



## **Antidiabetic Activity of Ethanolic Extract of Kale (*Brassica oleracea* var. *sabellica*)**

Afdrian Kusumawardianingrum\*, Novena Yety Lindawati

Pharmacy Study Program, National College of Health Sciences (STIKES Nasional), Surakarta, Indonesia

\*Corresponding author: [afdrianafaf@gmail.com](mailto:afdrianafaf@gmail.com)

Submitted: 16 August 2021

Accepted: 2 March 2022

Published: 26 April 2022

### **Abstract**

**Background:** Diabetes Mellitus is a disease caused by a disruption of the pancreas in producing insulin. The International Diabetes Federation (IDF) organization identified ten countries that have the highest cases of Diabetes Mellitus. Indonesia is ranked 7th out of 10 countries and is the only Southeast Asian country included in the list. Prevention of diabetes mellitus is a solution that must be done to reduce cases of diabetes mellitus in Indonesia., one of which is by consuming vegetables that have antidiabetic activity. **Objective:** This study was conducted to determine the activity of kale (*Brassica oleracea* var. *sabellica*) extract in reducing glucose levels. **Methods:** The qualitative test results showed that the kale extract contained positive flavonoids, triterpenoids, tannins and phenols. The antidiabetic activity test was carried out using the UV-Vis spectrophotometer and the Nelson Somogyi method. This test is carried out at the 25th minute operating time and a maximum  $\lambda$  of 745 nm. **Results:** The decrease in glucose levels in kale extract concentration of 4 ppm was  $2.23\% \pm 0.46$ , 6 ppm of  $16.47\% \pm 0.27$ , 8 ppm of  $30.62\% \pm 0.46$ , 10 ppm of  $41.88 \pm 0.27$ , 12 ppm of  $55.50 \pm 0.20$ . The concentration of kale extract in reducing glucose up to 50% ( $EC_{50}$ ) was 11.13 ppm. **Conclusion:** Kale extract (*Brassica oleracea* var. *sabellica*) can reduce glucose levels or have antidiabetic activity.

**Keywords:** activity antidiabetic, kale, nelson-somogyi

### **How to cite this article:**

Kusumawardianingrum, A., & Lindawati, N. Y. (2022). Antidiabetic Activity of Ethanolic Extract of Kale (*Brassica oleracea* var. *sabellica*). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 92-100. <http://doi.org/10.20473/jfiki.v9i12022.92-100>

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose levels due to impaired insulin production or use by body cells. Symptoms experienced by patients with diabetes mellitus are frequent eating (polyphagia), frequent drinking (polydipsia) and frequent urination (polyuria) (WHO, 2018). Diabetes mellitus is often associated with inflammation. The inflammatory response to an infection or virus is influenced by blood glucose levels in a person's body. Hyperglycemia in type 2 diabetes will stimulate macrophages to secrete the proinflammatory cytokine TNF- $\alpha$ . When patients with type 2 diabetes have high levels of TNF- $\alpha$  this will lead to more severe insulin resistance resulting in endothelial dysfunction resulting in disease complications (Yuniarti, 2017).

Prevention and control of diabetes mellitus is carried out to keep individuals healthy and people who already suffer or have risk factors for this disease can control their disease so as not to cause complications or death. The International Diabetes Federation Organization (IDF) projects the number of people with diabetes in the population aged 20 - 79 years in several countries in the world in 2019. Research from the IDF is able to show a list of 10 countries with the highest number of people with diabetes mellitus. Indonesia is ranked 7th among ten countries, with 10.7 millions of sufferers. Indonesia is the only country from Southeast Asia on the list, so it can be estimated that Indonesia's contribution to the prevalence of diabetes cases in Southeast Asia can be estimated (Kemenkes RI, 2020). With the high number of sufferers in Indonesia, it is necessary to prevent and control the disease preventing and controlling the disease is necessary.

Prevention and control of diabetes mellitus that everyone can do is to eat vegetables that contain polyphenols. Polyphenols, in particular flavonoids can be suggested as a better treatment therapy in diabetes mellitus as well as chronic complications associated with the disorder. Research from Arjadi & Susatyo (2010) states that flavonoid compounds can lower blood sugar levels by stimulating pancreatic  $\beta$  cells to produce more insulin. One of the vegetables in Indonesia that contains flavonoids is kale (*Brassica oleracea* var. *sabellica*). Kale is a vegetable with curly leaves that are dark green and belongs to the cabbage family. This vegetable kale abroad is nicknamed the Queen of Vegetable (Superfood) because it has many benefits, but some Indonesian people are not familiar with this vegetable. Based on research by Krumbein *et*

*al.* (2010), kale contains flavonoids, namely quercetin, kaempferol and isorhamnetin. Study from Cieslik *et al.* (2008) states that the polyphenol content in kale is higher than broccoli, brussels sprouts, and cauliflower, namely 773 mg/100 g.

Based on the data obtained from kale vegetables and the antidiabetic effects of kale that have not been studied the researchers were interested in testing the antidiabetic activity of kale extract (*Brassica oleracea* var. *sabellica*) using the Nelson-Somogyi method. The principle of the Nelson somogyi method is that Nelson's reagent in a sample that has been added to standard glucose will reduce  $\text{Cu}^{2+}$  ions to form gluconic acid and cuprous oxide precipitates. The copper oxide precipitate will react with the arsenomolybdate reagent to form a turquoise-blue copper and molybdate complex. The color created is then measured in its absorbance with a UV-Vis spectrophotometer at the maximum wavelength (Advistasari *et al.*, 2019). This study of glucose-lowering activity is expected to provide an initial study of the use of kale (*Brassica oleracea* var. *sabellica*) as an antidiabetic vegetable.

## MATERIALS AND METHODS

### Materials

The materials used in this study were kale vegetables from Pasar Gedhe Harjonagoro, Jebres District, Surakarta, Nelson reagent (Merck), 96% ethanol, glucose pa (Merck), arsenomolybdate (Merck), concentrated HCl (32% Technical HCl 500 mL), Mg powder Merck (Catalog 1.05815.1000), Technical HCl 2N (Colorless), aquadest, gelatin solution, 10% NaCl, Mayer reagent  $\text{HgI}_4\text{K}_2$  25 mL, Dragendroff reagent 10 mL, Wagner reagent 25 mL, glacial  $\text{CH}_3\text{COOH}$  100% Merck, concentrated  $\text{H}_2\text{SO}_4$  95 - 97% pa (Merck), Technical  $\text{FeCl}_3$  5% 100 mL.

### Tools

The tools used analytical scales (Ohaus Pioneer with a sensitivity of 0.0001 g and a minimum weighing of 0.1000 g), rotary evaporator (IKA RV 10 Digital V), waterbath WNB14RING 6 holes, volumetric flask 100 mL 50 mL 10 mL (pyrex), beaker glass 50 mL and 100 mL (pyrex), measuring cup (Merck IWAKI), measuring pipette 1 mL (Pyrex), test tube 17 mL Pyrex and test tube rack 12 hole, UV-Visible Spectrophotometer (Shimadzu UV mini-1240), Hellma Analytic cuvette type No. 100.600 QG Light parh lotum.

## Methods

### Sample determination

Identification of kale (*Brassica oleracea* var. *sabellica*) was carried out at the Research and Development Center for Medicinal and Traditional Medicinal Plants in Tawangmangu with the letter-number YK.01.03/2/545/2021.

### Preparation of kale powder

The kale vegetables collected from the Pasar Gedhe Harjonagoro are carried out wet sorting, which aims to separate the dirt from the kale, then continue washing it using running water to facilitate the loss of dirt is still attached to the kale. After that, it was chopped to speed up the drying time. Drying is carried out in an oven at 40°C until dry so that the moisture content in the material is reduced so that it can prevent the growth of microbes in the sample, then mashed using a blender and sieve no. 40 to get a fine kale powder. The kale powder obtained is stored in a tightly closed container.

### Preparation of extract

A total of 200 g of kale powder put into a maceration container, then (1 : 7.5) dissolved in 1500 mL of 96% ethanol. The solution left to stand for 3 days and stirred occasionally. After 3 days, the kale sample was filtered, the first filtrate was obtained. Furthermore, the remaceration was carried out with 500 mL of 96% ethanol solvent (1 : 2.5), left to stand for two days. Then the sample is filtered until a second filtrate is obtained. The results of the first and second filtrate that have been collected are subjected to a concentration process with the help of a rotary evaporator at a temperature of 40°C, then continued with concentration with an electric water bath at 40°C. After that, the yield was calculated, and phytochemical screening was carried out on kale extract. The extract obtained is a thick extract with a constant weight when weighed.

### Antidiabetic activity test

The antidiabetic activity test was carried out using the Nelson Somogyi method and the UV-Vis Spectrophotometry instrument. This test starts from determining the operating time, maximum wavelength, positive glucose control, and decreasing the glucose levels of kale extract.

The operating time was determined by pipetting 1 mL of glucose 100 ppm standard working solution, 1 mL of nelson reagent, and putting it in a test tube closed with a cotton swab. Then, heating the solution for 10 minutes in boiling water and cooling for 5 minutes, then transferred to a 5 mL volumetric flask.

The solution was added with 1 mL of arsenomolybdate reagent and diluted with distilled water to mark the limit and shake. Absorbance measured at the theoretical maximum wavelength until optimum time was stable (Aprizayansyah *et al.*, 2016).

The maximum wavelength and positive control of 20 ppm glucose were determined with the same treatment as the operating time. The two tests differ only in wavelength measurements. In the test, the maximum wavelength was suspended during operational time and measured at a 700 - 780 nm wavelength. Meanwhile, the treatment on the positive control test for glucose carried out by measuring the maximum wavelength of 745 nm.

The test for lowering the glucose level of kale extract was carried out by pipetting 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL from the 100 ppm working sample solution. The sample was pipetted into a test tube, added with a standard solution of 100 ppm glucose and 1 mL Nelson's reagent, and then covered with cotton. It was heated for 10 minutes in boiling water, cooled for 5 minutes, and then transferred to a 5 mL volumetric flask. 1.0 mL of arsenomolybdate reagent was added, diluted with distilled water to the mark, shaken and deferred for an operational time. The measurement results of the sample are read at the maximum wavelength, and the percentage of glucose reduction is calculated.

### Data analysis

#### Calculation of the percent decrease in glucose levels

The reduction in glucose levels is measured by the difference between the positive controls for glucose and residual glucose, then compared with intact glucose to get a percent decrease in glucose levels. The formula for this value is stated as follows:

$$A = \frac{B-C}{B} \times 100\%$$

Note:

A = % decrease in glucose levels

B = absorbance of glucose positive control

C = residual glucose absorbance

#### EC<sub>50</sub> calculation

The EC<sub>50</sub> value (Effective Concentration) is a value that describes 50% of the maximum effect of kale extract in reducing glucose levels. This calculation uses a linear equation from the relationship between the concentration of kale extract and the percent decrease in glucose levels.

$$Y = bx + a$$

Note :

y = percent decrease in glucose levels (50)

b = slope (slope)  
 a = intercept  
 x = concentration of kale extract

**RESULTS AND DISCUSSION**

**Determination**

The results showed that the vegetables taken from Pasar Gedhe were kale with the name simplisia *Brassica oleracea* var. *sabellica*. Vegetable kale is shown in Figure 1.



**Figure 1.** Kale

**Kale extraction**

Kale is carried out by making powder first before the extraction process is carried out. The kale powder was made using a blender and sieve no. 40 to get a fine kale powder. The use of sieve no. 40 is based on research by Nurhasnawati *et al.* (2017) which states that the mesh size of 40 on tiwai onion bulbs has a higher yield than mesh 20, 60, and 80.

Dry powder of kale is remacerated with 96% ethanol while stirring once every 8 hours. Remaceration is carried out to optimize the process of withdrawing the active compounds contained in kale powder because there is a possibility that in the

previous maceration process, the active substance was still left in the residue and stirring aims to maintain the difference in concentration inside and outside the cell so the filter fluid can still filter substances according to its properties polarity (Angraini & Damayanti, 2019).

The maceration method is a cold extraction method, this method is appropriate to use in research, where this test will take flavonoid compounds that have heat insoluble properties at temperatures > 50°C. The choice of 96% ethanol solvent was chosen because based on Bintang *et al.* (2014), it showed that the total flavonoids were more dissolved in 96% ethanol compared to 70% ethanol and water. This solvent also has the ability to extract compounds better because the high solvent concentration influences the amount obtained from the extraction. The extraction results obtained a thick brownish green color extract with 15.3%.

**Phytochemical screening**

Phytochemical screening is carried out to determine the presence of active compounds in kale extract which are expected to act as antidiabetic. The results of phytochemical screening on kale extract (*Brassica oleracea* var *sabellica*) can be seen in Table 1. The table shows that kale extract contains flavonoids, tannins, triterpenoids and phenols.

**Flavonoids**

Figure 2 below shows the flavonoid reactions occur because Magnesium and HCl powder will reduces flavonoids that have hydrogen bonds (-OH), resulting in a red or orange color (Agustina *et al.*, 2017).

**Table 1.** The phytochemical test of kale extract

Compound Group	Positive result based on reference	Test Result	Note
Flavonoids	Red or orange	Red	Positive flavonoids
Saponins	A stable foam is formed with a height of 1.5 cm	No foam is formed	Negative saponin
Tannins	Yellowish white precipitate	Yellowish white precipitate	Positive tannins
Mayer	White precipitate		
Alkaloids	Orange red precipitate	No precipitate is formed	Negative alkaloids
Dragendroff	Chocolate precipitate		
Wagner	Blue or green	Sample color does not change	Negative steroids
Steroids	Red or purple	Purple	Positive triterpenoids
Triterpenoids			
Phenols	Green, purple, blue, and black	Black	Positive phenols

**Tannins**

Kale extract also contains tannins because the sample is able to form a yellowish white precipitate. This happened because of the nature of tannins that can tanner proteins (gelatin) so as to form a water-insoluble precipitate. Protein is more difficult to dissolve at high salt concentrations, the addition of sodium chloride, a salt that aims to optimize gelatin deposition (Esteti, 2008).

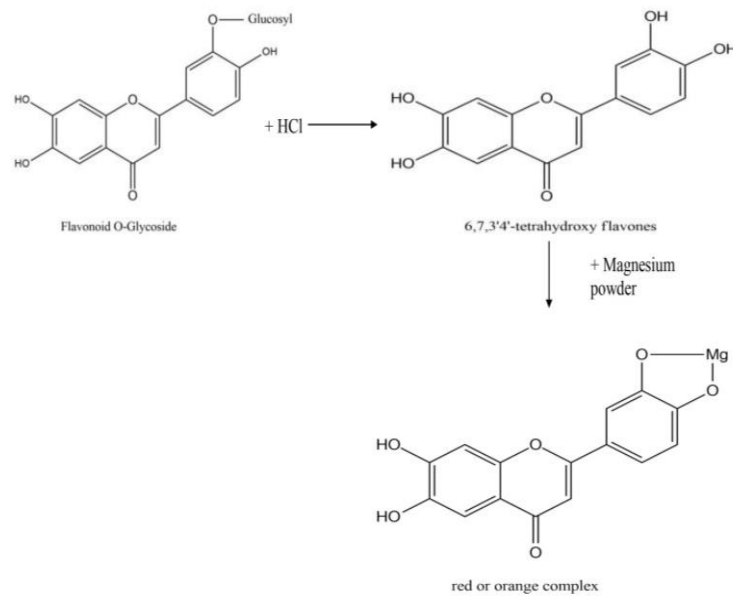
**Triterpenoids**

Triterpenoids which are hydrolyzed with concentrated sulfuric acid will produce hydroxyl groups and react with acetic anhydride (Souhoka et al., 2019). In the identification of triterpenoids, the testing

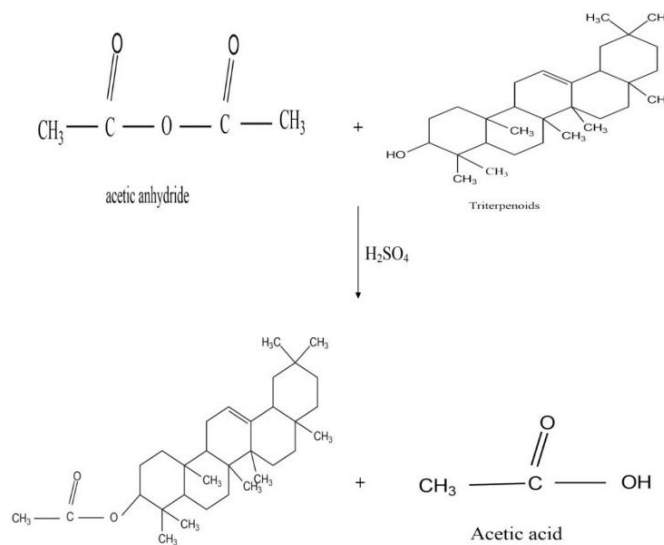
of this group of compounds was carried out with the reagent Liebermann-Burchard (acetic anhydrous- $H_2SO_4$ ). Figure 3 shows the reaction of triterpenoid with  $H_2SO_4$ .

**Phenols**

Kale extract contains phenols. Positive results are shown in black after adding reagent. This happens because  $FeCl_3$  is able to react with the hydroxyl groups present in phenol (Susanti *et al.*, 2017). The color change reaction is shown in Figure 4, where the reaction of  $FeCl_3$  with phenols produces a black complex.



**Figure 2.** Flavonoid reaction with magnesium powder and concentrated HCl



**Figure 3.** Reaction of triterpenoids with  $H_2SO_4$

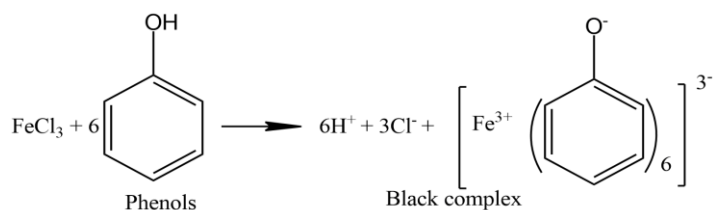


Figure 4. Reaction of phenol with FeCl<sub>3</sub>

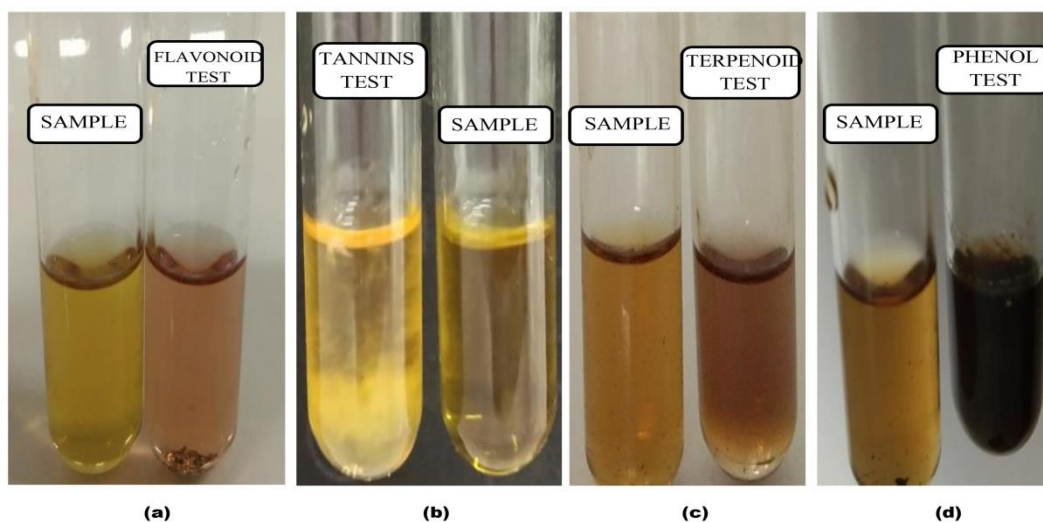


Figure 5. Results of phytochemical screening of flavonoids (a), tannins (b), triterpenoids (c) and phenols (d)

Figure 5 contains two tube names, where the tube labeled as sample contains kale extract without reagents and the second tube is sampled with each reagent. The positive result of favonoid in the test is shown in Figure 5 (a) where the kale sample that has not been added with the reagent is greenish yellow and after testing the tube II sample becomes red. Figure 5 (b) shows the difference in tube II (Sample) which was not tested and tube I (tannins) which after being tested with gelatin and NaCl formed a yellowish white precipitate. The test results in Figure 5 (c) there is a difference in the color, tube II is purple because the sample has been added Lieberman-Burchard reagent, which means kale extract contains triterpenoids. Tube II (phenols) in Figure 5 (d) shows that the kale sample that has been added with FeCl<sub>3</sub> can turn black, which means that the kale sample contains phenol.

#### Antidiabetic activity test

##### Operating time

Determination of the operating time is done with the aim of knowing the time required for the test solution to achieve a constant absorbance. This solution is in the form of a complex compound formation reaction between a whole glucose solution, Nelson's reagent and arsenomolybdate reagent which forms a greenish blue color. The operational time used

in this study is at 25 minutes in accordance with previous research by Hamdani *et al.* (2017).

##### Maximum wavelength

This determination aims to determine the maximum absorption area that can be produced from the 20 ppm glucose standard solution. According to the theoretical results, the measurement results obtained a maximum wavelength of 745 nm with an absorbance of 0.72, according to the theoretical results, namely 745 nm.

##### Positive glucose control

Positive control testing for glucose is very important in this test. This determination is used to determine the % value of glucose reduction so that we can find out what concentration of the sample has an effective value in reducing glucose levels (50%). In this determination, positive glucose control obtained an average absorbance of 0.75.

##### Antidiabetic activity

The antidiabetic activity test was carried out by Nelson Somogyi method. This method was chosen because the Nelson Somogyi method has specific properties for reducing sugars. The principle in this method remaining glucose which is not bound by the active compound in the kale extract will react with Nelson's reagent to form a brick-red precipitate which is then added with arsenomolybdate to form

molybdenum blue which is turquoise. Glucose reaction with Nelson can be seen in Figure 6.

The kale extract which added with glucose and Nelson's reagent subjected to a heating process for 10 minutes to accelerate the reaction of copper ions to form a brick red copper oxide precipitate. The cooling process for 5 minutes in this method is carried out so that the reaction can run stably, because there may be compounds that are not heat-resistant and volatile.

The brick-red precipitate will react with arsenomolybdate to form a turquoise color. This color results from the reduction of cuprooxide to cuprioxide. The smaller the residual glucose level, the smaller the absorbance value, because the color that is formed has a low concentration. A small absorbance value results in a large decrease in glucose levels.

The results of study in Table 2 start from the calculation of the decrease in glucose levels. The difference in positive control of glucose and absorbance of kale samples was calculated for each concentration, then the results of the difference were compared with the positive control of glucose. The final result is a percentage. The higher the concentration of added kale extract, the greater the percent decrease in glucose levels, and vice versa. The low concentration of kale extract contains glucose which is still high, so the absorbance value of the

sample read on uv-vis spectrophotometer is almost close to the value of the positive control of glucose.

The EC<sub>50</sub> value is obtained from the linear equation for the average decrease in glucose levels with the concentration of kale extract so that the value is 11.13 ppm. The value of this test shows that with a concentration of 11.13 ppm kale extract has produced a 50% maximum effect in reducing glucose levels. This ability is thought to be obtained from the flavonoid compounds identified in kale extract, where these compounds can react with glucose to form a glucose-flavonoid complex. The reaction for forming the glucose-flavonoid complex is shown in Figure 7.

The hydroxyl group on the flavonoids in kale extract will bind glucose so that the glucose level in the sample solution will decrease. The higher the kale extract concentration will make the flavonoids in it bind more glucose so that the remaining glucose is less, and the decrease in glucose levels in the kale extract gets bigger.

Flavonoids are compounds that have the ability to lower glucose through inhibition of metabolic enzymes, increase insulin secretion, reduce apoptosis, and reduce insulin resistance (Azzahra *et al.*, 2020). Kaempferol and quercetin found in kale also have a working mechanism: protecting beta cells from damage, supporting glycogen synthesis, and preventing alpha glucosidase (Adams *et al.*, 2015).

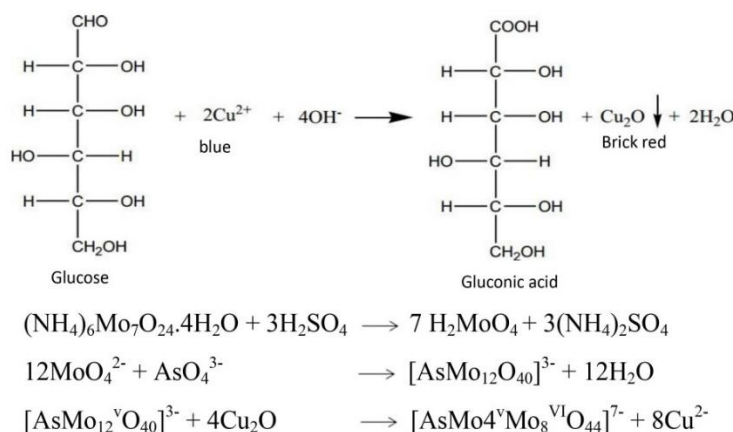
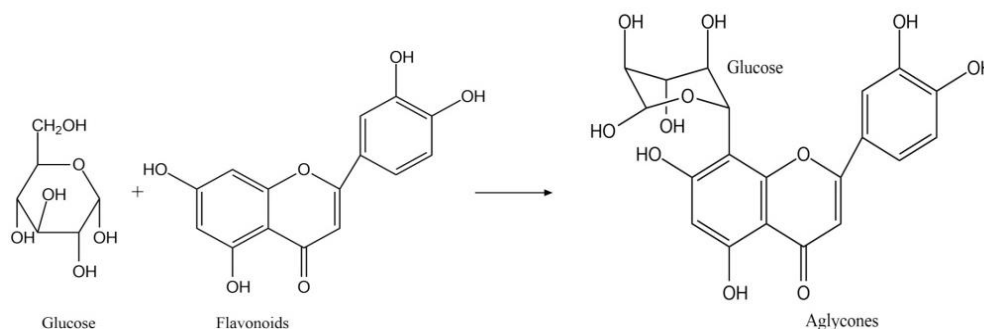


Figure 6. Glucose reaction with Nelson's reagent (Anggraini & Damayanti, 2019)

Table 2. Results of the percent decrease in glucose levels

Concentration (ppm)	% Decrease in Glucose Levels			Mean ± SD	Regression Equations	EC50 (ppm)
	Test I	Test II	Test III			
4	2.67 %	2.27 %	1.74 %	2.23% ± 0.46		
6	16.56 %	16.69 %	16.15 %	16.47% ± 0.27		
8	31.11 %	30.57 %	30.17 %	30.62% ± 0.46	y = 6.59x	11.13
10	42.19 %	41.79 %	41.66 %	41.88% ± 0.27	- 23.44	
12	55.54 %	55.27 %	55.67 %	55.50% ± 0.20		



**Figure 7.** Reactions of the formation of glucose complex compounds with flavonoids

## CONCLUSION

Phytochemical results show kale extract (*Brassica oleracea* var. *sabellica*) contains flavonoids, tannins, terpenoids, and phenols. Kale extract can reduce glucose levels or has antidiabetic activity with an EC<sub>50</sub> of 11.13 ppm.

## AUTHOR CONTRIBUTIONS

Conceptualization, A.K., N.Y.L.; Methodology, A.K., N.Y.L.; Software, A.K.; Validation, A.K., N.Y.L.; Formal Analysis, A.K., N.Y.L.; Investigation, A.K.; Resources, A.K.; Data Curation, A.K., N.Y.L.; Writing - Original Draft, A.K.; Writing - Review & Editing, A.K., N.Y.L.; Visualization, A.K.; Supervision, N.Y.L.; Project Administration, A.K., N.Y.L.; Funding Acquisition, A.K., N.Y.L.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

## REFERENCES

- Adams, A., Chen, J., Kimpe, N. D., Li, W., Mangelinckx, S. & Wang Z. (2015). Natural Flavonoids as Potential Herbal Medication for the Treatment of Diabetes Mellitus and its Complications. *Natural Product Communications*; 10; 187-200. doi: 10.1177/1934578X1501000140.
- Advistasari, Y. D., Vifta, R. & Wilantika. (2019). In Vitro Study of Antioxidant Potential and Antidiabetic Activity of the Ethyl Acetate Fraction of Parijoto Fruit (*Medinilla speciosa* B.). *Indonesian Journal of Medicinal Plants*; 12; 93-102. doi: 10.22435/jtoi.v12i2.1160.
- Agustina, W., Handayani, D. & Nurhamidah. (2017). Screening Phytochemicals and Antioxidant Activities Some Fractions of Distance Skin (*Ricinus communis* L.). *Journal of Education and Chemistry*; 1; 117-122.
- Anggraini, D. I. & Damayanti D. (2019). Antidiabetic Study of Combination of Cabbage (*Brassica oleracea* L.) and Tomato (*Solanum lycopersicum* L.) Extract by In Vitro. *As-Syifaa Journal of Pharmacy*; 11; 30-37. doi: 10.33096/jifa.v11i1.503.
- Aprizayansyah, A., Wiendarlina, I. Y. & Wardatun, S. (2016). Activity of In Vitro Decreasing Glucose Levels of Breadfruit Leaf Extract (*Artocarpus altilis* (Park.) Fosberg) and Its Correlation to Flavonoid Content. *Phytopharmaca*; 6; 1-11.
- Arjadi, F. & Susatyo, P. (2010). Regeneration of Langerhans Island Cells in Diabetic White Rats (*Rattus norvegicus*) Boiled Mahkota Dewa Meat (*Phaleriamacrocarp* (scheff.) Boerl. *Medica Journal of Medicine and Health*; 2; 1-11.
- Azzahra R., C., Nurmala, S., Risnanda H. D. & Yanuar, A. (2020). The Antidiabetic Potential of Orange Peels Flavonoids: A Review Regarding the Mechanism. Jakarta: Faculty of Pharmacy University of Indonesia.
- Bintang, M., Falah S. & Syafitri, N.E. (2014). Phytochemical Content, Total Phenol, and Total Flavonoids of Harendong Fruit Extract (*Melastoma affine* D. Don). *Current Biochemistry*; 1; 105-115.
- Cieslik, E., Florkiewicz, A. F., Leszczynska, T., Pisulewski, P. M. & Sikora, E. (2008). The Antioxidant Activity of Selected Cruciferous Vegetables Subjected to Aquathermal Processing. *Food Chemistry*; 107; 55-59. doi: 10.1016/j.foodchem.2007.07.023.
- Esteti, P. (2008). Isolation and Identification of Tannins in Purslane Herbs (*Portulaca oleracea* L.). *Thesis*; Faculty of Pharmacy University of Sanata Dharma, Yogyakarta.
- Hamdani, L. S., Wardatun, S. & Miranti, M. (2017). Sugar Level Reduction Activity and Antioxidant Potential of Dayak Onion (*Eleutherine palmifolia* (L.) Merr) Extract. Bogor: Faculty of



- Mathematics and Natural Science. Pakuan University.
- Kemenkes RI. (2020). InfoDATIN: Stay Productive, Prevent and Overcome Diabetes Mellitus. Jakarta: Kementerian Kesehatan Republik Indonesia.
- Krumbein, A., Kroh, L.W., Rohn, S., Schmidt, S., Schreiner, M. & Zietz, M. (2010). Identification of Complex, Naturally Occurring Flavonoid Glycosides in Kale (*Brassica oleracea* var. Sabellica) By High-Performance Liquid Chromatography Diode-Array Detection/Electrospray Ionization Multi-Stage Mass Spectrometry. *Rapid Communications in Mass Spectrometry*; 24; 2009–2022. doi: 10.1002/rcm.4605.
- Nurhasnawati, H., Putri, M. & Supriningrum, R. (2017). Determination of Flavonoid Levels of Ethanol Extract of Tiwai Onion (*Eleutherine palmifolia* (L.) Merr) Based on Simplicia Powder Size. Samarinda: Academy of Pharmacy Samarinda.
- Souhoka, A., Sunarty & Tulus, L.F. (2019). Utilization of Moringa Leaf Extract (*Moringa oleifera*, Lam) as Antioxidant in Coconut Oil. *Molluca Journal of Chemistry Education*; 9; 18-30.
- Susanti, N. M. P., Dewi, L. P. M. K., Harlina, S. M. & Wirasuta, I. M. A. G. (2017). Identification of Phenol Group Compounds from Ethanol Extract of Green Betel Leaf (*Piper betle* Linn) Using TLC-Spectro Photo Densitometry Method. *Metamorphosis Journal*; 4; 108-113.
- WHO. (2018). World Health Organization Diabetes mellitus. *World Health Organization*. <http://www.who.int/mediacentre/factsheets/fs138/en/>.
- Yuniarti, E. (2017). Differences in Tumor Necrosis Factor - Alpha Levels Between Controlled and Uncontrolled Type 2 Diabetes Mellitus. Padang: Faculty of Mathematics and Natural Sciences State University of Padang.