INTERNATIONAL JOURNAL OF FOOD SCIENCES and NUTRITION

http://informahealthcare.com/ijf
ISSN: 0963-7486 (print), 1465-3478 (electronic)

Int J Food Sci Nutr, 2015; 66(5): 553–558
 DOI: 10.3109/09637486.2015.1038223

informa healthcare

IN VITRO AND ANIMAL STUDIES

Study of *Stevia rebaudiana* Bertoni antioxidant activities and cellular properties

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Abstract

The aim of our study was to determine the antioxidant activities, cytotoxicity and proliferative properties in *Stevia rebaudiana* leaves and stems. Leaves extracts exhibited a higher antioxidant activity than stems extract, through oxygen radical absorbance capacity (ORAC) and cellular antioxidant activity (CAA) assays. Stevioside and rebaudioside A, the main sweetening metabolites in stevia leaves, exhibited a low ORAC value in comparison with plant extracts, while did not elicit any CAA. *Stevia rebaudiana* did not exhibit toxicity against HepG2 (hepatocellular carcinoma) human cells. No proliferative nor catalase modulations were observed in cells treated with such extracts. Our findings support the promising role of stevia that, apart from its sweetness, can act as a source of antioxidants, even at the intracellular level. This activity makes *S. rebaudiana* crude extract an interesting resource of natural sweetness with antioxidant properties which may find numerous applications in foods and nutritional supplements industries.

Keywords

Cellular antioxidant activity, ORAC, rebaudioside, stevia, stevioside, sweet herb

History

Received 17 November 2014
Revised 17 March 2015
Accepted 25 March 2015
Published online 26 May 2015

Introduction

Stevia rebaudiana Bertoni (stevia) is a native herb from South America appertaining to the sunflower family (*Asteraceae*). The Guaraní tribes from South America have been using this sweet herb since ancient times, the traditional use of stevia comprises various purposes, their leaves are known to be a natural sweetener and are also used to prepare medicinal teas with apparent therapeutic properties.

Presently, stevia is cultivated in a large-scale in several countries worldwide (Lemus-Mondaca et al., 2012), the leaf is several times sweeter than table sugar but has no calories (Goyal et al., 2010; Madan et al., 2010), it is used mainly as a natural sweetener useful to control the dietary intake of calories (Thomas & Glade, 2010).

Steviol glycosides are stevia's secondary metabolites with a sweet taste, they are diterpenes that mainly occur in leaves. Several steviol glycosides were characterized, being stevioside the most represented (4–14% w/w) followed by rebaudioside A (2–4% w/w) (Lemus-Mondaca et al., 2012; Yadav & Guleria, 2012). In addition, more than 30 less-represented steviol glycosides have been described (Wölwer-Rieck, 2012). Additionally, stevia is a good source of carbohydrates, crude fiber, protein and essential amino acids (Lemus-Mondaca et al., 2012; Wölwer-Rieck, 2012). Scientific literature shows that stevia leaves contain antioxidant compounds with diverse biochemical roles, comprising ascorbic acid (Kim et al., 2011), phenolic compounds (Shukla et al., 2009) including many flavonoids (Abou-Arab & Abu-Salem, 2010; Ghanta et al., 2007; Jahan et al., 2010; Rajbhandari & Roberts, 1983; Tadhani, 2007) and tannins (Savita et al., 2004). Antioxidant and preventive activity against DNA oxidative damage were reported *in vitro*, in extracts with crude methanolic and ethyl acetate from leaves (Ghanta et al., 2007).

Since little is known about *S. rebaudiana*'s antioxidative action, and data were so far obtained through merely chemical methods, we investigated the effects of stevia on the cellular availability and the protection against oxidation within human cells. The main purpose was the application of *in vitro* system for the evaluation of their antioxidant activity, by using different cellular screening methods, and in parallel to establish the cytotoxicity and the cellular proliferative potential, elicited by aqueous extracts from stevia.

The methods used in this study allowed a qualitative and quantitative analysis of the antioxidant potential of *S. rebaudiana* water extracts as well as its main sweetening metabolites. The efficiency of protection against peroxyl radicals assessed with a cell-based approach allowed evaluating the cellular uptake and its effectiveness under physiological conditions.

Materials and methods

Samples preparation

Dried leaves and stems samples from *S. rebaudiana*, stevioside and rebaudioside A extracts, were provided by different stevia extraction companies from Europe and are described in Table 1. Dry powders were obtained from the plant tissues by milling with a kitchen grinder. Extracts were prepared fresh by infusion of stevia powder in 100 °C distilled water, at 10 g/L final

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Table 1. Data summary of samples from *Stevia rebaudiana* and steviol glycosides.

Sample ID	Sample description	TP	SG (%)
L-DE	Dry leaves powder	17.49	10.90
L-DE-Org	Dry leaves powder from organic farming	89.49	10.93
L-SP-1	Dry leaves	195.30	13.86
L-SP-2	Dry leaves	149.06	14.30
L-SA	Dry leaves from Peru	60.19	8.41
S-SA	Dry stems powder from Peru	5.53	2.26
Rebaudioside A	Powder, purity >90% by HPLC	n.d.	97.10
Stevioside	Powder, purity >90% by HPLC	n.d.	100.65

TP = total polyphenols content expressed as mg of chlorogenic acid equivalents/g; SG = steviol glycosides content; n.d. = not determined.

concentration, allowed to stand for 10 min stirring every 5 min. Of the resulting infusions 1 mL was filtered and diluted immediately before use.

Standards and reagents

Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), quercetin dihydrate, DMSO, ethanol, methanol, potassium phosphate, potassium hydroxide, fluorescein sodium salt, 2′-7′-dichlorodihydrofluorescin diacetate (DCFH-DA), 2,2′-azobis[2-methylpropionamide] dihydrochloride (AAPH) were purchased from Sigma-Aldrich (Milan, Italy).

Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA solution 10X, trypan blue solution, glutamine–penicillin–streptomycin 10X solution were from culture grade and purchased from Sigma-Aldrich (Milan, Italy). Fetal bovine serum (FBS), Dulbecco's phosphate buffered saline (PBS) without Mg^{2+} and Ca^{2+} and Hank's balanced salts solution (HBSS) were purchased from Euroclone SpA (Milan, Italy).

All other chemicals were from analytical grade and purchased from common sources.

Preparation of stocks solutions

Phosphate buffer solution (75 mM, pH 7.4) was prepared and conserved at +4 °C. Fluorescein 2 μM stock solution was prepared in phosphate buffer and preserved at +4 °C protected from light. Trolox 10 mM stock solution was prepared in phosphate buffer. A 20 mM solution of DCFH-DA was prepared in methanol. For the CAA assay a 200 mM stock solution of AAPH was prepared in water. Quercetin dihydrate was resuspended in DMSO. Stock solutions aliquots were preserved at −20 °C and thawed once to prepare fresh working solutions immediately before use.

Cell culture

HepG2 cells from human origin were grown in DMEM high glucose medium supplemented with 10% heat inactivated FBS, 2 mM L-glutamine, 50 μg/mL penicillin and 50 μg/mL streptomycin. Cells were maintained at 37 °C under a humidified atmosphere of 5% CO_2.

Viability and cytotoxicity

Cell viability was determined at each experiment by trypan blue exclusion, within a hemocytometer.

The cytotoxicity of extracts toward HepG2 cells was assessed, by measuring the cytosolic lactate dehydrogenase activity, with the LDH cytotoxicity kit (Innoprot, Bizkaia, Spain) and according to the manufacturer's instructions. A dose of 1 mg/mL of each extract was administrated to cells up to 22 h. Untreated cells and 1% Triton X-100-treated cells served as negative and positive controls, respectively. Free LDH concentrations from two experiments were measured spectrophotometrically at 490 nm.

Cell proliferation ELISA

The antiproliferative effects of stevia extracts on cancer cells were determined by the BrdU incorporation assay (Roche Milan, Italy). Briefly, 5×10^3 HepG2 cells were plated in 96-well plates and grown overnight. The second day, 1 mg/mL of stevia extract was added in triplicate wells and cultured up to 24 h. After this incubation, a 19 h pulse of BrdU reagent was performed. The fourth day, the cell proliferation was quantified spectrophotometrically at 485 nm, based on the measurement of BrdU incorporation during DNA synthesis.

Determining antioxidant activities

ORAC assay

The chemical antioxidant capacities of stevia extracts were assessed by the oxygen radical absorbance capacity (ORAC) assay (Prior et al., 2005), with minor modifications (Bender et al., 2014). Briefly, stock samples were dissolved in phosphate buffer, and added in a 96-well black plate containing a 10 nM solution of fluorescein. The plate was incubated for 30 min at 37 °C, the background signal was determined and a 240 mM solution of AAPH was added into each well. Fluorescence measurements (Ex. 485 nm, Em. 520 nm) were taken for an hour at 37 °C (Fluostar Optima, BMG Labtech, Offenburg, Germany) and data were analyzed by MARS 2.0 software (Fluostar Optima, BMG Labtech, Offenburg, Germany). On each plate, different dilutions of Trolox (12.5–200 μM) were used as reference standard. ORAC values are expressed as mean ± standard error and as μmol of Trolox Equivalents (TEs) per gram of dry weight of two experiments.

CAA assay

HepG2 cells (6×10^4 cells/well) were seeded in a black 96-well plate with transparent bottom and incubated overnight at 37 °C and 5% CO_2, with 1 mg/mL of the respective samples diluted in complete growth medium (triplicate wells). Intracellular oxidation was estimated by using the CAA method (Wolfe & Liu, 2007) with minor modifications, as reported elsewhere (Bender et al., 2014). The raw data were analyzed with MARS 2.0 Optima Data Analysis software (Fluostar Optima, BMG Labtech, Offenburg, Germany). The integrated area under the fluorescence curve (AUC) was calculated from three experiments, for each sample, standards and controls (untreated cells) and corrected with blanks AUC. The CAA units of each sample were calculated according to the following formula: AUC unit = $100 - (AUC_{sample}/AUC_{control}) * 100$.

To associate ORAC values with CAA, the Quercetin equivalents (QEs) were calculated based on the reference standard curves and CAA values were expressed as μmol of QE per gram on dried basis.

Analysis of polyphenols

The stevia extracts were analyzed by UHPLC-UV. Equipment and experimental conditions are described elsewhere (Feuereisen et al., 2014) with the following modifications: the gradient started with 2% B and raised linearly to 36.3% within 20 min, then to 100% B within 1 min and holding for 2 min as a washing step; back to 2% B within 2 min and equilibrating for 2 min. Eluent A was water with 0.1% formic acid, eluent B was acetonitrile with 0.1% formic acid, the flow was 0.4 mL/min on a BEH Shield

RP18 column (150 mm × 2.1 mm, 1.7 μm) by Waters (Milford, MA) at 40 °C.

The peaks were identified by MS/MS, i.e. the hydroxybenzoic acid derivative acids according to Clifford et al. (2003, 2005) and Karaköse et al. (2011) and the flavonoids according to Fabre et al. (2001). The compounds that could be identified and had a visible UV signal were quantified using 5-*O*-caffeoyl quinic acid at 320 nm.

Steviol glycosides quantification

Steviol glycosides were quantified by HPLC as published elsewhere (Morlock et al., 2014; Zimmermann et al., 2011). Briefly, steviol glycosides were analyzed by HPLC-UV using a Hilic column (Nucleodur Hilic, 125 mm × 2.1 mm, 3 μm particle size; Macherey-Nagel, Düren, Germany) with a gradient elution and acetonitrile and water as eluents. The peaks were detected at 200 nm and quantified by external calibration with rebaudioside A as reference.

Catalase activity assay

HepG2 cells were treated up to 28 h, in triplicate wells and with 1 mg/mL of stevia extracts. The catalase activity was measured spectrophotometrically at 550 nm (Fluostar Optima, BMG Labtech, Offenburg, Germany) with the CAT assay kit (Innoprot, Bizkaia, Spain) according to the manufacturer's instructions and compared with untreated cells.

Total glutathione assay

HepG2 cells were cultured up to 48 h in the presence or absence of stevia extracts (100 μg/mL). After treatment, cell lysis was achieved and the total glutathione (GSH + GSSG) level was measured spectrophotometrically at 412 nm (Fluostar Optima, BMG Labtech, Offenburg, Germany) by means of the Total Glutathione assay kit (Innoprot, Bizkaia, Spain) following the producer's instructions. Results were compared with untreated cells and calculated per μM.

NAD/NADH assay

The NAD:NADH ratio was measured spectrophotometrically at 490 nm (Fluostar Optima, BMG Labtech, Offenburg, Germany) with the NAD/NADH assay kit (Innoprot, Bizkaia, Spain). Briefly, human hepatocytes were treated in triplicate wells for 24 h and with 1 mg/mL of stevia extracts. After incubation, cells were handled according to the producer's recommendations and the results were compared with untreated cells.

Statistical analysis

Statistical data analyses were aided by Daniel's XL Toolbox add-in for the Microsoft Excel (by Daniel Kraus, Würzburg, Germany). For the statistical evaluation between means, the ANOVA test followed by multiple comparisons by Bonferroni–Holm's test was applied. Probability values of significance were considered below $p < 0.05$.

Standard deviations were calculated for each test. The data are represented as the mean ± standard error of at least two experiments. Pearson's test was used to assess calibration curves linearity and correlation among different parameters.

Results

Cell viability and cytotoxicity

The cellular viability was estimated in confluent plates by trypan blue exclusion and resulted greater than 95% before each experiment.

Table 2. HepG2 cellular analysis after treatment with stevia extracts.

Sample ID	% Cytotoxicity	BrdU in corporation	Catalase	CAA unit
L-DE	9.91 ± 2.40	0.85 ± 0.04	1.08^{ns} ± 0.15	62.3*
L-DE-Org	2.27 ± 0.67	0.85 ± 0.06	1.08^{ns} ± 0.08	193.6*
L-SP-1	9.80 ± 0.12	1.89 ± 0.05	1.05^{ns} ± 0.06	155.6*
L-SP-2	3.79 ± 1.05	1.42 ± 0.06	1.10^{ns} ± 0.10	269.0*
L-SA	2.64 ± 0.21	0.44 ± 0.01	1.18^{ns} ± 0.10	187.2*
S-SA	6.99 ± 0.29	0.76 ± 0.05	0.77^{ns} ± 0.10	33.8*

Mean data are normalized against control (untreated) cells. ns: not significant.
*$p < 0.001$ versus control.

Cellular toxicity of stevia extracts was evaluated by LDH activity, the assay is based on the release of lactate dehydrogenase into the culture medium as a result of cellular membrane damage, upon treatment with toxic concentrations of a sample substance. Stevia extracts at 1 mg/mL concentration showed slight to no cytotoxic effect after 22 h of treatment (Table 2). Hence, no-cytotoxic concentrations of the samples were used in the cell-based assays.

LDH activity was also evaluated in HepG2 cells treated with stevioside and rebaudioside A purified extracts, no toxicity was observed at the tested concentrations (0.5, 1 and 2 mg/mL).

Cellular proliferation

To estimate the sensitivity of cancer cells to stevia extracts a proliferation assay was assessed. To this end, HepG2 cells were exposed to 43 h incubation with 1 mg/mL of stevia extracts, a pulse with BrdU were performed during the last 19 h of treatment. Absorbance readings indicated very little to no proliferative effect ($p > 0.05$) against carcinoma hepatocytes from human origin (Table 2).

Antioxidant activities

To test the ability of the extracts to scavenge free radicals a combination of H-ORAC and a cell-based method was used. The highest ORAC values were observed in extracts from dried leaves (Figure 1), ranging from 958.8 to 1071.1 μmol TE/g, except for sample L-DE that together with the stem sample S-SA showed the lowest ORAC values (278 and 215.7 μmol TE/g, respectively).

The CAA assay was performed to correlate the chemical antioxidant effect of stevia extracts with respect to their biological antioxidative capability, in a biological model characterized by HepG2 human hepatocytes. Stevia samples exhibited inhibition of AAPH-induced fluorescence indicating, even if at different rates, that the extracts were absorbed into cells. As illustrated in Figure 2, stevia extracts also demonstrated a remarkable capacity in scavenging peroxyl radicals at the intracellular level. The CAA values, reported as μmol QE/g, range from 61.3 to 35.6, being much lower in leaves sample L-DE and in stems sample S-SA (14.5 and 8 μmol QE/g, respectively).

Under our experimental conditions, the association between ORAC and CAA assays resulted positively correlated by means of regression analysis ($R^2 = 0.84$, $p < 0.01$).

Data from a previous study showed that green tea, a well-known antioxidant, has an ORAC value of 2412 μmol TE/g (Bender et al., 2014), thus 2.2–11.2-fold higher than the *S. rebaudiana*'s crude extracts. However, when assessed in the same cell model, the CAA value of green tea is in line

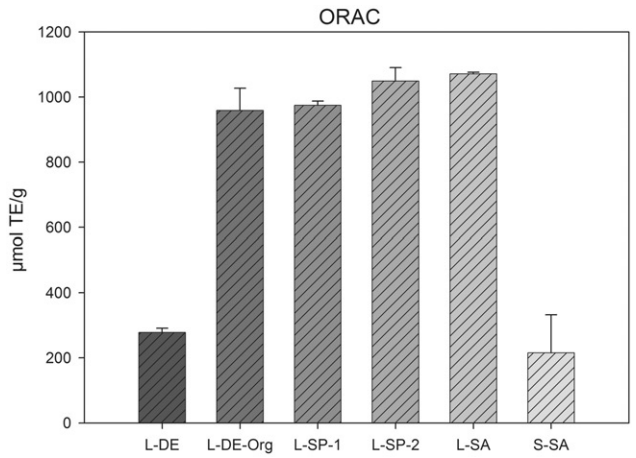

Figure 1. Antioxidant capacity of stevia extracts as evaluated by the ORAC assay. Data are expressed as mean ± SD of two experiments.

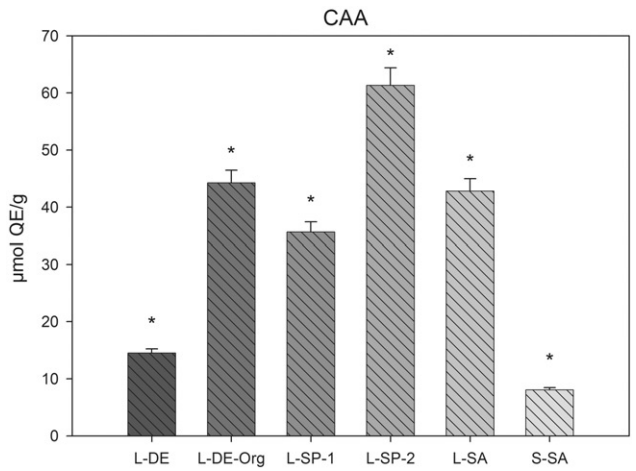

Figure 2. Intracellular antioxidant capacity of stevia extracts as evaluated by the CAA assay. Significant cellular antioxidant activity after 20 h treatment with stevia extracts, as evaluated by the CAA assay. Data are expressed as mean ± SD of three experiments. *$p < 0.001$ versus untreated cells.

Table 3. Phenolic compounds detected by HPLC-UV and expressed as concentration in the leaf in mg chlorogenic acid equivalents per gram.

	L-DE	L-DE-org	L-SP-1	L-SP-2	L-SA	S-SA
3-*O*-Caffeoyl quinic acid	0.445	2.116	5.426	4.576	1.431	0.101
5-*O*-Caffeoyl quinic acid	0.198	1.765	3.148	1.912	1.156	0.090
4-*O*-Caffeoyl quinic acid	4.807	21.217	41.562	36.185	6.547	1.039
5-*O*-Coumaroyl quinic acid	0.045	0.074	0.089	0.081	0.055	0.024
Caffeoyl shikimic acid	0.109	0.270	0.500	0.760	0.161	0.040
3,4-Di-*O*-caffeoyl quinic acid	0.344	2.245	6.182	3.578	2.145	0.224
3,5-Di-*O*-caffeoyl quinic acid	10.040	42.897	97.994	80.321	29.934	2.648
4,5-Di-*O*-caffeoyl quinic acid	1.509	18.910	40.399	21.648	18.769	1.367

(38.6 ± 17.2 µmol QE/g; $n = 5$) with the stevia values on weight basis. This, in accordance with some further data, indicates that a high ORAC value does not always guarantee a high CAA value and *vice versa*.

Stevioside and rebaudioside A purified extracts, the main sweetening molecules in *S. rebaudiana*, were also used to prove its antioxidant activity by means of both ORAC and CAA assays. Results showed low antioxidant activity measured by ORAC, being 23.8 and 22.8 µmol TE/g for stevioside and rebaudioside A, respectively. Nevertheless, by the CAA assay it was found that no intracellular antioxidant activity is elicited in HepG2 cells by these purified metabolites (data not shown).

Polyphenolic composition

In the stevia leaf extracts analyzed here, mainly hydroxybenzoic acid esters were found. The flavonoids described by others (as summarized by Wölwer-Rieck (2012)) could be found only in trace amounts, i.e. they were detectable by MS but not by UV. This can be explained by the polar extraction used here, while flavonoids are more efficiently extracted by organic solvents. Several compounds were found in quantifiable amounts among the hydroxybenzoic acid derivatives (Table 3); conversely other compounds were found in traces as said for the flavonoids. The predominant compounds were 4-*O*-caffeoyl quinic acid, 3,5-di-*O*-caffeoyl quinic acid and 4,5-di-*O*-caffeoyl quinic acid. These results are in agreement with published data (Karaköse et al., 2011).

Steviol glycosides

Amounts of steviol glycosides are reported in Table 4. The total quantity ranged from 8.4% to 14.3% for leaves, being much lower in the stem's sample (2.3%). As expected, the most abundant steviol glycosides present in dried leaves were stevioside (5.5–7.5 g/100 g) and rebaudioside A (2–4.7 g/100 g). Conversely, the stems extract showed a low stevioside content (0.8 g/100 g), being dulcoside A the main steviol glycoside observed (1.2 g/100 g).

Catalase activity assay

To investigate whether the stevia extracts could modulate the antioxidant enzyme production, the catalase activity was evaluated in HepG2 cells after 28 h of treatment (Table 2). Results revealed that the catalase activities in cells treated with stevia are not significantly modulated when compared to untreated cells ($p > 0.05$)

Total glutathione and NAD:NADH ratio

To assess if stevia may act as a GSH protector, thus preventing the depletion of endogenous GSH, total glutathione content was measured after a 48 h of treatment with 100 µg/mL of stevia extracts. We observed slightly high levels of GSH in five out of six samples. The cytosolic NAD:NADH ratio was calculated in a colorimetric assay where HepG2 cells were treated for 24 h with 1 mg/mL of stevia extracts. The large variability in the measurements due to the small number of samples does not allow assigning the statistical significance of the observed results when compared to control cells.

Conclusion

The antioxidant nature of *S. rebaudiana* was studied in water extracts from leaves and stems. The protection against peroxyl radical formation, assessed by ORAC, was positively correlated ($R^2 = 0.84$, $p < 0.01$) with the intracellular antioxidant activity,

Table 4. Steviol glycosides contents detected by UHPLC.

g/100 g dry weight	L-DE	L-DE-Org	L-SP-1	L-SP-2	L-SA	S-SA	Stevioside	Rebaudioside A
Rubusoside	<0.01	<0.01	1.12	1.80	<0.01	<0.01	0.59	<0.01
Steviolbioside	<0.01	<0.01	<0.01	0.80	<0.01	<0.01	3.13	<0.01
Dulcoside A	<0.01	0.22	<0.01	<0.01	<0.01	1.20	<0.01	<0.01
Reb B	<0.01	0.06	<0.01	<0.01	<0.01	<0.01	<0.01	3.07
Stevioside	7.10	7.54	7.29	6.90	5.46	0.76	90.21	2.73
Reb C	0.58	1.07	0.64	0.40	0.22	<0.01	<0.01	0.36
Reb F	0.21	<0.01	1.37	<0.01	<0.01	<0.01	0.71	<0.01
Reb A	2.90	2.04	3.27	4.70	2.35	0.27	5.89	90.61
Reb E	0.05	<0.01	0.08	<0.01	0.24	0.02	0.11	<0.01
Reb D	0.06	n.d	0.09	<0.01	0.14	0.01	<0.01	0.33
Steviol glycosides	10.90	10.93	13.86	14.30	8.41	2.26	100.65	97.10

Reb = rebaudioside; n.d. = not determined.

assessed by the CAA assay. The efficacy of the extracts from the leaves was higher than that of stems, while the capacity of stevioside and rebaudioside A, the most abundant sweeteners found in stevia, was much lower through ORAC (ranging from 2.1–10.7%). Furthermore, these data confirm the previous findings from Hajihashemi group (Hajihashemi & Geuns, 2013), who also found a better ROS scavenging capacity for crude extracts.

No antioxidant activity was registered by the CAA, this result suggests that steviol glycosides can hardly be absorbed by liver cells *in vitro*, therefore, any antioxidant property may not exert at the intracellular level. Indeed, it was previously reported that stevioside and rebaudioside A extracts undergo hydrolysis to the aglycone steviol (Gardana et al., 2003; Wheeler et al., 2008), a metabolism that takes place in the intestine by human microflora before the absorption occurs. Our result, obtained with the HepG2 cell model, supports previous evidence, indicating that the hepatocytes are not able to metabolize the stevioside and rebaudioside A purified extracts and thus no significant absorption can occur.

The total polyphenol content was not significantly associated to CAA and ORAC values. In agreement with our results, some further data obtained with different vegetables indicate that there is no correlation between the total phenolic content and the antioxidant capacity (Eberhardt et al., 2005). However, sometimes a positive correlation was found (Wolfe et al., 2008).

Although not surprising, our results indicate that there is no significant association between steviol glycoside content and ORAC and CAA values. Additionally, we observed that water extracts of stevia leaves and stems did not show significant differences in antioxidant enzyme activities when assayed in terms of catalase activity. Similarly, it was reported elsewhere (Vaško et al., 2014) that after the treatment of liver mitochondria with stevia extracts no significant effect was observed in the superoxide dismutase activity, another key antioxidant enzyme. Thus, the mechanisms of action of stevia's antioxidant capacity seem to be through its ability to directly reduce oxidizing free radicals, rather than a potential to modulate endogenous enzymatic antioxidant systems.

Evidences already reported indicate that *S. rebaudiana* extracts show high contents of total phenolic and flavonoids (Abou-Arab & Abu-Salem, 2010; Ghanta et al., 2007; Jahan et al., 2010; Kim et al., 2011; Rajbhandari & Roberts, 1983; Shukla et al., 2009; Tadhani et al., 2007). The results here obtained suggest that the high antioxidant activities observed in water extracts from stevia leaves were due to the presence of antioxidant agents that may contribute directly in the cellular defense by eliciting a protective role.

The aqueous extracts from stevia leaves and stem, at 1 mg/mL concentration, did not impart a significant proliferative effect on cancer cells, assessed by the BrdU incorporation assay.

Overall, our findings indicate that besides having sweetening properties, the crude aqueous extracts from *S. rebaudiana* leaves can exert a cellular scavenging activity against free radicals, and thus the potential antioxidant role of *S. rebaudiana* certainly merits further consideration. Due to their antioxidant activities, stevia leaves or crude extracts thereof might be considered not only as natural sweeteners but also resources for food preservation.

Acknowledgements

The authors express their thanks to Dr. Nadja Fitze (Institut Prof. Dr. Georg Kurz, GmbH, Köln, Germany) for kindly providing the plant material.

Declaration of interest

The authors declare no competing financial interests. This article does not contain any studies with human or animal subjects.

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