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| RESEARCH ARTICLE

Total Phenolic and Total Flavonoid Content, Antioxidant Activity, and Nutritional Profile of *Ziziphus mauritiana* Fruit Juice

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| ABSTRACT

Ziziphus mauritiana is a plant species belonging to the Rhamnaceae family. It is commonly found in tropical and subtropical regions of Asia, and in Indonesia, this plant grows wild and thrives on the islands of Bali, Lombok and Sumbawa. The fruits of *Z. mauritiana* are edible, however, in Indonesia, it is underutilized and has very low economic value. To evaluate its possibility to be developed as a functional food, this study was conducted to determine the total phenolics and flavonoids, dietary fiber, and nutrients contained in the fruit juice of *Ziziphus mauritiana*. Total phenols and flavonoids contents were determined using Folin Ciocalteu and AlCl3 methods, respectively. The in vitro antioxidant activity of the juice was evaluated using the DPPH method. The content of soluble and insoluble dietary fiber was determined using the standard AOAC analysis method, and proximates analysis was conducted in accordance with SNI 01-2891-1992. The results of this study revealed that freeze-dried juice of *Z. mauritiana* fruit has total phenols content of 1690 mg GAE/100g, total flavonoids of 15.10 mg QE/100g, soluble dietary fiber of 0.61%, and insoluble dietary fiber 2.03%. However, the antioxidant activity of *Ziziphus mauritiana* fruit juice was weak with IC_{50} 328.54 ppm. The proximate analysis revealed the crude fiber in the freeze-dried fruit juice was 0.56%, carbohydrates 82.43%, protein 3.34%, fats 0.31%, ash 3.62%, and water 10.30%. In conclusion, *Ziziphus mauritiana* fruit juice has a high content of total phenolic substances, high dietary fiber, and high nutritional value, therefore it has a good potential to be developed as a functional food.

| KEYWORDS

Ziziphus mauritiana fruits, nutritional profile, total phenols, flavonoids, antioxidant activities.

| ARTICLE INFORMATION

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1. Introduction

Indonesia has an abundant diversity of fruit plants, however, most of these fruits have not been utilized optimally. One species of these fruits is *Ziziphus mauritiana* which belongs to the Rhamnaceae family. *Z. mauritiana* is a plant that can be found in tropical and subtropical regions and is able to survive in moderate (semi-arid) to extreme (arid) climates (Muhammad *et al*., 2022). The fruit of *Z. mauritiana* is edible, the texture is soft, fleshy, crunchy, and has a sour or sweet taste, sometimes even very sweet, with red, yellowish brown or orange colour, with a pleasant aroma. The fruit varies in size and shape, round or oval, depending on the variety, with a diameter of 2-5 cm, with large seeds. *Z. mauritiana* is an upright tree with small thorns attached to tree branches and leaf gaps. The leaves are slightly oval in shape and green in color (Sodimu *et al*., 2020; Soraya *et al*., 2022). Although edible, *Z. mauritiana* fruits do not have high economic value in Indonesia. The fruit is only used as fodder along with the leaves, or

occasionally eaten by children in the villages. *Z. mauritiana* trees generally grow wild and are not cultivated commercially, so they can be categorized as underutilized fruits (Meghwal *et al*., 2021).

The chemical content of *Z. mauritiana* originating from outside Indonesia has already been analyzed. This fruit contains various bioactive compounds such as flavonoids, phenols, triterpenoids, alkaloids, saponins, glycosides, tannins, and vitamin C (Okala *et al*., 2014; Riaz *et al*., 2021). In addition, these fruit is a very good source of protein, carbohydrates, fats and micronutrients, such as zinc (Zn), iron (Fe), copper (Cu), phosphorus (P), sodium (Na), potassium (K), and calcium (Ca) (Butt *et al*., 2021; Sareen et al., 2020). However, the chemical content and antioxidant activity of Z. *mauritiana* fruit found in Indonesia have never been reported, even though this fruit is quite common in Indonesia.

To find out the potential utilization of Indonesian *Z. mauritiana* fruits, especially their potential as a functional food, it is necessary to carry out an investigation to determine the chemical content, antioxidant activity and nutritional profile of *Z. mauritiana* fruits in Indonesia. In this study, we determined the total phenolic and total flavonoids content, antioxidant activity, and nutritional profile of *Z. mauritiana* fruit juice originating from Medang Island in West Nusa Tenggara, Indonesia.

2. Literature Review

2.1 Botanical aspects of Ziziphus mauritiana

Ziziphus mauritiana known as jujube, Indian jujube, Indian plum, ber, bor, beri, narkeli kul, dongs, boroi, desert apple, Malay apple, or Chinese apple (Palejkar *et al*., 2012; Prakash *et al*., 2021). In Indonesia it is known as bidara, widara (Javanese, Sundanese), goal, rangga (Bima), kalangga (Sumba), bekul (Bali), or kom (Kupang) (Kurniawan & Pujiono, 2019; Raharjeng & Masliyah, 2020).

Z. mauritiana is a fruit tree that belongs to the Rhamnaceae family. It is an evergreen tree, that grows to a height of 3-15 meters, and the trunk diameter can reach more than 40 cm. *Z. mauritiana* is a thorny plant, its thorns are located on the branches. The leaves are green, alternate, elliptical or ovate, 2-9 cm long, with 3 prominent longitudinal veins. The upper surface of the leaf is glossy dark green and the underside is hairy greenish-gray. The flowers are small and yellowish in color with delicate petals and appear in the axils of the leaves. *Z. mauritiana* fruit is edible. The fruit has shape and size that varies depending on the cultivar or varieties. They are usually round, oval or oblong in shape, 2–5 cm long. The fruit flesh has a soft, crunchy texture and is white in color. The taste of the fruit is sour or sweet, but sometimes the taste is very sweet and has a pleasant aroma. The rind can be smooth or rough, shiny, or thin but firm. The unripe fruit is green to yellowish, while the ripe fruit is brownish-yellow to reddish, or blackish (Naaz *et al*., 2020; Raharjeng & Masliyah, 2020; Sodimu *et al*., 2020; Soraya *et al*., 2022).

***Figure*1. *Ziziphus mauritiana*: a. Flowers and leaves; b. Fruits**

2.2 Habitat and distribution of Ziziphus mauritiana

Ziziphus mauritiana can be found in tropical and subtropical regions and are able to survive in moderate (semi-arid) to extreme (arid) climates (Muhammad *et al*., 2022; Youl *et al*., 2019). This plant is known for its ability to withstand adverse conditions, such as salinity, drought, and waterlogging. Fruit quality is best under hot, sunny, and dry conditions, but there must be a rainy season to support growth extension and flowering (Orwa *et al*., 2009).

Z. mauritiana plants are spread in several tropical regions of the world, such as South Asia, East Africa, South Africa, Australia, India, China, Egypt, Kenya, Afghanistan, Algeria, Australia, Bangladesh, Iran, Libyan Arab Jamahiriya, Nepal, Pakistan, Thailand, Tunisia, Uganda, Vietnam, and Southeast Asia such as Malaysia, the Philippines, and Indonesia (El Maaiden *et al*., 2020; Naaz *et al*., 2020; Prakash *et al*., 2021). In Indonesia, *Z. mauritiana* thrives in Java, Bali, West Nusa Tenggara, East Nusa Tenggara, Sulawesi and Maluku (Kurniawan & Pujiono, 2019; Raharjeng & Masliyah, 2020).

2.3 Phytochemical content and nutrition of Ziziphus mauritiana fruit

Z. mauritiana fruit is a potential source of carbohydrates, protein, fiber, and micronutrients such as calcium (Ca), potassium (K), sodium (Na), phosphorus (P), copper (Cu), iron (Fe), and zinc (Zn) as shown in Table 1 (Butt *et al.,* 2021; Ghasham *et al*., 2017; Nyanga *et al*., 2013). Tajudeen *et al*., (2018) revealed the presence of carbohydrates (55.00 ± 0.24%), crude fat (5.13 ± 0.02%), crude

protein (17.50 ± 0.32%), crude fiber (11.40 ± 0.04 %), ash (6.27 ± 0.31%) and moisture (4.70 ± 0.25%) in *Z. mauritiana* fruits. Winarti *et al*., (2020) reported that *Z. mauritiana* fruit also has a fairly high water content, namely 81.6-83g/100g. The high water content causes the fruit that has been harvested to quickly decay.

Okala *et al*., (2014) showed that *Z. mauritiana* fruit extract contains flavonoids, glycosides, steroids, terpenoids, tannins and antioxidant substances. Riaz *et al*., (2021) showed that the total phenols in *Z. mauritiana* fruit were 207.6 mg GAE/100 g, while the total flavonoids were 102.9 mg QE/100 g. In addition, *Z. mauritiana* fruit also contains vitamins, such as vitamins A and B complex (Butt *et al*., 2021), as well as vitamin C, with levels ranging from 65.8 to 76.0 g per 100 g fresh *Z. mauritiana* fruit (Sareen *et al*., 2020).

2.4 Utilization and biological activity of Ziziphus mauritiana

Ziziphus mauritiana is a versatile tree grown primarily for its fruit. *Z. mauritiana* fruit can be eaten fresh and dried as dates, candied, or pickled (Orwa *et al*., 2009). According to Okala *et al*., (2014) *Z. mauritiana* fruit in India is processed into flour, butter, pasta, cakes, creams and drinks, as well as used for the manufacturing of candies. The young leaves of *Z. mauritiana* can be eaten and cooked as vegetables in Indonesia. The leaves are usually used as fodder for sheep and goats. The fruit and bark are used to make dyes and traditional medicines. The wood is reddish in color, fine textured, hard and durable, hence it can be used in country house construction, poles, tools, and used as firewood (Sodimu *et al*., 2020).

Several studies have reported the pharmacological activities of various plant parts such as fruit, leaves and stem as antioxidants, cytotoxic, antimicrobial, anti-diarrheal, antidepressant, immunomodulatory, anti-inflammatories and hepatoprotective (Prakash *et al*., 2021), and also preventing tumor development (Kaur *et al*., 2015; Marfu'ah *et al*., 2019). Sareen *et al*., (2020) revealed that *Z. mauritiana* fruit has the potential as a source of vitamin C which plays a very important role in boosting the immune system and counteracting free radicals. The dried fruit is used as an anodyne, anticancer, cooling agent, sedative, stomach ache, and tonic, and is believed to purify the blood and aid digestion (Palejkar *et al*., 2012). The fruit is also mixed with salt to treat indigestion and bile disorders (El Maaiden *et al*., 2020).

3. Methodology

3.1 Preparation of Fruit Juice

The material used in this study was ripe *Ziziphus mauritiana* fruit obtained from Medang Island, Sumbawa, West Nusa Tenggara, Indonesia. The plant and fruits were identified at Herbarium Bogoriense, Center for Biology Research, Bogor, Indonesia. The preparation of the fruit juice followed the work of Sinaga et al. (2021). The juice was made by processing the mesocarp of the fruits with slow-juicer. After that the fruit juice is immediately frozen, then freeze-dried at -43°C for 72 hours, and stored in the freezer.

3.2 Determination of total phenolics content

The total phenolics content of the juice was determined using the Folin-Ciocalteu method, following the method described by Sinaga et al. (2021) with slight modifications using gallic acid as standard. The standard solution concentration range was 0, 10, 30, 50, 70, and 100 ppm. Absorbance was measured at 730 nm against the reagent blank. The total phenolics content was determined as mg gallic acid equivalent per 100 g sample.

3.3 Determination of total flavonoids content

The total flavonoid content was determined by the $AlCl_3$ colorimetric method using quercetin as standard (Nayeem *et al*., 2022; Wairata *et al*., 2022). The total flavonoids content was determined as mg quercetin equivalent per 100 g sample (mg QE/100 g) using the quercetin calibration curve. Absorbance was measured at 434.2 nm using a spectrophotometer.

3.4 Determination of antioxidant activity

Antioxidant activity was approached by free radical scavenging activity measured using the DPPH (2,2-diphenyl-1-pikrylhidrazyl) reagent as described by Effendi *et al*. (2022) and Sukweenadhi *et al*. (2020) with slight modifications. Ascorbic acid was used as standard. The fruit juice was mixed with DPPH solution in ethanol. The mixture was vortexed thoroughly and stored in a dark place at room temperature for 30 minutes. The reaction was left for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. Antioxidant activity is represented in IC50, namely the concentration of the extract required to bind 50% of the DPPH free radical, calculated from the graph plot of the radical scavenging activity against the concentration of the freeze-dried juice.

3.5 Determination of dietary fiber content

The dietary fiber content of freeze-dried *Z. mauritiana* fruit juice was determined using the AOAC method as carried out by previous researchers (Kusumastuti *et al*., 2016; McCleary *et al*., 2012; Pratiwi & Krisbianto, 2019). Briefly, 0.5 grams of sample was put into Erlenmeyer flask, and then 50 mL of phosphate buffer and 0.1 mL of the alpha-amylase enzyme were added. The mixture

was then heated at 100°C for 30 minutes while stirring on a magnetic stirrer. Then the sample was cooled and added 20 mL of distilled water, 5 mL of 1N HCl and 1 ml of 1% pepsin solution. The mixture was then heated to 100°C for 30 minutes, and after cooling, 5 mL of 1N NaOH and 0,1 mL of the beta-amylase enzyme were added. The Erlenmeyer flask was closed and heated to 100°C for 1 hour, cooled, and the mixture was filtered. The residue was washed, 2 times with 10 mL of ethanol and 2 times with 10 mL of acetone. The sample was then dried in an oven at 105°C for 1 night, cooled in a desiccator and weighed for the final weight of insoluble dietary fiber. The 95% ethanol was added to the filtrate until the volume reach 100 mL and 400 mL, and was allowed to settle for 1 hour. The phytate was filtered using filter paper and washed twice with 10 mL of ethanol and twice with 10 mL acetone, then dried overnight using an oven at 105°C. After that, it was put into a desiccator and the final weight was weighed as soluble dietary fiber. Total dietary fiber content is the sum of the insoluble and soluble dietary fiber content of the juice.

3.6 Analysis of nutritional profile

Nutrition analysis of freeze-dried *Z. mauritiana* fruit juice was carried out by determining the moisture content, ash content, fat, *crude protein, crude fiber, and carbohydrates* refer to *SNI 01-2891-1992* as described by several researchers (Churun & Sulardiono, 2018; Dayu Putri & Dyna, 2019; Musfiroh *et al*., 2016) with minor modification.

4. Results and Discussion

4.1 Total phenolic and flavonoid content

Quantitative phytochemical analysis showed that *Ziziphus mauritiana* fruit juice had a total phenolic content of 1690 mg GAE/100g, and a total flavonoid content of 15,10 mg QE/100g. These results indicate that *Z. mauritiana* fruit juice contains a considerable amount of phenolic and flavonoid compounds indicating its potential as a functional food.

Table 1. Total phenolics and total flavonoids content of freeze-dried *Z. mauritiana* fruit juice

Parameter	Content
Total Phenolics	1690 mgGAE/100g
Total Flavonoids	15.10 mgQE/100g

The total phenolics content in *Z. mauritiana* fruit juice is in about the same range compared to other fruit juices, and even higher. For example, the total phenolic compounds in freeze-dried juice of *Syzygium cumini* fruit was 131.55 mg/100g (Suryani et al, 2022), while Beh et al. (2012) reported that the total phenolic compounds in freeze-dried juice of fresh apple, guava, lime, mango, and orange are 585±83, 1574±179, 1213±144, 926±77, and 1319±92 mg/100g respectively. This means that *Z. mauritiana* fruit juice phenolics content was around 1 to 12 times higher compared to these other fruit juices. Nevertheless, of course, there are also many fruit juices that have a higher content of phenolics, for example, the total phenolic compounds in freeze-dried juice of *Bouea macrophylla* fruit juice is 57,000 mg GAE/100g (Effendi *et al*., 2022) and in *Ecballium elaterium*, freeze-dried fruit juice is 7870 – 10,640 mg GAE/100g (Felhi *et al*., 2016). Phenolic compounds are natural compounds found in plants and are products of plant secondary metabolism. Phenolic compounds of plant origin have many positive health effects for humans attributed to their antioxidant, anti-inflammatory, antimicrobial, antitumor, and other properties (Grgić *et al*., 2020; Ha *et al*., 2020). This means that the higher content of phenolic compounds in a natural product indicates a higher medicinal potential of that natural product.

One group of compounds include in phenolic compounds is flavonoids. Flavonoids are the most abundant group of polyphenolic compounds found in plants (Grgić *et al*., 2020). The results of this study showed that the flavonoid compounds in *Z. mauritiana* fruit juice are 15.10 mg QE/100g, which means only about 0,9% of the total phenolic compounds in the juice. This indicates that the content of other phenolic compounds in *Z. mauritiana* fruit juice is more dominant, such as phenolic acids, tannin, stilbenes, and others. However, the levels of flavonoid compounds in *Z. mauritiana* fruit juice are in about the same range compared to other fruit juices, for example, the total flavonoids content of freeze-dried fruit juice of Gergalebong orange juice (*Citrus nobilis* L) is 5,58 mg QE/100g (Ramadhani *et al*., 2022), *Averrhoa bilimbi* is 29 mg QE/100g, apple (*Malus domestica*) is 206 mg QE/100g (Utami et al., 2019), and *Ecballium elaterium* has a total flavonoid content of 60 – 650 mg QE/100g (Felhi *et al*., 2016).

4.2. Antioxidant properties

The antioxidant activity of *Z. mauritiana* fruit juice was determined by the DPPH method, using ascorbic acid as the standard. The inhibition percentage of *Z. mauritiana* fruit juice and ascorbic acid at various concentrations is shown in Figure 2. Based on the inhibition percentage, a regression curve was made and the IC50 value was determined (Table 2).

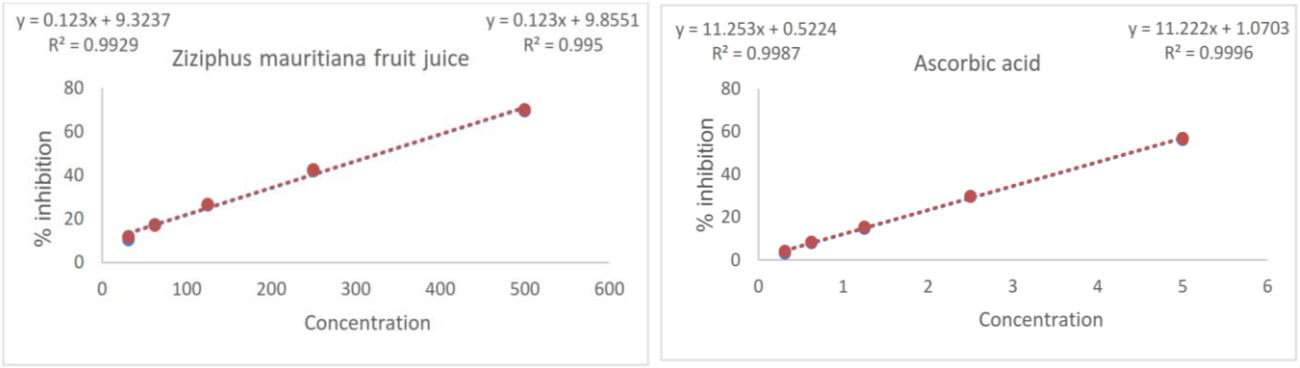

Figure 2. Antioxidant activity determined as DPPH radical scavenging capacity of *Z. mauritiana* freeze-dried fruit juice and ascorbic acid (standard)

Table 2. I
DPPH radical scavenging activity of *Z. mauritiana* fruit juice and ascorbic acid

Sample	IC_{50} (ppm)
Z. mauritiana freeze-dried fruit juice	328.54
Ascorbic acid	4.3785

Antioxidant activity is one of the properties of natural products that are often associated with medicinal potential. Prevention and healing of degenerative diseases are closely related to antioxidant activity, the higher the antioxidant activity, the greater the health potential of the natural product. The antioxidant activity of *Z. mauritiana* fruit juice is low with IC_{50} 328.54 ppm. The low antioxidant activity is usually found in fruit juice, especially raw or cloudy juice, which contains many other substances, including fiber which does not have antioxidant activity. Raw or cloudy juice, as *Z. mauritiana* fruit juice used in this work, was prepared by directly processing the fruit mesocarp without filtering process, therefore it contains a variety of compounds including fiber and other ingredients that do not have antioxidant activity. This makes the antioxidant activity of the fruit juice low because IC_{50} of the antioxidant activity is calculated per sample weight. So, the more other substances that do not have antioxidant properties in the sample, the lower the IC_{50} value of the sample. The low antioxidant activity of the fruit juices was reported by many researchers, for example, IC_{50} of freeze-dried juice of apple (*Malus domestica*) is 805.11 ppm, *Canarium sp.* is 370,84 ppm (Utami *et al.*, 2019), and *Bouea macrophylla* is 564.271 ppm (Effendi *et al.*, 2022).

4.3. Nutritional profile

4.3.1. Dietary Fiber

One of the important components in fruit juice that is beneficial to health is dietary fiber. The analysis of dietary fiber in this study was conducted by enzymatic gravimetric using the AOAC method. The results obtained from the study are shown in Table 3. Soluble food fiber (SDF) contained in *Z. mauritiana* fruit juice is 0.61% and insoluble food fiber (IDF) is 2.03%. It can be seen that the content of soluble dietary fiber is smaller than that of insoluble dietary fiber.

Table 3. Soluble and insoluble dietary fiber of Z. *mauritiana* fruit juice

Parameter	Content (%)
Soluble dietary fiber	0.61
Insoluble dietary fiber	2.03

Dietary fiber is the edible part of plants composed of carbohydrates that are resistant to digestion and absorption in the human small intestine and undergo partial or complete fermentation in the large intestine. Thus, dietary fiber is an edible part that cannot be hydrolyzed by digestive enzymes. In general, dietary fiber includes polysaccharides, oligosaccharides, lignin, cellulose, hemicellulose, and other plant-related compounds (DeVries *et al.*, 2001; Ionită-Mîndrican *et al.*, 2022; Williams *et al.*, 2019)**.** Based on its solubility in water, dietary fiber is divided into two types, namely soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF consists of pectin and its derivatives, gum, and mucilage, while IDF consists of cellulose, hemicellulose, lignin, and modified cellulose. Dietary fiber is an important part of the diet, that has a positive impact on health maintenance and disease prevention. Dietary fiber consumed in sufficient quantities can reduce cholesterol levels and cardiovascular disease, control body weight or obesity (obesity), control diabetes, prevent gastrointestinal disorders and prevent colon cancer (Santoso, 2011).

4.3.2. Proximate analysis of freeze-dried juice of Z. mauritiana fruit

Proximate analysis of *Z. mauritiana* fruit juice was carried out to determine the amount of nutrients contained therein. The parameters are moisture or water content, ash, crude fiber, carbohydrates, protein, and fat (Table 4). These analyses are important for the determination of food quality, microbial stability and can be used for nutritional labeling.

Table 4. Proximate analysis of freeze-dried juice of *Z. mauritiana* fruit

PARAMETER	CONTENT (%)
WATER	10.30
ASH	3.62
CRUDE FIBER	0.56
CARBOHYDRATE	82.43
PROTEINS	3.34
FAT	0.31

The results of the analysis showed that the moisture content of the freeze-dried fruit juice is 10.30%. Moisture content can be used as an index of microbial stability and susceptibility to microbial contamination and degradation. The highest content of this freeze-dried fruit juice is carbohydrates, 82,43%. The carbohydrate content in this juice is quite high because, in addition to containing soluble simple carbohydrates, like fructose, glucose, and lactose, this juice also contains soluble and insoluble complex carbohydrates, such as starch and cellulose. Fiber, which is quite high in this fruit juice, consists of complex carbohydrate compounds, such as cellulose, hemicellulose, lignin, modified cellulose pectin, gum, and mucilage. The ash content of this juice is quite high, 3.62%. The amount of ash present can be translated to the quantity of minerals present in the samples (Coimbra and Jorge, 2011). Ash is the inorganic residue remaining after the water and organic matter have been removed by food. The ash content is a measure of the total amount of mineral present within a food, whereas the mineral content is a measure of the amount of specific inorganic compounds present in the food. The protein and fat content of freeze-dried juice *Z. mauritiana* fruit are low as fruits are not a good source of protein and fat.

5. Conclusion

The conclusion of this research is *Z. mauritiana* fruit juice has a total phenol content of 1688 mg GAE/100g, total flavonoids of 15,01 mg QE/gr, the antioxidant activity of Ziziphus mauritiana fruit juice was weak (IC50 = 328,54 ppm), soluble dietary fiber 0,61%, insoluble dietary fiber 2,03%, and also high nutritional value. *Z. mauritiana* fruit juice has a good potential to be used as food and functional food.

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