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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(3): 515-517

www.thepharmajournal.com
Received: 17-01-2018
Accepted: 18-02-2018

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HPLC analysis of phenolic compounds from *Stevia rabaudiana* Bertoni leaves

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Abstract
Stevia rebaudiana leaves are used as a calorie-free natural sweetener in a variety of food products in many countries. In this study, phenolic compounds of *S. rebaudiana* have been investigated qualitatively and quantitatively by HPLC method. In sweetleaf rutin, hyperoside, luteolin, quercetin-3-D-glycoside and kaempferol were identified among flavonoids. Furthermore two hydroxycinnamic acids (chlorogenic and rosmarinic) were present in analyzed object. Six catechins were identified and quantified. The predominant was epigallocatechin.

Keywords: *Stevia rebaudiana* Bertoni (Sweetleaf), hydroxycinnamic acids, flavonoids, catechins, HPLC-method

1. Introduction
Stevia rebaudiana Bertoni (Family – Asteraceae) is one of 154 members of the genus Stevia and one of only two species that produce sweet steviol glycosides (mainly stevioside and rebaudioside), which have 250–300 times the sweetness of sugar [1]. These glycosides were discovered in 1931 by chemists M. Bridel and R. Lavielle [2]. Stevia rebaudiana is commonly known as candyleaf, sweetleaf or sugarleaf. It is native to subtropical and tropical regions from western North America to South America were it grows in sandy soils near streams on the edges of marshland, acid infertile sand or muck soils.
It reaches up to 65–80 cm in height, it has oval, lanceolate or spatulate leaves of 3–4 cm, arranged opposite (Chan *et al*., 2000). The stem is woody, while the five-petaled flowers are light violet or white [3].
Sweetleaf roots and leaves contain fructooligosaccharides and polysaccharides, which regulate lipid metabolism and control blood sugar level; fatty acids. Analyses of this plant detected such sterols as stigmasterol, beta-sitosterol, campesterol and daucosterol as well as flavonoid glycosides, including apigenin, quercetin, luteolin and kaempferol glycosides, alkaloids, water-soluble chlorophylls and xanthophylls [4-5]. Analysis has shown that Stevia also contains folic acid, vitamin C and all of the indispensable amino acids with the exception of tryptophan [6].
Antimicrobial, antioxidant, hypotensive, antitumor and anti-human rotavirus activities of *S. rebaudiana* leaf extracts were evaluated. The effects of stevia leaves and its extracted polyphenols and fiber on streptozotocin induced diabetic rats were also studied [7].
The aim of our research was to evaluate the content of phenolic compounds in sweetleaf. For this purpose, *S. rebaudiana* leaves were selected in the present study for the analysis of flavonoids, hydroxycinnamic acids and catechins.

2. Materials and Methods
2.1 Plant material
Stevia rebaudiana Bertoni leaves were harvested from research plots of M. M. Gryshko National Botanic Garden (Kyiv) during the flowering stage in 2016. The collected raw material was dried and stored for further analysis.

2.2 Sample preparation
50 ml of 60% aqueous methanol was added to 2.28 g of dried sample and refluxed in a water bath at 90 ºC for 30 min. After this the sample was treated with ultrasound for 10 min, cooled, filtered and made up to 100 ml with 60% methanol solution and injected to HPLC [8-9].

2.3 Chemicals and Methods

All chemical were of analytical grade (> 95% purity) and were purchased from Sigma–Aldrich (USA). High performance liquid chromatography (HPLC) method was applied for separation and quantification of flavonoids, hydroxycinnamic acids catechins and coumarines.

2.4 HPLC analysis

HPLC analysis was carried out on high performance liquid chromatograph Agilent 1200 3 D LC System Technologies, USA coupled with a four-channel vacuum degasser G1354 A, autosampler G1329 A, autosampler thermostat G1330 B, column thermostat G1316A, diode array detector (G1315C) in complex with PC software Agilent Chem Station (G2215 BA). The separation was achieved on a Discovery C 18, 250 mm x 4.6 mm x 5 µm (Supelco, № 505129) column with the precolumn of 20 mm at 25 °C temperature. The mobile phase consisted of distilled water with 0.005 N orthophosphoric acid and acetonitrile (solvent A) and 0.005 N orthophosphoric acid and acetonitrile (solvent B) – analysis of flavonoids, hydroxycinnamic acids; 0.1% solution of trifluoroacetic acid, 5% acetonitrile solution and water (solvent A) and 0.1% solution of trifluoroacetic acid with acetonitrile (solvent B) – analysis of tannic substances components. The flow rate was 0.8 ml/min and the injection volume was 10 µm. The monitoring wavelength was 320 and 330 nm for hydroxycinnamic acids; 255 and 340 nm for flavonoids; 255 and 280 nm for catechins [9-10].

Phenolic compounds were identified by comparison of their retention times with corresponding reference standards. Quantification was done by the peak area.

3. Results and Discussion

Pharmacological activities of a drug are contributed by the presence of secondary metabolites. In the class, phenolic compounds are known to have activity against several pathogens and therefore could suggest their traditional use for the treatment of various illnesses. It has been recognized that phenolic compounds are a class of antioxidant compounds which act as free radical terminators [11].

The HPLC chromatogram of hydroxycinnamic acids from *S. rebaudiana* showed the presence of chlorogenic and rosmarinic acids as presented in fig. 1. The leaves of *S. rebaudiana* revealed the presence of rutin, hyperoside, luteolin, quercetin-3-D-glycoside and kaempferol under wavelength 255 nm. A remarkable amount was for rutin (0.14%) followed by quercetin-3-D-glycoside (0.05%) and luteolin (0.03%) (fig.2). The HPLC chromatogram of catechins from *S.rebaudiana* leaves indicated the presence of five catechins at wavelength 280 nm: gallic acid (0.01%), gallocatechin (0.09%), epigallocatechin (0.72%), epicatechin (0.07%) and epicatechin gallate (0.03%) (fig. 3). Under 255 nm only ellagic acid was identified and quantified; its content was 0.02% as shown in fig. 4.

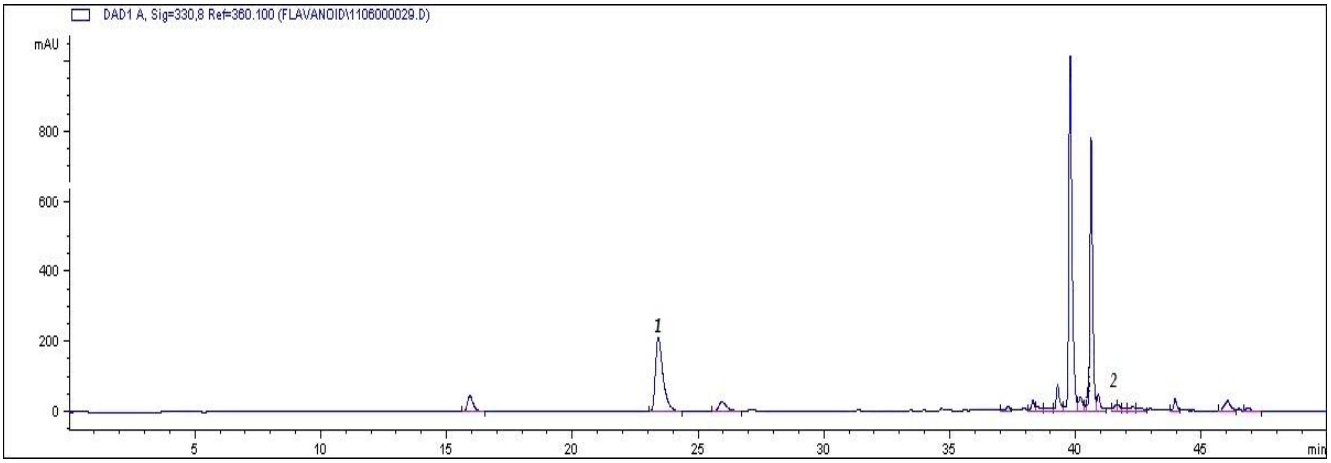

Fig 1: HPLC-chromatogram of hydroxycinnamic acids from *S. rebaudiana* leaves (wavelength 330 nm): 1- chlorogenic acid, 2- rosmarinic acid.

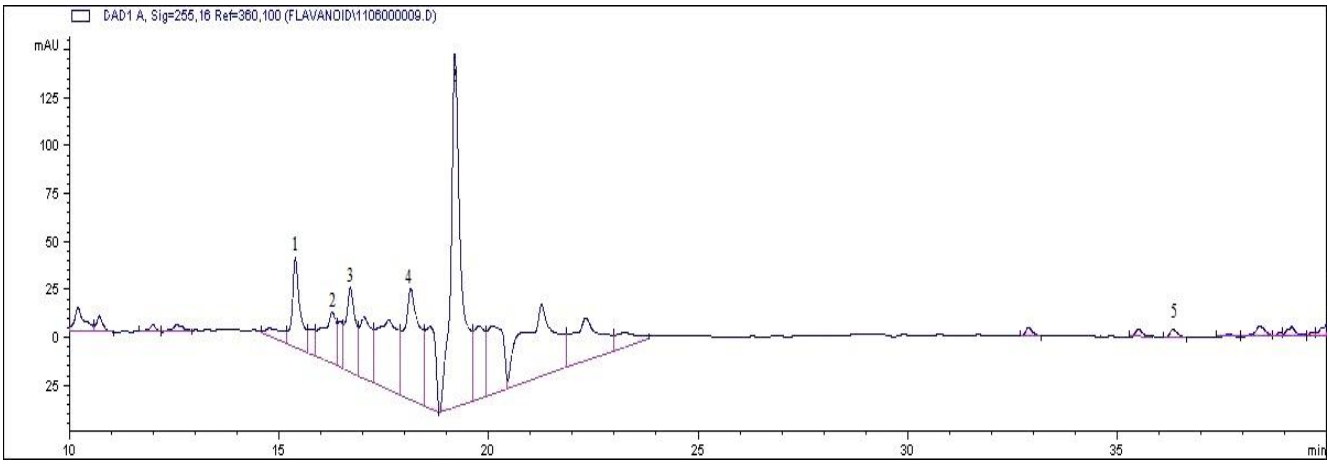

Fig 2: HPLC-chromatogram of flavonoids from *S. rebaudiana* leaves (wavelength 255 nm): 1- rutin, 2- hyperoside, 3- luteolin, 4- quercetin-3-D-glycoside, 5- kaempferol.

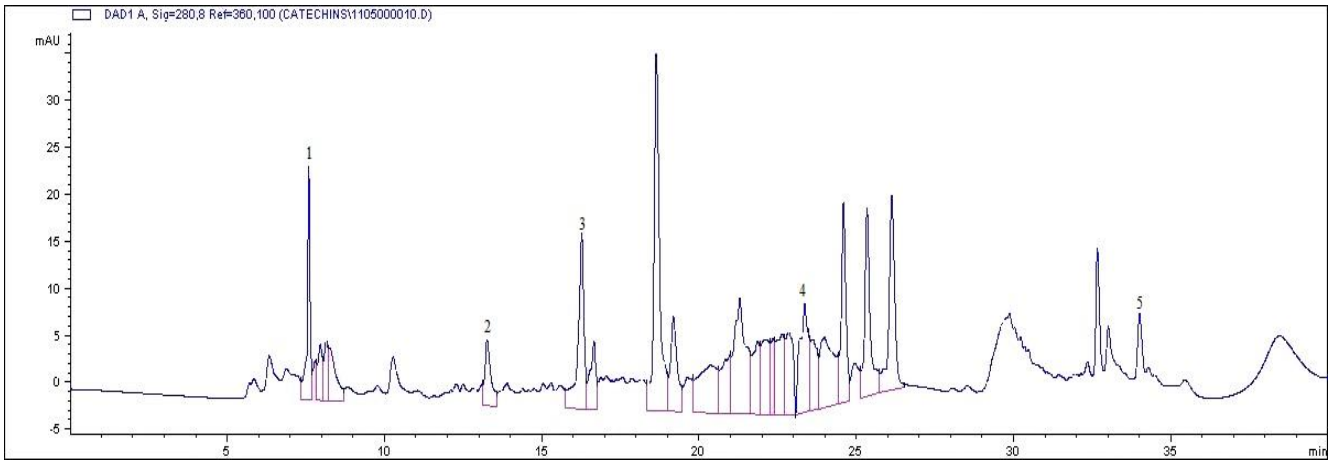

Fig 3: HPLC-chromatograms of catechins from *S. rebaudiana* leaves (wavelength 280 nm): 1- gallic acid, 2- gallocatechin, 3- epigallocatechin, 4- epicatechin, 5- epicatechin gallate.

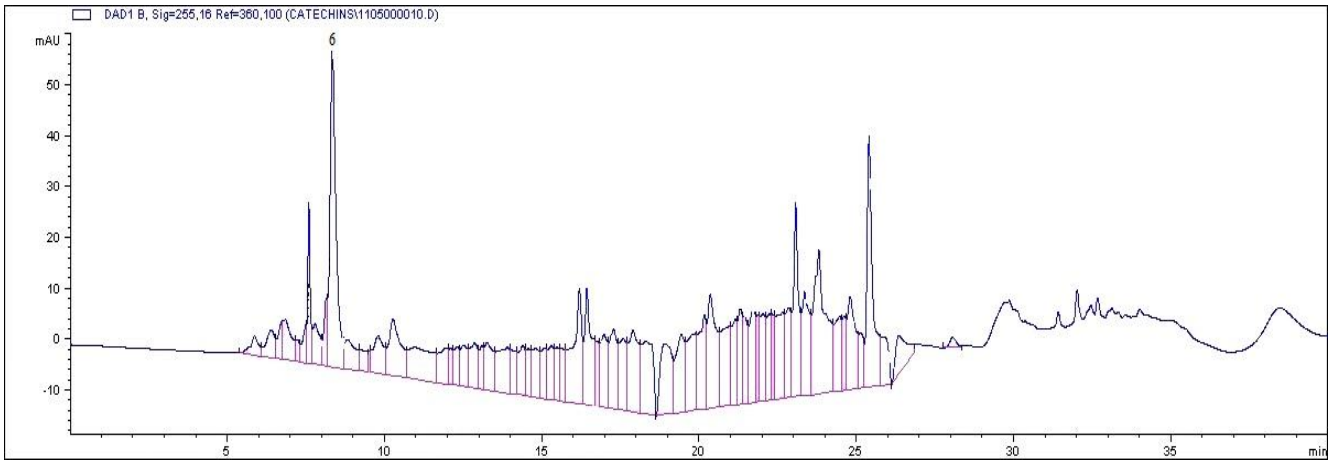

Fig 4: HPLC-chromatograms of catechins from *S. rebaudiana* leaves (wavelength 255 nm): 6- ellagic acid.

4. Conclusion

The HPLC method was used for the quantitative estimation of flavonoids, hydroxycinnamic acids and catechins in sweetleaf leaves. The HPLC assays showed a presence of significant amounts of rutin (0.14%), chlorogenic acid (0.97%) and epigallocatechin (0.72%) in the analyzed raw material. The presence of significant amount of respective bioactive components in this herb ensures its unequivocal recommendation for the use in the pharmaceutical and nutraceutical sector.

5. References

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