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GROWING OF SEEDLINGS OF STEVIA REBAUDIANA BERTONI (STEVIA) PLANT AND THE CHEMICAL COMPOSITION OF STEVIOSIDE

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Abstract
This article provides information about the stevia rebaudiana bext (stevia) plant and its chemical composition and use in medicine.

Keywords: Stevia rebaudiana bext, tocopherol, vitamin K, ascorbic acid, carbohydrate, cellulose, amino acid, potassium, phosphorus, magnesium, iron, calcium, zinc, bactericidal, antiseptic, antibacterial, immunity.

Introduction

For centuries, folk medicine has used decoctions and tinctures prepared from natural plants to treat various diseases. Research in the field of chemistry and pharmaceutical sciences has led to the creation of medicines and dosage forms based on new substances that do not occur in nature. However, the negative effects of the obtained synthetic medicines on the body have also been identified. Therefore, interest in natural medicines is currently increasing. The problem of synthesizing new biologically active substances, the treatment and prevention of viral diseases in medicine based on them, the creation of new medicines for immunodeficiencies of various etiologies is one of the main tasks of bioorganic chemistry today, due to the increasing prevalence of AIDS, hepatitis A, B, C, D, F and new respiratory virus infections. One of the promising ways to create new medicines is to search for new biologically active substances from existing natural compounds and their derivatives. One of the medicinal plants with unique properties is the stevia plant. The substance that gives the stevia leaf its main medicinal properties is its glycoside - stevioside. Medicines obtained from the stevia plant are the best substitute for sugar. It is non-toxic, has no negative effects, tastes good, and is inexpensive. All this is very convenient for people with diabetes and obesity. Dozens of medicines have been

created based on the stevia plant and are being effectively used in medical practice. [1-3]

Theoretical part

Stevia - (Stevia rebaudiana Bekht.) - Asteraceae - The stevia genus of the Asteraceae family includes more than 180 species of plants. Stevia - Stevia rebaudiana Bertoni is one of them. Stevia is an annual plant that can grow up to 1 meter tall. Its leaves are simple, opposite on the stem, and reach a length of 2-3 cm. The surface of the leaf blade is smooth, the front side is not pointed. The flowers are collected in 6-7 white complex inflorescences, 3-4 mm long. The fruit is 3-4 mm. It blooms from September to November. The seeds ripen in October-November (Figure 1). [1-5]

Figure 1. View of Stevia rebaudiana Bertoni plant.

The chemical composition of the stevia plant is unique, and its leaves contain a glycoside that gives it a sweet taste (stevioside). Stevioside is 300 times sweeter than a 0.4% sucrose solution (Figure 2) and 150 times sweeter than a 10% sugar solution. In addition, it contains flavonoids (quercetin, rutin), minerals (calcium, phosphorus, potassium, zinc, iron, chromium, magnesium, selenium, copper, manganese, cobalt), and vitamins (group B, A, C, E, etc.).

The medicinal properties of the Stevia rebaudiana Bertoni plant (antisclerotic, immunomodulatory, antihypertensive, anticarcinogenic effects) have been studied by Japanese and Russian scientists for 20 years. They contain 8 diterpene glycosides, vitamins A and C, 17 amino acids, flavonoids, polyunsaturated fatty acids and essential oils. However, at present, only the preparation “stevoside” has been introduced into production, which is a complex of diterpene glycosides and is widely used in the food and pharmaceutical industries as a natural sweetener. It is 300 times sweeter than sucrose, has no calories, is non-toxic, and is recommended for patients with diabetes. [4-6].

Stevioside is a white crystalline substance, a glycoside, obtained from the stevia plant. It has the chemical formula C38H60O18. Stevioside is widely used in the confectionery industry as a substitute for sugar in the preparation of soft drinks, juices, chewing gum, various sweets and preserves, and is used as a medicine to treat diabetes and other diseases associated with metabolic disorders. Stevioside is known as (β- D - Glucopyranosyl 13- [β- D -glucopyranosyl-(1→2)-β- D -glucopyranosyloxy] -5β, 8α, 9β, 10α, 13α-kaur-16-en-18-oat molecular weight). The chemical structural formula and conformational structure are shown in Figure 2.

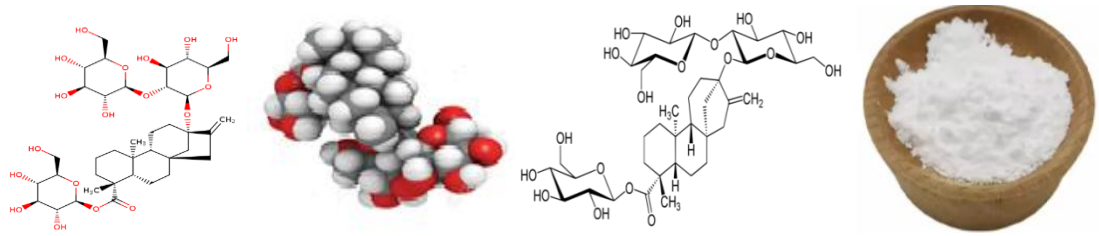

Figure. 2. Molecular formula and conformational structure of stevioside (β- D - Glucopyranosyl 13- [β- D -glucopyranosyl-(1→2)-β- D -glucopyranosyloxy]- 5b,8a,9b,10a,13a-kaur-16-en-18-oate).

The stevia plant has a general strengthening and tonic effect on the body in medicine. In ancient times, the Indians of Brazil and Paraguay used stevia as food, and sometimes in the treatment of gastrointestinal and kidney diseases. At the beginning of the 20th century, French scientists identified stevioside and rebaudioside in the composition of this plant [3-6].

The substances contained in the plant do not allow fat and other substances to accumulate on the teeth. This prevents tooth enamel from being destroyed by microbes. Stevia controls the level of sugar in the blood and plays a significant role in providing the body with sugar by supporting the activity of the hormone insulin. The biologically active substance contained in the stevia plant - glycoside (stevioside) - is one of the natural food substitutes in the body. Stevia products are also used in diseases associated with diabetes, the accumulation of excess fat in the body and metabolic disorders. In addition, it is used as a hypotensive (lowers blood pressure), cardiotonic, antibacterial, antifungal, antiviral, diuretic, tonic and effective in the treatment of wounds.

Considering that diabetic patients are not given sweets, it has been effectively used to satisfy the patients' need for sweets and to treat diabetes. China ranks second in the world in terms of diabetes incidence after the USA and Russia. India had the highest number of diabetics in 2008 (30 million). With the increase in demand for

stevia raw materials due to this disease, farmers began to establish stevia plantations on large areas. India is also one of the countries with very favorable soil and climatic conditions for growing stevia. Currently, the annual production of stevia in India is about 600 tons.

Also, in Japan, the stevia plant is used on a very large scale, with a profit of up to 270 thousand US dollars per hectare of land.

Although the sweetness of this plant is high, it does not cause any unpleasant effects in diabetic patients.

In folk medicine, substances obtained from stevia are used in diabetes. In this case, when the crushed (powdered) plant product is consumed, it destroys pathogenic microbes and has a positive effect on the immune system. It is also used in inflammation of the heart muscle, to improve the condition of the skin and hair, and to remove cholesterol from the body. In addition, stevioside is widely used in the confectionery industry (Japan) instead of sugar in the preparation of soft drinks and sweets. The important thing about stevia sugar is that its consumption rate is currently very unlimited, and no negative effects on organisms have been observed [1-7].

Discussion of the Results

A method for obtaining a dry purified extract from the leaves of Stevia rebaudiana Bertoni. A sample of 50 g of crushed Stevia rebaudiana Bertoni dried raw material to a constant weight was placed in a flask and 300 ml of 80% ethyl alcohol was added. The extraction was carried out at 20-25 °C with stirring for three hours. The combined alcoholic extracts were evaporated to dryness, and the dry residue was dissolved in 500 ml of water at 80 °C. The aqueous solution was extracted with 300 ml of ethyl acetate three times at 15-minute intervals. The ethyl acetate layers were separated and discarded in a separatory funnel. The aqueous layer was extracted three times with 100 ml of butanol. The combined butanol layers were evaporated to dryness, and the dry residue was dissolved in a minimum volume of methanol at 80 °C and left at 5 °C for 12 hours. The resulting precipitate was filtered, dried in a vacuum oven to constant weight and recrystallized from methanol. The resulting precipitate, containing the total fraction of diterpene glycosides, was filtered and dried in a vacuum oven to constant weight. Yield: 2.4 g ± 0.25 g of dry purified extract, containing an average of 67.1% stevioside. The scheme for obtaining dry purified stevioside extract from the leaves of Stevia rebaudiana Bertoni is presented in Figure 3.

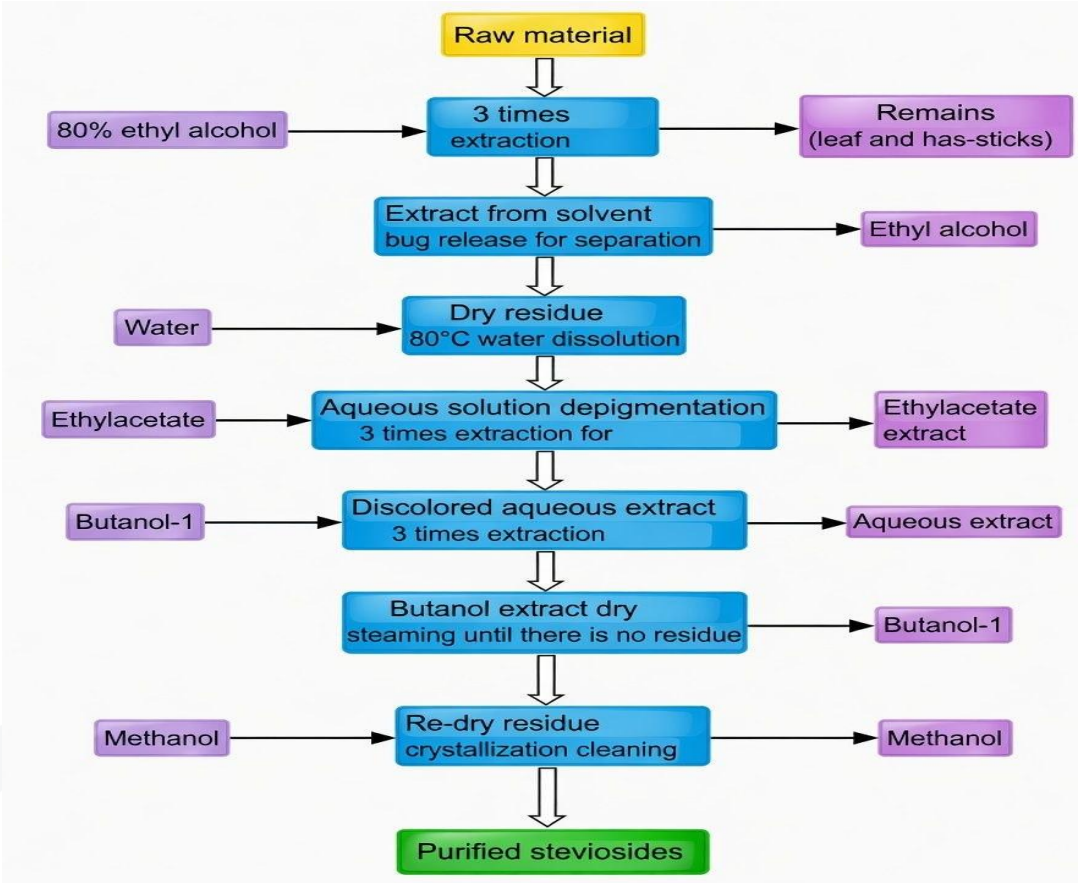

Figure 3. Scheme of obtaining dry purified stevioside extract from the leaves of Stevia rebaudiana Bertoni.

For the separation and determination of stevioside from the dry extract of Stevia rebaudiana Bertoni leaves by thin layer chromatography (TLC), 0.5 g of purified dry extract was dissolved in 6 ml of a 5:1 ethanol-water mixture and 0.2 ml of the solution was dropped onto the initial line of a 20 x 20 cm TLC plate with a sorbent layer thickness of 0.25 mm in a 10 cm line using a micropipette. A portion of a 1% stevioside standard solution in ethanol was dropped next to it. The plate was air-dried for 10 minutes and chromatographed in a chamber with a solvent system consisting of chloroform, methanol, and water in the ascending mode 10:5:1. After the solvent front had traveled approximately 15 cm, the plate was removed from the chamber and air-dried for 30 minutes. Then, the part of the plate containing the stevioside standard solution was sprayed with a 50% sulfuric acid solution. After drying the plate in a drying oven at 100°C for 15 minutes, a black spot corresponding to stevioside with Rf 0.37 was detected in the chromatogram. The sorbent layer with Rf 0.37 in the dry purified extract solution and located near the spot corresponding to stevioside was scraped off, dissolved in 4 ml of water, filtered through a paper filter and evaporated to dryness, and 7.5 mg of stevioside was obtained. The thin layer

chromatography of stevioside (1) and stevioside (2) in the purified dry extract obtained from the leaves of Stevia rebaudiana Bertoni is shown in Figure 4.

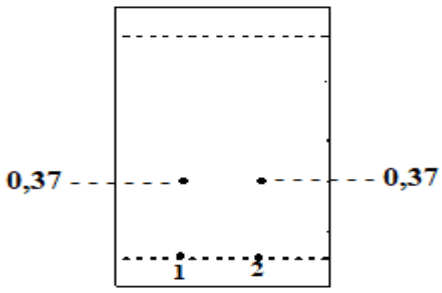

Figure 4. Thin layer chromatography of 1. Stevioside and 2. Stevioside in purified dry extract from the leaves of Stevia rebaudiana Bertoni.

Thin layer chromatography shows that stevioside and standard stevioside isolated from the Stevia rebaudiana Bertoni plant were dissolved in a 6:1 ethanol-water mixture and subjected to thin layer chromatography in a 10:5:1 chloroform, methanol, and water system. The Rf value was the same for standard stevioside and stevioside in the dry extract, Rf = 0.37.

To study the chemical composition of stevioside, stevioside was isolated from the Stevia rebaudiana Bertoni plant according to a known method in the literature, dissolved in a 6:1 ethanol-water mixture by thin layer chromatography (TLC) and determined in a 10:5:1 chloroform, methanol, and water system. The data are presented in Table 1.

Table 1 Comparison of Stevioside isolated from Stevia rebaudiana Bertoni according to a method known in the literature with standard Stevioside

№	**Substances**	**Solvent**	System	Rf system	IR spectrum, cm^{-1}	UV spectrum nm
1	Stevioside	6:1 ethanol-water	chloroform, methanol and water 10:5:1	0,37	1041 (COC); 1736 (CO) 2969 (CH_3); 3384 (OH)	219
2	Purified dry extract from the leaves of Stevia rebaudiana Bertoni	6:1 ethanol-water	chloroform, methanol and water 10:5:1	0,37		

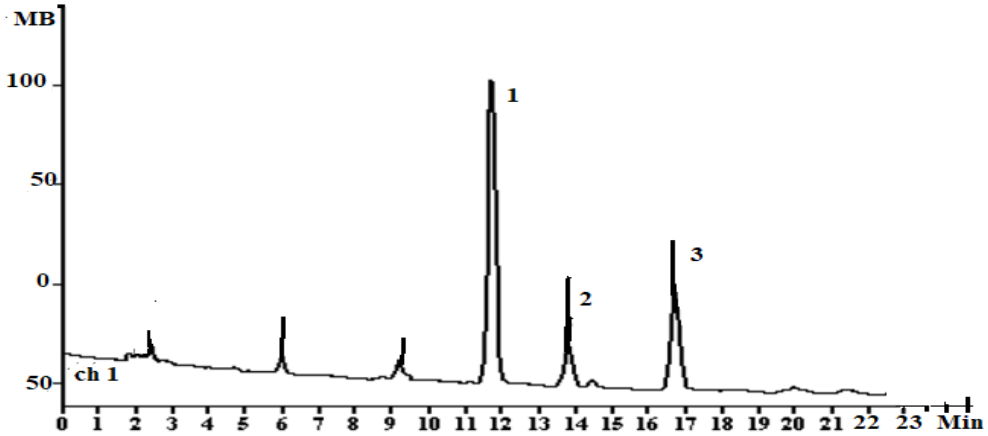

Figure 5. Purity chromatogram of stevioside content from a dry purified extract isolated from the leaves of Stevia rebaudiana Bertoni 1 - stevioside, 2 and 3 - diterpene glycoside mixtures.

As can be seen from the chromatogram, three main peaks were observed. The first of these peaks was related to stevioside, and the other two were related to other related diterpene glycosides, namely Rebaudioside-A and Rebaudioside-C. The results obtained showed that the purity of stevioside was 72%. Thus, 7.5 mg of stevioside was isolated from 0.5 g of dry extract, and the proportion of stevioside in the starting material was calculated.

$$\text{Stevioside (\%)}=\frac{\text{Stevioside mass extracted}}{\text{initial raw material mass}}\cdot 100\%$$

$$\text{Stevioside (\%)}=\frac{7{,}5\text{ mg}}{0{,}5\text{ gr}}\cdot 100\%=15\%$$

The results of the laboratory study showed that 15% stevioside was present in the dry extract. This result is consistent with the results reported in the literature (the literature states that stevioside constitutes 9-18% of the dry leaf).

In studying the structure of the obtained Stevioside substance, physical methods based on the interaction of organic molecules with electromagnetic radiation were widely used, in particular, their IR- (vibrational spectrum of atoms in the molecule, λ=10-4-10-2 cm) spectrum and UV- (electronic spectrum based on changes in the energy state of electrons in the outer shell, λ=10-6-10-4 cm) spectra. Using these methods, UV and IR-spectral data were used to analyze the structure of the substance.

In the UV spectrum of stevioside, an intense absorption maximum corresponding to the π-π* transition of the conjugated C=O to the π electrons of the ring was observed in the water : ethanol (1:1) system in the near UV region at a wavelength of 219 nm. The UV spectrum of stevioside is presented in Figure 6.

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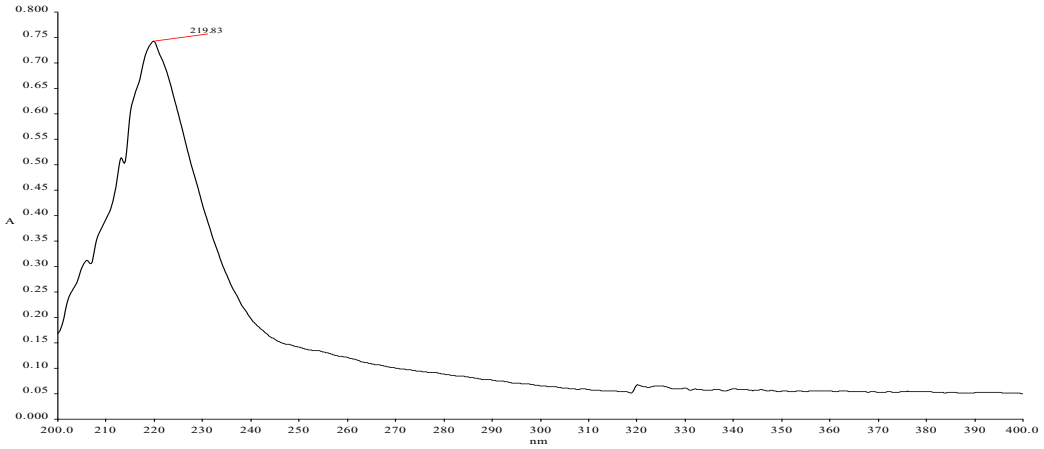

Figure 6. UV spectrum of stevioside.

In the IR spectrum, the valence vibration frequencies of the OH groups in the stevioside molecule formed broad "bands" in the region of 3374.28 cm−1, the valence vibration frequencies of the CH3 and CH2 groups appeared at 2946.68 cm−1, and the valence vibration frequencies belonging to the carbonyl part of the stevioside molecule were observed at 1773.22 cm−1 and 1609.62 cm−1. In the regions of 1043.04 cm−1, the valence vibration frequencies of the C-O-C and C-OH bonds in the molecule were observed in an intensive state. The valence vibration frequencies of the stevioside molecule in the IR spectrum at 3374.28 2946.68 1773.22 1609.62 and 1043.04 cm−1 are shown in Figure 7.

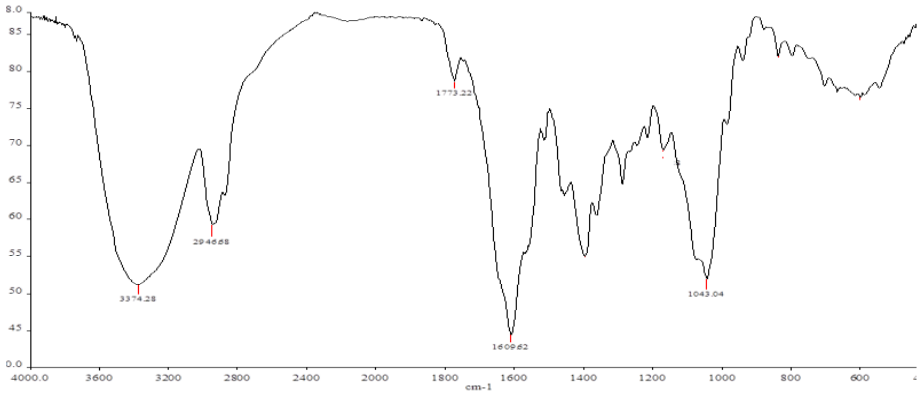

Figure 7. IR spectrum of stevioside.

Conclusion

1. Stevioside and standard stevioside isolated from Stevia rebaudiana Bertoni were dissolved in a 6:1 ethanol-water mixture and subjected to thin layer chromatography in a 10:5:1 chloroform, methanol and water system. The Rf value of standard stevioside and stevioside in the dry extract was uniform, Rf = 0.37.

2. When the stevioside isolated from Stevia rebaudiana Bertoni was studied in the UV spectrum, it was found that an intense absorption maximum corresponding to the π-π* transition of the conjugated C=O with the double bond in the ring was observed in the water:ethanol (1:1) system in the near UV region at a wavelength of 219 nm.

3. When stevioside isolated from the Stevia rebaudiana Bertoni plant was studied in the IR spectrum, it was found that the valence vibration frequencies in the molecule were observed intensively at 3374.28 cm-1, 2946.68 cm-1, 1773.22 cm-1, 1609.62 cm-1, and 1043.04 cm-1.

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