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Enhancing Nutraceutical Properties of Moringa: A Biotechnological Perspective

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Abstract

Moringa oleifera Lam. is recognized for its versatile nutritional and medicinal benefits, largely attributed to its rich polyphenolic content, which includes bioactive compounds with significant antioxidant, anticancer, antimicrobial, and antidiabetic properties. This study addresses the gap in knowledge regarding the enhancement of phenolic compounds in Moringa leaves through innovative biotechnological methods. The primary objective is to analyze the extraction and quantification of polyphenols from Moringa leaves subjected to boiling and fermentation processes to produce kombucha. Using the Folin-Ciocalteu method, quantified the total polyphenol content, revealing that boiled Moringa leaves contained 67.66 ± 2.79 mg/g of polyphenols, while the resulting kombucha tea exhibited a higher concentration at 70.66 ± 2.79 mg/g. These findings demonstrate the potential of Moringa leaves as not only a dietary supplement but also as a functional ingredient in health-promoting beverages, such as kombucha. The implications extend to enhancing the nutritional value of plant-based diets and establishing Moringa as a sustainable antioxidant source. This research underscores the importance of advanced biotechnological approaches in optimizing extraction and cultivation methods, thus potentially increasing phenolic yields. By providing crucial insights into the functional applications of Moringa leaves, this study contributes to the broader fields of plant biotechnology and food science, encouraging further exploration into their health benefits and utility in promoting sustainable nutrition.

Keywords : *M. Oleifera*, Polyphenols, Folin-Ciocalteu, Kombucha, Plant Biotechnology

1. Introduction

Moringa oleifera Lam. is increasingly recognized as a multi-functional plant due to its extensive nutritional and medicinal properties. Revered in many cultures, particularly in regions with tropical and subtropical climates, Moringa is often referred to as the "miracle tree" due to its rich array of vitamins, phytonutrients, and bioactive compounds. Its leaves contain significant quantities of polyphenols, including quercetin, kaempferol, and gallic acid, which contribute substantially to its antioxidant, anticancer, antimicrobial, and antidiabetic activities.[1] The health benefits associated with Moringa leaves have been documented extensively, reinforcing its role as a functional food capable of enhancing dietary quality and providing therapeutic effects against diverse health conditions.[2]

The process of optimizing the phenolic content in Moringa leaves through various processing techniques is critical for maximizing its health benefits. Approaches such as boiling and fermentation have been employed not only to alter the concentrations of these essential compounds but also to enhance their bioavailability. The boiling of Moringa leaves has been shown to yield a total polyphenol content of around 67.66 ± 2.79 mg/g, while subsequent fermentation into kombucha has resulted in an increased total polyphenol concentration of approximately 70.66 ± 2.79 mg/g.[3] This enhancement through fermentation is pivotal, as studies indicate that microbial activity can lead to the degradation of complex polyphenols into smaller, more bioavailable forms, thus improving their antioxidant properties.[4,5] This transformation underscores the importance of fermentation in enhancing the nutritional profile of Moringa leaves.

Kombucha, a fermented beverage known for its health benefits, offers a practical application for Moringa leaves beyond traditional consumption as a vegetable or supplement. The fermentation of Moringa-leaf-based kombucha not only maintains but amplifies bioactive properties, particularly antioxidant activities.[6,7] Prior research indicates that longer fermentation times positively correlate with increases in bioactive compound concentration which corroborates with the observed elevation of polyphenol levels during the kombucha fermentation process.[5] Moreover, the presence of organic acids and other metabolites produced during fermentation further contributes to the synergistic health benefits of kombucha, enhancing its therapeutic potential.[8]

Research has shown that the functional characteristics of kombucha can vary significantly based on the base substrate used for fermentation. Studies have demonstrated that kombucha derived from different substrates exhibits varying antioxidant capacities.[9,10] When Moringa leaves are combined with kombucha, the unique phytochemical profile of Moringa adds an additional layer of health benefits that may extend beyond those provided by traditional tea-based kombucha, ultimately enhancing consumer health through enriched nutrients and bioactive compounds in these beverages.[4]

In terms of methodology, utilizing the Folin-Ciocalteu reagent is a well-established approach for quantifying total polyphenolic content, providing reliable data regarding the antioxidant capacity of plant extracts.[11] This analytical technique supports the validity of findings regarding both boiled Moringa leaves and the resulting kombucha, affirming that the processing methodologies used in this study merit further exploration within the realm of plant biotechnology and food science.

The implications of these findings extend far beyond the laboratory, indicating substantial potential for the integration of Moringa-derived products into broader dietary frameworks. Moringa leaves processed into functional beverages like kombucha could serve as sustainable sources of antioxidants in people's diets, particularly in regions where access to diverse nutritional foods is limited. Additionally, the documented health benefits associated with Moringa, combined with the probiotic properties of kombucha, illustrate a unique opportunity to promote public health through dietary innovations.[12,13]

Changing dietary habits to incorporate functional foods such as Moringa leaves fermented into kombucha could contribute significantly to global health. [5] Given the growing interest in plant-based diets and functional foods, further research is warranted to investigate the full spectrum of health benefits associated with Moringa and its use as both a food supplement and a formulation ingredient in the development of health-enhancing beverages destined for the global market.[14]

Moreover, as this body of research progresses, continuous efforts must be directed toward optimizing cultivation and harvesting practices for Moringa to ensure that it can effectively meet growing demands as both a nutritional resource and a focal point of sustainable agriculture.[15,16] Biotechnological advancements in extraction and processing methodologies will play a critical role in sustaining quality and maximizing nutritional value, ensuring that Moringa remains a viable option for addressing dietary deficiencies globally.

This research highlights the importance of utilizing scientific and biotechnological methodologies to enhance the nutritional value of Moringa oleifera. The data suggests a promising avenue for future exploration into the development of functional foods that leverage the unique properties of Moringa leaves, particularly when processed through methods such as boiling and fermentation into kombucha. Continued investigation into these methodologies and their implications may offer significant contributions towards the improvement of dietary health and nutrition in various populations around the world.

2. Method

2.1 Materials

The materials required for this study were dried moringa leaves, a SCOBY (a Symbiotic Culture of Bacteria and Yeasts) as a kombucha starter culture obtained from Rumah Fermentasi, South Tangerang, Folin-Ciocalteau reagent, and gallic acid.

2.2 Drying Moringa Leaves

500 g of fresh moringa leaves were cleaned under running water and then air-dried. Once dry, they were placed in a sealed container for storage. Drying was carried out using an oven at 60°C. The oven drying time for the moringa leaves was 3 hours. Leaf color and texture were monitored, as higher temperatures can alter sensory quality and reduce some beneficial compounds.

2.3 Preparation of Gallic Acid Standard Solution

Weigh 25 mg of gallic acid and dissolve it in 25 mL of methanol p.a. to produce a 1000 ppm standard solution. Pipette 12.5 mL of the 1000 ppm standard solution and dilute it with methanol p.a. to 25 mL to produce a 500 ppm standard solution. A 500 ppm standard solution was pipetted into 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL volumes and diluted with methanol p.a. to 10 mL, resulting in standard solutions of 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm.

2.4 Preparation of Calibration Curves for Gallic Acid Standard Solutions

To standardize the calibration curve for gallic acid standard solutions, refer to the procedure. Gallic acid standard solutions were prepared at several concentrations: 50, 100, 150, 200, and 250 ppm in volumetric flasks. 5 mL of Folin-Ciocalteau reagent was added to each volumetric flask. Next, 4 mL of Na_2CO_3 solution was added and shaken until homogeneous. The mixture was then allowed to stand for 15 minutes at room temperature. The absorbance was then measured using a UV-Vis spectrophotometer at a wavelength of 770 nm, and a calibration curve was created between the gallic acid concentration and the absorbance obtained.

2.5 Determination of Total Phenolic Content

The total phenolic compound content was determined using a specific reagent, Folin-Ciocalteau. Two sample solutions were used: Moringa leaf decoction and Moringa leaf kombucha tea. 1 g of each sample was dissolved in 10 mL of methanol, then 0.5 mL of each sample was pipetted, and 5 mL of Folin-Ciocalteau reagent was added to each volumetric flask. 4 mL of Na_2CO_3 solution was added and shaken until homogeneous. The mixture was allowed to stand for 15 minutes at room temperature. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 770 nm. The phenolic compound content was indicated by the equivalent point of gallic acid.

3. Result

3.1 Phytochemical Screening

Phytochemical screening was conducted to identify the chemical compounds contained in the water extract and kombucha samples of Moringa leaves (Table 1). The results of the alkaloid test showed no alkaloid compounds in the boiled water and kombucha of moringa leaves.

3.2 Determination of Phenol Content from Water Extract and Moringa Leaf Kombucha

Gallic acid solution was then measured for its absorbance using UV-Visible spectrophotometry at a wavelength of 770 nm. Thus, a linear regression equation was obtained, y = 0.0077x + 0.1286 with R2 = 0.9983 and R = 0.999 approaching 1. From this linear regression equation, it can be used to calculate the phenol content in the sample (Figure 1).

The results of the phenolic content measurement in the boiled water sample were 67.66 ± 2.79 mg/g and in the kombucha sample were 70.66 ± 0.3 mg/g. After statistical analysis, the results of the statistical analysis showed that there was no significant difference in the polyphenol content of the boiled water of moringa leaves and kombucha tea of moringa leaves (P > 0.05) (Table 2).

Table 1. Phytochemical Screening of Moringa Oleifera Leaf (Moringa Oleifera Lam.) Crude Drug, Water Extract, And Kombucha

No.	Compound	Crude drug	Water extract	Kombucha
1.	Phenols	+	+	+
2.	Alkaloids	-	-	-
3.	Flavonoids	+	+	+
4.	Saponins	-	-	-
5.	Quinones	+	+	+
6.	Tannins	-	-	-

(+) = chemical compound present, (-) = no chemical compound present

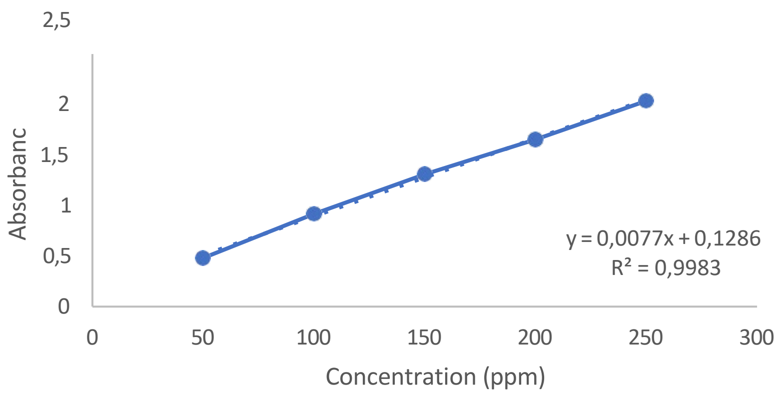

figure 1 Gallic Acid Calibration Curve

Table 2 Results of Phenolic Content of Water Extract and Kombucha of Moringa Leaves

Sample	Absorbance	Phenolic content (mg/g)	Mean (mg/g)
Water extract	0.673	70.7012	
	0.640	66.9350	67.66 ± 2.79
	0.631	65.3467	
Kombucha of moringa leaves	0.675	70.9610	
	0.670	70.3116	70.66 ± 0.3
	0.673	70.7012	

4. Discussion

The absence of alkaloid compounds in the aqueous extracts of Moringa oleifera leaves, specifically in boiled water and kombucha, presents a significant point of discussion in phytochemistry and nutritional studies. Moringa leaves are indeed known for their rich biochemical profile, which includes secondary metabolites like alkaloids, although not all extraction methods yield these compounds effectively.[2] The analysis of different extraction techniques can deepen our understanding of how alkaloid content may vary depending on the solvent or method used.

It has been established that Moringa oleifera leaves contain a diverse range of phytochemicals, including alkaloids, flavonoids, tannins, saponins, and phenolics. Emphasizes the presence of alkaloids among other phytoconstituents in Moringa leaves. Moreover, studies indicate that Moringa extracts exhibit biological activities attributed to these compounds, suggesting potential medicinal benefits such as antioxidative and antimicrobial activities.[17,18] However, the method of extraction can significantly influence the quantity and type of phytochemicals retrieved. Aqueous extraction methods, like boiling, are common due to their simplicity; however, they may not be efficient for extracting alkaloids. The thermal degradation of certain compounds during boiling can lead to their breakdown, reducing their final yield in the extract.[16] High temperatures can adversely affect the phytochemical profile of Moringa leaves, thus altering the alkaloid content observed in the extracted solution.[19]

Moreover, the extraction solvent plays a critical role in the solubility and stability of alkaloids. Alcoholic solvents have been found to be more effective for extracting alkaloids and other bioactive components compared to water. This highlights why the boiled water extracts from Moringa leaves may exhibit negligible alkaloid content.[20] In contrast, a study utilizing ethyl acetate extracts demonstrated significant alkaloid presence,[21] showcasing the importance of using appropriate solvents based on the desired phytochemical outputs. It is critical to note that while Moringa leaves are recognized for their potential health benefits due to their antioxidant activity, bioactivity can vary markedly depending on the extraction approach. Compounds such as phenolic acids and flavonoids often demonstrate significant antioxidative properties.[22,23] However, if primary constituents like alkaloids are absent or present in small amounts, the overall therapeutic potential of the extract could be misleadingly underestimated.

Research on Moringa's health effects predominantly focuses on its broader nutrient composition, with many studies highlighting its antioxidants, vitamins, and essential minerals, rather than specifically highlighting alkaloids.[24,25] This suggests that while alkaloids are a noteworthy part of Moringa's phytochemical landscape, they may not be the primary focus in every extraction method or study, particularly those emphasizing nutritional profiles over phytochemical diversity. Despite the absence of alkaloids in certain extracts from Moringa, the plant remains highly beneficial due to its numerous other pharmacologically active compounds, which exhibit various pharmacological properties such as anti-inflammatory and antibacterial effects essential in traditional medicine applications.[26,27]

The analysis of phenolic content in food samples, specifically in boiled moringa leaf water and kombucha tea, provides valuable insights into the health-promoting properties of these beverages. In a recent empirical study, the measured phenolic content in boiled water samples derived from moringa leaves was 67.66 ± 2.79 mg/g, while kombucha samples prepared from the same moringa leaves had a phenolic content of 70.66 ± 0.30 mg/g. Recognizing the nutritional value of moringa and kombucha, it is critical to examine their respective phenolic profiles and antioxidant activities. The phenolic compounds present in these samples are significant due to their association with antioxidative properties, attributed to their ability to scavenge free radicals. Higher total phenolic contents correlate with enhanced antioxidant activities in various plant extracts.[28,29] This suggests that both beverages may serve as important sources of antioxidants, essential for mitigating oxidative stress.

Furthermore, the consistency of phenolic levels across different extraction methods, including the use of hot water, is supported by findings in the literature. Hot water is recognized as a viable solvent for extracting bioactive compounds from plant materials rich in phenolics like moringa.[30] Previous studies have demonstrated that the choice of solvent significantly affects the yield of phenolic compounds,[31] indicating that boiling enhances the bioaccessibility and extraction of bioactive constituents from moringa leaves. Empirical research focusing on other matrices often illustrates that different varieties and preparations of plant foods can yield similar phytochemical profiles. A comparative study of catechins in green tea has revealed comparable antioxidant capacities, reinforcing the notion of parity in phenolic availability among varied tea preparation methods.[28] This connection aligns with the observed similarities between moringa preparations, highlighting the shared presence of phenolic compounds across comparable food products.

The observed phenolic contents are significant, as polyphenolic compounds offer varied health benefits, including anti-inflammatory and cardiovascular protective actions.[32,33] Kombucha has gained popularity, not only for its flavors and effervescence but also for its potential health benefits attributed to its bioactive compounds.[34] With similar phenolic content ranges, both moringa water and kombucha could be promoted as healthful beverages contributing to daily antioxidant intake. Additionally, the slight difference in phenolic concentration between the two beverages may reflect the interaction between microbial fermentation in kombucha and the naturally occurring compounds in moringa leaves, potentially producing synergistic health effects.[35] Further studies could explore these biochemical interactions during fermentation and their implications for overall phenolic concentrations.

The comparison of phenolic content in kombucha versus boiled leaf extracts raises qualitative inquiries about bioavailability and functional effects post-consumption. As indicated in the literature, the bioaccessibility of phenolic compounds can vary significantly between extraction methods and food matrices, which warrants attention in future nutritional studies.[34,36] This emphasizes the need for ongoing exploration of how processing methods or fermentation protocols may enhance or inhibit phenolic stability and bioactivity. While the current study highlights the richness of moringa leaf-based beverages, it also presents an opportunity to investigate the influence of environmental factors, growth conditions, and preparation methods on phenolic yield. Variations in phenolic profiles across plant cultivars and processing techniques suggest that a comprehensive approach to understanding these factors will better inform dietary recommendations surrounding these natural products.[31,37]

The study of Moringa oleifera not only highlights its impressive nutritional profile but also underscores the need for ongoing research into biotechnological advancements to optimize its extraction methods, health benefits, and overall usability in food systems. By bridging knowledge gaps and enhancing methods of incorporation into foods and beverages, particularly through fermentation processes, Moringa could play a pivotal role in supporting sustainable nutrition and health across diverse populations.

5. Conclusions

The study highlights significant advancements in the understanding of Moringa's nutraceutical properties through biotechnological approaches. However, several gaps in research remain, particularly in the areas of bioactivity testing and bioavailability studies. Future research should focus on conducting in vitro and in vivo bioactivity assessments to evaluate the health benefits of Moringa extracts and fermented products. This would provide a clearer understanding of their efficacy in various health contexts.

6. Acknowledgement

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