



Impact of Temperature and Duration on Green Extraction Yields and Antioxidant Activity of *Stevia rebaudiana*

Ismiarni Komala^{*1}, Mariyatul Qibtiyah¹, Muthoharoh¹, Flori R Sari² & Marvel¹

¹Department of Pharmacy, Faculty of Health Sciences, UIN Syarif Hidayatullah, Jakarta, Indonesia

²Department of Pharmacology, Faculty of Medicine UIN Syarif Hidayatullah, Jakarta, Indonesia

ABSTRACT: *Stevia rebaudiana* leaves are well-known for their intense natural sweetness, derived from steviol glycosides, which are 100-300 times sweeter than sugar. Besides being a natural sweetener, these leaves also hold the potential for various health benefits. This study compares two eco-friendly extraction methods: maceration using a water bath shaker (Dynamic maceration) and ultrasonic-assisted extraction in a water bath utilizing distilled water as the solvent. The objective was to assess their effects on extraction yield and antioxidant activity. Dynamic maceration was conducted at 25°C for 3 hours and 6 hours, yielding 14.2% and 15.5%, respectively, and at 75°C for 3 hours and 6 hours, yielding 18.6% and 16.7%, respectively. Ultrasonic-assisted extraction was carried out at 25°C for 30 minutes and 60 minutes, yielding 12.1% and 15.2%, respectively, and at 75°C for 30 minutes and 60 minutes, yielding 15.0% and 14.6%, respectively. The antioxidant activity of the extracts, assessed using the DPPH method at a concentration of 100 µg/mL, showed a linear trend corresponding to each extraction condition. For dynamic maceration, inhibition percentages were 33.4% (25°C, 3 h), 30.1% (25°C, 6 h), 25.9% (75°C, 3 h), and 16.7% (75°C, 6 h); for ultrasonic-assisted extraction, values were 39.9% (25°C, 30 min), 33.8% (25°C, 60 min), 31.8% (75°C, 30 min), and 27.1% (75°C, 60 min). The highest yield (18.6%) was obtained from dynamic maceration at 75°C for 3 hours, whereas the highest antioxidant activity (39.9%) was observed in ultrasonic-assisted extraction at 25°C for 30 minutes.

Keywords: *Stevia rebaudiana*; dynamic maceration; ultrasonic-assisted extraction; temperature; duration; antioxidant activity assay.

Introduction

The trend of choosing low-sugar and low-calorie foods is increasing as people become more aware of the link between high-sugar and high-calorie diets and health issues, such as diabetes and obesity. Therefore, the food industry is exploring replacing sugars and their derivatives with low-calorie sweeteners. The search for alternative sweeteners has led to the development of artificial sweeteners such as aspartame, acesulfame-K, cyclamates, neotame, saccharin, and sucralose. While these artificial sweeteners help to cut calories, some might not be suitable for managing diabetes and could raise safety concerns, including the potential risk of cancer [1-5]. Hence, further research is needed to identify natural foods and ingredients that can act as low-calorie, health-enhancing sweeteners.

Stevia Rebaudiana originates from Paraguay and neighboring countries like Brazil and Argentina. Indigenous communities have long used this plant for medicinal purposes and as a natural beverage sweetener. The leaves of *S. rebaudiana* are rich in steviol glycosides,

which give them distinct sweetness [6,7]. These glycosides make *S. rebaudiana* an ideal plant-based sweetener with no calories and sweetness that can be 100 to 300 times more intense than sugar [8]. It is an excellent choice for creating low-sugar, low-calorie foods and drinks. Stevia plants are commercially grown in Argentina, Paraguay, Colombia, Brazil, Japan, China, South Korea, Vietnam, Israel, Australia, Kenya, Malaysia, and the US. All major regulatory authorities worldwide approve high-purity steviol glycosides as sweeteners, and more than 150 countries have used them in food and drinks [7].

Stevia leaves (*S. rebaudiana*) are known for their sweet taste and contain various compounds that may have health benefits. These include sweetening agents like stevioside and rebaudioside (A, B, C, D, and E), as well as dulcoside, labdane diterpenes, triterpenes, stigmaterol, tannins, and essential oils [9,10]. Stevioside and rebaudioside A are the main contributors to the sweetness,

Article history

Received: 08 Sep 2024

Accepted: 12 May 2025

Published: 30 Jun 2025

Access this article



***Corresponding Author:** Ismiarni Komala

Department of Pharmacy, Faculty of Health Sciences,
UIN Syarif Hidayatullah Jakarta, Indonesia, 15412 | Email: ikomala@uinjkt.ac.id

with rebaudioside A being the sweetest [9]. However, too much stevioside can lead to a bitter aftertaste, which might affect how people enjoy the sweetener. Besides their use as a sweetener, stevia leaves may offer other health benefits, such as helping to manage diabetes, prevent tooth decay, and provide antioxidant, blood pressure-lowering, antimicrobial, anti-inflammatory, and anticancer effects [6,9–11].

Separating steviol glycosides from *S. rebaudiana* has become more challenging because of other components, including resins, proteins, organic acids, and pigments, in plant extracts [9]. In order to achieve a high yield of isolated compounds, the separation of steviol glycosides from plant extracts has been advanced using various extraction and isolation techniques, including conventional, ultrasonic, and microwave methods [12]. The Ultrasonic-assisted extraction of *S. rebaudiana* has been previously explored using different extraction conditions solvents [13–18]. Dynamic maceration employs rapid maceration with stirring and has been developed to extract components from *S. rebaudiana* using various solvents [9,19].

A systematic review and meta-analysis have shown that stevia leaf extracts are consistently better at restoring oxidative stress markers than pure glycosides [20]. While various extraction methods have been studied, the dynamic extraction method using a water-bath shaker for maceration has not yet been explored. Previous research focused on ultrasonic extraction using an ultrasound probe system with water, but not a water-bath ultrasonic approach [17]. This study aims to fill these gaps by developing and optimizing water-bath shaker maceration and ultrasonic-assisted extraction methods using distilled water as solvent. The objective is to evaluate their effects on yield and antioxidant activity, to create accessible, cost-effective, simple, and eco-friendly extraction methods for *S. rebaudiana* leaves.

Methods

Plant Material

S. rebaudiana leaves were obtained from PT Agro Jabar Bandung, West Java, Indonesia, 2019. The leaves were freshly taken from the plantation and dried naturally. Packed, dried leaves were sent to the Faculty of Medicine Universitas Islam Negeri Syarif Hidayatullah.

Procedure

Water-bath shaker extraction (dynamic maceration)

In an Erlenmeyer flask, 10 g of dried and ground leaves of *S. rebaudiana* were added to 100 ml of distilled

water and extracted using the water bath shaker technique at a strength setting 5. The extraction was conducted with variations in the duration (3 and 6 hours) and temperature (25° and 75°C). After completing the extraction, the extract was filtered through Whatman paper number 41, and the solvent was evaporated using a rotary evaporator at 80 °C. Finally, the obtained concentrate was freeze-dried to obtain a powdered extract. The extraction procedure was repeated three times.

Water-Bath Ultrasonic-Assisted Extraction

In an Erlenmeyer flask, 10 g of dried and ground leaves of *S. rebaudiana* were added to 100 ml of distilled water and extracted using an ultrasonic-assisted water bath at 20 kHz and 550 W, with variations in duration (30 and 60 minutes) and temperature (25 and 75°C). The subsequent steps were identical to those employed in the water bath shaker extraction.

Antioxidant Activity

The antioxidant activity was assessed using the DPPH method. A 0.25 mM solution of DPPH free radical was prepared following a previously described method [21]. Solutions of *S. rebaudiana* leaf extract and ascorbic acid (positive control) were prepared at a concentration of 100 µg/mL. Both the sample and ascorbic acid solution were mixed with the DPPH solution. The mixture was homogenized using a vortex mixer for approximately 2 minutes. The homogenized solution was then incubated at 25°C for 30 minutes in the dark (the tube was wrapped with aluminum foil). The absorbance of the solutions was measured using a UV-visible spectrophotometer at a wavelength of 517 nm. The percentage of inhibition (I%) was then calculated using the equation below.

$$I\% = (A-B)/A \times 100 \%$$

where A is the absorbance of the blank, and B is the absorbance of the sample or the ascorbic acid solution.

Statistical Analysis

The statistical significance of each variation of the dynamic maceration method was evaluated using the Kruskal-Wallis test, followed by a post hoc Mann-Whitney test for non-parametric datasets. P-values less than 0.05 were considered statistically significant. The statistical significance of each variation of the ultrasonic-assisted maceration method was evaluated using an ANOVA test and a post hoc Bonferroni test for parametric datasets.

Result and Discussion

Evidence suggests that the leaves of *S. rebaudiana* contain a mixture of steviol glycosides, resins, proteins, organic acids, and pigments. We aimed to develop an environmentally friendly extraction method using dynamic extraction with a water bath shaker and water-bath ultrasonic-assisted maceration. The goal was to find the most efficient, cost-effective, and environmentally friendly method for extracting *S. rebaudiana*. Distilled water was chosen as the solvent for the green chemical extraction approach. Extraction time is a critical parameter in any extraction technique, impacting time and cost. However, extraction temperature can influence the stability of the chemical composition of the extract. Therefore, in this study, we examined the effect of varying the extraction time and temperature on the yield and antioxidant activity of the extract.

Table 1 illustrates the effects of different extraction durations and temperatures on the yield and antioxidant activity of extracts obtained using a water bath shaker and ultrasonic-assisted maceration. The yields of both extractions were statistically analyzed to determine the significance of the effects of variations in the temperature and extraction time. In the case of water bath shaker maceration (dynamic extraction) at an extraction temperature of 25 °C, the impact of the extraction time on the yield was found to be non-significant ($p > 0.05$), as shown in Tables 1 and 2. Changing the extraction duration from 3 to 6 hours at 25 °C resulted in a 1.3% increase in the yield. This is because the diffusion-based solute displacement process in the matrix reaches a saturation point, at which no additional external energy is available

to boost the system's kinetic energy. Consequently, the capacity of the solvent to extract solutes from the matrix is limited. The processes of diffusion and osmosis are temperature-dependent [22]. The effect of temperature on solute extraction using water bath shaker maceration was evident when comparing treatments 1 and 3. Higher temperatures and shorter extraction periods notably influenced yield, leading to an increase in yield. The relationship between temperature and extraction capability was noticeable, wherein higher temperatures resulted in higher yields. However, the impact of high temperatures combined with extended extraction times (Treatments 2 and 4) did not induce significant changes. Notably, at high temperatures (75°C) with extraction times of 3 and 6 hours, a significant reduction in yield ($p < 0.05$) by 1.9% was observed.

The duration of extraction during water-bath ultrasonic-assisted maceration at 25°C significantly impacted yield ($p < 0.05$), as outlined in Tables 1 and 2. Changing the extraction duration from 30 to 60 minutes at 25°C (treatments 1 and 2) led to an increase in the yield from 12.1% to 15.2% (3.1%). This increase can be attributed to the prolonged extraction duration, facilitating better solvent penetration into the substance. The solubility of the components within the material is proportional to the extraction time. Moreover, when the extraction temperature was maintained at 75°C and the extraction time was extended from 30 to 60 min (treatments 3 and 4), there was no significant increase in yield ($p > 0.05$). However, the yield decreased by 0.4% from 15.0% to 14.6%. Based on these findings, it is evident that higher temperatures and longer extraction times lead to a decrease in extraction

Table 1. Effect of variation of temperature and extraction time on total yield and antioxidant activity of *S. rebaudiana* Leaf Extract.

Method	Treatment	Temperature	Time (minutes)	Extract (g)	Yield (% w/w)	% inhibition of DPPH*
Dynamic maceration	1	25°C	180	1.42	14.2 ± 0.3	33.4 ± 0.2
	2	25°C	360	1.55	15.5 ± 0.9	30.1 ± 2.5
	3	75°C	180	1.86	18.6 ± 0.1	25.9 ± 3.4
	4	75°C	360	1.67	16.7 ± 1.6	16.7 ± 1.0
Ultrasonic assisted maceration	1	25°C	30	1.21	12.1 ± 0.5	39.9 ± 7.4
	2	25°C	60	1.51	15.2 ± 1.1	33.8 ± 1.1
	3	75°C	30	1.50	15.0 ± 0.3	31.8 ± 1.9
	4	75°C	60	1.46	14.6 ± 0.9	27.1 ± 3.9
Vitamin C						49.3 ± 2.3

Note: *The concentration of each extract and vitamin C is 100 µg/mL

Data were generated from three experiments (n = 3) and presented as a mean with a standard deviation.

Table 2. Summary of the significant value of post Hoc test result of yield using a variation of time and temperature extraction.

Dynamic maceration				
Treatment	1	2	3	4
1 (25°C,180 mins)		0.275	0.005*	0.127
2 (25°C,360 mins)	0.275		0.050*	0.275
3 (75°C,180 mins)	0.050*	0.050*		0.050*
4 (75°C,360 mins)	0.127	0.275	0.050*	
Ultrasonic Maceration				
Treatment	1	2	3	4
1 (25°C,30 mins)		0.028*	0.038*	0.077
2 (25°C,60 mins)	0.028*		1.000	1.000
3 (75°C,30 mins)	0.038*	1.000		1.000
4 (75°C,60 mins)	0.077	1.000	1.000	

*Significance different ($p < 0.05$)

yield. The extraction temperature significantly affected the resulting extract. Previous studies on stevia leaf extraction using a water bath ultrasonic method with ethanol indicated that an intermediate temperature of 50°C was optimal for achieving higher yields. At this temperature, the solvent's vapor pressure is low, which allows cavitation bubbles to collapse more forcefully, breaking matrix bonds and disrupting cell tissues effectively. [16].

Previously, it has been reported that stevia leaf extracts were more effective at restoring oxidative stress markers than pure glycosides [20]. This highlights the need to optimize extraction methods to boost antioxidant activity. To further investigate this, we examined how extended extraction durations and varying temperatures, using different extraction techniques, impact the chemical stability and antioxidant efficacy of *S. rebaudiana* extracts. Alongside steviol glycosides, the extract from *S. rebaudiana* leaves contains an array of secondary metabolites, including flavonoids, phenolic acids, fatty acids, proteins, and vitamins [23]. In this research, we evaluate the antioxidant activity of each extract at 100 µg/mL and compare the percentage of inhibition in the variation of the extraction conditions.

The data in Table 1 demonstrate that extracting *S. rebaudiana* leaves using dynamic maceration (water-bath shaker) at an elevated temperature of 75°C yields a reduced inhibition percentage compared to extracts procured at lower temperatures (25°C). Delving into the percentage of inhibition against the DPPH radical derived from the antioxidant activity assay of *S. rebaudiana* leaf extract, it

became evident that escalated extraction temperatures and extended extraction times contributed to a diminished inhibition percentage. At an extraction temperature of 25°C for 3 hours the inhibition percentage of the extract against the DPPH radical was 33.4%. Extending the extraction time to 6 hours while maintaining the same temperature resulted in 30.1% inhibition.

This phenomenon holds even for extracts obtained using the ultrasonic extraction method. Heightening the extraction duration while maintaining a constant temperature (treatments 1 and 2) reduced antioxidant activity. Similarly, increasing the temperature while maintaining a steady extraction duration leads to decreased antioxidant activity. Hence, it can be reasonably deduced that elevating the temperature and extending the extraction duration via ultrasonic extraction might disrupt the stability of the chemical constituents present in the extract, supported by the observed decline in antioxidant activity.

Heightened extraction temperatures are anticipated to facilitate the extraction of a more significant number of compounds from the biological matrix (plants), owing to an augmented diffusion coefficient (mass transfer) and solubility (equilibrium). The heating process can soften plant tissues and weaken the bonds between phenol and polysaccharides, thereby fostering the migration of compounds from the matrix into the solvent, particularly flavonols often encountered as glycosides [24]. However, elevated temperatures can potentially inflict damage through compounds' oxidation and degradation reactions.

The decline in antioxidant activity with prolonged extraction can be attributed to the antioxidant activity of the extracted compounds, a phenomenon reliant on time [25].

When comparing water-bath shaker (dynamic maceration) and water-bath ultrasonic-assisted maceration for extracting *Stevia rebaudiana*, notable differences in yield and antioxidant activity emerge. Dynamic maceration generally achieved higher yields, with the peak yield of 18.6% at 75°C for 180 minutes, while ultrasonic-assisted maceration produced slightly lower but consistent yields, with the highest at 15.2% at 25°C for 60 minutes. Regarding antioxidant activity, ultrasonic-assisted maceration outperformed dynamic maceration, showing greater DPPH inhibition across all conditions, with the highest inhibition of 39.9% observed at 25°C for 30 minutes. While effective in yield, dynamic maceration showed decreased antioxidant activity at higher temperatures, with the lowest inhibition recorded at 75°C for 360 minutes. Therefore, dynamic maceration is more suitable for maximizing yield, especially at higher temperatures, whereas ultrasonic-assisted maceration is preferable for obtaining higher antioxidant activity, particularly at lower temperatures

Conclusion

This study elucidated the significant influence of extraction parameters on the yield and antioxidant activity of *S. rebaudiana* leaf extracts. Notably, dynamic maceration at 75°C for 3 hours resulted in the highest extraction efficiency (18.6%), whereas ultrasonic extraction at 25°C for 30 minutes exhibited the highest antioxidant activity (39.9%). Temperature and duration emerged as critical factors, with elevated temperatures generally increasing yield but concomitantly decreasing antioxidant activity. Prolonged extraction times also led to diminished antioxidant potential. This research enhances our understanding of how extraction conditions intricately influence the yield and antioxidant properties of *S. rebaudiana* extracts, providing valuable insights for developing natural sweeteners with augmented health-promoting attributes. Moreover, the emphasis on environmentally sustainable extraction methods aligns with the contemporary drive for eco-conscious practices in food production, positioning *S. rebaudiana* as a promising candidate for crafting healthier food products in response to evolving consumer demands.

Acknowledgment

The authors would like to thank PT. Agro Jabar for the sample (*S. rebaudiana* leaves) which was given for this study.

References

- [1]. Purohit V, Mishra S. The truth about artificial sweeteners – Are they good for diabetics? Indian Heart J. 2018;70(1):197–9.
- [2]. López-Carbón V, Sayago A, González-Domínguez R, Fernández-Recamales Á. Simple and Efficient Green Extraction of Steviol. Foods. 2019;8(402):1–10.
- [3]. Dyduch-Siemnińska M, Najda A, Gawroński J, Balant S, Świca K, Żaba A. Stevia Rebaudiana Bertoni, a Source of High-Potency Natural Sweetener—Biochemical and Genetic Characterization. Molecules. 2020;25(4):780.
- [4]. Debras C, Chazelas E, Srour B, Druesne-Pecollo N, Esseddik Y, de Edelenyi FS, et al. Artificial sweeteners and cancer risk: Results from the NutriNet-Santé population-based cohort study. PLoS Med. 2022;19(3):1–20.
- [5]. Orellana-Paucar AM. Steviol Glycosides from Stevia rebaudiana: An Updated Overview of Their Sweetening Activity, Pharmacological Properties, and Safety Aspects. Molecules. 2023;28(3).
- [6]. Formigoni M, Milani PG, da Silva Avincola A, dos Santos VJ, Benossi L, Dacome AS, et al. Pretreatment with ethanol as an alternative to improve steviol glycosides extraction and purification from a new variety of stevia. Food Chem [Internet]. 2018;241(July 2017):452–9.
- [7]. Samuel P, Ayoob KT, Magnuson BA, Wölwer-Rieck U, Jeppesen PB, Rogers PJ, et al. Stevia Leaf to Stevia Sweetener: Exploring Its Science, Benefits, and Future Potential. J Nutr. 2018;148(7):1186S-1205S.
- [8]. Salehi B, López MD, Martínez-López S, Victoriano M, Sharifi-Rad J, Martorell M, et al. Stevia rebaudiana Bertoni bioactive effects: From in vivo to clinical trials towards future therapeutic approaches. Phyther Res. 2019;33(11):2904–17.
- [9]. Martins PM, Thorat BN, Lanchote AD, Freitas LAP. Green extraction of glycosides from Stevia rebaudiana (Bert.) with low solvent consumption: A desirability approach. Resour Technol. 2016;2(4):247–53.
- [10]. Borgo J, Laurella LC, Martini F, Catalán CAN, Sülsen VP. Stevia genus: Phytochemistry and biological activities update. Molecules. 2021;26(9):1–45.
- [11]. Iatridis N, Kougioumtzi A, Vlataki K, Papadaki S, Magklara A. Anti-Cancer Properties of Stevia rebaudiana; More than a Sweetener. Molecules. 2022;27(4):1–20.
- [12]. Yılmaz FM, Görgüç A, Uygun Ö, Bircan C. Steviol glycosides and polyphenols extraction from Stevia rebaudiana Bertoni leaves using maceration, microwave-, and ultrasound-assisted techniques. Sep Sci Technol. 2021;56(5):936–48.
- [13]. Liu J, Li J wei, Tang J. Ultrasonically assisted extraction of total carbohydrates from Stevia rebaudiana Bertoni and identification of extracts. Food Bioprod Process [Internet]. 2010;88(2–3):215–21.
- [14]. Gasmalla MAA, Yang R, Hua X. Extraction of rebaudioside-A by sonication from Stevia rebaudiana Bertoni leaf and decolorization of the extract by polymers. J Food Sci Technol. 2015;52(9):5946–53.
- [15]. Periche A, Castelló ML, Heredia A, Escriche I. Influence of Extraction Methods on the Yield of Steviol Glycosides and Antioxidants in Stevia rebaudiana Extracts. Plant Foods Hum Nutr. 2015;70(2):119–27.
- [16]. Raspe DT, Ciotta SR, Zorzenon MRT, Dacome AS, da Silva C, Milani PG, et al. Ultrasound-assisted extraction of compounds from Stevia leaf pretreated with ethanol. Ind Crops Prod. 2021;172(September):1–9.
- [17]. Žlabur JŠ, Voča S, Dobričević N, Brnčić M, Dujmić F, Brnčić SR. Optimization of ultrasound assisted extraction of functional ingredients from Stevia rebaudiana Bertoni leaves. Int Agrophysics. 2015;29(2):231–7.

- [18]. Rouhani M. Modeling and optimization of ultrasound-assisted green extraction and rapid HPTLC analysis of stevioside from *Stevia Rebaudiana*. *Ind Crops Prod* [Internet]. 2019;132(December 2018):226–35.
- [19]. Martins PM, Lanchote AD, Thorat BN, Freitas LAP. Turbo-extraction of glycosides from *stevia rebaudiana* using a fractional factorial design. *Rev Bras Farmacogn* [Internet]. 2017;27(4):510–8.
- [20]. Papaefthimiou M, Kontou PI, Bagos PG, Braliou GG. Antioxidant Activity of Leaf Extracts from *Stevia rebaudiana* Bertoni Exerts Attenuating Effect on Diseased Experimental Rats: A Systematic Review and Meta-Analysis. *Nutrients*. 2023;15(15).
- [21]. Komala I, Azrifitria, Yardi, Suzanti BO, Muliati F, Ni'mah M. Antioxidant and anti-inflammatory activity of the Indonesian ferns , *Nephrolepis Falcata* and *Pyrrosia Lanceolata*. *Int J Pharm Pharm Sci*. 2015;7(12):162–5.
- [22]. Gallo M, Formato A, Formato G, Naviglio D. Comparison between two solid-liquid extraction methods for the recovery of steviol glycosides from dried *stevia* leaves applying a numerical approach. *Processes*. 2018;6(8):1–15.
- [23]. Gawel-Bęben K, Bujak T, Nizioł-Lukaszewska Z, Antosiewicz B, Jakubczyk A, Karaś M, et al. *Stevia rebaudiana* Bert. leaf extracts as a multifunctional source of natural antioxidants. *Molecules*. 2015;20(4):5468–86.
- [24]. Mokrani A, Madani K. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit [Internet]. Vol. 162, *Separation and Purification Technology*. Elsevier B.V.; 2016. 68–76 p.
- [25]. Rauf A, Nawaz H, Shad MA. Effect of solvent polarity and extraction time on in vitro antioxidant properties of *Brassica oleracea* Convar *Capitata* Var L. Seeds. *Pak J Pharm Sci*. 2018;31(5):1889–97.



Copyright © 2025 The author(s). You are free to share (copy and redistribute the material in any medium or format) and adapt (remix, transform, and build upon the material for any purpose, even commercially) under the following terms: Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use; ShareAlike — If you remix, transform, or build upon the material, you must distribute your contributions under the same license as the original (<https://creativecommons.org/licenses/by-sa/4.0/>)