INTRODUCTION Free radicals are naturally generated in all living cells as a part of normal cell functions. However, excessive free radicals, either from endogenous and exogenous sources, can be harmful to biological molecules such as protein, lipid, and DNA (Phaniendra et al. 2015). Cellular damages such as lipid peroxidation of the membrane lipid and biomolecules have been linked to the pathogenesis and progression of various chronic and degenerative diseases, such as cardiovascular diseases and diabetes mellitus.

However, the harmful effects of oxidative stress can be prevented by the consumption of antioxidants (Ayala et al., 2014; Lobo et al., 2010). In this case, antioxidant compounds may reduce oxidative stress conditions by stabilizing free radicals by donating protons or electrons or chelating pro-oxidant metal ions (Tan et al., 2018; Kurutas, 2016). Type 2 Diabetes mellitus (T2DM) is a metabolic disorder characterized by a high level of postprandial blood glucose.

This condition can be due to insufficient insulin secretion, or resistance to insulin action, or a combination of both (American Diabetes Association, 2009). It has been known that persistent hyperglycemia induces oxidative stress through multiple interacting pathways, including activation of protein kinase C, activation of the polyol pathway, and increased formation of the advanced glycation end product (Giacco & Brownlee, 2010; Mohora et al., 2007). Besides, the resulting oxidative stress may further damage the pancreatic β-cells, which produce insulin.

In addition to oxidative stress, studies have shown that obesity could also increase the risk for T2DM (Stokes et al., 2018). Current management in T2DM and obesity include inhibition of key enzymes related to carbohydrate and lipid metabolisms. Examples of this type of medication include acarbose and orlistat for T2DM and obesity, respectively (Vieira et al., 2019). However, these synthetic inhibitors seem to exert significant adverse side effects that potentially interfere with their clinical uses, such as abdominal discomfort, liver problems, and lactic acidosis (Saha & Verma, 2012).

Consequently, there is a need for other alternatives. One possible option could come from natural inhibitors of plant origin, including vegetables and fruit. They have gained global considerations for screening bioactive compounds of medicinal attributes, including antioxidant, antidiabetic, and anti-obesity activities (Choudhury et al., 2018). Besides, high consumption of vegetables and fruit has been recognized to positively correlate with decreased risk of chronic and nondegenerative diseases, such as cardiovascular disease, cancer, and diabetes mellitus (Carter et al., 2010; van’t Veer et al., 2000). Ipomoea reptans (synonym: Ipomoea aquatica), locally
knows as ‘kangkung,’ in Indonesia is a green leafy vegetable distributed widely in the South and Southeast Asia region Indonesia, Malaysia, and India. It belongs to the family Convolvulaceae. Ipomoea reptans is an aquatic plant, easily cultivated in muddy or moist soil. It has a long, hollow, and tender shoot. The leaves are long, heart-shaped, and rich with high nutrients, including vitamin A and C and essential minerals such as calcium and iron (Dewanjee et al., 2015; Rahman & Parkpain, 2004).

Ipomoea reptans leaves are frequently consumed and are one of the popular choices in the Indonesian diet. However, there is limited information regarding the bioactivities of I. reptans leaves. Previously, I. reptans leaves have been reported for their in vivo antioxidant and antihyperglycemic activities (Saha et al., 2008). A recent study showed that the antidiabetic activity could be due to the protective effect of I. reptans on the pancreatic β-cells (Hayati et al., 2017). Furthermore, I. reptans extract was prepared as a nano-emulsifying drug and showed antihyperglycemic activity using the zebrafish (Danio rerio) model (Hayati et al., 2018).

The present study sought to investigate antioxidant activity and possible inhibition on α-glucosidase, lipase, and trypsin by I. reptans leaves and its fractions in several solvent systems using in vitro methods. MATERIALS AND METHODS Materials Spectrophotometer measurements were carried out using a Biochrom Libra-S22 (Cambridge, UK). All solvents and chemicals used in the experiments were of analytical grade. Folin & Ciocalteu’s phenol reagent, α-glucosidase from Saccharomyces cerevisiae (EC 3.2.1.20), p-nitrophenyl-α-D-glucopyranoside, acarbose, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), and 3,5-di-tert-butyl-4-hydroxytoluene (BHT), porcine pancreatic trypsin (EC 3.4.21.4), orlistat, rutin, and sodium diclofenac were purchased from Sigma-Aldrich (St. Louis, US). Gallic acid was obtained from Santa Cruz Biotechnology (Dallas, US). Sodium carbonate (Na2CO3) was purchased from Merck (Darmstadt, Germany). Plant material and extract preparation The leaves of I. reptans were collected from the Tangerang, Banten, in April 2019. The plant was identified by one of the authors (AWS) based on its vegetative morphological organs (stems, leaves, and roots) (Madani et al., 2015). A voucher specimen (UKKW.D.007) was deposited at the Krida Wacana Herbarium Centre. As much as 15 g dried powdered leaves were soaked in 400 mL ethanol and left for eight days with intermittent shaking. Extraction was repeated three times.

The filtered supernatant was concentrated by a rotary evaporator (Rotavapor R3, Buchi Labortechnick AG, Switzerland) to obtain ethanol crude extract. The crude extract was then partitioned using hexane and ethyl acetate to obtain hexane, ethyl acetate, and ethanol fractions. Each fraction was reduced to dryness under reduced pressure, then
used for the preparation of stock solution for the various analyses.

**Determination of phenolic and flavonoid contents**

Estimation of total phenolic content

Total phenolic content (TPC) of each fraction was determined based on a Folin-Ciocalteu’s method reported previously (Khatoon et al., 2013). Gallic acid (12.5 – 200 µg/mL) was used to generate a standard curve. Results were presented as mg gallic acid equivalent (mgGAE)/g dried biomass. Total flavonoid content Total flavonoid content (TFC) of each fraction was determined based on an AlCl₃ colorimetric method, as reported previously (Simamora et al., 2018a). Quercetin (3.20 – 200 µg/mL) was used to generate a standard curve. Results were presented as mg quercetin equivalent (mgQE)/g dried biomass.

**Determination of antioxidant activities**

**DPPH radical scavenging assay**

The ability of different I. reptans fractions to scavenge DPPH radicals were evaluated based on a reported method (Simamora et al., 2018b). An aliquot of 1 mL from each fraction solution was added with 3 mL of 0.6 mM DPPH solution. The reaction mixture was incubated in the dark at ambient temperature for 30 minutes. The absorbance was measured at 517 nm. The absorbance of DPPH in ethanol was used as a control solution, and those of ascorbic acid and BHT were used as positive controls.

The percentage of scavenging activity was calculated as \((A - B)/A \times 100\), where \(A\) is the absorbance of the control solution, and \(B\) is the sample solution's absorbance. Radical scavenging activity was presented as IC50 values. CuPRAC assay

Cupric ion reducing antioxidant capacity (CuPRAC) assay was carried out based on a method described previously (Aktumsek et al., 2013). A reaction mixture was prepared to contain 1 mL of 10 mM CuCl₂, 1 mL of 7.5 mM neocuproine in ethanol, and 1 mL of 1 M NH₄OAc buffer (pH 7.0). Into this mixture was added 0.5 mL extract solution and 0.6 mL water to make a total volume of 4.1 mL.

The reaction mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 450 nm. Trolox (10 – 320 µg/mL) was used to prepare a standard curve, and results were reported as mg Trolox equivalent (mgTE)/g dried material. Total antioxidant assay

Each fraction's total antioxidant activity was determined by a phosphomolybdenum method described previously (Prieto et al., 1999). A phosphomolybdenum reagent was prepared, containing 4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM trisodium phosphate. The reaction mixture was prepared in a capped tube, consisting of 3 mL of phosphomolybdenum reagent and 0.3 mL of the test solution.

The tube was incubated in a boiling water bath for 90 minutes and after that was let to
cool at room temperature. Absorbance was measured at 695 nm on a spectrophotometer. Trolox (40 – 1000 µg/mL) was used to generate a standard curve, and results were expressed as mg Trolox equivalent (mgTE)/g dried biomass. Reducing power assay Reducing power assay was carried out based on a ferric thiocyanate method reported previously (Gülçin et al., 2012). Reaction mixture was prepared containing 1 mL test solution, 2.5 mL of 0.2 M phosphate buffer pH 6.6, and 2.5 mL of 1% (w/v) potassium ferric cyanide K3[Fe(CN)6]. The reaction mixture was incubated in a water bath at 50°C for 20 minutes and then cooled at room temperature. This mixture was added with 2.5 mL of 10% (w/v, water) trichloroacetic acid, followed by centrifugation of the mixture at 3000 rpm for 10 minutes. The upper layer (2.5 mL) was taken out and mixed with 0.5 mL of 1% (w/v, water) FeCl3 and 2.5 mL water. The absorbance was measured at 700 nm on a spectrophotometer. Ascorbic acid (1.56 – 100 µg/mL) was used to generate a standard curve, and results were reported as mg ascorbic acid equivalent (mgAAE)/g dried biomass.

α-Glucosidase inhibition assay Inhibition of α-glucosidase was assayed by a previously reported method (Simamora et al., 2019). In this assay, p-nitrophenyl-α-D-glucopyranoside (pNPG) was used as a substrate. The reaction mixture contained 50 µL of test solution of different concentrations, 50 µL of 0.5 U/mL α-glucosidase, and 50 µL of 0.05 M phosphate buffer (pH 6.8). After pre-incubating for five minutes at 37°C, the mixture was added with 100 µL of 1 mM pNPG to start the reaction. The reaction was incubated for 20 minutes at 37°C, and 750 µL of 0.1 M Na2CO3 was added to terminate the reaction. Absorbance was measured at 405 nm on a spectrophotometer. The control solution was measured by replacing the sample with phosphate buffer. Acarbose was used as a positive control. The percentage of inhibition was calculated as (A – B)/A x 100, where A is the absorbance of the control solution, and B is the sample solution’s absorbance. Inhibition activity was presented as IC50 (µg/mL). Qualitative test for lipase inhibition Inhibition on lipase was assayed by a qualitative method of a phenol red agar plate reported previously (Gupta et al., 2015), with some modifications. In this method, agar (2%, w/v) was suspended with phenol red indicator (0.01%, w/v) and olive oil as a substrate (1%, v/v). The test solution was prepared by mixing in a 1 : 1 ration of each extract or orlistat (a synthetic lipase inhibitor) and porcine pancreatic lipase solution (200 U/ml in 0.05 M tris buffer pH 8.0 and NaCl 0.03 M). A 50 µL of this test solution was suspended into a circular well in the agar, and the reaction was incubated for ten minutes at 37°C. Lipolytic degradation releases fatty acids from the substrate, which changes the indicator color from yellow to red.
Qualitative test for trypsin inhibition activity Inhibition activity of I. reptans leaves fractions on trypsin was evaluated based on a qualitative method as reported before (Vijayaraghavan & Vincent, 2013), with some modifications. An agar plate was prepared by dissolving agar (1.5%, w/v) added with skimmed milk (5%, w/v). The agar solution was poured into Petri dishes and let to solidify. Wells of 5 mm were punched. The test solution was prepared by mixing trypsin solution (10 mg in 10 mL of 100 mM tris buffer HCl pH 7.6) with fractions of I. reptans leaves or natrium diclofenac (positive control) in a 1 : 1 ratio.

A 50 µL of each test solution was loaded into each well and incubated overnight at 37°C. Trypsin inhibition was observed by a decrease in zone diameter in the presence of inhibitors. Statistical analysis All experiments were conducted in three replicates, and results were presented as mean ± SD. The significance of difference among multiple averages was determined by analysis of variance (ANOVA), followed by a Tukey post hoc test at a 5% significance level.

RESULTS AND DISCUSSION Total phenolic and flavonoid contents In this study, the leaves of I. reptans were extracted using ethanol, and partitioned by hexane and ethyl acetate. Fractions were tested for their TFC (by aluminum chloride method with quercetin standard) and TPC (by Folin-Ciocalteu’s method with gallic acid standard). The TPC and TFC of I. reptans leaves concerning solvents used for fractionation are presented in Table I. It is clear that solvents significantly affected TPC and TFC obtained (p <0.05).

Among three different solvents, ethyl acetate appeared to be the best solvent to extract phenolic compounds, followed by ethanol and hexane. However, in TFC results, maximum TFC was obtained in ethanol, followed by ethyl acetate and hexane. It was reported previously that the use of a polar solvent such as methanol and water resulted in high phenolic content but low flavonoid content (Dasgupta & De, 2007; Prasad et al., 2005). In the present study, a less polar solvent such as ethyl acetate was effectively extracting phenolics, whereas ethanol was more effective for flavonoids. The majority of flavonoids contain phenolic groups that would be identified in the phenolic assay. Thus, it was expected that the phenolic contents of the extracts would be higher than their flavonoids, such as those observed for ethyl acetate and hexane extracts. However, for the ethanol extract, phenolic content was found to be lower than flavonoid content. It might be that the use of a polar extraction solvent contributes to the observed results. Previous studies have also reported similar findings in which higher TFC than TPC was obtained (Srisupap & Chaicharoenpong, 2021; Ling et al., 2019).
However, researchers have not found a more definitive explanation for why this phenomenon can occur and open the possibility of further research to find the cause. Table I. **Total phenolic and flavonoid contents** of I. reptans leaves fractions Parameters  

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I. reptans fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>_ _ _ ethanol _ethyl acetate _hexane _ TPC (mgGAE/g dried biomass)</td>
<td></td>
</tr>
<tr>
<td>_0.86±0.00 _2.19±0.11 _0.11±0.00 _ TFC (mgQE/g dried biomass)</td>
<td></td>
</tr>
<tr>
<td>_1.45±0.00 _0.35±0.00 _0.03±0.00</td>
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</table>

In vitro antioxidant activities One of the antioxidant mechanisms of action is removing free radicals, which can be achieved by transferring protons or electrons from antioxidant compounds to the free radicals (Lobo et al., 2010).

In the present study, the radical scavenging activity of I. reptans fractions was evaluated using stable DPPH radicals. The use of DPPH radicals may be relevant to represent a lipophilic radical initiated by lipid auto-oxidation (Shukla et al., 2016). It was proposed that the scavenging mechanism for DPPH to form the non-radical DPPH-H is predominantly through proton transfer (Marxen et al., 2007). The scavenging capacity of I. reptans leaves fractions on DPPH radicals was presented as % inhibition and IC50 values as shown in Table II. All fractions exhibited inhibition on DPPH radicals in a concentration-dependent manner.

This is indicative of the proton donating capacity of all fractions. Based on their IC50 values, it is worth noting that the **DPPH radical scavenging** activities of ethanol and hexane samples did not differ significantly (p >0.05). The order, as seen in the table, is ethanol > hexane > ethyl acetate. Table II. **DPPH radical scavenging activity of I. reptans leaves fractions**  

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (mg/mL)</th>
<th>Inhibition (%)</th>
<th>IC50 (mg/mL)</th>
</tr>
</thead>
</table>
| _Ethanol _0.41 0.62 0.83 1.03 _20.82 ± 0.67 29.62 ± 2.53 48.72 ± 0.75 53.28 ± 3.49 _1.12 ± 0.02 _Ethyl acetate _1.25 2.50 3.75 5.00 _17.05 ± 3.41 34.10 ± 0.33 51.23 ± 0.25 67.94 ± 2.96 _3.76 ± 0.08 _Hexane _0.44 0.88 1.75 _26.88 ± 0.35 42.95 ± 0.40 60.59 ± 1.15 _1.33 ± 0.04 _Ascorbic acid _0.01 0.02 0.04 0.08 _8.56 ± 0.11 15.28 ± 0.12 40.48 ± 1.28 75.96 ± 0.18 _0.05 ± 0.00 _BHT _0.003 0.007 0.013 _0.027 _12.65 ± 1.04 23.15 ± 0.20 40.99 ± 0.59 56.28 ± 1.86 _0.02 ± 0.00 _Phenolic and flavonoid compounds are known to be strong proton donors (Paixão et al., 2007). Quercetin derivative isolated from I. reptans was shown to have a potent **DPPH radical scavenging activity** (Prasad et al., 2005).

The present study suggests that antioxidant activity may not solely be attributed to **phenolic and flavonoid compounds** and that other compounds may also contribute to the scavenging activity. A previous study has reported that non-phenolic compounds isolated from plants had antioxidant activity (el-Sayed et al., 2008). In addition to radical scavenging activity, I. reptans extracts' antioxidant activity was also evaluated by their reducing capacity. Compounds having reducing capacity indicate their ability to act as...
an electron donor to any oxidized intermediates to form more stable species of lower oxidation states.

In the present study, CuPRAC, phosphomolybdenum, and ferric thiocyanate methods were employed to investigate the reducing potential of ethanol, ethyl acetate, and hexane fractions from I. reptans. These methods are different in terms of reaction conditions and the metal ions used. However, in all methods, higher values indicate a stronger reducing capacity. Results in Table III suggested that I. reptans had reducing capacities. This indicates that all fractions' antioxidant compounds can transfer an electron to metals of higher oxidation number and reduce them to lower oxidation states.

In this case, Cu(II) to Cu(I), Mo(VI) to Mo(V), and Fe(III) to Fe(II), for CuPRAC, Total antioxidant, and Ferric thiocyanate activities, respectively. Results also indicated that the solvent had a pronounced effect on the reducing capacity, as indicated by significantly different activities observed among extracts. Based on the CuPRAC assay, hexane fraction exhibited the highest activity, followed by ethanol and ethyl acetate fractions. The same order was observed based on reducing power assay.

However, a slight difference was observed using the phosphomolybdenum assay, where the order follows ethanol > hexane and ethyl acetate fractions. The difference could be due to each metal ion's sensitivity used in the respective method (Choirunnisa et al., 2016). It is known that the ease of each metal ion to be reduced to a lower oxidation state depends on the redox potential of each metal ion. Table III. Reducing capacity of I. reptans leaves fractions as measured by three different methods Parameters _I. reptans _ _ethanol _ethyl acetate _Hexane _ _Phosphomoly-bdenum (mgAAE/g dried biomass) _5.04 ± 0.03 _3.09 ± 0.02 _3.96 ± 0.08 _ _CuPRAC (mgTE/g dried biomass) _9.58 ± 0.19 _6.72 ±0.54 _13.79 ± 0.30 _ _Reducing power (mgAAE/g dried biomass) _2.00 ± 0.01 _1.90 ± 0.01 _8.03 ± 0.01 _ A previous study reported the antioxidant activity of methanol extract of I. reptans leaves using an animal model (Saha et al., 2008). This study used STZ induced diabetic rats and observed a decrease in MDA level and an increase in GSH level in the liver, pancreas, and kidney tissue of extract-treated rats, indicating a lower oxidative stress condition to extract treatment. These results complement those observed in DPPH scavenging activity; thus, I. reptans can act as radical scavengers and reducing agents.

In vitro antidiabetic activity As α-glucosidase hydrolyzes the catalytic degradation of polysaccharides or oligosaccharides into glucose, this enzyme has become a therapeutic target for regulating blood glucose levels. In vitro antidiabetic activity of ethanol, ethyl
acetate, and ethanol fractions of I. reptans was evaluated by examining their inhibition effect on α-glucosidase activity. In this study, acarbose, a standard α-glucosidase inhibitor, was used as a positive control. Results in Table IV shows that only hexane and ethanol fractions exhibited inhibition activity on α-glucosidase.

In both cases, % of inhibition activities increased with increasing extract concentrations. However, hexane showed significantly stronger activity than ethyl acetate and acarbose (p >0.05). On the other hand, ethyl acetate showed no inhibition activity on α-glucosidase. Table IV. α-Glucosidase inhibition activity of I. reptans leaves fractions

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (mg/mL)</th>
<th>Inhibition (%)</th>
<th>IC50 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.14 0.21 0.28 0.34 3.24 ± 2.82 8.47 ± 5.09 60.07 ± 2.12 89.18 ± 1.68 0.26 ± 0.01 _</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3.13 6.25 12.50 2.99 1.88 5.76 _</td>
<td>No activity _</td>
<td>_</td>
</tr>
<tr>
<td>Hexane</td>
<td>0.01 0.02 0.03 0.05 6.34 ± 1.14 10.15 ± 6.15 24.03 ± 4.39 57.66 ± 8.09 0.04 ± 0.00 _</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Acarbose</td>
<td>0.03 0.05 0.08 0.10 _</td>
<td>_</td>
<td>0.10 ± 0.00 _</td>
</tr>
</tbody>
</table>

This finding was supported by a previous study reporting antihyperglycemic activity of methanol extract of I. reptans leaves using STZ induced diabetic rats (Saha et al., 2008).

The observed hypoglycemic activity in this animal model may be due to the inhibition activity of I. reptans leaves on α-glucosidase. Various phenolic and flavonoid compounds have been reported to inhibit α-glucosidase in vitro (Limanto et al., 2019; Yin et al., 2014). However, the lack of activity observed in ethyl acetate indicates that α-glucosidase inhibition activity is not only attributed to phenolic and flavonoid compounds. In vitro anti-lipase activity In vitro anti-obesity activity for ethanol and ethyl acetate fractions were conducted based on inhibition activity on lipase.

In the present study, a qualitative method using a phenol red agar plate was used, and the results can be seen in Figure 1. Figure 1 (well A) shows an olive oil – lipase system in the absence of an inhibitor. The strong intensity in the red halo and large halo diameter indicate no inhibition on lipase. However, in the presence of inhibitors, orlistat (well B), ethyl acetate (well C), and ethanol fractions (well D), positive inhibitions on lipase were observed. In each case, a decrease in halo diameter, and less intense red halo was observed. Inhibition of lipase by hexane extract could not be determined due to poor solubility in the system.

Inhibition of lipase decreased lipolytic degradation of olive oil, thus generating fewer fatty acids. Previously, many plants have been studied for their anti-lipase activity (Rajan et al., 2020). However, to date, no studies yet reported on the anti-lipase activity of I. reptans. The present study's finding may serve as a preliminary screening for further investigations on the anti-obesity activity of I. reptans leaves. / Figure 1. Anti-lipase activity assay, olive oil and lipase without inhibitor (A), in the presence of orlistat (B),
ethyl acetate (C), and ethanol fractions (D). In vitro anti-trypsin activity Trypsin has been studied for its role in treating obesity (de Lima et al., 2019). The previous result in an in vivo study suggested that treatment with synthetic trypsin inhibitor reduced intake and meal size of obese rats, possibly by modulating satiety hormone such as cholecystokinin (McLaughlin et al., 1983).

In the present study, fractions of I. reptans were evaluated for their potential trypsin inhibition activity, and results were presented in Figure 2. Figure 2 (Well A) shows the trypsin system in the absence of an inhibitor, exhibited by a maximum zone diameter. However, in the presence of inhibitors, zone diameter decreases were observed, as can be seen for wells B, C, and D, indicating trypsin activity inhibition.

Trypsin inhibition activity for hexane extract could not be determined due to the extract’s low solubility in the reaction system. To date, very few studies reported on the anti-trypsin activity of plant extracts (Miedzianka et al., 2020). The positive results obtained in this study can be a starting point to further study the trypsin inhibition effect of I. reptans, in conjunction with an anti-obesity activity through in silico or in vivo studies. / Figure 2. Anti-trypsin activity assay, without inhibitor (A), ethyl acetate (B), ethanol fractions (C), and Natrium Diclofenac (D) CONCLUSION The present study proved the antioxidant, antidiabetic, and anti-obesity properties of I. reptans leaves.

The study revealed that solvents used in the partition significantly influenced the antioxidant and a-glucosidase inhibition activity. The study can conclude that I. reptans leaves could be a natural resource for antioxidant, antidiabetic, and anti-obesity agent.

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