



doi: 10.4103/2221–1691.250857

©2019 by the Asian Pacific Journal of Tropical Biomedicine.

Fruiting increases total content of flavonoids and antiproliferative effects of *Cereus jamacaru* D.C. cladodes in sarcoma 180 cells *in vitro*

Jean Carlos Vencioneck Dutra¹✉, Judá Ben–Hur de Oliveira¹, Vanessa Silva dos Santos¹, Paula Roberta Costalonga Pereira¹, Jean Moisés Ferreira², Maria do Carmo Pimentel Batitucci¹

¹Laboratório de Genética Vegetal e Toxicológica, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, 29075–910, Vitória, Espírito Santo, Brazil

²Laboratório de Biologia Molecular e Expressão Gênica, Departamento de Ciências Biológicas, Universidade Federal de Alagoas, 57309–005, Arapiraca, Alagoas, Brazil

ARTICLE INFO

Article history:

Received 23 December 2018

Revision 9 January 2019

Accepted 23 January 2019

Available online 1 February 2019

Keywords:

Mandacaru

Flavonoid content

DPPH

ABTS

Fe²⁺ ion chelating activity

MTT assay

Sarcoma 180 cells

ABSTRACT

Objective: To evaluate the influence of fruiting phenological stage on total flavonoid content, antioxidant activity, and antiproliferative effects of *Cereus jamacaru* (*C. jamacaru*) (mandacaru) cladodes and fruit. **Methods:** Fruit and cladodes at vegetative and fruiting stage of *C. jamacaru* were collected. The fruit was dissected and bark, pulp, and seeds were separated. Vegetative and fruiting cladodes, together with bark, pulp, and seeds were used to obtain five hydroalcoholic extracts. The extracts were investigated for total flavonoid content, using AlCl₃ colorimetric method, antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging capacity and Fe²⁺ ion chelating activity, and *in vitro* antiproliferative effects (sarcoma 180 cells) by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. **Results:** The extract of *C. jamacaru* cladodes at the fruiting stage showed higher flavonoid content compared to the other extracts. Seed extracts showed the highest antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assays, and the extract of cladodes at vegetative stage showed better antioxidant activity in Fe²⁺ ion chelating activity. The extract of fruiting cladodes promoted higher antiproliferative effects compared to the other extracts. **Conclusions:** These findings suggest that fruiting increases the content of flavonoids and antiproliferative effects of *C. jamacaru* cladodes. Data reinforce the potential use of *C. jamacaru* cladodes and fruits as natural antioxidants and potent anticancer agent.

1. Introduction

Cereus jamacaru (*C. jamacaru*) D.C. (mandacaru) is a Cactaceae native from Brazil, found mainly on arid areas and is used as food and, in traditional medicine, to treat urinary infection, kidney inflammation, and rheumatism[1]. The antioxidant and antiproliferative actions of *C. jamacaru* have already been reported in the literature, which motivated the conduction of more researches on the effective use of this plant as a natural source of antioxidants and for the development of new drugs[2–4].

Optimization of the uses of natural products is not restricted to the

identification of the vegetal actives and their biological effects. Many studies have aimed to evaluate the influence of genetic and environmental factors, crop management, and phenology on the production of phytochemicals[5–8]. The influence of phenology on the frugivory, dispersion, and germination of seeds of *C. jamacaru* has been reported[9]. However, there are no studies demonstrating the influence of phenology on the biological activities of this cactus,

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2019 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow

How to cite this article: Dutra JCV, de Oliveira JBH, dos Santos VS, Pereira PRC, Ferreira JM, Batitucci MCP. Fruiting increases total content of flavonoids and antiproliferative effects of *Cereus jamacaru* D.C. cladodes in sarcoma 180 cells *in vitro*. Asian Pac J Trop Biomed 2019; 9(2): 66–72.

✉First and corresponding author: Jean Carlos Vencioneck Dutra, Laboratório de Genética Vegetal e Toxicológica, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, 29075–910, Vitória, Espírito Santo, Brazil.

E-mail: jeanvencioneck@gmail.com

Foundation project: This work was supported by grants from FAPES (Fundação de Amparo à Pesquisa e Inovação do Espírito Santo) – term of grant 225/2015.

such as the antioxidant and antiproliferative activities.

In recent years, there has been a growing use of *in vitro* techniques to evaluate the antioxidant and antiproliferative potential of natural products. These techniques have been used for multiple and complementary measures of antioxidant and cytotoxicity activities[5,6,10,11]. In this context, antioxidant assays, such as 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Fe²⁺ ion chelating activity, and *in vitro* antiproliferative assays using cell lines, such as sarcoma 180 (murine cancer), have been efficiently used as screening tools for natural products of plant origin with potential use for health promotion and development of new drugs[5–8].

Therefore, the aim of this study was to evaluate the influence of fruiting on the total flavonoids content of extracts of *C. jamacaru* cladodes at the vegetative stage and fruiting, as well as their fruits, and to relate these results to the *in vitro* antioxidant activities and antiproliferative effects.

2. Materials and methods

2.1. Chemicals

The chemicals used in this study included Ficoll® Paque Plus (Sigma-Aldrich); RPMI 1640 culture medium (Cultilab); fetal calf serum (Gibco); Cisplatin (Fauldcispla®, Libbs); DPPH (Sigma-Aldrich); ABTS (Sigma-Aldrich); potassium persulfate (Sigma-Aldrich); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma-Aldrich); 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4'-disulfonic acid Na-salt (Sigma-Aldrich); iron chloride II (Sigma-Aldrich); 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma-Aldrich).

2.2. Plant material

The fruit and cladodes of *C. jamacaru* in the vegetative stage (without flowers or fruits) and fruiting stage (with fruit) were collected in the state of Alagoas, northeastern Brazil (9° 40' 44.7" S, 36° 41' 21.9" W), in July 2018. The thorns of cladodes were removed and the cladodes were later sliced with a blade. The fruit was collected together with cladodes in the fruiting stage, dissected and the bark, pulp, and seeds were obtained. The plant material was oven-dried at 50 °C for 24 h and vegetative and fruiting cladodes, bark, pulp, and seeds were used to obtain five hydroalcoholic extracts.

2.3. Hydroalcoholic extract

Dried plant material was powdered, macerated in EtOH/H₂O (70:30 v/v) solution (dry plant: EtOH/H₂O - 1:5 w/v) at room temperature (25-30 °C), protected from the light for 3 d, filtered and stored. Vegetal material was submitted to the same conditions of maceration (plus three days of maceration) for exhaustion of the compound extraction, totaling six days of maceration. The solvent was evaporated under reduced pressure at 60 °C to obtain the crude extracts of *C. jamacaru*. The extracts were stored at 6-10 °C and protected from the light until its utilization. The yield of the extracts

were calculated by the formula[12]:

$$\text{Total extract yield (\%)} = \text{FM/IM} \times 100$$

Where "FM" = final mass of dry extract (g); "IM" = initial mass of dry plant (g).

2.4. Total flavonoid content

The colorimetric method described by Jia *et al.*[13] was used to determine the total flavonoid content of *C. jamacaru* extracts. The experiment was performed in triplicate and the absorbance was detected at 430 nm, using an ELISA reader. Rutin was used as a standard for flavonoids and for this purpose a series of methanolic dilutions of rutin (500.0, 250.0, 125.0, 62.5, 31.3, 15.6, and 7.8 µg/mL) were prepared and assayed. The total flavonoid content in the extracts was expressed as rutin equivalent (µg/mL).

2.5. Antioxidant activity

2.5.1. DPPH

DPPH radical reduction method was used to evaluate the antioxidant activity of *C. jamacaru* extracts and ascorbic acid standard[14]. The absorbance was taken at 517 nm by ELISA reader. The test was performed in triplicate and the percentage of reduction of DPPH' radical was calculated by the equation:

$$\% \text{ inhibition} = [(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}}] \times 100$$

Where "% inhibition" is the percentage of inhibition capacity of DPPH'; "Abs_{Control}" is the absorbance of DPPH' reaction of the control; and "Abs_{Sample}" is the absorbance of the sample.

2.5.2. ABTS

ABTS capturing method was performed to evaluate the total antioxidant activity of *C. jamacaru* extracts and ascorbic acid standard[15]. The absorbance was taken at 734 nm by ELISA reader. The experiment was performed in triplicate and the percentage of scavenging inhibition capacity of ABTS'' of the *C. jamacaru* extract was calculated by the following equation:

$$\% \text{ inhibition} = [(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}}] \times 100$$

Where "% inhibition" is the percentage of scavenging inhibition capacity of ABTS''; "Abs_{Control}" is the absorbance of ABTS'' reaction of the control; and "Abs_{Sample}" is the absorbance of the sample.

2.5.3. Fe²⁺ chelation ions

Chelating activity of ferrous ions (Fe²⁺) was assayed by inhibition of ferrous-ferrozine complex formation after treatment with *C. jamacaru* extracts and EDTA standard[16]. The absorbance was taken at 562 nm by ELISA reader. The experiment was performed in triplicate and the percentage of ferrous ion chelating effect was calculated using the following equation:

$$\% \text{ chelating effect} = [(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}}] \times 100$$

Where "% chelating effect" is the percentage of ferrous ions (Fe²⁺) chelating effect; "Abs_{Control}" is the absorbance of Fe²⁺ reaction of the control; and "Abs_{Sample}" is the absorbance of the sample.

2.6. Cytotoxicity in vitro

2.6.1. Sarcoma 180 cells

Sarcoma 180 cells (murine sarcoma) were acquired from Banco

de Células do Rio de Janeiro. In 96-well plates, sarcoma 180 cells were plated with 2×10^5 cells per well. The cells that received *C. jamacaru* extracts were diluted with water at 10.0, 50.0, 100.0, 200.0, 300.0, 400.0, and 500.0 $\mu\text{g/mL}$ and untreated cells (0.0 $\mu\text{g/mL}$ of extract) were used as a control. Sarcoma 180 cells were cultured for 24 h, 48 h, or 72 h to evaluate the anticancer effect of *C. jamacaru* extracts. All protocols were approved by the Research Ethical Committee of Universidade Federal do Espírito Santo (certificate 89/2015).

2.6.2. Cell culturing methods

Culture medium RPMI 1640, supplemented with gentamicin (50.0 mg/L), amphotericin B (2.0 mg/L), and 10% of fetal calf serum at 37 °C, 5% of CO₂ saturation and humid atmosphere was used to grow the sarcoma 180 cells. The cells were cultured under these conditions 24 h before starting the treatments. At the end of the treatment, the cell viability was evaluated.

2.6.3. MTT assay

MTT assay was used to determine cell viability of sarcoma 180. At the end of the treatment, the plates were centrifuged at 860 rcf for 10 min, the supernatant was discarded and 20 μL of MTT at 5 mg/mL were added to each well. 3 h later, 100 μL of DMSO were added in each well and the absorbance was detected at 590 nm by ELISA reader. The results were expressed as a relative percentage of cell viability in comparison to the untreated cells. The experiment was performed in triplicate.

2.7. Statistical analysis

Normality of the data was evaluated a priori and results were expressed as mean \pm standard deviation. To quantify the total content of flavonoid in *C. jamacaru* extracts, a linear curve relating rutin concentrations and absorbances was performed and the equation and R^2 were obtained. The total flavonoid content in *C. jamacaru* extracts was compared by multiple *t* test ($P < 0.05$). The antioxidant capacity of the extracts at different concentrations was compared by ANOVA *post hoc* Tukey test ($P < 0.05$). To evaluate the anticancer activity of *C. jamacaru* extracts *in vitro*, the cell viability of sarcoma 180 treated with extracts at various concentrations was compared to untreated cells (0.0 $\mu\text{g/mL}$ of extract) by ANOVA *post hoc* Dunnett's test ($P < 0.05$). Principal component analysis (PCA) and Pearson correlation were performed to visualize relationships between the total flavonoid content, antioxidant activities, and anticancer effects of *C. jamacaru* extracts. In the PCA analysis and Pearson correlation, the values of antioxidant activities and anticancer effects at the concentration of 500.0 $\mu\text{g/mL}$ were used as this concentration presented the best results for the biological activities tested.

3. Results

3.1. Flavonoid content

Table 1 summarizes the total yield and total flavonoid content of *C. jamacaru* extracts. The total flavonoid content was calculated using the equation $y = 0.003345x + 0.1212$ ($R^2 = 0.9948$; $P < 0.0001$). The highest total yield was observed in the pulp extract (70.34%) and *C.*

jamacaru cladode extract at the fruiting stage presented the highest total content of flavonoids [(201.45 \pm 16.54) $\mu\text{g/mL}$] among all extracts, approximately 10 times higher than the *C. jamacaru* cladode extract at the vegetative stage [(20.85 \pm 3.78) $\mu\text{g/mL}$].

Table 1

Total extract yield and total flavonoid content of *C. jamacaru* extracts.

Extract	Initial plant dry mass (g)	Final extract dry mass (g)	Total extract yield (%)	Flavonoid content ($\mu\text{g/mL}$) (mean \pm SD)
Vegetative cladode	43.44	5.25	12.08	20.85 \pm 3.78 ^b
Fruiting cladode	53.04	8.28	15.61	201.45 \pm 16.54 ^a
Bark	4.92	1.29	26.22	9.54 \pm 1.43 ^c
Pulp	2.10	1.48	70.48	4.69 \pm 0.17 ^d
Seeds	1.29	0.09	6.98	15.09 \pm 2.10 ^b

The values of plant and extract mass are the observed weight. The total flavonoid content is presented as mean \pm standard deviation (SD). The comparison between the total content of flavonoids in *C. jamacaru* extracts was performed by the *t* test ($P < 0.05$) - means followed by different lowercase letters in the column (^{a, b, c, d}) differ statistically.

3.2. Evaluation of antioxidant activity

The results of the antioxidant evaluation of extracts are presented in Table 2. In the DPPH test, *C. jamacaru* seeds extract presented better antioxidant activity when compared to the other extracts, which was similar to the results of the ascorbic acid as a standard in some tested concentrations. In comparison to the other extracts, the *C. jamacaru* fruit pulp extract showed lower antioxidant activity in DPPH assay and no antioxidant activity was detected at concentrations of 62.5 and 125.0 $\mu\text{g/mL}$.

For the ABTS assay, *C. jamacaru* cladode extract at the fruiting stage was the one with the better antioxidant activity when compared to the other extracts. The extract of seeds showed antioxidant activity similar to the standard ascorbic acid at the concentration of 500.0 $\mu\text{g/mL}$. Moreover, similar to the results observed in the DPPH assay, the *C. jamacaru* fruit pulp extract presented the lowest antioxidant power in the ABTS assay and no antioxidant activity was detected at concentrations of 62.5 and 125.0 $\mu\text{g/mL}$.

At the evaluation of the chelating activity of Fe²⁺ ions, the *C. jamacaru* cladode extract at the vegetative stage presented the highest antioxidant power in comparison to the other extracts. At concentrations of 62.5 and 125.0 $\mu\text{g/mL}$, no antioxidant activity was detected in the *C. jamacaru* fruit bark extract. However, at all tested concentrations of *C. jamacaru* seeds extract, no antioxidant activity was detected. The values of DPPH, ABTS, and Fe²⁺ chelation, for fruiting cladodes, are higher for 250.00 $\mu\text{g/mL}$ compared to 500.00 $\mu\text{g/mL}$ concentration (Table 2).

3.3. Anticancer activity in vitro

Figure 1 shows the cytotoxicity of *C. jamacaru* extracts in sarcoma cells 180 *in vitro*. Compared to the group of untreated cells (0.0 $\mu\text{g/mL}$ of extract), it was observed that all *C. jamacaru* extracts significantly reduced the viability of tumor cells after 24 h (Figure 1A), 48 h (Figure 1B), and 72 h of treatment in all concentrations (Figure 1C). At the end of 72 hours of treatment (Figure 1C), it was found that the *C. jamacaru* cladode extract at the fruiting stage was able to promote a more pronounced reduction in the viability

Table 2Antioxidant activity of *C. jamararu* extracts and standards by DPPH, ABTS, and Fe²⁺ chelation ions assays (mean±SD).

Assay	Concentration (µg/mL)	% inhibition						
		Vegetative cladode	Fruiting cladode	Bark	Pulp	Seeds	Ascorbic acid	EDTA
DPPH	62.50	9.36 ± 4.26 ^{IB}	45.25 ± 5.08 ^{CB}	2.16 ± 0.60 ^{CC}	nd	58.29 ± 2.12 ^{BB}	89.01 ± 0.17 ^{AA}	–
	125.00	8.41 ± 1.62 ^{EB}	58.67 ± 3.68 ^{BB}	6.06 ± 0.23 ^{CC}	nd	87.78 ± 0.19 ^{AA}	90.09 ± 0.23 ^{AA}	–
	250.00	13.71 ± 0.14 ^{AB}	71.64 ± 2.97 ^{BA}	15.00 ± 0.60 ^{BB}	2.66 ± 0.14 ^{AA}	87.27 ± 0.13 ^{AA}	91.85 ± 0.17 ^{AA}	–
	500.00	20.88 ± 1.80 ^{EA}	69.83 ± 9.69 ^{BA}	31.18 ± 2.34 ^{CA}	2.45 ± 0.92 ^{AA}	86.34 ± 0.54 ^{AA}	92.29 ± 1.13 ^{AA}	–
ABTS	62.50	7.36 ± 1.94 ^{BD}	43.90 ± 1.32 ^{BC}	2.47 ± 1.85 ^{CC}	nd	25.50 ± 1.44 ^{CD}	89.48 ± 0.44 ^{AA}	–
	125.00	20.82 ± 0.78 ^{DC}	68.92 ± 1.06 ^{BB}	3.70 ± 1.40 ^{CC}	nd	43.85 ± 2.57 ^{CC}	91.89 ± 0.60 ^{AA}	–
	250.00	37.33 ± 3.44 ^{DB}	83.24 ± 1.00 ^{BA}	8.33 ± 1.89 ^{BB}	0.36 ± 0.04 ^{AA}	52.86 ± 0.77 ^{BB}	92.73 ± 0.94 ^{AA}	–
	500.00	55.15 ± 1.03 ^{EA}	83.20 ± 3.65 ^{BA}	22.55 ± 1.53 ^{AA}	0.63 ± 0.02 ^{EA}	91.19 ± 1.23 ^{AA}	93.57 ± 0.35 ^{AA}	–
Fe ²⁺ chelation	62.50	14.48 ± 4.20 ^{DD}	46.02 ± 0.60 ^{BB}	nd	2.70 ± 0.71 ^{DD}	nd	–	96.47 ± 0.05 ^{AA}
	125.00	35.01 ± 1.71 ^{CC}	56.83 ± 0.61 ^{BA}	nd	10.97 ± 1.00 ^{CC}	nd	–	96.97 ± 0.05 ^{AA}
	250.00	59.48 ± 3.76 ^{BB}	60.70 ± 2.64 ^{BA}	11.93 ± 5.44 ^{DB}	25.94 ± 1.02 ^{BB}	nd	–	96.89 ± 0.12 ^{AA}
	500.00	87.64 ± 1.38 ^{BA}	59.86 ± 3.21 ^{CA}	36.27 ± 3.58 ^{AA}	51.32 ± 1.70 ^{CA}	nd	–	96.92 ± 0.08 ^{AA}

The percentage of inhibition in each antioxidant assay is presented as mean ± standard deviation (SD). The comparison between the percentage of inhibition exhibited for each concentration of *C. jamararu* extracts was performed by ANOVA *post hoc* Tukey test ($P < 0.05$) – values followed by different lowercase letters in the row ^(a, b, c, d, e, f) or followed by different capital letters ^(A, B, C, D) in the column differ statistically. nd = not detected.

of the tumor cells at the concentrations of 200.0, 300.0, 400.0, and 500.0 µg/mL. Likewise, the seed extract was able to promote a more pronounced reduction in the viability of tumor cells at concentrations of 300.0, 400.0, and 500.0 µg/mL.

3.4. Explorative analyses: Correlations between total flavonoid content and antioxidant and anticancer assays

PCA and Pearson correlation coefficient were used to correlate the results obtained in different assays. In PCA, it was accounted 52.23% and 25.92% in the first and second principal components, respectively, and total variance of 78.16% (Figure 2). PC1 was dominated by the following variables: total flavonoid content, DPPH, ABTS, and MTT assay after 48 h and 72 h of treatment. PC2 was dominated by the variables: total flavonoid content, Fe²⁺ chelating activity, and MTT assay after 24 h of treatment. These results suggested that flavonoid content, DPPH, and ABTS antioxidant assays were correlated with anticancer effects after 48 h and 72 h of treatment, while Fe²⁺ chelating activity was correlated with anticancer effect after 24 h of treatment. *C. jamararu* cladode extract at fruiting stage presented the highest total flavonoid content level in comparison to the other extracts and it seemed to be the main factor related to its antioxidant activity (DPPH and ABTS assays) and anticancer effects. DPPH and ABTS assays were the main factor related to the anticancer activity exhibited for seeds extract. Added to this, Fe²⁺ chelating activity seemed to be the main factor related to the anticancer effects exhibited for *C. jamararu* cladode extract at vegetative stage and fruit pulp extract.

Table 3 presents the results of Pearson correlation analysis. Values followed by negative sign indicate an inversely proportional relation between the factors. Thus, considering that MTT assay was used to verify the viability of tumor cells after treatment with *C. jamararu* extracts, negative correlations between the MTT assay and the total flavonoid content or antioxidant activities suggest increasing anticancer effect. A moderate negative correlation between anticancer effect after 24 h of treatment (MTT 24 h) and total flavonoid content level was demonstrated. Moreover, a moderate negative correlation between anticancer effect after 48 h of treatment (MTT 48 h) and total flavonoid content, DPPH, and ABTS activities was observed. The anticancer effect after 72 h of treatment (MTT 72 h) showed a moderate negative correlation with the DPPH activity and moderate positive correlation with Fe²⁺ chelating activity.

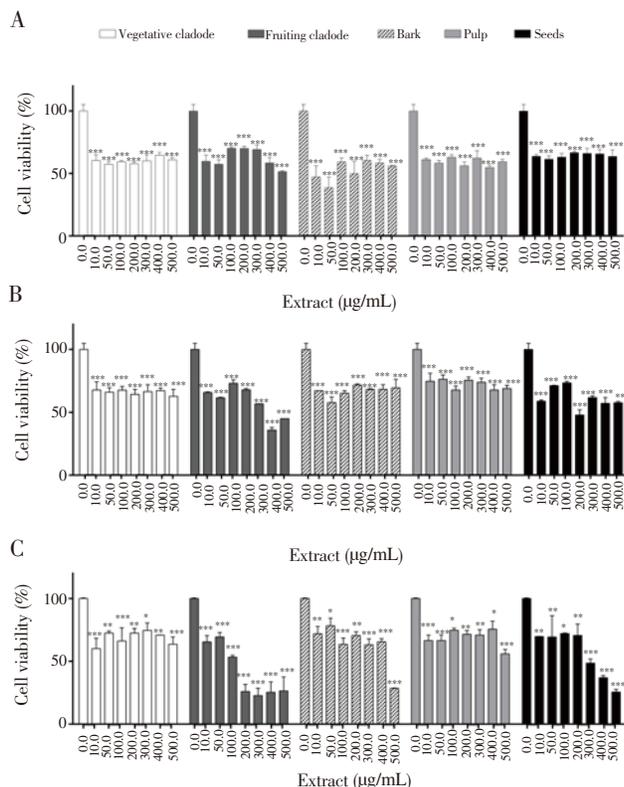


Figure 1. Cytotoxicity of *C. jamararu* extracts in sarcoma 180 cells *in vitro* by MTT assay.

Percentage of viable cells is presented as mean ± standard deviation (SD). Sarcoma 180 cells were treated with *C. jamararu* extracts for 24 h (A), 48 h (B), or 72 h (C). The comparison between untreated cells (0.0 µg/mL) and cells treated with *C. jamararu* extracts was performed by ANOVA *post hoc* Dunnett's test – * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$ vs. untreated cells.

Table 3

Pearson correlation analysis between total flavonoid content, antioxidant activities (DPPH, ABTS, and Fe²⁺ chelation), and anticancer effects (MTT assay) of *C. jamacaru* extracts.

	Flavonoid	DPPH	ABTS	Fe ²⁺ chelation	MTT 24 h	MTT 48 h	MTT 72 h
Flavonoid	1						
DPPH	0.453 0	1					
ABTS	0.513 1	0.876 8	1				
Fe ²⁺ chelation	0.147 4	-0.656 6	-0.292 0	1			
MTT 24 h	-0.602 5	-0.052 7	0.013 2	-0.183 7	1		
MTT 48 h	-0.799 9	-0.653 6	-0.732 0	0.046 6	0.253 3	1	
MTT 72 h	-0.388 5	-0.742 1	-0.451 6	0.709 7	0.247 0	0.304 6	1

To evaluate anticancer effects, MTT assay was performed after 24 h of treatment (MTT 24 h), 48 h of treatment (MTT 48 h), and 72 h of treatment (MTT 72 h). Values of antioxidant activities and anticancer effects at the concentration of 500.0 µg/mL were used to perform Pearson correlation.

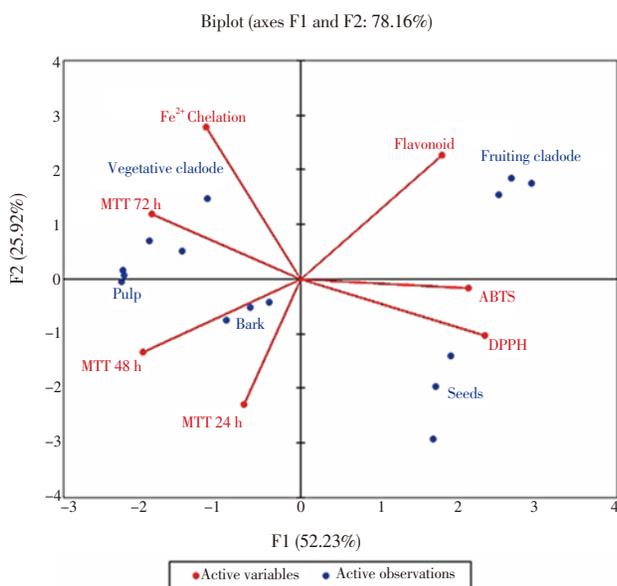


Figure 2. PCA (scores and loading plots, biplot) based on total flavonoid content analyzed in *C. jamacaru* extracts (vegetative and fruiting cladodes, bark, pulp, and seed fruit extracts) and their antioxidant activities (DPPH, ABTS, and Fe²⁺ chelating activity) and anticancer effects (MTT assay).

To evaluate anticancer effects, MTT assay was performed after 24 h of treatment (MTT 24 h), 48 h of treatment (MTT 48 h), and 72 h of treatment (MTT 72 h). Values of antioxidant activity and anticancer effect at the concentration of 500.0 µg/mL were used to perform PCA analysis.

4. Discussion

Phytochemicals production and accumulation in plants may differ due to genetic, environmental, and phenological factors[5,17]. The phytochemicals can accumulate in different parts of the plants[18], while the variety, processing, cooking, and growing conditions may determine the level of phytochemicals accumulated in plant tissues[19].

Studies on the phenological aspects of cacti are scarce and usually address factors related to ecology or increase in fruit and plant biomass production, as the studies of Gomes *et al.*[9] and Arba *et al.*[20]. Gomes *et al.*[9] performed a study relating phenology, frugivory, dispersion and germination of *C. jamacaru* seeds, and Arba *et al.*[20] investigated the effect of irrigation on plant biomass at different phenological stages in cactus of the genus *Opuntia* produced for commercial purposes.

C. jamacaru is a succulent plant adapted to arid regions and the

fruiting stage seems to play an important modulating role in the production of flavonoids. The total flavonoid content in cladodes extract of *C. jamacaru* at vegetative stage (20.85 µg/mL) was higher than that observed by de Sousa Araújo *et al.*[21] in methanolic extract (0.59 µg/mL) and Dutra *et al.*[4] in hydroalcoholic extract (0.51 µg/mL). For the content of flavonoids in the bark and pulp, the values found in our study differ from those observed by Lima[22], evaluating the total yellow flavonoid in *C. jamacaru* fresh fruits, 3.20 and 2.35 mg/100 g in bark and pulp, respectively.

The plant material used in our study was collected in July 2018, after the fructification peak of June 2018[9]. Considering that plants grown in natural environments may show heterogenous flowering and fruiting, it was possible to find and collect in the same population *C. jamacaru* plants in vegetative and fruiting stage.

Compared to previous studies conducted with *C. jamacaru* cladodes at the vegetative stage[4,21], our study found that there is an increase in the total production of flavonoids during the fruiting stage. Our study also showed that the extract of *C. jamacaru* cladodes in the vegetative stage had relatively high total flavonoid content (20.85 µg/mL) compared to what Dutra *et al.* (0.51 µg/mL) described using *C. jamacaru* cladodes collected at the same region and phenological stage in September 2016[4]. Fluctuations in flavonoid content may occur due to environmental changes, as suggested by Pretti *et al.*[5]. However, we believe that fruiting increases plant flavonoid production and that the relatively high level of flavonoids in cladodes at the vegetative stage may have occurred due to post-fructification stage.

Several methods can be employed to assess the antioxidant capacity of vegetable extracts *in vitro*[23,24]. The use of a single method of antioxidant evaluation provides basic information about antioxidant properties of a sample. Therefore, the use of different methods of antioxidant evaluation is recommended, since it allows a better understanding of antioxidant properties and mechanism of action of a sample[23,25].

DPPH, ABTS, and Fe²⁺ chelation ions assays were used to evaluate the antioxidant activity of *C. jamacaru* extracts in this work. A low antioxidant activity in pulp and bark extracts was observed, and in some concentrations the antioxidant activity was not detected (62.5 and 125.0 µg/mL) for DPPH and ABTS assays using pulp extract and for Fe²⁺ chelation assay using bark extract. The studies with fruits showed that plant phenology and physiological responses to biotic and abiotic factors were able to modify the properties of potential antioxidant, such as under climate scenarios[7,26,27]. *C. jamacaru* is a cactus adapted to semi-arid conditions, surviving in high temperatures[28]. However, the high temperatures in which this cactus develops may degrade the phytochemicals found in the fruits

and reduce their antioxidant capacity[26], as observed in our study.

Bark extract presented higher flavonoid content and better antioxidant activity in DPPH and ABTS than pulp extract, similar to that observed by Lima[22], evaluating the flavonoid content and ABTS antioxidant activity of fresh fruits of *C. jamacaru* collected in the same biome. The flavonoids are phenolic compounds reported as the main factor responsible for the chelating activity[29]. The extract of *C. jamacaru* cladodes at the fruiting stage presented higher flavonoid content, but it did not show the best results in chelating activity. Therefore, no direct relationship was observed between total flavonoid content and chelating activity, suggesting that, as observed by dos Santos *et al.*[6], the antioxidant ability of plant extracts may be related to the chemical structure of flavonoids.

For the conditions tested in our study, all extracts of *C. jamacaru* were able to promote *in vitro* antiproliferative effects, demonstrating that the cladodes and the fruit of this cactus present potential use for the prevention/treatment of cancer. *In vivo* antiproliferative effects of cladodes extract of *C. jamacaru* at vegetative stage were reported in the literature[2–4]. The aqueous extract of the cladodes induces antiproliferative effects and increases cellular aberrations in *Allium cepa* test system cells[3] and hydroethanolic extract from the cladodes promotes antiproliferative effect on sarcoma 180 tumor cells in rodents[2,4].

Phenological stage, as well as the variation of climate, soil, and time conditions, seem to exert an important influence on the secondary metabolism, modifying the quality and properties of plant compounds, inducing changes in chemical composition[30], and interfering with the production of phenolic compounds, such as flavonoids[5,6].

Secondary metabolism products provide plant protection against herbivores, pathogen attack, protection of abiotic stresses, and can act as modulators of gene expression and signal transduction, influencing the content/availability of phytochemicals[31,32]. In addition, flowering and fruiting are also able to modulate the secondary metabolism of plants and alter production of these compounds and the biological activities related to them[6,18].

Among more than 200 000 different types of compounds produced by plants[33], there are many colored substances, pigments, such as flavonoids found in different plant organs. These secondary metabolites belong to the class of phenylpropanoids and present a wide color range, from pale-yellow to blue. The color development in fruits is considered an important evolutionary trait and is a factor that interferes in fruit quality and market value[34].

The fruits of *C. jamacaru* present reddish-brown barks when ripe. The pigmentation of the bark is related to compounds such as anthocyanins, a class of flavonoids responsible for the colors ranging from orange to blue found in many flowers, leaves, fruits, seeds, and other matrices[35]. Anthocyanins are used as food dyes mainly in the beverage industry and in recent years the consumption of these substances has increased due to the public concern about the possible adverse effects of synthetic food dyes[36].

Following the comparison of *C. jamacaru* extracts at different developing stages, we suggest that the differences between the flavonoid contents and biological activities observed are related to the modulations of the primary and secondary metabolic pathways in the plants. In addition, the diversity of phenolic compounds in plants is a result of derivations in the shikimic acid pathway, a process mediated by the enzyme L-phenylalanine ammonia-lyase (PAL), an enzyme that acts on a branch point between the primary and secondary metabolism, shikimic acid and phenylpropanoid production pathways,

respectively[37].

PAL acts on a limited set of molecular structures, producing several phenolic compounds, such as flavonoids, the most abundant class of plant polyphenols synthesized by the combination of the shikimic acid and acyl polyolate pathways[37]. The enzymatic action of PAL mediates the synthesis of L-phenylalanine, a compound used in the production of trans-cinnamic acid, a molecule from which most of the phenolic compounds derive[38,39]. Thus, modulations in the pathway of secondary plant metabolism may induce both the production of phytochemicals and the increase in the content and/or accumulation of these substances.

Our findings indicate that during the fruiting stage there is modulation of the secondary metabolic pathway of *C. jamacaru*, leading to an increase in total flavonoid content. The extracts of the cladodes and the fruit of *C. jamacaru* presented antioxidant activity and antiproliferative effects, however no direct relationship between the flavonoid content and the biological activities was observed. This study also suggests the potential use of *C. jamacaru* cladodes and fruits as a source of natural antioxidants and as potent anticancer agents. These data reinforce the effectiveness of the biological activities of natural products, such as those observed in our study, favor the extensive use of *C. jamacaru* in cooking, industry, drug development and also assist in understanding the role of secondary metabolite pathway in metabolites synthesis.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

We would like to express our gratitude to Universidade Federal do Espírito Santo.

Funding

This work was supported by grants from FAPES (Fundação de Amparo à Pesquisa e Inovação do Espírito Santo) - term of grant 225/2015.

References

- [1] de Lucena CM, Alves CAB, Pereira DD, Nunes EN, Costa GM, da Silva Costa GG, et al. Use and knowledge of Cactaceae in Northeastern Brazil. *J Ethnobiol Ethnomed* 2013; **9**(1): 62.
- [2] Souza I, Lima MCA, Melo UBC, Higino JS. Antitumour properties of *Cereus jamacaru* on an experimental model of cancer *in vivo*. *Fund Clin Pharmacol* 2001; **15**(Suppl 1): 148. Doi: <https://doi.org/10.1046/j.1472-8206.2001.00113.x>.
- [3] da Silva Darci DM, Ronielson FR de SC, Gabriel AGM, Peron AP. Cytotoxic action of the stem aqueous extract of the stem of *Cereus jamacaru* DC.(mandacaru). *Rev Cuba Plantas Med* 2015; **19**(2): 226-234.
- [4] Dutra JCV, Moisés Ferreira J, Pereira PRC, Ben-Hur de Oliveira J, Vitorino Gervásio S, Bernardes Xavier M, et al. *Cereus jamacaru* DC hydroalcoholic extract promotes anti-cytotoxic and antitumor activity. *Pharmaceuticals* 2018; **11**(4): 130.

- [5] Pretti IR, da Luz AC, Jamal CM, Batitucci MCP. Variation of biochemical and antioxidant activity with respect to the phenological stage of *Tithonia diversifolia* Hemsl. (Asteraceae) populations. *Ind Crops Prod* 2018; **121**: 241-249.
- [6] dos Santos PC, Dutra JCV, Delarmelina JM, Tose LV, Romão W, Jamal CM, et al. *Coriandrum sativum* grown under organic or chemical fertilizer effectively prevents DNA damage: Preliminary phytochemical screening, flavonoid content, ESI (-) FT-ICR MS, *in vitro* antioxidant and *in vivo* (mice bone marrow) antimutagenic activity against cyclophosphamide. *Asian Pac J Trop Biomed* 2018; **8**(6): 292.
- [7] Oliveira JB de, Dutra JCV, Gervásio SV, Xavier MB, Pereira PRC, Mota MM, et al. *Bauhinia* sp. sob diferentes condições de sazonalidade induz atividade anticâncer em sarcoma-180 *in vitro*. In: Natiéli Piovesan VBV (ed). *Produção e controle de produtos naturais*. Atena Editora: Ponta Grossa; 2018, p. 42-59.
- [8] Santos VS, Dutra JCV, Gervásio SV, Pereira PRC, Mota MM, Santos PC, et al. *Coriandrum sativum* em estágio vegetativo e floração induz atividade anticâncer *in vitro*. In: Natiéli Piovesan VBV (ed). *Produção e controle de produtos naturais*. Atena Editora: Ponta Grossa; 2018, p. 69-82.
- [9] Gomes VGN, Quirino ZGM, Araujo HFP. Frugivory and seed dispersal by birds in *Cereus jamacaru* DC. ssp. *jamacaru* (Cactaceae) in the Caatinga of Northeastern Brazil. *Brazilian J Biol* 2014; **74**(1): 32-40.
- [10] Fuertes MA, Castilla J, Alonso C, Prez JM. Cisplatin biochemical mechanism of action: From cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. *Curr Med Chem* 2003; **10**(3): 257-266.
- [11] Belcavello L, Dutra JCV, de Freitas JV, Aranha IP, Batitucci MCP. Mutagenicity of ipriflavone *in vivo* and *in vitro*. *Food Chem Toxicol* 2012; **50**(3-4): 996-1000. Doi:10.1016/j.fct.2011.12.013.
- [12] Santana AF. *Composição química e atividades antioxidante e biológica das frações clorofórmica e hidrometanólica do extrato da raiz de Cereus jamacaru DC. (Cactaceae)*. Dissertation Thesis. Feira de Santana State University; 2016.
- [13] Jia ZS, Tang MC, Wu JM. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 1999; **64**(4): 555-559.
- [14] Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Pérez-Jiménez J, et al. Comunicado técnico-metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. *Fortaleza: Embrapa* 2007; **1**(1): 1-4.
- [15] Rufino M, Alves RE, de Brito ES, de Morais SM, Sampaio C de G, Pérez-Jimenez J, et al. Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS*. *Embrapa Agroindústria Trop Comun Técnico* 2007; **1**(1): 1-4.
- [16] Soler-Rivas C, Espín JC, Wichers HJ. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochem Anal An Int J Plant Chem Biochem Tech* 2000; **11**(5): 330-338.
- [17] Cirak C, Radusiene J, Ivanauskas L, Jakstas V, Çamaş N, Kurt D. Phenological changes in the chemical content of wild and greenhouse-grown *Hypericum pruinatum*: Hypericins, gyperforins and phenolic acids. *J Bot* 2015; **4**(3): 37-47.
- [18] Okunlola GO, Jimoh MA, Olatunji OA, Olowolaju ED. Comparative study of the phytochemical contents of *Cochorus olitorius* and *Amaranthus hybridus* at different stages of growth. *Ann West Univ Timisoara Ser Biol* 2017; **20**(1): 43.
- [19] King AMY, Young G. Characteristics and occurrence of phenolic phytochemicals. *J Am Diet Assoc* 1999; **99**(2): 213-218.
- [20] Arba M, Falisse A, Choukr-Allah R, Sindic M. Effect of irrigation at critical stages on the phenology of flowering and fruiting of the cactus *Opuntia* spp. *Brazilian J Biol* 2018; **78**(4): 653-660.
- [21] de Sousa Araújo TA, Alencar NL, de Amorim ELC, de Albuquerque UP. A new approach to study medicinal plants with tannins and flavonoids contents from the local knowledge. *J Ethnopharmacol* 2008; **120**(1): 72-80.
- [22] Lima RKB. *Caracterização e potencial antioxidante do fruto da palma (Tacinga inamoena) e do mandacaru (Cereus jamacaru)*. Dissertation Thesis. Federal Rural University of the Semi-Arid; 2016.
- [23] Genskowsky E, Puente LA, Pérez-Álvarez JA, Fernández-López J, Muñoz LA, Viuda-Martos M. Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry. *J Sci Food Agric* 2016; **96**(12): 4235-4242.
- [24] Číž M, Čížová H, Denev P, Kratchanova M, Slavov A, Lojek A. Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control* 2010; **21**(4): 518-523.
- [25] Shahidi F, Zhong Y. Measurement of antioxidant activity. *J Funct Foods* 2015; **18**: 757-781.
- [26] Torres N, Goicoechea N, Morales F, Antolín MC. Berry quality and antioxidant properties in *Vitis vinifera* cv. *Tempranillo* as affected by clonal variability, mycorrhizal inoculation and temperature. *Crop Pasture Sci* 2016; **67**(9): 961-977.
- [27] Sadras VO, Moran MA. Nonlinear effects of elevated temperature on grapevine phenology. *Agric For Meteorol* 2013; **173**: 107-115.
- [28] Kavamura VN, Taketani RG, Lançon MD, Andreote FD, Mendes R, de Melo IS. Water regime influences bulk soil and rhizosphere of *Cereus jamacaru* bacterial communities in the Brazilian Caatinga biome. *PLoS One* 2013; **8**(9): e73606.
- [29] Sumaya-Martínez MT, Cruz-Jaime S, Madrigal-Santillán E, García-Paredes JD, Cariño-Cortés R, Cruz-Cansino N, et al. Betalain, acid ascorbic, phenolic contents and antioxidant properties of purple, red, yellow and white cactus pears. *Int J Mol Sci* 2011; **12**(10): 6452-6468.
- [30] Korres NE, Norsworthy JK, Tehranchian P, Gitsopoulos TK, Loka DA, Oosterhuis DM, et al. Cultivars to face climate change effects on crops and weeds: A review. *Agron Sustain Dev* 2016; **36**(1): 12.
- [31] Misra A. Studies on biochemical and physiological aspects in relation to phyto-medicinal qualities and efficacy of the active ingredients during the handling, cultivation and harvesting of the medicinal plants. *J Med Plants Res* 2009; **3**(13): 1140-1146.
- [32] Manikandaselvi S, Vadivel V, Brindha P. Studies on physicochemical and nutritional properties of aerial parts of *Cassia occidentalis* L. *J Food Drug Anal* 2016; **24**(3): 508-515.
- [33] Fiehn O. Metabolomics-the link between genotypes and phenotypes. *Plant Mol Biol* 2002; **48**(2): 155-171.
- [34] Kayesh E, Shangguan L, Korir NK, Sun X, Bilkish N, Zhang Y, et al. Fruit skin color and the role of anthocyanin. *Acta Physiol Plant* 2013; **35**(10): 2879-2890.
- [35] Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *Plant J* 2008; **54**(4): 733-749.
- [36] McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, et al. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: A randomised, double-blinded, placebo-controlled trial. *Lancet* 2007; **370**(9598): 1560-1567.
- [37] Piccolella S, Crescente G, Pacifico F, Pacifico S. Wild aromatic plants bioactivity: A function of their (poly) phenol seasonality? A case study from Mediterranean area. *Phytochem Rev* 2018; **17**(4): 785-799.
- [38] Dewick PM. The shikimate pathway: Aromatic amino acids and phenylpropanoids. *Medicinal natural products: A biosynthetic approach*. 3rd ed. Nottingham, U.K; John Wiley & Sons; 2009, p. 137-186.
- [39] Vogt T. Phenylpropanoid biosynthesis. *Mol Plant* 2010; **3**(1): 2-20.