


## Research Article

**Antioxidant, Antidiabetic, and Anti-obesity Potential of *Ipomoea reptans* Poir Leaves**Hendrik Kurniawan <sup>1</sup>Ermenilda Sonia Dacamis <sup>2</sup>Adelina Simamora <sup>1,4\*</sup> Priscilla Sari Dianauli Lumban Tobing <sup>2</sup>Ali Hanapiah <sup>2</sup>Adit Widodo Santoso <sup>3,4</sup> 

<sup>1</sup>Department of Biochemistry, Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, West Jakarta, Jakarta Capital Special Region, Indonesia

<sup>2</sup>Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, West Jakarta, Jakarta Capital Special Region, Indonesia

<sup>3</sup>Research Unit, Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, West Jakarta, Jakarta Capital Special Region, Indonesia

<sup>4</sup>Centre for Enzyme Research in Health and diseases (CERiH), Universitas Kristen Krida Wacana, West Jakarta, Jakarta Capital Special Region, Indonesia

\*email: [adelina.simamora@ukrida.ac.id](mailto:adelina.simamora@ukrida.ac.id)

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$\alpha$ -Glucosidase inhibition

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*Ipomoea reptans*

**Abstract**

*Ipomoea reptans* Poir or kangkung is a popular leafy vegetable, a favorite to people in Asian countries. However, limited information is available on their bioactivities. In the present study, the antioxidant, antidiabetic, and anti-obesity potential of *I. reptans* leaves were investigated. Different fractions (ethanol, ethyl acetate, and hexane) of *I. reptans* leaves were evaluated for their scavenging activity on DPPH radicals, whereas their reducing potential was investigated by cupric reducing antioxidant capacity (CuPRAC), total antioxidant, and reducing power assays. The antidiabetic potential was investigated by their inhibition effect on  $\alpha$ -glucosidase. Total phenolic and flavonoid contents of *I. reptans* leaves were solvent dependent. Ethyl acetate contained the highest phenolic content, followed by ethanol and hexane fractions. However, for flavonoid content, the order was ethanol > ethyl acetate > hexane. All fractions showed DPPH scavenging activity in a concentration-dependent manner, with activities weaker than standards ascorbic acid and BHT, in the order of ethanol > ethyl acetate > hexane. All fractions showed reducing capacity, but only hexane and ethanol fractions of *I. reptans* leaves showed inhibition on  $\alpha$ -glucosidase, with hexane showed more potent inhibition compared to acarbose. The study also found that fractions of *I. reptans* inhibit lipase and trypsin, enzymes related to lipid metabolism. Findings in this study offer a prospect for *I. reptans* leaves as a functional food source for antioxidant, antidiabetic, and anti-obesity purposes.

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**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  $\beta$ -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 known 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  $\beta$ -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  $\alpha$ -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,  $\alpha$ -glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), p-nitrophenyl- $\alpha$ -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 ( $\text{Na}_2\text{CO}_3$ ) 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 Laborotechnick 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  $\mu\text{g}/\text{mL}$ ) 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  $\text{AlCl}_3$  colorimetric method, as reported previously (Simamora *et al.*, 2018a). Quercetin (3.20 – 200  $\mu\text{g}/\text{mL}$ ) 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  $\text{IC}_{50}$  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  $\text{CuCl}_2$ , 1 mL of 7.5 mM neocuproine in ethanol, and 1 mL of 1 M  $\text{NH}_4\text{OAc}$  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  $\mu\text{g}/\text{mL}$ ) 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  $K_3[Fe(CN)_6]$ . 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)  $FeCl_3$  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.

#### $\alpha$ -Glucosidase inhibition assay

Inhibition of  $\alpha$ -glucosidase was assayed by a previously reported method (Simamora *et al.*, 2019). In this assay, *p*-nitrophenyl- $\alpha$ -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  $\alpha$ -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  $Na_2CO_3$  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  $(A - B)/A \times 100$ , where A is the absorbance of the control solution, and B is the sample solution's absorbance. Inhibition activity was presented as  $IC_{50}$  (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  $\mu$ L 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  $\pm$  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	<i>I. reptans</i> fractions		
	ethanol	ethyl acetate	hexane
TPC (mgGAE/g dried biomass)	0.86 $\pm$ 0.00	2.19 $\pm$ 0.11	0.11 $\pm$ 0.00
TFC (mgQE/g dried biomass)	1.45 $\pm$ 0.00	0.35 $\pm$ 0.00	0.03 $\pm$ 0.00

### 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 IC<sub>50</sub> 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 IC<sub>50</sub>

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

Samples	Concentration (mg/mL)	Inhibition (%)	IC <sub>50</sub> (mg/mL)
Ethanol	0.41	20.82 ± 0.67	1.12 ± 0.02
	0.62	29.62 ± 2.53	
	0.83	48.72 ± 0.75	
	1.03	53.28 ± 3.49	
Ethyl acetate	1.25	17.05 ± 3.41	3.76 ± 0.08
	2.50	34.10 ± 0.33	
	3.75	51.23 ± 0.25	
	5.00	67.94 ± 2.96	
Hexane	0.44	26.88 ± 0.35	1.33 ± 0.04
	0.88	42.95 ± 0.40	
	1.75	60.59 ± 1.15	
Ascorbic acid	0.01	8.56 ± 0.11	0.05 ± 0.00
	0.02	15.28 ± 0.12	
	0.04	40.48 ± 1.28	
	0.08	75.96 ± 0.18	
BHT	0.003	12.65 ± 1.04	0.02 ± 0.00
	0.007	23.15 ± 0.20	
	0.013	40.99 ± 0.59	
	0.027	56.28 ± 1.86	

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>I. reptans</i>		
	ethanol	ethyl acetate	Hexane
Phosphomolybdenum (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  $\alpha$ -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  $\alpha$ -glucosidase activity. In this study, acarbose, a standard  $\alpha$ -glucosidase inhibitor, was used as a positive control.

Results in **Table IV** shows that only hexane and ethanol fractions exhibited inhibition activity on  $\alpha$ -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  $\alpha$ -glucosidase.

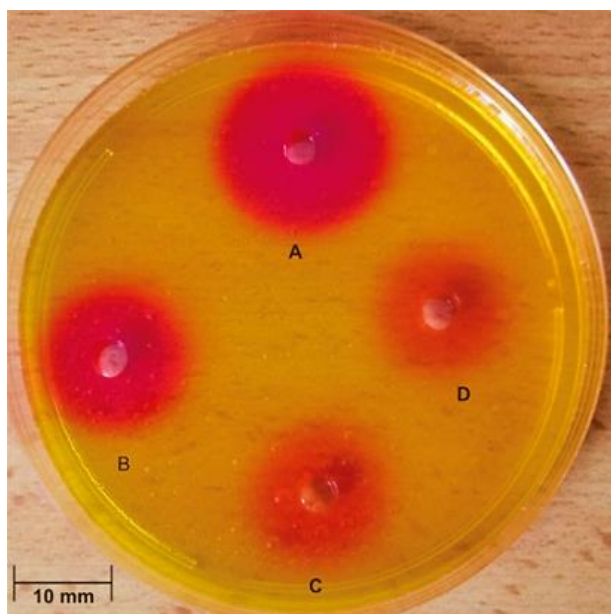
**Table IV.**  $\alpha$ -Glucosidase inhibition activity of *I. reptans* leaves fractions

Samples	Concentration (mg/mL)	Inhibition (%)	IC <sub>50</sub> (mg/mL)
Ethanol	0.14	3.24 ± 2.82	0.26 ± 0.01
	0.21	8.47 ± 5.09	
	0.28	60.07 ± 2.12	
	0.34	89.18 ± 1.68	
Ethyl acetate	3.13	2.99	No activity
	6.25	1.88	
	12.50	5.76	
Hexane	0.01	6.34 ± 1.14	0.04 ± 0.00
	0.02	10.15 ± 6.15	
	0.03	24.03 ± 4.39	
	0.05	57.66 ± 8.09	
Acarbose	0.03	19.42 ± 3.41	0.10 ± 0.00
	0.05	30.09 ± 2.52	
	0.08	40.27 ± 3.35	
	0.10	50.07 ± 2.85	

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  $\alpha$ -glucosidase. Various phenolic and flavonoid compounds have been reported to inhibit  $\alpha$ -glucosidase in vitro (Limanto *et al.*, 2019; Yin *et al.*, 2014). However, the lack of activity observed in ethyl acetate indicates that  $\alpha$ -glucosidase inhibition activity is not only attributed to phenolic and flavonoid compounds.

#### *In vitro* anti-lipase activity

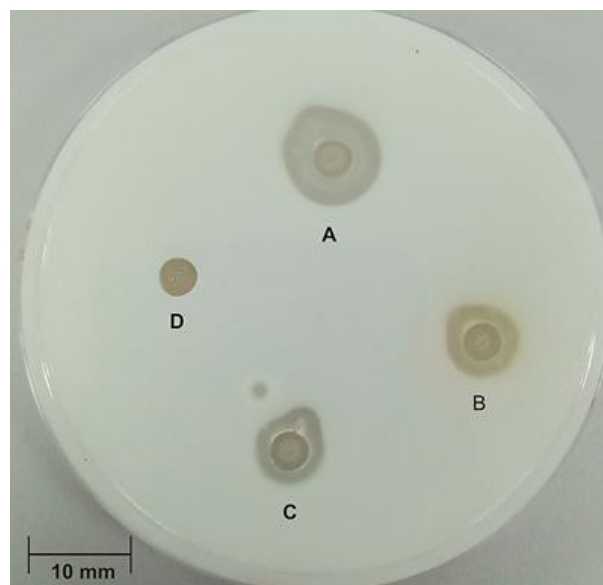
*In vitro* anti-obesity activity for ethanol and ethyl acetate fractions were conducted based on inhibition activity on lipase. In the present study, a qualitative method using a phenol red agar plate was used, and the results can be seen in **Figure 1**. **Figure 1** (well A) shows an olive oil – lipase 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  $\alpha$ -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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