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EVALUATION OF INHIBITORY EFFECTS OF SOME BRAZILIAN CERRADO PLANT EXTRACTS ON CYP3A *IN VITRO* ACTIVITY

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ABSTRACT

The Cerrado is a Brazilian biome with over 10,000 species of plants, many with therapeutic activities. Despite the great diversity, there is still little knowledge about the flora. Among these plants, four stand out for wide popular and traditional use: *Stryphnodendron adstringens* Mart (Barbatimao) and *Lafoensia pacari* A.St. Hil. (Pacari), and the oils of *Copaifera langsdorfii* Desf (Copaíba) and *Pterodon emarginatus* Vogel (Sucupira). This study evaluated the inhibitory effects of *Stryphnodendron adstringens*, *Copaifera langsdorffii*, *Lafoensia pacari* and *Pterodon emarginatus* on CYP3A activity in rat liver microsomes by HPLC-PDA. The method used to prepare the microsomes and the incubation method were suitable to assess the *in vitro* inhibition of CYP3A. Results showed that dry extracts of *S. adstringens* and *L. pacari*, *C. langsdorfii*o leoresin and *P. emarginatus* essential oil did not inhibit CYP3A activity.

Key words: Cytochrome P450 3A (CYP3A), Inhibition, Cerrado plants, Rat liver microsomes.

INTRODUCTION

Drug and xenobiotic metabolism is a fundamental process in pharmacokinetics, once it transforms them into more hydrophilic and, often, less toxic metabolites facilitating elimination and detoxification (Audi and Pussi, 2000; Wilkinson, 2005; Fan and Lannoy, 2014).

The liver is the main organ involved in the metabolism, presenting several enzymes responsible for drug biotransformation, being the cytochrome P450 system (CYP) the one most involved in these reactions(Audi and Pussi, 2000; Wilkinson, 2005; Lamattina and Golan, 2009). CYP is constituted by 41 subfamilies, which gather 57 isoenzymes. The main isoenzymes involved in drug metabolism are: 3A4, 2D6, 2C9 and 1A2. The isoform 3A4 is fundamental for biostransformation, as it metabolizes approximately 50% of known drugs (Audi and Pussi, 2000; Wilkinson, 2005).

Some compounds inhibit CYP3A4, which may result in drug interaction, once for individuals who need to use more than one drug during treatment, such property may result in the reduction one of the drugs' metabolism, increasing its plasmatic concentration, prolonging its effect, as well as possibly provoking adverse reaction/intoxication (Gibbs and Hosea, 2003; Wilkinson, 2005).

An important way to evaluate possible drug interactions due to enzymatic inhibition is obtaining data from *in vitro* inhibition studies, which are able to predict the interaction potential due to the presented inhibition (Guest *et al*., 2010). Cytochrome P450 inhibition tests have been performed more often, not only with isolated drugs, but also with medicinal plants, plant derivatives

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and herbal medicine (Lee *et al*., 2012; Djuv and Nilsen, 2012).

Midazolam is a widely used drug as a substrate to evaluate CYP3A *in vitro* activity that is intensively biotransformed into 1-hydroxymidazolam and 4-hydroxymidazolam, easily quantified by high-performance liquid chromatography - photodiode array detection (HPLC-PDA) (Elbarbry *et al*., 2009; Li *et al*., 2010; FDA, 2011). In humans, midazolam is metabolized by CYP3A4 and in rats by CYP3A1/3A2, being considered an ideal substrate to evaluate CYP3A activity, for synthetic substances or those of natural origin, including medicinal plant extracts and fractions (Ghosal *et al*., 1996; Li *et al*., 2010; Wang *et al*., 2010).

The Cerrado biome contains hundreds of plant species potentially useful and viable for economic exploration as food, for oilseeds, fibers, forage, fruits, medicine such as sucupira (*Pterodon emarginatus* Vogel), barbatimao (*Stryphnodendron barbatiman* Mart), pacari (*Lafoensia pacari* A.St.-Hil), pequi (Caryocar brasiliense Camb.), araticum (*Annona crassiflora* Mart.), araca (Psidium sp.), tarumã (Vitex cymosa Bert), mangaba (*Hancornia speciosa* Gomez), murici (*Byrsonisa verbascifolia* Rich), coco buriti (*Mauritia vinifera* Mart.), macaúba (*Acrocomia aculeata* Mart.), baru (*Dipterix alata* Vog), cagaita (*Eugenia dysenterica* DC) and other species, which are used by the local population and present therapeutic potential on several diseases that affect both man and domestic animals (Ribeiro *et al*., 1994; Souza and Felfili, 2006; Simon *et al*., 2009).

In this article four vegetable species with wide popular use and scientific documentation are highlighted, being three part of the Febaceae Family: *Stryphnodendron adstringens* (Mart.) Coville (barbatimão), *Copaifera langsdorffii* Desf. (copaíba), *Pterodon emarginatus* Vogel(sucupira); and one part of the Lythraceae family: *Lafoensia pacari* A.St.-Hil (pacari).

S. adstringens is popularly utilized as anti-inflammatory, astringent, for wound, varicose ulcer and vaginal infection treatment (Souza and Felfili, 2006; Ishida *et al*., 2006; Rodrigues and Carvalho, 2001). It is reported that barbatimão peel contains between 10 and 37 % of tannins, as well as flavonoids, proanthocyanidins and prorobinetinidins (Teixeira *et al*., 1990; Mello *et al*., 1996).

Ardisson *et al*., (2002) describe the action of barbatimao in wound cutaneous scarringand suggest that this effect occurs due to the high level of tannins present in the plant bark. Ishida and collaborators (2006) stress the antifungal action of *S. adstringens* against *Candida albicans*. Anothe study with barbatimao reports the efficacy of its crude ethanolic extract on *Trypanosoma cruzi* parasitaemia (Herzog-Soares *et al*., 2002), in addition to antimicrobial activity of hydro alcohol extract on *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Gonçalves *et al*., 2006) and, moreover, the effect against *Escherichia coli, Streptococcus pyogenes, Providencia spp., Proteus mirabilis, Shigella sonnei, Staphylococcus aureus* and *Staphylococcus spp* (Gonçalves *et al*., 2005).

*C. langsdorffii*is popularly utilized in the treatment of airway and urinary tract affections, to stimulate scarring, as an anti-inflammatory, in contusions and as antitumor drug (Rodrigues and Carvalho, 2001; Souza and Felfili, 2006). The bark oil contains high levels of sesquiterpenes (more than 40) and diterpenes (28 in total) (Veiga Jr. *et al*., 1997; Veiga Jr. and Pinto, 2002; Gelmini *et al*., 2013).

Copaíba oleoresin presents anti-inflammatory (Paiva *et al*., 2002a; Paiva *et al*., 2002b; Montes *et al*., 2009; Souza *et al.* 2011), antioxidant, antilipoperoxidative, antipsoriatic and antitumor effects against Walker sarcoma (Ohsaki *et al*., 1994; Paiva *et al*., 1998; Paiva *et al*., 2002a; Gelmini *et al*., 2013), antiulcerogenic (Paiva *et al*., 1998), damaged intestine cell protective, gastroprotective, cercaricidal, anthelmintic (Pellegrino, 1967; Galdino *et al*., 1972), mosquiticidal effects against *Aedes aegypti* (Mendonça *et al*., 2005) and, lastly, it also contains antimicrobial and antibacterial activities (Maruzella e Sicurella, 1960; Opdyke, 1976; Gonçalves *et al*., 2005; Brancalion *et al*., 2012).

Pacari (*L. pacari*) is porpularly known as dedaleiro, dedal, mangabeira-brava or pacari in Brazil (Mundo and Duarte, 2007). It can be widely found in altitude forests, mainly the Cerrado, and in city landscaping trees. It is utilized as a febrifuge, scarring drug, tonic (Mundo and Duarte, 2007) and antidiarrheal (Coelho *et al*., 2005), however, it also presents effects such as antifungal, analgesic, antipyretic, anti-edema, anti-inflammatory and antidepressant, and also against gastric disorder and cancer (Vila Verde *et al*., 2003; Rogerio *et al*., 2006; Souza and Felfili, 2006; Galdino *et al*., 2009; Naruzawa and Papa, 2011).

The methanolic extract of the bark of pacari shows recorded antioxidant activity and ellagic acid is the main substance responsible for the potent antioxidant activity of the methanolic extract and fractions (Solon *et al*., 2000). The aqueous extract of the bark inhibits inlerleukin-5 (IL-5) liberation in a toxocariasis model induced in mice without presenting antiparasitic activity (Rogerio *et al*., 2003). The aqueous extract of pacari bark, rich in ellagic acid, presents anti-inflammatory, antinoceptive and antiedematogenic activities in a murine model (Rogerio, 2006). However, the methanolic extract of pacari bark does not present activity in the eradication of *Helycobacter pylori* in a double-blind randomized clinical trial, despite reducing the dyspeptic symptomatology of a large number of treated patients (Menezes, 2006).

The ethanolic extract of *L. pacari* leaves was active against eight different varieties of *Staphylococcus*

aureus Gram+, *Proteus mirabilis* Gram-and acid-resistant bacilli *Mycobacterium phlei, M. fortuitum* e *M. smegmatis*. The ethanolic extract of *L. pacari* bark presented activity against *S. aureus* (Lima *et al*., 2006).

In another study, ethanolic extracts of *L. pacari* leaves and bark presented anti-inflammatory and analgesic activity, discarding the possibility of the antinoceptive activity being related to effects in central nervous system (CNS), such as anxiolytic, depressing or myorelaxing action (Guimarães, 2008).

The main chemical constituents described for *L. pacari* belong, mostly, to the class of phenolic compounds, such as tannins, flavonoids, and ellagic acid (Solon *et al*., 2000; Guimaraes, 2008), in addition to saponins in the bark and the leaves of the plant (Sampaio and Leão, 2007).

Pterodon emarginatus Vogel (Fabaceae), also called white sucupira, is a common tree in Brazil in the estates of Goias, Tocantins, Piaui, Bahia and the northwest region of Minas Gerais, in the cerrados or cerradoes (Lorenzi, 2014).

Sucupira fruit oil is described as medicinal, as well as the infusion and extracts of the bark. Therapeutic properties such as antimicrobial, anti-inflammatory, analgesic, anti-asthmatic, antioxidant, antiulcer and anti-rheumatic are described for this plant (Vieira and Martins, 2000; Dutra *et al*., 2008; Dutra *et al*., 2009; Bustamante *et al*., 2010). Popularly, it is used to treat sore throat, airway affections, as an anti-inflammatory, analgesic e depurative drug, for children in fortifying medicine or appetite stimulants (Mascaro *et al*., 2004; Dutra et al., 2009; Bustamante *et al*., 2010).

The objective of this study is to increase the knowledge on extracts, essential oils and oleoresins of the aforementioned plants, with the development of enzymatic inhibition trials of the most important family of drug oxidative metabolizing system, CYP3A.

MATERIALS AND METHODS

Plant collection, identification and authentication

Samples of dried extracts, oleoresin and essential oils were kindly provided by the Natural Product Research Laboratory of the Federal University of Goias - Laboratório de Pesquisa em Produtos Naturais da Universidade Federal de Goiás (LPPN-UFG)– in May 2014.

The dried extract of *S. adstringens* bark was prepared from the raw matter acquired from the company Paladar Condimentos. The biomarker for this extract was epigallocatechin gallat eat the concentration between 0.77 and 0.94% (m/m), quantified by HPLC- PDA (Sousa, 2014). *L. pacari* barks were collected in the municipality of Niquelandia in Goias at 14° 20' 50.58'' south latitude, 48° 04' 26.53'' west longitude and 1837 m of altitude. Identification was performed by Professor Dr. José Realino de Paula and an exsiccate can be found at the Federal University of Goias herbarium under registration n°43186. The dried extract was prepared and the ellagic acid quantified by HPLC- PDA, as biomarker at 3.84% concentration (Cardoso, 2013).

*C. langsdorfii*oleo resin was prepared from the raw-material provided by Nutragyn Industria de Alimentos Inc. The marker found for oleoresin was β-caryophylleneat 19.90% (m/m) concentration, quantified by HPLC- PDA (Faria, 2014).

P. emarginatus essential oil was prepared from fruits collected by 11 individuals in 5 cities. Identification was performed by Professor Dr. José Realino de Paula and an exsiccate can be found at the Federal University of Goiás herbarium under registration n°41714. The marker found for oleoresin was β-caryophyllene at 20.30% (m/m) concentration, quantified by HPLC-PDA (Alves, 2012).

Reagents, equipment and other materials

It was utilized for chromatographic analyses and for inhibition trials the standards of midazolam (Sigma) and its metabolites 1-hydroxymidazolam (Sigma) e 4-hydroxymidazolam (Sigma), HPLC solvents (Scharlau), dimethylsulfoxide (DMSO) (Quimis) and ultrapure water 18.2 MΩ (Gehaka). For midozam and its metabolites quantification, it was used a C18 250 mm x 4.6 mm x 5µm column (Shim-pack, Shimadzu) and HPLC equipment with PDA detector, SPDM-20 (HPLC-PDA) Shimadzu^{TM}, with a quaternary pump and anauto sampler.

Preparation of rat microsomes

Liver lysate was prepared from the livers of eight male adult Wistar rats, weighing from 380 g ± 40 g. The animals were kept in a controlled-temperature environment (25 ± 2ºC), 12 h dark/light cycle and free access to feed and water.

The animals were euthanized in a closed chamber with carbon dioxide and livers were surgically removed, immediately after the animal's death was verified (Li *et al*. 2010).

Each liver was washed in an ice cold Tris/KCl 0.02 M/0.2M buffer solution (pH 7.4). All following procedures were performed at 4ºC. Livers were weighed, cut with a scalpel and then ground with a glass mortar and pestle forming a pool. An ice cold Tris/KCl 0.02 M/0.2 M buffer (pH 7.4) was added (approximately 4ºC) and it was centrifuged at 9,000 g, 4ºC, for 20 min. The supernatant was centrifuged once again at 22,200 g for 80 min, being the second centrifugation's supernatant re-suspended in 5ml of the ice cold Tris/KCl buffer and once more centrifuged at 22,200g for 40 min to remove hemoglobin. The microsomes (micro vesicles present in the liver

endoplasmic reticulum where CYP450 isoforms can be found) were aliquoted in 5 mL of 0.1 M sodium phosphate buffer containing 0.8 mM EDTA, 1 mM dithiothreitol (DTT) and20 % glycerol. Proteins were determined with the biuret method and samples were stored in a liquid nitrogen tank (Li *et al*., 2010).

The project was approved by the Animal Use Ethics Committee (CEUA) of UFG under protocol number 051/13.

Inhibition of CYP3A assay

A solution of ketoconazole (20 µg/mL) in 1% methanol was used as positive control of CYP3A inhibition. The literature reports that methanol utilized in this concentration does not interfere with microsome activity (Li *et al*., 2010; Chauret *et al*., 1998).

Microsome incubation test was adapted from (Li *et al*., 2010).Initially it was performed a 5-minute pre-incubation witha 200-µL potassium phosphate buffer (pH 7.4) containing 36 µM of midazolam and 480 µM of NADPH (Table 1).

A volume of 180 µL of the inhibition solution (20 µg/mL ketoconazolein 1% methanol)or potassium phosphate buffer (pH 7.4) was added and remained at 37ºC for 10 min. The reaction began with the addition of 400 µL of liver lysate. After 60 minutes, the reaction was interrupted by adding200 µL of ice cold methanol and the internal standard (diazepam 5 µg/mL) was added. The sample was homogenized and centrifuged at 13,000 g for 10 min. The supernatant was filtered with a Millex Durapore membrane (0.22µm pore) filtration system and used for HPLC-PDA analysis.

Tests were performed with the dried extracts of *S. adstringens* (Barbatimao) and *L. pacari* (Pacari) barks, *C. langsdorfii* (Copaíba) oleoresin and the essential oil of *P. emarginatus* (Sucupira). All samples were dissolved in 5%DMSO and evaluated in the final concentrations of 1,000, 500 and 100 µg/mL.

The incubation was performed with 180 µL of 5% DMSO (white control) to assess if this solvent causes any inhibiting effect that might interfere with the assay (negative control).

In order to calculate the inhibition percentage, the concentration of midazolam obtained in the incubation assay was subtracted from the concentration of metabolized midazolam without the inhibitor (considered as maximum metabolized quantity (minimum midazolam quantity found).

Validation of HPLC-PDA analytical method for quantification of midazolam, 1-hydroxymidazolamand 4-hydroxymidazolam

Midazolam and its metabolites were quantified by HPLC-PDA, in column (C18 250 mm x 4.6 mm x 5µm), isocratic method, mobile phase composed by acetonitrile, methanol and ultra-pure water (20:40:40 v/v), flow of 1.0 mL/min, room temperature, injection volume of 20 µL and PDA detector under a wave length of 230 nm and diazepam as internal standard (5 µg/mL).

The analytical method was previously validated according to the rules of Board Resolution – RDC nº 27/2012 and Resolution – RE nº 899/2003 from National Sanitation Sanitation Agency (ANVISA) and ICH of 2005 (Brazil, 2003; Brazil, 2012; ICH, 2005).

RESULTS AND DISCUSSION

The method used to quantify midazolam, 1-hydroxymidazolam and 4-hydroxymidazolam showed linearity, as the calibration curves in solution and in liver lysate (matrix) presented correlation coefficient (r) higher than 0.99 in all performed assays (Brazil, 2003). The limits of detection and quantification were established at 50ng/mL and 200 ng/mL, respectively. The method did not show residual effect, did present post-processing stability and when the matrix effect was analyzed, it showed acceptable interference of liver hepatic lysate components under the intensity of chromatographic peaks (Brazil, 2003; ICH, 2005; Brazil, 2012). In the 20-minute analysis period, all the peaks eluted adequately (Figure 1), showing good parameters of system suitability (Table 2).

From the liver lysate, it was obtained, on average, 5.57 mg ± 0.9 mg/mL of protein, quantified by the biuret method. In the literature, there are reports of utilization of different protein concentrations in the liver lysate for the execution of inhibition assay, being the mean value of 5.57 mg/mL considered adequate, since it can be diluted to the necessary amount for assays accomplishment. There are descriptions of tests using proteins at concentrations of 0.2 mg/mL (Liu *et al*., 2006; Pan *et al*., 2010; Qiao *et al*., 2014); 0.5 mg/mL (Takahashi *et al*., 2003; Mikasa *et al*., 2013), 2.0 mg/mL (Pekthong *et al*., 2009) and 4.0 mg/mL (Usia *et al*., 2005), among other values.

It was performed an incubation test of midazolam in the microsomes without ketoconazole (positive control) and one with ketoconazole. Ketoconazole was used as a positive control for the CYP3A inhibition assay because it is a well reported drug for the inhibition of this specific isoform, besides being recommended by the FDA to be used as a positive control in CYP3A inhibition assays (Obach, 2000; FDA, 2011).

The positive control assay showed no metabolization, since midazolam was completely recovered, which indicates total enzyme inhibition.

When midazolam was solely incubated, it was observed the formation of 1-hydroxymidazolamand4-hydroxymidazolam metabolites.

The 1-hour period of incubation was adequate, as midazolam metabolization was observed, which permitted its and its metabolites quantification. When the 5-minute

incubation period was tested, according to (Li *et al*., 2010), the substrate metabolization did not occur. On the other hand, the 2-hour period did not increase metabolization, being equal to the 1-hour period, which was considered adequate for the assay (Li *et al*., 2010).

DMSO is a commonly utilized solvent to solubilize extracts in inhibition assays (Pekthong *et al*., 2009; Sieniawska *et al*., 2013; Larson *et al*., 2014). In the present study, a 5% DMSO was used to dissolve the extracts and the oils to accomplish the inhibition assays. In order to guarantee no interference of the solvent in the study, substrate incubation was performed and there was no midazolam metabolism alteration in the presence of 5% DMSO, indicatingthat, at this concentration, it is adequate to be utilized in *in vitro* inhibition assays.

The plant materials (essential oil, oleoresin and dried extracts) were assessed at three concentrations: 100, 500and 1,000 µg/mL, being 1,000 µg/mL a value considered high for inhibition tests using plants, a kind of cut off value for inhibition properties (Appiah-Opong *et al*., 2008; Hanapi *et al*., 2010). Most of the articles consulted present plant extract IC_{50}values below 300 µg/mL (Li *et al*., 2010; Hanapi *et al*., 2010; Pandit *et al*., 2012).

IC_{50} is the sample concentration that inhibits 50% of the enzyme, that is, which causes a 50% reduction in its activity regarding the control. It is generally calculated from the analysis of the sample concentration linear regression graph vs the control's activity percentage. It is a widely utilized parameter for the evaluation of plant extract enzymatic inhibition and other products such as drugs or drug prototypes (Usia *et al*., 2006; Hanapi *et al*., 2010).

None of the four plants tested had the calculated IC_{50}, once there was no CYP3A inhibition in the utilized concentrations. Hanapi et al.(2010) consider that inhibitions lower than 20% at the high concentration (1,000 µg/mL) may be ignored and, from the four samples evaluated, only copaíba (*Copaifera langsdorfii*) oil, at the 1,000 µg/mL concentration inhibited less than 10% of midazolam metabolismo, which is not considered a significant value (Table 3).

Therefore, the accomplished assay demonstrated that *S. adstringens* and*L. Pacari* extracts, *C. langsdorfii* oleoresin and*P. Emarginatus* oil did not inhibit Wistar rats' CYP3A isoenzyme.

The absence of inhibitory activity of the extracts, oil and oleoresin studied on CYP3A isoenzyme adds an important piece of information about the non-interference of traditional use of plants on the metabolism of alarge quantity of biostransformed drugs by the subfamiy mentioned, such as midazolam, erythromycin, buspirone as to assure more precise information for the likely use of these medicinal plants' derivatives and, possibly, of medicinal herbs that may be produced using as raw-material the plants assessed in this study (Lamba *et al*., 2012; Olivares-Morales *et al*., 2015).

Table1. Conditions of microsome incubation with midazolam substrate for CYP3A4 enzymatic inhibition study

	Midazolam metabolism assay	**Midazolam inhibition assay (positive control)**	**Extract / Oil inhibition assay**
Pre-incubation 5 min	200 µL of potassium phosphate buffer pH 7.4 36 µM of midazolam 480 µM of NADPH	200 µL of potassium phosphate buffer pH 7.4 36 µM of midazolam 480 µM of NADPH	200 µL of potassium phosphate buffer pH 7.4 36 µM of midazolam 480 µM of NADPH
10 min (37ºC)	180 µL of potassium phosphate buffer pH 7.4	180 µL of inhibition solution (ketoconazole in 1% methanol)	180 µL de extractor oil in 5% DMSO
60 min (37ºC)	400 µL of liver lysate	400 µL of liver lysate	400 µL of liver lysate
After 60 min	200 µL of ice cold methanol 20 µL of internal standard	200 µL of ice cold methanol 20 µL of internal standard	200 µL of ice cold methanol 20 µL of internal standard
10 min	Centrifuge at 13,000 g	Centrifuge at 13,000 g	Centrifuge at 13,000 g

Table 2. System suitability ICH parameters for HPLC-PDA method

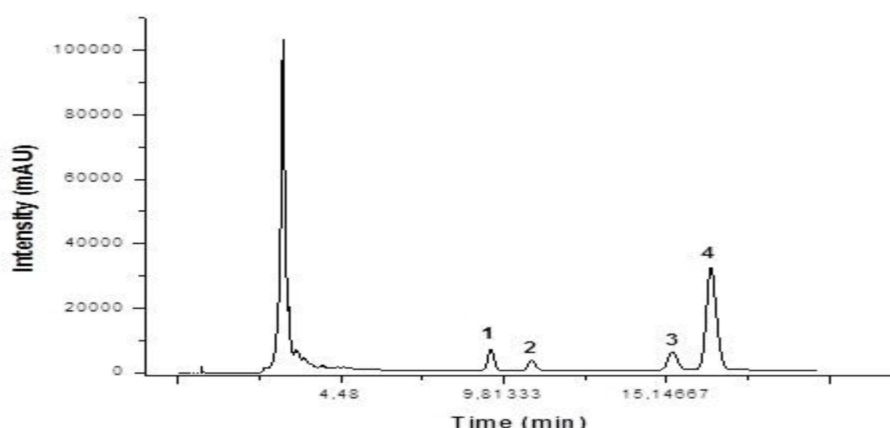
Parameters	**ICH***	**4-MDZ**	**1-MDZ**	**Midazolam**	**Diazepam**
Number of theoretical plates	≥ 2000	6662	8631	10532	12487
Resolution	≥ 2.0	----	3.07	11.28	2.46
Symmetry	≤ 2.0	1.09	1.22	1.17	1.04

* Reference values recommended by ICH (International Conference on Harmonization). 1-MDZ = 1-hydroxymidazolam; 4-MDZ = 4-hydroxymidazolam.

Table 3. Inhibition percentage of Wistar rats' liver CYP3A, *Stryphnodendron adstringens* and 3 *Lafoensia pacari* extracts, *Copaifera langsdorfii* and *Pterodon emarginatus* fixed oils

Extract/Oils	% of inhibition
*C. langsdorffii*1.000 µg/mL	8.78
C. langsdorffii 500 µg/mL	4.40
C. langsdorffii 100 µg/mL	0.00
P. emarginatus 1.000 µg/mL	0.00
P. emarginatus 500 µg/mL	0.00
P. emarginatus 100 µg/mL	0.00
S. adstringens 1.000 µg/mL	6.37
S. adstringens 500 µg/mL	0.00
S. adstringens 100 µg/mL	0.00
L. pacari 1.000 µg/mL	0.00
L. pacari 500 µg/mL	0.00
L. pacari 100 µg/mL	0.00
Cetoconazol (20 µg/mL)	100.00
DMSO (5% v/v)	0.00

Fig 1. Chromatogram of 4-hydroxymidazolam (1), 1-hydroxymidazolam (2), midazolam (3) and diazepam (4) in the liver lysate of Wistar rats



Other plants that do not inhibit CYP3A are reported in the literature, such as*Eurycoma longifolia*, *Sida rhombifolia*, *Alstonia scholaris*, *Bidens pilosa*, *Citrus limon*, *Coleus blumei*, *Cassia siamea*, *Morinda lucida* (Appiah-Opong *et al*., 2008; Hanapi *et al*., 2010; Larson *et al*., 2014).

CONCLUSION

The utilized method to prepare microsomes and the incubation method were proven adequate in order to assess Wistar rats' CYP3A *in vitro* inhibition. After analyzing the dried extracts of *Stryphnodendron adstringens* and *Lafoensia pacari*, the oleoresin of *Copaifera langsdorfii* and the essential oil of *Pterodon emarginatus*, it was observed that they did not inhibit CYP3A. It is important to perform other studies to evaluate if there is inhibition of any other enzyme, *in vitro* and *in vivo*, as well as induction studies, to verify if these plants are inductors of P450 cytochrome isoforms.

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