ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392202103642

ORIGINAL ARTICLE

# Phenolic and flavonoid content and *in vitro* inhibitory effect of some Amazonian fruit juices on CYP3A4 activity

Edna Márcia Almeida COSTA[1], Mariana Brasil DE ANDRADE FIGUEIRA[1], Daniel Blascke CARRÃO[2], Maísa Daniela HABENSCHUS[2], Anderson Rodrigo Moraes DE OLIVEIRA[2], Leonard Domingo Rosales ACHO[3], Emerson Silva LIMA[3], Igor Rafael DOS SANTOS MAGALHÃES[1]*

[1] Universidade Federal do Amazonas, Faculdade de Ciências Farmacêuticas, Núcleo de Estudos em Farmacocinética, 69077-000, Manaus, AM, Brazil

[2] Universidade de São Paulo, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Departamento de Química, 14040-901,Ribeirão Preto - SP, Brazil

[3] Universidade Federal do Amazonas, Faculdade de Ciências Farmacêuticas, Laboratório de Atividade Biológica, 69077-000, Manaus, AM, Brazil

* Corrresponding author: imagalhaes@ufam.edu.br; https://orcid.org/0000-0002-0651-696X

## ABSTRACT

Many foods interact with drugs and may cause changes in the pharmacological effects of the co-administered therapeutic agent. The enzyme CYP3A4, which belongs to the cytochrome P450 enzyme complex, is responsible for the metabolism of most drugs currently on the market and is involved in many drug interactions. Hence, the interaction of this enzyme with juices of some fruits, such as grapefruit, can affect the pharmacokinetics of various drugs. However, native fruits from the Amazon region have not yet been the target of this type of research. We determined total polyphenols and flavonoids of the Amazonian fruits açaí (*Euterpe precatoria*), buriti (*Mauritia flexuosa*), camu-camu (*Myrciaria dubia*), cubiu (*Solanum sessiliflorum*), cupuaçu (*Theobroma grandiflorum*), jenipapo (*Genipa americana*), and taperebá (*Spondias mombin*) and evaluated the effects of each fruit juice on CYP3A4 activity, using the star fruit (*Averrhoa carambola)* juice as positive control. Açaí juice presented the highest content of total polyphenols and flavonoids (102.6 ± 7.2 µg gallic acid equivalent (GAE) per mL and 7.2 ± 0.6 µg quercetin equivalent (QE) per mL, respectively). All juices were able to inhibit the activity of CYP3A4. There was no residual activity of the drug-metabolizing enzyme for açai, buriti, cubiu, camu-camu, and taperebá juice, while for cupuaçu, jenipapo and the positive control, the residual activity was 44.3, 54.3 and 20.2%, respectively. Additional studies should identify the phytocompound(s) responsible for this inhibition activity, to clarify the mechanisms involved in this phenomenon.

**KEYWORDS:** edible plants, metabolism, enzyme inhibition, drug interactions

# Conteúdo fenólico e flavonoídico e efeito inibitório *in vitro* de alguns sucos de frutas da Amazônia sobre a atividade de CYP3A4

## RESUMO

Muitos alimentos interagem com medicamentos e podem causar alterações nos efeitos farmacológicos do agente terapêutico coadministrado. A enzima CYP3A4, que pertence ao complexo enzimático do citocromo P450, é responsável pelo metabolismo da maioria dos medicamentos atualmente no mercado e está envolvida em muitas interações medicamentosas. Assim, a interação dessa enzima com sucos de algumas frutas, como a toranja, pode afetar a farmacocinética de vários medicamentos. No entanto, frutas nativas da região amazônica ainda não foram alvo desse tipo de pesquisa. Determinamos polifenóis e flavonoides totais dos frutos amazônicos de açaí (*Euterpe precatoria*), buriti (*Mauritia flexuosa*), camu-camu (*Myrciaria dubia*), cubiu (*Solanum sessiliflorum*), cupuaçu (*Theobroma grandiflorum*), jenipapo (*Genipa americana*) e taperebá (*Spondias mombin*) e avaliamos os efeitos de cada suco de fruta sobre a atividade de CYP3A4, utilizando o suco de carambola (*Averrhoa carambola*) como controle positivo. O suco de açaí apresentou o maior teor de polifenóis totais e flavonóides (102,6 ± 7,2 µg equivalente de ácido gálico (GAE) por mL e 7,2 ± 0,6 µg equivalente de quercetina (QE) por mL, respectivamente). Todos os sucos foram capazes de inibir a atividade de CYP3A4. Não houve atividade residual da enzima metabolizadora de fármacos para os sucos de açaí, buriti, cubiu, camu-camu e taperebá, enquanto para cupuaçu, jenipapo e o controle positivo, a atividade residual foi de 44,3, 54,3 e 20,2%, respectivamente. Estudos adicionais devem identificar o(s) fitocomposto(s) responsável(is) por esta atividade de inibição, para esclarecer os mecanismos envolvidos neste fenômeno.

**PALAVRAS-CHAVE:** plantas comestíveis, metabolismo, inibição enzimática, interações medicamentosas

**CITE AS:** Costa, E.M.A.; De Andrade Figueira, M.B.; Carrão, D.B.; Habenschus, M.D.; De Oliveira, A.R.M.; Acho, L.D.R.; Lima, E.S.; Dos Santos Magalhães, I.R. 2022. Phenolic and flavonoid content and *in vitro* inhibitory effect of some Amazonian fruit juices on CYP3A4 activity. *Acta Amazonica* 52: 172-178.

## INTRODUCTION

The Brazilian Amazon has a wide diversity of native fruit species. Many of these fruits are becoming increasingly popular in Brazil and in other countries. Edible fruits marketed in traditional fairs throughout the Amazon region remain to be explored as sources of nutrients for food and pharmaceutical applications, but their chemical profile and effects on health is still poorly known and deserving investigation (Araújo *et al.* 2021).

Amazonian fruits are usually consumed fresh, frequently in the form of juice (Rabelo 2012). Besides being highly appreciated for their taste, juices facilitate easier access to several bioactive compounds with beneficial effects to human health (Anderle *et al.* 2004; Chen *et al.* 2018). Fruits and their juice can also alter the disposition of drugs, mainly by acting on cytochrome P450 enzymes (CYP), especially CYP3A4 (Korhonen *et al.* 2007; Iwata *et al.* 2015; Ohkubo *et al.* 2017).

Several studies have shown that grapefruit (*Citrus paradisi* Macfad.) juice has an inhibitory effect on CYP3A4 leading to an increased bioavailability of drugs metabolized by this enzyme and, consequently, greater systemic exposure to orally administered drugs (Marco *et al.* 2002; Yin *et al.* 2010). The mechanism of this interaction involves irreversible inhibition of CYP3A4 in the small intestine (Girennavar *et al.* 2006; Girennavar *et al.* 2007). Other fruits that have been described to inhibit this enzyme are the star fruit (*Averrhoa carambola* L.) (Hidaka *et al.* 2004; Zhang *et al.* 2007; Hosoi *et al.* 2008), the pomegranate (*Punica granatum* L.) (Hidaka *et al.* 2005), and the wild grape (*Vitis vinifera* L.) (Kim *et al.* 2006).

According to some authors, the polyphenolic compounds found in fruits are responsible for inhibiting CYP3A4 (Girennavar *et al.* 2006; Girennavar *et al.* 2007; Hosoi *et al.* 2008). Thus, the presence and interaction of these compounds in fruit juices on CYP3A4 must be evaluated properly. The *in vitro* interaction of dietary polyphenols and CYP3A4 activity is variable due to structural and physicochemical characteristics such as the number of hydroxyl groups, stereostructure, molecular weight and lipophilicity (Basheer and Kerem 2015). The structure-activity relationship of flavonoids towards CYP3A4 has been recently established by computational models, in order to predict some possible drug interactions (Li *et al.* 2018).

One of the interactions described in the literature is for curmumin, a polyphenol extracted from turmeric (*Curcuma longa* L.), which can induce pharmacokinetic changes in several drugs by inhibiting cytochrome P450 isoenzymes (Bahramsoltani *et al.* 2017). Green tea (*Camelia sinensis* L.) polyphenols may also interact with CYP2C8, CYP2B6, CYP3A4, CYP2D6, and CYP2C19 enzymes, promoting overdose or underdosing of various drugs (Teschke and Xuan 2019).

Despite the wide diversity and amount of native fruit juices consumed by the local population in the Amazon region, to our knowledge, currently there is no study available in the scientific literature which evaluated drug-food interaction by Amazonian fruit juices. Therefore, the aim of this study was to determine the content of phenolic compounds and flavonoids in seven popular Amazonian fruit juices, as well as their *in vitro* inhibitory effect on CYP3A4 activity.

## MATERIAL AND METHODS

### Materials

Chromatographic grade methanol and acetonitrile were acquired from Panreac (Barcelona, Spain). Diethylamine and hydrochloric acid were purchased from Synth (Diadema, Brazil). The components of the NADPH generation system, nicotinamide adenine dinucleotide phosphate, glucose-6-phosphate and glucose-6-phosphate dehydrogenase enzyme, as well as midazolam (MI) and diazepam (IS), were acquired from Sigma-Aldrich (St. Louis, USA). The 1-hydroxymidazolam (1-HMI) was obtained from the Cayman Chemical Company (Ann Arbor, USA). Gallic acid, Folin-Ciocalteu and quercetin reagents were purchased from Sigma-Aldrich (St. Louis, USA). Human liver microsomes (HLMs) were acquired from Corning Life Science (Arizona, USA) and stored at -80 °C until use.

### Fruit samples

The analyzed fruits were chosen among the widely consumed fruit juices in the Amazon region which were harvested and marketed at the time of execution of this study (Rabelo 2012). Samples of açaí, *Euterpe precatoria* Mart. (Arecaceae); buriti, *Mauritia flexuosa* Mart. (Arecaceae); camu-camu, *Myrciaria dubia* (Kunth) McVaugh (Myrtaceae); cubiu, *Solanum sessiliflorum* Dunal (Solanaceae); cupuaçu, *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. (Malvaceae); jenipapo, *Genipa americana* L. (Rubiaceae); and taperebá, *Spondias mombin* L. (Anacardiaceae) (Figure 1) were acquired from local markets in the city of Manaus, Amazonas state, Brazil, from May to June and September to October 2015. The star fruit (Oxalidaceae), which is not an Amazonian fruit, was chosen as a standard positive control, as the juice of this fruit has already been reported to inhibit CYP3A4 (Hidaka *et al.* 2004; Zhang *et al.* 2007).

### Preparation of the juices

The fruits were sanitized in 2% sodium hypochlorite solution (Yuyama *et al.* 2011), and stored at -20 °C until use. Each sample was subjected to a turbolysis process (2 min), using ultra-pure water as solvent (40% m $v^{-1}$) and the obtained juices were centrifuged at 4000 rpm for 5 min (Girennavar *et al.* 2007). The supernatant was collected, filtered, and lyophilized (Hidaka *et al.* 2004). For analysis, the lyophilized

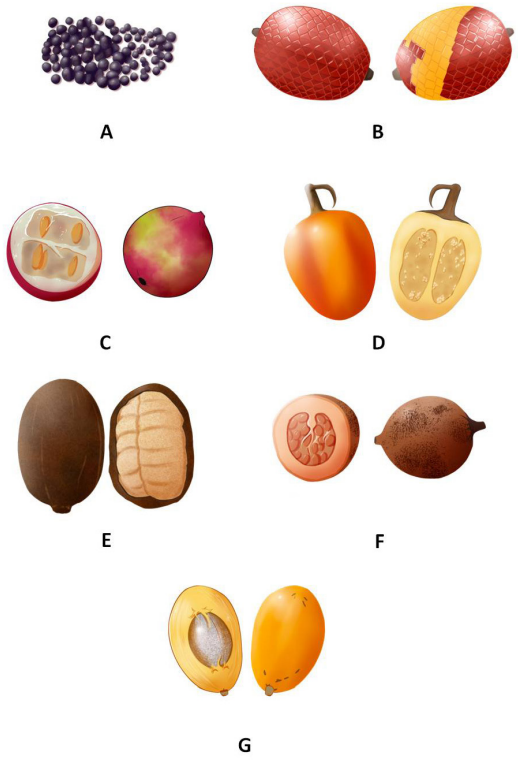


**Figure 1.** Amazonian fruit species studied: A – *Euterpe precatoria* (açaí); B – *Mauritia flexuosa* (buriti); C – *Myrciaria dubia* (camu-camu); D – *Solanum sessiliflorum* (cubiu); E – *Theobroma grandiflorum* (cupuaçu); F – *Genipa americana* (jenipapo); G – *Spondias mombin* (taperebá). This figure is in color in the electronic version.

juices were resuspended in water and centrifuged at 3000 rpm for 10 min (Girennavar *et al.* 2007). The fruit part used in each case is described in Table 1.

## Determination of total polyphenols and flavonoids

The assessment of the phenolic and flavonoid content of edible fruits is important since these compounds are generally linked to biological activities (Becker *et al.* 2018). Total polyphenol content was determined using Folin–Ciocalteu reagent, as described by Bonoli *et al.* (2004). For quantification, a standard curve with gallic acid solution was used at the range 7.8125-500 μg $mL^{-1}$. The total polyphenol content was assayed in quadruplicates and expressed as μg gallic acid equivalent (GAE) $mL^{-1}$.

Total flavonoids were determined according to Chang *et al.* (2002). The quantification was performed using a standard curve with quercetin solution in the range 7.8125-500 μg $mL^{-1}$. The total flavonoid content was assayed in quadruplicates and expressed as μg quercetin equivalent (QE) $mL^{-1}$.

A Beckman Coulter microplate reader, model DTX800 (Brea, USA) operating at the wavelengths of 620 nm and 405 nm was used for the determination of polyphenols and flavonoids, respectively. Values were expressed as the mean ± standard deviation.

## Analysis of CYP3A4 activity marker

The CYP3A4 activity marker (1-HMI) was analyzed with an HPLC equipment coupled to a diode-array detector (DAD) acquired from Shimadzu (Kyoto, Japan). The analysis was carried out by using a Gemini C18 analytical column (250 mm x 4.6 mm, 5 μm particle size, Phenomenex, Torrance, USA) and mobile phase composed of acetonitrile: ultra-pure water with 0.1% diethylamine (45:55, v/v). The flow rate was set at 1.2 mL $min^{-1}$ and the column temperature was kept at 30 °C. The injection volume was 50 μL. Analytes were monitored at 260 nm (for 1-HMI) and 239 nm (for diazepam - IS). The data were analyzed using the LC Solution software, version 1.25 SP1 (Shimadzu).

## Enzymatic kinetics of midazolam

The reaction time, protein concentration, and concentration range of MI were determined in advance of sample analysis to ensure that all reactions take place under conditions of V0, which is the initial rate of the enzymatic reaction. This rate corresponds to a linear decrease of substrate concentration in relation to protein concentration and incubation time.

**Table 1.** Content of total polyphenols equivalent to gallic acid (μg GAE $mL^{-1}$) and total flavonoids equivalent to quercetin (μg QE $mL^{-1}$) in the juice of seven Amazonian fruits (and star fruit as positive control) and their effect on midazolam 1-hydroxylation in human liver microsomes.

| Fruits species | Common name | Plant part used | Total polyphenols (μg GAE $mL^{-1}$) | Total flavonoids (μg QE $mL^{-1}$) | Residual activity of CYP3A4 (%) |
|---|---|---|---|---|---|
| *Averrhoa carambola* L. | star fruit | Pulp | * | * | 20.20 ± 0.05 |
| *Euterpe precatoria* Mart. | açaí | Pulp | 103 ± 7 | 7.2 ± 0.6 | 0.0 |
| *Myrciaria dubia* (Kunth) McVough | camu-camu | Pulp and peel | 70 ± 3 | n.d. | 0.0 |
| *Mauritia flexuosa* L.f. | buriti | Pulp | 35 ± 1 | 1.6 ± 0.2 | 0.0 |
| *Solanum sessiliflorum* Dunal | cubiu | Pulp | 20 ± 3 | n.d. | 0.0 |
| *Spondias mombin* L. | taperebá | Pulp and peel | 20 ± 1 | n.d. | 0.0 |
| *Genipa americana* L. | jenipapo | Pulp and seed | 19 ± 2 | n.d | 54.30 ± 0.04 |
| *Theobroma grandiflorum* (Will ex Spreg) | cupuaçu | Pulp | 18 ± 1 | n.d. | 44.30 ± 0.03 |

Values are the mean ± standard deviation of three replicates; n.d. = not detected; *: not analyzed.

For the enzyme kinetics, the MI concentrations of 1, 2, 5, 7, 10, 30, 50, 70, 100, 200, 250, 300 and 350 µM (0.32, 0.65, 1.62, 2.28, 3.25, 6.51, 9.77, 16.28, 22.80, 32.57, 65.15, 81.44, 97.73 and 114.02 µg $mL^{-1}$) were evaluated. The formation of the metabolite 1-HMI was employed to determine the enzyme kinetics. Thus, the samples were analyzed in quintuplicate and quantified by HPLC-DAD with the aid of an analytical curve of 1-HMI in the concentrations of 0.035, 0.04, 0.1, 0.5, 1, 2 and 4.5 µM (0.011, 0.013, 0.017, 0.034, 0.170, 0.256, 0.341, 0.683, 1.367 and 1.537 µg $mL^{-1}$). The enzyme reaction rate for each concentration of MI was determined by the quotient of the concentration of 1-HMI found, the protein concentration and the incubation time, according to Equation 1

$$Rate\ of\ reaction = \frac{\frac{[1 - hydroxymidazolam\ obtained]}{0.1\ mg\ mL^{-1}}}{10\ min}$$

(Equation 1)

The results obtained were plotted on a graph of enzyme reaction velocity by the concentration of MI and analyzed by non-linear regression by using the GraphPad Prism 6 program (GraphPad Software, San Diego, USA).

After determining the enzyme kinetics, the $K_M$ value – Michaelis-Menten constant (substrate concentration in which V0 is equal to half of $V_{MAX}$) was calculated. For the inhibition studies of the fruit juices, midazolam concentration (5 µM) was used, with a value close to its $K_M$ value.

### Inhibitory effect of fruit juices on CYP3A4 activity

The inhibition of CYP3A4 by the fruit juices was evaluated by the formation of 1-HMI, according to Fujita *et al.* (2003) and Hidaka *et al.* (2004). The assay was performed in microtubes using a 5 µM MI solution (5 µL), 5 mg $mL^{-1}$ lyophilized juice resuspended in PBS solution 0.1 M pH 7.4 (125 µL), NADPH generator system (50 µL) containing 5 mmol $L^{-1}$ glucose-6-phosphate, 0.25 mmol $L^{-1}$ nicotinamide adenine dinucleotide phosphate, and 0.5 unit $mL^{-1}$ glucose-6-phosphate dehydrogenase enzyme. A volume of HLMs of 20 µL was used to a final volume of 200 µL. The samples were pre-incubated in a water bath with stirring for 5 min at 37 °C. The metabolism was initiated by the addition of HLMs, with a reaction time of 10 min at 37 °C. Then, chilled acetonitrile (200 µL), was added to stop the reaction followed by the IS addiction (30 µL of a solution containing 1.4 µg $mL^{-1}$). The mixture was then centrifuged at 3500 rpm for 10 min. The supernatant was collected (200 µL) and samples were analyzed using HPLC-DAD. Under these specific conditions, the term *residual activity* was regarded as the enzyme activity that remains after exposure of the enzyme to the potential inhibitors.

## RESULTS

The determination coefficient obtained for the quantification of total polyphenols was 0.993. Total polyphenol levels varied between 19.57 and 102.56 µg GAE $mL^{-1}$. The determination coefficient for the quantification of total flavonoids was 0.999. The levels of total flavonoids were determined only for açaí and cupuaçu (7.16 and 1.64 µg QE $mL^{-1}$, respectively) as the results obtained for the other juices were below the limit of quantification of the method used (Table 1). Açaí presented the highest polyphenol and flavonoid concentrations (Table 1).

The total analysis time for the chromatographic separation of 1-HMI, MI and the IS was less than 15 min (Figure 2). The retention time of 1-HMI, MI and IS was 6.2, 9.6 and 11.8

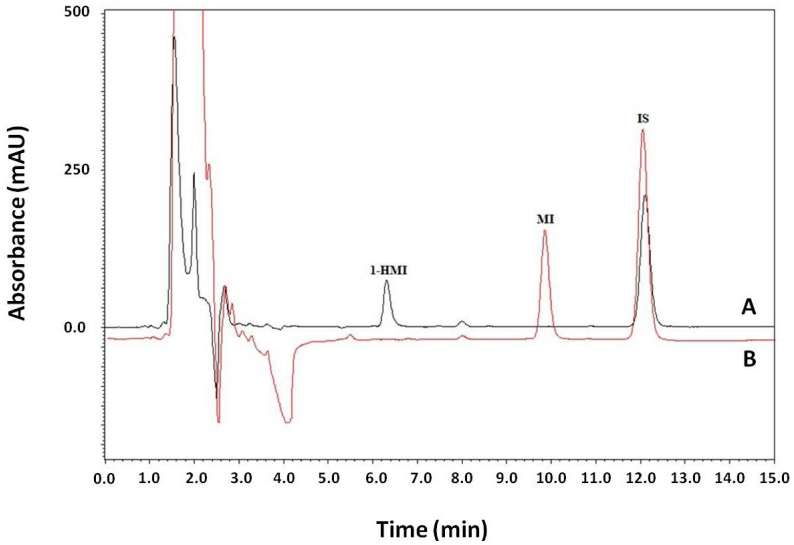


**Figure 2.** Representative chromatogram of 1-hydroxyimidazolam (1-HMI), midazolam (MI) and diazepam (IS). A – 260 nm; B – 239 nm. Chromatographic conditions are described in Materials and Methods. This figure is in color in the electronic version.

min, respectively. The method showed to be selective, since no interferents from the microsomal medium were observed in the retention time of the analytes (data not shown).

The enzyme kinetics of the MI metabolism by HLMs conformed to a Michaelis-Menten kinetic profile (Figure 3). The enzyme parameters were $V_{MAX}$ = 0.59 ± 0.01 nmol mg protein $min^{-1}$ and $K_M$ = 5.0 ± 0.5 µmol $L^{-1}$. The control activity of midazolam hydroxylation at 5 µM by HLM determined in the absence of juice was 0.11 ± 0.02 nmol mg protein $min^{-1}$.

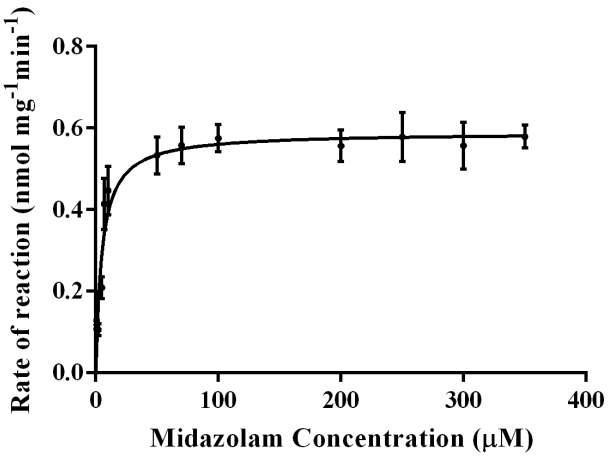


**Figure 3.** Enzyme kinetics of midazolam metabolism by the CYP3A4 form by using human liver microsomes (HLMs). The enzyme parameters were $V_{MAX}$ = 0.59 ± 0.01 nmol mg protein $min^{-1}$ and $K_M$ = 5.0 ± 0.5 µmol $L^{-1}$. The control activity of midazolam hydroxylation at 5 µM by HLM determined in the absence of juice was 0.11 ± 0.02 nmol mg protein $min^{-1}$. $R^2$ = 0.89; df = 59; absolute sum of squares = 0.18; Sy.x = 0.05.

All juices were able to inhibit the activity of the CYP3A4. No residual activity of the drug-metabolizing enzyme was observed for açai, buriti, cubiu, camu-camu, and taperebá, which was below the value observed for star fruit (Table 1). Only cupuaçu and jenipapo showed residual activity above that of star fruit.

## DISCUSSION

The highest contents of both phenolic and flavonoid were found in açaí samples. Several studies have reported the antioxidant potential of açaí due to the presence of phenolic compounds, especially flavonoid anthocyanins, both present in both *E. precatoria* and *E. oleracea Mart* (Yuyama *et al.* 2011; Yamaguchi *et al.* 2015; De Moura and Resende 2016; Martins *et al.* 2018).

Phenolic and flavonoid contents in fruit juice are generally determined for quality control purposes and not to assess inhibitory effects on CYP450 (Zhang *et al.* 2007). Some authors evaluated the impact of phenolic and flavonoid contents on other biological activities. For example, freeze-dried *E. oleraceae* and *E. precatoria* fruits was added to NIH-31 rodent chow for modulation of neurochemical parameters in rat brain. The polyphenol content varied between the two açaí species, with most glycosidic anthocyanins, flavonoids and phenolic acids about two to 50 times higher in *E. precatoria*, except delphinidin, quercetin and vanillic acids, which were considerably higher in *E. oleraceae*, resulting in better behavioral outcomes for the diet supplemented with the latter species (Poulose *et al.* 2017).

Camu-camu, which had the second highest content of polyphenols, is known to have a high concentration of phenolic compounds (Neves *et al.* 2012; Tauchen *et al.* 2016). Likewise, buriti, which had the third highest content, is also considered as rich in antioxidant compounds, such as carotenoids, ascorbic acid and phenolic compounds (Melo *et al.* 2008; Tauchen *et al.* 2016). The levels of total polyphenols obtained for cubiu and jenipapo were close to those reported by Tauchen *et al.* (2016), who also reported higher values for these compounds in cupuaçu from Peru.

All evaluated fruit juices interacted with CYP3A4, however only açaí, camu-camu, buriti, cubiu and taperebá juices inhibited 100% of the enzymatic activity (remaining activity 0%). The residual activity of CYP3A4 in the presence of jenipapo and cupuaçu juices was higher than that of the positive control. Despite the low concentration of polyphenols in comparison to other fruits, cubiu and taperebá juices also resulted in the absence of residual CYP3A4 activity. Unlike our result, Hidaka *et al.* (2004) determined a residual activity of 0.1% for star fruit juice. The procyanidin B1 and B2 stereoisomers and/or catechin/epicatechin found in star fruit are suggested as responsible for the inhibition of CYP3A4 (Hosoi *et al.* 2008). The discrepancy with the average value of 20.2% obtained in our study is likely owed to variation in the level of phytochemicals, which depends on ripeness, plant part used, geographical origin and environmental conditions of the samples, collection period, harvest conditions, cultivation practices and storage process (Kårlund *et al.* 2014). Although the levels of phenolic and flavonoid compounds were not determined for our star fruit juice samples, Zhang *et al.* (2007) observed that large variations in the contents of these chemicals did not result in substantial differences of their inhibitory effect, suggesting the participation of other substances in inhibition mechanisms.

Several limitations might have influenced our results. The fruits were acquired at local fairs, so that we had no precise information regarding geographical origin, ripening and harvesting of the fruits. Furthermore, only one concentration of each juice was analyzed. Further studies should determine the half maximum inhibitory concentration ($IC_{50}$), i.e., the concentration necessary to decrease the enzyme activity in 50%. Taken together, these facts limit the generalizations that can be drawn from this study, which was devised as a first-step

screening step to determine possible drug-food interactions *sensu* Burkina *et al*. (2016).

The interpretation of our results also deserves caution, as *in vitro* drug interaction studies with plant products, though being relatively easy to perform, inexpensive compared to studies in animals or humans, and allowing controlled test conditions, are not easily reproducible. This is due to insufficient data regarding the absorption process of unknown botanical components, uncertainty about relevant concentrations for the assay, and difficulties in standardizing samples with complex phytochemical profiles, among other aspects. However, despite these limitations, *in vitro* studies are a viable screening alternative to identify compounds that may represent a risk of drug interactions and, serve as indicators for conducting *in vivo* studies (Markowitz and Zhu 2012). In this sense, our study provided guiding data on the potential for *in vivo* drug interactions of several popular Amazonian fruit juices, indicating those requiring further clinical studies (Showande *et al*. 2019).

## CONCLUSIONS

We report the first data on the inhibitory effect of seven popular Amazonian fruit juices on the CYP3A4 enzyme activity. Our results indicate that all the juices may interact with drugs metabolized by CYP3A4, which may cause further changes in the bioavailability of the associated drug. However, additional studies are needed to investigate the clinical relevance of the interaction of these juices with CYP3A4 *in vivo* and to identify the phytocompound(s) responsible for the observed inhibition effects and to clarify the mechanisms involved.

## ACKNOWLEDGMENTS

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) for research grants to Edna Márcia Almeida Costa, and for the funding of the project (Edital Universal Amazonas FAPEAM # 021/2011). The authors are grateful to Felipe Miguel and Cléber Paes Pereira for the assistance in the experiments and for providing the illustrations of the fruits studied, respectively.

## REFERENCES

Anderle, P.; Huang, Y.; Sadee, W. 2004. Intestinal membrane transport of drugs and nutrients: genomics of membrane transporters using expression microarrays. *European Journal of Pharmaceutical Sciences,* 21: 17–24.

Araújo, N.M.P.; Arruda, H.S.; Marques, D.R.P.; Oliveira, W.Q.; Pereira, G.A.; Pastore, G.M. 2021. Functional and nutritional properties of selected Amazon fruits: A review. *Food Research International,* 147: 110520.

Bahramsoltani R.; Rahimi R.; Farzaei M.H. 2017. Pharmacokinetic interactions of curcuminoids with conventional drugs: A review. *Journal of Ethnopharmacology,* 209: 1-12.

Basheer, L.; Kerem, Z. 2015. Interactions between CYP3A4 and dietary polyphenols. *Oxidative Medicine and Cellular Longevity*, 2015: 854015.

Becker, M.M.; Mandaji, C.M.; Catanante, G.; Marty, J.-L.; Nunes, G.S. 2018. Mineral and bromatological assessment and determination of the antioxidant capacity and bioactive compounds in native Amazon fruits. *Brazilian Journal of Food Technology,* 21: e2018022.

Bonoli, M.; Verardo, V.; Marconi, E.; Caboni, M.F. 2004. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *Journal of Agricultural and Food Chemistry,* 52: 5195–5200.

Burkina, V.; Zlabek, V.; Halsne, R.; Ropstad, E.; Zamaratskaia, G. 2016. In vitro effects of the citrus flavonoids diosmin, naringenin and naringin on the hepatic drug-metabolizing CYP3A enzyme in human, pig, mouse and fish. *Biochemical Pharmacology,* 110-111: 109-116.

Chang, C.; Yang, M.; Wen, H.; Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis,* 10: 178-182.

Chen, M.; Zhou, S.; Fabriaga, E.; Zhang, P.; Zhou, Q. 2018. Food-drug interactions precipitated by fruit juices other than grapefruit juice: An update review. *Journal of Food and Drug Analysis,* 26: SS61-SS71.

De Moura R.S; Resende Â.C. 2016. Cardiovascular and metabolic effects of açaí, an Amazon plant. *Journal of Cardiovascular Pharmacology,* 68: 19-26.

Fujita, K.; Hidaka, M.; Yamasaki, K.; Takamura, N.; Iwakiri, T.; Okumura, M.; Kodama, H.; Yamaguchi, M.; Ikenoue, T.; Arimori, K. 2003. Inhibitory effects of citrus fruits on cytochrome P450 3A (CYP3A) activity in humans. *Biological & Pharmaceutical Bulletin,* 26: 1371–1373.

Girennavar, B.; Poulose, S.M.; Jayaprakasha, G.K.; Bhat, N.G.B.; Patil, B.S. 2006. Furocoumarins from grapefruit juice and their effect on human CYP 3A4 and CYP 1B1 isoenzymes. *Bioorganic & Medicinal Chemistry,* 14: 2606–2612.

Girennavar, B.; Jayaprakasha, G.K.; Patil, B.S. 2007. Potent inhibition of human cytochrome P450 3A4, 2D6, and 2C9 isoenzymes by grapefruit juice and its furocoumarins. *Journal of Food Science,* 72: C417-421.

Hidaka, M.; Fujita, K. I.; Ogibuko, T.; Yamasaki, K.; Iwakiri, T.; Okumura, M.; Kodama, H.; Arimori, K. 2004. Potent inhibition by star fruit of human cytochrome P450 3A (CYP3A) activity. *Drug Metabolism and Disposition,* 32: 581–583.

Hidaka, M.; Okumura, M.; Fujita, K.; Ogibuko, T.; Yamasaki, K.; Iwakiri, T.; Setoguchi, N.; Arimori, K. 2005. Effects of pomegranate juice on human cytochrome P450 3A (CYP3A) and carbamazepine pharmacokinetics in rats. *Drug Metabolism and Disposition,* 33: 644–648.

Hosoi, S.; Shimizu, E.; Arimori, K. Okumura, M.; Hidaka, M.; Yamada, M.; Sakushima, A. 2008. Analysis of CYP3A inhibitory

components of star fruit (*Averrhoa carambola* L.) using liquid chromatography–mass spectrometry. *Journal of Natural Medicines,* 62: 345–348.

Iwata, H.; Yamaguchi, K.; Takeshita, Y.; Kubota, A.; Hirakawa, S.; Isobe, T.; Hirano, M.; Kim, E.Y. 2015. Enzymatic characterization of in vitro-expressed Baikal seal cytochrome P450 (CYP) 1A1, 1A2, and 1B1: Implication of low metabolic potential of CYP1A2 uniquely evolved in aquatic mammals. *Aquatic Toxicology,* 162: 138–151.

Kårlund, A.; Moor, U.; Sandell, M.; Karjalainen, R. O. 2014. The impact of harvesting, storage and processing factors on health-promoting phytochemicals in berries and fruits. *Processes*, 2: 596-624.

Kim, H.; Yoon, Y.J.; Shon, J.H.; Cha, I.J.; Shin, J.G.; Liu, K.H. 2006. Inhibitory effects of fruit juices on CYP3A activity. *Drug Metabolism and Disposition*, 34: 521–523.

Korhonen, L.E.; Turpeinen, M.; Rahnasto, M.; Wittekindt, C.; Poso, A.; Pelkonen, O.; Raunio, H.; Juvonen, R.O. 2007. New potent and selective cytochrome P450 2B6 (CYP2B6) inhibitors based on three-dimensional quantitative structure-activity relationship (3D-QSAR) analysis. *British Journal of Pharmacology,* 150: 932–942.

Li, Y., Ning, J.; Wang, Y.; Wang, C.; Sun, C.; Huo, X.; *et al*. 2018. Drug interaction study of flavonoids toward CYP3A4 and their quantitative structure activity relationship (QSAR) analysis for predicting potential effects. *Toxicology Letters,* 294: 27-36.

Marco, M.P.D.; Edwards, D.J.; Wainer, I.W.; Ducharme, M.P. 2002. The effect of grapefruit juice and seville orange juice on the pharmacokinetics of dextromethorphan: The role of gut CYP3A and P-glycoprotein. *Life Sciences,* 71: 1149–1160.

Markowitz, J. S.; Zhu, H.-J. 2012. Limitations of in vitro assessments of the drug interaction potential of botanical supplements. *Planta Med,* 78: 1421–1427.

Martins, I.C.V.S.; Borges, N.A.; Stenvinkel, P.; Lindholm, B.; Rogez, H.; Pinheiro, M.C.N. *et al*. 2018. The value of the Brazilian açai fruit as a therapeutic nutritional strategy for chronic kidney disease patients. *International Urology and Nephrology,* 50: 2207-2220.

Melo, E.A.; Maciel, M.I.S.; Lima, V.L.A.G.; Leal, F.L.L.; Caetano, A.C.S.; Nascimento, R.J. 2006. Capacidade antioxidante de hortaliças usualmente consumidas. *Ciência e Tecnologia de Alimentos,* 26: 639–644.

Neves, L.C.; De Campos, A.J.; Benedette, R.M.; Tosin, J.M.; Chagas, E.A. 2012. Caracterização da atividade antioxidante de frutos nativos da região Amazônica Brasileira. *Revista Brasileira de Fruticultura,* 34: 1165-1173.

Ohkubo, A.; Chida, T.; Kikuchi, H.; Tsuda, T.; Sunaga, K. 2017. Effects of tomato juice on the pharmacokinetics of CYP3A4-substrate drugs. *Asian Journal of Pharmaceutical Sciences,* 12: 464-469.

Poulose, S.M.; Bielinski, D.F.; Carey, A.; Schauss, A.G.; Shukitt-Hale, B. 2017. Modulation of oxidative stress, inflammation, autophagy and expression of Nrf2 in hippocampus and frontal cortex of rats fed with açaí-enriched diets. *Nutritional Neuroscience,* 20: 305-315.

Rabelo, A. 2012. *Frutos Nativos da Amazônia: Comercializados nas Feiras de Manaus – AM*. 1st ed, Editora INPA, Manaus, 390p.

Showande, S.J.; Fakeye, T.O.; Kajula, M.; Hokkanen, J.; Tolonen, A. 2019. Potential inhibition of major human cytochrome P450 isoenzymes by selected tropical medicinal herbs — Implication for herb–drug interactions. *Food Science and Nutrition*, 7: 44-55.

Tauchen, J.; Bortl, L.; Huml, L.; Miksatikova, P.; Doskocil, I.; Marsik, P.; *et al*. 2016. Phenolic composition, antioxidant and anti-proliferative activities of edible and medicinal plants from the Peruvian Amazon. *Brazilian Journal of Pharmacognosy,* 26: 728-737.

Teschke, R.; Xuan, T.D. 2019. How can green tea polyphenols affect drug metabolism and should we be concerned? *Drug Metabolism Toxicology*, 15: 989-991.

Yamaguchi, K.K.L.; Pereira, L.F.R.; Lamarão, C.V.; Lima, E.S.; Veiga-Junior, V.F. 2015. Amazon açaí: Chemistry and biological activities: A review. *Food Chemistry,* 179: 137-151.

Yin, O.Q.P.; Gallagher, N.; Li, A.; Zhou, W.; Harrell, R.; Schran. H. 2010. Effect of grapefruit juice on the pharmacokinetics of nilotinib in healthy participants. *The Journal of Clinical Pharmacology,* 50: 188-194.

Yuyama, L.K.O.; Aguiar, J.P.L.; Filho, D.F.S.; Yuyama, K.; Varejão, M.J.; Fávaro, D.I.T.; Vasconcellos, M.B.A.; Pimentel, S.A.; Caruso, M.S.F. 2011. Caracterização físico-química do suco de açaí de *Euterpe precatoria* Mart. oriundo de diferentes ecossistemas amazônicos. *Acta Amazonica,* 41: 545-552.

Zhang, J.; Liu, Y.; Cheng, J.; Li, W.; Ma, H.; Liu, H.; *et al*. 2007. Inhibition of human liver cytochrome P450 by star fruit juice. *Journal of Pharmacy & Pharmaceutical Sciences,* 10: 496-503.

**RECEIVED:** 29/12/2021
**ACCEPTED:** 23/05/2022
**ASSOCIATE EDITOR:** Emiliano Barreto, Jorge Mauricio David