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Measurement of Natural and Artifical Radionucludies of *Stevia Rebaudiana* Bertoni Extract

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Abstract

Present study was carried out to measure the natural and artificial radionuclides of two different extract of *Stevia Rebandiana* Bertoni and a standard sugar solution. Factor affecting low-background Germanium Spectroscopy with HPGE Detectors, which is sensitive to all Gamma energies have been used for the measurement of natural and artifical radionuclides in *stevia* extracts and sugar solution. By analyzing two different types of *Stevia* extract samples and standard sugar solution sample, no significant presence of natural radionuclides were observed compared with background count. We also didn't find any artificial radionuclides in these three samples. So based on above observation we conclude that the leaves of *Stevia Rebaudiana* Bertoni extract is very safe to use as substitute of sugar as sweetener in foods and beverages for diabetics and obese people due to their potent sweetener stevioside and free of artifical radionuclides.

Key Words: Natural radionuclides, Artificial radionuclides, Stevia extract, Sweetener, Food additives

Introduction

Radioactive substances are harmful to normal tissues of human body because of high attenuation power. Radionuclides are consumed in trace amounts by living species through their food chain and from the environment (Young 1958) but it is necessary to maintain a maximum permissible level of radiation in food (Smith 1991) at present soft drinks are very common item in our daily food menu and there are various kinds of soft drinks in the market supplied by various companies.

The second main ingredient is sugar, whch makes up 7-12% of a soft drink (Sen *et al.* 2006). Liquid from sugar adds sweetness and body to the beverage, enhancing the 'mouth fell' an important component for consumer enjoyment of a soft drink, sugar also balances flavors and cids. A well-known low-nutritive sweetener, aspartame has been used from 1981 in food additives and in soft drink during 1983. Large amount of the radioactive-carbon lebel from oral aspartme intake have been detected in DNA (Roberts 2004). On the other hand stevia extract is widely used daily in many countries as a non-caloric sugar substitute. Its sweetening power is higher than that of sugar by approximately 70 times (Commission of the European Communities, 1999), being extensively employed as a household sweetener or added to beverages and food products (Commission of the European Communities, 1999). This sweetener contains many organic molecules, minerals, water etc. But at the same time these also contain a little amount of radioactive substances. These radioactive substances pose great hazard by emiting many daughter particles. These particles may cause damage to normal tissues of various organ by their chemical and radioactive toxicity effect (Fleisch *et al.* 1975). For this reason measurement of natural and artificial raionuclides have been done in two types of stevia extract and a standard sugar solution sample. Many research work have been carried out on this types of sweetener (Commission of the European Communities, 1999 and Maitree 1993). But radioactivity test is necessary to investigate the role of radionuclides emitter in causing various diseases, especially cancer.

Material and Method

Plant collection

The herbs of *Stevia Rebaudiana* Bertoni have been collected from BCSIR laboratories campus, Chittagong.

Extract preparation

The chlorophyll of the leaf has been removed by means of n-hexane (Pasquel *et al.* 2000). Then the leaves were dried

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again. The leaves (15g) have been extracted with rectified sprit for five times. The extract was concentrated by means of rotary evaporator under reduced pressure and low-temperature. The concentrated extract was then dried freeze dryer. The freeze dried extract was dissolved in 1000ml water.

Secondly, dried leaves (15g) were mixed with 250 ml distilled water and heated at 70-75^{O}C for half an hour then cooled and filtered. The filtrate was collected. The filtering process was carried out four times. The total volume of the filtered extract was 1000ml.

A standard sugar solution of same concentration was also made.

Radionuclide measurement

The detection and measurement of radionuclides (Keyser 1990) in the sample were carried out by Gamma-spectrometry using a 100 cm^3 intrinsic P-type coaxial HPGE detector with a relative efficiency of 20% and resolution of 1.83 Kev for the peak of 1332 Kev of ^{60}Co. The detctor was connected to an 8192- Channel computer analyzer. Efficiency calibration was carried out using standard samples (AQCS 1996) The ^{226}Ra activity was determined from Gamma-ray energy of ^{214}Pb at ^{352}Kev and ^{214}Bi at 609 Kev; ^{232}Th activity was measured from gammaray energy of ^{228}Ac at 911 and 969 Kev; ^{228}Th measured from ^{208}TI at 583.19 Kev; 40K activity at 1460 kev; and ^{137}Cs activity was measured at 661 Kev gamma-ray energy (Debertin and blemer, 1988)

Numerical Analysis of the peak

In exceptionally fine spectrometers the peaks are essentially Gaussian, (Leo 1994) throughthis depends on the settings of the amplifier and on the count rates. Obtaining really symmetric peaks is more difficult at high count rates.

For a Gaussian peak superimposed upon a continuum b_i, the count in channel I will be

$$C_i = npeak\ exp\ \{xi - \langle x \rangle^2 / 2\sigma\} + b_i \quad (1)$$

By simple counting statistics, the fractional uncertainty in the total number of counts (c_i) in a bin is simply $\sigma_{bi} = c_i^{-0.5}$ if the number of background counts (b_i) is known with relative accuracy σ_{bi} then $n_i = c_i - b_i$, the net count in channel i is known with fractional accuracy

$$\sigma_{ni} = \{c_i + (b_i \sigma_{bi})^2\}^{0.5} / (\ c_i - (b_i) \quad (2)$$

The best estimate of the mean energy, x of the peak is readily obtained from by weighing the bin contents by the reciprocals of their squared fractional error, i.e.

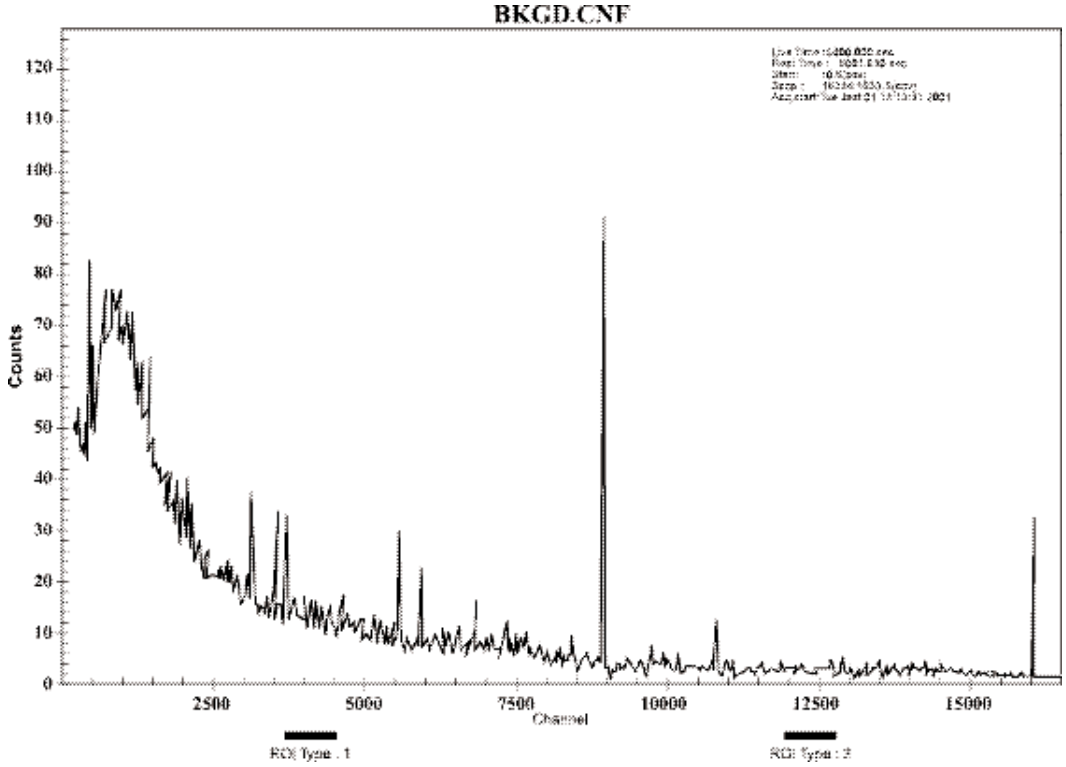

Fig. 1. Spectrometer background

$$\langle x\rangle = \Sigma_i\{x_i / \sigma_{ni}^2 \ / \ \Sigma_i\{1/\sigma_{ni}^2\} \quad (3)$$

It is sometimes difficult to estimate b_i under a peak, but luckily small errors of judgment in estimating σ_{bi} or b_i are often insignificant.

The best estimate of the Gaussian width σ is given by the root mean square of $x_i - \bar{x}$:

$$\sigma^2 = \Sigma_i\{(x_i - \langle x\rangle^2 / \sigma_i^2\}/\Sigma_i(1/\sigma_i^2) \quad (4)$$

The best estimate of the uncertainty in (x) is given by

$$\sigma_{(x)} = \sigma/\{\Sigma_i(1/\sigma_i^2\}^{0.5} \quad (5)$$

If the spectral peaks are not Gaussian, a carefull centriod analysis (equation 4) is sti ll called for, with weighing given by equation 3. The determination of widths must be based on scientific judgment as it will depend on the line shape.

Results and Discussion

Results of the present study are depicted in (fig-01) that the spectrum of background counts of HPGE detector, which describes the spectrum of energy counts without sample.

M = First peak in a multiplet region
m = Other peak in a multiplet region
F = Fitted singlet
Error quoted at 1.000 sigma

Peak analysis data of background is indicating the count rate of energy of the HPGE detector background. The values of energy and cts/sec are the determinants of natural and artificial radionuclides.

M = First peak in a multiplet region
m =n Other peak in a multiplet region
F = Fitted singlet
Error quoted at 1.00 Sigma

The background counts (Table I) is a basement of measuring artificial radionuclides. We compared others energy values (Table, II, III, IV) with counts (Table I). No energy value of artificaila radionuclides exists in background counts. We can get easily the attifical radionuclides with eye observation by comparing with that scale (Table I) based on the energy value. Peak analysis data of Alcoholic extract (Table II) indicates us as mentioned above that natural radionuclides are not significantly higher than the background counts. The peak analysis data (Table I) also indicates that there are no artifical radionuclides present in this sample.

M = First peak in a multiplet region
m = Other peak in a multiplet region
F = Fitted singlet
Error quoted at 1.00 sigma

Peak analysis data of water extract ios given below. No significant energy count rate was observed in water extract compared with alcoholic extract that indicates significant natural radionuclides and artificial radionclides are not present in this sample.

Table I. Peak analysis data for Background

Pk	Energy	Area	Cts/Sec	%err	Fit
M 1	74.26	18	3.5E-003	139.4	1.8
m 2	75.32	247	4.29E-002	20.1	1.8
M 3	84.62	221	4.4E-002	20.5	3.0
m 4	85.44	105	2.1E-002	39.2	3.0
5	239.09	211	4.2E-002	28.4	0.0
6	351.94	76	1.5E-002	49.7	0.0
7	511.06	355	7.1E-002	15.7	0.08
8	583.54	218	4.4E-002	18.5	0.0
9	609.39	189	3.8E-002	18.9	0.010
10	911.12	178	3.6E-002	18.5	0.011
11	1119969.12	101	2.0E-002	24.5	0.012
12	0.90	117	2.3E-001	24.9	0.0
13	1461.10	1093	2.2E-001	3.6	0.0
14	1592.54	3	1.0E-002	31.1	1.2
M15	1763.78	50	2.1.11.01E-002	27.1	1.2
m16	1765.54	55	2.1.1E-002	27.1	1.2
M 17	2615.61	21	4.3E-003	100.8	14.1
m 18	2616.92	724	1.4E-001	2.8	14.1

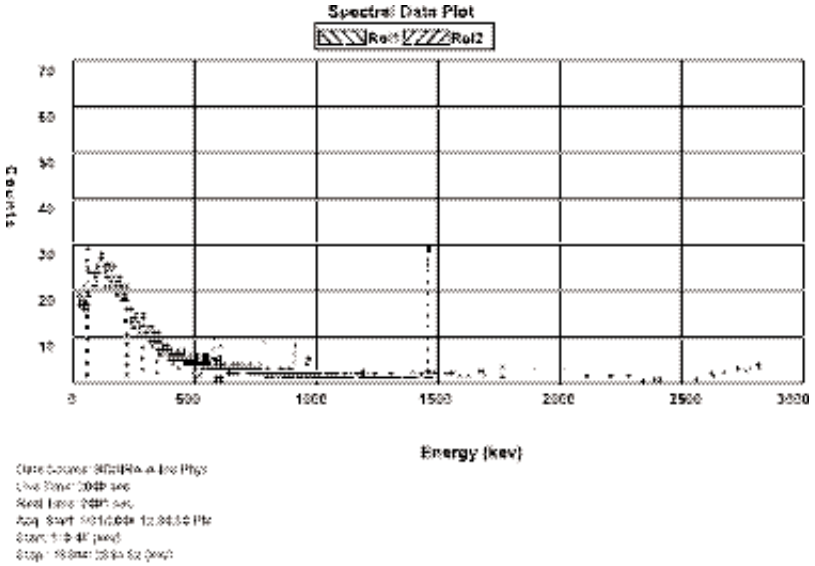

Fig. 2. Spectral data plot of Alcoholic extract of stevia

M = First peak in a multiplet region
m = Other peak in a multiplet region
F = Fitted singlet
Error quoted at 1.000 sigma

The peak analysis of standard sugar solution (table-4) given us a clear concept in same way is that there is no artificial radionuclides in sugar solution and natural radionuclides (over dose) are not significantly higher than that of Stevia rabaudiana Bertoni extract samples and the background counts of HPGE detector.

Table II. Peak analysis date for alcoholic extract of stevia

Pk		Energy (kev)	Area	Cts/Sec	%err	Fit
F	1	71.93	63	3.2 E- 002	66.5	0.2
F	2	230.38	66	3.3E- 002	53.0	0.1
F	3	294.94	72	3.6 E- 002	41.4	0.2
F	4	352.51	44	2.2 E- 002	49.9	0.2
F	5	510.71	106	5.3 E- 002	26.4	0.4
F	6	582.95	143	7.2 E- 002	13.0	0.3
F	7	609.50	93	4.6 E- 002	19.9	0.2
F	8	726.85	47	2.3 E- 002	28.8	0.52
F	9	863.27	77	3.8 E- 002	15.6	0.4
F	10	894.31	47	2.3 E- 002	14.2	0.2
F	11	911.16	101	5.0 E- 002	13.5	0.3
F	12	968.56	46	2.3E- 002	41.6	1.9AA
M	13	1043.46	33	1.6 E- 002	10.2	0.4
M	14	1046.30	37	1.9 E- 002	16.1	0.4
F	15	1460.56	437	2.2 E- 001	4.8	0.0
F	16	1764.32	35	1.7 E- 002	15.3	0.1
F	17	2615.42	122	6.1 E- 002	9.0	0.1

Table III. Peak analysis data of water extract of stevia

Pk	Energy	Area	Cts/Sec	%err	Fit
F 1	71.78	225	1.1 E- 001	23.2	0.5
F 2	81.99	94	4.7 E- 002	41.1	0.1
F 3	352.36	47	2.4 E- 002	55.9	0.1
F 4	497.55	21	1.0 E- 002	103.3	0.7
F 5	511.75	195	9.8 E- 002	18.1	0.4
F 6	583.55	81	4.1 E- 002	21.8	1.0
F 7	609.12	77	3.8 E- 002	25.9	0.4
F 8	633.91	35	1.8 E- 002	49.7	0.4
F 9	679.33	32	1.6 E- 002	35.5	0.8
F 10	714.11	101	5.0 E- 002	30.2	0.7
M 11	738.37	33	1.6 E- 002	30.2	0.7
M 12	742.80	39	2.0 E- 002	32.1	0.7
M 13	745.93	43	2.2 E- 002	20.1	0.7
14	827.00	20	9.8 E- 003	56.1	0.0
F 15	859.63	68	3.4 E- 002	15.9	0.2
F 16	910.67	120	6.0 E- 002	11.3	0.6
M 17	968.17	72	3.6 E- 002	44.5	2.3
M 18	969.98	26	1.3 E- 002	44.5	23
19	1015.41	28	1.4 E- 002	18.9	0.0
F 20	1460.58	406	2.0 E- 001	5.0	0.1
F 21	2615.49	120	6.0 E- 002	8.9	0.1

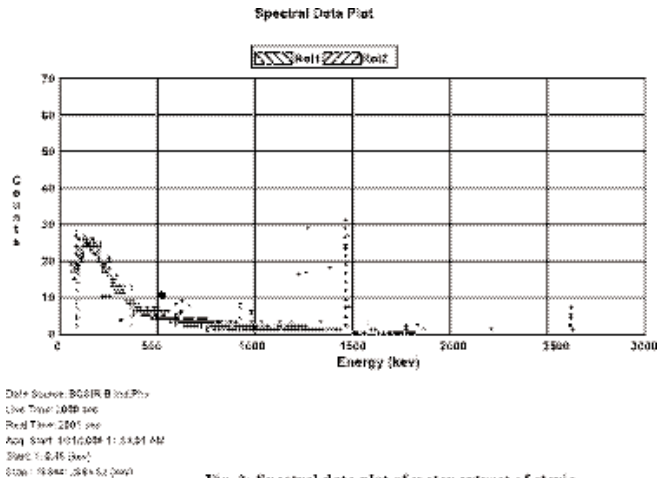

Fig. 3. Spectral data plot of water extract of stevia

Also, comparing with standard scale (Table V) of Radionuclides, the values of energy for diffferent sample data do not cross the international Commission on Radiological Protection (ICRP) limit on public exposure.

Table IV. Peak Analysis data of Sugar Solution

Nuclide	Energy (kev)	Nuclide	Energy (Kev)	Nuclide	Energy (kev)
^{73*}en (N)	53.6	^{208}TI(A)	583.16	^{232}Th (N)	238.6
^{241}Am(A)	59.53		860.56		583.1
^{57*}Fe(A)	122.1		2614.53		2614.7
^{238}U(N)	145.3	^{214}Bi(N)	609.13	^{125}Sb(A)	427.9
^{226}Ra(N)	186.2		1120.28		600.6
^{212}Pb(N)	295.2		1238.28	^{60}Co(N)	1173.23
^{214}Pb(N)	352.0		1764.49		1332.50
^{106}Ru(A)	635.9		2204.21	^{133}Ba(A)	356.01
^{137}Cs(A)	661.66	^{238}U(N)	295.2	^{40}K(N)	1460.53
^{228}Ac(N)	727.10		351.9	^{22}Na(N)	1274.53
			609	β^{+}annihilatio	511.03
			1764	n(N)	

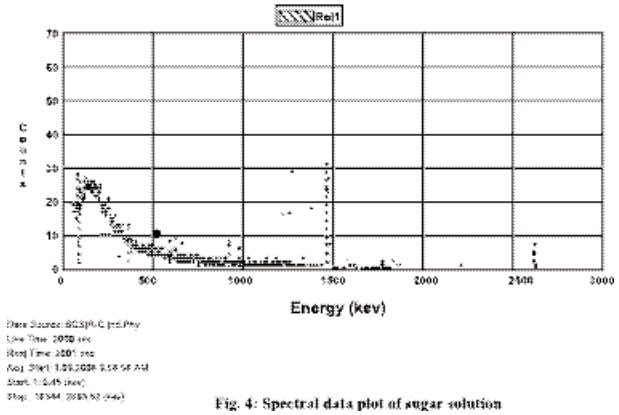

Fig. 4: Spectral data plot of sugar solution

Table V. Standard scale of radiounuclides

Pk		Energy	Area	Cts/Sec	%Err	Fit
F	1	73.05	82	4.1 E-002	41.2	0.0
F	2	239.16	44	2.2 e-002	67.9	0.0
F	3	352.38	72	3.6 e-002	38.6	0.0
F	4	406.14	11	5.6 e-003	233.2	0.0
F	5	583.33	147	7.3E-0002	26.0	0.0
F	6	583.33	86	4.3 E-0002	27.6	0.0
F	7	608.86	61	3.0 E-002	40.1	0.0
F	8	692.28	42	2.1 E-002	34.0	0.0
F	9	794.54	20	1.0 E-002	67.4	0.0
F	10	911.31	90	4.5 E- 002	19.5	0.0
M	11	933.63	1	7.1 E-004	822.7	0.0
m	12	1024.62	15	7.6 E-003	73.4	0.0
m	13	1460.81	458	2.3 E-001	4.7	0.0
	14	1764.61	38	1.9 E-002	16.2	0.0
F	15	261577	129	6.5 E-002	8.8	0.0

Conclusion

From Stevia extracts and standard sugar solution analysis it has been observed (eye observation) that there is no far difference between Stevia extracts and standard sugar solution. Both are free form harmful radionuclides. This experiment indicates that there are no significant natural radionuclides and artifical radionuclides are present in the investigated Stevia extracts. This natural sweetener is safe for the human consumptionl. The research break through will assist the Government and other agency while dealing with export Stevia products to other countries. the data may be useful guideline for researchers working on radioecology and health physics.

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References

Commission of the European Communities, Food Science and Techniques, Report of the Scientific committee for food, Stevioside as a sweetener (1999), **1**. 17 June.

Fleisch, R. L., Prece P. B. and Walker R. M. (1975) Nuclear Track in Solids: Principal and Application, University of California press, Berkelely, pp 210-215.

Jianguo Chen M. (2007) Effects of Stevioside on Pancreatic beta cell function during glucotoxicity, lipoxicity and glibenclamide secondary failure: Studies *in vitro*. Danish Medical Bulletin, NO, August **54** :230.

Keyser R., Toney T. and Wagner S. (1990) Benefits of using Super large Germanium Gamma-ray detectors for5 the quantative deternination of Environmental radionuclides, *Radioactivity and Radiochemistry.***1**(2): 46-56

Leo W. R. (1994) Techniques for Nuclear and Particle physics experiment" Springer-verlage QC-793, pp;46-246.

Maitree S., Usanee .V, Umnat M. and Duang B. (1993) Mutagenicity and human chromosomal effect of Stevioside, a Sweetener from *Stevia rebaudiana* Bertoni Environmental health Perspective supplements, **101** (suppl. 3): 53-56.

Pasquel A., Meireles M. A. A, Marques M. O. M. and Petenate A. J. (2000) Extraction of Stevia glycosides with CO_2+ water, Co_2+ ethanol and CO_2+ water + ethanol Braz. *J Chem. Eng.***17**(2):, Sa o Paulo set

Roberts H. J. (2004) Aspartame Disease: An Ignored Epidemic (Paperback). Sunshine Sentinel press Inc, pp; 201-210.

Sen J. K, Alam M. M, Kabir M. I., Asaduzzaman K. and Rafiuddin M. (2006) Mesurement of Alpha emitter's concentration in some soft drinks, *Bangladesh J. Phy.* **2**(1): 35-40

Smith H. (1991) International Commission on Radiological Protection (ICRP).Publication 60. Recommendation ICRP 1990, Oxford pregaonon press: **21**: pp; 79-89

Veros D., Srnka D., Slesinger J., Zanoucky D., Stehro J., Severijins N. and Van Geert A. (1995) Performance of HPGE detectors in Temperature range 2-77k, Nucl. *Inst & Math in Phy. Res.* **A365**: pp-419-423.

Young D. A. (1958) Etching Radiation damage in life Nature. Nature publishing group, item notes: **182:** 375-377

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