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Effect of Extraction Time and Plant Part Variations on the Characteristics of Crude Stevia Extracts

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ABSTRACT. Stevia is a natural, high-intensity sweetener that has gained increasing attention as a promising alternative for the production of low-calorie sweeteners. Maceration extraction is a simple and cost-effective method for recovering steviol glycosides from stevia. This study investigated the effects of extraction time and different stevia plant parts on the extraction performance, as evaluated by yield and Brix value. Distilled water was used as the extraction solvent, while extraction time (30, 60, 90, and 120 min) and plant part (leaves, stems, and a mixture of leaves and stems) were employed as experimental variables. Prior to extraction, the stevia samples were dried in a vacuum oven to a moisture content below 10%. Maceration extraction was performed at 60°C with continuous stirring at 100 rpm. The extract was subsequently concentrated using a rotary evaporator to obtain crude stevia extract. The results demonstrated that both extraction time and plant part significantly affected the extraction yield and °Brix value. The highest extraction yield (60.14%) was obtained from leaf samples extracted for 90 min, whereas the highest Brix value (89.5%) was achieved from leaf samples extracted for 30 min. These findings indicate that stevia leaves are the most suitable plant part for aqueous maceration extraction, with longer extraction favoring higher yield and shorter extraction preserving higher soluble solids.

1. INTRODUCTION

Sweeteners are one of the essential daily needs of the human body, with a recommended daily consumption of 50 grams or approximately 5–9 teaspoons. The primary sources of sweeteners commonly consumed include granulated sugar and artificial sweeteners. However, excessive consumption of sweeteners can negatively impact human health. Sugar, classified as a simple carbohydrate, dissolves in water and is rapidly absorbed by the body to produce energy [1]. In humans, overconsumption of sugar can lead to diabetes mellitus, a condition where the body is unable to effectively absorb glucose, resulting in elevated blood sugar levels. This condition can cause various organ disorders and, if left untreated, may lead to life-threatening complications for diabetes patients. To prevent such health issues, natural sweeteners can be an alternative. One example of a natural sweetener is stevioside, a compound found in steviol glycosides derived from stevia leaves. Stevioside has a sweetness level 200 to 300 times that of sucrose and is considered a safer option. Furthermore, several studies have shown that steviol glycosides contain very low to zero calories, making them suitable for individuals with diabetes or those following a calorie-restricted diet.

Stevia possesses several distinct properties, including its hygroscopic nature, a melting point of 198°C, solubility in water, and the ability to maintain a clear color during cooking. Additionally, stevia is 300 times sweeter than sucrose, making it an efficient and potent natural sweetener [2]. Beyond its sweetness, stevia offers various health benefits and advantages over other sweeteners. It helps stabilize blood sugar levels in individuals with diabetes and regulates blood pressure in those with hypertension. Stevia also enhances calcium absorption, which supports the treatment of osteoporosis. Its antimicrobial properties aid in alleviating symptoms of gingivitis and inhibiting plaque formation on teeth. Furthermore, stevia improves digestion, relieves stomach discomfort, and addresses scalp conditions, restoring vitality to dull hair. In skincare, it helps reduce wrinkles and promotes healthier skin. Notably, stevia has an extremely low caloric value, making it ideal for individuals mindful of their calorie intake [3]. Glycosides are molecules composed of a sugar component (glycone) bound to a non-sugar molecule (aglycone or genin). Stevia extract is rich in diterpene glycosides, primarily in the form of steviol glycosides. The most abundant sweeteners in stevia leaves include stevioside, rebaudioside A, and rebaudioside C, which are the key compounds responsible for the sweet taste of stevia. In addition to glycosides, stevia also contains several other compounds, such as proteins, water, fats and carbohydrates [4]. To date, the utilization of stevia as a natural sweetener has primarily focused on the leaves, whereas the potential of the stems as a source of sweet-tasting compounds has received considerably less attention [5][6]. Exploring the stems as an alternative source of sweetener-related compounds therefore represents an important opportunity to broaden the utilization of the whole stevia plant and enhance its value as a natural sweetener resource.

Maceration extraction is one method for extracting steviol glycosides. The maceration technique involves soaking the material in a suitable solvent to selectively dissolve the active compounds. This extraction can be performed with or without mild heating. The effectiveness of the maceration process depends heavily on the selection of the appropriate solvent, which should be chosen based on the solubility of the target compound [7], [8]. Compared with more sophisticated extraction techniques, maceration offers a simple and relatively low-cost process that requires minimal specialized equipment, making it readily applicable not only at laboratory scale but also for larger-scale and industrial extraction processes [7], [9]. The factors influencing the extraction performance investigated in this study were extraction time and the specific part of the stevia plant used. Extraction time plays an important role in determining extraction yield by affecting the duration of contact between the plant matrix and solvent; however, prolonged extraction does not necessarily result in a higher yield, as excessive extraction time may lead to degradation or changes in extractable compounds. Additionally, steviol glycosides are predominantly concentrated in the leaves, as stevia plants tend to accumulate these sweet-tasting compounds mainly in leaf tissues. Consequently, the economic value and utilization of stevia have traditionally been associated with its leaves. In contrast, the stems remain relatively underexplored, particularly regarding their potential as a source of sweet-tasting compounds. Therefore, investigating different plant parts may provide opportunities for broader utilization and valorization of the stevia plant. This study was conducted to investigate the effects of extraction time and plant part variation during maceration extraction on the characteristics of stevia extracts, particularly yield and Brix value.

2. MATERIALS AND METHODS

2.1 Material and Tools

The materials used are *Stevia Rebaudiana Bertoni* leaves and stems from PT. Indoalgae Aquaculture (Batu, East Java) and distilled water. The tools used in this study include a stirred glass extractor IKA/LR-2.ST Package 2, oven (Binder-Germany/ED 53), desiccator, digital balance, vacuum pump, Buchner funnel, Rotary evaporator (Buchi/R-215), moisture balance (Kern/MLS503HA), water bath (Memmert/MEM-WNE29FCLO), vacuum oven, and hand refractometer for sugar (ATC).

2.2 Raw Material Preparation

The stevia plants were initially dried using a vacuum oven at 55°C until the moisture content reached 8-10%. The leaves and stems were then separated, as they required different treatments in the subsequent steps. The leaves of the stevia plant were ground using a blender until a powder was formed. The stems of the stevia plant were subjected to size reduction using scissors to achieve a length of 2-3 cm. The material was then ground using a blender. The powder of leaves and stems were then sieved to achieve the particle size that passed through an 80-mesh sieve.

2.3. Maceration Extraction

The procedure begins by adding 50 grams of sample (various plant parts: leaves, stems, leaves and stems) powder to 500 mL of distilled water in a stirred reactor. The extraction is carried out at varying times of 30, 60, 90, and 120 minutes, at a temperature of 60°C and a stirring speed of 100 rpm. After the maceration extraction process, the residue and filtrate are separated. The extraction filtrate was evaporated using a rotary evaporator at 57°C and a pressure of 68 mBar to separate the crude extract from the solvent. The crude stevia extract is then stored in a refrigerator at approximately 2°C

2.4 Yield Calculation

The yield is calculated by comparing the mass of the crude stevia extract to the mass of the dry material, according to equation 1 [10], [11].

$$\text{Yield (\%)} = \frac{\text{the mass of the crude stevia extract (g)}}{\text{the mass of the dry material (g)}} \text{ x } 100\% \quad (1)$$

2.5 Determination of Brix Value

The Brix value is determined by testing with a hand refractometer. The Brix value indicates the total dissolved solids in the crude stevia extract. During the first use, the refractometer needs to be calibrated with pure water (deionized water). Deionized water is placed on the surface of the refractometer prism, and the refractometer is then closed. Natural light or a lamp can be used to assist in reading the refractometer. If the contrast line (the boundary between blue and white) is not at the zero position, the screw on the refractometer should be adjusted until the line is at the zero position [12]. The crude stevia extract is diluted with a 10-fold dilution factor, and the Brix value is measured three times to obtain accurate results. The calculation of the Brix value of the crude stevia extract is done using equation (2) [13], [14].

$$\text{Brix (\%)} = \text{Brix measurement result x dilution factor} \quad (2)$$

2.6 Statistical Analysis

Data on brix values were analyzed using SPSS 27. First, normality tests were performed. For normally distributed data, one-way ANOVA followed by Tukey HSD was applied. Samples with the same letter notation indicated no significant difference, while different notations signified significant differences [15].

3. RESULTS AND DISCUSSION

3.1 Effect of Extraction Time and Plant Part Variations on the Yield of Crude Stevia Extract

The extraction yield of stevia varied substantially with both extraction time and plant part, indicating that the efficiency of solid–liquid extraction (maceration) was strongly influenced by the characteristics of the plant material and the duration of contact between the plant matrix and solvent (Figure 1). In general, based on Figure 1 the yield did not increase linearly with extraction time, but exhibited different trends depending on the plant part used. For the leaf part, the yield increased from 33.82% at 30 min to 44.08% at 60 min, followed by a further increase to the highest yield of 60.14% at 90 min. However, extending the extraction time to 120 min resulted in a substantial decrease in yield to 37.34%. The initial increase in yield with extraction time can be attributed to the greater opportunity for solvent penetration into plant tissues and the diffusion of soluble compounds from the plant matrix into the extraction medium [16]. As extraction proceeds, the concentration gradient between the plant tissue and solvent facilitates mass transfer, resulting in a greater amount of extractable material being recovered [17]. The highest yield at 90 min suggests that this duration provided sufficient time for the release of soluble constituents from the leaves. The subsequent decrease at 120 min may indicate that prolonged extraction does not necessarily improve recovery. Alternatively, changes in the extract matrix during prolonged extraction may also contribute to the lower apparent yield [18].

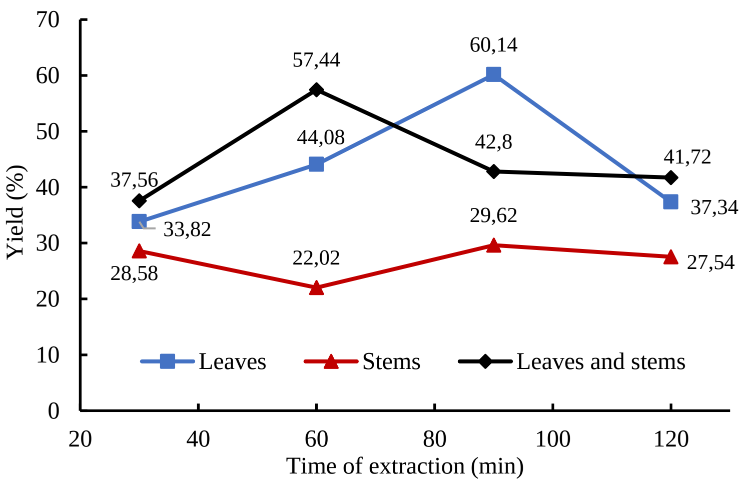

Figure 1. Effect of Extraction Time and Plant Part Variations on the Yield of Crude Stevia Extract

In the stem part extract, the yield decreased from 28.58% at 30 min to 22.02% at 60 min, before increasing to 29.62% at 90 min and subsequently decreasing slightly to 27.54% at 120 min. The relatively low yield throughout the extraction period compared with the leaves is likely related to differences in the physical and chemical characteristics of the stem tissue. Stem tissues generally contain a greater proportion of structural components, such as cellulose, hemicellulose, and lignin, which can restrict solvent penetration and the release of soluble compounds [19], [20]. Consequently, the extraction of stems may require longer contact times or more intensive mass-transfer conditions to achieve efficient recovery. The fluctuations in yield observed with increasing extraction time further suggest that the extraction behavior of stem tissue differs from that of leaves.

The leaves and stem part showed that the yield increased from 37.56% at 30 min to 57.44% at 60 min, reaching a high yield at 60 min. However, the yield subsequently decreased to 42.80% at 90 min and 41.72% at 120 min. Interestingly, the yield obtained from the combined leaves and stems material was higher than that of leaves alone at 30 and 60 min, despite the relatively low yield obtained from stems alone. This result may indicate that combining different plant tissues can alter the extraction behavior of the overall matrix, potentially through differences in tissue structure, solvent accessibility, and the distribution of soluble compounds [18]. Overall, the results demonstrate that the best extraction time depended strongly on the plant part. For leaves, the highest yield was obtained at 90 min (60.14%), whereas the leaves and stem part produced its highest yield at 60 min (57.44%). The stem treatment exhibited a considerably lower maximum yield of 29.62% at 90 min. These differences indicate that extending extraction time can enhance mass transfer up to a certain point, beyond which the extraction process may reach an equilibrium or the recovered extract may be affected by degradation or other physicochemical changes. Therefore, a longer extraction time should not automatically be regarded as more effective [18], [21].

3.2 Effect of Extraction Time and Plant Part Variations on the Brix Value of Crude Stevia Extract

The Brix value indicates the concentration of total dissolved solids in a solution, representing the refractive index calibrated against dissolved sucrose on a weight percentage basis [12], [22]. Because the refractive index of a solution is a function of dissolved solids concentration, all dissolved solutes beyond merely the sugar content contribute to the overall Brix reading. Steviol glycosides comprise a glycone (sugar component) and an aglycone core. The glycone portion consists primarily of rhamnose, fructose, glucose, xylose, and deoxyribose, while the aglycone portion comprises steviol [23]. Based on Table 1, the extraction time and plant part variations affect the Brix values. Brix value is commonly associated with the concentration of soluble solids in an extract; therefore, variations in Brix values may reflect differences in the amount of soluble compounds extracted from the plant material [24], [25]. For the leaf part, the highest Brix value was obtained after 30 min of extraction (89.5 ± 1.91%), which was significantly higher ($p < 0.05$) than those obtained at 60, 90, and 120 min. The Brix value subsequently decreased to 71.0 ± 1.15% after 60 min and reached the lowest value of 53.5 ± 1.00% at 90 min. A slight increase to 76.5 ± 1.91% was observed at 120 min. This non-linear trend suggests that prolonged extraction did not necessarily increase the concentration of soluble solids in the crude extract. Although longer extraction can facilitate the transfer of soluble compounds from plant tissues into the solvent, extended extraction may also promote degradation or transformation of certain soluble constituents. In addition, prolonged exposure to extraction conditions may alter the composition of the extract and consequently affect its measured Brix value [23], [26].

Table 1. Brix Values of Crude Extract

Plant Part Variations	Extraction Time (min)	Brix Values (%)
Leaves	30	89.5 ± 1.91^{a}
	60	71.0 ± 1.15^{c}
	90	53.5 ± 1.00^{e}
	120	76.5 ± 1.91^{b}
Leaves and stems	30	61.0 ± 1.15^{d}
	60	43.0 ± 1.15^{g}
	90	62.5 ± 1.00^{d}
	120	61.5 ± 1.00^{d}
Stems	30	49.0 ± 1.15^{f}
	60	63.0 ± 1.15^{d}
	90	48.5 ± 1.91^{f}
	120	44.5 ± 1.00^{g}

Note: Values are presented as means ± standard deviations. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$)

A similar variation was observed for the leaves and stem part, although the Brix values were generally lower than those of the leaves alone. The highest value was observed at 30 min (61.0 ± 1.15%), followed by 62.5 ± 1.00% at 90 min and 61.5 ± 1.00% at 120 min, whereas the lowest value was obtained at 60 min (43.0 ± 1.15%).

The relatively lower Brix values compared with the leaf extract may be associated with differences in the chemical composition of the plant tissues. Leaves are generally considered the major source of steviol glycosides, whereas stems contain comparatively lower concentrations of these compounds. The incorporation of stems may therefore dilute the concentration of soluble extractable constituents and contribute to the lower Brix values observed [27], [28]. For the stem part, the Brix value increased from 49.0 ± 1.15% at 30 min to 63.0 ± 1.15% at 60 min, followed by a decrease to 48.5 ± 1.91% and 44.5 ± 1.00% at 90 and 120 min, respectively. The increase observed between 30 and 60 min indicates that extending the extraction time initially enhanced the release of soluble compounds from the stem tissue. However, further extension of the extraction time resulted in a decrease in Brix value. This result indicates that an extraction time of 60 min may be more favorable for obtaining soluble solids from stems, whereas prolonged extraction may not provide additional benefits [21].

Overall, plant part had a pronounced effect on the Brix value of the crude stevia extract. The results also demonstrate that extraction time did not exhibit a consistent linear relationship with Brix value. The leaf part produced the highest Brix value, particularly at 30 min, whereas the extracts containing stems generally exhibited lower values. This finding indicates that the distribution of soluble compounds in stevia is not uniform throughout the plant. The higher Brix value obtained from leaves may be related to their greater concentration of soluble phytochemicals, including steviol glycosides and other water-soluble compounds. Meanwhile, stems may contain a higher proportion of structural components, such as cellulose and other insoluble materials, resulting in a lower concentration of soluble solids in the extract.

4. CONCLUSION

The results showed that extraction time and plant part variations influenced both yield and Brix value of crude stevia extract. The leaf part extracted for 90 min produced the highest yield (60.14%), while the leaf part extracted for 30 min resulted in the highest Brix value (89.5 ± 1.91%). The fluctuations observed with increasing extraction time could be attributed to changes in mass transfer between the plant matrix and the solvent. Prolonged extraction may promote the release of soluble compounds; however, it may also induce the degradation or transformation of some compounds, resulting in non-linear extraction behavior. The Brix value represents total soluble solids and does not directly quantify steviol glycosides, thus further compositional analysis would be necessary to determine whether the higher Brix value corresponds to a higher concentration of the target sweetening compounds such as quantitative analysis of steviol glycosides using HPLC.

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