Extraction of Antioxidant Compounds from *Eugenia uniflora L.* Leaves by Energized Dispersive Guided (EDGE) System

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The leaves of Pitanga (*Eugenia uniflora L.*) present recognized antioxidant potential that has practical applications in pharmaceuticals, food, perfume, agrochemical, and cosmetic industries. The Energized Dispersive Guided Extraction (EDGE) system, which combines dispersive solid-phase extraction with the Accelerated Solvent Extraction system, was employed in "combined mode" with extraction cycles to obtain antioxidant compounds from Pitanga leaves. The results showed that extraction cycles with different solvents produce similar extraction yields. At the same time, their ability to scavenge free DPPH radicals exhibited variations according to the polarity of the extraction solvent. Thus, acetone was the optimal solvent for extracting antioxidant compounds from *E. uniflora* leaves in the combined mode.
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Introduction

The species of *Eugenia uniflora L.*, also known as pitanga, Brazilian cherry, or purple cherry, is a plant native to the Amazon rainforest in South America, with high economic and pharmacological potential evidenced by various scientific studies. Its extracts are rich in bioactive compounds such as terpenoids, tannins, and flavonoids with recognized antioxidant potential [1,2]. Based on this assumption, using the genus *Eugenia* in traditional medicine has been encouraged by several studies emphasizing its bioactive compounds and biological potential associated with antioxidant activity related to this species [3,4].

In general, antioxidant compounds can interact with free radicals before vital molecules are damaged, playing a crucial role against various diseases, including chronic inflammation, atherosclerosis, cancer, and cardiovascular diseases, as well as slowing biochemical aging processes [5,6]. Such constituents exhibit biological properties that the pharmaceuticals, food, perfume, agrochemical, and cosmetic industries can use. However, choosing the ideal extraction method for these compounds represents a significant analytical challenge [7,8].

The extraction of bioactive compounds can be carried out through traditional techniques, including liquid-liquid and solid-liquid extraction, as well as modern methods like ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), subcritical fluid extraction (SFE), and accelerated solvent extraction (ASE). However, most extraction protocols utilizing these techniques have drawbacks, such as large amounts of organic solvents, multiple operational steps, extended analysis time, and potential thermal degradation, which can impact the yield and quality of the obtained extracts [9,10].

Recently, novel techniques have been developed to efficiently extract bioactive compounds from natural sources. These technologies are environmentally friendly due to reduced consumption of organic solvents, shorter operation times, and improved yield and extract quality [11].

The Energized Dispersive Guided Extraction (EDGE) system, introduced by CEM Corporation in October 2017, was developed to combine

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dispersive solid-phase extraction with the ASE system, being considered "faster than Soxhlet, more automated than QuEChERS, and simpler than other solvent extractions" [12].

The EDGE uses a two-piece open sample vessel called "Q-Cup" to hold the sample. During an extraction, the solvent is added to the sample in the Q-Cup, and the sample and solvent are pressurized and heated to the selected temperature and time. When the extraction is finished, the extract passes through the bottom of the Q-Cup through the filter and moves through the fluidic pathway of the system, including a cooling coil, to be dispensed into the collection vial. The final extract is at room temperature, filtered, and ready for analysis [13].

This system has some successful applications for extracting pesticides from natural sources, such as strawberries, cucumbers, tomatoes, green peppers, phthalates and additives from plastics, or the recovery of fats from foods [14]. Since then, some authors have shown the excellent potential of this system to extract target compounds from fruits and drugs from feeding stuff [15,16]. This study aimed to extract antioxidant compounds from Pitanga leaves using the EDGE system in "combined mode". The process can be divided into multiple cycles collected in the same vial for each solvent in this extraction mode. Each cycle involves extracting Pitanga leaves using 10 mL of solvent to maximize the extraction of desired compounds.

Materials and Methods

Collection, Identification, and Preparation of Plant Material

Leaves of *Eugenia uniflora L.* (Pitanga) were collected on private property in the city of Aracaju, Sergipe, Brazil, according to the geographical coordinates: latitude 10°59'15.8" S and longitude 37°04'03.8 "W. The plant material was herborized, preserved, and identified in the plant collection of the Biology Department at the Federal University of Sergipe at the number 145362-82 in the UFS-AJU Herbarium. The plant material was cleaned with distilled water to remove impurities and dried at 40 ºC using a circulating air oven for 4 days. The leaves were ground using a knife mill, sieved to achieve a particle size between 32 and 60 mesh, and stored in a dry place, protected from light and heat.

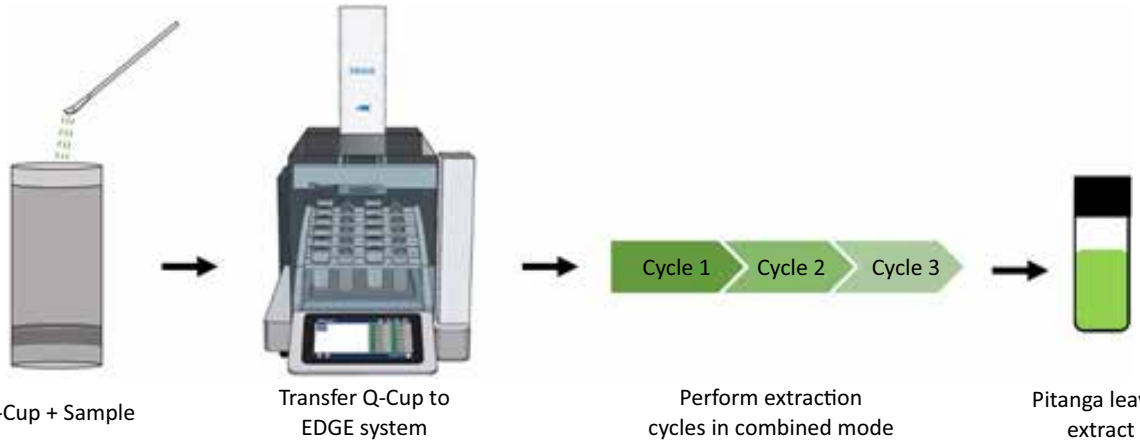
EDGE Extraction

Figure 1 schematized the extraction process. Firstly, dry leaves (1g) were directly weighed into a Q-Cup containing S1 filters, and the Q-Cups were placed on the removable rack of the EDGE system, accompanied by collection vials with a maximum volume of 40 mL. The extracts were obtained in combined mode, extracting Pitanga leaves with three extraction cycles with 10 mL of hexane, dichloromethane, or acetone added from the top of the Q-Cup and heated at 75 ºC for 120 seconds. After each extraction, a washing step was performed by passing 10 mL of the respective extracting solvent in the system at 100 °C for 30 seconds, preparing the EDGE instrument for the following sample. The percentage yields (weight to weight) were calculated in triplicate according to the extract dry weight basis.

DPPH Radical Scavenging Assay

The antioxidant activity was measured in a UV–vis spectrophotometer (Hach DR 5000) using a quartz cuvette (45 mm × 12.5 mm × 12.5 mm) with a 1.00 cm optical path at room temperature (25 °C), according to modified DPPH free radical scavenging method developed by Brand- Williams and colleagues [17]. Around 100 μL of methanolic solutions at different concentrations of the extracts (200, 400, and 600 ppm) were diluted in 3.9 mL of a methanolic solution of DPPH (0.06 mM). Methanol was used as a blank solution. After 1 h of incubation at 25 °C, the absorbance was measured at 515 nm. Free radical DPPH inhibition in percentage (I %) was calculated as follows

Figure 1. Extraction process in combined mode with EDGE system.



[Equation (1)]:

$$I\,\% = \left(\frac{A\ control\ -\ A\ sample}{A\ control}\right) \times 100$$

in which: a control is the absorbance of the control solution and A sample is the absorbance of the tested extract.

Results and Discussion

The EDGE process starts with the automated transfer of the Q-Cup into a sealed extracting chamber, where a smaller amount of solvent (0 - 10 mL) can be added between the extraction cell and the chamber walls or from the top of the cell, where a more considerable amount of solvent (0 - 30 mL) can also be added. The chamber walls are heated (Tmin: 25°C - Tmax: 200°C), causing the solvent to expand and disperse into the sample through the holes at the bottom of the Q-Cup. As the system is sealed, temperatures above the solvent's boiling point can be applied, and the volatilized product does not escape from the cell (Pmax: 200 psi) [12]. Thus, the extraction efficiency is governed by several critical parameters, including solvent type, temperature, pressure, time, and, obviously, extraction technique [13]. In this experimental study, extraction time, temperature, and solvent volume were kept constant for all cycles with the organic solvents tested, aiming to achieve lower IC50 values by DPPH assay. The results showed that extraction cycles with different solvents produce similar yields (Acetone: 3.76 % > Dichloromethane: 3.27 % > Hexane: 3.07 %) (Figure 2). The similarity observed in the yields obtained using different organic solvents at the same extraction conditions can be attributed to the fact that the EDGE is a fully automated system, which reduces human error associated with the extraction process. However, despite the similar extraction yields obtained by applying the EDGE system with extraction cycles in "combined mode", its capacity to scavenge free DPPH radicals exhibited variations depending on the utilized solvent (Figure 3).

The average IC50 values were obtained by DPPH scavenging assay with acetone. (0.47 mg L^{-1}), dichloromethane (9.77 mg L^{-1}) and hexane (24.52 mg L^{-1}) correspond to the same order of the dielectric constant of the solvents (εacetone = 20.7 > εdichloromethane = 8.93 > εhexane = 1.88), indicating that the polarity of extraction solvent highly influenced the extraction yields and recovery of antioxidant compounds from Pitanga leaves. In the EDGE system, extraction cycles represent the number of extractions performed with the same sample. In this context, prolonged exposure of the matrix to the extracting solvent improves the solvent's penetration into the sample's interstice, facilitating contact with the analytes. Various techniques have been employed to obtain antioxidant compounds from plant leaves. However, this work showed higher DPPH scavenge capability than

Figure 2. Extraction yields were obtained by the EDGE system with Pitanga leaves.

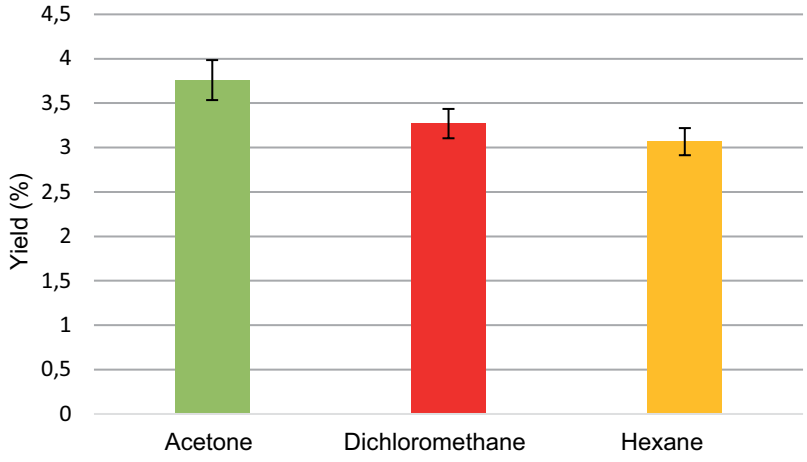
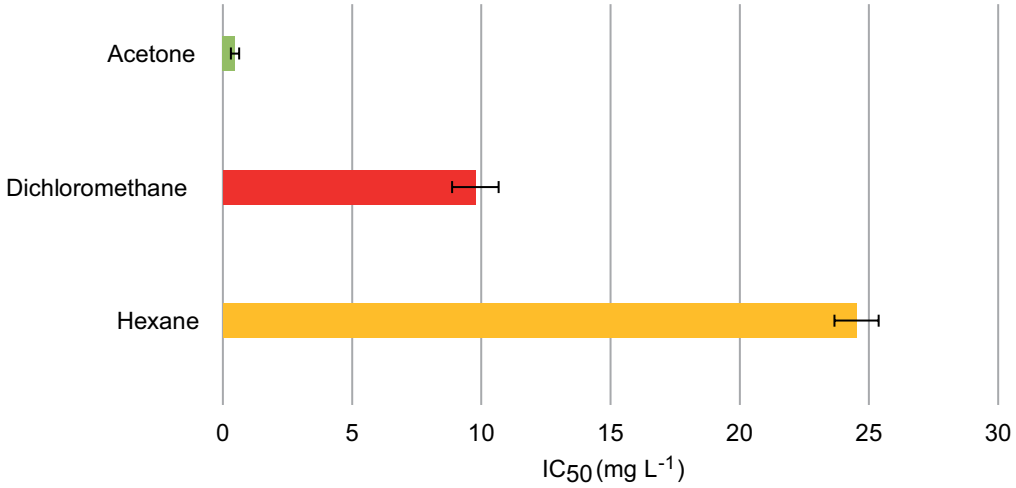


Figure 3. IC50 (mg L^{-1}) for Pitanga leaf extract with different solvents.



that described in the literature by the same assay. Zhu and colleagues [18] reported that the antioxidant activity of the aqueous extract of *S. oleracea* leaves obtained by MAE is 365 mg L^{-1}. In comparison, the aqueous extract of A. sativum leaves reported by Liu and colleagues [19] showed 421 mg L^{-1}. Chang and colleagues [20] reported that the aqueous extract of C. sativa leaves obtained by maceration had IC50 values of 361 mg L^{-1}, attributed to various phytochemicals acting synergistically to neutralize free radicals. Based on these results, the EDGE system emerges as a new, fully automated technique for recovering antioxidant compounds from plants, offering advantages such as reduced solvent and time consumption and enhanced reproducibility compared to techniques commonly described in the literature.

Conclusion

In this study, the innovative EDGE system was applied in a combined mode to extract antioxidant compounds from Pitanga leaves. The extraction time, temperature, and solvent volume (acetone, dichloromethane, or hexane) were kept constant for all cycles with the organic solvents tested, aiming to achieve lower IC50 values on the DPPH

assay. The results obtained showed that extraction cycles produce similar extraction yields (acetone: 3.76 % > dichloromethane: 3.27 % > hexane: 3.07 %); moreover, despite the similar extraction yields, its capacity to scavenge free DPPH radicals exhibited variations according to the solvent utilized (acetone: 0.47 mg L^{-1}, dichloromethane: 9.77 mg L^{-1} and hexane: 24.52 mg L^{-1}), indicating that the polarity of extraction solvent highly influenced the process. Among the tested solvents, acetone was the most suitable to extract antioxidant compounds from E. uniflora leaves. The EDGE has proven to be an efficient, fully automated technique for recovering antioxidant compounds, offering several advantages such as lower solvent and time consumption and good reproducibility.

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