Literature Review: Antidiabetic Activities and Phytochemical Screening of Broccoli Plants *(Brassica oleracea* L. Var. *italica)*

Alya Chairunnisa and Haryoto(✉)

Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Jl. A. Yani Tromol Pos I, Pabelan, Kartasura 57162, Sukoharjo, Indonesia
har254@ums.ac.id

Abstract. Diabetes mellitus is a metabolic disease that is characterized by hyperglycemia which is closely related to pancreatic β cell dysfunction and insulin resistance. *Brassica oleracea* L. var. *italica* is a member of the Brassicaceae family which has antidiabetic properties. These effects are caused by the presence of various phytochemical compounds in broccoli plants, flavonoids, tannins, saponins, alkaloids, steroids, glucosinolates, isothiocyanates, and phenolics. The aim of this review is to find out the antidiabetic activity of broccoli plants and the mechanism of action of phytochemical compounds in broccoli plants that support these benefits. The preparation of this review was sourced from articles obtained from the PubMed and Google Scholar databases with the interval 2012–2021 publication year. Based on the selected articles, it shows that the content of phytochemical compounds in broccoli plants has an effect on reducing blood sugar levels through various mechanisms. Therefore, the results of this study are expected to be used as support for the use of broccoli plants as antidiabetic agents.

Keywords: Brassica oleracea L. var. Italica · antidiabetic · phytochemical · in vivo

1 Introduction

Diabetes mellitus is one of the most common diseases in Indonesia. According to [1] diabetes mellitus is a condition of hyperglycemia characterized an absolute reduction in insulin or reduced sensitivity of cells to insulin. Insulin is a hormone that is produced by pancreatic cells that works as a regulator of the balance of sugar levels in the blood. However, too much carbohydrate intake in individuals can cause insulin to not work properly, causing hyperglycemia [2]. A person is diagnosed with diabetes if the results of laboratory examinations reveal a value that exceeds the normal value, such as a fasting plasma glucose value > 126 mg/dL; plasma glucose 2 h after OGTT > 200 mg/dL; plasma glucose when > 200 mg/dL; and HbA1c > 6.5% [3].

In the treatment of degenerative diseases such as diabetes, synthetic drugs are often used for a long time. Not a few of the patients then experience various unpleasant

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side effects. For example, water retention and hyponatremia can be caused by sulfonylureas; and gastrointestinal disturbances can be caused by alpha-glucosidase inhibitors or biguanids [4]. The use of medicinal plants can be used as an alternative treatment because medicinal plants tend to be safer when compared to synthetic drugs [5]. At present, the use of plants as an effort to treat disease has been widely carried out by the community, both to avoid the side effects of synthetic drugs and to save costs. Various plants with blood sugar-lowering effects have been widely studied by the public, one of which is broccoli *(Brassica oleracea* L. var. *Italica).* Broccoli contains various compounds such as flavonoids, alkaloids, saponins, and steroids [6], as well as antioxidants [7]. Broccoli can lower blood sugar levels by various mechanisms, such as inhibition of alpha-amylase and alpha-glucosidase enzymes, increased expression of GLUT4, inhibition of DPP-IV *(Dipeptidyl peptidase-4)* enzymes, and Nrf2 activation [8–14].

Previous research has been conducted regarding giving steamed broccoli for four weeks to prediabetic women and showed that there was a significant decrease in fasting blood sugar levels of 11.73 mg/dL [15]. In addition, research on broccoli extract has been carried out by [16], and it was found that there was a decrease in blood sugar in rats with type 2 diabetes mellitus induced by Streptozotocin. Various compounds in broccoli are thought to be the cause of this effect through a variety of different mechanisms. Therefore, this review was prepared with the aim of knowing the activity of phytochemical compounds in broccoli plants as antidiabetic also their previously studied mechanisms so that it can be used as a support for the use of broccoli plants as antidiabetic agents.

2 Method

This research was conducted using the Literature Review method. The literature search strategy used two types of databases, namely PubMed and Google Scholar with the keywords "broccoli or *Brassica oleracea* L.var *italica* and antidiabetic and phytochemicals". The various literatures obtained were then identified and examined for feasibility. The selection of articles was carried out using two criteria, namely inclusion criteria and exclusion criteria. The inclusion criteria used were free full text articles regarding the phytochemical content and/or antidiabetic effects of broccoli plants, as well as articles on in vivo antidiabetic experiments on broccoli plants with a publication year range of 2012–2021.

In the first stage, screening for duplication of titles and abstracts was carried out. At this stage, articles with the same title and articles that were not related to phytochemicals and antidiabetic effects of broccoli were not selected. Then proceed with the stage of screening the contents of the article. In this stage, the research samples and the categories of research conducted are checked. Articles that did not use broccoli as an experimental sample and were not in vivo antidiabetic studies were not selected. At the final stage of selection, a quality assessment is carried out. At this stage, further analysis was carried out regarding the completeness of the explanation of the results along with the experimental analysis carried out by the researchers, so that 8 articles were obtained which were then used in writing a literature review.

3 Result and Discussion

3.1 In Vivo Antidiabetic Activity of Broccoli Plants

Broccoli plant is known to be able to be used as an alternative treatment for diabetic patients because it has benefits in lowering blood sugar levels. This has been proven through in vivo experiments by several researchers (Table 1). These experiments used different solvents and plant parts. However, the experiment still gave positive results, that is a decrease in blood sugar levels in animals. This proves that the broccoli plant has a real action as an antidiabetic agent.

Research on the antidiabetic effect of broccoli has been carried out by [17] using broccoli ethanol extract samples. In this study, experimental animals were used in the form of rats that were induced with Streptozotocin-Nicotinamide until blood sugar levels were $>$ 100 mg/dL. The results obtained were the percentage decrease in blood sugar levels of 7.8% in rats given a dose of 75 mg/kgBW of broccoli ethanol extract; 16.5% in rats given a dose of 150 mg/kgBW broccoli ethanol extract; and 38.1% in rats given broccoli ethanol extract 300 mg/kgBW. Based on these results, it is known that there is an increase in the percentage of blood sugar decrease levels of rats which is proportional to the increase in the dose of broccoli ethanol extract given. The results obtained were then tested statistically using One Way ANOVA and the resulting p-value < 0.05 for the entire dose group which can be concluded that there is a significant difference.

Then in vivo research on the antidiabetic activity of broccoli plants was also carried out by [18]. He researched using three different types of extract solvents; broccoli aquadest extract, broccoli ethanol extract, and broccoli chloroform extract. In this study, alloxan was used as a diabetes inducer. The experimental results obtained that all samples were able to provide a significant decrease in blood sugar levels, as evidenced by the overall sample having a significance value of ANOVA test $p < 0.05$. The highest reduction in sugar content was produced by samples of broccoli aquadest extract, which was

Table 1. In Vivo Researches of Antidiabetic Activity of Broccoli Plants

References	Sample	Method	Sample Conc.	% Lower Blood Sugar Levels
[17]	Broccoli ethanol extract	In vivo	75 mg/kgBW	7.80%
			150 mg/kgBW	16.50%
			300 mg/kgBW	38.10%
[18]	Broccoli water extract	In vivo	300 mg/kgBW	43.57%
	Broccoli ethanol extract	In vivo	300 mg/kgBW	35.83%
	Broccoli chloroform extract	In vivo	300 mg/kgBW	14.16%
[19]	Broccoli sprout methanol extract	In vivo	50 mg/kgBW	41.86%
			100 mg/kgBW	57.45%
			200 mg/kgBW	72.26%

43.57%; then followed by broccoli ethanol extract by 35.83%; and broccoli chloroform extract 14.16%.

Experiments on the antidiabetic effect of broccoli were also carried out with different plant parts by [19]. In his research, the sample used was broccoli root extracted in methanol solvent. The test animals used were rats that were induced using Streptozotocin to achieve diabetic blood sugar levels. In this experiment, three experimental groups were made; the doses of broccoli root methanol extract 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW. The percentage reduction in blood sugar levels obtained from each experimental group was 41.86%, 57.45%, and 72.25%, respectively. One Way ANOVA statistical test was used to determine the significance of each experimental group and the results showed that the experimental group at a dose of 100 mg/kgBW and 200 mg/kgBW had the best significance value with $p < 0.001$ and the experimental group at a dose of 50 mg/kgBW had a significant p-value. < 0.01.

3.2 Chemical Compounds in Broccoli Plants

The ability of broccoli plants to decrease blood sugar levels is caused by various chemical compounds-contained therein. The following describes the results of phytochemical screening using broccoli plant as the samples (Table 2). Research by [6] regarding the phytochemical test of broccoli was carried out using the tube test method. In this study, samples of broccoli ethanol extract were used which were made by maceration using 96% ethanol. The results obtained from each of these tests are as follows: positive results for flavonoids which are indicated by the presence of yellow color in the sample; a positive result of tannin which is indicated by the presence of a blackish brown color in the sample; positive results for saponins were indicated by no loss of foam when 1% HCl was added; positive results for alkaloids which are indicated by the presence of an orange precipitate when reacted with Dragendorf's reagent and a yellow precipitate when reacted with Mayer's reagent; and a positive result of steroids which is indicated by the presence of bluish-green color in the sample.

In a study conducted by [20], it was found that broccoli methanol extract contains glucosinolate compounds (glucoraphanin, glucoiberin, glucoraphenin, glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin); isothiocyanates (sulphoraphane, iberin, and indole-3-carbinol); and phenolic compounds (chlorogenic, synapic, ferulic acid derivatives).

It is different from the research conducted by [21], who conducted a test tube on two samples, there were broccoli methanol extract and broccoli aquadest extract. The broccoli methanol extract contained flavonoids, tannins, saponins, alkaloids, and phenols, while the broccoli aquadest extract did not show positive results for the saponin test. The research was then continued to determine the phenol and flavonoid content of each extract and the results showed that the highest phenolic content was found in broccoli methanol extract, which was 247.79 mg gallic acid equivalent (GAE)/g while the highest total flavonoid content was contained in broccoli aquadest extract with levels of 104.20 mg quercetin equivalent (QE)/g.

Then research on the content of phytochemical compounds in broccoli plants was also carried out by [18]. In this study, samples of broccoli extract were macerated in different solvents, there were sample 1 was broccoli aquadest extract; sample 2 broccoli

Table 2. Broccoli Extract Phytochemical Screening Results

References	Method	Sample	Chemical compund
[6]	Tube test	Broccoli ethanol extract	Flavonoids, tannins, saponins, alkaloids, and steroids.
[20]	HPLC-DAD; UHPLC-QqQ-MS/MS	Broccoli methanol extract	Glucosinolates, Isothiocyanates, Phenols.
[21]	Tube test	Broccoli methanol extract	Flavonoids, tannins, saponins, alkaloids, and phenols.
		Broccoli water extract	Flavonoids, tannins, alkaloids, and phenols.
	Spectrophotometer	Broccoli methanol extract	TPC = 247.79 mg GAE/g
			TFC = 97.92 mg QE/g
		Broccoli water extract	TPC = 110.43 mg GAE/g
			TFC = 104.20 mg QE/g
[18]	Microplate reader	Broccoli water extract	TPC = 161 µg GAE/mg E
			TFC = 139.7 µg QE/mg E
		Broccoli ethanol extract	TPC = 184 µg GAE/mg E
			TFC = 160.9 µg QE/mg E
		Broccoli chloroform extract	TPC = 146.4 µg GAE/mg E
			TFC = 133.8 µg QE/mg E
[22]	UHPLC	Broccoli seed ethanol extract	TPC = 480.4 mg FAE/100 g.
			TFC = 216.9 mg CE/100 g
			TSC = 18.6 mg soy saponin BE /100 g
		Broccoli sprout ethanol extract	TPC = 385.4 mg FAE/100 g
			TFC = 206.9 mg CE/100 g
			TSC = 27.0 mg soy saponin BE/100 g

ethanol extract; and 3 samples of broccoli chloroform extract. All samples were checked for phytochemical content using a microplate reader. The results obtained were the highest total phenolic content (TPC) and total flavonoid content (TFC) were obtained by samples of broccoli extract macerated using ethanol solvent, with the results of TPC = 184 μg gallic acid equivalent per mg extract (μg GAE/mg E) and TFC = 160.9 μg quercetin equivalent per mg extract (μg QE/mg E). This was because ethanol is a solvent that has the widest extracting power, making it the main solvent of choice for screening research [23].

The research was then carried out by [22] but with different methods and plant parts. In this study, samples were used in the form of ethanol extract of broccoli seeds and broccoli roots which were made by maceration using 70% ethanol as solvent. Each extract was then tested to determine the total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC) using UHPLC. The test results obtained are as follows: in the sample of broccoli seed ethanol extract, the TPC value = 480.4 mg ferulic acid equivalent per 100 g of sample (mg FAE/100 g); TFC = 216.9 mg catechin equivalent per 100 g of sample (mg CE /100 g); TSC = 18.6 mg soy saponin B equivalent per 100 g of sample (mg soy saponin BE/100 g); and in samples of broccoli root extract, the TPC value = 385.4 mg ferulic acid equivalent per 100 g of sample (mg FAE/100 g); TFC = 206.9 mg catechin equivalent per 100 g of sample (mg CE /100 g); TSC = 27.0 mg soy saponin B equivalent per 100 g of sample (mg soy saponin BE/100 g).

3.3 Antidiabetic Mechanisms of Broccoli Plants

To follow up on the results of in vivo study about antidiabetic activity from broccoli plants, an analysis was carried out on the mechanism of action of the chemical compounds contained in broccoli plants in lowering blood sugar (Table 3).

Table 3. Antidiabetic Mechanisms of Broccoli Plants

Mechanism of Action	Chemical compound	References
Inhibition of Alpha-Amylase and Alpha-Glucosidase Enzymes	Isothiocyanate	[8]
	Flavonoids	[9]
	Polyphenol	
	Glucosinolates	[10]
	Phenolic	
Enhanced GLUT4 Expression	Flavonoids	[11]
	Isothiocyanate	[12]
DPP-IV Enzyme Inhibition	Peptide	[13]
Nrf2 Activation	Isothiocyanate	[14]

3.3.1 Mechanism of Inhibition of Alpha-Amylase and Alpha-Glucosidase Enzymes

Alpha-amylase is a hydrolytic enzyme that has a role in starch hydrolysis. Alpha-amylase enzymes are produced by humans, plants, bacteria, fungi, and also animals. In humans, the alpha-amylase enzyme is located in the salivary glands which are then able to secrete the enzyme into the mouth, and also located in the pancreas which secretes it into the small intestine. This enzyme has a role in the metabolism process of carbohydrates into monosaccharides [24]. It hydrolyzes α-(1,4)-glycosidic bonds in starch molecules to produce maltose, maltotriose, maltotetraose, maltodextrin, and glucose. Then the alpha-glucosidase enzyme is an enzyme that has a role in catalyzing the hydrolytic breakdown of disaccharides such as maltose and sucrose into monosaccharides such as glucose and fructose. Alpha-glucosidase enzymes can be found in microorganisms, plants, animals, and also human tissues. More precisely, the enzyme is present on the luminal surface of enterocytes which is then secreted in the small intestine [25]. In short, alpha-amylase is related to the breakdown of long-chain carbohydrates, while alpha-glucosidase is related to the breakdown of starch and saccharides into monosaccharide glucose [26]. Inhibition of these two enzymes is one of the treatment strategies for patients with diabetes mellitus. By inhibiting these two enzymes, the rate of glucose absorption is delayed which is then able to prevent an increase in postprandial plasma glucose levels [27].

A study conducted by [8] proved that 100; 250; and 500 μg of broccoli extract containing sulforaphane had inhibitory values of the alpha-amylase enzyme, respectively, 11.65; 36.38; and 46%. Furthermore, research by [9] proved that broccoli juice with a concentration of 10% (v/v) containing polyphenols and flavonoids was able to inhibit the alpha-glucosidase enzyme by 36%. According to [28], the structure of flavonoid compounds is similar to the structure of natural glucosidase substrates so that it causes an inhibitory mechanism in the form of competitive inhibition in which inhibitor compounds (flavonoids) will compete with natural substrates by occupying the active site of the enzyme. In addition, the glucosinolate and phenolic compounds in broccoli also can inhibit alpha-amylase and alpha-glucosidase enzymes through the activation of the Nrf2 pathway [10]. Pancreatic beta cells are known to have the low antioxidant capacity, with the activation of Nrf2, they can increase the antioxidant capacity which then leads to protection against cell death and increased glucose absorption [29].

3.3.2 Enhanced GLUT4 Expression

GLUT4 (Glucose transporter 4), a protein encoded by the GLUT4 gene, is a glucose transporter that is regulated by insulin. GLUT4 is found in the body, especially in adipose and striated tissue [30]. In the state of insulin resistance, tissues that are involved in the process of glucose homeostasis as well as those that are sensitive to insulin (eg liver, muscle, adipose tissue) no longer respond well to insulin. That condition causes the liver to continue to synthesize and release glucose, while muscle and adipose tissue are only able to transport glucose in small amounts [31]. The uptake of glucose is the task of GLUT4, it moves glucose from the blood circulation as well as a regulator of glucose homeostasis in the body [32].

Sulforaphane is an isothiocyanate compound that is widely contained in Cruciferous or Brassicaceae plants. Through research conducted by [12], the sulforaphane content in broccoli can stimulate glucose uptake through the activation mechanism of Akt and translocation of GLUT4 to the plasma membrane. Sulforaphane was able to improve glucose intolerance in the in vivo experiments on obese rats. In this experiment, it was found that sulforaphane was able to improve insulin resistance by measuring the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) parameter and it was also proven to decrease the area under curve (AUC) of obese diabetic rats compared to other experimental groups.

Kaempferol is a flavonol found in cruciferous vegetables or Brassicaceae [33]. Through in vivo experiments, it was found that there was an increase in GLUT4 protein expression in skeletal muscle and adipose tissue. Orally administered kaempferol has been shown to significantly reduce blood glucose levels, HbA1c levels, and improve insulin resistance. Then in vitro studies found that kaempferol can restore glucose absorption and glycogen synthesis which is associated with increased GLUT4 expression [34].

3.3.3 DPP-IV Enzyme Inhibition

Incretin hormones are intestinal peptides that are secreted when there is an intake of nutrients that enter the body or when a person eats. Incretin hormones consist of GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1) [35]. GLP-1 and GIP can increase beta cell mass, increase insulin secretion and maintain normal blood glucose levels [36]. The presence of DPP-IV in the body can inactivate both hormones by cleaving the dipeptide from the amino terminus which includes alanine or proline residues at position 2. That causes a very short half-life of GIP and GLP-1. Therefore, incretin therapy is an option that is widely used in the treatment of diabetes.

The therapy consists of two types, namely dipeptidyl peptidase IV (DPP-IV) inhibitors and glucagon like peptide-1 (GLP-1) receptor agonists [37]. DPP-IV inhibitors work by inhibiting the DPP-IV enzyme in degrading GLP-1. That way, the half-life of GLP-1 can be extended and the role of GLP-1 in controlling blood sugar can run well [36]. The peptide content in broccoli has been shown to have an inhibitory effect on the DPP-IV enzyme [13]. The peptide Leu-Pro-Gly-Val-Leu-Pro-Val-Ala (LPGVLPVA) had a DPP-IV IC_{50} value of 392 ± 24 μM and Tyr-Leu-Tyr-Ser-Pro-Ala-Tyr (YLYSPAY) had a DPP-IV IC_{50} $181 \pm \mu 4$ M.

3.3.4 Nrf2 Activation

The development of diabetes mellitus is followed by an increase in oxidative stress on beta cells which then leads to oxidative damage and decreased function of beta cells. Oxidative stress is a condition where there is an increase in the production of free radicals or a decrease in antioxidant activity or both. The occurrence of oxidative stress is often associated with Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Hyperglycemia conditions can stimulate the production of superoxide and excess nitric oxide in the mitochondria. This can encourage the formation of peroxynitrite oxidants that can damage DNA. DNA damage will stimulate the activation of polynuclear

polymerase (PARP) enzymes and decrease glyceraldehyde-3-phosphate dehydrogenase (GADPH) activity and produce endothelial dysfunction that causes complications in diabetes mellitus patients [38].

Nrf2 is known to be a major regulator in the fight against oxidative stress levels. Nrf2 can improve beta cell function, promote beta cell proliferation, and increase beta cell survival. Another benefit of activating Nrf2 is an increase in insulin sensitivity which is then associated with a decrease in the possibility of complications in diabetics [39]. Sulforaphane is known to be a strong Nrf2 activator that can be used to prevent complications of diabetes mellitus. Based on research conducted by [14], it was found that administration of broccoli extract containing sulforaphane was able to increase Nrf2 levels in rat liver and reduce levels of oxidative stress marker, 4-hydroxy-2-noneal (4-HNE)., 3-nityotyrosine (3-NT), and malondialdehyde (MDA).

Based on the discussion above, we can conclude that broccoli extract was able to reduce blood sugar levels with the largest percentage reduction was 72.26% by broccoli sprout methanol extract at the dose of 200mg/kgBW and the smallest reduction percentage was 7.80% by broccoli ethanol extract at the dose of 75mg/kgBW; broccoli plants contain flavonoids, tannins, saponins, alkaloids, steroids, glucosinolates, isothiocyanates, and phenolic compounds; and then the antidiabetic mechanisms of broccoli are the inhibition of alpha-amylase and alpha-glucosidase enzymes, enhancement of GLUT4 expression, inhibition of DPP-IV enzymes, and also activation of Nrf2.

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