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ISOLATION AND EXTRACTION OF ARTIFICIAL SWEETNER (STEVIA)

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ABSTRACT

Stevioside is extracted from the leaves of Stevia rebaudiana Bertoni (asteraceae or compositae) plant. Due to its sweet taste it is used as a natural non caloric sweetener. These glycosides are 300 times sweeter than sugar and also exhibit wide therapeutic activity. Stevia plants are also rich in carbohydrates (61.93% d.w), protein(11.41% d.w), crude fibre (15.52% d.w), minerals(K-21.15; Ca-17.7; Na-14.93; Mg-3.26 mg/100gm d.w; Cu-0.73; Mn-2.89; Fe-5.89; Zn-1.26 mg/100gm d.w) and also essential amino acids. It is used as a substitute for sucrose in food & beverages in many countries. In the present experiment isolation of stevioside from stevia leaves had been done by low cost methods. Isolated stevioside was analyzed by TLC method. The RF values of TLC were 0.50 & 0.67.

KEYWORD: Stevia rebaudiana, *asteraceae*, stevioside, non caloric sweetener, extraction, TLC.

INTRODUCTION

In recent years, there has been a wide range of demand for natural non-nutritive intensity sweeteners, exhibits wide therapeutic activity [1] and also essential amino acids [2] with low-calorific value as a substitute for sucrose [1]. Diabetes is the most common and major increasing health problem in India and many other countries. According to IDF, the number of diabetic persons will rise from 40 million to 70 million by 2025. The country with largest diabetic people will be India followed by china and USA by 2030[3]. The study of plants

chemistry is referred to as phytochemistry. Stevia rebaudiana Bertoni a perennial plant (a member of the Asteraceae or compositae family) commonly called as sweat herb which is gaining a significant and vital importance as a natural sweetener. It is a rich and high source of herbal sugar, stevioside and rebaudioside. It is also called as honey leaf. It is native plant of South America. In Egypt, the gap between sugar production (1.757 million tons) and consumption (2.6 million tons) issue represents a serious problem, since it was estimated to be 0.843 million tons and this problem was solved by introducing stevia in that region. Now a day, attention is concentrated upon using stevia in food industries, in order to reduce the difference between the production and consumption [2]. Stevioside can be degraded to its major metabolite, steviol, by intestinal bacterial micro flora of human being [6-8]. steviol glycoside is isolated from the leaves of Stevia rebaudiana possessing a 250-300 times higher sweetening character than sucrose, and they also used for treating carbohydrate metabolic disorders like diabetes mellitus, obesity, hypertension and stimulates cell regeneration[1]. Stevia extracts generally possess a high percentage of the glycoside diterpenes stevioside (CAS no. 57817-89-7) and rebaudioside-A (CAS no. 58543-16-1), the principal and vital sweetening compounds, and smaller amounts of other steviol glycosides. The composition of leaf extracts mainly depend on the composition of the leaves, influenced by soil and climate and on the extraction and purification methods and applications used [7]. Toxicological studies have shown that stevioside does not have mutagenic, teratogenic or carcinogenic effects and no allergic reactions have been observed when it is used as sweetener. So, stevia and stevioside have been applied as substitutes for sucrose for the treatment of diabetes mellitus, obesity, hypertension and carries prevention [2]. It has been observed and reported to have anti bacterial,[8] anti microbial[9] ,anti-retoviral,[10] anti-oxidant[11] & hypoglycemic activity[12]. It has a great property to reduce premature aging of skin due to its rejenuating property. It is widely used in the manufacturing of cosmetics and other beauty products. Thus, these are also extensively used in food, beverages, medicine, wine making, cosmetics etc. Due to its bitterness in taste, it is limited for human consumption. Stevia extract mainly contains stevioside, rebaudioside A. There is a rapid growth in the interest towards natural antioxidants because of the trend toward the use of natural additives in food and cosmetics worldwide. Natural antioxidants which are plant based especially phenolics and flavanoids have gained great importance in therapeutic potential and has been exploited commercially either as antioxidant additives or as nutritional supplements [13-15]. The objective of the

present paper is to isolation of stevioside from stevia leaves by low cost methods and common laboratory chemicals.

MATERIALS & CHEMICALS

Leaves of stevia plant were ground into powder & preserved in an air tight polythene bag. Chemicals and solvents used in this process were commercial solvents. The chemicals are Butanol, Ethyl acetate: methanol: water (7.5: 1.5: 10), conc.H_2So_4: Ethyl alcohol (1: 10).

METHODS

Preparation of Stevia Extract

100gm of stevia leaves were powdered and was extracted with 1 litre of water stirring at 60^0c for 4 hours. The process was repeated again with the spent obtained from first extraction for the complete extraction of stevioside. The 2 filters were concentrated together by distillation using rotary evaporator to get the residue.

Isolation of Stevioside from the Residue

The residue should be washed with butanol for 3 washes to isolate stevioside from the water into the butanol with a separating funnel.

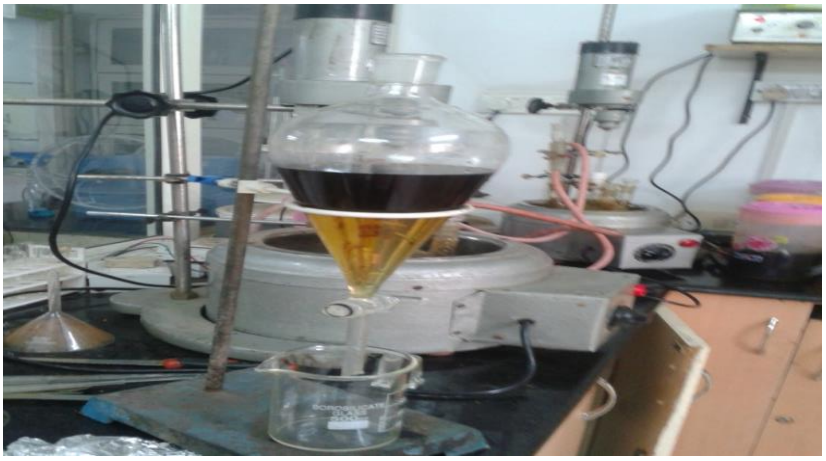

Figure-1 Isolation of Stevioside

Thin Layer Chromatography of Isolated Stevioside

The Thin Layer Chromatography (TLC) was performed using mobile phase i.e., Ethyl acetate: methanol: water (7.5: 1.5: 10). The visualization of spot was done using a detecting agent i.e., conc.H_2So_4: Ethyl alcohol (1: 10).

TABLE 1 - Result of TLC

S.no	Compound	Mobile phase(mp)	Detecting agent(da)	Rf values(cm)
1	Stevioside (solute A)	Ethyl-acetate: Methanol: Conc.H_2So_4(7.5:1.5:10)	Conc.H_2SO_4: ethyl alcohol (1 : 10)	0.50
2	stevioside (solute B)	Ethyl-acetate: Methanol: Conc.H_2So_4=(7.5:1.5:10)	Conc.H_2SO_4: ethyl alcohol= (1 : 10)	0.67

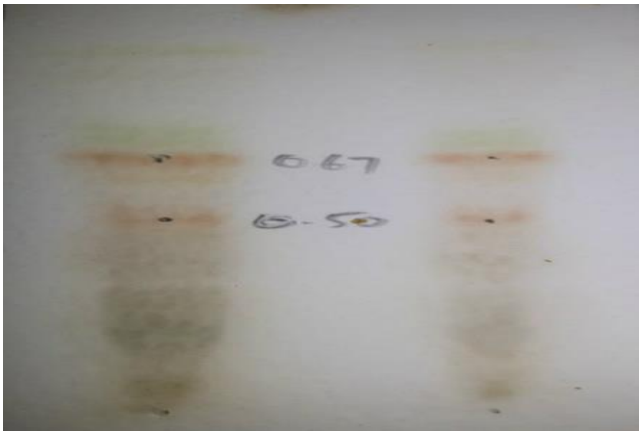

Figure-2 TLC Paper

Purification & Crystallization

The isolated stevioside is concentrated to get dry residue and is dissolved in methanol. The residue is treated with charcoal & cellite for the removal of unwanted impurities and colour. It is re-concentrated and crystallized to get the powdered stevioside.

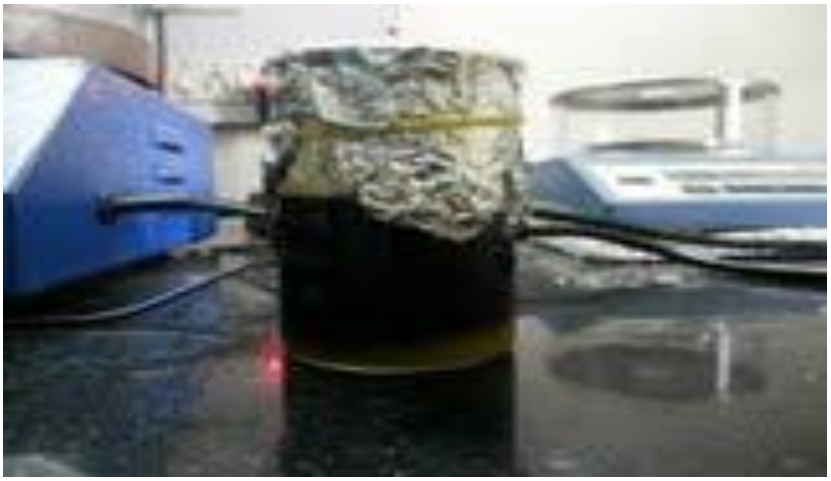

Figure-3 crystallization of stevioside

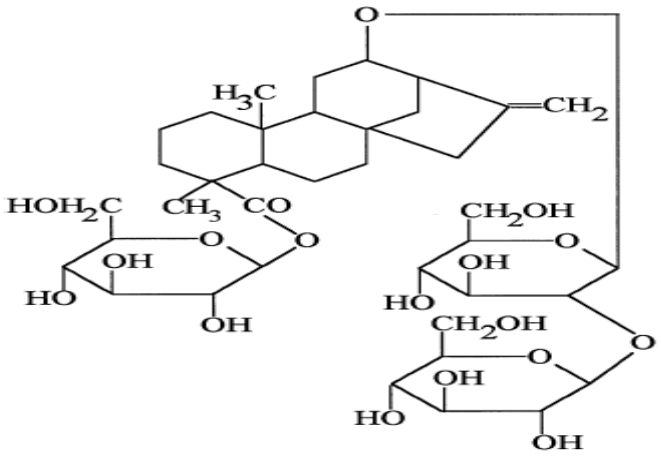

Figure-4 Structure of Stevioside and Chemical Formula: C_{38} H_{60} O_{18}

LOD- validation

Limit of detection was done as to know whether the required amount of stevioside is preserved in our extract.

RESULTS AND DISCUSSION

Stevioside was successfully isolated by using two different solvents with Rf values 0.50 And 0.67 respectively on TLC it was further purified and recrystallised.

CONCLUSION

Steviosides are low calorie steviol glycoside present in the leaves of stevia rebaudiana Bertoni. They are widely used as natural sweetener for the diabetic patients. The data obtained from TLC shows the band of stevioside which is confirmed by comparing the Rf value of standard (0.50) with that of the samples.

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