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# Isolation of Stevioside and related compounds from two types of *Stevia rebaudiana (Bertoni)* species from Bangladesh.

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***Abstract*** *— The sweet Stevia rebaudiana (Bert.) leaf of South American region mainly contains Stevioside and rebaudioside-A which are two commercially important diterpenoid glycoside. Stevioside is 300 times sweeter than sugar, while rebaudioside-A is 1.6 to 1.8 times sweeter than stevioside. Some of the modern techniques of extraction and purification have been found to be complex and expensive. Therefore, the aim of the work reported here was to extract and purify stevioside and rebaudioside A using easy, simple and less expensive way. A solvent Extraction method has been chosen to evaluate the individual effect of different solvents (i.e. water, acidic, basic, methanol and ethanol solvent), time, temperature, pH. Dried leaves were blended to make fine powder and macerated by using different solvents at different temperature and time. Best result was found with 70% ethanol solution which procedure is less time consuming and can be carried out at room temperature. The stevia powder to ethanol solvent ratio was 1:15. Percentage of yield obtained after completing extraction steps was 24.71% which needs to undergo further purification process to obtain pure stevioside and rebaudioside A crystals. This green extraction process provided a simple and cost effective path to obtain almost pure stevioside and rebaudioside A.*

***Keywords*** *— **Stevia rebaudiana, Stevioside, Rebaudioside A, Methanolic extracts.***

## I. INTRODUCTION

Stevia is a natural sweetener which found in South American region specially in Paraguay and Brazil. Stevia genus have almost 150-300 species, distributed in the south western United States to the northern Argentina [1,2] and only S. rebaudiana gives the sweetest essence [25]. It is known as sweet leaf or honey leaf [3,4]. Stevia plant is perennial, endemic and is of Asteraceae family. The leaves are green in colour and contain approximately 10% of stevioside which are intensely sweet compound. The demand of stevioside is increasing widely due to its non caloric nature. It is 300-400 times sweeter than sugar and rebaudioside A is 1.6 to 1.8 times sweeter than stevioside [5-7]. Stevioside has bitter taste but rebaudioside A has no bitterness. The main advantage of stevioside over other sweeteners is that it is stable at 100°C [26]. Stevioside is used as a low caloric sweetening substance in several parts of the world. Stevia has been approved for several years in Brazil, Argentina, and Paraguay, as well as in China, Korea, and Japan to sweeten soft drinks, soy sauce, yogurt, and other foods, whereas in the United States they are used as dietary supplements since 1995. Although Stevia has been in use in Asia and Europe for years, it was only in the past couple of years that it has really started to capture attention in our market as a healthy alternative sweetener to sugar. Rather than sweetening properties, S. rebaudiana leaves also has therapeutic properties like antimicrobial [8], anti cariogenic [9], antifungal [10], antihypertensive [11], anti tumour [12], , diuretic, antidiarrheal, antihuman rotavirus activities [13], anti hyperglycaemic [14,15], anti HIV [16], anti-inflammatory [17], antiviral [18], hepatoprotective [19] , and immunomodulatory [20].

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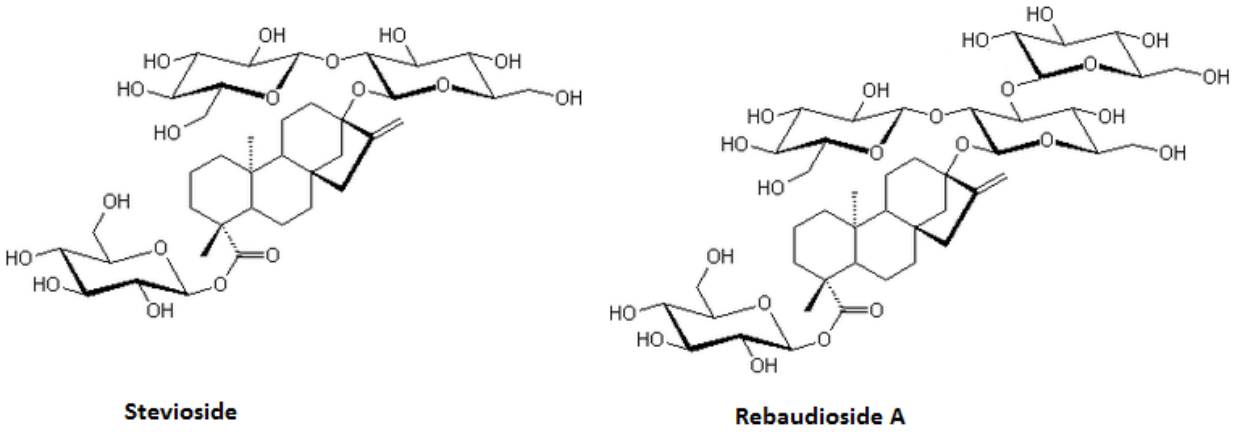


*Fig. 1: Chemical structure of Stevioside and Rebaudioside A*

There are various techniques for extraction and purification of active chemicals from plant extracts. There are several published literatures on extraction and purification process of stevioside and rebaudioside A using different solvents, solvent plus decolorizing agent, adsorption and ion exchange chromatography, supercritical fluid extraction and ultra and nano membrane filtration [21-24]. Solvent extraction of stevioside from S. rebaudiana leaves had been optimized using different solvents and improved yield was found due to use of alcohol (70% Ethanol) at room temperature and pressure. Stevioside yield mixed with other impurities was found 24.71% by Stevia powder: Solvent (1:15) ratio.

## II. MATERIAL AND METHODS

The study was conducted in the laboratories of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. A number of equipments i.e. Blender, Magnetic heating stirrer, pH meter, Rotary evaporator, Fridge drier etc. have been used. Methanol, Ethanol, Sodium hydroxide and Hydrochloric acid have been used as required chemicals in this process. Solvent extraction method has been followed for extraction of sweet compounds from stevia.

### 2.1 Preparation and Procurement of Material

Dried stevia leaves were procured from Khagrachari district, Bangladesh. Stevia leaves were crushed with bare hands and then crushed leaves were blended to make fine powder with a blender. Coarse parts were discarded and only fine powder was kept for use in extraction.

### 2.2. Experimental Design for Solvent Extraction Process

Solvent Extraction method has been followed for this extraction process. The ground leaves of Stevia rebaudiana were mixed with water and other solvents. In case of water, basic and acidic solvent, the mixture was heated. The extract was allowed to cool at room temperature. Then the aqueous extract was separated by filtering. On the other hand, for the treatment with alcoholic solvent, dry powder of stevia leaves was soaked in the solvent for two hours. The process was done in triplicate for all solvents but in case of water, it was done for 13 times.

In hot water extraction method, a specific ratio (1:5) of leaf powder to water (w/v) was measured and the stevia leaves powder was mixed with water. This sample was exposed to a temperature range of 75-80 °C for a fixed duration of 2 hour. After the termination of the heating process, the stevia extract was allowed to cool and then filtered by using a Whatman filter paper. The solid part was treated again with the same procedure. The process was repeated 13 times to remove all the sweetness from the stevia powder. A Magnetic heating was used to control the temperature in this process. Similar to the extraction using water, basic solvent extract was prepared by mixing a specific ratio (1:10) of leaf powder to Sodium hydroxide solution (w/v). pH was measured with a pH meter. This sample was also exposed to a temperature range of 75-80 °C for a fixed duration of 2 hour. After the termination of the heating process, the stevia extract was allowed to cool and then filtered. The solid part was treated again with the same procedure. The process was repeated 3 times but all the sweetness was not removed from the stevia powder. Acidic solvent extract was prepared by mixing same ratio of (1:10) for leaf powder to Hydrochloric acid solution (w/v). Same process was done as basic solvent extraction process. Alcoholic solvent extract was prepared by soaking a specific ratio (1:10) of leaf powder to 70%

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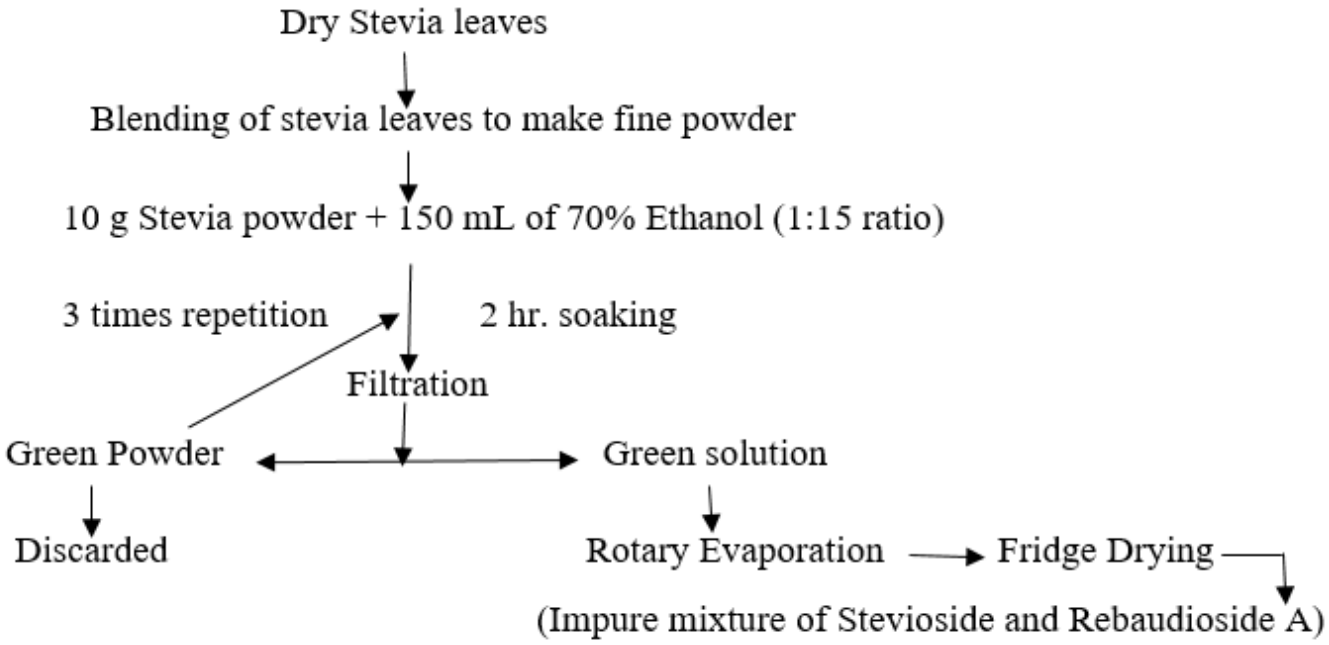
Methanol solution (w/v) for 2 hour at room temperature. After filtration, the solid part was treated again with the same procedure. The process was repeated 3 times but all the sweetness was not removed from the stevia powder but it was less in amount. Same process like methanol treatment was done by using ethanol solution as a solvent by soaking a specific ratio (1:15) of leaf powder to 70% Ethanol solution (weight to volume) for 2 hour at room temperature. Then this solution was filtered. The solid part was treated again with the same procedure. The process was repeated 3 times and all the sweetness was removed properly from the stevia powder. This process is most acceptable among all of the processes.

Flow chart is given below:




**2.3. Rotary Evaporation and Fridge Drying**

Rotary evaporation was done for the green solution obtained from Ethanol extraction process to remove ethanol from the solution. After this a green concentrated solution was obtained containing stevia extract and water. The temperature of water bath was 40-45 °C and rotation was 80 rev./min. Fridge drying was done to remove water from the solution obtained after evaporation of ethanol to make a dry product containing stevioside crystal mixed with other impurities. A fridge drier (Model: ALPHA 1-2 LD plus) has been used in this process. The product was kept in a refrigerator in an air tight bag to avoid fungi attack.

## III. RESULTS AND DISCUSSIONS

This research was carried out to wake of commercial importance of Stevioside in Bangladeshi market as well as outside of this country. Stevia extract was isolated from blended stevia leaves by solvent extraction method using different solvents i.e. water, basic and acidic solvents, different percentage of methanol and ethanol solvents. Different process parameters viz. leaf to solvent ratio, temperature, extraction time and percentage of alcohol in solvents, pH was optimized on the basis of quality parameters. Using hot water as a solvent, its needed thirteen time repeatations from starting stevia powder to end the process of removing all the sweetness from the stevia powder. It is time consuming abd will not be commercially beneficial. In case of both basic and acidic solvent of NaOH of pH 9.2 and HCl of pH 2.0 respectively, triplicate of procedure was unable to remove sweetness. So, these solvents were avoided for this extraction process of stevioside and related compounds from stevia leaf. The alcoholic solvent treatment process undergoes using 70% methanol was also not suitable because sweetness was not removed from stevia leaf powder after three repeatation of whole procedure. Methanol treatment is also not feasible for us due to its harmful effect on our body. So it is better to avoid this solvent for stevioside extraction.

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Use of 70% ethanol the two main glycosides present in stevia leaf could be completely extracted in a three steps of dynamic maceration for 2 hour each at room temperature. Rotary evaporation with an evaporator was done and all of the ethanol was evaporated from the solution. Fridge drying in a fridge drier was done due to remove rest of the water present. Ethanol 70% resulted in stevioside and rebaudioside A contents mixed with other impurities of 24.71%. This result can be compared with the tests conducted by authors [28,29], which can be explained by the polarity of 70% ethanol, favorable extraction of components of stevia leaf like the glycoside terpenes, flavonoids, chlorophyll, xanthophyll, alkaloids, amino acids, organic acids, oligosaccharides and lipids [30]. Higher percentage of yields were observed with 70% ethanol which is adequate and has the advantage of evaporation to make concentrated solution. This process is most feasible and can be highly acceptable. In our research, we will also try to remove green colour from this product to obtain pure crystals of stevioside and rebaudioside A.

## IV. CONCLUSIONS

The extraction of stevioside and rebaudioside A from S. rebaudiana (Bert.) leaves by multistep dynamic maceration showed that 70% ethanol solvent gives better results than water, acidic, basic solvents and 70% methanol solvent. At room temperature, 2 hour soaking of stevia powder with 70% ethanol extracts almost all the sweetness from leaves after third repeatation of the process. In case of water, it needs 13 repeatations at a temperature range of 75-80 °C. Acidic and basic solvents also require 3 repeatations at same temperature range alike water extraction but these solvents can not remove sweetness from stevia leaves. 70% methanol solvent is also unable to remove sweetness with 3 repeatations compared to 70% ethanol solvent and methanol is also not feasible at all. So ethanol was taken as a green solvent for this study and product obtained after fridge drying contains a mixture of stevioside and rebaudioside A with other impurities. This product can be used for further purification to obtain pure crystals of stevioside and rebaudioside A. Further purification will be continue for isolation of pure stevioside and rebaudioside A through HPLC.

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