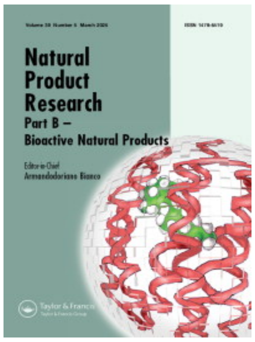

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Mangaba (*hancornia speciosa*): exploring potent antifungal and antioxidant properties in lyophilised fruit pulp extract through in vitro analysis

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ABSTRACT

Mangaba is a fruit native to Brazil, rich in bioactive compounds. To evaluate physicochemical composition, bioactive compounds, antioxidant and antifungal activity of mangaba fruit pulp. Moisture, ash, protein, lipid, energy values and phenolic compounds were determined. Antioxidant activity was determined by capture of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Evaluation of antifungal activity was performed by Minimum Inhibitory Concentration, according to protocols M07-A9 and M27-S3, and minimum fungicidal concentration. Freeze-dried mangaba pulp presented high levels of carbohydrates, low levels of lipids, and high energy density. Phenolic analysis demonstrated that chlorogenic acid was found in the highest concentration, followed by p-coumaric acid and ferulic acid. Mangaba extract showed antioxidant activity like BHT. Mangaba extract inhibited the growth of *Candida albicans* (ATCC 90028), *Cryptococcus gattii* (AFLP4), *Candida guilliermondii* (ATCC 6260) and *Candida albicans* (MYA 2876). Freeze-dried mangaba inhibited fungal activity associated with antioxidant effect due to presence of phenolic compounds.

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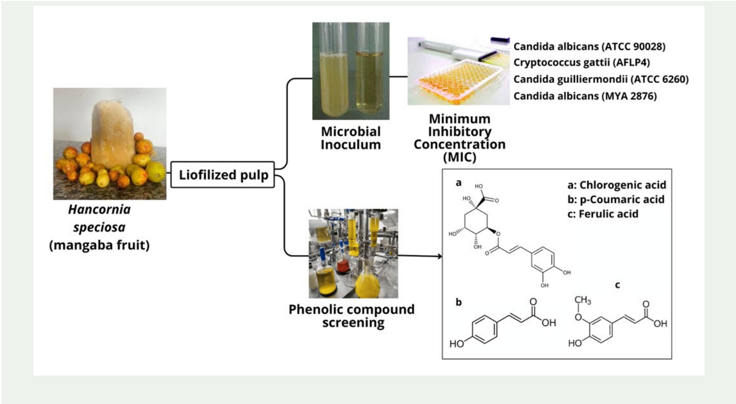

1. Introduction

The mangabeira (*Hancornia speciosa* Gomes) is a known tree of the Brazilian Cerrado and Pantanal region belonging to the Apocynaceae family. Mangabeira fruits, known as mangaba, are berry typed, weighing between 30 and 260 g in this region. Ripe fruits present yellowish-green to pinkish-green peel and whitish and viscous pulp (Soares et al. 2016; Reis et al. 2022). *Mangabeira* presents high socioeconomic potential and is characterised by using almost all its parts (bark, seed, latex, leaves and fruits) (Junior et al. 2018).

Despite the great potential associated with mangabeira, similarly to other Brazilian plant species, the tree is poorly known and explored. In the state of Mato Grosso do Sul, mangaba became popular due to its culinary use, from artisanal production of sweets, juices, ice cream, jellies, cookies and liqueurs to industrial application of frozen pulp. The other parts of the tree are used to obtain rubber, extracts and, more recently, seed bio-oils (Junior et al. 2018; Reis et al. 2019). Thus, although its use has been made mainly through extractivism, mangaba presents great economic and nutritional potential, including generation of new products such as nutraceuticals, generating income and local development (Bailão et al. 2015).

In traditional medicinal use, the different parts of the mangabeira have been studied in hypertension, gastric disorders, hyperglycaemia and weight loss treatments (da Silva et al. 2019). Regarding health effects, the use of mangabeira parts extracts (bark, leaves, latex and fruit) are associated with antioxidant action *in vitro* and animals studies (Endringer et al. 2009; Santos et al. 2016; Barbosa et al. 2019; Reis et al. 2022).

More than thirty compounds present in different parts of mangabeira have already been identified, such as high levels of total phenols such as rutin, quinic and chlorogenic acid (Endringer et al. 2009; Endringer et al. 2010; Barbosa et al. 2019), catechin, isoquercitin (Santos et al. 2016), ascorbic acid and carotenoids (Almeida et al. 2011). Animal studies using mangaba pulp showed antioxidant activity in liver and lung lesions (Santos et al. 2016; de Oliveira Yamashita et al. 2020).

Mangaba pulp is mainly composed of carbohydrates and lipids, in addition to phenolic compounds and carotenoids (Reis et al. 2022). In addition to nutrients and bioactive compounds, microbiological components were identified in the fruit, such as fungi (Trindade et al. 2004), suggesting potential postbiotic property of the fruit (Reis et al. 2022), by killing pathogenic microorganisms. However, to our knowledge, no previous study was found regarding antifungal activity of mangabeira parts. Thus, the aim of the present is to evaluate antifungal activity of mangaba pulp.

2. Results and discussion

2.1. Proximate composition of mangaba pulp and phenolic composition

The present study aimed to characterise the nutrients in freeze-dried mangaba pulp and verify its antifungal effect. The results obtained from the proximate analysis (Association of Official Analytical Chemists. 1990; Association of Official Analytical Chemists. 2000; Bligh & Dyer 1959) of freeze-dried mangaba pulp showed high carbohydrate concentration, as shown in Table S1. Experimental results showed that mangaba pulp contains $276.4 \pm 0.99\,\mu g{\cdot}g^{-1}$ of total phenolic compounds, including chlorogenic, p-coumaric and ferulic acids in the concentrations 106.3 ± 1.5; 79.0 ± 2.3 and $42.4 \pm 0.4\,\mu g{\cdot}g^{-1}$, respectively. Phenolic compounds chromatograms can be found in Figure S1. The analyzes (Gupta & Gupta 2011; Clinical and Laboratory Standards Institute 2008) found significant levels of bioactive acids, such as chlorogenic, ferulic and p-coumaric acid. High energy density was also observed, containing, in 100 grams, more than half of the daily carbohydrate recommendation for an adult man, but low amount of proteins (Institute of Medicine 2003).

The nutrient results found in the freeze-dried pulp are higher than in the fresh pulp results described in the literature, as expected. The freeze-drying process guarantees an increase in nutrient concentration of the fruit pulp, as all the water is removed to obtain a dry extract. The nutrient content in the present study differed from those described in the literature for proteins and total ash, being lower than those obtained in the present study (Santos et al. 2012). However, a previous study of our group using fresh pulp also obtained a higher protein value when compared to the literature (Rodrigues and Rafacho 2021). These differences might be explained due to the geographical region and also the state of maturation of the samples, factors that can directly interfere with the compounds found in fruits (Msaada et al. 2009).

2.2. Antioxidant and antifungal activity

Mangaba pulp presented antioxidant activity similar to BHT, as shown in Table S3. The results obtained from the antifungal analysis of lyophilised mangaba pulp showed potential for inhibiting the growth of *Candida albicans* (ATCC 90028), *Cryptococcus gattii* (AFLP4), *Candida guillermondii* (ATCC 6260) and *Candida albicans* (MYA 2876) strains visible in the microplate, as shown in Table S4. Table regarding antifungal activity, mangaba extract showed the ability to inhibit the development of certain strains, such as *Candida albicans* (ATCC 90028), *Cryptococcus gattii* (AFLP4), *Candida guilliermondii* (ATCC 6260) and *Candida albicans* (MYA 2876) evaluated in microplates. It is interesting to note that no previous reports of the antifungal capacity of the pulp of *Hancornia speciosa* Gomes were found. So, to our knowledge, our study is the first report of the antifungal activity of the mangaba fruit pulp.

Previously, Santos et al. evaluated the antimicrobial action of mangabeira leaf extracts against some species of Candida ssp (Santos et al. 2016). Despite Candida ssp are fungus naturally present in the human microbiota, found in the oral, vaginal and intestinal mucosa, the genus causes concern because it might turn to opportunistic microorganism and might be related to cases of serious infections in hospital environments and has increased in recent years (Yan et al. 2013; Aldholmi et al. 2019). Thus, antifungal natural product screening is an important field of research in infection treatment.

A previous study describe that Mangabeira leaf extracts contain high concentration of quercetin and condensed tannins, commonly present in mangabeira specie, which might have lead to antifungal effect through the inactivation of adhesion proteins and their enzymes, making their survival unfeasible (Costa et al. 2008; Santos et al. 2016). The Apocynaceae family is highly diverse and numerous, with previous studies addressing the alkaloid content of its fruits (Rahman et al. 2016; Barny et al. 2021). However, it is worth highlighting that this is the first study that reports antifungal activity related to mangaba pulp. In the present study, phenolic acids were identified (Table S2), as previously reported (Panontin et al. 2022), which may explain the antifungal effect found, as described below.

Chlorogenic acid is capable of suppressing spore growth through a mechanism of inducing cell lysis of fungal cells (Martínez et al. 2017). Also, p-coumaric acid has been studied as an association for antifungal treatments, given its *in vivo* potential against vaginal candidiasis (Ferreira et al. 2021). The same is described for ferulic acid, which has the ability to induce apoptosis in strains of the Candida genus (Canturk 2018). Since these acids are present in several plant species, their antifungal potential can be explained due to the need to inhibit possible fungi that may grow on the tree and consequently on the fruit (Arnoso et al. 2019).

It is also worth noting that in our study, antifungal activity was associated with antioxidant activity. Mangaba is traditionally known for potential antioxidant activity (Bailão et al. 2015). Our findings corroborate previous results of the antioxidant effect of mangaba in different experimental models (de Oliveira Yamashita et al. 2020; Santos et al. 2022). Our hypothesis is that the antifungal effect is due to the observed antioxidant potential, similar to that reported with the mangaba leaf (Santos et al. 2016). Thus, the phenolic compounds found may be responsible for the observed effect, since they were previously described as inhibitors of fungal growth and free radical scavengers (Miao and Xiang 2020; Singh et al. 2020; Li et al. 2021). At last, our results suggest that mangaba pulp can be an alternative for the control of fungal species with potential application by the pharmaceutical industry, valuing local biodiversity.

3. Conclusion

The freeze-dried mangaba showed antifungal activity against species such as *Candida albicans* (ATCC 90028), *Cryptococcus gattii* (AFLP4), *Candida guilliermondii* (ATCC 6260) and *Candida albicans* (MYA 2876). The antifungal effect can be explained by the presence of bioactive compounds chlorogenic acid, ferulic acid and p-coumaric acid, associated with the antioxidant effect of the fruit pulp.

Disclosure statement

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