

## Identification of quercetin compounds in cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) from Malang Regency

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### ABSTRACT

Free radicals are chemical molecules with an unpaired electron in their outermost orbit. Free radicals are often referred to as Reactive Oxygen Species (ROS) which have reactive and labile properties so they tend to capture electrons from other molecules. Unhealthy lifestyles have a very high risk of increasing free radicals in the body. The imbalance of antioxidants with free radicals entering the body will cause oxidative stress. Consuming healthy foods, one of which is vegetables, is an effort that can be made to maintain a healthy body. The abundant production of cabbage and mustard greens in Malang Regency is expected to provide good benefits to help ward off excess free radicals. Quercetin compounds are one of the most common compounds found in plants and contain good antioxidants. The purpose of this study was to identify the content of quercetin compounds in cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) in Malang Regency. The method used is the quercetin compound test, with measurement performed using a UV-Vis spectrophotometer to determine the levels of compounds in cabbage and mustard greens extracts. The results of the study showed that after adding HCl reagents and Mg powder to cabbage and mustard greens, a dark red color change occurred, indicating that the results were positive for quercetin compounds. Measurements using a UV-Vis spectrophotometer obtained quercetin compound levels in cabbage (*Brassica oleracea* var. capitata L.) of 358.34 ppm or 3.5% and mustard greens (*Brassica juncea* L.) of 360.87 ppm or 3.61%.

**Keywords:** Cabbage, mustard greens, quercetin, Reactive Oxygen Species (ROS), UV-Vis spectrophotometer

### INTRODUCTION

Free radicals are chemical molecules that have unpaired electrons in their outer orbit (Pratama & Busman, 2020). Free radicals are often also called *Reactive Oxygen Species* (ROS) which have highly reactive and labile properties, so they tend to capture electrons from other molecules. The imbalance of antioxidants with free radicals entering the body will cause oxidative stress (Sinaga, 2016). The balance of each individual's body against exposure to free radicals depends on the lifestyle they lead (Nurdyansyah, 2017).

An unhealthy lifestyle has a very high risk of increasing free radicals in the body. Free radicals can come from metabolic disorders and the environment outside the body (Santosa & Baharuddin, 2020). Nowadays, many unhealthy

habits occur around us, which trigger an increase in free radical levels in the body, including exposure to X-rays, smoking, alcoholic beverages, air pollution, chemicals, lack of sleep, sunlight (UV), drugs, fast food, and so on (Pratiwi et al., 2023). The effects of the many sources of triggers to increase free radical levels in the body will cause cell damage to various serious health problems such as stroke, alzheimer's, diabetes, parkinson's, cancer, neurodegenerative diseases, heart disease, lung disease, autoimmune diseases, and others (Prasetyaningsih et al., 2023; Zhang et al., 2018).

Consuming healthy food is one of the things that can be done to maintain the body with the entry of good nutrients. Vegetable is a food that comes from plants and has many benefits (Heldawati, 2016). Malang Regency is

one of the districts in East Java that has abundant agricultural sector products (Widhaswara & Sardjito, 2017). According to Novita (2022), the production of cabbage (*Brassica oleracea* var. capitata L.) amounted to 72491.1 kilogram and mustard greens (*Brassica juncea* L.) amounted to 35804.5 kilogram. The abundant production of cabbage and mustard greens from Malang Regency is expected to provide good benefits to help ward off excess free radicals in the body with its properties.

Flavonoid compounds are one class of secondary metabolites that are widely available in nature. The content of flavonoid compounds has several functions, one of which is as an antioxidant so that it can capture free radicals and inhibit lipid oxidation (Zuraida et al., 2017). The type of flavonoid that has the best antioxidant properties is quercetin (Ningsih et al., 2023). Noer et al., (2018) determining flavonoid levels as quercetin in inggu leaf extract (*Ruta angustifolia* L.) using UV-Vis obtained flavonoid levels as quercetin 1.67% which includes the number of moderate levels. Quercetin levels in ethanol extracts of melasti kosta leaves amounted to 0.18% which is quite low (Melanie et al., 2023).

Based on previous research on the content of quercetin compounds in plants, researchers want to identify cabbage and mustard greens in Malang regency because of the benefits of natural plant bioactive compounds that have low toxicity and are environmentally friendly. This study was conducted to identify quercetin compounds in cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) from Malang Regency. The method used to determine the presence or absence of flavonoid compounds (quercetin) is by giving reagents and to determine the levels of flavonoids (quercetin) using a UV-Vis spectrophotometer.

## METHOD

This research is descriptive-exploratory

research. To see the presence or absence of flavonoid compounds (quercetin) in cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) from Malang Regency using flavonoid (quercetin) with HCl reagent, Mg powder and using a UV-Vis spectrophotometer.

The research was conducted in the chemistry laboratory of Muhammadiyah University of Malang. The research time began in August 2023 – March 2024. The samples used in this study were cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) from Malang Regency.

The tools are glassware: Erlenmeyer, beaker glass, glass funnel, measuring cup, test tube, and dropper pipette. The tools are non-glassware: oven, blender, sieve, filter paper, stirring rod, analytic balance, rotary vacuum evaporator, test-tube rack, label paper, aluminum foil, analytical scales, test tube racks, volumetric flasks, and cuvette. Instrumentation uses a UV-Vis spectrophotometer.

Materials for extraction and qualitative tests include 96% ethanol, 2N HCl, Mg powder, cabbage (*Brassica oleracea* var. capitata L.), and mustard greens (*Brassica juncea* L.) vegetables. Materials for measurements using UV-Vis spectrophotometer include quercetin, aluminum (III) chloride ( $\text{AlCl}_3$ ) 10%, sodium acetate ( $\text{CH}_3\text{COONa}$ ) 1 M, ethanol 96%, aquades, cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) vegetable extracts.

## Simplisia preparation

Vegetable samples of cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) were collected and then washed thoroughly using running water to remove dirt or dust attached to vegetable samples. After that, drain and sort wet on the part to be used. The sorted samples are chopped and dried. After drying until dry, the sample was pulverized using a blender until it became a fine powder and sieved using and sieve no. 60 mesh (Andriani & Anggraini, 2023).

### Extract preparation

Preparation of ethanol extract was carried out using the maceration method. Beginning with weighing the simplisia powder from cabbage vegetables of as much as 100 grams, the mustard greens of as much as 100 grams, then extracted by soaking the sample in 96% ethanol solvent in a ratio of 1:10. After that, it was allowed to stand for 3x4 hours without being exposed to sunlight while occasionally stirring. The extract was then filtered using filter paper (whatman paper no 2) and the filtrate was obtained. Furthermore, it was concentrated using a rotary vacuum evaporator at a temperature of 60°C and continued by evaporating in a water bath until a thick extract was obtained (Bangun et al., 2021).

### Test of flavonoid (quercetin)

Take 2 ml of ethanol extract from each sample. Then add a few drops of concentrated 2N HCl and 0.2 grams of Mg powder. A dark red (magenta) color change for 3 minutes indicates a positive flavonoid result. Testing of flavonoid compounds using three repetitions with the same extract volume between the three repetitions (Bangun et al., 2021).

### Preparation of standard solution (quercetin)

Make quercetin solution in 1000 ppm by weighing 25 mg of quercetin standard raw material, then dissolve in a 25 ml volumetric flask with 96% ethanol solvent until the line mark and then homogenized. Then, quercetin was diluted to 100 ppm by taking 5 ml of stock quercetin into a 50 ml flask, then added with ethanol solvent until the mark if the flask limit and homogenized. After that, it was diluted again to 0, 10, 20, 30, 40, and 50 ppm in a 25 ml flask by pipetting from quercetin stock 2.5; 5; 7.5; 10; and 12.5 ml then homogenized (Lindawati & Ma'ruf, 2020).

### Measurement of standard solution (quercetin)

The stock solution of each concentration was taken as much as 0.5 ml into a test tube.

Then 0.1 ml of 10%  $\text{AlCl}_3$ , 0.1 ml of 1M  $\text{CH}_3\text{COONa}$ , 1.5 ml ethanol, and 2.8 ml of aquades were added. Then, incubation was carried out for 30 minutes. After that, the absorbance was measured using a UV-Vis spectrophotometer under a wavelength of 430 nm using an absolute ethanol blank. After all, concentrations are measured, the absorbance value and standard curve are obtained, which will be used to determine the flavonoid standard of the sample (Lindawati & Ma'ruf, 2020).

### Measurement of total flavonoid (quercetin) level using UV-Vis spectrophotometer

Add 0.5 ml of ethanol extract of both samples into a test tube, then add 0.1 ml of 10%  $\text{AlCl}_3$ , 0.1 ml of 1M  $\text{CH}_3\text{COONa}$ , 1.5 ethanol, dan 2.8 ml of aquades. Then, incubation was carried out for 30 minutes. After that, the absorbance was measured using a UV-Vis spectrophotometer under a wavelength of 430 nm using an absolute ethanol blank. After all, concentrations are measured, the absorbance value and standard curve are obtained, which will be used to determine the flavonoid standard of the sample (Lindawati & Ma'ruf, 2020).

## RESULTS AND DISCUSSION

### Test of flavonoid compounds (quercetin)

The results of the research that (Table 1) has been carried out in the qualitative test of flavonoid compounds to determine the presence or absence of flavonoid compounds in cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) vegetables are positive for flavonoid compounds which can be observed in color changes to dark red (magenta) (Figure 1 and Figure 2). The reagent added is 2N HCl and Magnesium (Mg) powder until a color change to dark red (magenta) occurs. The addition of HCl serves to break flavonoid compounds into their aglycones with the hydrolysis of O-glycosyl which will be replaced by acidic  $\text{H}^+$  derived from HCl because it has electrophilic properties (Ikalinus et al., 2015). According to Estikawati & Lindawati (2019) concentrated HCl added to the extract will detect

compounds that have benzopyrillium (flavilium) salts (Lindawati & Ma'ruf, 2020). The results of flavonoid compounds using qualitative tests on

cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) showed positive results containing flavonoid compounds.

Table 1. Test of flavonoid compounds (quercetin)

Sample	Repetition	Reagents	Results	Conclusion (+)/(-)
Cabbage ( <i>Brassica oleracea</i> var. capitata L.)	U1	2N HCl and Mg powder	Discoloration occurs	+
	U2	2N HCl and Mg powder	Discoloration occurs	+
	U3	2N HCl and Mg powder	Discoloration occurs	+
Mustard Greens ( <i>Brassica juncea</i> L.)	U1	2N HCl and Mg powder	Discoloration occurs	+
	U2	2N HCl and Mg powder	Discoloration occurs	+
	U3	2N HCl and Mg powder	Discoloration occurs	+

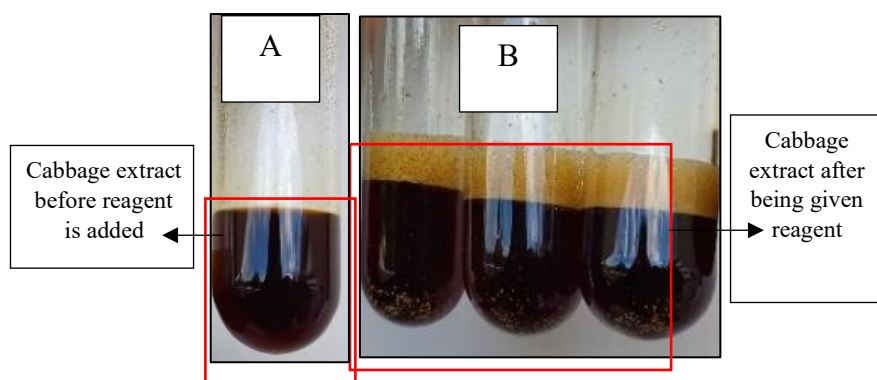


Figure 1. Identification of flavonoid compounds (quercetin) in cabbage; A) Control, B) Treatment.

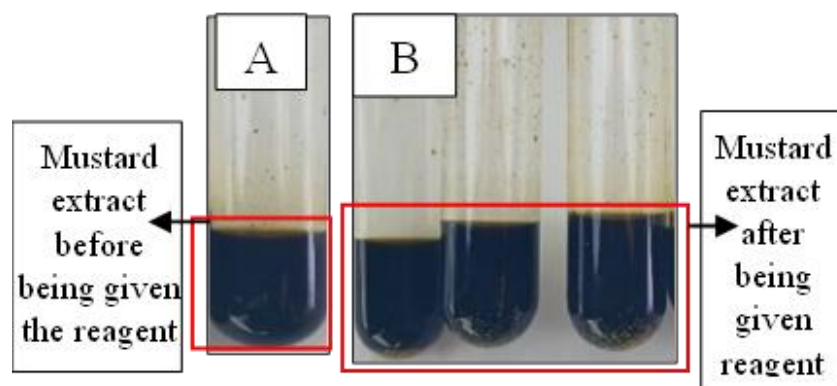


Figure 2. Identification of flavonoid compounds (quercetin) in mustard greens; A) Control, B) Treatment.

### Measurement of total flavonoid compound levels

The results of measurement using a UV-Vis spectrophotometer of flavonoid standard solution namely quercetin, obtained the absorbance value of each concentration at a wavelength of 430 nm. Based on the absorbance value produced, the higher the concentration the greater the absorbance value produced.

From the Table 2, the absorbance value of quercetin standard solution is obtained which

Table 2. Absorbance value of quercetin standard solution

Concentration (ppm)	Absorbance
0	0.000
10	0.100
20	0.145
30	0.167
40	0.252
50	0.403

can be depicted in the calibration curve of the standard solution in the form of a graph of concentration and absorbance.

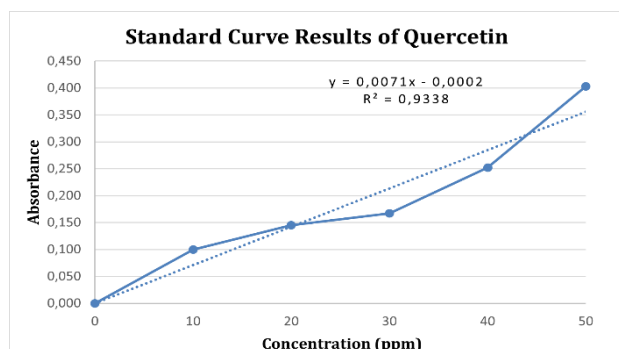


Figure 3. Quercetin curve results

The standard curve (Figure 3) shows a linear relationship between absorbance and concentration by the resulting linear regression equation of  $y = 0.0071x - 0.0002$  and the correlation coefficient ( $r^2$ ) of 0.9338. The correlation coefficient value is close to the value of 1, so it can be said that there is a linear relationship between absorbance which will be directly proportional to concentration.

Based on the qualitative test of flavonoid compounds in cabbage and mustard greens extracts in Malang Regency, the results showed positive results containing flavonoid compounds (quercetin) which were marked by a change in color to dark red (magenta). To determine the levels of flavonoids in cabbage and mustard greens extracts, measurements were made using a UV-Vis spectrophotometer using quercetin standard solution because one of the flavonoids comes from the flavonol group which has a keto group at C-4 and has a hydroxyl group at atoms C-3 or C-5 (Aminah et al., 2017). The quercetin standard solution used in this study has a concentration series of 0, 10, 20, 30, 40, and 50 ppm and the higher the concentration has a greater absorbance value. This follows the Lambert-Beer law which states that there is a linearity relationship between absorbance and concentration of the sample solution (Melanie & Legasari, 2022). In addition to using quercetin as a standard solution to determine the total flavonoid content, other solutions added are  $AlCl_3$  and  $CH_3COONa$ . The addition has a function to react with quercetin which can form a complex bond between aluminum chloride and quercetin so that there is a wave shift towards

the visible and is marked by a change in color to more yellow. Meanwhile, the addition of sodium acetate ( $CH_3COONa$ ) in the solution to maintain the wavelength in the visible region (Suwartini et al., 2021).

The measurement of flavonoid compounds (quercetin) comes from the calculation of the absorbance value of the standard solution which will produce a standard curve of quercetin and the resulting linear equation  $y = 0.0071x - 0.0002$  and the correlation coefficient ( $r^2$ ) is 0.9338. The correlation coefficient of this study is categorized as not too strong to see a linear relationship between concentration and absorbance value. Human error is one of the factors that cause the correlation coefficient on the standard curve to be less than the resulting standard grains. The total flavonoid content in cabbage extract (*Brassica oleracea* var. capitata L.) was 358.338 ppm or 3.503% and the total flavonoid content in mustard greens extract (*Brassica juncea* L.) was 360.873 ppm or 3.608% (Table 3 and Table 4).

Table 3. Absorbance measurement of cabbage extract

Repetition	Sample Volume (ml)	Absorbance	Average Absorbance	Total Flavonoids (ppm)	Total Flavonoids (%)
U1	0.5	2.517	2.562	360.873	3.608%
U2	0.5	2.607			

Table 4. Absorbance measurement of mustard greens extract

Repetition	Sample Volume (ml)	Absorbance	Average Absorbance	Total Flavonoids (ppm)	Total Flavonoids (%)
U1	0.5	2.492	2.544	358.338	3.503%
U2	0.5	2.596			

### Benefits of quercetin compound against Reactive Oxygen Species (ROS)

Cabbage extract is dark brown (very dark), so when given the reagent it does not experience much color change to dark red and the resulting change is bubbles (foam) at the top of the extract

after Magnesium (Mg) powder is given. The addition of Magnesium (Mg) powder and hydrochloric acid (HCl) will produce a reaction that forms bubbles in the form of H<sub>2</sub> gas (La et al., 2021). Likewise, in mustard green extract with a dark green color, it is rather difficult to observe the color change to dark red to determine whether or not there are flavonoid compounds. However, the cabbage and mustard green extracts showed positive flavonoid compounds with a dark red color change that was not very clear but there was a difference with the extract that had not been given the reagent (Figure 4).

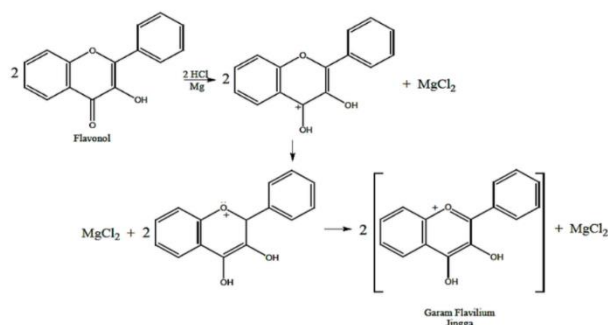


Figure 4. Flavonoid reaction with HCl and Mg

Based on the results of experiments that have been carried on through qualitative tests and UV-Vis spectrophotometer measurements, the results show the cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) contain flavonoid compounds (quercetin). Flavonoids are one of several secondary metabolite compounds produced by plants. Antioxidant is one of the roles of flavonoid compound to protect the body against Reactive Oxygen Species (ROS). ROS in normal amounts will intervene in metabolic processes in the body, but if in excessive amounts will be neutralized naturally by the body's antioxidant system. Exposure of the body to various activities and unhealthy lifestyles is increasingly occurring in the surrounding environment resulting in high exogenous factors of oxidative stress. Excessive formation of ROS in the body and decreased antioxidant capacity can lead to

worse damage to various biomolecules including lipids, proteins, and nucleic acid (Seo et al., 2023; Rinninella et al., 2019).

The compound that is most widely obtained in food through vegetables and fruits is quercetin (Zharani et al., 2023; Agustin., 2020). The results of the identification of cabbage and mustard greens show that they contain flavonoid compounds as quercetin can add insight into the consumption of foods that can help the body in balancing free radicals that enter the body. Free radicals that are very much in the body are closely related to cell damage, uncontrolled growth of cancer cells, and decreased immune system to other chronic diseases.

## CONCLUSION

The results of the research that has been done can be concluded that the qualitative test of flavonoid compounds (quercetin) in cabbage (*Brassica oleracea* var. capitata L.) and mustard greens (*Brassica juncea* L.) extract samples shows positive results by producing a color change to dark red (magenta). Furthermore, the results of quantitative tests of flavonoid compounds (quercetin) using a UV-Vis spectrophotometer can be concluded that the levels of flavonoid compounds (quercetin) in cabbage (*Brassica oleracea* var. capitata L.) amounted to 358.338 ppm or 3.503% and mustard greens (*Brassica juncea* L.) amounted to 360.873 ppm or 3.608%.

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