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Lipase inhibitory activity of *Carica papaya, Chrysophyllum cainito, Corcorus olitorius, Cympogon citrates* and *Syzygium cumini* extracts

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Abstract

The lipase inhibitory action of *Carica papaya, Chrysophyllum cainito, Corcorus olitorius, Cymbopogon citrates* and *Syzygium cumini* were evaluated to explore for the presence of anti-obesity compounds and their potential weight-lowering activity. Enzyme inhibition results of the alcoholic extracts of the five plants showed that *C. cainito* has the highest percent inhibition at 74.91% while *S. cumini, C. citratus, C. olitorius* and *C. papaya* obtained less than 50% average inhibition. *C. cainito* was partitioned using hexane and ethyl acetate to further concentrate the bioactive compounds. The lipase inhibition assay of hexane and ethyl acetate extracts showed 92.11% inhibition and 21.9% inhibition, respectively. The greater activity in the former may imply that majority of potential anti-lipase constituents are found in the hexane portion.

1. Introduction

According to the 2014 data of the World Health Organization, more than 1.9 billion adults are overweight and 600 M of which are obese (WHO, 2016). Obesity has been considered as a global epidemic that needs immediate prevention and control (Brug and Crawford, 2009). It results from an imbalance between energy intake and energy expenditure (Abete *et al*., 2010). The imbalance leads to an abnormal weight gain and increases the risk for chronic illnesses including cardiovascular diseases (Lavie *et al.*, 2016), cancer (Noureddin and Rinella, 2015) and diabetes (Genser *et al*., 2016).

Despite the growing awareness of the importance of active lifestyle and healthy diet, obesity is still widespread and is now a common health problem (Lim *et al*., 2012). Although physical activity and lifestyle change are crucial for reducing body weight and elevating average daily metabolic rate, these approaches do not offer long-term effects. It is ideal to combine these approaches with inhibitors of fat absorption (Garza *et al*., 2011). The most commonly marketed weight loss agent is Orlistat, an irreversible inhibitor of gastric and pancreatic lipase, which is the key enzyme for dietary triacylglycerol digestion (Kaila and Raman, 2008). By inhibiting these lipases, systemic fat absorption can be significantly reduced.

Pharmaceutical drugs such as Orlistat may moderately reduce fat absorption, but side effects like steatorrhea, oily stools, diarrhea, cholelithiasis, and incontinence render them as undesirable means of weight management (Flippatos *et al*., 2008). Several researchers have focused on screening plant extracts for potential lipase inhibition activities since medicinal plants have been used as dietary supplements for weight control (Lee *et al*., 2015; Seo *et al*., 2015; Belfeki *et al*., 2016; Chien *et al*., 2016; Glisan *et al*., 2017).

Since food is the major source of unwanted calories, this study focuses on plants used for human consumption. *Syzygium cumini* (black plum), *Chrysophyllum cainito* (star apple), *Cymbopogon citratus* (lemon grass), *Corchorus olitorius* (bush okra) and *Carica papaya* (papaya) were evaluated for their potential to inhibit lipase activity. These plants were chosen due to their abundance, availability, and ease of access in the local setting.

There is limited literature on lipase inhibition activities of the aforementioned plants. However, plants from the same family and genus have been previously studied. Ado *et al.* (2013) pointed out that the methanol leaf extract of *Syzygium malaccense* can inhibit up to 99% of pancreatic lipase activity, while *Psidium guajava* from the same family *Myrtaceae* can inhibit lipase up to 85.6%. Another species of the *Chrysophyllum* genus

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namely *Chrysophyllum roxburghii* showed significant dose-dependent activity against lipase (Prashith *et al*., 2013). Some members of *Poaceae* family, where *C. citratus* belongs, were also found to have lipase inhibitors (Sharma *et al*., 2005). Using 80% methanol solvent, *Setaria italica* extracts exhibited 80.3% lipase inhibition at 0.2 mg/mL solution concentration. Extracts of *Eriochloa villosa* showed 83% inhibition of lipase activity. *Hibiscus sabdariffa (*Malvaceae) also showed moderate anti-lipase activity at 60.9% (Ado *et al*., 2013). *Abroma augusta* from *Malvaceae* family gave 88.6% inhibition at 100 mg/mL. The activity of plant was also concentration-dependent (Gupta *et al*., 2012). In this work, the lipase inhibitory activities of *S. cumini*, *C. cainito*, *C. citratus*, *C. olitorius* and *C. papaya* have been evaluated.

2. Materials and methods

2.1 Collection of samples

S. cumini, *C. cainito, C.citratus* and *C. papaya* leaves were collected from the University of the Philippines Diliman campus and submitted to the Jose Vera Memorial Herbarium, Institute of Biology, University of the Philippines-Diliman for authentication and verification. *C. olitorius* leaves were purchased at Regalado Wet Market and also submitted to the herbarium for authentication and verification. The voucher specimen number for *S. cumini* is 9469, for *C. cainito* is 019337 , for *C. citratus* 14618, for *C. papaya* 21403 and for *C. olitorius* 9398.

2.2 Crude extraction and solvent partitioning

The leaves were washed and air-dried. Grinding and homogenization of samples were done using a kitchen blender. Methanol, hexane, and ethyl acetate solvents used were singly distilled prior to use. Ground samples were soaked in methanol. The methanol solvent was later filtered out and concentrated *in vacuo* using a rotary evaporator at 40°C.

The concentrate with the highest inhibition activity was partitioned using hexane and ethyl acetate. The methanol extract was re-dissolved in water for partitioning with hexane followed by ethyl acetate. Approximately 10 g of the methanol extract was dissolved in 100 mL distilled water and 600 mL hexane. The mixture was allowed to settle until a distinct separation between the organic and aqueous layer was obtained. The organic layer was collected and concentrated *in vacuo* using a rotary evaporator at 40^{o} C. The process was repeated two more times. A similar process was done for the partitioning with ethyl acetate.

2.3 Lipase inhibition assay

Lipase inhibition assay was adapted from Kim *et al*. (2007). 3-morpholinopropane-1-sulfonic acid (MOPS)-ethylenediaminetetraacetic acid (EDTA), porcine pancreatic lipase solution, Tris-HCl buffer, *p*-nitrophenyl butyrate (*p*-NPB) substrate solution were freshly prepared. Lipase from porcine pancreas (Sigma L3126) was used. Orlistat served as the positive control. Samples were placed on a 96 well-quartz microplate. The total reaction volume inside one well is 200 mL. Fifteen mL each of lipase and p-NPB were placed in each well. For the uninhibited reaction, 170 mL of buffer was added. For the positive control, 150 mL of the buffer was added with 20 mL of Orlistat prepared from 1 mg of Orlistat in 1 mL of DMSO. For the sample reaction, 150 mL of buffer was used with 20 mL of plant extract prepared from 1 mg of extract dissolved in 3% DMSO solution.

The reaction was monitored using a Multiskan^{TM} GO UV/Vis Microplate Spectrophotometer. The samples were then incubated at 37°C for 10 min to allow interaction of samples with the enzyme. After incubation, the *p*-NPB substrate was added to the samples. Solutions were shaken for 15 seconds and scanned at 405 nm for 40 times at 30-second intervals. For each extract, 2 trials were performed with 2 replicates per trial.

Percent inhibition was calculated using the equation

$$\% \text{ Inhibition} = 100 - \left[A_{Sample} / A_{Uninhibited} \right] x100$$

where A_{sample} is the activity of the solution with inhibitor and $A_{uninhibited}$ is the activity of the solution without the inhibitor.

3. Results and discussion

The methanol extracts of the samples were obtained and the percent yields are shown in Figure 1 with the percent inhibitory activities of the samples against pancreatic lipase. *C. cainito* had the highest activity at 74.91% while *C. olitorius* had the lowest anti-lipase activity 37.33%. The plants that were frequently studied for their possible anti-obesity effects were *C. olitorius*, *C. papaya* and *C. cainito*; however, the first two plants exhibited moderate activity (<50% inhibition). It is possible that the anti-obesity mechanism of *C. olitorius* and *C. papaya* is different from the inhibition of the pancreatic lipase enzyme. *C. olitorius* contains the flavonol glycoside Q3MG which is responsible for the antioxidant activities that subdue increase in body

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adiposity, reduces plasma and hepatic lipid levels and triggers lipid catabolism in the liver (Wang *et al*., 2011). These effects are attained via alteration of the gene expression of specific enzymes that are linked to lipid and glucose metabolism (Honda *et al*., 2009). Suppression of NADPH oxidase expression and enhancement of b-oxidation are claimed to be one of the targets of polyphenolic compounds with regards to obesity control (Dávalos *et al.,* 2009).

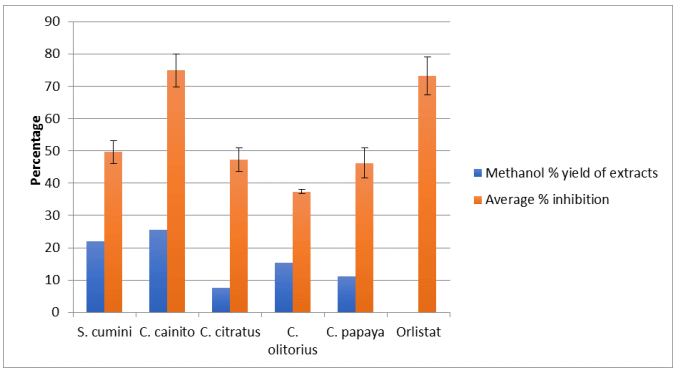

Figure 1. Percent yield of methanol extracts and their corresponding lipase inhibitory activity

C. papaya has constituents that follow the same anti-obesity mechanism as *C. olitorius* extracts. Studies showed regulation in total cholesterol, triglycerides, phospholipids and free fatty acid in manifested in high-diet fed mice (Manjula *et al*. 2014). Athesh *et al.* (2012) suggested that the substantial reduction of total cholesterol is by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase which limits cholesterol biosynthesis. In addition, increase in endothelium-bound lipoprotein lipase may have caused the decrease in total triglyceride count. Heightened activity of lipoprotein lipase can then lead to activation of b-oxidation in the liver, along with the decline in gene expression for fatty acid synthesis (Athesh *et al*., 2012).

C. cainito methanol extract was further partitioned using hexane and ethyl acetate and the inhibitory activity are shown in Figure 2. The hexane extract showed high inhibitory activity at 92.11% while the ethyl acetate extract is lower (21.93%) than the methanol extract (74.91%). Results suggest that the pancreatic lipase inhibitory agents were concentrated in the hexane extract. Phytochemical screening of *C. cainito* leaves showed the presence of alkaloids, flavonoids and sterols (N'guessan *et al.,* 2009). The triterpenoids ursolic acid, b-sitosterol and lupeol were isolated from the leaves, along with the phenolic compound gallic acid (Shailajan and Gurjar, 2014). Flavonoids and alkaloids have been previously shown to inhibit lipase activity (Ruiz *et al*., 2006). Cyclic di-terpenoids from *Calotropis procera* were found to competitively inhibit pancreatic lipase (Patil *et al*., 2015). It is possible that the *C. cainito* extracts contain similar secondary metabolites that affect the pancreatic lipase activity.

Future *in vivo* studies will be carried out to further explore the potential of *C. cainito* as dietary supplements or consumed for weight management. Current efforts include the isolation and identification of compounds responsible for the lipase inhibitory activity.

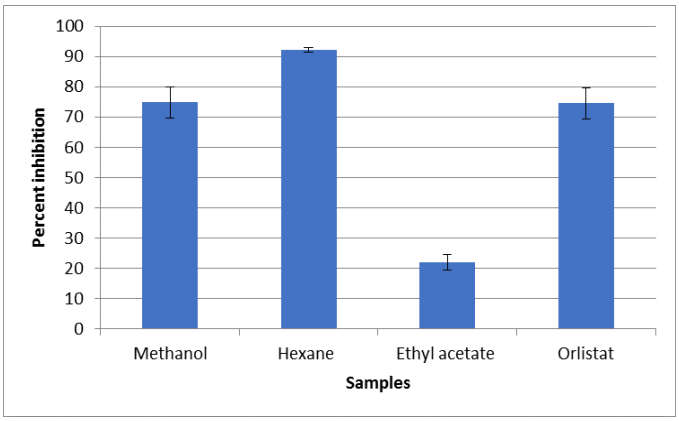

Figure 2. Lipase inhibitory activity of methanol, hexane and ethyl acetate extracts of *C. cainito*

Conflict of Interest

The authors declare no conflict of interest.

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