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Application of supercritical technology in the recovery of bioactive compounds from murici (*Byrsonima crassifolia*)

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ABSTRACT

The murici (*Byrsonima ssp*.) is a fruit that is spread throughout the Amazon region and presents marked sensory characteristics and therapeutic properties associated with the presence of lutein, quercetin, fatty acids, unsaturated fatty acids and long-chain triglycerides of the fruit. Although these antioxidants are naturally present in murici, processing and the storage of the fruit affects its content, activity, and bioavailability. Therefore, supercritical fluid extraction of murici pulp appears as an alternative promising for applications of various products acquired by green technology obtaining desired characteristics such as high selectivity of bioactive compounds, high purity, shorter extraction times, higher yields compared to other types of extraction and absence of toxic organic solvents. Through this technology, it is possible to lead future perspectives for the application of murici products in food, cosmetics, and pharmaceutical industries.

Keywords: Murici pulp, Sequential supercritical extraction, Lutein, Quercetin, Fatty acids, Triglycerides, Bioactive properties, Biological properties, Future perspectives

INTRODUCTION

The murici (*Byrsonima ssp*., Malpighiaceae) is a native fruit from tropical America, distributed throughout the Amazon (Rezende et al., 2003). Due its marked sensory characteristics and therapeutic properties associated with the presence of bioactive compounds, murici has been used as a food and in medicinal treatments since pre-Hispanic times (Martínez-Vázquez et al., 1999).

Among the majority compounds found in murici pulp, it can be highlighted lutein in the class of carotenoids, quercetin in the class of phenolic compounds and unsaturated fatty acids, constituting the molecules of the long chain triglycerides of the fruit (Mariutti et al., 2013, Mariutti et al., 2014; Pires et al., 2021a).

Regardless these antioxidants are naturally present in the murici, fruit processing and storage can reduce carotenoid and phenolic compounds levels by losing the integrity of the cell structure, as well as oxidize and modify unsaturated fatty acid chains, affecting their content, activity, and bioavailability (Soethe et al., 2016; González-Saucedo et al., 2019).

Thereby, the supercritical fluid extraction of Murici pulp (*B. crassifolia*) appears as a promising alternative for applications of various products attained by this green technology, obtaining desired characteristics such as high selectivity of bioactive compounds, high purity, shorter extraction times, higher yields compared to other types of extraction and absence of toxic organic solvents (Pires et al., 2017; Pires et al., 2019; Pires et al., 2021a; Pires et al., 2021b; Menezes et al., 2022).

MURICI (*Byrsonima crassifolia*)

The Murici is a fruit of the genre Byrsonima, Malpighiaceae family, cultivated in several regions of Central and South America. In Brazil, the tree is found in the states of Mato Grosso, Goiás and Minas Gerais, and throughout the Brazilian Amazon, including the North and Northeast coast of Brazil (Martínez-Vázquez et al., 1999; Rezende et al., 2003).

The fruit owns different varieties, because it comes from different regions, and can be differentiated by soil characteristics, humidity, and climatic condition (Medina-Torres et al., 2004; Morzelle et al., 2015). Far greater than the variety of different types, murici is also distinguished by its colors and places of occurrence, known as yellow murici, white murici, red murici, white flower murici, red flower murici, murici from the plateau, murici from the woods, murici from the mountain range, murici from the capoeiras, murici from the field, murici from the bog, murici from the beach, among others (Ferreira et al., 2005).

The murici tree is characterized by being a small tree, reaching at most 5 meters high. Due to its seasonality, fructification occurs between the months of December and May. Mature *B. crassifolia* fruits have a yellow color, oval to slightly elongated morphology with length and diameter around 1.5 cm to 2.4 cm, the pulp constitutes about 89%-95% of the fruit and has a strong characteristic scent that resembles fruity and cheese aromas (Alves et al., 2003; Agredano et al., 2021).

The fruit pulp is a nutritious food, consisting of carbohydrates (68.90% d.b.) and lipids (14.14% d.b.), as well as being rich in minerals such as potassium, magnesium, calcium, phosphorus, and iron (Souza et al., 2012; Cunha et al., 2020).

BIOACTIVE COMPOUNDS OF MURICI PULP

The fruits of *B. crassifolia* have been the object of several studies about the content of bioactive compounds, as well as the activities and therapeutic effects associated with these compounds (Uekane et al., 2017; González-Saucedo et al., 2019; de Souza et al., 2020). This is mostly, owed to its use in popular medicine for the treatment of skin infections, gastrointestinal and gynecological inflammations (Belisário et al., 2013; Pires et al., 2017).

The fruit pulp of *B. crassifolia* presents elevated content of bioactive compounds including carotenoids, fatty acids, and phenolic compounds. In the midst of the majority compounds found in the murici pulp it can be highlighted lutein, oleic, palmitic and linoleic acids, and quercetin (Mariutti et al., 2013; Mariutti et al., 2014; Santos et al., 2018).

Lutein

The Global Market of carotenoids is continuously expanding estimated to be worth USD 1079.5 million in 2022 and achieve USD 1187.4 million by 2028 (Market Watch, 2022). Among the various carotenoids, lutein stands out, that act as antioxidants and blue light filters that protect the eye from light-induced oxidative stress (Arunkumar et al., 2020; Mitra et al., 2021). Lutein (β, ε-carotene-3,3’-diol) is one of the essential nutrients promising benefits against numerous health issues, including neurological disorders, eye diseases, skin irritation, among others, since is distributed in the human brain in regions that controls various aspects of cognition, in particular executive function, language, learning and memory (Hammond et al., 2013; Mitra et al., 2021).

Lutein represents about 67% of the carotenoid content in the fruit of *B. crassifolia* (Mariutti et al., 2013). Due to this, several studies have been developed to extract/concentrate this compound so industrially valued, since it is being increasingly consumed in the form of food supplement intended for eye health (Pires et al., 2021b). One of the methods recently used to extract/concentrate this compound into extracts of *B. crassifolia* is supercritical technology (Pires et al., 2019, Pires et al., 2021a).

In the study by Pires et al. (2019), lutein levels of *B. crassifolia* oil and ethanolic extract were concentrated at about 367% and 372% respectively, using sequential supercritical extraction with CO_2 and CO_{2+} ethanol at pressures of 15 to 42 MPa and temperatures of 323.15 to 333.15 K. In the study by Pires et al. (2021a), lutein levels of *B. crassifolia* oil and ethanolic extract were concentrated at about 398% and 429%, respectively, using sequential supercritical extraction with CO_2 and CO_{2+} ethanol at pressures of 22 to 49 MPa and temperature of 343.15 K. This makes it possible to say that the best condition studied for the recovery of lutein in murici oil was 49 MPa/ 333.15 K with supercritical CO_2 and for lutein recovery in murici ethanolic extract was 22 MPa/ 333.15 K with supercritical CO_{2+} ethanol

(90:10%). Supercritical technology has also been used for the extraction and development of lutein-based products (Zhao et al., 2017; Derrien et al., 2018; Araus et al., 2019).

Furthermore, all the powerful health benefits to the intake of lutein are attributed to the chemical and structural features, therefore several articles have indicated that a high lutein intake, either by food or as nutritional supplements is well established from epidemiological, clinical, and interventional studies (Liu et al., 2021), such as cataract (Buscemi et al., 2018), age-related macular degeneration (Sawa et al., 2020), and central obesity (Zhou et al., 2020). Lately, studies have suggested that lutein has a positive influence on inflammatory behavior, neuroprotection, reduction of the incidence of coronary disorders as well as systemic disorders (Mitra et al., 2021). Besides that, regarding the pandemic situation involving Covid-19 not only scientists, but also community has shown a high interest in dietary supplements with bioactive compounds by reason of immune boosting, anti-inflammatory, antioxidant, and antiviral properties, for the treatment and prevention of the virus (Hamulka et al., 2020). Thereby, lutein may be another sourced carotenoid underexplored novel antiviral treatments, such as fucoxanthin, siphonaxanthin used against SARS-CoV-2 Virus (Yim et al., 2021) and C50 carotenoids against viral hepatitis (Hegazy et al., 2020).

Triglycerides and Fatty Acids

Fatty acids have an influence on human health being associated with metabolism, helping in the treatment of cardiovascular diseases, neurological diseases, non-alcoholic fatty liver diseases, allergic diseases, besides having anti-inflammatory and neuroprotective effects (Chen et al., 2020).

The fatty acid market assumes a steady growth rate of \$26.69 billion in 2020 to \$51.21 billion in 2028 (Globenweswire, 2022).

These compounds are found in nature bound in triglyceride molecules that constitute the oils. The extraction of vegetable oils has been the subject of several studies using supercritical CO_2 extraction (Campalani et al., 2020; Melgosa et al., 2021; Menezes et al., 2022). In supercritical extraction studies of *B. crassifolia* pulp oil, the major fatty acids observed were oleic, palmitic, and linolenic acids, both using pressures of 15 to 42 MPa and temperatures of 323.15 and 333.15 K, when using pressures of 22 to 49 MPa and temperature of 343.15 K (Pires et al., 2019, Pires et al., 2021a). In the study performed by Santos et al. (2018), a similar profile of fatty acid composition was also obtained.

This demonstrate that the murici oil obtained by supercritical technology is stable, since there have been no changes in the fatty acids compositions using different process conditions, which makes it possible to say that, regardless of the process conditions, the oil maintains its activities and antithrombotic, anti-hypercholesterolemic and antiatherosclerogenic properties, which characterizes a product with functional quality (Ulbricht et al., 1991; Santos-Silva et al., 2002; Pinto et al., 2018; Pires et al., 2019).

Oleic and palmitic acids have applications mainly in the pharmaceutical and cosmetic industry, used in the manufacture of skin products such as lipsticks and shampoos (Nengroo et al., 2019). Linoleic acid can be used in the preventive treatment of chronic migraine and as a dietary supplement, since it can reduce the chances of developing cardiovascular diseases and obesity (Duhan et al. 2020), thus serving the pharmaceutical and food industries (Santos et al., 2018).

Regarding the composition of triglycerides present in murici oil, the majorities were the following long chain triglycerides: POLi (Palmitic, Oleic, and Linoleic), POO (Palmitic, Oleic, and Oleic), PPO (Palmitic, Palmitic, and Oleic), and LiOO (Linoleic, Oleic, and Oleic) constituting 58% of the triglyceride profile, which are long chain triglycerides (Pires et al., 2021a).

It is important to emphasize that long-chain triglycerides are beneficial for carotenoids absorption such as lutein, by reason of form mixed structures with a large hydrophobic nucleus, with oxidation functionality and stability characteristics that are directly related to unsaturation degree (Lykke et al., 2021). This property is very important for the development of murici-based products for eye health, since in the study by Downie et al. (2019) the beneficial influence of linoleic acid on dry eye disease was noticed. Therefore, the presence of linoleic acid and lutein in murici oil increases the potential to produce food supplement.

Phenolic Compounds

The phenolic compounds are considered one of the classes of bioactive molecules most studied by the scientific community since these molecules have well-reported health benefits. Various epidemiological studies have shown that a high intake has the capability to decrease the incidence of chronic diseases such as cardiovascular diseases, cancer, obesity, diabetes, among others (de la Rosa et al., 2019). Hereby, the Global Phenolic Compounds Antioxidant market size is evaluated to achieve USD 2240.8 Million in 2028, growing at a compound annual growth rate of 4.0% over the analysis duration (MarketWatch, 2022b).

In the murici, the most abundant phenolic compound is quercetin. Quercetin (3-(3,4-dihydroxyphenyl)-3,5,7-trihydroxyromo-4-one) is a flavonoid commonly found in plants and are active ingredients in many herbal medicines, stimulating great interest for its beneficial pharmacological properties and antioxidant properties (Shi et al., 2019).

Pires et al. (2021a) performed a sequential extraction with supercritical CO_2 and CO_{2+}ethanol, for the recovery of quercetin from *B. crassifolia* defatted pulp, concentrating the level of quercetin in ethanolic extracts up to 108%. Supercritical technology has also been used to extract or obtain various products based on quercetin, such as the production of micro and quercetin nanoparticles (Dong et al., 2019; Guastaferro et al., 2020) and quercetin-laden aerogel (Baldino et al. 2021).

Additionally, studies nowadays have shown that this compound has multifaceted biological functions on human health, such as: neurodegenerative protection (treated with 50 and 100 mg/kg per day) (Amanzadeh et al. 2019), cardiovascular protection (Patel et al., 2018), antiulcer (treated with 50 mg/kg quercetin per body weight) (Alkushi et al., 2017), antiallergic (Jafarinia et al., 2020), anticancer (Vafadar et al., 2020), anti-diabetic and anti-hyperglycemic (administration of quercetin (25, 50, and 75 mg/kg body weight) (Srinivasan et al., 2018). In addition, there is evidence that quercetin have antiviral and anti-inflammatory properties against COVID-19, reduce inflammation as well as decrease the chances of being infected (Di Pierro et al., 2021b, a).

MURICI BIOLOGICAL/BIOACTIVE PROPERTIES

As well as being used as food, the murici (*B. crassifolia*) has also been used in folk medicine due to its great potential of therapeutic action and various studies address its pharmaceutical properties as anti-oxidant, anti-fungal, anti-microbial, anti-hyperlipidemic, anti-depressant, anti-toxic, photochemoprotective, anti-inflammatory and anti-diabetic activities (Almeida et al., 2018).

Studies have shown that fresh pulp, defatted pulp, oil and ethanolic extract of *B. crassifolia* obtained by sequential extraction with supercritical CO_2 and CO_{2+} ethanol indicate antioxidant activities, where the oil presented hypercholesterolemic, anti-atherosclerogenic and antithrombotic effects. The cytoprotective and cytotoxic effects of oil and ethanolic extract were also tested in HepG2 cells treated with H_2O_2, in concentrations of 0.20 to 0.01 kg/m^3 for the cytoprotective effect and in the concentration of 0.05 kg/m^3 with addition of 1.00 10^{-8} m^3 of hydrogen peroxide for cytotoxic effect. In this study, oil and ethanolic extract showed no cytotoxic effect and only the oil presented cytoprotective effect at 72 hours of exposure, indicating that both extracts qualify for food and pharmaceutical applications (Pires et al., 2019, Pires et al., 2021a).

Another research showed that ethanolic extract from the fruit of *B. crassifolia* showed inhibitory activity on the fungus *Fusarium solani and Sclerotinia sclerotiorum*, where concentrations of 800 and 1600 µg/100 mL inhibited 38% of the mycelial growth of *F. solani* and, 2400 µg/100 mL concentrations inhibited 37.5% of mycelial growth of S. sclerotiorum, where antifungal potential was attributed to the presence of phenolic compounds and triterpene derivatives (Andrade et al., 2017).

The hyperglycemic effects of *B. crassifolia* fruit extracts were also evaluated in severe diabetic rats and induced by streptozotocin, as for the antidiabetic effect was examined for blood glucose and others. The extracts exhibited significant inhibitory activity against forming advanced final glycation products with IC 50 values ranging from 94.3 to 138.7 µg/mL, demonstrating that murici has anti-hyperglycemic effect and may improve hyperlipidemia and hyperinsulinemia in diabetic rats induced by streptozotocin, being considered a potential safe antidiabetic agent (Perez-Gutierrez et al., 2010).

Pro-apoptotic effects of *B. crassifolia* fruit extract has been tested for its effect on the viability of the A2780 ovarian cancer cell line and its cisplatin-resistant derived cell line called ACRP. The murici extract presented strong cellular bioactivity inhibiting cell viability A2780 and ACRP in 76.37% and 78.37%, besides modulating the cell cycle and inducing cell death by apoptosis. That brings new perspectives for the development of therapeutic strategies using murici extract to sensitize ovarian cancer cells to current chemotherapeutic options (De Souza et al., 2019).

Thus, these studies provide evidence of support for the therapeutic potential of murici fruits that may be useful to prevent and/or delay several diseases outbreaks.

SEQUENTIAL SUPERCRITICAL EXTRACTIONS

In the growing demand for environmentally clean separation technologies with efficient recovery of bio-composts, Supercritical Fluid Extraction (SFE) stands out for being a scalable technique that uses green solvents in supercritical state to extract bioactive compounds from vegetable raw materials (Brunner, 1994).

Among the SFE techniques, sequential extraction is an effective method for obtaining concentrated products with substances of different polarities using the same raw material, to improve the quality of the extracts in terms of high concentration of target compounds (Lefebvre et al., 2021).

In the studies by Pires et al. (2019, 2021a) processes of sequential extraction of the lyophilized pulp of *B. crassifolia* with supercritical CO_2 were developed (steps 1 to 9) followed by re-extraction of the defatted pulp with CO_{2+} supercritical EtOH (steps 1 to 12) as shown in Figure 1, where the difference in the chemical compositions of the extracts was confirmed. Different conditions of pressure (15 to 49 MPa) and temperature (323.15 to 343.15 K) were used in the studies. Oil from murici pulp obtained from supercritical CO_2 extraction showed lutein (224.77 μg/g), fatty acids, triglycerides, and antioxidant activity. On the other hand, the ethanolic extract of murici defatted pulp obtained by CO_{2+} supercritical EtOH extraction showed lutein (242.16 μg/g), phenolic compounds (20.63 mg GAE/g) and flavonoids (0.65 mg QE/g), with greater antioxidant activity than the oil. Likewise, compared to the two studies, it was observed that the use of the co-solvent only in the period of static extraction, at higher temperatures, increased the solubilization of polar bioactive compounds and consequently the yield of the extract, due to the longer contact time between the solutes and the solvents that facilitated the solubilization of the compounds (Pereira et al., 2010).

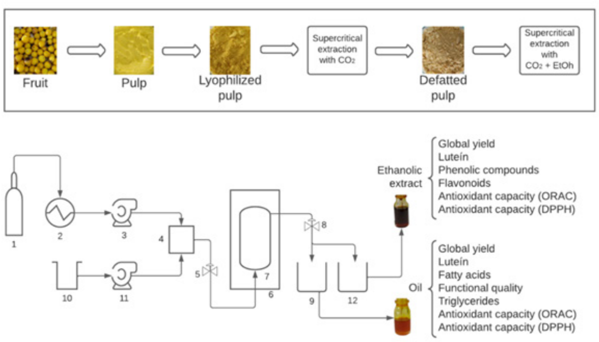

Figure 1: Technological configuration of the equipment for sequential extraction in two steps. Stage 1-SC-CO2 extraction to obtain murici oil (Step 1 to 9); Stage 2-SC-CO_{2+} EtOH to obtain murici ethanolic extract (Step 1 to 12). Note: 1. CO_2 storage cylinder; 2. Heat exchanger (condenser); 3. Solvent pump; 4. Solvent mixer; 5. Solvent and co-solvent flow control valve; 6. Oven; 7. Extraction cell; 8. Extract flow control valve; 9. Oil collection bottle; 10. EtOH storage container; 11. Co-solvent pump; 12. Ethanolic extract collection bottle.

Consequently, the sequential supercritical extraction process of *B. crassifolia* increased the utilization of biodiversity and reduced the waste of organic matter dumped in the environment, adding value to a by-product extraction, and highlighting the production of extracts with different chemical compositions for application in various industrial segments.

FUTURE PROSPECTS FOR APPLYING MURICI-BASED PRODUCTS FROM SUPERCRITICAL TECHNOLOGY

Thanks to its peculiar sensory characteristics of taste, color and odor, the fruit pulp is already widely used in cooking and gastronomy for the preparation of ice cream, sweets, juices, etc. (Guilhon-simplicio et al., 2011). The most common way of marketing the fruit is fresh or as frozen pulp. Yet, due to the information about bioactive composition of murici, the development of new products from the fruit is valid. Correlated to this, there is currently a global scenario for bioeconomy, where it is encouraged to use natural resources in a sustainable way. Therefore, the supercritical extraction of Murici can be considered a bioeconomic process (Pilařová et al., 2019).

Bioactive/functional potentials, sensory characteristics, and the absence of toxicity of murici extracts and defatted pulp obtained by supercritical technology allow such products to be used in the food industries, pharmaceuticals, cosmetics and animal feed (Pires et al., 2019, Pires et al., 2021b, Pires et al., 2021a).

In view of the presence of lutein in murici oil and extract, these can be incorporated into foods such as cheeses, since some carotenoids such as β-carotene are added to these products as dyes and antioxidants, whereas being of natural origin, do not have allergic reactions, unlike artificial dyes (dos Santos Gouvea et al., 2019).

The lutein present in the products also enables the use of them for the manufacture of food supplements aimed at eye health, which is one of the products widely marketed in the market (Khalil et al., 2012; Research and Markets, 2016; Buscemi et al., 2018).

Another field that it can also be used is in the chocolates formulation, where the incorporation of murici oil and extract could disseminate exotic fruity and cheese flavor and scent, as well as nutraceutical properties to products (Rezende et al., 2003; Dias et al., 2016; Dordoni et al., 2019).

In the cosmetics industry, murici oil and extract can be incorporated into anti-aging products, body creams, body oils, shampoos, lipsticks, among others, since it is an expanding market that uses several bioactive ingredients from the Amazon (Pharmaceutica Jr, 2020; Harhaun et al., 2020; Sebrae, 2021).

The murici defatted pulp can be used in animal feed formulations, being a potential source of fibers and phenolic compounds, which provides current market demand (Georganas et al., 2020; Tolve et al., 2021).

Therefore, due to all its possible industrial applications and therapeutic benefits, murici is among the plant species with high productive potential, of economic interest and that present a promising future (Embrapa, 2016).

CONCLUSION

The presence of lutein, quercetin, unsaturated fatty acids and long chain triglycerides, and peculiar sensory characteristics of the colour, aroma and taste of murici pulp contribute to the verticalization of the fruit production chain and future agro-industrial investments for its better use and improvement.

In addition, the use of environmentally friendly technologies for the development of new products from the murici provide 100% high quality natural products without generate negative impacts to the environment, stimulating the bioeconomy.

Therefore, the application of supercritical technology in the recovery of bioactive compounds from murici pulp (B. crassifolia) expands the industrial possibilities of using in a sustainably way this natural resource of great bioactive, therapeutic and economic potential.

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