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The Potential of α- glucosidase Inhibition from Endophytic Fungi Associated in *Portulaca oleracea* L.

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ABSTRACT

Introduction: The discovery of endophytic microbes, which are microorganisms that reside within plant tissues and can produce bioactive compounds with similar properties to their host plants, is one possible solution to this issue. Endophytic fungi are capable of manufacturing antibacterial, antifungal, antiviral, anticancer, antimalarial, and antidiabetic agents. Portulaca oleracea L. is a weed that has spread globally. Portulaca oleracea L.has been utilized as a nutritious and medicinal plant for countless centuries. **Aims**: This study seeks to assess the antidiabetic potential of the endophytic fungi Portulaca oleracea L. **Methods**: In this study, isolation, macroscopic testing, fermentation, and antidiabetic activity testing were conducted using the alpha-glucosidase method. Twelve isolates with macroscopically distinct characteristics were obtained from of isolating the endophytic fungi. The procedure then advances to the fermentation and ethyl acetate extraction phases. **Result**: The results showed that there was inhibition of the α-glucosidase enzyme in hydrolyzing substrates into glucose by secondary metabolite extracts of fungi associated with the plant *Portulaca oleracea* L. with the highest percentage inhibition on the lab scale in the 12th isolate (83.92%). **Conclusion**: This measurement gives good results as an α-glucosidase inhibitor, so its potential as a source of antidiabetic drug substances is very high.

KEYWORDS: *Portulaca oleracea* L., endophytic fungi, antidiabetic, inhibition, and α -glucosidase..

INTRODUCTION

Diabetes Mellitus is a metabolic disorder characterized by hyperglycemia and abnormalities in carbohydrate, lipid, and protein metabolism resulting from decreased insulin secretion, decreased insulin sensitivity, or both. Due to the insufficient quantity and potency of insulin, glucose is not utilized by the cells and instead accumulates in the blood (Paramitha, 2016). Type 2 diabetes is the most prevalent form of the disease, accounting for approximately 90% of all cases worldwide (IIDF, 2019). According to the International Diabetes Federation, after China, India, the United States, Brazil, and Mexico, Indonesia has the sixth-most diabetes patients on the globe. In 2017, there were approximately 10.3 million diabetics in Indonesia; by 2045, this figure is projected to reach 16.7 million. Approximately 7.6 million (73.7%) of these 10.3 million individuals are undiagnosed, putting them at risk of developing complicati-

ions unknowingly and without intervention (IIDF, 2017).

Alpha-glucosidase is a crucial enzyme involved in the hydrolysis of carbohydrates into glucose. By inhibiting carbohydrate-digesting enzymes such as alpha-amylase and alfa-glucosidase, this enzyme has the effect of delaying glucose assimilation (Yuniarto & Selifiana, 2018). Inhibition of the carbohydrate-digesting enzymes alpha-glucosidase and alfa-amylase can significantly reduce postprandial blood glucose increases after a carbohydrate-rich meal and may be important in the postprandial management of blood sugar levels in patients with type 2 diabetes and borderline patients (Subramanian *et al.,* 2008). Acarbose and miglitol, which inhibit glycosidases such as glucosidase and amylase, are currently used clinically as inhibitors. Nevertheless, many hypoglycemic agents have drawbacks, namely that they cause adverse effects and exacerbate diabetes complications. The primary adverse effects of the beta-glucosidase inhibitor on the gastrointestinal tract include bloating, nausea, diarrhea, and flatulence, among others. Natural glucosidase inhibitors derived from natural constituents can be used to treat hyperglycemia because they have fewer adverse effects and are less expensive than synthetic antihyperglycemic medications (Sudha *et al.,* 2011).

Portulaca oleracea L. is a popular plant that has been studied for its anti-diabetic effects. More than 120 species comprise the family Portulacaceae, which includes Portulaca oleracea L. (Azuka *et al.,* 2014). Various compounds have been isolated from Portulaca oleracea L plants, including flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, vitamins, proteins, and minerals, which contain omega-3 fatty acids typically found in fish fat (Zhou *et al.,* 2015). These compounds exhibit an array of pharmacological effects, including antibacterial, antiulcerogenic, anti-inflammatory (Agyare, 2015), antioxidant, and anti-diabetic properties (Gao *et al.,* 2010).

Zhang *et al*. (2015) discovered that the Portulaca oleracea L root has a pharmacological effect that plays a crucial role in the glucose metabolism of HepG2 liver cells. A sodium-glucose cotransporter (SGLT) that regulates blood glucose levels is present in HepG2 cells. SGLT mutations result in hyperglycemic conditions in diabetic patients. Flavonoids have a C-aryl glucoside group that inhibits SGLT through a mechanism that breaks the glycoside bond on SGLD. flavonoids are effective as alpha-glucosidase, which regulates sugar homeostasis, they are considered potential anti-diabetes agents (Hummel *et al*. 2012).

Portulaca oleracea L. is widely used as a traditional medicine, specifically as a heat reducer, antiseptic, and vermifuge. In addition, numerous studies demonstrate a variety of pharmacological effects, such as antibacterial, anti-ulcerogenic, anti-inflammatory, antioxidant, and wound-healing properties

The potential of α- glucosidase inhibition

(Zhou *et al.,* 2015).

Endophytic fungi are a solution to land and time constraints, as well as the conservation of plants because the use of microbes does not necessitate large amounts of land or a lengthy harvesting period. Endophytic microbes are plant-damaging microorganisms that are alive (Kharwar and colleagues, 2008; Prabavathy & Nachiyar, 2013). According to Tan & Zou (2001), endophytic microbes that reside in plants can produce the same secondary metabolite compounds as their hosts due to long-standing genetic exchanges and evolutionary connections. Previous research has demonstrated that alpha-glucosidase inhibitors can be produced by isolating endophytic fungi from a variety of plants. Results of the antibacterial activity test showed that isolates of endophytic fungi from Portulaca oleracea L. had activity in inhibiting the growth of Staphylococcus aureus and Escherichia coli (Nuryanti *et al.,* 2023). In a previous study, Pahriyani *et al*. (2019) discovered that Endophytic fungi isolated from turmeric rump produce metabolites that inhibit alpha glucosidase.

This study is a preliminary investigation into the potential of the endophytic fungi Portulaca oleracea L to produce antidiabetic compounds. As a preliminary study, inhibition tests of alpha-glucosidase activity were used to evaluate the antidiabetic activity of a substance.

METHODS

Chemicals and Instrument

This research employed the oven, scissors, autoclave, Erlenmeyer, LAF (laminar air flow), chemical glassware, rotary shakers, petri cups, refrigerators, incubators, microscopes, fermenters, centrifuges, digital scales, incubators, volumetric pipets, pinsets, microplate 96, and micropipettes.

The materials used in this study are the study sample of *Portulaca oleracea L* obtained from the City of Makassar Province of South Sulawesi, water, 70% ethanol, aquades, PDAC medium (Potato Dextrosa Agar Cloramfenicol), PDA medium (Potato dextrose Agar), PDY (Potato Dextrose Yeast), ethyl acetate, glucosidase enzyme, pH phosphate buffer 7, mM-paranitrofenyl-Dukopiranoside, DMSO (Dimethyl Sulfoxide), Na_2CO_3, and p-nitrophenol.

Isolation of the Endophytic Fungi *Portulaca oleracea* L

Fresh *Portulaca oleracea L* is washed with running water to remove soil and adhering dirt, then reduced in size to 2 cm. The sample was put into a 250 mL Erlenmeyer glass, to which 70% ethanol was added until submerged, then shaken gently and sterilized for 2 minutes. The 70% ethanol solution was discarded and then rinsed with sterile distilled water three times, each for 1 minute. Sterilization was carried out in an aseptic manner in the LAF. These materials were drained in a sterile Petri dish, then cut with a sterile scalpel knife into a size of 1 cm^2. The sections were grown on PDAC

medium in sterile Petri dishes at room temperature (25°C) for three days. After three days, the growth of the fungi occurred, and then it was isolated to obtain a pure culture. Pure cultures of endophytic fungi were grown on PDA medium in Petri dishes (Deponda *et al.*, 2019)

Purification and Macroscopic Endophytic Fungi

The medium used for the purification of endophytic fungi is PDA medium. Endophytic fungi growing on PDAC medium were purified on PDA medium, respectively. Then incubated for three days at 25 °C, the shape and colour of the colonies were observed on PDA medium. Each colony with a different colour was transferred again to the oblique PDA medium until a pure colony was obtained (Deponda *et al.*, 2019).

Extraction of Secondary Metabolites

Fermentation was carried out on Potato Dextrose Yeast (PDY) medium. Two pieces of fungal mycelium measuring 1 x 1 cm were put into 100 mL of PDY medium and incubated at room temperature for seven days at a centrifugal speed of 120 rpm. The fermented supernatant, which had been separated from the fungi by centrifugation, was then extracted using ethyl acetate solvent with a ratio of extract to solvent of 1:3 (Deponda *et al.*, 2019).

Alpha-glucosidase inhibition activity test

An in vitro antidiabetic activity test was carried out using the glucosidase method. A total of 1 mg of glucosidase was dissolved in 1000 µL of phosphate buffer (pH 7). Then 12 µL of the enzyme solution was diluted in 30 µL of phosphate buffer before being used for testing. 250 µL of 20 mM paranitrophenyl-D glucopyranoside, 475 µL of 100 mM phosphate buffer, and 25 µL of sample solution were dissolved in DMSO. After the homogeneous solution was incubated for 5 minutes at 37°C, 250 µL of α-glucosidase enzyme solution was added, and the incubation was continued for 25 minutes. The reaction was stopped by adding 1 mL of 0.2 M Na_2CO_3. The amount of p-nitrophenol released was measured at 410 nm. Next, the inhibition ability is calculated. The OD test shows the absorbance of the sample with the addition of enzyme; the blank OD is the absorbance of the sample without the addition of enzyme; the COD absorbance test is the control with the addition of enzyme; and the COD blank is the absorbance of the control without the addition of enzyme. Inhibition (%) = (OD test-OD blank) / (COD test-COD blank) 100% (Saijyo, *et al.*, 2008).

RESULTS AND DISCUSSION

Plants, as traditional medicinal ingredients, have been widely used for treatment. One example of a plant that can be efficacious in medicine is purslane (*Portulaca oleracea L.*). According to the results of studies on the pharmacological effects of purslane plants as antidiabetic (Zhang et al., 2015), anti-inflammatory, antioxidant, and anti-tumor

(Rahimi et al., 2019), empirically, the purslane plant is used as traditional medicine by the community, including as a febrifuge, antiseptic, vermifuge, and wound healer (Zhou et al., 2015). Diabetes mellitus (DM) is a group of diseases caused by a relative or absolute insulin deficiency and characterized by increased blood glucose levels in the body. Purslane contains chalcone-type flavonoids, which are considered potential anti-diabetics because they are effective as glucosidase, which regulates sugar homeostasis (Hummel et al., 2012).

Endophytic fungi are fungi that live in plant tissues for a certain period of time and are able to form colonies in the tissues without endangering the host itself. Endophytic fungi are important microorganisms and a source of the same bioactive compounds as their host plants or other compounds. In this study, the alpha-glucosidase enzyme inhibition activity test was carried out on the ethyl acetate extract of the endophytic fungi *Portulaca oleracea L.* to know the potential of the ethyl acetate extract of the endophytic fungi in inhibiting the -glucosidase enzyme, which is one of the mechanisms of drug action. antidiabetic based on the percent inhibition value.

This research began by isolating the endophytic fungi from *Portulaca oleracea L.* by planting them in sterilized medium containers. The results of the isolation of endophytic fungi from different plant parts of the same host plant also contain different types of isolates. Isolation was carried out on eight

The potential of α- glucosidase inhibition petri dishes as media, and 12 isolates of endophytic fungi were obtained from the isolation process. The mechanism of adaptation of endophytes to specific microecological and physiological conditions can cause more than one endophytic fungi to be isolated from one living tissue of a plant, as obtained in this research.

After purification, the isolate is fermented. Endophytic fungi fermentation was carried out by liquid fermentation using PDY (potato dextrose yeast) media. This fermentation aims to obtain a suspension of endophytic fungi colonies and produce secondary metabolites. Optimization of the fermentation media needs to be done to get the maximum secondary metabolites (Gandjar et al., 2006). Furthermore, fermentation was carried out using a rotary shaker at a speed of 120 rpm at room temperature at intervals of 14 days because the endophytic fungi had reached the stationary phase, namely the phase where the number of cells increased and the number of cells that died were relatively balanced. The curve in this phase is a horizontal straight line. Many secondary metabolites can be harvested in this phase, namely the 14th- day phase (Gandjar et al., 2006). These cotton-like forms are single spores or conidia that have grown into mycelium. After that, vacuum filtration separated the fermentation results.

At this study stage, endophytic fungi metabolites were extracted using ethyl acetate. Extraction is the process of withdrawing the active substance components from a material

using a solvent to obtain specific active components. Extraction with different levels of solvent polarity will produce different extracts. The activity of α-glucosidase inhibitors was determined by measuring the absorbance of the blank, control of the blank, acarbose (comparison), acarbose control (control), and samples of the ethyl acetate extract endophytic fungi *Portulaca oleracea L* at 410 nm. In this investigation, quantitative analysis is conducted using UV-Vis spectrophotometers. The advantages of the UV-Vis Spectrophotometer are that it can be used to analyze numerous organic and anorganic substances, is selective, has high accuracy with a relative error of 1%–3%, can be performed quickly and precisely, and can be used to determine the amount of a substance required (Yahya, 2013).

In standard control, acarbose and ethyl acetate extracts of metabolites of the endophytic fungi *Portulaca oleracea L.* are used as a correction factor for the absorbance values of the samples and blanks, as the color of the extract can also influence the absorbance values at these wavelengths.

Acarbose was used as a comparison in this study. Acarbose is used because it inhibits the glucosidase enzyme in the small intestine wall. Acarbose is an oligosaccharide derived from the fermentation of *Actinoplanes utahensis*. While the function of p-nitrophenyl-D-glucopyranosida as a substrate resembles that of carbohydrate in the body, the enzyme degrades the substrate into glucose and p-nitrophenol. This test measures enzyme activity using an indicator based on the absorbance results of p-nitrophenol, which is produced during the hydrolysis of p-nitrophenyl-D-glucopyranoside. Because the potential of a plant component to inhibit the glucosidase enzyme determines the amount of p-nitrophenol produced, this is distinguished by a change in the substrate's pigment, which takes on a faded yellow hue. Tables 1 display the results of the α-glucosidase inhibitor activity test using an ethyl acetate extract of metabolites from the endophytic fungi *Portulaca oleracea L* and acarbose comparators.

According to Table 1, twelve metabolite extracts of the endophytic fungi *Portulaca oleracea L* exhibited alpha glucosidase inhibitory activity. The results of the percentage of inhibition differed significantly between the metabolites extracted with ethyl acetate for 14 days, as evidenced by the wide range of observed values. The ethyl acetate extract of the metabolite of the endophytic fungi *Portulaca oleracea L* isolated on day 14 exhibited the lowest inhibition percentage of 7.16 % and the maximum inhibition percentage of 83.92 %. On day 14, isolate 12 demonstrated the highest inhibitory activity of an ethyl acetate extract of metabolites from endophytic fungi, at 83.92 %. In table 1, the inhibitor compound acarbose was used as a comparison; however, compared to acarbose, which has a very high inhibition of 97.24 %, the inhibition of isolate 12 was

Tabel 1. Results of testing of the activity of the α-glucosidase enzyme inhibitor with a sample of ethyl acetate extract of the metabolite Endophytic *Fungi Portulaca oleracea L.*

Isolate	% inhibition
1	30.54
2	7.16
3	39.64
4	54.53
5	69.59
6	63.70
7	59.51
8	40.40
9	59.13
10	44.33
11	82.29
12	83.92
Acarbose	97.24

relatively high given that the samples tested were only supernatants, not purified compounds, if not only supernatants. The probability of inhibition is likely to be higher than that of acarbose when tested. Therefore, the twelve isolate has tremendous potential if it is further investigated in additional studies.

CONCLUSION

This study indicated that the extract of the endophytic fungi *Portulaca oleracea L* demonstrated antidiabetic activity. The 12th isolate produced the highest score, 83.92%, compared to acarbose's 97.24%.

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