

1       **A preliminary study of the cytotoxicity of the protein extract of *Chrysobalanus***  
2       ***icaco* L. and *Eugenia astringens* Cambess., commercialized in markets**

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11  
12       **Abstract**

13       The use of plants and their products for medical treatment is very common  
14       procedure in Brazil, especially for treatment of diabetes. In fact, several plants can  
15       demonstrate hypoglycemic effects in vitro assays. However, the use for human  
16       treatment requires the knowledge of their toxicological properties. The aim of this study  
17       was to evaluate the effect of protein extracts of *Chrysobalanus icaco* collected from  
18       natural habitats and of *Eugenia astringens* acquired from the market of Rio de Janeiro  
19       on the viability and migration of fibroblasts. *E. astringens* has a similar morphology as  
20       *C. icaco* and it is sold as *Chrysobalanus* in a popular market in Rio de Janeiro. Being a  
21       different plant, *E. astringens* expresses different proteins, and its protein extract has  
22       proved to possess higher toxic properties than *C. icaco* does. Cytotoxicity assays  
23       indicated that, as the protein extract concentration increases, fibroblast viability  
24       decreases. Only the *E. astringens* extract displayed cytotoxicity at all concentrations, in  
25       addition to reduced fibroblast migration. The results obtained in this study demonstrates  
26       that it's necessary integrative policies for rational use of medicinal plants and their  
27       commercialization, since the current use of medicinal plants may be inadequate and it is  
28       of great importance for Public Health.

29       **Keywords**

30       Cytotoxicity; Protein extract; Hypoglycemic plant; *Chrysobalanus icaco*; *Eugenia*  
31       *astringens*; popular markets

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Abbreviations: MAD, Mercado de Madureira; PG, Praia Grande; RMA, Restinga de Massambaba; AL, Marechal Deodoro; MTT, 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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## 32 **1. Introduction**

33

34 Several plants are widely used for medical purposes by the population, but this  
35 use is most often made from a lay indication, without knowing the risks of toxic effects.  
36 Besides, there is no guarantee of the provenance and proper storage of these supposedly  
37 “medicinal plants”. It is clear that there is a lack of incentive and scientifically-based  
38 information to integrative and complementary practices and actions to promote the safe  
39 and rational use of medicinal plants, including information on how the species should  
40 be prepared and used by the population (Bochner *et al.*, 2012).

41 The leaf extract (tea) of the plant *Chrysobalanus icaco* L., a species of restinga  
42 popularly known as abajerú, is used in folk medicine because it exerts biological  
43 activities, such as the decrease of blood sugar levels, being indicated for the treatment of  
44 diabetes, besides be diuretic and antioxidant (Venancio *et al.*, 2018). Another plant  
45 (*Eugenia astringens*, Cambess.) which is morphologically similar to *C. icaco*, also is  
46 known by the same popular name of abajerú and is commercialized as *C icaco* (Bochner  
47 *et al.*, 2012; Silva and Peixoto, 2009). These two species may not possess the same  
48 therapeutic and toxicological properties, which are of concern to Public Health. The  
49 attribution of hypoglycemic activity to *E. astringens* may indicate a misconception  
50 since other species of Myrtaceae have hypoglycemic potential (Silva and Peixoto,  
51 2009). So, in order to clarify the toxicological aspects of the extract obtained from these  
52 2 plants, a cytotoxic assay was performed.

53 For cytotoxicity studies in animal cells several techniques, using distinct cell  
54 types as a target, are available. Cytotoxicity means the determination of any toxic effects  
55 at the cellular level, such as changes in membrane permeability, cell death or enzymatic  
56 inhibition resulted from exposure to a toxicant, in this case, the studied plants or plant  
57 products (Stockert *et al.*, 2012).

58 Cell viability can be evaluated by several methods, among which the one which  
59 involves the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium  
60 bromide (MTT) to formazan by mitochondrial reactivation in active-living cells (Zandi  
61 *et al.*, 2016). The MTT assay is a standard colorimetric assay that estimates the  
62 cytotoxic potential of the samples, in addition to measuring the cellular proliferation of  
63 drug agents. Cell viability is expressed as a percentage of live cells from the tested  
64 material, comparing with the percentage of cells of the cytotoxicity positive control  
65 (Stockert *et al.*, 2018).

66 Another test to evaluate the toxicity of the plant extract is the Scratch Wound  
67 Healing Assay, which allows measuring the migration of cells that is a phenomenon  
68 present in the healing process. It is a method in which a crack imitates a wound in a  
69 monolayer of confluent cells so that the cells at the edge gradually move towards the  
70 crack (Manoj *et al.*, 2009). Wound healing is the process of repairing and regenerating  
71 the dermis and epidermis that accompanies the lesions (Liang *et al.*, 2007; Pitz *et al.*,  
72 2016). The evaluation of the healing activity of plant extracts is scarce at the cellular  
73 level. Fibroblast cultures have been proposed as a method for the investigation of  
74 wound healing activity, since these cells are the main source of extracellular connective  
75 tissue matrix and the migration of fibroblasts is considered vital for rapid and effective  
76 skin repair damaged (Manoj *et al.*, 2009).

77 The lack of data about the toxicity of the protein extract of these 2 plants  
78 commercialized as abajerú (*C. icaco* and *E. astringens*), protein extracts of  
79 *Chrysobalanus icaco* collected directly from its natural habitats and of *Eugenia*  
80 *astringens* acquired from the market of Rio de Janeiro was performed using the viability  
81 and migration of fibroblasts assay.

82

## 83 **2. Material and methods**

84

### 85 *2.1 Plant sampling*

86

87 *Chrysobalanus icaco* leaves were collected directly from its natural habitats,  
88 Praia Grande – Arraial do Cabo- RJ (PG; -22,9696606, -42,0302859), Restinga de  
89 Massambaba – RJ (RMA; -22,9337727, -42,4267012), Marechal Deodoro – AL (AL; -  
90 9,7823233, -35,852364), as shown in the map (Fig. 1). *Eugenia astringens* leaves were  
91 purchased on the market Mercado de Madureira located in the North zone of Rio de  
92 Janeiro city.



93

94 **Fig. 1.** Sampling sites. AL - Marechal Deodoro, MAD – Mercadão de Madureira, RMA  
95 - Restinga de Massambaba, PG - Praia Grande (PG).

96

## 97 2.1 Protein extraction

98

99 About 10 mg of each lyophilized sample were weighed into microtubes in  
100 triplicate. Samples were incubated in the presence of 400  $\mu$ l lysis buffer (4% SDS 0.1 M  
101 Tris-HCl buffer pH 7.6) at 95 °C for 15 min in a thermomixer. The lysate extract was  
102 frozen at -80 °C for further quantification of total proteins by the Lowry method (Lowry  
103 et al., 1951), using bovine serum albumin (2.0 mg/mL) as the standard for the analytical  
104 curve. Samples (2  $\mu$ L) and analytical curve (0, 10, 20, 30, 40, 50, 60 and 70  $\mu$ g/mL)  
105 were read in a Jasco V-530 spectrophotometer at the wavelength of 750 nm.

106

## 107 2.2 Cytotoxicity evaluation of protein extracts

108

109 This assay was performed as follows:

110

### 111 2.2.1 Cell culture

112

113 Fibroblasts (3T3 cell line) were kept in medium Dulbecco's Modified Eagle  
114 Medium (DMEM), containing 10 % fetal bovine serum, L-glutamine (2 mM) and  
115 gentamicin (40  $\mu$ g/mL) in incubator at 37 °C and 5 % CO<sub>2</sub>.

116

#### 117 2.2.1.1 Cell viability assay

118

119 The effect of the protein extracts of *Eugenia astringens* (Mercadão de  
120 Madureira) and *Chrysobalanus icaco* (Restinga de Massambaba - RJ, Marechal - AL,  
121 Praia Grande - RJ) on fibroblasts viability was evaluated through the MTT assay  
122 (Mosmann, 1983).

123 The cells were seeded in a 96 well plates and placed in CO<sub>2</sub> incubator overnight.  
124 The cells were then treated with different sample solutions (1, 5, 10 and 20 µg/mL) in  
125 four replicates. The control group was treated only with the medium (DMEM). After  
126 treatment, a solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium  
127 bromide) (5 mg/mL in phosphate buffered saline - 1X PBS pH 7.4) was added to each  
128 well and incubated for 4 hours. Subsequently, the supernatant was discarded and 150 µl  
129 of dimethyl sulfoxide were added for solubilization of the formazan crystals. The  
130 absorbance was measured using a microplate spectrophotometer (DTX 880 Multimode  
131 Detector, Beckman Coulter), adjusted to 595 nm, and the optical density was calculated  
132 (Equation 1).

133

134 Equation 1 – Optical density of cells submitted to the cell viability assay.

135

$$A = \frac{DO_t}{DO_{nt}} \times 100$$

136

DO<sub>t</sub> – optical density of treated cells

137

DO<sub>nt</sub> – optical density of non-treated cells

138

#### 139 2.2.1.2 Scratch wound healing assay

140

141 The effect of the protein extracts of *Eugenia astringens* (Mercadão de  
142 Madureira), *Chrysobalanus icaco* (Restinga de Massambaba - RJ, Marechal - AL, Praia  
143 Grande - RJ - branch) on fibroblast migration was evaluated through cell migration  
144 technique, method described by Liang *et al.* (2007).

145

146 Cells (7 x 10<sup>4</sup> cells / well, measured by the Newbauer's chamber) were seeded in  
147 24-well plates and maintained overnight for cell adhesion and formation of a monolayer  
148 at approximately 80% confluency. A small part of the monolayer was removed in the  
149 middle of the plate with a 200 µL pipette tip (a scratch is placed on the monolayer and  
150 the part removed is discarded). Cells were washed with phosphate buffered saline and  
151 treated with 5 µg/mL of the samples or culture medium (control) in triplicate. This  
152 exposure concentration at which some effects started to be observed in the cell viability  
153 assay was chosen to perform the present assay. Cell migration was assessed by  
photomicrographs at 0- and 24-hours post-exposure using an inverted microscope

154 (Olympus IX70) with digital camera to measure the area of wound closure. The  
155 photomicrographs were analyzed using Image J software and cell migration was  
156 expressed as the area in pixels, so that the percentage of closure of the initial area  
157 formed was determined quantitatively (Equation 2).

158

159 Equation 2 – Migration rate of fibroblasts submitted to the cell migration assay.

$$\% \text{ migration} = \frac{(A_0 - A_t)}{A_0} \times 100$$

160

161

$A_0$  – original area (time = 0 h)

162

$A_t$  – area after the scratch (time = 24 h).

163

## 164 2.4 Statistical analysis

165

166 The results of the cell viability and migration tests were expressed as mean  $\pm$   
167 standard error, performed in triplicate and analyzed statistically using analysis of  
168 variance (ANOVA), followed by Newman-Keuls post-test. The results were considered  
169 significant when  $p < 0.05$ . All results were analyzed using GraphPad Prism® software  
170 version 5.01 (GraphPad Software Inc, San Diego CA, USA).

171

## 172 3. Results

173

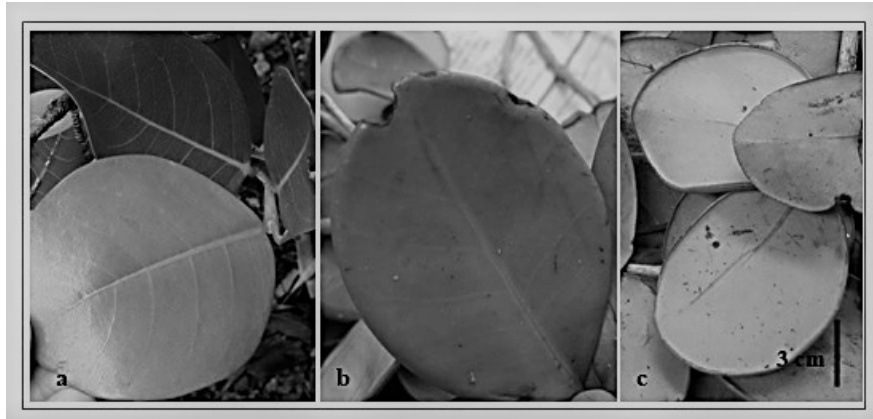
### 174 3.1 Plant identification and protein concentration

175

176 The identification of the studied plants was performed by a botanist from the  
177 Jardim Botânico do Rio de Janeiro. The plant purchased on the market (Mercadão de  
178 Madureira) was identified as *Eugenia astringens* Cambess., of family Myrtaceae, and  
179 the plants collected in Marechal Deodoro, Massambaba and Praia Grande as  
180 *Chrysobalanus icaco* L., plant from family Chrysobalanaceae.

181 Chrysobalanaceae can be morphologically differentiated from the Brazilian  
182 Myrtaceae species, by some characteristics, such as phyllotaxia, which is alternating  
183 (and opposite in Myrtaceae). Nevertheless, the similar form of the leaves of *C. icaco*  
184 and *E. astringens* can cause confusion (Fig. 2), the *E. astringens* leaf has a fold around  
185 it facing the abaxial part (Fig. 2c) not found in *C. icaco*.

186



187

188 **Fig. 2.** Comparison between the branches of *Chrysobalanus icaco* L.  
189 (Chrysobalanaceae) (a) and *Eugenia astringens* Cambess (Myrtaceae) (b). Abaxial part  
190 of *E. astringens* leaf (c). Source: Photos by the author.

191

192 This misconception has been previously reported (Bochner *et al.*, 2012; Silva  
193 and Peixoto, 2009), claiming that the trade of medicinal plants is not a safe source of  
194 sale, as it may be difficult for both the trader and the consumer to correctly identify a  
195 desirable plant. And yet there is the problem that different plants known by the same  
196 popular name are commercialized without proof of their pharmacological properties and  
197 toxicological safety (Bochner *et al.*, 2012), besides the adulteration possibilities.  
198 Unfortunately, in Brazil, the supervision of trade of medicinal plants by regulatory  
199 agencies is still incipient.

200 Total protein concentrations ranged from 30.18 to 54.95  $\mu\text{g } \mu\text{L}^{-1}$  in *Eugenia*  
201 *astringens*, from 28.01 to 43.88  $\mu\text{g } \mu\text{L}^{-1}$  in *Chrysobalanus icaco*.

202

### 203 3.2 Cytotoxicity evaluation of protein extracts

204

205 Fibroblasts (3T3 cell line) were submitted to the cell viability assay, exposed to  
206 different concentrations of protein extract and to the cell migration assay, exposed to a  
207 determined concentration of this extract.

208

#### 209 3.2.1 Cell viability assay

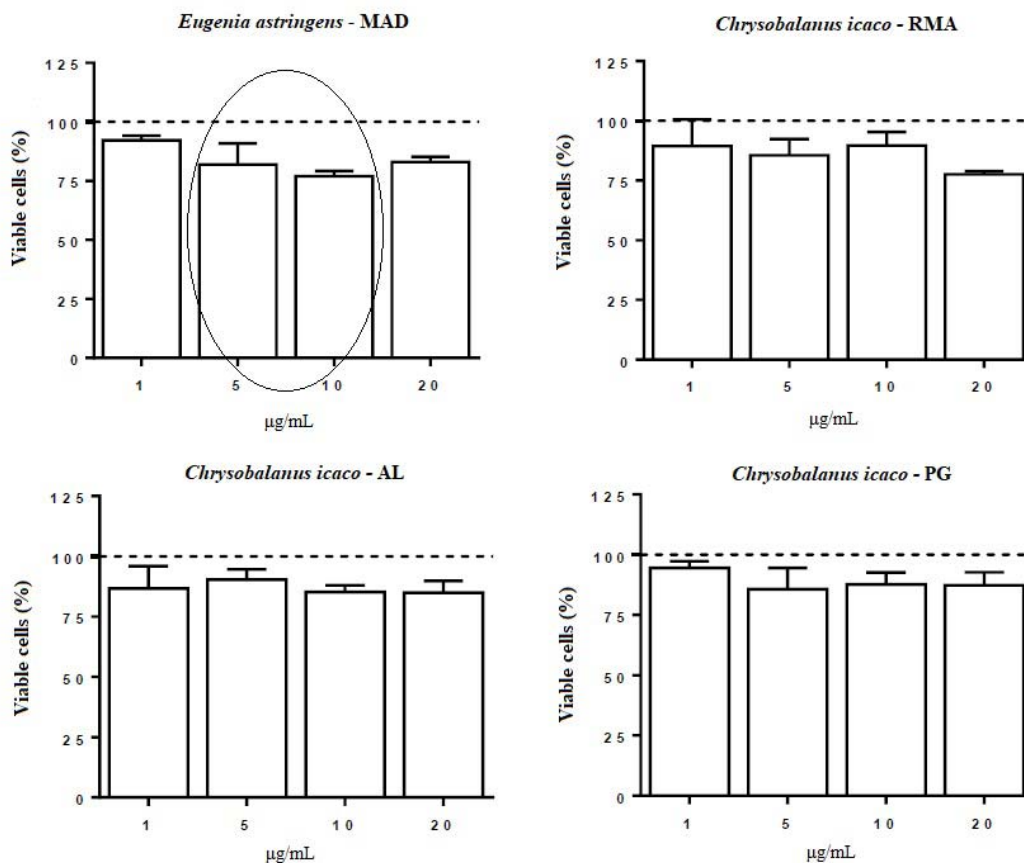
210

211 To evaluate the effects of extracts of *E. astringens* (MAD), *C. icaco* (RMA), *C.*  
212 *icaco* (AL) and *C. icaco* (PG) on fibroblast viability, the MTT assay was performed.



213 The results for the cell viability assay are shown in Fig. 3, in which it can be  
214 observed and compared the reduction of fibroblasts viability among the species and  
215 protein extract concentration.

216 Treatment with *E. astringens* (MAD) at all concentrations tested, reduced cell  
217 viability, decreasing by 8.8% (1  $\mu\text{g/mL}$ ), 19.2% (5  $\mu\text{g/mL}$ ), 23% (10  $\mu\text{g/mL}$ ) and 17%  
218 (20  $\mu\text{g/mL}$ ) the percentage of viable cells. Exposure with *C. icaco* (RMA) at  
219 concentrations of 1, 5 and 10  $\mu\text{g/mL}$  did not alter significantly the fibroblasts viability.  
220 On the other hand, the increase in concentration resulted in a decrease in the percentage  
221 of viable cells, leading to a reduction of 22.4% ( $P < 0.001$ ) in cell viability when the  
222 highest concentration (20  $\mu\text{g/mL}$ ) was used. Treatment with *C. icaco* (AL), in turn,  
223 induced a decrease in cell viability (8-16 %) at all concentrations tested, when compared  
224 to the control group. In addition, treatment with *C. icaco* (PG) at 1  $\mu\text{g/mL}$  did not alter  
225 the viability of fibroblasts, whereas treatment with the other concentrations induced a  
226 decrease in cell viability (12-14 %).  
227



228



229 **Fig. 3.** Effect of protein extracts of *Eugenia astringens* and *Chrysobalanus icaco* on  
230 fibroblasts viability. MAD – Mercadão de Madureira, RMA - Restinga de Massambaba,  
231 AL - Marechal Deodoro, PG - Praia Grande (PG). The dashed line represents the control  
232 group (100 %). The circle indicates high reduction on fibroblast viability for *E.*  
233 *astringens* treatment. Results are mean  $\pm$  S.E.M. n = 4.

234

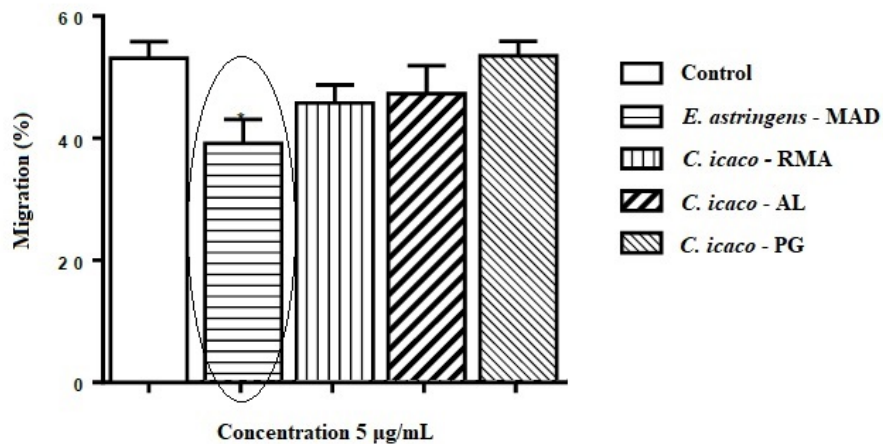
### 235 3.2.2 Scratch wound healing assay

236

237 To evaluate the effects of extracts of *E. astringens* (MAD), *C. icaco* (RMA), *C.*  
238 *icaco* (AL) and *C. icaco* (PG) on fibroblast migration, the cell migration assay (Scratch  
239 Wound Healing Assay) was performed.

240 As shown in Figure 4, treatment with *C. icaco* (RMA), *C. icaco* (AL) and *C.*  
241 *icaco* (PG) was not able to alter the migration rate of fibroblasts. On the other hand, it  
242 can be noted that the treatment with *E. astringens* led to a significant reduction in the  
243 migration of these cells by 26.04% (p <0.05), comparing to the control.

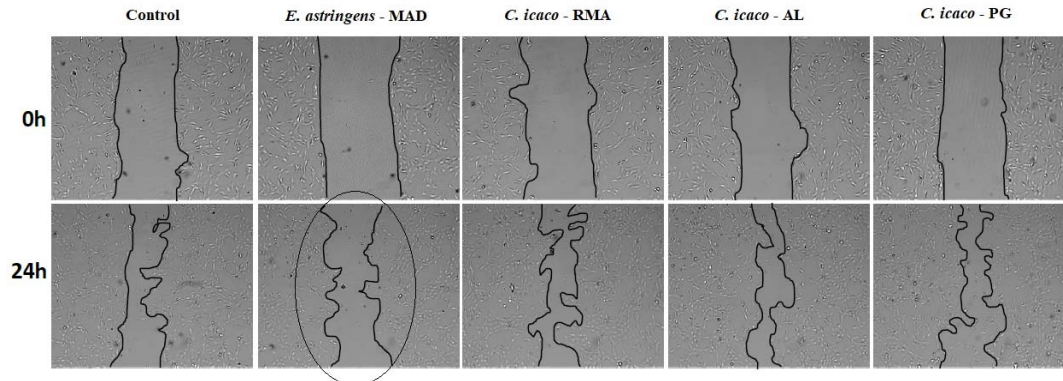
244 The migration of fibroblast is illustrated in Fig. 5, in which can be observed a  
245 lower migration of these cells when treated with *E. astringens* protein extract than the  
246 *C. icaco* treatments, slowing wound closure.



247

248 **Fig. 4.** Effect of the extracts of *Eugenia astringens* and *Chrysobalanus icaco* on the  
249 migration of fibroblasts at times 0 and 24 hours. MAD – Mercadão de Madureira, RMA  
250 - Restinga de Massambaba, AL - Marechal Deodoro, PG - Praia Grande (PG). Circle  
251 indicates significant reduction on fibroblast migration for *E. astringens* treatment.  
252 Results are mean  $\pm$  S.E.M. One-way Anova, followed by Newman-Keuls post-test, \*p  
253 <0.05. n = 3.

254



255  
256 **Fig. 5.** Effect of the extracts of *Eugenia astringens* and *Chrysobalanus icaco* on the  
257 migration of fibroblasts (3T3) at 0 h and 24 hours. MAD – Mercado de Madureira,  
258 RMA – Restinga de Massambaba, AL – Marechal Deodoro, PG - Praia Grande. Circle  
259 indicates significant reduction on fibroblast migration for *E. astringens* treatment. n=3.

260

#### 261 4. Discussion

262

263 There is a misunderstanding regarding the sale of abajerú in Mercado de  
264 Madureira, where *Eugenia astringens*, of the same popular name, is sold in place of  
265 *Chrysobalanus icaco*. This is of great concern to Public Health, because *C. icaco* is  
266 popularly used as medicinal plant for treating diabetes, due to its hypoglycemic  
267 potential. Meanwhile the population consumes tea from the leaves of *E. astringens*,  
268 coming from these markets instead of *C. icaco*. Medicinal plants are widely used due to  
269 their easy accessibility, but they usually not have their efficacy and safety well  
270 established (Bochner *et al.*, 2012). This fact can become a risk to those who use them  
271 since they can cause more deleterious effects than bring health benefits. It is of prime  
272 importance to inspect qualified individuals, traders, distributors and producers for  
273 regularization of the sale of medicinal plants.

274 Silva and Peixoto (2009) raised three hypotheses regarding the introduction of  
275 *Eugenia astringens*, replacing *Chrysobalanus icaco* in popular marketing. First, it  
276 would be a strategy of the merchants to circumvent the competent oversight, by having  
277 the same popular name, but neither could it distinguish. A second hypothesis would be  
278 related to the difficulty in the recognition of the species by the collectors and sellers, as  
279 well as the consumers, due to the similar morphology. The last hypothesis would be the  
280 attribution of hypoglycemic activity to *E. astringens* by herbivores, since other species  
281 of Myrtaceae, such as pitanga (*Eugenia uniflora* L.), jambo (*Eugenia jambos* L.) and  
282 *Eucalyptus*, are used by the population for this purpose and have antioxidant, antifungal

283 and antibacterial properties (Queiroz *et al.*, 2015). Also, the natural environments to  
284 which *C. icaco* occurs are restinga-type vegetation sites, which are usually areas of  
285 environmental protection, which makes it difficult to collect specimens of this species.  
286 Therefore, this could also be a hypothesis regarding the introduction of *E. astringens*,  
287 replacing *C. icaco* in popular marketing. This species is not hypoglycemic like *C. icaco*,  
288 which can lead to intoxication in those people who buy erroneously, thinking that they  
289 are acquiring the correct abajerú plant.

290 Since medicinal plants may also have unknown toxicological properties, the  
291 evaluation of toxicity, through in vitro tests, is required. Cytotoxicity of the extracts of  
292 medicinal plants, including those that are hypoglycemic, can affect cellular processes  
293 like healing that is crucial for diabetic patients. Hyperglycemia alters leukocyte  
294 function, increasing the risk of bleeding and impairing inflammatory and healing  
295 processes (Negri, 2005; Aquino *et al.*, 2019). This difficulty in healing occurs due to  
296 cardiovascular complications, which cause blockage or decrease of blood circulation,  
297 and due to excess glucose, which can impair the functioning of the immune system.  
298 That is, diseased vessels decrease blood flow, especially to the legs and feet, harming  
299 the healing process and high glycemic levels incapacitate the body's defense cells (Hu *et*  
300 *al.*, 2002).

301 Zandi *et al.* (2016) verified the viability of fibroblasts (ovine line) in extracts of  
302 different plants (*Aloe vera*, hena, camomile, licorice, myrtle, mint, cinnamon, ginger  
303 and cedar) and that at the minimum concentration (6.25 µg/mL), the viability of dermal  
304 fibroblasts by MTT assay increased significantly in cedar ( $p < 0.05$ ). Combination of  
305 *Aloe vera*, mint extract and licorice significantly increased the viability of dermal  
306 fibroblasts ( $p < 0.05$ ). *Aloe vera*, which is also known for its hypoglycemic activity, has  
307 the ability to stimulate proliferation of L929 fibroblasts (Manoj *et al.*, 2009). Calloni *et*  
308 *al.* (2016) tested the phenolic extract of *Plinia trunciflora* from the same family as *E.*  
309 *astringens* on human lung fibroblast cells in the presence and absence of amiodarone, a  
310 drug used to treat arrhythmia, but which causes toxicity in the lungs. The extract rich in  
311 polyphenols was able to prevent the decrease of cellular viability (MTT test) and the  
312 ATP biosynthesis.

313 There are no studies testing the viability of fibroblasts exposed to protein  
314 extracts of *Chrysobalanus icaco*, but ethanolic extracts of these species prove to be  
315 important in cellular processes. Silva *et al.* (2017) evaluated the antifungal activity of  
316 the *C. icaco* ethanolic extract, noting the inhibition of growth of *Candida albicans* and

317 *C. parapsilosis*, strains exposed to this extract.

318 Pitz *et al.* (2016) evaluated the in vitro activity of ethanolic extract of *Plinia*  
319 *peruviana* bark, the same family as the *E. astringens*, in healing processes and  
320 antioxidant activity in urinary fibroblasts (L929 cell line). The cell migration assay  
321 (Scratch Wound Healing Assay) indicated that none of the tested shell concentrations  
322 (0.5, 5, 25, 50 and 100 µg/ml) was able to increase the migration rate after 12 hours of  
323 incubation. These results demonstrate a positive effect of the peel on the wound healing  
324 process in the L929 fibroblast cell line, probably due to the antioxidant activity  
325 exhibited by phytochemicals in the extract. Manoj *et al.* (2009) verified the effect of  
326 germplasm of *Aloe vera*, which is also hypoglycemic in L929 fibroblasts, through the  
327 cell migration assay, confirming the increase in fibroblast migration, which is important  
328 for regeneration and skin repair in case of injury.

329 There are no studies testing the viability and migration of fibroblasts exposed to  
330 protein extracts of *C. icaco* and *E. astringens*, however ethanolic extracts are used in  
331 studies to test toxicity of *Eugenia* species. The in vitro antioxidant activity of the  
332 ethanolic extract of *Eugenia uniflora* was determined by the inhibition of spontaneous  
333 autoxidation in brain homogenate, with the LD<sub>50</sub> of 5.93 g/kg in mice (Auricchio *et al.*,  
334 2007). In the phytotoxicity test of the *Eugenia catharinae* extract, it was observed that  
335 ethyl acetate and hexane fractions inhibited seed germination, while the hexane fraction  
336 showed higher inhibition of lettuce seedlings. *E. catharinae* demonstrated a  
337 considerable toxic activity, encouraging the search for the compounds responsible for  
338 this activity (Colla and Brighente, 2011).

339

## 340 **Conclusion**

341

342 The assays to evaluate the toxicity of the protein extracts of the plants studied  
343 served to make aware of the sale and use of the *Eugenia astringens* plant, sold in place  
344 of *Chrysobalanus icaco*, since it reduced cell viability at all concentrations of the extract  
345 and decreased the fibroblast migration rate. These results showed that *E. astringens* can  
346 cause cytotoxic effects if consumed in larger doses.

347 The present work demonstrated the importance of research in the area of Public  
348 Health and the dissemination and communication to society of the results of scientific  
349 works since, due to the confounding of the use of medicinal plants, diabetic patients  
350 may opt for natural products in therapeutic use for the treatment of diabetes, in the

351 wrong way.

352

### 353 **Acknowledgements**

354

355 The authors are thankful to CNPq, to CAPES and to Dra. Viviane Kruehl from the  
356 Research Institute of Botanical Garden of Rio de Janeiro for the identification of the  
357 specimens collected. The cytotoxicity assays were performed at the Laboratory of Cell  
358 Biology of the Federal University of Alagoas under the supervision of Dr. Emiliano  
359 Barreto. This study was carried out with financial support from the Coordination and  
360 Improvement of Higher Level or Education Personnel – CAPES (PhD's grant).

361

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363

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Alagoas

Marechal Deodoro (AL)

Rio de Janeiro

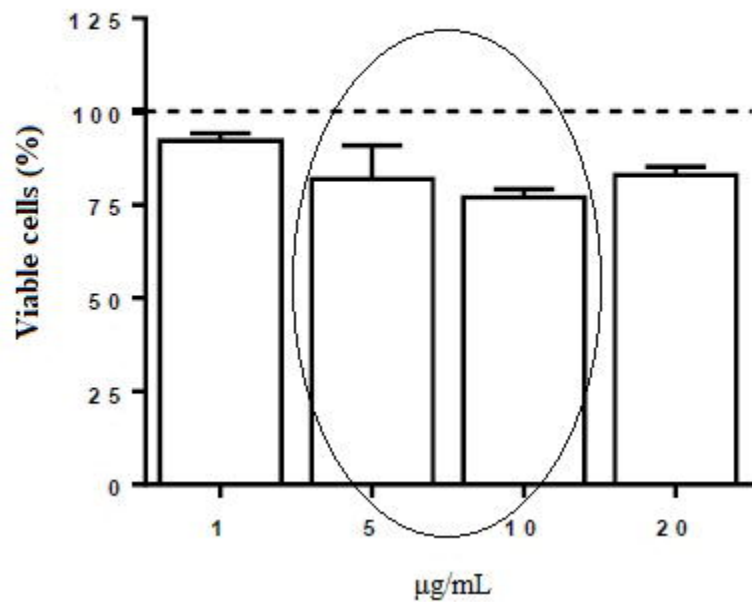
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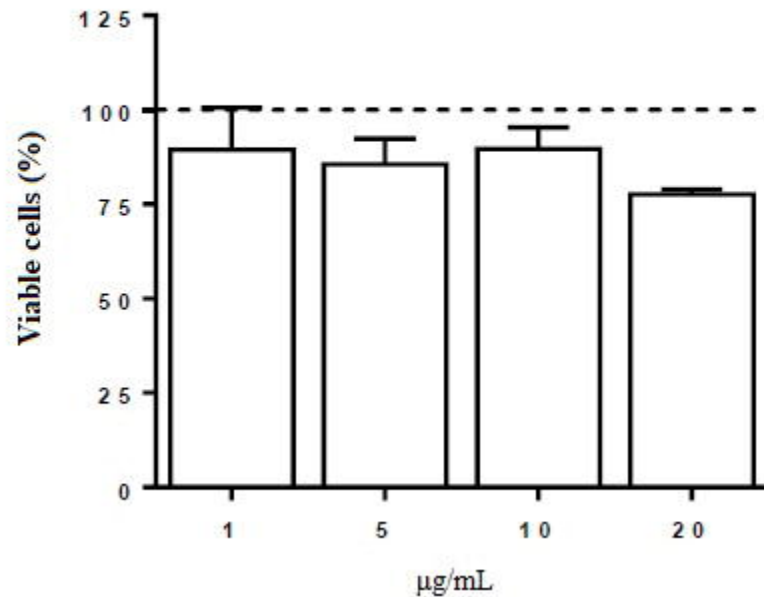
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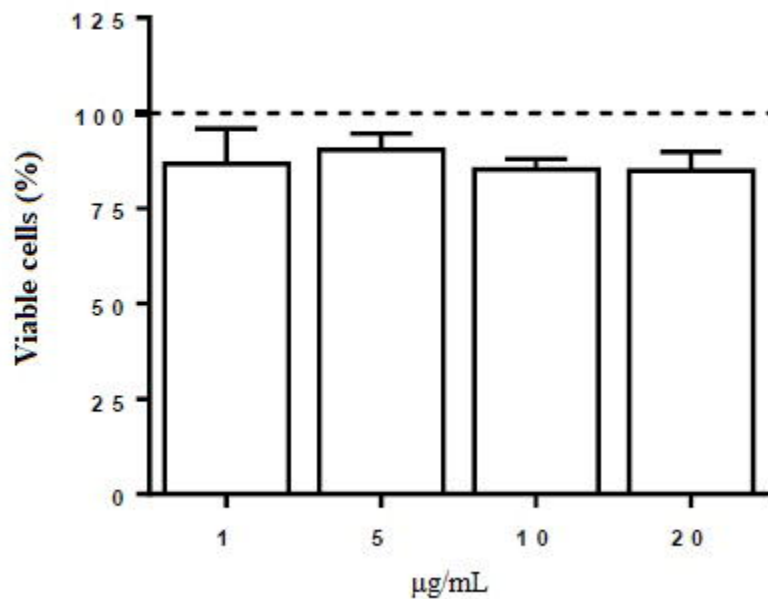
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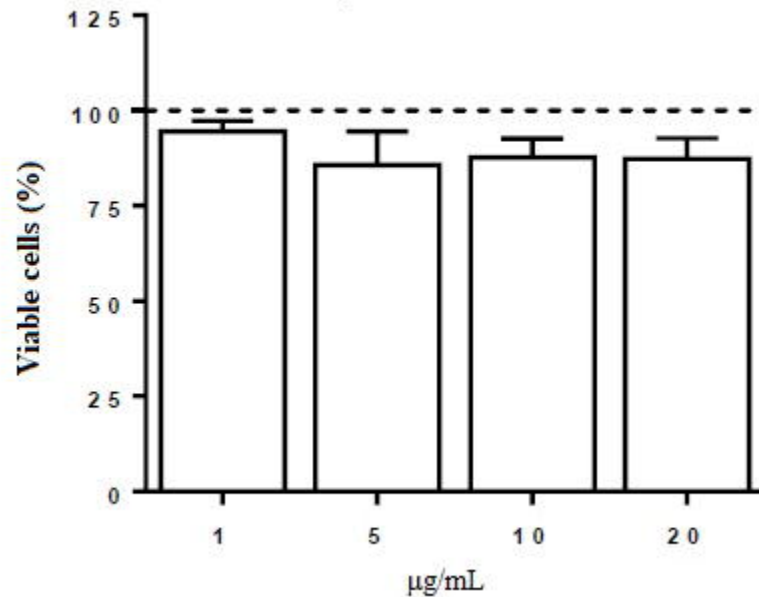
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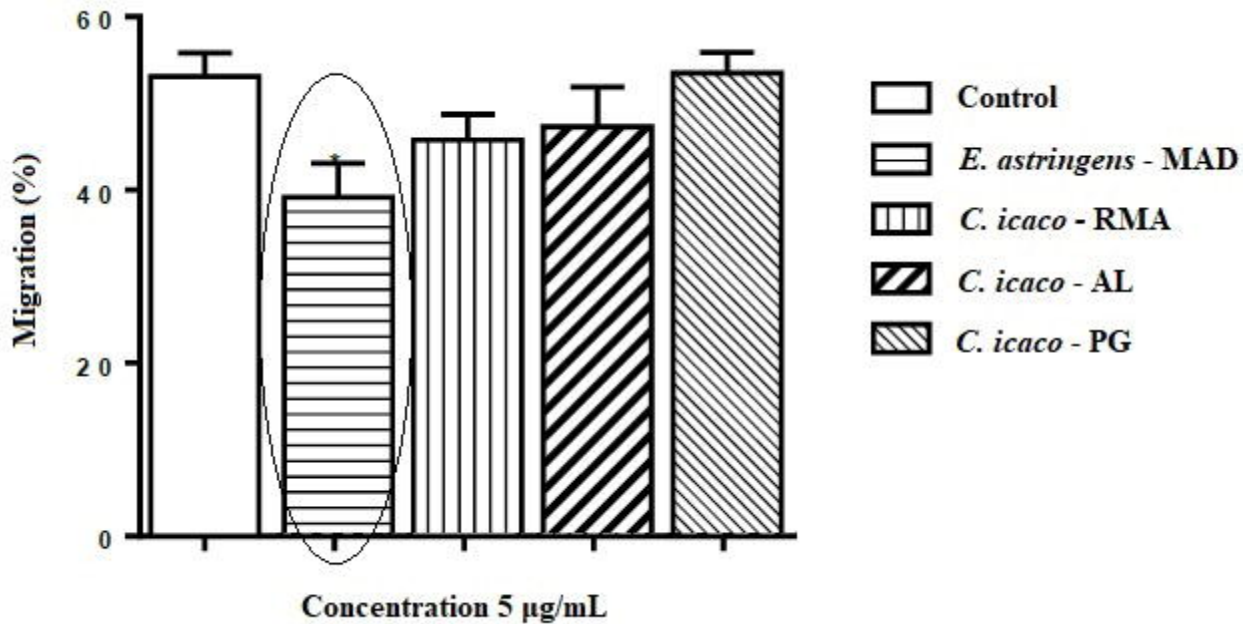


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*Chrysobalanus icaco* - PG





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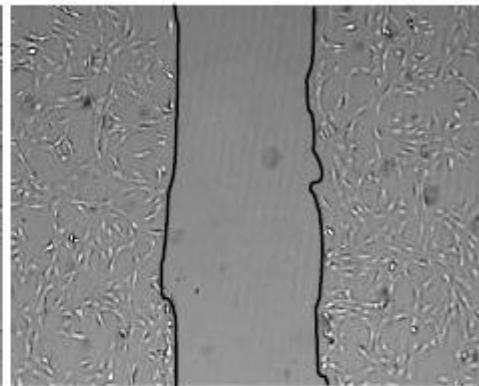
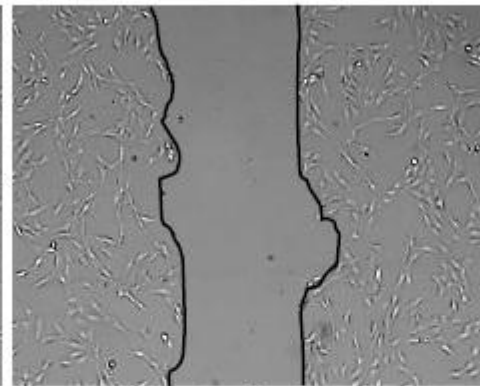
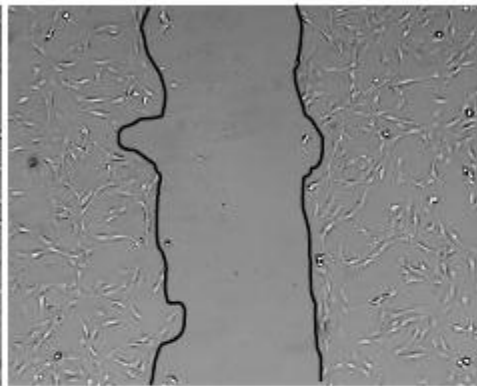
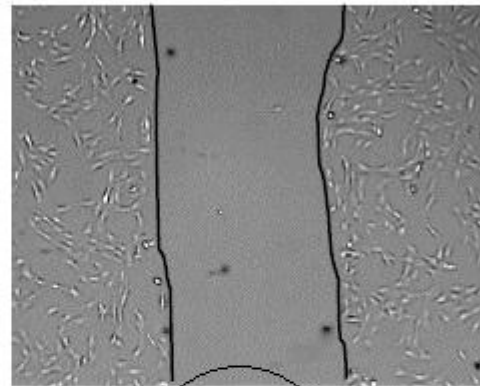
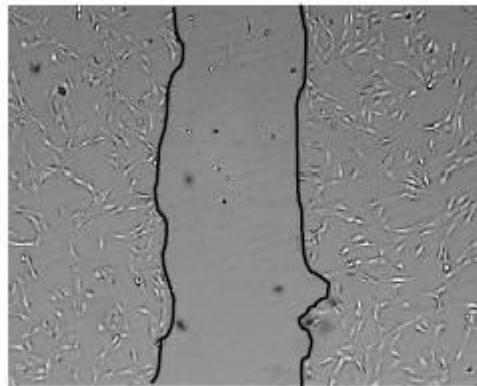
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*C. icaco* - RMA

*C. icaco* - AL

*C. icaco* - PG

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