PROCEEDING
2st International Conference of Health Polytechnic of Jambi 2023
icon@poltekkesjambi.ac.id
http://journal.poltekkesjambi.ac.id/index.php/ICoHPJ

2nd ICoHPJam 2023
Transforming Future Health Research : Current Challenge Technology and Inovation
ISSN 2988-7356

QUALITY ASSESSMENT OF *MORINGA OLEIFERA* LEAF JUICE AS AN IMMUNE BOOSTER AGAINST ENDEMIC DISEASES IN EAST NUSA TENGGARA

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ABSTRACT

Background: Moringa leaf juice is a natural beverage with high antioxidant properties, making it a potential candidate for traditional medicine and food additive applications. This study aimed to identify the classes of chemical compounds present in moringa leaf juice and evaluate its antioxidant activity, hedonic test and storage stability.
Method: Three different moringa juice formulations were prepared using variations of fresh leaf powder and evaluated for color, taste, aroma, phytochemical content (to confirm the presence of alkaloids flavonoids, saponins, and tannins), antioxidant activity, and storage stability. The optimal formulation was selected based on a panel of experts' sensory evaluation.
Result: The optimal moringa juice formulation consisted of 120 g of fresh moringa leaves, 50 mL of wild honey, 50 g of local guava, 800 mL of UHT milk, and 200 mL of purified water. Phytochemical testing revealed the presence of alkaloids, flavonoids, saponins, and tannins in the moringa leaf juice. The antioxidant activity was 14,339,974 ppm, indicating that moringa leaf juice is a potent antioxidant. The moringa juice remained stable at room temperature for 6 hours and in cold storage for 24 hours, maintaining the same taste and color as when initially prepared.
Conclusion: Moringa leaf juice is a natural beverage with high antioxidant activity and a rich phytochemical profile, including alkaloids, flavonoids, saponins, and tannins. The good storage stability of moringa leaf juice at room temperature and in cold storage suggests that it is a viable option as an antioxidant beverage.

Keywords: Antioxidants, Moringa juice, Moringa oleifera, Phytochemistry

INTRODUCTION

Immune system disorders can be managed with the administration of specific drugs such as antihistamines, anti-inflammatories, and corticosteroids. However, the long-term use of these medications can lead to undesirable side effects. Recently, combination therapy using immunostimulants has been explored, but synthetic drugs come with numerous side effects, are expensive, and have limited availability, making them less accessible to the general public (Arora et al., 2013). Immunostimulants derived from natural sources can be a preferable option due to their lower cost, minimal long-term side effects, and easy accessibility by the public (Aldi et al., 2015).

The moringa tree has numerous benefits, earning it the nickname "the miracle tree," and it is well-known by a significant portion of the global population (Mahmood et al., 2010). In East Nusa Tenggara, especially on the Timor mainland, this plant thrives abundantly, is easily obtainable, and boasts second-best quality globally just after Spain, making it highly sought after by international buyers(Stohs & Hartman, 2015). Chemical constituents of moringa leaves, serving as immunostimulants, have been identified including flavonoids, phenolic compounds, carotenoids, triterpenoids, vitamin A, and vitamin C (Biswas et al., 2012). Some pre-clinical trials on experimental animals have shown that moringa leaf extracts exhibit immunostimulant activity, and the discovery of new compounds such as ethyl 12,15-octadecatrienoate, 6-octadecenoic acid, cis-vaccenic acid, and 2-octyl-cyclopropane-octanal in methanol extracts of moringa leaves

strengthens the potential of moringa leaves as an immunostimulant (Biswas et al., 2012;Xiao et al., 2020).

Commonly, people process moringa leaves using conventional methods such as boiling or steaming (Gunathilake et al., 2018). Boiling, which involves heating, can reduce the active compound content in moringa leaves. The preparation of moringa leaf juice is one approach that can minimize the degradation of active compounds while offering practical advantages in utilization (Mahmood et al., 2010). Moringa, rich in benefits including its role as an immunostimulant, underscores the importance of immunostimulants in disease prevention. Coupled with the abundant availability of moringa leaves in the NTT region, these factors justify the significance of this research (Suhirman et al., 1985).

Previous studies have created moringa leaf juice as a dietary option, but data related to its immunostimulant effects remain unavailable (Netea et al., 2019). This study aims to create moringa leaf juice by adding UHT milk and guava, with the aim of enhancing its immunostimulant effects and imparting a unique flavor profile. The moringa juice preparation will then undergo qualitative and quantitative testing, as well as *in vitro* and *in vivo* immunostimulant evaluations.

METHODS

The samples used in this study were moringa (*Moringa oleifera L.*) leaves obtained from the East Nusa Tenggara region. The research commenced with the preparation of three different moringa juice formulations, incorporating variations of fresh leaf powder. The optimal moringa juice formulation, which was well-received by the panelists based on color, taste, and aroma (Acceptance test,Hedonic test). Subsequently, phytochemical testing was conducted to detect the presence of active compounds such as alkaloids, flavonoids, saponins, and tannins. The antioxidant activity of moringa leaf juice was evaluated to assess its overall antioxidant capacity. Shelf life testing is conducted to determine the duration during which a product can be stored without significant quality deterioration, including taste, color, texture, and nutritional content.

Qualitative phytochemical testing was conducted to detect the presence of active compounds.

Identification of Alkaloids

A total of 0.20 g moringa (*Moringga oleifera L.*) powder was mixed with 3 mL of distilled water and 3 mL of chloroform. The mixture was then boiled for 15 minutes until it formed two phases. These two phases were separated into different reaction tubes. An appropriate amount of the aqueous phase was pipetted into another reaction tube and then supplemented with a sufficient amount of Mg powder and 5 drops of HCl. A positive result was indicated by the formation of a red, yellow, or orange color (Aksara et al., 2013).

Identification of Flavonoid

A total of 0.20 g moringa (*Moringga oleifera L.*) powder was placed into a reaction tube, followed by the addition of a few drops of 2 N HCl and 9 mL of distilled water. The mixture was then heated on a water bath for 2 minutes, cooled, and filtered. The filtrate used for the flavonoid test was as follows: Three drops of the filtrate were added to 2 drops of Mayer's reagent, indicated by the formation of a white or slightly yellowish precipitate. Three drops of the filtrate were added to 2 drops of Bouchardat's reagent, indicated by a brown precipitate, ranging from reddish-brown to dark brown. Three drops of the filtrate were added to 2 drops of Wagner's reagent, indicated by the formation of a yellow precipitate (Nugraha et al., 2017).

Identification of Tannins

A total of 0.20 g Moringa (*Moringa oleifera L.*) powder was placed into a reaction tube, and 1-2 drops of 1% ferric (III) chloride reagent were added. The presence of tannins was indicated by a change in the color of the

filtrate to green or dark blue (Dwika et al., 2016).

Identification of Saponins

A total of 0.20 g Moringa (*Moringa oleifera L.*) powder was placed into a reaction tube, and then 5 mL of destilled hot water was added (Dwika et al., 2016).

Antioxidant Activity Test of Moringa Leaf Juice

The antioxidant activity of moringa leaf juice (*Moringa oleifera L.*) was evaluated to assess its overall antioxidant capacity. Based on qualitative testing, moringa leaf juice contains flavonoids, compounds known to enhance the body's antioxidant defense against free radicals. Moringa leaf juice is expected to be used as an immune booster against endemic diseases in East Nusa Tenggara. This research determined the antioxidant activity of moringa leaf juice, measured by the IC_{50} value using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The principle of this method is the reduction in color intensity or absorbance of the DPPH solution, which is proportional to the increase in the concentration of antioxidant compounds. Moringa leaf juice was prepared using Formula I (fresh moringa leaves 90 g, local guava 50 g, wild honey 50 mL, UHT milk 800 mL, purified water 200 mL), Formula II (fresh moringa leaves 120 g, local guava 50 g, wild honey 50 mL, UHT milk 800 mL, purified water 200 mL) and Formula III (moringa leaves 150 g, local guava 50 g, wild honey 50 mL, UHT milk 800 mL, purified water 200 mL). The research was conducted by thoroughly washing each ingredient. Subsequently, fresh moringa leaves were blanched to remove bacteria, blended with guava and UHT milk, and the resulting juice mixture was filtered and then supplemented with honey. The prepared juice was used as the antioxidant test sample at concentrations of 10,000 ppm, 15,000 ppm, 20,000 ppm, 25,000 ppm, and 30,000 ppm.

Shelf Life Test of Moringa Leaf Juice

Moringa (*Moringa oleifera L.*) is a medicinal plant known for its antioxidant properties and is expected to be used as an immune booster against endemic diseases in East Nusa Tenggara. Erma Nur Faujan's research concluded that moringa leaf extract is effective in increasing hemoglobin levels in adolescent girls. Currently, fresh moringa leaves are being developed into juice, supplemented with local guava, UHT milk, and local honey from Semau Island. To ensure the quality of the juice, a storage test for moringa leaf juice is necessary. Asselerated Shelf Life Test (ASLT)is the Method.The shelf life test was carried out by placing the bottle of Moringa juice at closed room temperature (25-30^{0}C) for 8 hours, room temperature (30-34 ^{0}C) or 6 hours and also storing at a cold temperature of 2-8^{0}C for 24 hours.

Hedonic Test of Moringa Leaf Juice

Moringa (*Moringa oleifera L.*) is a plant with numerous benefits. Moringa grows easily in tropical regions like Indonesia and is commonly consumed as food. Nowadays, moringa leaves are processed into various forms, such as tea, face masks, juice and more. This research transforms fresh moringa leaves into juice, incorporating local guava, UHT milk, and local honey from Semau Island. To assess the quality and preference of 60 panelists in terms of color, texture, aroma, and taste regarding moringa leaf juice, a hedonic test was conducted. The hedonic scale is used to measure the level of liking for a product, ranging from "very much liked" score 5, "liked" score 4, "neutral" score 3, "disliked" score 2, "strongly disliked" score 1. This testing is employed to gauge consumer reactions to a product or evaluate consumer responses to tested samples. Hedonic test refers to several previous tests (Hastuti et al., 2016; Rustamaji & Ismawati, 2021; Winnarko & Mulyani, 2020).

RESULTS AND DISCUSSION

RESULTS

Data collection for the formulation, qualitative screening of flavonoids, and shelf

life testing were conducted in the laboratory. The hedonic test was performed by distributing questionnaire forms to the respondents, while antioxidant activity testing was carried out using the DPPH method.

Preparation of Moringa Leaf Juice Formulas

Through several experiments, three Moringa leaf juice formulas that were deemed good by the researchers were obtained. The results of the formulations are shown in **Table 1** below.

Table 1. Moringa Leaf Juice Formulations

Composition	Formula I	Formula II	Formula III
Fresh moringa leaves	90 g	120 g	150 g
Semau honey	50 ml	50 ml	50 ml
Local guava	50 g	50 g	50 g
Uht milk	800 ml	800 ml	800 ml
Purified water	200 ml	200 ml	200 ml

In principle, we only made variations in the composition of fresh Moringa leaves, while the composition of the other ingredients remained unchanged, all the same in each formula.

Qualitative Testing of Moringa Leaf Juice

Qualitative screening of Moringa leaf juice was conducted for alkaloid, flavonoid, tannin, and saponin content. The test results are shown in **Table 2** below.

Table 2. Qualitative/Screening Results of Chemical Content in Moringa Leaves

Secondary Metabolite	**Reagent**	**Result**
Alkaloid	Mayer	(+)
	Wagner	(+)
	Bounchard	(+)
Flavonoid	Aquades+ Chloroform+ Mg Powder+ Concentrated HCl	(+)
Saponin	HCl	(+)
Tanin	$FeCl_3$	(+)

Based on the results of the qualitative testing, we found positive results for alkaloids, flavonoids, saponins, and tannins in Moringa leaves samples.

Quantitative Testing of Moringa Leaf Juice and Antioxidant Activity Test

Subsequently, an antioxidant activity test has conducted on Moringa leaf juice. We found that Moringa leaf juice Formula II exhibited antioxidant activity with an IC_{50} value of 14.339 ppm, while Moringa leaf juice Formula III exhibited antioxidant activity with an IC_{50} value of 17.049 ppm.

Shelf Life Test of Moringa Leaf Juice

The results showed that the shelf life of moringa leaf juice at room temperature lasted for 8 hours with the same taste and color as during the production process. The shelf life at outdoor temperature lasted for 6 hours with the same taste and color as during production. Remarkably, the shelf life of moringa leaf juice at cold temperatures (2-8°C) can extend for 24 hours with the taste and color remaining the same as during the production process.

Hedonic Test of Moringa Leaf Juice

Based on the results of the hedonic test of moringa leaf juice combined with guava and honey, considering aspects such as color, aroma and taste. it can be concluded that the juice most preferred by the panelists is Formula II juice, which consists of (Moringa leaves 120 g, guava 50 g, UHT milk 800 ml, honey 50 ml, and purified water 200 ml). The results of the hedonic test are shown in **Table 3** below.

Table 3. Average Results of Hedonic Quality Test for Moringa Leaf Juice

Specification	Formula I	Formula II	Formula III
Color	4.12	5.32	4.77
Aroma	4.5	5.75	4.75
Taste	4.63	5.58	4.75

Note:
Formula I: Addition of 50 g of moringa powder
Formula II: Addition of 90 g of moringa powder
Formula III: Addition of 120 g of moringa powder

DISCUSSION

Moringa (*Moringa oleifera* L.) is renowned for its high nutritional content. Its leaves contain vitamin A, which is equivalent to 10 times the amount in carrots, 17 times the amount of calcium in milk, 15 times the amount of calcium in bananas, 9 times the protein found in yogurt, and 25 times the iron content of spinach (Jonni, 2008). Research by Haryadi (2011) also reported that every 100 g of dried Moringa leaves contain 0.075% water, 2.05% calories, 0.382% carbohydrates, 0.271% protein, 0.023% fat, 0.192% fiber, 20.03% iron, 8.7% sulfur, and 13.24% potassium(Biswas et al., 2012)(Stohs & Hartman, 2015).

Color is a critical category because it significantly influences the appearance of a food product and affects panelists preference. Based on the results of the hedonic quality test, Formula I, Formula II, and Formula III received an average score of 4.12; 5.32; and 4.77 respectively for color. This difference is attributed to the varying amounts of Moringa leaf powder added, resulting in different colors. The highest average score for color among the three formulas was achieved by Formula 2. This finding is supported by the research of Widyawatiningrum et al. (2018), which stated that the more Moringa leaves added to chicken nugget dough, the greener the product becomes. This green color is due to the high chlorophyll content in Moringa leaves reaching 6890 mg/kg, which is four times more than wheatgrass(Winnarko & Mulyani, 2020).

Aroma plays a crucial role in how panelists perceive food products. It can indicate whether a product is pleasant or not. Based on the hedonic quality test results, Formula I received an average score of 4.5, Formula II received a score of 5.75, and Formula III received a score of 4.75. These differences are attributed to the varying amounts of Moringa leaf powder added, resulting in different aromas. The highest average score for aroma among the three formulas was achieved by Formula 2. This is in line with Widyaningrum's statement that factors influencing the taste of food ingredients include smell, oral stimulation, and taste(Widyawatiningrum et al., 2018).

The taste results from the combination of food ingredients and is perceived by the sense of taste. It is the most crucial factor in determining the final decision regarding the acceptance of a food product. Based on the hedonic quality test results, Formula I received an average score of 4.63 for taste, Formula II received a score of 5.58, and Formula III received a score of 4.75. Panelists perceived a stronger taste of Moringa leaves in Formula III due to the higher quantity of Moringa leaves added, however the highest average score for taste among the three formulas was achieved by Formula II. As more Moringa leaves are added, the taste of Moringa becomes more pronounced.

The study also conducted shelf-life tests on the Moringa leaf juice produced. The results showed that the juice can maintain its quality at room temperature for 6 hours and also at refrigerator temperatures (2-4°C) for 24 hours. Several previous studies have reported that Moringa can resist microbial spoilage due to its tannin and saponin content. The qualitative test results in our study indicated the presence of tannin and saponin in Moringa juice(Akmi et al., 2022; Kusumawardani et al., 2018).

Furthermore, we found that Moringa leaf juice exhibits high antioxidant activity. The antioxidant activity of Moringa has been extensively reported in previous studies. Nurulita *et al.* found that Moringa leaf extract inhibits the β-carotene bleaching process (BCB inhibitor) and collagenase enzyme inhibition by 47%. Moringa leaf body butter has potential anti-aging effects through antioxidant mechanisms and collagenase inhibition(Nurulita et al., 2019). Fitriana *et al.* reported that the ethyl acetate phase exhibited antioxidant activity of 85.4% in the DPPH assay and 92.12% in the ABTS assay. The antioxidant activity of the ethyl acetate phase is influenced by the phenolic compound content in Moringa leaves, such as quercetin, flavonoids, and kaempferol(Fitriana et al., 2015).

The studies conducted by Yuliani *et al.*, as well as Meigaria *et al.*provide valuable insights into the antioxidant activity of Moringa leaf extracts compared to vitamin C. Yuliani et al. concluded that the antioxidant activity of Moringa leaf infusion is lower than vitamin C, with an IC_{50} value of 2.151 ppm for Moringa leaf infusion and 3.454 ppm for vitamin C. This suggests that Moringa leaf infusion has a weaker antioxidant activity compared to vitamin C(Yuliani & Dienina, 2008). In line with those study, Meigaria *et al.* reported that the IC_{50} value of Moringa leaf

acetone extract is 427.49 µg/mL, while the IC_{50} value of vitamin C is 35.52 µg/mL. This significant difference in IC_{50} values indicates that Moringa leaf acetone extract has relatively weaker antioxidant activity compared to vitamin C(Komang Mirah Meigaria, I Wayan Mudianta, 2016). Furthermore, the results of the antioxidant activity test for the ethanol extract of Moringa leaves in this study showed an IC_{50} value of 89.305 ppm, while vitamin C, used as a comparative compound, had an IC_{50} value of 8.374 ppm(Hasanah et al., 2017).

CONCLUSION

In conclusion, the best formulation of Moringa leaf juice, which was well-received by the panelists in terms of color, taste, and aroma, consists of 120g of fresh Moringa leaves, 50 mL of Semau honey, 50g of local guava, 800 mL of UHT milk, and 200 mL of distilled water. The antioxidant activity of Moringa leaf juice was determined to have a value of 14.339 ppm.

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