



# Investigation of antioxidant activity from beetroot juice (*Beta vulgaris* L) as a healthy drink for the prevention of non-communicable disease

*Uji aktivitas antioksidan pada jus buah bit (*Beta vulgaris* L) sebagai minuman kesehatan untuk pencegahan penyakit tidak menular*

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## Abstract

Non-communicable diseases can cause oxidative stress, which affects their pathophysiology. One of the recommended diets for preventing complications of non-communicable diseases (NCD) is a rich antioxidant diet. One of the foods rich in antioxidants is beetroots. This study aimed to assess the antioxidant content of locally obtained beetroot juice. Laboratory experimental methods were used to test the antioxidant activity of beetroot juice. Before the laboratory test, the panelists carried out a hedonic test. The beets were then juiced and checked for DPPH' (2,2-diphenyl-1-picrylhydrazyl). The beetroot was then juiced and checked for DPPH' (2,2-diphenyl-1-picrylhydrazyl). Statistical analysis was performed using the Kruskal–Wallis test. Results: The hedonic test showed that panelists preferred beetroot juice with lemon juice, with a 'like' range for the aroma and viscosity and a 'neutral-like' range for the taste and color of beetroot juice. The antioxidant content in 300 ml of beetroot juice was  $53,34 \pm 0,13\%$ . In conclusion, beetroot juice has a high antioxidant content and health benefits in preventing NCD.

**Keywords:** Acceptance test, antioxidant, beetroot, diet, health product

## Abstrak

Penyakit tidak menular dapat menimbulkan stres oksidatif yang berperan dalam patofisiologi penyakit tidak menular. Salah satu pola makan yang dianjurkan untuk mencegah komplikasi penyakit tidak menular adalah diet kaya antioksidan. Salah satu makanan yang kaya akan antioksidan adalah buah bit. Penelitian bertujuan untuk menilai kandungan antioksidan pada jus buah bit yang diperoleh secara lokal. Penelitian analitik menggunakan metode eksperimen laboratorium untuk menguji aktivitas antioksidan dari jus buah bit. Sebelum dilakukan uji laboratorium dilakukan uji hedonik. dilakukan oleh panelis. Bit kemudian diperas dan diperiksa DPPH' (2,2-difenil-1-pikrilhidrazil). Sampel diambil dari bit yang dibeli dari pasar buah lokal dan diuji di Layanan Uji Laboratorium Departemen Teknologi Pangan dan Hasil Pertanian Universitas Gadjah Mada pada Juni 2023. Buah bit kemudian dijus dan diperiksa DPPH' (2,2-difenil-1-pikrilhidrazil). Analisis statistik menggunakan uji Kruskal Wallis. Hasil, tingkat kesukaan panelis lebih menyukai jus buah bit penambahan perasan jeruk lemon dengan rentang 'suka' pada aroma dan kekentalan dan rentang 'netral-suka' pada rasa dan warna jus buah bit. Kadar antioksidan pada 300 ml jus buah bit sebesar  $53,34 \pm 0,13\%$ . Kesimpulan, Jus buah bit memiliki kandungan antioksidan yang tinggi dan memiliki manfaat kesehatan untuk mencegah PTM.

**Kata Kunci:** Antioksidan, buah bit, diet, produk kesehatan, uji terima

## Introduction

The World Health Organization (WHO) defines non-communicable diseases (NCDs) as non-infectious diseases caused by various combinations of genetic, physiological, behavioral, and environmental factors, which tend to last a long time and develop slowly (Finlay, 2020). Non-communicable diseases are the leading cause of death in the world (Luna & Luyckx, 2020). Cardiovascular disease, cancer, and diabetes are the most common non-communicable diseases (Martinez et al., 2020). In Indonesia, the most common non-communicable diseases are cancer, stroke, and chronic kidney disorders (Al Rahmad, 2021; Purnamasari, 2018).

Non-communicable diseases resulted in an increase in premature deaths from 23.1 million in 1990 to 34.5 million in 2017 (Martinez et al., 2020). Various factors that can increase the risk of non-communicable diseases include unhealthy diet, low physical activity, and use of tobacco and alcohol (Allen et al., 2017). The symptoms of non-communicable diseases are slowly progressive until clinical disorders appear which result in damage to important organs such as the heart and brain (Senapati et al., 2015).

Several studies show that non-communicable diseases can cause oxidative stress which causes changes in the body's biochemistry and plays a role in the pathophysiology of non-communicable diseases (Seyedsadjadi & Grant, 2021). Oxidative stress that arises in non-communicable diseases arises due to increased free radicals in chronic diseases (Al-Hadlaq et al., 2022). Increased levels of free radicals can form reactive oxygen species (ROS) as a result of aerobic cell damage by free radicals and damage genes in cell DNA which leads to pathological conditions of non-communicable diseases (Al-Hadlaq et al., 2022). This condition leads to cell damage that occurs progressively due to limited antioxidant components in cell defense against free radicals (Adnan et al., 2022). Cell damage that occurs in non-communicable diseases will activate a low-level inflammatory response that runs chronically and results in final organ damage (Al-Hadlaq et al., 2022). To prevent complications from damage to the body's important organs due to non-communicable diseases, an effective preventive approach is needed to reduce damage to the body (Budreviciute et al., 2020).

Lifestyle interventions, a healthy diet, and improving access to health can prevent non-communicable diseases (Budreviciute et al., 2020). One type of diet recommended to prevent complications of non-communicable diseases is a diet rich in antioxidants (Gantenbein & Kanaka-Gantenbein, 2021). Antioxidants can come from endogenous (in the body) or exogenous (outside the body) (Ayoka et al., 2022). Antioxidants function to neutralize ROS formed due to oxidative stress while preventing the formation of new ROS (Ayoka et al., 2022). Food sources rich in antioxidants come from fruits and plants which are rich in natural antioxidants such as vitamins, carotenoids, and polyphenols (Ayoka et al., 2022; Rahayu et al., 2023). Several existing studies show the benefits of consuming a diet rich in antioxidants in reducing oxidative stress as a risk factor for non-communicable diseases (Bruins et al., 2019). One of the recommended fruits that has a high antioxidant content is beetroot (*Beta vulgaris L. subsp. Vulgaris*).

Beetroot (*Beta vulgaris L. subsp. vulgaris*) is a type of fruit from the Chenopodiaceae family originating from Eastern and Southern Europe and North Africa (Wruss et al., 2015). Beetroot has a high micronutrient content including vitamin A (20 IU), vitamin C (10 mg), calcium (27 mg), iron (1 mg), phosphorus (43 mg), and various phytochemicals such as betalains, betaine, and nitrates (Babarykin et al., 2019). Several studies have found the health benefits of beets for lowering blood pressure, improving physical and cognitive performance, and cardiovascular health (Zamani et al., 2021). These findings are important for providing alternative food ingredients that are useful for preventing non-communicable diseases that develop from unhealthy diets, considering that there has been a shift in the diseases that dominate Indonesian society, namely non-communicable diseases such as coronary heart disease, stroke, and diabetes mellitus (Afshin et al., 2014; Purnamasari, 2018).

Beetroot can also be used in various forms of processed food which can increase the value of beetroot (Chhikara et al., 2019). One of the most common forms of processed beetroot is beetroot juice because it is easy to consume and does not require a long manufacturing process (Wruss et al., 2015). In existing studies, beets have the benefit of lowering blood pressure in both healthy individuals and populations with NCDs, thereby potentially reducing the risk of cardiovascular

events in the future (Ocampo et al., 2018). In addition, the high antioxidant content is useful for improving cell function in the body from the effects of inflammation in NCDs (Liliana & Oana-Viorela, 2020).

However, the antioxidant content in beetroot juice in several studies varies which may be due to differences in the conditions and environment of the beetroot (Wruss et al., 2015). Based on the study above, this research aimed to assess the antioxidant content in beetroot juice obtained locally.

## Methods

This research is a preliminary study with experimental research using an *in vitro* laboratory to determine the levels of antioxidant activity in beetroot juice. In this study, the independent variable was antioxidant levels with beetroot juice as the dependent variable. Beetroot was obtained from a local fruit market and then tested for antioxidant activity at the Laboratory Testing Service of the Department of Food Technology and Agricultural Products, Gadjah Mada University. The antioxidant activity test was carried out on June 19, 2023. This research used 150 grams of beetroot which was processed into 300 mL of beetroot juice.

Testing antioxidant activity levels in this study used the DPPH' (2,2-diphenyl-1-picrylhydrazyl) method which is the most commonly used method for measuring antioxidant activity in food ingredients (Permanasari et al., 2021). The mixture was read at a maximum wavelength of 516 nm with a UV-Vis spectrophotometer. Testing was carried out at the Food Technology and Agricultural Products Testing Laboratory, Gadjah Mada University with sample number 436/PS/06/23. This research has received approval from the Ethics Committee of the Faculty of Medicine, Sebelas Maret University with code 119/UN27.06.11/KEP/EC/2023.

DPPH, or 2,2-diphenyl-1-picrylhydrazyl, is the free radical most often used to assess the antioxidant activity of natural component extracts and is stable at room temperature. The DPPH method was chosen because it allows a fast, accurate, easy, and sensitive evaluation of the antioxidant activity of natural ingredients. This method can evaluate materials quickly and has a good level of sensitivity for determining antioxidant activity in samples.

The EC50 (effective concentration) or IC50 (inhibitory concentration) value is a parameter used to indicate antioxidant activity. The IC50 value is the concentration of antioxidant substances that can cause a 50% loss of radical properties with DPPH or the concentration of antioxidant substances that provide 50% inhibition which is obtained through the regression equation (Permanasari et al., 2021).

The IC50 value of the DPPH test compound is usually compared with the value of vitamin C, vitamin E, or quercetin, which are natural antioxidant compounds. The lower the IC50 or EC50 value of the test compound, the more effective the compound is as an antidote to free radicals (Permanasari et al., 2021)

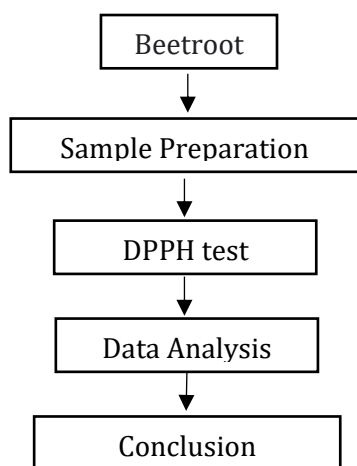
Previously, beetroot fruit juice samples had been tested for acceptability on treatment respondents with a total of 35 panelists. The beet juice formula is made of 3 types each; the first formula (without lemon juice), the second formula plus 2 tablespoons (Tsp) of lemon juice, and the third formula one tablespoon of lemon juice plus frambozen essence with each formula coded JB01, JB02, JB03. Measurements were carried out on a numerical scale of 1 – 9 which indicates "very very like" for a value of 9 and "very much dislike" for a value of 1. Based on the results of the acceptability test analysis, it was found that the panelists preferred the second formula of beetroot juice with the code JB02 or fruit juice beets with added 2 tablespoons (Tbsp) lemon juice. The formula was then analyzed for antioxidant content with DPPH.

## Instruments and Work Procedures

Some of the ingredients needed are 150 grams of beetroot, 150 ml of water, 2 tablespoons of lemon juice, and sugar without calories. Apart from that, the tools needed are a juicer (BL-102-PL), a filter, and a 300 ml plastic bottle. The work procedure in this research began with sample preparation, where 150 grams of beetroot were washed, sorted, and cut into small pieces then added with 150 ml of water and 2 tablespoons of lemon mashed using a blender. Then the juice is filtered with a filter and packaged in 300 ml bottles.

The next stage is to determine the free radical scavenging activity of DPPH, namely 300 ml of beet juice whose concentration has been determined is added with a solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The change in color of the solution from purple to yellow indicates the efficiency of free radical scavengers.

Next, it was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer.



**Figure 1.** Research flow.

Determination of the antioxidant concentration of beetroot juice was then adjusted to the IC50 strength classification according to Table 1.

**Table 1.** Classification of antioxidants (Novatama et al., 2016).

IC50	Antioxidants
<50	Very strong
50 - 100	Strong
101 - 150	Currently
151 - 200	Weak

**Table 2.** Hedonic test score results.

category	Mean ±SD (95% CI)			p-value	Description
	JB01	JB02	JB03		
Color	6,77 ± 0,42 (6,63 - 6,92)	6,94 ± 0,33 (6,83 - 7,06)	6,83 ± 0,38 (6,70 - 6,96)	0,179	Like
Aroma	5,69 ± 1,10 (5,31 - 6,07)	7,03 ± 0,74 (6,77 - 7,29)	7,09 ± 1,01 (6,74 - 7,43)	0,000*	Neutral -Like
Flavor	5,03 ± 1,80 (4,41 - 5,65)	7,06 ± 0,99 (6,71 - 7,40)	6,94 ± 0,76 (6,68 - 7,21)	0,000*	Neutral -Like
Thickness	6,74 ± 0,65 (6,52 - 6,97)	6,89 ± 0,79 (6,61 - 7,16)	6,74 ± 0,81 (6,46 - 7,02)	0,609	Like

Notes:

JB 01: Beetroot juice formula 1, JB 02: Beetroot juice formula 2, JB 03: Beetroot juice formula 3.

\*Significant at 95% CI (p < 0.05)

From 150 grams of beetroot, we got a 300 mL sample of beetroot juice which was then analyzed for antioxidant levels using DPPH examination. Based on examination with DPPH, we found antioxidant levels in beets of 53.34 ± 0.13%. Based on the criteria, the antioxidant content in beetroot juice has strong antioxidant power (Table 3).

Statistic analysis was carried out using the Kruskal Wallis test via SPSS software to test the significance of data between hedonic tests. The test results are then calculated and presented along with the mean and standard deviation (determining the quality value by finding the average result for each panelist at the 95 percent confidence level).

## Result and Discussion

Based on the hedonic test that was carried out, each percentage value of the beetroot juice formula was obtained. JB 01: Beetroot juice formula 1, JB 02: Beetroot juice formula 2, JB 03: Beetroot juice formula 3.

The percentage of beet juice formulas JB01, JB02, and JB03 based on the color preference test was 49,11%; 57,8%, and 52,09% respectively. Then the percentage values based on the aroma preference test were 31.5%, 64,6%, and 63,34% respectively. The percentage values based on the taste preference test were 39,01%, 64,94%, and 63,97%. The percentage values based on the viscosity preference test were 50,14%; 56,47%, and 53,39% respectively. From these results, it was found that on average the panelists liked the JB02 formula as beetroot juice.

The results are not much different from the research of Desseva et al (2020) on examining the antioxidant activity of red beetroot juice 56.71 ± 1.66 (Desseva et al., 2020). The DPPH method is a bio-chemical examination using a stable DPPH radical reagent (Firdausia et al., 2023). When it reacts with an

antioxidant, the DPPH reagent will change color from purple to yellow (Firdausia et al., 2023). DPPH examination is interpreted by the percentage of the antioxidant's ability to react with free radicals, with the smaller the percentage indicating the stronger the antioxidant activity against free radicals (Baliyan et al., 2022; Firdausia et al., 2023). The results of this study found that beetroot juice has a strong antioxidant effect, and is very good as a health drink.

**Table 3.** Beet juice concentration levels

Test Sample	Mean $\pm$ SD (% wb)
Antioxidant activity	56,71 $\pm$ 1,66

The antioxidant content of beet juice differs from other forms of preparation (Guldiken et al., 2016). In a study by Guldiken et al (2016), the antioxidant content of beets in juice form was lower than in the form of fruit or other processed products such as jam or puree (Guldiken et al., 2016). Changes in phytochemical and nutritional content in beets can be influenced by processing activities which include changes in temperature, pH, oxygen levels, and water as a solvent in the beet processing method (Liliana & Oana-Viorela, 2020). The process of managing beetroot can also affect the betalain content, the main bioactive components in beetroot can reduce their concentration due to high temperatures (> 100), water activity and radiation which can degrade the betalain content (Fu et al., 2020).

The most abundant antioxidant in beets is betanin, which is included in the bioanthin group, one of the main groups of betalains (Esatbeyoglu et al., 2015). Betanin has been widely studied as having high antioxidant activity because it has a phenolic hydroxy group and a benzene group with unsaturated bonds (Chen et al., 2021). The betanin component can counteract oxidative stress activity which can impact human DNA by being mediated by the nuclear factor (erythroid-derived 2)-2 (Nrf-2) gene (Chen et al., 2021). In addition, consuming beets has the effect of increasing levels of superoxide dismutase, a natural antioxidant in the body, and repairing them after exposure to free radicals (Chen et al., 2021). The discovery of the betanin content in beets plays a role in providing a protective effect on important

organs in the body due to the influence of oxidative stress on various chronic diseases such as cardiovascular disease (Silva et al., 2021).

Several studies show that the betanin content in beets has an antidiabetic effect by influencing glucose metabolism in the body and preventing damage to the liver and pancreas (Abedimanesh et al., 2021). In research by Dhananjayan et al (2017) and Abedimanesh et al (2021) in rats induced diabetes with streptozocin, intervention with betanin had a significant effect on reducing glucose levels, and at higher doses, betanin improved insulin levels and profiles lipids (Abedimanesh et al., 2021; Dhananjayan et al., 2017). Betanin also has a protective effect on the liver and pancreas from damage caused by streptozocin through activation of the sirtuin-1 (SIRT1) and Adenosine 5'-monophosphate-activated protein kinase (AMPK) genes which act as anti-inflammatory and protect against oxidative stress (Abedimanesh et al., 2021; Dhananjayan et al., 2017). In a study by Aliahmadi et al (2021) on a population with type 2 diabetes, giving raw beets for 8 weeks had antidiabetic and hepatoprotective effects (Aliahmadi et al., 2021). Apart from that, beets can also increase the total antioxidant capacity in diabetes patients and improve cognitive function (Aliahmadi et al., 2021; Dainy & Yunieswati, 2023).

Another ingredient in beet juice is Nitric Oxide (NO) (Arazi & Eghbali, 2021). The NO content in beets also varies depending on the processed product (Baião et al., 2020). In a review by Baião et al (2020), the NO content in juice form was 4.10 mmol per 100 grams, which is lower than in other processed forms such as cereal, gel, or chips (Baião et al., 2020). The NO consumed can be absorbed by the upper digestive tract (Baião et al., 2020). In human physiological conditions, NO has an antioxidant effect which is useful for vasodilation and preventing blood vessel damage (Baião et al., 2020; Saxena et al., 2018). In addition, NO can improve the immune system and neurotransmitters that are useful for the nervous system (Baião et al., 2020).

Several studies show that consuming beetroot juice over a certain period has antihypertensive effects (Ocampo et al., 2018). In a review by Bonilla Ocampo et al (2018), the amount of nitric oxide was between 300 and 500 mg in studies with beetroot juice, which is higher

than the acceptable daily intake as defined by WHO (3.7 mg/kg body weight per day) (Ocampo et al., 2018). In hypertensive conditions, the availability of NO decreases due to oxidative stress in blood vessels, thereby damaging the ability of vasodilation and endothelial dysfunction (Pinheiro et al., 2017). Because NO has a short half-life (1-2 ms) when in the bloodstream, additional NO supplementation can improve NO levels in the body and repair blood vessels (Ocampo et al., 2018; Pinheiro et al., 2017). Giving NO can also reduce blood pressure and reduce the risk of complications due to hypertension such as stroke and coronary heart disease (Ahmad et al., 2018; Ocampo et al., 2018).

Several existing studies also show the benefits of consuming beetroot juice, although the existing studies were conducted on limited population samples and the results varied. In a study by Gilchrist et al (2013), consumption of beetroot juice in patients with type 2 diabetes mellitus and hypertension had the effect of reducing diastolic blood pressure although there was no significant difference when compared to the control group (Gilchrist et al., 2013). Further research by Karimzadeh et al (2022) shows that consuming beets has a significant blood pressure-lowering effect in diabetes patients for 12 weeks without a diet high in nitrates which is generally recommended for patients with metabolic syndrome (Karimzadeh et al., 2022). Therefore, further research is needed to assess the effectiveness of beetroot antioxidants for health. In addition, it is necessary to compare the antioxidant content in other processed beet products.

This research still has several limitations and weaknesses. Because this research is a preliminary study, it cannot be concluded regarding the effect of giving beetroot juice on various body metabolic parameters. We also did not test the phytochemical components contained in each beetroot juice formula, so further research is needed regarding the phytochemical composition of beetroot juice and its benefits for the body. In addition, tests on humans are needed to assess the health effects of beetroot juice on the metabolic parameters of the human body.

## Conclusion

The potential of beet juice as a health drink has high antioxidant activity. The most antioxidant content in beets is bioxanthin which is one of the

phytochemicals in beets and nitric oxide. The antioxidant content has many benefits for the body because it can prevent non-communicable diseases such as diabetes and hypertension.

Suggestions, further research on beetroot products is needed to compare the product content and its benefits for body health by assessing the effect of beetroot on components of body metabolism such as blood pressure, cholesterol, and uric acid, which are indicators of non-communicable diseases. Collaboration is needed between research agencies in the research and health fields and the community to utilize research results on the benefits of beetroot as a beverage product to reduce the problem of NCDs in the future.

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