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Anti-Obesity Activity of *Tradescantia spathacea* Leaves Through Pancreatic Lipase Inhibition

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Obesity is a prevalent global metabolic disorder that impacts societies worldwide. To the best of our knowledge, no studies have been investigated on the anti-obesity effects of *Tradescantia spathacea* leaves. The aim of this study is to evaluate the pancreatic lipase (PL) inhibitory effects of *Tradescantia spathacea* leaves. Orlistat was used as positive control. Ultrasonic-assisted extraction, solvent partitioning and fractionation techniques were used to obtain hexane, dichloromethane, and ethyl acetate crude extracts and their subfractions. The extracts and subfractions were then analyzed for PL inhibitory activity. The results show that the dichloromethane crude extract exhibits the highest PL inhibition rate of 97.73±0.59%, followed by the ethyl acetate crude extract (90.89±0.05%) and the hexane crude extract (43.80±1.80%) at 2.5 mg/mL. The dichloromethane and ethyl acetate extracts were chosen to be further isolated into their respective fractions. Three dichloromethane subfractions exhibited IC_{50} values of 0.39±0.06, 0.45±0.01, and 0.54±0.08 mg/mL while four ethyl acetate fractions exhibited IC_{50} values of 0.13±0.03, 0.28±0.12, 0.33±0.11, and 0.59±0.27 mg/mL, respectively. *T. spathacea* exhibits potent pancreatic lipase inhibitory activity, suggesting it as a potential source of anti-obesity compounds and is amenable to further purification processes to isolate their bioactive components that contribute to the PL inhibitory effects.

Keywords: Commelinaceae; *Tradescantia spathacea*; antiobesity; pancreatic lipase inhibition; Ultrasonic-assisted extraction.

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Obesity has become the most prevalent metabolic disorder of the 21st century in numerous countries, which includes middle- and lower-income countries such as Malaysia [1]. It can damage the quality of life physiologically, economically, and psychologically because of its correlation with many major non-communicable diseases (NCDs). Although synthetic drugs used for long-term treatment of overweight and obesity that have been approved by FDA, including orlistat, lorcaserin, phentermine-topiramate, bupropion-naltrexone and liraglutide [2,3], have been launched, high price and adverse side effects are still under concerned. However, plant-derived bioactive compounds offer a safe and cost-effective way to target this anomaly.

Pancreatic lipase (PL) is an enzyme secreted by the pancreas which can be found in visceral and subcutaneous adipose (fat) cells. Its function is to break down dietary fat (triglycerides) into fatty acids and glycerol which will then be used by the body. When there is excess of them, they will be stored in the adipose tissue as energy storage [4]. One of the ways used to treat obesity is PL inhibition by preventing the absorption of triglycerides through the small intestine. This mechanism directly targets the root cause of obesity. Once catalytic activity is inhibited, the digestion and absorption of lipids in food, together with the accumulation of adipose tissue will be reduced and thus achieve the effect of preventing and treating obesity. An example of PL inhibitor, orlistat, which is one of the FDA-approved anti-obesity drugs, has been reported that is able to reduce the absorption of dietary fat up to 30% [5]. Furthermore, treating obesity by PL inhibition has been proven to be relatively safe since it does not cause long-term effects

in the human body, as the lipase inhibitor will be excreted along with the lipase to which it is bound after the action is completed. Another supporting fact is that PL inhibitors neither enter human blood vessels or the nervous system, nor affect the balance of body's minerals and bone circulation [4]. Hence, the discovery and development of anti-obesity drugs for the treatment and even prevention of obesity and related diseases rely heavily on the inhibition of PL.

Tradescantia spathacea Sw. (Commelinaceae) is a small, clumping, and luscious herbaceous plant that grows easily and low in maintenance to cultivate [6-8]. It grows natively throughout Mexico, Central America, and the West Indies, typically in woods and urban areas [9]. Importantly, it is a perennial herb that grows robustly and flowers all year round under optimal conditions, which makes it highly available to be picked all year round and used fresh or dried [6]. It is widely used globally as ethnopharmacology due to its incredible medicinal properties like antioxidant, antimicrobial, anticancer, antiviral, anti-tuberculosis, and anti-inflammatory activities [9-11]. In fact, strong pancreatic lipase (PL) inhibitory effects are seen in plant extracts from other plant species that show high antioxidant activity [12, 13]. This proposes a strong correlation between antioxidant and anti-obesity activities. Evidence of the strong antioxidant activity of *T. spathacea* [10, 11, 14-18] suggests that it could also be a valuable source of anti-obesity compounds, thus justifying further investigation.

EXPERIMENTAL

Chemicals and Materials

Analytical grade ethanol (99.9%), hexane, dichloromethane and ethyl acetate were bought from ChemSoln were used in this study. Other chemicals and materials used were Silica Gel 60 F_{254} aluminium plates (Merck, 1.05554.0001), lipase from porcine pancreas (Type II, 125 units/ mg protein, Sigma Aldrich), 4-nitrophenyl butyrate (>98%, Sigma Aldrich, N9876), Orlistat (Pharma grade, Sigma Aldrich, PHR1445), analytical grade dimethyl sulfide (DMSO) (ChemSoln), and potassium phosphate buffer solution (0.1 mM, pH 8.0).

The leaves of *T. spathacea* were freshly harvested in October 2023 from a plantation in Kepong, Kuala Lumpur, Malaysia. The identity of the plant has been authentically identified and confirmed by the Phytochemistry Group of Universiti Pendidikan Sultan Idris (UPSI). The voucher specimen (TM1053) is deposited in the herbarium of UPSI, Malaysia. Plant samples were cleaned with distilled water to remove dirt and subsequently freeze-dried to remove moisture. Dried leaf samples were ground into fine powder with a mechanical grinder until a uniform fine texture and stored in hermetic plastic bags under refrigerated conditions prior to extraction.

Methodology

The plant was identified, freeze-dried, and pulverized. The dried material (616 g) was extracted with 1 L of 80% ethanol (v/v) by ultrasonic-assisted extraction method (50 Hz, 25°C) three times, with each for the duration of four hours to reduce extraction temperature and time [19]. The EtOH extract obtained was concentrated by a rotary evaporator before freeze-drying. The dried EtOH extract was subsequently portioned with hexane (Hex), dichloromethane (CH_2Cl_2), and ethyl acetate (EA). These extracts were then dried for PL inhibitory activity. The bioactive crude extract (>80 % PL inhibition at 2.5 mg/mL against 125 U/mL of enzyme) was fractionated by silica gel column chromatography using solvent gradient series into 10 to 15 subfractions. The extract and its subfractions were then dried for bioassay.

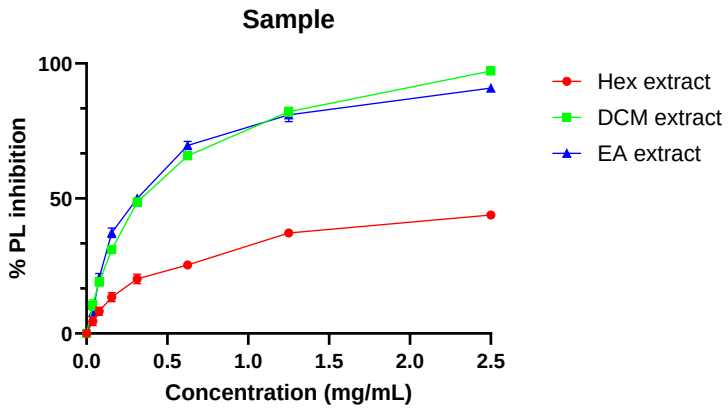

Figure 1. Dose response relationship between *T. spathacea* crude extracts.

Table 1. PL inhibitory activity of crude extracts with comparison to orlistat.

Sample	IC_{50} values (mg/mL)	% PL inhibition at 2.5 mg/mL
Hexane crude extract	> 2.5	43.80 ± 1.80
DCM crude extract	0.35 ± 0.04	97.73 ± 0.59
EA crude extract	0.31 ± 0.01	90.89 ± 0.05
Orlistat (positive control)	58.63 ± 3.14 µM	79.65 ± 1.92 at 200 µM

*Data are expressed as mean ± relative standard deviation, with n = 3. For the last column, values followed by the different letters are significantly different at $p \leq 0.05$, as measured by the one-way ANOVA and Tukey's post-hoc test.

The PL inhibitory activity was carried out at TAR UMT by using the modified method previously reported by Chang et al. [20] with validation under our specific assay conditions. The assay parameters, including temperature and enzyme concentration, were optimized and verified to ensure reproducibility and comparability with the reported method. The PL activity of the extracts was determined by measuring the hydrolysis of *p*NPB to p-nitrophenol, measured at the bandwidth of 405 nm using 96-well plates on a microplate reader (Tecan, Infinite M200 PRO). The lipase inhibitory assays were performed by incubating the crude extracts and the subfractions at concentrations ranging between 31.25 to 1000 µg/mL (in a 6-point dose response format) with PL (5 mg/mL) and *p*NPB (5 mM) in the reaction buffer (50 mM potassium phosphate buffer, pH 8.0) for 20 min. *p*NPB was solubilized with DMSO of the final volume and then diluted with the reaction buffer to a final concentration of 2.5 mM in a 100 µL reaction. The negative control consisted of a reaction mixture without the enzyme, in which the amount of DMSO was the same as in the other experimental groups. The final concentration of DMSO in all reactions was maintained at 1% (v/v) to avoid interfering with enzyme activity. All assays were run at 37°C and the reported results are the average of three biological replicates. Orlistat was used as a positive control. The activity was also examined with and without the crude extract. Blank subtraction was performed by deducting the absorbance values of the corresponding negative controls (without enzyme) to correct for background and color interference from the substrate or extracts. PL inhibition (%) was calculated according to the following formula: PL inhibition (%) $= 100 - [(B - b)/(A - a) \times 100]$; where, A is the activity without inhibitor, a is the negative control without inhibitor, B is the activity with inhibitor, and b is the negative control with inhibitor, also known as blank. IC_{50} value, the concentration of the extract that results in 50% inhibition of maximal activity, was also determined. Data are expressed as mean ± SD (n = 3), and statistical comparisons between extracts (e.g., DCM and EA) were performed using one-way ANOVA, followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Among the crude extracts, the DCM extract showed the highest % PL inhibition (97.73 ± 0.59 %), followed by the EA extract (90.89 ± 0.05 %) and lastly the hexane extract (43.80 ± 1.80 %), at the concentration of 2.5 mg/mL, respectively. Comparing their IC_{50} values, the EA extract exhibited the lowest IC_{50} value (0.31 ± 0.01 mg/mL), followed by the DCM (0.35 ± 0.04 mg/mL) and the hexane extract (>2.5 mg/mL). These results demonstrate that the DCM and EA extracts both could be sources of PL inhibitors with higher efficacy and potency, although the EA extract was slightly more potent than the DCM extract but the other way round in terms of efficacy. This makes the DCM extract (0.53%, w/w) and the EA extract (0.90%, w/w) highly interesting to be further isolated using column fractionation and using their fractions for testing PL inhibition assay.

After the first column fractionation, samples were collected and tested for TLC profiling under UV 254 nm. Those with a similar chemical profiling pattern were combined into one fraction. After combined, a total of seven DCM fractions were tested for PL inhibition assay. Among them, only three fractions showed strong PL inhibitory activity with good IC_{50} values. DCM fraction 2 (DF2) exhibited the most favorable IC_{50} value (0.39 ± 0.06 mg/mL) and % PL inhibition (96.88 ± 1.42 %). This suggests a high interest in investigating these three DCM fractions for the isolation of bioactive compounds which may help in developing anti-obesity drug therapies.

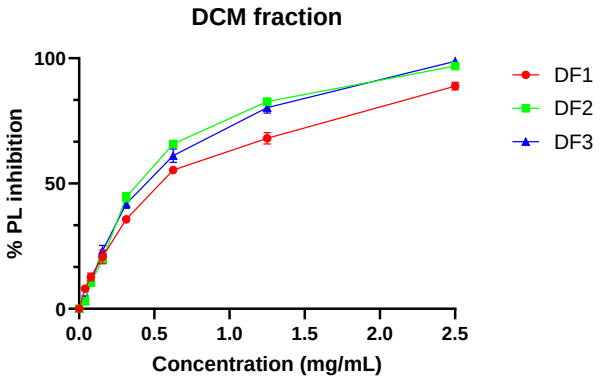

Figure 2. Dose response relationship between DCM fractions.

Table 2. PL inhibitory activity of DCM fractions.

Sample	IC_{50} values (mg/mL)	% PL inhibition at 2.5 mg/mL
DF1	0.54 ± 0.08	88.83 ± 1.58
DF2	0.39 ± 0.06	96.88 ± 1.42
DF3	0.45 ± 0.01	98.80 ± 1.18

*Data are expressed as mean ± relative standard deviation, with n = 3. For the last column, values followed by the different letters are significantly different at $p \leq 0.05$, as measured by the one-way ANOVA and Tukey's post-hoc test.

Same goes to the EA extract, a total of four EA combined fractions were collected after the first column fractionation and tested for PL inhibition assay. Among them, only four fractions showed strong PL inhibitory activity with good IC_{50} values. EF fraction 1 (EF1) exhibited the most favorable IC_{50} value (0.33 ± 0.11 mg/mL) and % PL inhibition (77.92 ± 2.03%). These four fractions are highly recommended for the isolation of bioactive compounds.

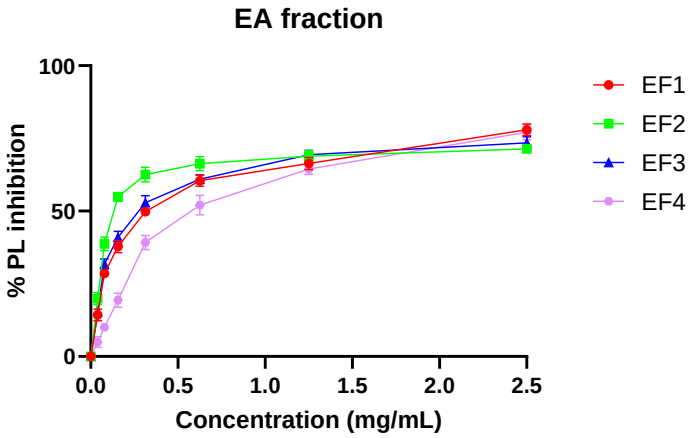

Figure 3. Dose response relationship between EA fractions.

Table 3. PL inhibitory activity of EA fractions.

Sample	IC_{50} values (mg/mL)	% PL inhibition at 2.5 mg/mL
EF1	0.33 ± 0.11	77.92 ± 2.03
EF2	0.13 ± 0.03	71.41 ± 1.44
EF3	0.28 ± 0.12	73.44 ± 2.13
EF4	0.59 ± 0.27	77.13 ± 2.61

*Data are expressed as mean ± relative standard deviation, with n = 3. For the last column, values followed by the different letters are significantly different at $p \leq 0.05$, as measured by the one-way ANOVA and Tukey's post-hoc test.

CONCLUSION

The DCM and EA crude extracts were identified as the most interesting candidates for subsequent analyses. Three DCM fractions and four EA fractions showed good IC_{50} values (0.13 - 0.59 mg/mL) with strong PL inhibitory activity (71.41 - 98.80%) at 2.5 mg/mL. For future perspective, molecular networking could be incorporated into the study for guiding the isolation of bioactive compounds. Future studies involving in-silico molecular docking and enzyme kinetic analyses will be essential to rationalize the observed inhibitory activity. Further research is needed to explore their mechanism of action and optimize their efficacy.

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