Chemical evaluation and biological activity of bioactive compounds from 
Campomanesia xanthocarpa Berg

Avaliação química e atividade biológica de compostos bioativos da Campomanesia xanthocarpa Berg

Evaluación química y actividad biológica de compuestos bioactivos de Campomanesia xanthocarpa Berg

Received: 06/09/2022 | Reviewed: 06/26/2022 | Accept: 07/01/2022 | Published: 07/20/2022

Viviane Dalastra
ORCID: https://orcid.org/0000-0002-9852-7542
Universidade Federal da Fronteira Sul, Brazil
E-mail: vivianedalastra@outlook.com

Caroline Kael
ORCID: https://orcid.org/0000-0003-4649-0247
Instituto Pequeno Príncipe, Brazil
E-mail: carol_kael@gmail.com

Sandro José Ribeiro Bonatto
ORCID: https://orcid.org/0000-0001-7262-3766
Instituto Pequeno Príncipe, Brazil
E-mail: sandrobonatto@gmail.com

Ricardo Key Yamazaki
ORCID: https://orcid.org/0000-0003-2922-5572
Universidade Federal da Fronteira Sul, Brazil
E-mail: ricardo.yamazaki@ufsc.edu.br

Abstract

Campomanesia xanthocarpa Berg. (Myrtaceae), also known as “guabiroba”, is widely distributed in the South and Southern regions of Brazil and has been used by local families as folk medicine to treat several diseases. This study aimed to evaluate the bioactive compounds of guabiroba fruits and leaves, such as flavonoids, carotenoids, chlorophyll, total phenolics, tannins, and coumarins. The biological effect of different parts of C. xanthocarpa (leaf and pulp) was also investigated for the immunomodulatory activity on RAW 264.7 macrophages, and anti-cancer effects on H295R adrenocortical cells under different concentrations and extraction protocols. Extracts from guabiroba leaves and pulp using 70% ethanol and n-hexane as a solvent by magnetic stirring, as well as ethanolic extracts from leaves and pulp obtained by heat reflux extraction were produced. Most of the bioactive compounds were found in the leaves rather than the fruit pulp. The in vitro assays showed that the leaf extraction with hexane affected the viability assay in the adrenocortical cell line H295R, while the pulp extracted with hexane activated the macrophage cell line RAW 264.7. Therefore, C. xanthocarpa leaves and pulp have proven to affect macrophage and adrenocortical cell lines in a dose-dependent manner.

Keywords: Guabiroba; Functional foods; Anti-inflammation; Anticancer.

Resumo

Campomanesia xanthocarpa Berg. (Myrtaceae), também conhecida como “guabiroba”, é amplamente distribuída nas regiões Sul e Sudeste do Brasil e tem sido utilizada por famílias locais como remédio popular para o tratamento de diversas doenças. Este trabalho teve como objetivo avaliar os compostos bioativos dos frutos e folhas da guabiroba, como flavonóides, carotenóides, clorofila, fenólicos totais, taninos e cumarinas. O efeito biológico de diferentes partes de C. xanthocarpa (folha e polpa) também foi investigado quanto à atividade imunomoduladora em macrófagos RAW 264.7, e efeitos anticâncer em células H295R adrenocorticais sob diferentes concentrações e protocolos de extração. Extratos de folhas e polpa de guabiroba utilizando etanol 70% e n-hexano como solvente por agitação magnética, bem como extratos etanólicos de folhas e polpa obtidos por extração por refluxo térmico foram produzidos. A maioria dos compostos bioativos foi encontrada nas folhas e não na polpa do fruto. Os ensaios in vitro mostraram que a extração de folhas com hexano afetou o ensaio de viabilidade na linhagem de células adrenocorticais H295R, enquanto a polpa extraída com hexano ativou a linhagem de macrófagos RAW 264.7. Portanto, as folhas e polpa de C. xanthocarpa demonstraram afetar macrófagos e linhagens de células adrenocorticais de maneira dose-dependente.

Palavras-chave: Guabiroba; Alimentos funcionais; Anti-inflamação; Anticâncer.
Resumen
Campomanesia xanthocarpa Berk. (Myrtaceae), también conocida como “guabiroba”, está ampliamente distribuida en el sur y suroeste de Brasil y ha sido usado por las familias locales como medicamento popular para el tratamiento de diversas enfermedades. Este trabajo tiene como objetivo evaluar los compuestos bioactivos de los frutos y hojas de guabiroba, tales como flavonoides, carotenoides, clorofila, fenoles totales, taninos y cumarinas. También se investigó el efecto biológico de diferentes partes de C. xanthocarpa (hoja y pulpa) en cuanto a actividad inmunomoduladora en macrófagos RAW 264.7 y efectos anticancerígenos en células H295R adrenocorticales bajo diferentes concentraciones y protocolos de extracción. Se produjeron extractos de hojas y pulpa de guabiroba utilizando etanol al 70% y n-hexano como solvente por agitación magnética, así como extractos etánolícos de hojas y pulpa obtenidos por extracción por reflejo térmico. La mayoría de los compuestos bioactivos se encontraron en las hojas y no en la pulpa de la fruta. Los ensayos in vitro mostraron que la extracción de hojas con hexano afectó el ensayo de viabilidad en la línea celular adrenocortical H295R, mientras que la pulpa extraída con hexano activó el linaje de macrófagos RAW 264.7. Por lo tanto, se ha demostrado que las hojas y la pulpa de C. xanthocarpa afectan a los macrófagos y líneas de células adrenocorticales de manera dependiente de la dosis.

Palabras clave: Guabiroba; Alimentos funcionales; Anti-inflamación; Anticáncer.

1. Introduction

It is well known that fruits and vegetables are major sources of essential nutrients such as vitamins and minerals (Rein et al., 2013). Food-derived bioactive compounds have gained attention due to their biological effects such as anti-inflammatory, anti-cancer, and neuroprotective properties (Neri-Numa et al., 2018). The antioxidation capacity of several bioactive compounds can also have application in food science and technology to extend the shelf-life of food products (Nanditha & Prabhasankar, 2009), as well as in biological science due to their protective effect against damages caused by oxidative stress (Neha et al., 2019).

Tropical countries present a large number of native fruits with the potential to be exploited from a nutritional, therapeutical, and commercial point of view. Although Brazilian flora is very rich in edible fruits, most of them have not been studied or consumed by the population (Teixeira et al., 2019). The Campomanesia genus comprises about 30 species, mainly used in folk medicine to combat and prevent diseases, including fever, diabetes, hypercholesteremia, obesity, and urinary disturbances. Scientific studies have shown the anti-inflammatory properties of the extracts from Campomanesia velutina (Michel et al., 2013) and Campomanesia adamantium leaves (Ferreira et al., 2013). C. xanthocarpa leaves can contribute to reducing cholesterol levels (8), and inhibiting platelet aggregation, without demonstrating cytotoxicity. The leaves also showed fibrinolytic activity (Klaftke et al., 2010) and antioxidant activity, due to the reducing power of the phenolic group, which reduces free radicals and produces the phenoxy radical (M. da S. Santos et al., 2012). Antidiabetic effects were also reported through restoration or preservation of hepatic glycogen, probably due to a possible stimulus of the extract in the release of insulin from β cells or the insulin-mimetic effect (Vinagre et al., 2010). The leaves also exhibited anti-ulcer activity due to the antioxidant properties of flavonoids and tannins, which protect against ulcer development in the gastric mucosa, although this mechanism has not yet been elucidated (Markman et al., 2004).

Interestingly, most of those authors did not investigate the mechanism of action of the C. xanthocarpa extracts. More recently, a study found that extracts from C. xanthocarpa leaves can reduce the incidence of atherosclerosis by modulating atherosclerotic-related molecules such as CD14, ICAM-1, VCAM-1, and CD105 in the THP-1 human monocytic cell line (Cunha et al., 2020). The positive effect of this plant on hypercholesterolemia was found to be related to the reduction in AOPPs (Advanced Oxidation Protein Products), a biomarker of protein oxidation and an inflammatory mediator, and CRP (C Reactive Protein) related to the decrease of endothelial nitric oxide synthase mRNA expression and bioactivity in human aortic endothelial cells (Viecili et al., 2014). Pectin extracted from guabiroba pulp also presented an anticancer effect on U251-MG and T98G human glioblastoma cell lines, associated with the increase in cellular ROS levels (Amaral et al., 2019).
Guabiroba contains high contents of carbohydrates (pectin), vitamin C, and minerals (potassium, calcium, sodium, phosphorus, iron, manganese, and zinc) (de Oliveira Raphaelli et al., 2021). The phenolic compounds found in the fruits and leaves of guabiroba tree are related to the solvent used for extraction. Anthocyanins (cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, and pelargonidin-3,5-diglucoside), phenolic acids (cafeic acid, chlorogenic acid), and flavonoids (quercetin, myricitrin, rutin) were also found in high concentrations in this plant (de Oliveira Raphaelli et al., 2021). Some authors fractionated the C. xanthocarpa extracts and identified various components, including 2’,4’-dihydroxy-5’-methyl-6’-methoxychalcone; 2’,4’-dihydroxy-3’,5’-dimethyl-6’-methoxychalcone; 2’-hydroxy-3’-methyl-4’,6’-dimethoxychalcone; 2’,6’-dihydroxy-3’-methyl-4’-methoxychalcone; 5-hydroxy-7-methoxy-8methylflavanone; and 7-hydroxy-5-methoxy-6-methylflavanone (Salmazzo et al., 2019).

Although C. xanthocarpa is often used in folk medicine in some Brazilian regions, further studies are necessary to support their potential in food and therapeutical applications. Thus, this study aimed at investigating the bioactive compounds from C. xanthocarpa leaves and pulp, as well as their immunomodulatory and anti-cancer effects in vitro. Macrophages are important cells involved in the inflammatory process, a condition that is commonly found in hypercholesterolemia and diabetes. The present study on RAW 264.7 macrophage cell lines can contribute to the elucidation of the effects of C. xanthocarpa on inflammatory processes. The investigation of the cytotoxicity effect on H295R cells, a human adrenocortical cell line, can contribute to the search of new molecules with the potential to treat adrenocortical tumors, mainly adrenocortical carcinoma, which is a very rare type of cancer with often poor prognosis and lack of effectiveness by the conventional chemotherapies. Thus, these cellular studies can play an important role in the discovery of new anticancer agents and anti-inflammatory molecules.

2. Methodology

C. xanthocarpa leaves and mature fruits were collected in the city of Laranjeiras do Sul, Parana State, Brazil, in the geographic coordinates latitude 25° 24’ 28” S and longitude 52° 24’ 57” W. The fruits were stored at -80 °C and leaves were dried in an air circulation oven at 35°C for 37 h, and milled in a hammer mill. The extraction protocol, the chemical determinations, and the biological assays are summarized in Figure 1.

**Figure 1.** Extraction, chemical determinations, and biological assays.

![Diagram](source: Authors.)

Source: Authors.
The extracts were obtained using 70% ethanol or n-hexane, under magnetic agitation, as described by (de Oliveira Fernandes et al., 2015). For that, 10 g of leaves and 20 g of pulp were mixed with 100 mL ethanol (70% v:v) or n-hexane, under magnetic stirring at 37 oC for 1 hour. Then, the samples were filtered on filter paper and this process was carried out twice. A rotary evaporator under reduced pressure was used to obtain the crude dry extract of each sample.

For the Soxhlet extraction, 10 g of leaves and 20 g of pulp were mixed with 70% ethanol (v/v) (100 mL), maintaining reflux for 3h, as described by Seigler (Skalicka-Woźniak & Głowniak, 2012). After this period, the extracts were filtered and concentrated in a rotary evaporator under reduced pressure.

All extracts were stored in Ambar flasks, protected from light, humidity, and high temperatures for later analysis. The samples were identified as follows:

- **L-et** (ethanolic extract of leaves by magnetic stirring)
- **L-hex** (hexane extract of leaves by magnetic stirring)
- **P-et** (ethanolic extract of pulp by magnetic stirring)
- **P-hex** (hexane extract of pulp by magnetic stirring)
- **SL-et** (ethanolic extract of leaves by Soxhlet)
- **SP-et** (ethanolic extract of pulp by Soxhlet)

The total phenolics content was determined using the Folin-Ciocalteu reagent, as described elsewhere (Bucić-Kojić et al., 2007) with modifications. The dried extracts were diluted in the respective solvents and Folin-Ciocalteu reagent at 10% was added. The samples were placed in 96-well microplates, and 7.5% sodium carbonate solution was added. The microplates were incubated in the dark, for 2 hours, followed by absorbance readings at 765 nm. The total phenolics content was determined using a gallic acid standard curve with concentrations ranging from 0 to 100 mg of GAE/L.

The chlorophyll and carotenoids contents were determined by Lichtenthaler method (Lichtenthaler, 1987). Briefly, 2 mg of each extract was homogenized in 5 mL of acetone (80% v:v). The sample was centrifuged at 1788.80 g for 10 minutes and the supernatant was transferred and diluted in 25 mL of acetone (80%). Absorbance readings were performed at 647, 663, and 470 nm for chlorophyll a, chlorophyll b, and total carotenoids contents, respectively. The results were expressed in ng/g of extract.

The flavonoids content was determined using the aluminum chloride method, as described by (Zhishen et al., 1999).

The coumarin content was determined as described by Do Amaral et al. (Do Amaral, M. D. P. H et al., 2009), diluted in methanol (80%), and absorbance readings at 275 nm. The results were expressed in ug Equivalent of Coumarin of extract (w/w). The tannin content was determined as described by (Seigler et al., 1986), using Folin-Denis reagent, followed by the addition of sodium carbonate solution (8%). Absorbance readings were performed at 725 nm, and the results were expressed in ug Equivalent of Tannic Acid/g.

DPPH free radical scavenging activity was determined as described by (Brand-Williams et al., 1995) with slight modifications. A solution of Trolox 4000 uM diluted in methanol was used for the calibration curve. The volumes were adjusted for microplate readings at 515 nm, and the results were expressed in µg Equivalent of Trolox/g. ABTS radical scavenging activity was determined as described by (Re et al., 1999). The extracts were diluted in a freshly prepared ABTS solution, and the mixture was incubated at room temperature for 6 minutes. Absorbance readings were performed at 734 nm, and the results were expressed in µg Equivalent of Trolox/g.

The H295R cell line is considered to be a good model of adrenal cortical cells. The cells were cultured in Dulbecco's modified Eagle's (DMEM) supplemented with fetal bovine serum protein (10%) and 1 % antibiotics (streptomycin and penicillin). MTT assay was used to evaluate the cytotoxic activity, as described elsewhere (Stockert et al., 2018). This colorimetric method is based on the ability of the enzyme mitochondrial succinate dehydrogenase to convert the yellow water-
soluble tetrazolium salt MTT to the purple formazan crystal, which is insoluble in water. After 3 hours of incubation at 37º C with MTT solution (0.5 mg/mL), DMSO was added to the wells, incubated for 5 minutes, and absorbance readings were performed at 595 nm.

The RAW 264.7 cell line, a murine macrophage cell line, was maintained in a 95% air/5% CO2 atmosphere in Dulbecco’s modified Eagle's medium (DMEM) plus 10% (v/v) FBS (Fetal bovine serum protein) and 100 U/mL penicillin, and 100 µg/mL streptomycin. The activity of these cells was identified by phagocytosis, adhesion, and neutral red uptake.

Phagocytic activity was measured according to (Pipe et al., 1997) The cells were incubated in 96-well flat-bottomed tissue culture plate for 1 hour at 37 ºC. Then 10 µL of neutral-red-stained zymosan was added to each well. After incubation for 30 minutes, the cells were fixed with Baker formol-calcium solution for another 30 minutes. The cells were washed twice and centrifuged at 2501 x g for 5 minutes. The samples were solubilized in extraction solution (10 % acetic acid, and 40% ethanol in distilled water), and absorbance readings were performed at 550 nm after 30 minutes.

The uptake of the cationic dye neutral red was used to assess the total content of the cationic vesicles of macrophages, as described by (Pipe et al., 1997). For that, 100 µL of cell suspension was incubated for 1 hour, followed by the addition of 20 µL neutral-red at 2% in each well and incubation for 30 minutes. Then, 100 µL of the extraction solution was added and incubated for 30 minutes at 37 ºC. The absorbance readings were performed at 550 nm.

Macrophage adhesion was assayed according to (Rosen & Gordon, 1987). The cells were incubated for 1 hour at 37 ºC. After incubation, the plates were washed twice with PBS, and the adherent cells were fixed with methanol. Cells were stained with 100 µL Giemsa solution at 0.2% for 40 minutes at room temperature. The cells were washed twice with PBS and solubilized with m 50% methanol. After 30 minutes of incubation at room temperature, the absorbance was measured at 550 nm.

Data were presented as mean ± SE. Statistical analysis was performed by One-Way ANOVA, followed by a post-hoc Tukey test, at p <0.05, using the software GraphPad Prism, version 5.0 (Graphpad Software, San Diego, California).

3. Results and Discussion

The extraction yields of the crude extracts from leaves and pulp of guabiroba plant are shown in Table 1. Higher extraction yields were observed for the extraction with 70% ethanol, with higher percentages for the leaf extract when compared to the pulp extract. The results showed that the majority of the bioactive compounds were detected in the leaves, which was also observed for the antioxidant activity determined by DPPH and ABTS assays. The ethanolic extract of the leaves presented a higher concentration of phenolic compounds and more efficient scavenging of DPPH and ABTS free radicals. The L-hex, P-et, and P-hex extracts did not show antioxidant activity by DPPH assay, while the highest antioxidant activity was observed for the P-hex group by the ABTS assay (Table 1).
Table 1. Extraction yields of the crude extracts (g and %), quantification of bioactive compounds, and determination of the antioxidant activity of Campomanesia xanthocarpa Berg.

<table>
<thead>
<tr>
<th></th>
<th>L-et</th>
<th>L-hex</th>
<th>P-et</th>
<th>P-hex</th>
<th>SL-et</th>
<th>SP-et</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction yield (g and %)</td>
<td>5.25 (52.5%)</td>
<td>0.1 (1.5%)</td>
<td>4.1 (20.8%)</td>
<td>0.3 (1.5%)</td>
<td>0.5 (5.7%)</td>
<td>0.4 (3.9%)</td>
</tr>
</tbody>
</table>

Quantification of Bioactive Compounds

<table>
<thead>
<tr>
<th>Component</th>
<th>L-et</th>
<th>L-hex</th>
<th>P-et</th>
<th>P-hex</th>
<th>SL-et</th>
<th>SP-et</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic Compounds (µg/g)</td>
<td>228.1±3.77</td>
<td>3.61±0.2</td>
<td>25.71±0.51</td>
<td>0.1±0.01</td>
<td>151.1±1.81</td>
<td>50.35±0.77</td>
</tr>
<tr>
<td>Chlorophyll (ng/g)</td>
<td>33.19±0.95</td>
<td>88.11±1.7</td>
<td>12.28±1.46</td>
<td>7.13±0.93</td>
<td>34.68±3.31</td>
<td>16.77±3.09</td>
</tr>
<tr>
<td>Carotenoids (ng/g)</td>
<td>8±0.32</td>
<td>16.48±0.83</td>
<td>7.63±0.59</td>
<td>5.52±0.17</td>
<td>6.84±0.30</td>
<td>8.08±0.29</td>
</tr>
<tr>
<td>Flavonoids (µg/g)</td>
<td>60.2±1.24</td>
<td>17.57±1.12</td>
<td>5.23±0.51</td>
<td>8.43±0.61</td>
<td>52.64±1.22</td>
<td>7.18±0.44</td>
</tr>
<tr>
<td>Coumarins (µg/g)</td>
<td>270.6±2.25</td>
<td>146.9±4.55</td>
<td>31.25±0.77</td>
<td>8.75±0.1</td>
<td>243.8±6.16</td>
<td>38.13±3.37</td>
</tr>
<tr>
<td>Tannins (µg/g)</td>
<td>585.2±8.48</td>
<td>36.74±1.42</td>
<td>57.8±2.01</td>
<td>0.24±0.2</td>
<td>543.6±9.8</td>
<td>67.27±2.85</td>
</tr>
</tbody>
</table>

Antioxidant Capacity in vitro through the free radical scavenging assay

<table>
<thead>
<tr>
<th>Radical</th>
<th>DPPH radical (µmol of Trolox/g)</th>
<th>ABTS radical (µmol of Trolox/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4690±100.4</td>
<td>2329±20.21</td>
</tr>
<tr>
<td></td>
<td>76.85±1.29</td>
<td>242.5±4.4</td>
</tr>
<tr>
<td></td>
<td>79.96±1.47</td>
<td>270.4±4.97</td>
</tr>
<tr>
<td></td>
<td>95.29±1.4</td>
<td>291.9±4</td>
</tr>
<tr>
<td></td>
<td>4641±36.77</td>
<td>2462±48.89</td>
</tr>
<tr>
<td></td>
<td>85.64±0.33</td>
<td>261±3.95</td>
</tr>
</tbody>
</table>


Several authors have reported higher concentrations of phenolic compounds in fruits of the Campomanesia family (Alves et al., 2013; Rocha et al., 2011) when compared with the present study. Other authors also found higher concentrations of this compound in the leaves of C. adamantium (Coutinho et al., 2008). The differences between those findings may be due to differences in territory regions, climatic conditions, and type of solvent extraction. Chlorophyll and its derivatives have shown several biological properties, and some authors have reported higher chlorophyll contents in the Myrtaceae family (Batista et al., 2017; Carneiro et al., 2014). Concerning the carotenoids content, some authors found higher levels in other Myrtaceae family plants when compared to the present study (Batista et al., 2017; Carneiro et al., 2014; M. D. S. Santos et al., 2013). The flavonoids content can also vary in different Campomanesia species (Donado-Pestana et al., 2015; Malta et al., 2013). In turn, coumarin is considered an important bioactive compound and is usually found in guaco leaves (de Melo & Sawaya, 2015). This compound was found in Campomanesia lineatifolia, while the present study was the first to quantify coumarin in the Campomanesia xanthocarpa species. Bioactive compounds were found in the leaves of guabiroba tree, such as flavonoids, tannins, triterpenoids, heterosids, anthocyanins, and saponins (Markman et al., 2004). The discrepancy between the results from other studies may be due to seasonality, development stage, temperature, irradiation, altitude, mechanic, or pathogen stimuli, among other factors (Gobbo-Neto & Lopes, 2007).

Several studies have shown the antioxidant activity of leaves, pulps, and seeds of Eugenia, Myrciaria, Syzygium, and Psidium genus, all belonging to the Myrtaceae family (Annadurai et al., 2012; Chai et al., 2018; Figueirôa et al., 2013; Stewart et al., 2013; Wang et al., 2014). High antioxidant activity was reported for the methanolic extract of C. xanthocarpa pulp (Souza et al., 2018), while Donado-Pestana et al. (Donado-Pestana et al., 2015) correlated the antioxidant activity of ethanolic extracts of C. phae with the total phenolics content. Interestingly Muller et al. (2011) reported no relationship between the antioxidant activity, measured by DPPH assay, and the carotenoids content. In the present study, the leaf extracts exhibited higher antioxidant activity when compared to the pulp extracts. These
results corroborate with the concentration of phenolic compounds found in the ethanolic extract of the leaves, which was also found in other studies (do Amarante et al., 2017).

MTT assay allows measuring cell proliferation, cell viability, and cytotoxicity. Four extracts were tested in the H295R cell line at concentrations of 0, 25, 50, 75, and 100 µg/mL. The results showed that only the group Leaf-hexane (Figure 2B) had a cytotoxic effect at the concentration of 75 µg/mL, reducing cell viability at 17% when compared with the control group. The other extracts did not present statistical differences (Figures 2A, 2C, and 2D).

Figure 2. MTT- Toxicity of extracts from C. xanthocarpa Berg against the H295R cell line (adrenal cortex tumor)

Neri-Numa et al. (Neri-Numa et al., 2013) reported no antiproliferative effect of the ethanolic extract of E. stipitata, which is a plant from the Myrtaceae family, on several cell lines at the concentration tested. Hexane extract of the Psidium guajava (Myrtaceae) leaves had cytotoxic effects on the OV2008 and Kasumi-1 cell lines in a dose-dependent manner (Levy & Carley, 2012), while C. adamantium aqueous extract caused cytotoxicity dose-dependent in the Jurkat cell line (Campos et al., 2017). Although the hexane extract of the C. xanthocarpa leaves of the present study showed a low cytotoxic effect at the concentration of 75 µg/mL, this research is the first study to investigate the effects of C. xanthocarpa on the adrenal cell line H295R.

Macrophage function in RAW 264.7 cell line was investigated by phagocytosis, lysosomal volume, and cell adhesion. Phagocytosis increased by 49%, 50%, and 42%, when cells were cultured with P-hex extract at 50, 75, and 100 µg/mL, respectively (Figure 2D). No significant differences were observed for the other extractions (3A, 3B, and 3C). An increase of 8% in lysosomal volume was observed for the extract concentration of 75 µg/mL (Figure 4D), with no effect for the other extracts. The results of the adhesion assay showed an increase in cell adhesion of 30% and 28% for the extract concentrations
of 25 and 75µg/mL, respectively, for the P-et group when compared to the control. The P-hex group reduced macrophage adhesion by 33 and 40%, at 75 and 100 µg/mL, respectively (Figure 5D).

**Figure 3.** Phagocytic activity of the macrophage lineage (RAW 264.7) against different concentrations of *C. xanthocarpa* Berg extracts

Note: values are expressed as a percentage. Leaf extraction with 70% Ethanol (L-et), Leaf extraction with n-hexane (L-hex), Pulp extraction with 70% ethanol (P-et), and Pulp extraction with n-hexane (P-hex). % change when compared to the Control group (100.5 ± 5.8). Where *p* <0.05 compared to the control. Source: Authors.
Figure 4. Capture of Neutral Red (Lysosomal Retention) of the macrophage lineage (RAW264.7) against different concentrations of *C. xanthocarpa* Berg extracts.

Note: values are expressed as a percentage. Leaf extraction with 70% Ethanol (L-et), Leaf extraction with n-hexane (L-hex), Pulp extraction with 70% ethanol (P-et) and Pulp extraction with n-hexane (P-hex). % Change when compared to the Control group (105 ± 4.3). Where *p* <0.05 compared to the control. Source: Authors.
Figure 5. Adhesion of macrophages from the macrophage lineage (RAW 264.7) against different concentrations of C. xanthocarpa Berg extracts.

Note: Values are expressed as a percentage. Leaf extraction with 70% Ethanol (L-et), Leaf extraction with n-hexane (L-hex), Pulp extraction with 70% ethanol (P-et), and Pulp extraction with n-hexane (P-hex). % changes when compared to the Control group (99.5 ± 4.7). Where *p < 0.05 when compared to the control and the concentration 75 µg/mL; **p < 0.05 when compared to concentration 100µg / mL, in the extract P-et (C). Source: Authors.

Macrophages are important cells of the immune system involved in pro-inflammatory and anti-inflammatory responses (Bowdish et al., 2007). These cells also play an important role in tumor development (Coussens & Werb, 2002). Some studies have shown the activation of macrophage phagocytosis in extracts of the Myrtaceae family and Campomanesia genus (Hmoteh et al., 2018; Park et al., 2015). The inflammatory response depends on the expression of several inflammatory proteins such as cytokines, enzymes, receptors, and adhesion molecules (Barnes, 1994). Some authors reported that phenolic compounds from C. phae reduced macrophage recruitment in the adipose tissue and the expression of inflammatory markers (Donado-Pestana et al., 2015). A similar effect was observed in this study for the effect of the P-hex extract on the macrophage cell line RAW 264.7, while the P-et extract led to an increase in macrophage adhesion for the majority of the concentrations tested.

4. Conclusion

Higher concentrations of bioactive compounds were found in the extracts from C. xanthocarpa leaves obtained with 70% ethanol. The ethanolic extract also showed a higher antioxidant activity by DPPH and ABTS assays. Leaf extracts obtained using hexane showed an effect on the viability of the H295R cell line, while the hexane extract of the pulp activated the RAW 264.7 macrophage cell line, as identified by phagocytic and volume lysosomal assays. Further investigations on the
cell lines under study are required to identify other molecules, methodologies, and concentrations.

Our results show potential of this plant in health and food industry. Nevertheless, future studies are necessary to investigate the mechanisms of action as well as future application in food industry.

Acknowledgments

This work was financially supported by Instituto de Pesquisa Pelé Pequeno Príncipe; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação Araucária; and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [Finance code 001]. The sponsors did not play any role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

References


