Diocléa Almeida Seabra Silva
(Organizadora)

# Agronomia: Elo da Cadeia Produtiva 6

Atena Editora
Ano 2019

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Editora Chefe: Profª Drª Antonella Carvalho de Oliveira
Diagramação: Natália Sandrini
Edição de Arte: Lorena Prestes
Revisão: Os Autores





Atena Editora
Ano 2019

**Dados Internacionais de Catalogação na Publicação (CIP)**
**(eDOC BRASIL, Belo Horizonte/MG)**

A281 Agronomia [recurso eletrônico] : elo da cadeia produtiva 6 / Organizadora Diocléa Almeida Seabra Silva. – Ponta Grossa, PR: Atena Editora, 2019. – (Agronomia: Elo da Cadeia Produtiva; v. 6)

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-85-7247-825-0
DOI 10.22533/at.ed.250190312

1. Agricultura – Economia – Brasil. 2. Agronomia – Pesquisa – Brasil. I. Silva, Diocléa Almeida Seabra. II. Série.

CDD 630.981

**Elaborado por Maurício Amormino Júnior – CRB6/2422**

Atena Editora
Ponta Grossa – Paraná - Brasil
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Atena Editora
Ano 2019

# APRESENTAÇÃO

A cadeia produtiva é um termo amplo que define com clareza onde cada segmento tem seu grau de importância seja na produtividade de frutos, venda de semente de capineira, na pesca, na aquicultura, na formação de resíduos para a indústria, no controle determinado de vírus, bactérias, nematóides para a agricultura e até mesmo na comercialização de espécies florestais com potencial madeireiro. Na verdade, o termo cadeia produtiva é um conjunto de ações ou processos que fazem presente em estudos científicos que irá dar imagem para o avanço de um produto final.

A imagem de um produto final se torna possível quando trabalhamos todos os elos da cadeia, como por exemplo: para um produtor chegar a comercializar o feijão, ele precisará antes preparar seu solo, ter maquinários pra isso, além de corririr o solo com corretivo, definindo a saturação de base ideal, plantar a semente de boa qualidade, adubar, acompanhar a produção fazendo os tratos culturais adequados, controlando pragas, doenças e ervas daninhas, além de encontrar mercados para que o mesmo possa vender sua produção. Esses elos são essenciais em todas as áreas, ao passo que na produção de madeira será necessário técnicas sofisticadas de manejo que começa na germinação de sementes, quebra de dormência para a formação de mudas, e além disso padronizar espaçamento, tratos silviculturais para a formação de madeira em tora para exportação.

Na pesca a cadeia produtiva segue a vertente do ganho de peso e da qualidade da carne do pescado, que está vinculada a temperatura, pH da água, oxigenação, alimentação e o ambiente para que haja produção. Também a cadeia se verticaliza na agregação de preço ao subproduto do pescado como o filetamento para as indústrias, mercado de peixe vivo e etc.

Na cadeia cujo foco são os resíduos da indústria açucareira, há mercados para a queima de combustível no maquinário da indústria, através da qualidade deste resíduo, além de mercados promissores para a fabricação de combustíveis, rações e até mesmo resíduo vegetal para incorporação nos solos, com a finalidade de manter ou melhorar as características químicas, físicas e biológicas, além de controlar erosão e elevar os níveis de produtividade nas áreas agrícolas, através da adição de nutrientes.

Contudo, sabemos que todos os elos que compõem a cadeia produtiva são responsáveis por agregar valor e gerar de maneira direta e indireta renda aos produtores e pescadores, possibilitando-os na melhoria da qualidade de vida, além da obtenção de produtos de alta qualidade. No entanto, aqui se faz presente a importância das pesquisas mostradas neste E-Book, v. 6 – Agronomia: Elo da Cadeia Produtiva para que o leitor possa perceber novidades que são contextualizadas, através dos trabalhos aqui publicados.

Diocléa Almeida Seabra Silva

## SUMÁRIO

# CAPÍTULO 10

# NOVA VARIEDADE SEMINAL DE *STEVIA REBAUDIANA*: OBTENÇÃO DE FRAÇÕES COM ALTO POTENCIAL ANTIOXIDANTE DE FOLHAS

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**RESUMO:** O objetivo deste estudo foi determinar a composição e o potencial antioxidante de folhas de uma nova variedade de *Stevia rebaudiana* (Stevia UEM-13). Folhas de estévia UEM-13 contêm rebaudiosídeo A como o glicosídeo principal, enquanto que a maioria das plantas de estévia selvagens contêm o esteviosídeo. Além disso pode ser multiplicado por semente, o que reduz o custo de técnicas de cultura de plantas como outras variedades clonais são multiplicados por botões, que exigem sistemas de produção de plântulas sofisticados e dispendiosos. O etanol e o metanol foram usados na extração para determinar os compostos bioativos. O extrato metanólico foi fracionado sequencialmente com hexano, clorofórmio, acetato de etilo e isobutanol, e a concentração mais elevada de compostos fenólicos e flavonoides foi obtido na fracção acetato de etilo (524,20 mg de ácido gálico equivalente / g; 380,62 µg quercetina equivalente / g). O conteúdo de glicosídeos variou bastante entre as frações (0,5% - 65,3%). O maior potencial antioxidante foi encontrado no extrato metanólico e a fração de acetato de etila com 93,5% e 97,32%, respectivamente. Além de ser uma excelente fonte para a obtenção de extratos ricos em glicosídeos, esta nova variedade pode também ser usada como matéria-prima para a produção de extratos ou frações com uma quantidade significativa de

atividade antioxidante e potencial para serem utilizados como aditivos em alimentos.
**Palavras-chave:** Compostos bioativos, glicosídeos, extratos de folhas, compostos fenólicos, nova cultura de célula.

## NEW SEMINAL VARIETY OF *STEVIA REBAUDIANA*: OBTAINING FRACTIONS WITH HIGH ANTIOXIDANT POTENTIAL OF LEAVES

**ABSTRACT:** The aim of this study was to determine the composition and antioxidant potential of leaves of a new variety of *Stevia rebaudiana* (Stevia UEM-13). Stevia leaves of UEM-13 contain rebaudioside A as the main glycoside, while most wild Stevia plants contain stevioside. Furthermore, can be multiplied by seed, which reduces the cost of plant culture techniques as other clonal varieties are multiplied by buds, requiring sophisticated and expensive seedling production systems. Ethanol and methanol were used in the extraction to determine the bioactive compounds. The methanolic extract was fractionated sequentially with hexane, chloroform, ethyl acetate and isobutanol, and the highest concentration of phenolic compounds and flavonoids was obtained in the ethyl acetate fraction (524.20 mg galic acid equivalent/g; 380.62 $\mu$g quercetin equivalent/g). The glycoside content varied greatly among the fractions (0.5% - 65.3%). Higher antioxidant potential was found in the methanol extract and the ethyl acetate fraction with 93.5% and 97.32%, respectively. In addition to being an excellent source for obtaining of extracts rich in glycoside, this new variety can also be used as raw material for the production of extracts or fractions with a significant amount of antioxidant activity and potential to be used as additives in food.
**KEYWORDS:** Bioactive compounds, glycosides, leaf extracts, phenolic compounds, new cultivar of Stevia.

## 1 I INTRODUCTION

The chapter in question was previously published as an article in the journal Anais da Academia Brasileira de Ciências, volume 89 (3), no ano de 2017 (doi 10.1590/0001-3765201720170174). Plants are an important source of structurally different active natural products and biological properties. These active ingredients can play adjuvant and important roles in traditional medicine in many countries (Tadhani *et al.*, 2007). The beneficial effects are produced by different active ingredients and other compounds of low molecular weight such as vitamins, carotenoids, flavonoids, anthocyanins, tannins and other phenolic compounds (Tadhani *et al.*, 2007; WöLwer-Rieck, 2012), which have different mechanisms of action. It is known that phenolic compounds and flavonoids are important for the normal development and protection of plants (WöLwer-Rieck, 2012), but their high antioxidant activity as food additives for humans must still be further investigated.

*Stevia rebaudiana* Bertoni, a plant from the Asteraceae family, is native to

Paraguay and it is cultivated in many countries, including Brazil, Argentina, Japan, China, the United States and European countries (Dacome *et al.*, 2005). It is important in the food industry because the leaves contain sweet-tasting diterpene glycosides, among which stevioside and rebaudioside A (RebA) are included (Carakostas *et al.*, 2008). These glycosides are 450 times sweeter than sucrose (Williams e Burdock, 2009), which has led to commercial interest, mainly for food and beverages. The Stevia plants have more than 100 compounds (WöLwer-Rieck, 2012) and extracts from the leaves have been traditionally used in the treatment of diseases such as diabetes (Tadhani *et al.*, 2007; Shukla, S. *et al.*, 2009). Stevia extracts can exert beneficial effects on human health, including anti-hypertension (Chan *et al.*, 2000) and anti-hyperglycemic effects (Abudula *et al.*, 2008), antiviral activities against the human rotavirus, it is not cariogenic and has beneficial properties for glucose metabolism and renal function (Shukla, Shruti *et al.*, 2009). The beneficial effects of these compounds on human health have been previously studied. Research aimed at obtaining Stevia extracts containing significant levels of sweeteners and these compounds (Kaushik *et al.*, 2010; Gaweł-Bęben *et al.*, 2015). The results showed that there is still no consensus on the ideal method of obtaining both compounds (sweeteners and bioactive compounds) in significant concentrations.

The composition of Stevia leaf extracts and the concentration of these bioactive compounds vary according to the solvents used and the different types of extraction methods. Significant results were found in the ethanol and methanol extracts and ethyl acetate solvents (Shukla, Shruti *et al.*, 2009; WöLwer-Rieck, 2012). However, further studies should be conducted on different varieties of plants and improved extraction methods need to be developed for better results. In addition, more studies should be conducted in order to obtain the best method and the best fractions of the bioactive compounds from stevia are present in higher concentrations. The Center for Studies in Natural Products (NEPRON), State University of Maringá (UEM) started in 1990 studies in order to obtain sweeteners or stevia extracts with high levels of RebA, sweetener better sensory profile that stevioside. The first projects ("Biotechnological Applications of *Stevia rebaudiana* Products (Bert.) Bertoni") resulted in the development of a selection of stevia plants with high content of this glycoside. The research resulted in obtaining two varieties M1 Alvarez and Stevia UEM-320, the latter forward three times as RebA compared with a variety of wild-type plant, and therefore the majority glycoside in the plant selected (Alvarez e Couto, 1984).

The cultivation of these clonal varieties, made by cutting method, presented some disadvantages over the years, as difficulties and rooting problems, increased susceptibility to environmental variations and attack predators, and need maintenance ideal conditions for plant growth. Currently the researchers NEPRON are developing projects in order to obtain varieties of stevia third generation that can be reproduced and grown for seed minimizing costs, increasing the viability of

farming, reproducibility and marketing, and decreasing the mortality of plants. Thus, this study was developed from a new seminal variety of Stevia rebaudiana called Stevia UEM-13.

The aim of this study was to present the glycoside composition, bioactive compounds and antioxidant potential of leaves from a new variety of *Stevia rebaudiana* (Stevia UEM-13). Was evaluated the concentration of these bioactives in different extracts and fractions produced by different extraction methods, and also identified in which these fractions have greater functional potential for use in food, pharmaceutical and cosmetic products.

## 2 I EXPERIMENTAL SECTION

### 2.1 Experimental material

The plants of *Stevia rebaudiana* of the seminal variety UEM-13 grown at NEPRON (UEM) were collected at maximum vegetative growth stage. These shrubs were previously dried in an oven at 60°C and the leaves were subsequently separated from the stems and branches, placed in polyethylene bags, and stored at -18°C before developing different extracts. The chemical reagents were purchased from Sigma-Aldrich.

### 2.2 Aqueous extract from the stevia leaves UEM-13

A 2.0 g sample of previously ground Stevia leaves UEM-13 were transferred to a 250 ml beaker; 100 mL of deionized distilled water was added and boiled for five minutes. After this procedure, the material was decanted and filtered under vacuum. The procedure was repeated twice with 100 ml and 50 ml of water, respectively. After the last filtration, the extract was transferred to a 250 ml volumetric balloon and filled up with distilled water. We used this extract to analyze glycosides, phenolic compounds and flavonoids.

#### *2.2.1 Ethanol extract of leaves by maceration (EELM)*

The extraction system was established using 500 g of previously ground *Stevia rebaudiana* leaves of the seminal variety UEM-13, placed in a 2.0 L glass beaker and 1.5 L of P.A absolute ethanol (99.5%) was added. The system stood for 24 hours in the dark at room temperature, to obtain the first fraction. The same procedure was repeated to obtain 7 fractions (extraction until exhaustion). The extracts were combined and dried in a rotary evaporator (Buchi) at 50 °C under vacuum. The powder obtained was used to evaluate the glycosides, phenolic compounds, total flavonoids and antioxidant activity.

### 2.2.2 Ethanol extract of the leaves by Soxhlet (EELS)

The previously dried and ground *Stevia rebaudiana* leaves (100 g) of the seminal variety UEM-13 were placed in 500 ml of absolute ethanol (99.5%) and added to the system using the Soxhlet apparatus. The extract was filtered and dried in a rotary evaporator (Buchi) at 50°C under vacuum and the dry powder was analyzed.

### 2.2.3 Methanol extract of the leaves by Soxhlet (MELS)

The previously dried and ground *Stevia rebaudiana* leaves (100 g) of the seminal variety UEM-13 were placed in 500 ml of P.A methanol and added to the system using the Soxhlet apparatus (extraction until exhaustion). The extract was filtered and dried in a rotary evaporator (Buchi) at 50 °C under vacuum and the powder was analyzed. Extract fractionated with different solvents. The fractionation was performed as follows, as shown in figure 1.

### 2.2.4 Hexane fraction (HF)

A 100 g sample of dried *Stevia rebaudiana* leaves of the seminal variety UEM-13 were extracted with 400 ml of methanol were packaged in apparatus Soxhlet for 4 hours. The extraction was repeated to obtain a colorless methanolic extract (extraction until exhaustion). The extracts were combined and dried in a rotary evaporator (Büchi) at 50 °C. A sample of 35.8 g of dried methanolic extract in a rotary evaporator (Büchi) was dissolved with distilled and deionized water (400 mL). The aqueous solution was extracted with 1000 mL of hexane and the mixture was separated in a separation funnel. The process was repeated. The aqueous and hexane fractions were obtained. The hexane fractions were pooled and dried in a rotary evaporator (Büchi) at 50 °C under vacuum and the fluid (HF) was analyzed.

### 2.2.5 Chloroform fraction (CF)

The resulting aqueous fraction from the hexane extraction was extracted with 1000 mL of chloroform in duplicate. The mixture was separated in a separation funnel and the aqueous and chloroform fractions were obtained. The chloroform fraction was dried in a rotary evaporator (Buchi) at 50 °C under vacuum. The resulting powder (CF) was analyzed.

### 2.2.6 Ethyl acetate fraction (EF)

The resulting aqueous fraction from the chloroform extraction was extracted with 1000 mL of ethyl acetate in duplicate. The mixture was separated in a separation funnel and the ethyl acetate (organic) fraction obtained was dried in a rotary evaporator

(Buchi) at 50 °C under vacuum. The powder (EF) was analyzed.

#### *2.2.7 Isobutanol fraction (IF)*

The resulting aqueous fraction from the ethyl acetate extract was extracted with 1000 mL of isobutanol in duplicate and the fractions were separated in a separation funnel. The fraction with isobutanol dried using rotary evaporator (Buchi) at 50 °C under vacuum. The resulting powder (IF) was analyzed.

#### *2.2.8 Aqueous fraction (AF)*

The quantity of the fraction referred to as IF (isobutanol) was reduced in a rotary evaporator and then dried on a bench-top spray dryer (Spray Dryer, Buchi) and the resulting powder (aqueous fraction - AF) was analyzed.

### 2.3 Analysis

#### *2.3.1 2Glycosides*

The glycosides compounds of leaves, extracts and fractions were assayed according to the method described by Dacome *et al*. (2005).

#### *2.3.2 Total phenolic compounds*

The phenolic compounds of leaves, extracts and fractions were assayed according to the method described by Singleton *et al.* (1999) (with modifications). A solution of 1 mg/mL of aqueous extract of the leaves was prepared to analyze the leaves. For the extract analysis, a solution of absolute ethanol (99.5%) was prepared at a concentration of 0.5 mg/mL of ethanolic leaf extract by maceration, ethanolic leaf extract through Soxhlet and methanolic leaf extract through Soxhlet. The hexane, chloroform and isobutanol fractions were prepared at a concentration of 1 mg/mL of absolute ethanol. The ethyl acetate fraction was solubilized in ethanol at a concentration of 0.1 mg/mL. The concentration of phenolic compounds was expressed as mg of gallic acid equivalents (GAE) per extract/g using a standard curve prepared with water for the analysis of leaves (aqueous extract) (0-30 µg/mL) and in ethanol for analysis of extract fractions (0-80 µg/mL).

#### *2.3.3 Total flavonoids*

The quantification of total flavonoids was determined by Zhishen *et al.* (1999) (with modifications). All extracts and fractions were prepared at a concentration of

1 mg/mL of ethanol, except the ethyl acetate fraction (0.5 mg/mL). The absorbance reading of samples was 510 nm. Data were expressed as rutin and quercetin equivalnts.

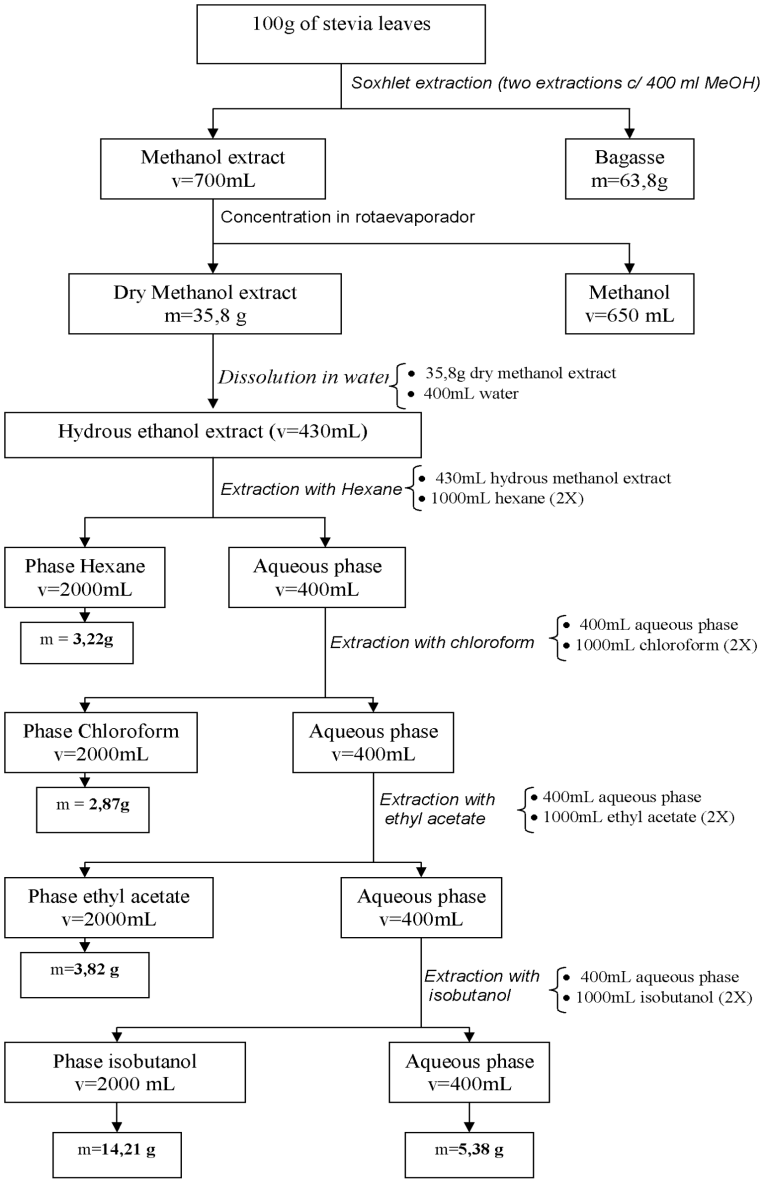


Figure 1 - Flow chart for obtaining stevia extract.

### 2.3.4 Antioxidant activity

The elimination of free radical activity from the extracts and *Stevia rebaudiana* fractions of the seminal variety UEM-13 was measured by the ability to eliminate DPPH (Blois, 1958). The results were expressed as inhibition percentage of free radicals by the sample according to the following formula: % Inhibition = $(A_0 - A_t)/A_t$ x 100. The symbol $A_0$ stands for control absorbance and $A_t$ for absorbance of each extract or fraction. All analyses were performed in triplicate and results expressed as mean values. Gallic acid was used as the standard.

### 2.4 Statistical analysis

Each analysis was performed in triplicate. The results of all the analyses were expressed as mean ± standard error of the mean and submitted to ANOVA followed by Tukey's test ($p<0.05$). We used the Statistical Analysis System (SAS, Institute Inc., Cary, North Carolina, United States, 2006) statistical software version 9.1.

## 3 I RESULTS AND DISCUSSION

### 3.1 Total glycosides

Table I shows the glycoside content (stevioside and rebaudioside A and C), total phenolics and flavonoids pooled from the leaves used in this study. Importantly, the Stevia leaves UEM-13 contain a higher content of rebaudioside A than of stevioside, and it is a variety of elite that can be reproduced by seeds, also increasing its advantage over crop varieties by cuttings. This is pioneering work with this cultivar. Stevia UEM-13 therefore presented a ratio RebA/Stev much higher (2.27) than the wild varieties that have inferiors values to 1 (Kovylyaeva *et al.*, 2007; Gardana *et al.*, 2010; Madan *et al.*, 2010; Gupta *et al.*, 2013) . The new cultivar has significant levels of bioactive compounds when compared with other wild varieties (Pasquel *et al.*, 2000; WöLwer-Rieck, 2012; Gaweł-Bęben *et al.*, 2015).

The Stevia leaves UEM-13 used in this study presented 14.8% of total glycosides. This seminal variety showed high rebaudioside A content, indicating to be an elite variety. Table II shows the extraction yield of glycosides observed in the extraction methods, total glycoside content (stevioside and rebaudioside A, C and D), phenolic compounds (gallic acid equivalents) and total flavonoids (quercetin equivalent). The ethanol extraction method by maceration presented low yield and low percentage of glycosides, when compared with the extraction method in Soxhlet apparatus. Both extracts showed significant amounts of phenolic compounds and flavonoids and a significant percentage of antioxidant activity; these results indicate that treatment with alcoholic solvents may contribute to the extraction of other substances present in the stevia leaves, extracting substantial amounts of glycosides. Periche *et al*. (2015) study effectives extraction procedures for maximization of the yield of steviol glycosides and total phenolic compounds as well as antioxidant activity in stevia extracts. The results showed significant correlations and experimental conditions greatly influence this achievement. The choice of solvent and extraction method, temperature, time and ratio changes the performance of the

| | Compound phenolics | Total flavonoids | Glycosides | Stev | Reb A | Reb C | RebA/Stev |
|---|---|---|---|---|---|---|---|
| Leaves | 2.36 % | 17.63 % | 14.8 % | 4.0 % | 9.1 % | 1.7 % | 2.27 |

TABLE I: Total glycoside content (stevioside and rebaudioside A and C) from Stevia UEM-13.

Stev= Stevioside; Reb A= Rebaudioside A; Reb C= Rebaudioside C.

extraction. This work indicates that the fractioning with different solvents can change the proportion of sweeteners and antioxidants, showing in most of them a negative correlation.

Pasquel *et al.* (2000) show that pretreatment of stevia leaves with solvents such as ethanol and CO2 prior to the conventional extraction process can improve the quality of the sweetener obtained, contributing to the reduction of the bitter aftertaste. Hexane, chloroform and ethyl acetate fractions do not contain significant concentrations of glycosides, unlike isobutanol that extracted 60.3% of sweeteners, making this the best solvent to extract higher yields of sweeteners in the industrial processes.

## 3.2 Total phenolic compounds

The content of phenolic compounds present in the Stevia leaves UEM-13 (aqueous extract of leaves) was 23.61 mg/g GAE (Gallic Acid Equivalent). Figure 2 shows the concentration of phenolic compounds of the *Stevia rebaudiana* leaf extracts and fractions of the variety UEM-13. These results demonstrate the high antioxidant potential of the extracts from the Stevia leaves, particularly the methanol and ethyl acetate extracts (107.08 mg/g, 524.2 mg/g, respectively).

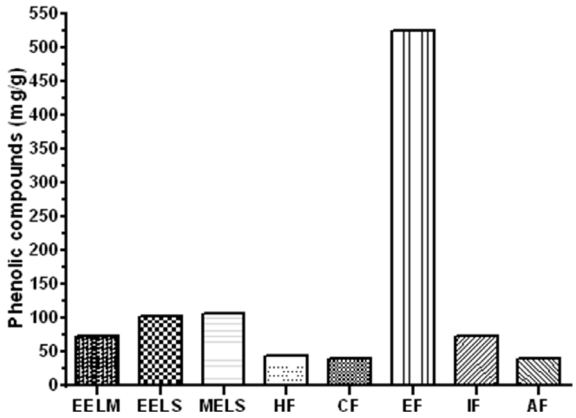


Figure 2 - Total phenolic extracts from Stevia leaves UEM- 13 expressed as mg of gallic acid equivalents per extract/g. The letters represent the following: EELM: Ethanolic Extract of Leaves (maceration); EELS: Ethanolic Extract of Leaves (Soxhlet); MELS: Methanolic Extract of Leaves (Soxhlet); HF: Hexane Fraction; CF: Chloroform Fraction; EF: Ethyl acetate Fraction; IF: Isobutanol Fraction, AF = Aqueous Fraction.

SHUKLA, S. *et al*. (2009) found 61.5 mg/g GAE from the ethanol extract of Stevia leaves through Soxhlet. (Gaweł-Bęben *et al.*, 2015) found similar values of phenolic compounds in ethanol extract of dried leaves from *Stevia rabaudiana*. The

values found in the present study indicate that these compounds should be further evaluated for their antioxidant potential in food and drinks, and further demonstrates that the ethyl acetate fraction has the greatest potential for will provide additional benefits in terms of oxidative.

| Type of extract | Extraction yield (g/100g) | Total glycosides (g/100g) | Phenolics compounds (g/100g) | Total flavonoids (g/g) |
|---|---|---|---|---|
| EELM | 6.13 ± 0,01 | 26.0 ± 0,01 | 7.27 ± 0,01 | 0.28 ± 0,02 |
| EELS | 29.50 ± 0,01 | 31.8 ± 0,01 | 10.26 ± 0,02 | 0.32 ± 0,01 |
| MELS | 38.82 ± 0,02 | 30.5 ± 0,05 | 10.70 ± 0,05 | 0.35 ± 0,05 |
| HF | 8.99 ± 0,01 | 0.1 ± 0,01 | 4.47 ± 0,01 | 0.29 ± 0,01 |
| CF | 8.01 ± 0,01 | 1.1 ± 0,04 | 3.96 ± 0,03 | 0.26 ± 0,02 |
| EF | 10.67 ± 0,02 | 0.05 ± 0,01 | 52.42 ± 0,03 | 0.38 ± 0,01 |
| IF | 39.69 ± 0,01 | 70.8 ± 0,02 | 7.29 ± 0,01 | 0.24 ± 0,01 |
| AF | 15.02 ± 0,01 | 1.14 ± 0,01 | 2.33 ± 0,01 | 0.07 ± 0,01 |

**TABLE II: Total compounds present in the *Stevia rebaudiana* extracts and fractions.**

EELM: Ethanolic Extract of Leaves (maceration); EELS: Ethanolic Extract of Leaves (Soxhlet); MELS: Methanolic Extract of Leaves (Soxhlet); HF: Hexane Fraction; CF: Chloroform Fraction; EF: Ethyl Acetate Fraction; IF: Isobutanol Fraction, AF: Aqueous Fraction.

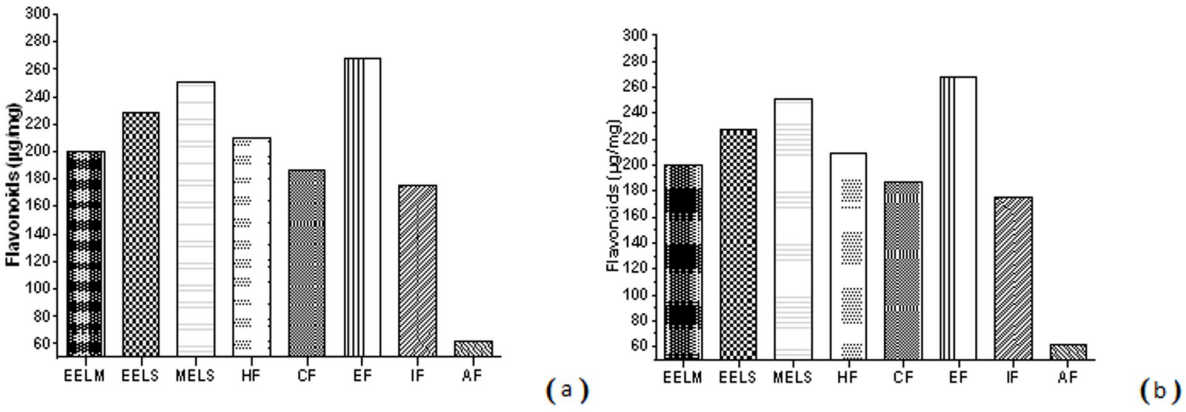


**Figure 3 -** Total flavonoid extracts from the Stevia leaves UEM-13 expressed as µg of quercetin equivalent per extract/mg (**a**) and µg of rutin equivalents per extract/mg; (**b**) µg of quercetin equivalents per extract/mg. The letters represent the following: EELM: Ethanolic Extract of Leaves (maceration); EELS: Ethanolic Extract of Leaves (Soxhlet); MELS: Methanolic Extract of Leaves (Soxhlet); HF: Hexane Fraction; CF: Chloroform Fraction; EF: Ethyl acetate Fraction; IF: Isobutanol Fraction, AF = Aqueous Fraction.

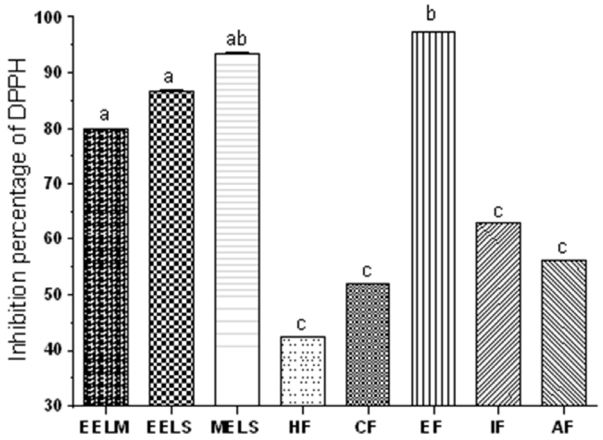


**Figure 4 -** Percentage of inhibition of DPPH radicals from leaf extracts of Stevia UEM-13 (1 mg/ml). Mean values with different superscript letters indicate significant differences ($P < 0.05$). The letters represent the following: EELM: Ethanolic Extract of Leaves (maceration); EELS: Ethanolic Extract of Leaves (Soxhlet); MELS: Methanolic Extract of Leaves (Soxhlet); HF: Hexane Fraction; CF: Chloroform Fraction; EF: Ethyl acetate Fraction; IF: Isobutanol Fraction, AF = Aqueous Fraction.

### 3.3 Total flavonoids

Different concentrations of these compounds can be found in leaves, callus, and different *Stevia rebaudiana* extracts, depending on the conditions, type of solvent and extraction method used (Madan *et al.*, 2010). In the present study the concentration of flavonoids present in the Stevia leaves UEM- 13 (aqueous extract) was 176.33 µg/mg. After the extraction process, significant concentrations of flavonoids were found in the methanol and ethyl acetate extract, demonstrating the antioxidant potential of the extract (Figures 3a and 3b).

### 3.4 Antioxidant activity

Studies show that Stevia leaf extracts may exhibit high antioxidant potential (Periche *et al.*, 2015). The antioxidant activity of the aqueous extract from the leaves of Stevia UEM-13 was 42.26% (1 mg/mL). The Figure 4 shows the percentages of inhibition of DPPH radicals of the extracts evaluated in this study. It can be seen that, in general, all extracts showed important antioxidant potential, particularly the methanol extract of leaves obtained by extraction and through Soxhlet. The methanol extract and ethyl acetate fraction showed inhibition of DPPH radical of the 93.5% and 97.32% at a concentration of 1 mg/ml. These extracts had higher levels of phenolic compounds and flavonoids which indicates high antioxidant activity. The $IC_{50}$ for each extract (EEMF, EESF, MESF, HF, CF, EF, IF, AF) were 626.5 µg/mL, 576.70 µg/mL, 534.75 µg/mL, 1175 µg/mL, 963.02 µg/mL, 513.76 µg/mL, 792.64 µg/mL, 889.20 µg/mL, respectively. The fraction with the highest potential is the ethyl acetate.

## 4 I CONCLUSIONS

This study shows for the first time the glycosides content, phenolic compounds

and antioxidant activity of seminal variety from *Stevia rebaudiana*, Stevia UEM-13, confirming that a variety with high RebA content and can be reproduced by seeds.

The evaluation of extraction and fractionation of this plant using different solvents and methodologies resulted in extracts of different antioxidant capacity, which can be used to enrich the literature and contribute to their application in foods, pharmaceuticals and cosmetics. A diet rich in antioxidants may contribute significantly to prevent degenerative diseases, cardiovascular and metabolic diseases. Thus, natural and industrialized foods enriched with flavonoids and phenolic compounds contribute to the reduction of these diseases. The present study showed that ethanol, methanol and ethyl acetate extracts of *Stevia rebaudiana* leaves (Stevia UEM-13) have a great potential antioxidant and so can be used as possible additives to enhance the functionality of food and beverages.

## ACKNOWLEDGMENTS

The authors thank the State University of Maringa and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) agency. The authors declare that they have no conflict of interest.

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## SOBRE A ORGANIZADORA

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ISBN 978-85-7247-825-0