibited the greatest IC_{50} of anti-pancreatic lipase activity (mean of IC_{50} (±S.D.) = 0.20 mg mL^{-1} ± 0.010). *Cinnamomum sp*. (S42) has the most substantial α-amylase activity with a mean IC_{50} (±S.D.) = 2.68 mg mL^{-1} ± 0.471. S19 (*Glochidion rubrum*) was the most effective antioxidants (mean of IC_{50} (±S.D.) = 0.011 mg mL^{-1} ± 0.004) among all the investigated samples. Interestingly, three plant extracts were found (S6-*Buchanania sp*.; S22-*Vitex negundo* and S42-*Cinnamomum sp*.) to exhibit inhibition activity in anti-pancreatic lipase, α-amylase and antioxidant assays. The bioactivities of plant extracts have been closely related to the content of phytochemicals, as in earlier studies. Thus, plants have the potential to serve as supplements and nutraceuticals for obesity and other related complications.

Keywords: Alpha-amylase; antioxidant; anti-pancreatic lipase; obesity; hyperglycemia.

Received on November 29, 2020.
Accepted on February 16, 2021.

Introduction

Obesity is one of the most concerning metabolic syndromes, where the global prevalence has nearly tripled since 1975 (World Health Organization [WHO], 2020). It is alarming that an estimated of 38.2 million children under five years old are overweight or obese, with almost half of them living in Asia (WHO, 2020). Obesity arises from the imbalance of consumed calories and expended calories which creates excess body weight through fat absorption. The imbalance of calories was due to high-fat diet intake, low metabolic rate, the low energy cost of physical activity, low-fat oxidation capacity and increased lipase activity (Little, Horowitz, & Feinle-Bisset, 2007). Among the various lipases, the pancreatic lipase hydrolyses 50-70% of total dietary fats (Birari & Bhutani, 2007). Fat absorption is reduced due to the inhibition by pancreatic lipase, which reduces obesity (Ahn et al., 2012).

Obesity is associated with an increased risk of chronic conditions such as diabetes, a condition where the blood sugar level is beyond the normal range (Carnethon, Rasmussen-Torvik, & Palaniappan, 2014). It has been reported that 18-20% of diabetic patients are obese, highlighting the association between diabetes and obesity prevalence (Menke, Casagrande, Geiss, & Cowie, 2015). Diabetes is categorized into type 1 and type 2, characterized by chronic hyperglycemic due to an impaired insulin secretion or activity (American Diabetes Association [ADA], 2015). Furthermore, approximately 85.2% of type 2 diabetic patients are overweight or obese (Centers for Disease Control and Prevention [CDC], 2004). Elevated glucose levels can be lowered by delaying glucose absorption through the reduction of starch digestion rate (Gallaher & Schneeman, 1986). α-amylase is the main enzyme that catalyses the hydrolysis of polysaccharides such as starch into oligosaccharides in the intestine and further degraded to glucose and absorbed into the bloodstream. Potential inhibitors of α-amylase were shown to significantly reduce blood glucose levels, suggesting the importance of this enzyme in regulating blood glucose levels (Wang, Huang, Shao, Qian, & Xu, 2012).

In addition, obesity and diabetes have been linked to increased oxidative stress (Furukawa et al., 2004; Wright Jr., Scism-Bacon, & Glass, 2006). Oxidative stress is the imbalance of antioxidants and pro-oxidants

such as reactive oxygen species (ROS) caused by various factors such as disease implication, drug actions or aging (Sies, 1985). ROS is a highly reactive molecule that damages healthy cells, leading to further complications of diseases (Marseglia et al., 2014). The ability to neutralize ROS by antioxidants presents a promising solution in alleviating the negative impacts of ROS. For example, coenzyme Q10 was reported to improve blood glucose levels in diabetes patients (Hodgson, Watts, Playford, Burke, & Croft, 2002). Moreover, plants have been reported to contain high antioxidants level, thus making them a potential remedy for various diseases (Jamous, Abu-Zaitoun, Akkawi, & Ali-Shtayeh, 2018).

Sabah is located at the northern region of the Borneo archipelago. It has several unique landscapes, such as the Crocker Range which is the longest conservation areas spanning from Kudat to Sipitang and the Kinabatangan river (Suleiman, Masundang, & Akiyama, 2017). There are approximately 5,000 to 6,000 species of vascular plants, where roughly 2,500 species are trees. Sabah is home to a highly diverse flora; one of the richest in the world (Ministry of Energy and Natural Resources [NRE], 2006). Plants contain various phytochemicals such as flavonoids, alkaloids, anthocyanins that carry various beneficial properties such as reducing the risk of cancer, type 2 diabetes and prevent obesity (Alakolanga, Kumar, Jayasinghe, & Fujimoto, 2015; Velu, Palanichamy, & Rajan, 2018; Wu et al., 2013). Plants rich in antioxidants also reduce the free-radical formation and lowers oxidative stress (More & Makola, 2020). Hence, this study was conducted to investigate the anti-pancreatic lipase, anti-amylase and antioxidant properties in selected plants collected from Sabah.

Material and methods

Sample collection and processing

A total of 46 plant extracts from different species and parts of plants from Sabah (Table 1), such as leaf, stem, root, flower and fruit were collected around Kota Kinabalu, Mantanani Island and Kota Belud, Sabah and identified by a botanical expert through observation of several plant features such as shape, leaves, color, number of petals, presence of thorns or hair. The samples were collected at random hours from May to December 2010. Plant extracts were prepared by drying the sample and ground into powder. Then, a ratio of 1 part of the sample and 5.0 mL of 99.9% (v v^{-1}) methanol was mixed thoroughly and subjected to sonication for 30 mins. The mixture was left overnight and filtered before evaporated using a rotary evaporator at a temperature of 40°C. Then, the sample residue was re-extracted twice before the dried filtrate from three independent extractions was mixed and stored at 4°C.

Table 1. List of plant species collected and extracts demonstrated > 50% inhibition in assays tested.

No	Plant parts	Family	*Genus*	*Species*	> 50% inhibition		
					α-amylase	Anti-pancreatic lipase	Antioxidant
1	L	Apiaceae	*Anethum*	*A. graveolens*	√	√	
2	St	Apiaceae	*Anethum*	*A. graveolens*			
3	L	Passifloraceae	*Turnera*	*T. ulmifolia*		√	√
4	L	Ebenaceae	*Diospyros*	sp.			
5	St	Ebenaceae	*Diospyros*	sp.			√
6	L	Anacardiaceae	*Buchanania*	sp.	√	√	√
7	L	Flacourtiaceae	*Flacourtia*	sp.			√
8	L	Verbenaceae	*Premna*	sp.			√
9	L	Oleaceae	*Chionanthus*	*C. pluriflorus*			√
10	St	Oleaceae	*Chionanthus*	*C. pluriflorus*			√
11	L	Lauraceae	*Litsea*	sp.			√
12	L	Rhamnaceae	*Colubrina*	*C. asiatica*			
13	Fr	Rhamnaceae	*Colubrina*	*C. asiatica*			
14	L	Moraceae	*Ficus*	sp.			
15	L	Hernandiaceae	*Hernandia*	*H. peltata*			
16	L	Calophyllaceae	*Calophyllum*	*C. inophyllum*			√
17	L	Apocynaceae	*Cerbera*	*C. odollam*			√
18	L	Sapotaceae	*Pouteria*	*P. obavata*			
19	L	Euphorbiaceae	*Glochidion*	*G. rubrum*		√	√
20	L	Apocynaceae	*Kopsia*	sp.	√		
21	L	Apocynaceae	*Nerium*	*N. oleander*	√		√
22	L	Lamiaceae	*Vitex*	*V. negundo*	√	√	√

No	Plant parts	Family	*Genus*	*Species*	> 50% inhibition		
					α-amylase	Anti-pancreatic lipase	Antioxidant
23	L	Apocynaceae	*Kopsia*	sp.	√		
24	St	Apocynaceae	*Kopsia*	sp.			
25	L	Moraceae	*Ficus*	sp.			
26	L	Moraceae	*Ficus*	sp.			
27	L	Moraceae	*Ficus*	sp.			
28	L	Rubisceae	Unknown	Unknown			
29	L	Lecythidaceae	*Barringtonia*	*B. asiatica*			
30	L	Verbenaceae	*Premna*	*P. obtusifolia*			√
31	L	Piperaceae	*Piper*	sp.			
32	St	Piperaceae	*Piper*	sp.			
33	L	Fabaceae	*Desmodium*	*D. umbellatum*			
34	L	Gesneriaceae	*Cyrtandromoea*	*C. grandis*			√
35	L	Pedaliaceae	*Sesamum*	*S. indicum*			√
36	W	Gleicheniaceae	*Dicranopteris*	*D. linearis*			√
37	W	Nephrolepidaceae	*Nephrolepis*	*N. biserrata*			
38	W	Dryopteridaceae	*Dryopteris*	*D. filix-mas*		√	
39	W	Pteridaceae	*Acrostichum*	*A. aureum*			
40	L	Plantaginaceae	*Plantago*	*P. major*			
41	L	Acanthaceae	*Clinacanthus*	*C. nutans*			
42	B	Lauraceae	*Cinnamomum*	sp.	√	√	√
43	L	Verbenaceae	*Lantana*	*L. camara*		√	√
44	L	Zingiberaceae	*Alpinia*	*A. galanga*			
45	St	Zingiberaceae	*Alpinia*	*A. galanga*			
46	L	Asteraceae	*Eupatorium*	*E. odoratum*			√

Note: B – Bark parts, L – leaf parts, St – stem parts, W – whole parts. Extracts exhibit > 50% in all three tests were bolded.

Anti-pancreatic lipase assay

The pancreatic lipase activity was determined as described by Kim et al. (2007), with slight modifications. In brief, enzyme buffer was prepared by mixing 90 µL of porcine pancreatic lipase (2.5 mg mL^{-1} in 10 mM of MOPS and 1 mM of EDTA, pH 6.8) to 2535 µL of Tris buffer (100 mM of Tris-HCl and 5 mM of $CaCl_2$, pH 7.0). Next, extracts (0.5 mg mL^{-1}) were screened for percentage (%) of lipase inhibition activity as the following. Approximately, 300 µL of extracts at were dissolved in ethanol and mixed with 2625 µL of enzyme buffer and incubated at 37°C for 15 min. Then, 75 µL of substrate solution (10 mM of p-nitrophenyl butyrate in dimethyl formamide) was added, followed by incubation at 37°C for 30 min. After that, the lipase activity was measured at 405 nm using Multiskan GO Spectrophotometer (Thermo Scientific, USA). The results were expressed as percentage (%) of pancreatic lipase inhibition activity by 0.5 mg mL^{-1} of plant extract and the concentration needed to inhibit the enzymatic activity by half (IC_{50}). Orlinstat was used as a positive control. The plant extracts which exhibited more than 50% inhibition during the initial screening was subjected to the determination of IC_{50} value.

α-Amylase inhibition assay

The anti-amylase assay was performed as described previously with slight modifications (Wang et al., 2012). Firstly, 1% starch solution (w v^{-1}) was prepared by boiling and stirring 1 g of potato starch in 100 mL of sodium phosphate buffer for 30 mins. Then, α-amylase enzyme mixture (50 unit 1 mL^{-1}) was prepared in 20 mM sodium phosphate buffer and 0.0006 mM sodium chloride. A total of 40 mL indicator solution containing 96 mM of 3,5-dinitrosalicylic acid, 5.31 M of sodium potassium tartrate and 2 M of sodium hydroxide was prepared. Acarbose was used as a positive control.

The α-amylase inhibition activity was initially screened at a concentration of 10 mg mL^{-1} for all plant extracts and expressed as a percentage (%) of inhibition as the following. Plant extracts (250 µL) at concentration ranges of 0.625 mg mL^{-1} to 10.0 mg mL^{-1} were mixed with 250 µL of enzyme mixture and incubated at 25°C for 10 min., followed by the addition of 250 µL of starch solution and incubated at 25°C for 10 min. Then, 500 µL of indicator solution was added and incubated at 85°C for 5 min. After that, the solution was diluted with 5 mL distilled water, and the absorbance was measured at 540 nm using Multiskan GO Spectrophotometer (Thermo Scientific, USA). The α-amylase inhibition activity was expressed as a percentage (%) of α-amylase inhibition by 10 mg mL^{-1} plant extract and the amount of plant extract required to exhibit half inhibition activity (IC_{50}). The extracts that exhibited more than 50% of inhibition were subjected to IC_{50} value determination.

Antioxidants assay

The extract's antioxidant activity was determined using DPPH free radical scavenging test (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006). Firstly, 0.2 mM DPPH solution was prepared in 99% methanol. Several concentrations of extracts and positive control (butylated hydroxytoluene, BHT) were prepared. Initial screening of antioxidant activity was performed at 0.125 mg/mL for all plant extracts and expressed as a percentage (%) of inhibition activity. Approximately 100 µL of the sample was mixed with an equal volume of DPPH and incubated for 30 min. at room temperature. The test was performed in triplicates. Then, the negative control containing methanol and positive control containing butylated hydroxytoluene (BHT) were prepared. The absorbance reading at 517 nm of the mixture was recorded using Multiskan GO Spectrophotometer (Thermo Scientific, USA). The results were expressed as a percentage (%) of inhibition at 0.125 mg mL^{-1} of plant extract and the concentration of plant extract required to reduce the enzymatic assay by half (IC_{50}). The plant extracts which exhibited more than 50% antioxidant activity were subjected to IC_{50} value determination. IC_{50} values were illustrated as bar charts using mean (±S.D.)

Statistical analysis

One-way ANOVA and post hoc Tukey were performed to determine the significance inhibition between the samples and control in anti-pancreatic lipase, α-amylase and antioxidant assays on SPSS v27.0 software with statistical significant at *p-value* < 0.05..

Results and discussion

Anti-pancreatic lipase activity

The screening of anti-pancreatic lipase activity using a 0.5 mg mL^{-1} of all 46 plant extracts revealed that eight plant extracts demonstrated > 50% of inhibition activity (Table S1; Figure 1). The plant extract which shows the most dramatic mean of IC_{50} (in mg mL^{-1}) (±S.D.) of anti-pancreatic lipase activity were S43 (IC_{50} = 0.20 ± 0.010, 62%), followed by S19 (IC_{50} = 0.22 ± 0.038, 62%), S38 (IC_{50} = 0.24 ± 0.004, 65%), S42 (IC_{50} = 0.25 ± 0.017, 66%), S6 (IC_{50} = 0.26 ± 0.006, 71%), S3 (IC_{50} = 0.27 ± 0.006, 70%), S1 (IC_{50} = 0.32 ± 0.012, 59%), and S22 (IC_{50} = 0.45 ± 0.008, 52%) (Figure 1). Significant differences at *p-value* < 0.05 were observed in all the plants extracts when compared to the positive control (Figure 1). However, there were no significant statistical differences between the anti-pancreatic lipase activity of S43 when compared with S1, S3, S6, S19, S22, S38 and S42 with *p-value* > 0.05.

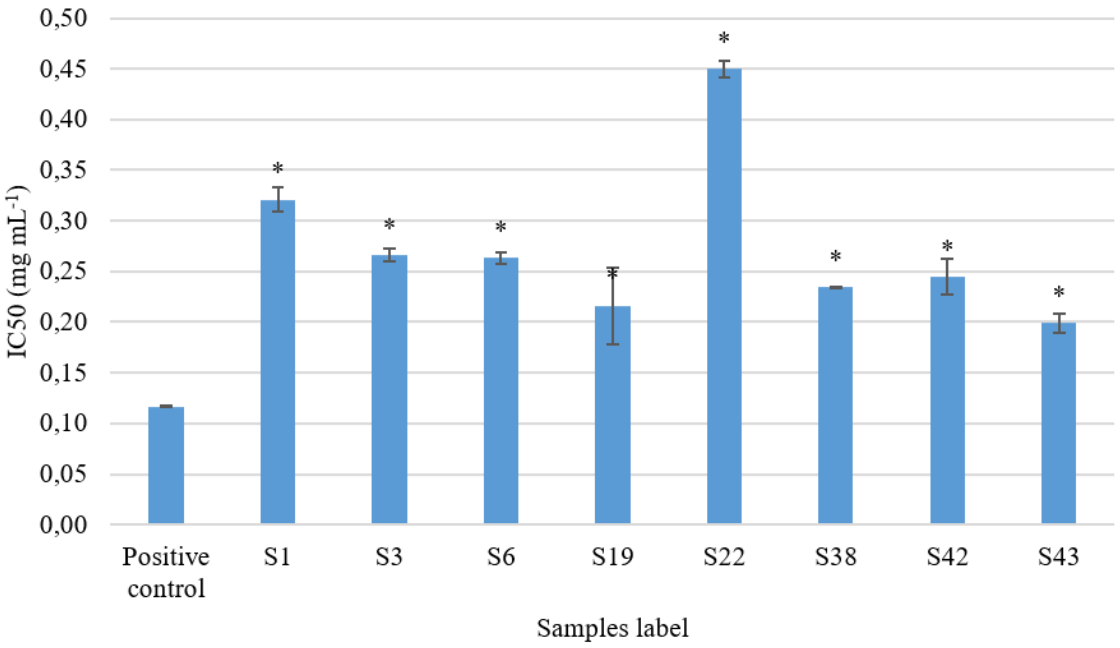

Figure 1. The mean IC_{50} (±S.D.) of eight plant extracts that exhibited > 50% inhibition and positive control in anti-pancreatic lipase activity. One-way ANOVA followed by post hoc Tukey statistical analysis were performed to compare the significant difference of IC_{50} (±S.D.) between plant extracts and positive control using SPSS Version 27 software. '*' indicated statistical significance at *p-value* < 0.05 when compared to the positive control.

S43 is the leaf of *Lantana camara* (*L. camara*), which exhibited the greatest IC_{50} of anti-pancreatic lipase activity, an important enzyme in regulating lipid levels in obesity. Extracts of *L. camara* have been shown to reduce fat cells in hyperlipidemia mice models (Gundamaraju, Mulaplli, & Ramesh, 2012). Phytochemical screening revealed that *L. camara* contains alkaloids, flavonoids, tannins and glycosides such as tricin, lantanilic acid, betulinic acid and a new flavone compound named gautin (Gundamaraju et al., 2012; Patil, Khare, Huang, & Lin, 2015). Therefore, the potential role of *L. camara* in lipid regulation was proven in the present study.

α-Amylase inhibition activity

The initial screening of α-amylase inhibition at 10 mg mL^{-1} of plant extracts showed that seven out of 46 plant extracts exhibited > 50% inhibition activities (Table S2; Figure 2). The mean of IC_{50} mg mL^{-1} (±S.D.) of seven plant extracts were determined (Figure 2). It was found that S42 (IC_{50} = 2.68 ± 0.471, 84%) has the most drastic α-amylase suppression followed by S1 (IC_{50} = 3.51 ± 0.260, 86%), S23 (IC_{50} = 3.60 ± 0.187, 84%), S6 (IC_{50} = 3.71 ± 0.275, 79%), S20 (IC_{50} = 4.86 ± 0.182, 76%), S22 (IC_{50} = 5.38 ± 0.135, 75%) and S21 (IC_{50} = 5.92 ± 0.196, 69%). Statistically significant differences were observed in S6, S20, S21, and S22 when compared with positive control at *p-value* < 0.05 (Figure 2). Further statistical analysis showed that the α-amylase inhibition activity of S43 were significantly different when compared with S20, S21 and S22 at *p-value* < 0.05. However, no significant difference were observed when compared with S1, S6 and S23 with *p-value* > 0.05.

Cinnamomum sp. (S42), commonly known as cinnamon, had the greatest IC_{50} suppression on α-amylase. This finding was in line with earlier studies that reported the α-amylase inhibition effect by several species (Hayward et al., 2019). Furthermore, the cinnamon extract has significantly reduced blood glucose in db/db mice (Kim, Hyun, & Choung, 2006). Moreover, a randomized, placebo-controlled clinical trial demonstrated that cinnamon consumption significantly reduced blood glucose level in diabetic patients, further supporting its benefit in regulation blood glucose level (Sahib, 2016). Therefore, it can be concluded that cinnamon reduces glucose levels through α-amylase in diabetic patients.

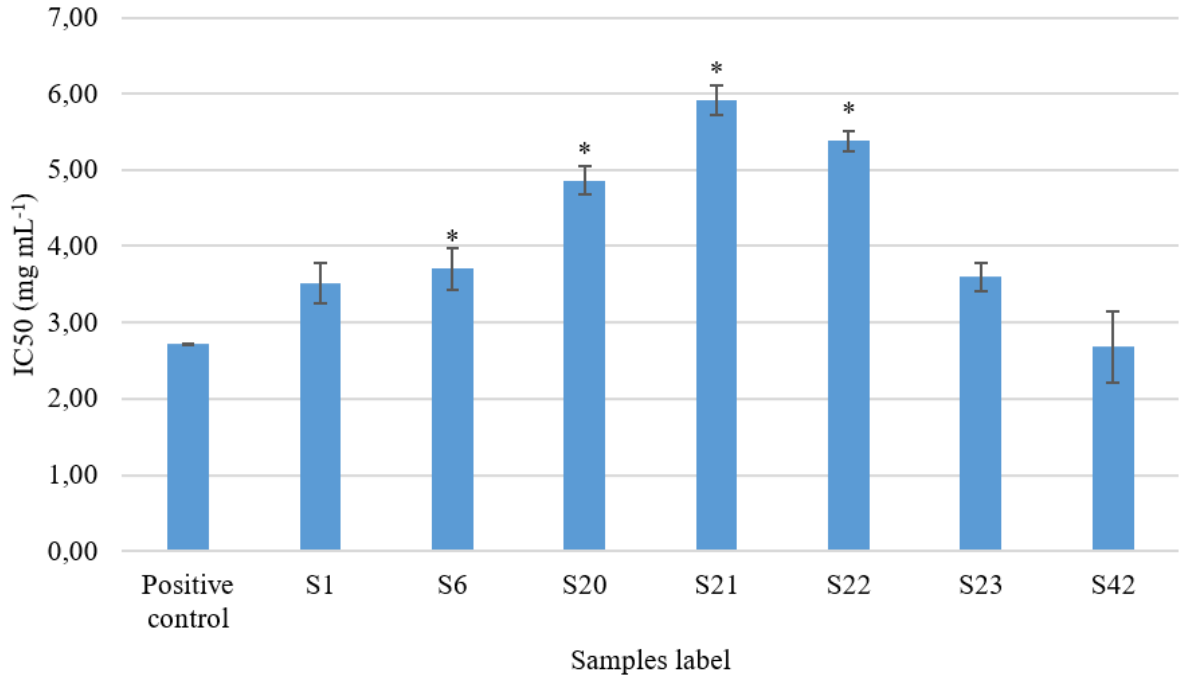

Figure 2. The mean IC_{50} (±S.D.) of seven plant extracts exhibited > 50% inhibition compared to positive control in α-amylase inhibition. activity. One-way ANOVA followed by post hoc Tukey statistical analysis were performed to compare the significant difference of IC_{50} (±S.D.) between plant extracts and positive control using SPSS Version 27 software. '*' indicated statistical significance at *p-value* < 0.05 when compared to the positive control.

Antioxidant activity

Preliminary screening of 46 plant extracts at 0.125 mg mL^{-1} demonstrated that 20 out of 46 plant extracts exhibited > 50% antioxidant activity (Table S3; Figure 3). The mean of IC_{50} (mg mL^{-1}) (±S.D.) of 20 potential plant extracts were determined and illustrated in Figure 3.

The most effective antioxidants were S19 (IC_{50} = 0.011 ± 0.004, 85%) followed by S3 (IC_{50} = 0.013 ± 0.00005, 92%), S6 (IC_{50} = 0.031 ± 0.001, 94%), S35 (IC_{50} = 0.037 ± 0.0007, 91%), S43 (IC_{50} = 0.005 ± 0.0008, 92%), S7 (IC_{50} = 0.053 ± 0.001, 91%), S36 (IC_{50} = 0.057 ± 0.001, 85%), S21 (IC_{50} = 0.060 ± 0.0004, 85%), S8 (IC_{50} = 0.065 ± 0.001, 83%), S34 (IC_{50} = 0.065 ± 0.0006, 81%), S9 (IC_{50} = 0.066 ± 0.0007, 82%), S16 (IC_{50} = 0.070 ± 0.001, 70%), S42 (IC_{50} = 0.073 ± 0.0005, 79%), S46 (IC_{50} = 0.073 ± 0.0006, 79%), S11 (IC_{50} = 0.078 ± 0.0014, 72%), S30 (IC_{50} = 0.083 ± 0.001, 69%), S10 (IC_{50} = 0.089 ± 0.001, 66%), S5 (IC_{50} = 0.096 ± 0.0008, 59%), S17 (IC_{50} = 0.109 ± 0.002, 55%), and S22 (IC_{50} = 0.111 ± 0.001, 55%). Statistical significance was observed between S3, S5, S6, S7, S8, S9, S10, S11, S16, S17, S22, S30, S34, S35, S42, S43, S46 and positive control at *p-value* < 0.05 (Figure 3). On the other hand, no significant difference was observed between S19, S21 and S36 when compared with positive control with *p-value* > 0.05. Subsequent analysis revealed S19 was statistically significant different (*p-value* < 0.05) with all plant extracts except S9, S21, S36 (*p-value* > 0.05).

S19 (*Glochidion rubrum*) was the most effective antioxidants among all the investigated samples. This finding was consistent a previous study that demonstrated the leaf extracts of *Glochidion sp.* exhibited antioxidant activity through several types of phenolic compounds such as gallic acid, methyl gallate, flavone-C-glycosides and isoorientin (Anantachoke, Kitphati, Mangmool, & Bunyapraphatsara, 2015). Furthermore, *Glochidion sp*. extract exhibited significant cytotoxic activity in the brine shrimp lethality bioassay, suggesting that the high antioxidant activity could lead to significant cytotoxic activity (Azam, Hasan, Uddin, Masud, & Hasan, 2012). Thus, the significant antioxidant activity of *G. rubrum* indicates its potential as an antioxidant that can regulate cell cytotoxicity.

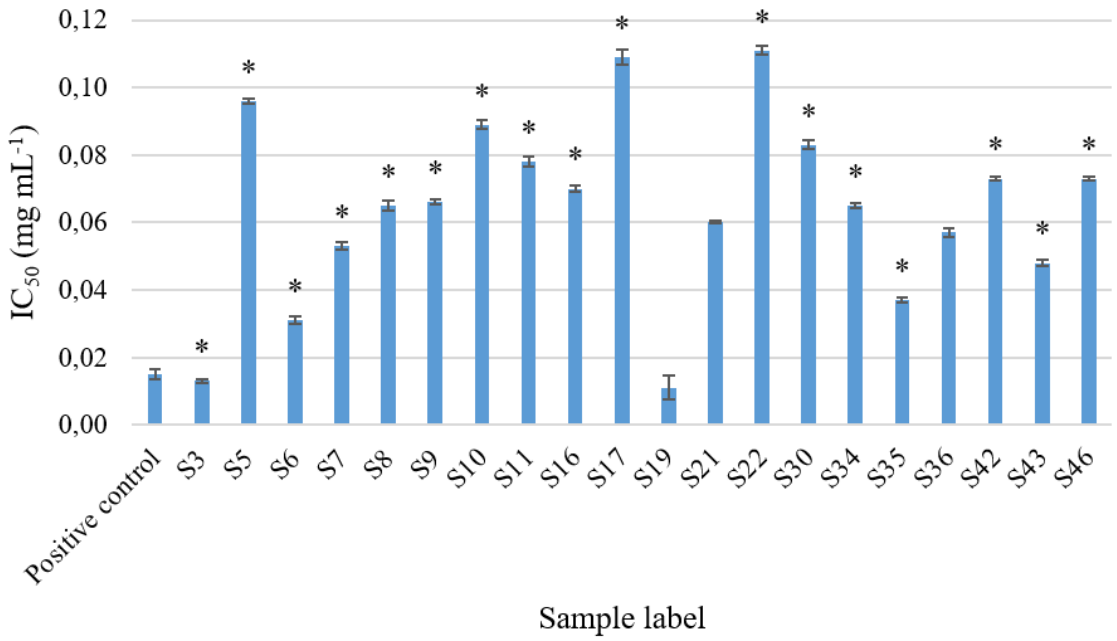

Figure 3. The mean IC_{50} (±S.D.) of 20 plant extracts exhibited > 50% inhibition compared to positive control in antioxidant activity. One-way ANOVA followed by post hoc Tukey statistical analysis was performed to compare the significant difference of IC_{50} (±S.D.) between plant extracts and positive control using SPSS Version 27 software. '*' indicated statistical significance at *p-value* < 0.05 when compared to the positive control.

Plants that show all inhibition activities

Among the 46-total extracts investigated, three (S6-*Buchanania sp*.; S22-*Vitex negundo*; S42-*Cinnamomum sp*.) exhibited inhibition activities in anti-pancreatic lipase, α-amylase and antioxidant assays (Figure 4). These plant extracts contains collective benefit towards curbing obesity, reducing glucose and oxidations that are harmful to human. In addition, reports have highlighted that these plant extracts contain various levels of phytochemical and secondary metabolites, contributing to their beneficial response (Siddiqui, Chowdhury, & Prasad, 2015). For example, S6-*Buchanania sp.* was reported to contain flavonoids, saponin, amino acid, and carbohydrates responsible for the antioxidant activities (Siddiqui et al., 2015). Similar phytochemicals were also found in S22-*Vitex negundo*, including flavonoids, lignans, terpenoids and steroids, which have promising bioactivities such as antioxidant, anti-hyperglycemic, antimicrobial, anti-inflammatory and anti-

tumour (Zheng et al., 2015). Furthermore, S42-*Cinnamomum sp.* contains various chemical constituents such as cinnamyl alcohol, anthocyanin, and coumarin, which exhibited antibacterial, anti-diabetic, antioxidant and anti-thrombotic activities (Al-Dhubiab et al., 2012). Thus, the plants studied in this research have great potential to act as an anti-glycemic, anti-obesity and antioxidant agents.

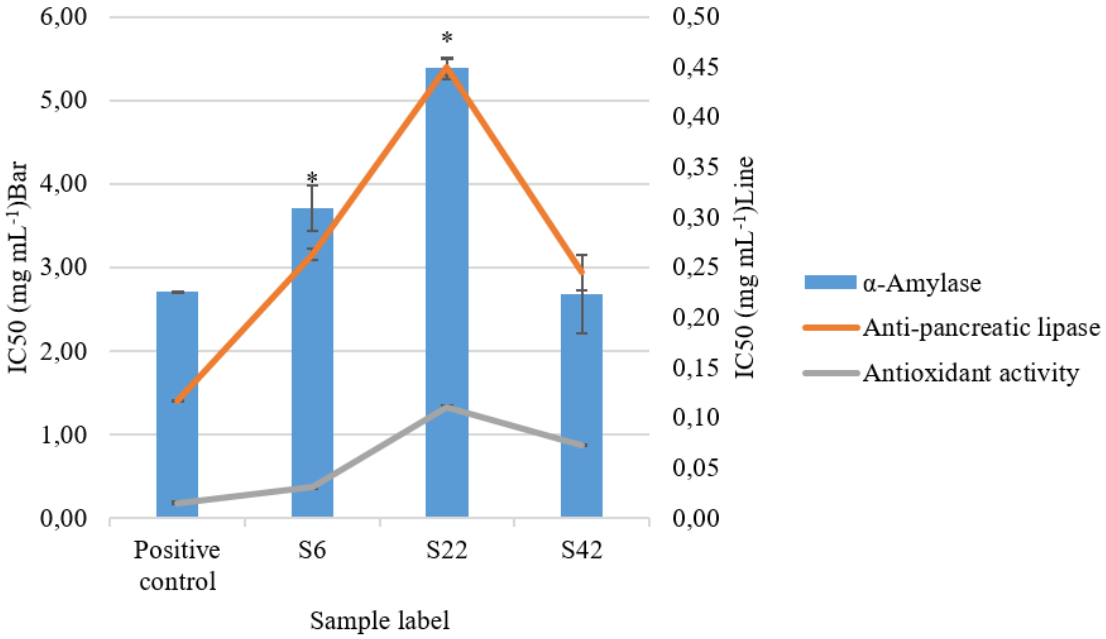

Figure 4. The plant extracts that showed significant IC_{50} activities in anti-pancreatic lipase, α-amylase, and antioxidant inhibition assays. One-way ANOVA followed by post hoc Tukey statistical analysis were performed to compare the significant differences of IC_{50} (±S.D.) between plant extracts and positive control using SPSS Version 27 software. '*' indicated statistical significance at *p-value* < 0.05 of samples in all three assays when compared to the positive control.

Conclusion

This study has demonstrated the potential of several plant extracts in inhibiting the key enzymes responsible for obesity, hyperglycemia and oxidation. Therefore, the consumptions of S6-*Buchanania sp*., S22-*Vitex negundo*, and S42-*Cinnamomum sp*. are promising in managing obesity and diabetic-related complications. The added value of these three plants indicated the potential for developing novel food supplements and nutraceuticals. However, several limitations in this study that could be addressed in future studies are the chemical constituents in the extracts that can be further analyzed and conduct alternative antioxidant assays to evaluate the potential mechanism of action.

Acknowledgements

This study was supported by Faculty of Science and Natural Resources, Universiti Malaysia Sabah and Malaysia Ministry of Science, Technology, and Innovation (Science Fund 02-01-10-SF0107).

References

Ahn, J. H., Liu, Q., Lee, C., Ahn, M.-J., Yoo, H.-S., Hwang, B. Y., & Lee, M. K. (2012). A new pancreatic lipase inhibitor from *Broussonetia kanzinoki*. *Bioorganic & Medicinal Chemistry Letters, 22*(8), 2760–2763. DOI: https://doi.org/10.1016/j.bmcl.2012.02.088

Alakolanga, A. G. A. W., Kumar, N. S., Jayasinghe, L., & Fujimoto, Y. (2015). Antioxidant property and α-glucosidase, α-amylase and lipase inhibiting activities of *Flacourtia inermis* fruits: characterization of malic acid as an inhibitor of the enzymes. *Journal of Food Science and Technology, 52*(12), 8383–8388. DOI: https://doi.org/10.1007/s13197-015-1937-6

American Diabetes Association (ADA). (2015). 2. Classification and diagnosis of diabetes. *Diabetes care, 38*(Supplement 1), S8–S16. DOI: https://doi.org/10.2337/dc15-s005

Anantachoke, N., Kitphati, W., Mangmool, S., & Bunyapraphatsara, N. (2015). Polyphenolic compounds and antioxidant activities of the leaves of Glochidion hypoleucum. *Natural Products Communications, 10*(3), 479-482. DOI: http://doi.org/10.1177/1934578X1501000325

Azam, A. T. M. Z., Hasan, A. A., Uddin, Md. G., Masud, M. M., & Hasan, C. M. (2012). Antimicrobial, antioxidant and cytotoxic activities of *Glochidion multiloculare* (Roxb. ex Willd.) Mull. Arg. (Euphorbiaceae). *Dhaka University of Journal of Pharmaceutical Science, 11*(2), 117-120.

Birari, R. B., & Bhutani, K. K. (2007). Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discovery Today, 12*(19–20), 879–889. DOI: https://doi.org/10.1016/j.drudis.2007.07.024

Carnethon, M. R., Rasmussen-Torvik, L. J., & Palaniappan, L. (2014). The obesity paradox in diabetes. *Current Cardiology Reports, 16*(446). DOI: https://doi.org/10.1007/s11886-013-0446-3

Centers for Disease Control and Prevention (CDC). (2004). Prevalence of overweight and obesity among adults with diagnosed diabetes—United States, 1988-1994 and 1999-2002. *MMWR Morbidity and Mortality Weekly Report, 53*(45), 1066-1068.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., ... Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation, 114*(12), 1752–1761. DOI: https://doi.org/10.1172/jci21625

Gallaher, D., &, Schneeman, B. O. (1986). Nutritional and metabolic response to plant inhibitors of digestive enzymes. In M. Friedman (Ed.), *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods-Advances in Experimental Medicine and Biology* (Vol. 199, pp. 167–184). Cham, SW: Springer US.

Gundamaraju, R., Mulaplli, S. B., & Ramesh, C. (2012) Evaluation of anti-obesity activity of *Lantana camara Var Linn*. by progesterone induced obesity on albino mice. *International Journal of Pharmacognosy and Phytochemical Research, 4*(4), 213-218.

Hayward, N. J., McDougall, G. J., Farag, S., Allwood, J. W., Austin, C., Campbell, F., ... Ranawana, V. (2019). Cinnamon shows antidiabetic properties that are species-specific: effects on enzyme activity inhibition and starch digestion. *Plant Foods for Human Nutrition, 74*(4), 544–552. DOI: https://doi.org/10.1007/s11130-019-00760-8

Hodgson, J. M., Watts, G. F., Playford, D. A., Burke, V., & Croft, K. D. (2002). Coenzyme Q_{10} improves blood pressure and glycaemic control: a controlled trial in subjects with type 2 diabetes. *European Journal of Clinical Nutrition, 56*(11), 1137–1142. DOI: https://doi.org/10.1038/sj.ejcn.1601464

Jamous, R. M., Abu-Zaitoun, S. Y., Akkawi, R. J., & Ali-Shtayeh, M. S. (2018). Antiobesity and antioxidant potentials of selected palestinian medicinal plants. *Evidence-Based Complementary and Alternative Medicine, 2018*(8426752), 1–21. DOI: https://doi.org/10.1155/2018/8426752

Kim, J. H., Kim, H. J., Park, H. W., Youn, S. H., Choi, D.-Y., & Shin, C. S. (2007). Development of inhibitors against lipase and α-glucosidase from derivatives of monascus pigment. *FEMS Microbiology Letters, 276*(1), 93–98. DOI: https://doi.org/10.1111/j.1574-6968.2007.00917.x

Kim, S. H., Hyun, S. H., & Choung, S. Y. (2006). Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *Journal of Ethnopharmacology, 104*(1–2), 119–123. DOI: https://doi.org/10.1016/j.jep.2005.08.059

Little, T. J., Horowitz, M., & Feinle-Bisset, C. (2007). Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. *The American Journal of Clinical Nutrition, 86*(3), 531–541. DOI: https://doi.org/10.1093/ajcn/86.3.531

Marseglia, L., Manti, S., D'Angelo, G., Nicotera, A., Parisi, E., Di Rosa, G., ... Arrigo, T. (2014). Oxidative stress in obesity: a critical component in human diseases. *International Journal of Molecular Sciences, 16*(1), 378–400. DOI: https://doi.org/10.3390/ijms16010378

Menke, A., Casagrande, S., Geiss, L., & Cowie, C. C. (2015). Prevalence of and trends in diabetes among adults in the United States, 1988-2012. *JAMA, 314*(10), 1021-1029. DOI: https://doi.org/10.1001/jama.2015.10029

Ministry of Energy and Natural Resources [NRE]. (2006). *Biodiversity in Malaysia*. Retrieved on November 1, 2020 from https://www.ketsa.gov.my/ms-my/pustakamedia/Penerbitan/Biodiversity%20in%20Malaysia.pdf

More, G. K., & Makola, R. T. (2020). *In-vitro* analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by *Solanum sisymbriifolium* extracts. *Scientific Reports, 10*(6493). DOI: https://doi.org/10.1038/s41598-020-63491-w

Patil, G., Khare, A. B., Huang, K-F., & Lin, F-M. (2015). Bioactive chemical constituents from the leaves of *Lantana Camara L. Indian Journal of Chemistry, 54B*, 691-697. DOI: http://doi.org/10.13140/RG.2.1.4247.4402

Sahib, A. S. (2016). Antidiabetic and antioxidant effect of cinnamon in poorly controlled type-2 diabetic Iraqi patients: a randomized, placebo-controlled clinical trial. *Journal of Intercultural Ethnopharmacology, 5*(2), 108-113. https://doi.org/10.5455/jice.20160217044511

Siddiqui, M. Z., Chowdhury, A. R., & Prasad, N. (2015). Evaluation of phytochemicals, physico-chemical properties and antioxidant activity in gum exudates of *buchanania lanzan*. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 86*(4), 817–822. DOI: https://doi.org/10.1007/s40011-015-0539-4

Sies, H. (1985). *Oxidative stress*. Florida, FL: Elseview.

Suleiman, M., Masundang, D. P., & Akiyama, H. (2017). The mosses of crocker range park, Malaysian borneo. *PhytoKeys, 88*(8), 71–107. DOI: https://doi.org/10.3897/phytokeys.88.14674

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*(6–7), 669–675. DOI: https://doi.org/10.1016/j.jfca.2006.01.003

Velu, G., Palanichamy, V., & Rajan, A. P. (2018). Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. In S. M. Roopan & G. Madhumitha (Eds.), *Bioorganic Phase in Natural Food: An Overview* (p. 135–156). Cham, SW: Springer International Publishing.

Wang, Y., Huang, S., Shao, S., Qian, L., & Xu, P. (2012). Studies on bioactivities of tea (*Camellia sinensis L.*) fruit peel extracts: antioxidant activity and inhibitory potential against α-glucosidase and α-amylase *in vitro*. *Industrial Crops and Products, 37*(1), 520–526. https://doi.org/10.1016/j.indcrop.2011.07.031

World Health Organization (WHO). (2020). Obesity and overweight. Recovered on 2020 October, 23 from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wright Jr., E., Scism-Bacon, J. L., & Glass, L. C. (2006). Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. *The International Journal of Clinical Practice, 60*(3), 308–314. DOI: https://doi.org/10.1111/j.1368-5031.2006.00825.x

Wu, T., Tang, Q., Gao, Z., Yu, Z., Song, H., Zheng, X., & Chen, W. (2013). Blueberry and mulberry juice prevent obesity development in C57BL/6 mice. PLoS ONE, *8*(10), e77585. DOI: https://doi.org/10.1371/journal.pone.0077585

Zheng, C-J., Li, H-Q., Ren, S-C., Xu, C-L., Rahman, K., Qin, L-P., & Sun, Y-H. (2015) Phytochemical and pharmacological profile of *Vitex negundo*. *Phytotherapy Research*, *29*(5), 633-647. DOI: https://doi.org/10.1002/ptr.5303

Supplementary material

Table S1. List of plant species collected and extracts demonstrated >50% inhibition in assays tested.

No	Plant parts	Family	*Genus*	*Species*	>50% inhibition		
					α-amylase	Anti-pancreatic lipase	Antioxidant
1	L	Apiaceae	*Anethum*	*A. graveolens*	√	√	
2	St	Apiaceae	*Anethum*	*A. graveolens*			
3	L	Passifloraceae	*Turnera*	*T. ulmifolia*		√	√
4	L	Ebenaceae	*Diospyros*	sp.			
5	St	Ebenaceae	*Diospyros*	sp.			√
6	**L**	**Anacardiaceae**	***Buchanania***	**sp.**	**√**	**√**	**√**
7	L	Flacourtiaceae	*Flacourtia*	sp.			√
8	L	Verbenaceae	*Premna*	sp.			√
9	L	Oleaceae	*Chionanthus*	*C. pluriflorus*			√
10	St	Oleaceae	*Chionanthus*	*C. pluriflorus*			√
11	L	Lauraceae	*Litsea*	sp.			√
12	L	Rhamnaceae	*Colubrina*	*C. asiatica*			
13	Fr	Rhamnaceae	*Colubrina*	*C. asiatica*			
14	L	Moraceae	*Ficus*	sp.			
15	L	Hernandiaceae	*Hernandia*	*H. peltata*			
16	L	Calophyllaceae	*Calophyllum*	*C. inophyllum*			√
17	L	Apocynaceae	*Cerbera*	*C. odollam*			√
18	L	Sapotaceae	*Pouteria*	*P. obavata*			
19	L	Euphorbiaceae	*Glochidion*	*G. rubrum*		√	√
20	L	Apocynaceae	*Kopsia*	sp.	√		
21	L	Apocynaceae	*Nerium*	*N. oleander*	√		√
22	**L**	**Lamiaceae**	***Vitex***	***V. negundo***	**√**	**√**	**√**
23	L	Apocynaceae	*Kopsia*	sp.	√		
24	St	Apocynaceae	*Kopsia*	sp.			
25	L	Moraceae	*Ficus*	sp.			
26	L	Moraceae	*Ficus*	sp.			
27	L	Moraceae	*Ficus*	sp.			
28	L	Rubisceae	Unknown	Unknown			
29	L	Lecythidaceae	*Barringtonia*	*B. asiatica*			
30	L	Verbenaceae	*Premna*	*P. obtusifolia*			√
31	L	Piperaceae	*Piper*	sp.			
32	St	Piperaceae	*Piper*	sp.			
33	L	Fabaceae	*Desmodium*	*D. umbellatum*			
34	L	Gesneriaceae	*Cyrtandromoea*	*C. grandis*			√
35	L	Pedaliaceae	*Sesamum*	*S. indicum*			√
36	W	Gleicheniaceae	*Dicranopteris*	*D. linearis*			√
37	W	Nephrolepidaceae	*Nephrolepis*	*N. biserrata*			
38	W	Dryopteridaceae	*Dryopteris*	*D. filix-mas*		√	
39	W	Pteridaceae	*Acrostichum*	*A. aureum*			
40	L	Plantaginaceae	*Plantago*	*P. major*			
41	L	Acanthaceae	*Clinacanthus*	*C. nutans*			
42	**B**	**Lauraceae**	***Cinnamomum***	**sp.**	**√**	**√**	**√**
43	L	Verbenaceae	*Lantana*	*L. camara*		√	√
44	L	Zingiberaceae	*Alpinia*	*A. galanga*			
45	St	Zingiberaceae	*Alpinia*	*A. galanga*			
46	L	Asteraceae	*Eupatorium*	*E. odoratum*			√

Note: B – Bark parts, L – leaf parts, St – stem parts, W – whole parts. Extracts exhibit >50% in all three test were bolded.

Table S2. α-amylase inhibition screening results of all plant extracts at 10 mg mL^{-1}.

Sample	Absorbance reading			Average absorbance value	Inhibition (%)
	1	2	3		
1	**0.031**	**0.049**	**0.040**	**0.040**	**86.27**
2	0.110	0.102	0.108	0.107	47.55
3	0.239	0.236	0.230	0.235	19.24
4	0.203	0.207	0.205	0.205	29.56
5	0.191	0.194	0.195	0.193	33.68
6	**0.055**	**0.069**	**0.062**	**0.062**	**78.69**
7	0.207	0.203	0.202	0.204	30.61
8	0.224	0.211	0.219	0.218	39.22
9	0.248	0.243	0.249	0.247	31.20
10	0.187	0.185	0.195	0.189	47.31
11	0.210	0.205	0.209	0.208	42.01
12	0.202	0.205	0.205	0.204	43.18
13	0.251	0.257	0.259	0.256	28.69
14	0.279	0.281	0.277	0.279	22.28
15	0.244	0.244	0.246	0.245	31.75
16	0.316	0.314	0.325	0.318	11.42
17	0.195	0.193	0.196	0.195	32.99
18	0.241	0.238	0.239	0.239	17.87
19	0.326	0.330	0.338	0.331	7.80
20	**0.069**	**0.065**	**0.073**	**0.069**	**76.29**
21	**0.083**	**0.075**	**0.095**	**0.084**	**69.12**
22	**0.065**	**0.069**	**0.067**	**0.067**	**75.37**
23	**0.042**	**0.047**	**0.044**	**0.044**	**83.82**
24	0.222	0.225	0.220	0.222	38.16
25	0.199	0.195	0.194	0.196	45.40
26	0.202	0.208	0.205	0.205	29.55
27	0.195	0.186	0.184	0.188	35.40
28	0.228	0.235	0.233	0.232	35.38
29	0.265	0.269	0.265	0.266	29.82
30	0.176	0.172	0.177	0.175	40.27
31	0.218	0.214	0.215	0.216	26.28
32	0.195	0.182	0.185	0.187	36.18
33	0.215	0.210	0.211	0.212	27.65
34	0.249	0.247	0.250	0.249	15.31
35	0.273	0.268	0.266	0.269	8.19
36	0.145	0.149	0.144	0.146	28.43
37	0.184	0.189	0.186	0.186	36.52
38	0.162	0.159	0.164	0.162	44.71
39	0.212	0.205	0.209	0.209	31.22
40	0.119	0.128	0.113	0.120	41.18
41	0.176	0.166	0.181	0.174	40.61
42	**0.039**	**0.045**	**0.057**	**0.047**	**83.85**
43	0.151	0.152	0.159	0.154	24.51
44	0.188	0.182	0.182	0.184	9.80
45	0.177	0.177	0.183	0.179	12.25
46	0.184	0.189	0.187	0.187	8.33

Note: Extract showing >50% inhibition was bolded.

Table S3. Anti-pancreatic lipase assay screening results of all plant extracts at 0.5 mg mL^{-1}.

Sample	Absorbance reading			Average absorbance value	Inhibition (%)
	1	2	3		
1	**0.266**	**0.271**	**0.267**	**0.268**	**59.46**
2	0.714	0.712	0.719	0.715	16.08
3	**0.234**	**0.232**	**0.228**	**0.231**	**70.19**
4	0.435	0.428	0.422	0.428	35.25
5	0.645	0.640	0.638	0.641	24.77
6	**0.217**	**0.225**	**0.222**	**0.221**	**71.48**
7	0.375	0.369	0.372	0.372	43.72
8	0.442	0.440	0.447	0.443	32.98
9	0.538	0.528	0.524	0.530	19.82
10	0.465	0.464	0.469	0.466	29.50
11	0.478	0.474	0.473	0.475	28.14
12	0.641	0.649	0.643	0.644	2.57
13	0.385	0.381	0.392	0.386	41.60
14	0.359	0.355	0.357	0.357	45.99
15	0.604	0.610	0.621	0.612	7.41
16	0.561	0.568	0.559	0.563	14.83
17	0.412	0.407	0.414	0.411	37.82
18	0.535	0.530	0.531	0.532	19.52
19	**0.173**	**0.171**	**0.175**	**0.173**	**62.22**
20	0.662	0.665	0.668	0.665	21.95
21	0.589	0.591	0.599	0.593	10.29
22	**0.222**	**0.218**	**0.217**	**0.219**	**52.18**
23	0.478	0.478	0.472	0.476	27.99
24	0.616	0.632	0.618	0.622	5.90
25	0.574	0.575	0.570	0.573	13.31
26	0.374	0.370	0.369	0.371	43.87
27	0.575	0.579	0.571	0.575	13.01
28	0.408	0.415	0.413	0.412	37.67
29	0.625	0.619	0.618	0.621	27.11
30	0.586	0.587	0.579	0.584	31.46
31	0.551	0.533	0.550	0.545	36.03
32	0.618	0.615	0.615	0.616	27.70
33	0.592	0.599	0.610	0.600	29.58
34	0.619	0.612	0.614	0.615	27.82
35	0.649	0.651	0.653	0.651	23.59
36	0.556	0.549	0.554	0.553	35.09
37	0.526	0.523	0.517	0.522	38.73
38	**0.264**	**0.256**	**0.246**	**0.255**	**64.97**
39	0.534	0.537	0.540	0.537	36.97
40	0.544	0.549	0.542	0.545	36.03
41	0.572	0.581	0.58	0.575	32.51
42	**0.238**	**0.245**	**0.243**	**0.242**	**66.76**
43	**0.277**	**0.282**	**0.281**	**0.280**	**61.54**
44	0.582	0.586	0.578	0.582	31.69
45	0.702	0.707	0.703	0.704	17.37
46	0.555	0.559	0.561	0.558	34.51

Note: Extract showing >50% inhibition was bolded.

Table S4. Antioxidant screening results of all plant extracts at 0.125 mg mL^{-1}.

Sample	Absorbance reading			Average absorbance reading	Inhibition (%)
	1	2	3		
1	0.350	0.341	0.342	0.344	48.36
2	0.475	0.468	0.471	0.471	28.42
3	**0.050**	**0.053**	**0.049**	**0.051**	**92.30**
4	0.395	0.400	0.392	0.396	40.18
5	**0.328**	**0.331**	**0.330**	**0.330**	**58.96**
6	**0.033**	**0.041**	**0.053**	**0.042**	**93.66**
7	**0.058**	**0.060**	**0.053**	**0.057**	**91.07**
8	**0.106**	**0.109**	**0.103**	**0.106**	**83.39**
9	**0.116**	**0.115**	**0.113**	**0.115**	**81.97**
10	**0.217**	**0.215**	**0.223**	**0.218**	**65.83**
11	**0.174**	**0.175**	**0.181**	**0.177**	**72.26**
12	0.563	0.569	0.559	0.564	12.83
13	0.502	0.511	0.508	0.507	21.63
14	0.331	0.341	0.327	0.333	48.53
15	0.500	0.509	0.495	0.501	22.57
16	**0.193**	**0.196**	**0.193**	**0.194**	**70.02**
17	**0.299**	**0.291**	**0.304**	**0.298**	**54.78**
18	0.503	0.529	0.517	0.516	21.70
19	**0.093**	**0.102**	**0.099**	**0.098**	**85.12**
20	0.535	0.532	0.542	0.536	33.33
21	**0.100**	**0.102**	**0.099**	**0.100**	**84.83**
22	**0.296**	**0.304**	**0.291**	**0.297**	**54.93**
23	0.532	0.535	0.529	0.532	18.28
24	0.520	0.513	0.522	0.518	20.43
25	0.328	0.325	0.322	0.325	49.77
26	0.558	0.550	0.544	0.551	15.36
27	0.835	0.821	0.830	0.829	12.45
28	0.679	0.680	0.674	0.678	28.41
29	0.679	0.692	0.679	0.681	28.09
30	**0.226**	**0.230**	**0.228**	**0.228**	**69.35**
31	0.586	0.584	0.598	0.589	20.83
32	0.588	0.590	0.585	0.588	20.97
33	0.554	0.560	0.555	0.556	25.27
34	**0.125**	**0.122**	**0.126**	**0.124**	**80.66**
35	**0.057**	**0.059**	**0.062**	**0.059**	**90.80**
36	**0.097**	**0.110**	**0.119**	**0.109**	**85.47**
37	0.403	0.407	0.399	0.403	37.13
38	0.424	0.435	0.425	0.428	33.23
39	0.582	0.574	0.575	0.577	30.40
40	0.590	0.609	0.601	0.600	25.37
41	0.596	0.600	0.593	0.596	28.11
42	**0.142**	**0.139**	**0.141**	**0.141**	**78.57**
43	**0.063**	**0.059**	**0.063**	**0.062**	**91.73**
44	0.602	0.608	0.593	0.601	19.87
45	0.536	0.549	0.544	0.543	27.60
46	**0.148**	**0.135**	**0.141**	**0.141**	**78.57**

Note: Extract showing >50% inhibition was bolded.