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Herbal-drug interaction through the pregnane X receptor

[Interacción de medicamentos y hierbas a través del receptor de pregnanos X]

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Abstract: The interaction potential of *Cynara scolymus* L., *Mikania glomerata* Spreng., *Rhamnus purshiana* DC and *Uncaria tomentosa* (Willd. Ex Roem. & Schult.) with conventional drugs metabolized by the CYP3A4 metabolic route was tested in HeLa cell lines, using the *in vitro* model of the hPXR. The herbal medicines *C. scolymus* (1.5 mg/mL dry extract) did not affect the receptor. On the other hand, *M. glomerata* (5.5 mg/mL dry extract), *R. purshiana* (1.5 mg/mL dry extract), and *U. tomentosa* (2.0 mg/mL dry extract) showed to be hPXR agonist, suggesting a potential interaction with the conventional drugs metabolized by the same isoforms of the CYP superfamily. The results from this study contribute to the use safer and more effective of these herbal medicines.

Keywords: Cynara; Mikania; Rhamnus; Uncaria; Pregnane X receptor.

Resumen: Se evaluó el potencial de interacción de *Cynara scolymus* L., *Mikania glomerata* Spreng., *Rhamnus purshiana* DC y *Uncaria tomentosa* (Willd. Ex Roem. & Schult.) con fármacos convencionales metabolizados por la ruta metabólica CYP3A4 en líneas celulares HeLa, utilizando el modelo *in vitro* del hPXR. Las hierbas medicinales *C. scolymus* (1,5 mg/mL de extracto seco) no afectaron al receptor. Por otro lado, *M. glomerata* (5.5 mg/mL de extracto seco), *R. purshiana* (1.5 mg/mL de extracto seco) y *U. tomentosa* (2.0 mg/mL de extracto seco) mostraron ser agonistas de hPXR, lo que sugiere una potencial interacción con los fármacos convencionales metabolizados por las mismas isoformas de la superfamilia CYP. Los resultados de este estudio contribuyen a un uso más seguro y eficaz de estos medicamentos a base de hierbas medicinales.

Palabras clave: Cynara; Mikania; Rhamnus; Uncaria; Receptor de Pregnano X

ABBREVIATIONS

CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2E1, and CYP3A4, CYP isoforms; CYP450, cytochrome P450 superfamily; DMEM, Dulbecco's Modified Eagle Medium with a pH of 7.0; HeLa, Human cervical adenocarcinoma cell line; HPLC/DAD, high-performance liquid chromatography with diode array detection; hPXR, human pregnane X receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NP/PEG, diphenylboryloxyethylamine (NP) and polyethylene glycol (PEG); TLC, thin-layer chromatography.

INTRODUCTION

The global drug market is worth approximately $ 1.1 trillion annually, of which 35% originate from natural products, including plants (25%) (Calixto, 2019). Herbal supplement sales in the United States increased by an estimated 9.4% in 2018 (Smith *et al*., 2019). Unfortunately, this increase is due to either self-medication or indiscriminate medical prescriptions (Silveira *et al*., 2008).

Several reports document that there are interactions between conventional drugs and medicines derived from plants (Galera *et al*., 2008; Rosenkranz *et al*., 2012; Mazzari & Prieto, 2014; Bordes *et al*., 2020). The scarcity of pharmacokinetic studies, the lack of attention to interactions between herbals and conventional drugs, and the ingrained concept that "natural does not hurt," induce the population to overuse products derived from plants, often concomitantly with conventional drugs. As a result, the increase in medicinal plants' use is often closely linked to an increase in reports of adverse reactions (Silveira *et al*., 2008; Mazzari & Prieto, 2014).

In healthcare, herbal medicines play a vital role in the treatment of diseases. However, more information about the combination of conventional drugs and herbals is needed. This information can be subsidized by clinicians and other professionals who make informed clinical decisions on safe combinations of herbal products with conventional medicines (Asokkumar & Ramachandran, 2020).

Herbal medicines can show an agonistic or antagonistic effect on the nuclear receptors related to drug metabolism, such as those on the enzymes of the cytochrome P450 superfamily (Tari *et al*., 2010; Rosenkranz *et al*., 2012). As in the conventional drug pharmacokinetics, herbal medicines, characterized by a complex mixture of pharmacologically active phytochemicals, can modify the bioavailability of a drug concurrently administered by two general interaction mechanisms; directly or indirectly (Rosenkranz *et al*., 2012).

In the direct interaction, an enzyme responsible for the metabolism of a specific drug can be inhibited or induced directly by the presence of a second drug. This interaction can cause a decrease or increase in enzyme activity, and as a consequence, it increases the bioavailability of the second drug or the more pronounced induction of its biotransformation. This inhibition or induction of metabolic enzymes can cause adverse effects or treatment failure. The indirect interaction, however, occurs by inducing or inhibiting nuclear regulatory receptors for the metabolism of xenobiotics which are aggregated transcription factors (co-activators or co-repressors) that act on the expression of metabolic enzymes (Tari *et al*., 2010).

Usually, the transcriptional induction of CYP450 is mediated by nuclear receptors that activate transcription factors. The human pregnane X receptor (hPXR) is considered one of the regulatory keys for CYP3A4 (Chang & Waxman, 2006). All isoforms (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2E1, and CYP3A4) are known to be induced by xenobiotic compounds, thus providing a mechanism for potential drug interactions. Interestingly, the expression of these isoforms can be induced by activation of the hPXR (Moore & Kliewer, 2000; Tolson & Wang, 2010).

Four traditional herbal medicines were selected in this study: *Cynara scolymus* L., *Mikania glomerata*, Spreng. *Rhamnus purshiana DC.,* and *Uncaria tomentosa* (Willd. Ex Roem. & Schult.). These plants are used traditionally for a long time, but there are few or no studies about their potential interaction with conventional drugs. *Cynara scolymus* L. (artichoke) has been used in folk medicine against hepatic dysfunctions (Lima *et al*., 2016; Rodrigues *et al*., 2018; Roos *et al*., 2019). *Mikania glomerata* Spreng. (Guaco) have been used, in Brazilian folk medicine, for several inflammatory and allergic conditions, particularly in respiratory system, such as flu and bronchitis conditions (Napimoga & Yatsuda, 2010). *Rhamnus purshiana* DC. (Cascara Sagrada) is traditionally used as a laxative medicine, general tonic, and digestive aid (Fisher *et al*., 2019). *Uncaria tomentosa* (Willd. Ex Roem. & Schult.) (cat's claw)

have been used by Ashaninka Peruvian rain forest people to treat various inflammatory diseases mainly (Keplinger *et al*., 1999).

To evaluate the interactions and contribute to the safety of herbal medicine used in Brazil, this study aimed to assess the interaction of herbal medicine by measuring the activation of the human pregnane X receptor (hPXR). The interactions can occur by inducing or inhibiting nuclear regulatory receptors for the metabolism of xenobiotics, as hPXR, which are aggregated transcription factors that act on the expression of metabolic enzymes (Tari *et al*., 2010).

MATERIALS AND METHODS

Herbal medicines

Herbal medicines were obtained from a pharmaceutical establishment in Brasília, Distrito Federal, Brazil. Four herbal medicines were used: capsule containing 300.0 mg of *Cynara scolymus* L dry extract and in this extract was present 1.5 mg cynarin, oral solution containing 81.5 mg/mL *Mikania glomerata* Spreng. dry extract and in this extract was present 0.3 mg coumarin, capsule containing 75.0 mg *Rhamnus purshiana* DC. dry extract and in this extract was present 12.0 mg hydroxyanthracene derivatives, and capsule containing 100 mg *Uncaria tomentosa* (Willd. Ex Roem. & Schult.) dry extract and in this extract was present 4.5-5.5 mg alkaloids. The characteristics of the herbal medicines, according to the manufacturer are described in Table No. 1.

TableNo. 1
Characteristics of herbal medicines

Herbal medicine	Dry extract	Phytochemical marker (labeled value)	Pharmaceutical form
***Cynara scolymus* L.**	**300.0 mg/capsule**	**1.5 mg cynarin**	**Capsule**
***Mikania glomerata* Spreng.**	**81.5 mg/mL**	**0.3 mg coumarin**	**Oral Solution**
***Rhamnus purshiana* DC.**	**75.0 mg/capsule**	**12.0 mg hydroxyanthracene derivatives**	**Capsule**
***Uncaria tomentosa* (Willd. Ex Roem. & Schult.)**	**100.0 mg/capsule**	**4.5-5.5 mg alkaloids**	**Capsule**

Data obtained from the medicine leaflets

This project was registered at SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge), under the number A3D8451.

Phytochemical marker analysis

Herbal medicines from *C. scolymus* L., *M. glomerata* Spreng., and *U. tomentosa* (Willd. Ex Roem. & Schult.) were subjected to analysis using high-performance liquid chromatography with diode array detection (HPLC-DAD) to quantify the main phytochemical markers. Only *R. purshiana* was analyzed qualitatively by thin-layer chromatography (TLC) since there was no standard phytochemical marker to compare in the HPLC-DAD analysis.

Liquid chromatography (HPLC) (LaChrom Elite®, Hitachi) coupled with a diode array detector (L2455 DAD detector, Hitachi, Japan) was used. Data were acquired using the Agilent EZChrom Elite® software (version 3.3.2 SP1 Scientific Software, Inc.). All solvents were HPLC grade from Sigma-Aldrich® and Tedia®. The water was obtained using a Millipore Mili-Q System.

The compounds were identified by retention time and UV spectra in comparison to research-grade standards. The data obtained from the standard were used to calculate the phytochemical marker content in the sample, comparing the peak areas of standards with known concentrations with equivalent areas in the sample. For non-pharmacopeial methods were established linearity range using linear regression of data by the least squares method, whereupon the y is the area of the chromatographic peak, and x the concentration of the standard solution. The analysis was performed in triplicate.

Cynara scolymus L. capsule

The chlorogenic acid (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxy-cyclohexane-1-carboxylic acid) content of the *C. scolymus* L. capsules was determined using the method described in the monograph for vegetable drugs in the 7th edition of the European Pharmacopoeia, with modifications according to de Assis Carneiro *et al*. (2017). The phytochemical marker used was chlorogenic acid (3-O-caffeoylquinic acid) obtained from Sigma-Aldrich®.

The sample of dry extract was prepared in ultrapure water at a concentration of 4.0 mg/mL. The chlorogenic acid standard was prepared in methanol at a concentration of 10.0 µg/mL.

Mikania glomerata Spreng oral solution

The *Mikania glomerata* Spreng oral solution was analyzed using HPLC-DAD to quantify the coumarin (2H-1-benzopyran-2-one) content. For this, a purospherStar RP C18e (150 × 4.6 mm, 5 mm, Merck®) column was used, coupled to a pre-column with the same characteristics, at 25ºC. An isocratic elution system was used. The mobile phase was composed of 37.0% phosphoric acid (1%) and 63.0% acetonitrile, with a flow rate of 0.6 mL/min. The injection volume was 10.0 µL and a chromatogram was extracted at 276 nm.

The samples were prepared at a dilution ratio of 1:5 using a solution made up of 35% acetonitrile and subsequently centrifuged for five min at 25ºC at 1200 rpm. The coumarin standard (Sigma-Aldrich®) was prepared in methanol, at a concentration of 100.0 µg/mL.

Uncaria tomentosa (Willd. Ex Roem. & Schult.) capsule

The alkaloid content of *U. tomentosa* (Willd. Ex Roem. & Schult.) capsules was determined according to the United States Pharmacopeia (USP-39).

The capsule contents, equivalent to 50.0 mg of alkaloids calculated as mitraphylline (methyl (1S,4aS,5aS,6R,10aR)-1-methyl-2'-oxospiro-[1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indolizine-6,3'-1H-indole]-4-carboxylate), were solubilized with 50.0 mL of methanol. In test tubes containing 300.0 mg of polyamide, 3.0 mL of the alkaloid solution were transferred and stirred. The supernatant was filtered through a 0.45 µm membrane and analyzed.

The total alkaloid content [speciophylline (methyl (1S,4aS,5aR,6S,10aS)-1-methyl-2'-oxospiro [1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indole-zine-6,3'-1H-indole]-4-carboxylate), uncarine F (methyl(1S,4aS,5aR,6R,10aS)-1-methyl-2'-oxospiro [1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indo-le-zine-6,3'-1H-indole]-4-carboxylate), mitraphylline (methyl(1S,4aS,5aS,6R,10aR)-1-methyl-2'-oxospi-ro[1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indo-lezine-6,3'-1H-indole]-4-carboxylate), isomitraphylline (methyl(1S,4aS,5aS,6S,10aR)-1-methyl-2'-oxospiro[1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indo-le-zine-6,3'-1H-indole]-4-carboxylate), pteropodine (methyl(1S,4aS,5aS,6R,10aS)-1-methyl-2'-oxospiro[1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indolizine-6,3'-1H-indole]-4-carboxylate), isopteropodine (methyl(1S,4aS,5aS,6S,10aS)-1-methyl-2'-oxospiro[1,4a,5,5a,7,8,10,10a-octahydropyrano[3,4-f]indolizine-6,3'-1H-indole]-4-carboxylate), rhynchophylline (methyl (E)-2-[(3R,6'R,7'S,8'aS)-6'-ethyl-2-oxospiro[1H-indole-3,1'-3,5,6,7,8,8a-hexahydro-2H-indolizine]-7'-yl]-3-methoxyprop-2-enoate), and isorhynchophylline (methyl (E)-2-[(3S,6'R,7'S,8'aS)-6'-ethyl-2-oxospiro[1H-indole-3,1'-3,5,6,7,8,8a-hexa-hydro-2H-indolizine]-7'-yl]-3-methoxyprop-2-enoate)] was calculated by comparing the peak area of the isopteropodine standard with the peak area corresponding to each marker. The peak area of each marker was identified according to the standardized extract certificate issued by the USP (USP 39, 2016).

Rhamnus purshiana DC. capsule

The major compounds [cascaroside A ((10S)-1-hydroxy-3-(hydroxymethyl)-10-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-8-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-hydroxyme-thyl)oxan-2-yl]oxy-10H-anthracen-9-one), cascara-side B ((10R)-1-hydroxy-3-(hydroxymethyl)-10-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxyme-thyl)oxan-2-yl]-8-[(2S,3R,4S,5S,6R)-3,4,5-trihydro-xy-6-(hydroxymethyl)oxan-2-yl]oxy-10H-anthracen-9-one), cascaroside C ((10S)-1-hydroxy-3-methyl-10-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxyme-thyl)oxan-2-yl]-8-[(2S,3R,4S,5S,6R)-3,4,5-trihydro-xy-6-(hydroxymethyl)oxan-2-yl]oxy-10H-anthracen-9-one), and cascaroside D ((10R)-1-hydroxy-3-methyl-10-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-8-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-10H-anthracen-9-one)] in the *R. purshiana* DC. capsules were identified using the TLC method. The

eluent was composed of mixture of ethyl acetate, methanol, and water in the ratio 39:7:4. The stationary phase consisted of aluminum plates covered with silica gel (Sigma-Aldrich®). For detection, the NP/PEG reagent was used, consisting of the methanolic solution of 1.0% diphenylboryloxyethylamine (NP) and the ethanolic solution of 5.0% polyethylene glycol 4000 (PEG), as described by Wagner & Bladt (1996).

Cell culture

Human cervical adenocarcinoma cell line (HeLa) cells were used for the cytotoxicity and luciferase reporter gene assays. The cells were obtained from the Rio de Janeiro Cell Bank (code 0100 - lot 001419). These cells were cultured in Dulbecco's Modified Eagle Medium with a pH of 7.0 (DMEM, Thermo Fisher Scientific®), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific®), 3.7 g/L of sodium bicarbonate (Sigma®), 100 IU/mL of penicillin (Sigma®), and 100.0 µg/mL of streptomycin (Sigma®). Culture conditions were set at a temperature of 37°C with 95.0% atmosphere air and 5.0% carbon dioxide (CO_2).

Cytotoxicity assay

Cytotoxicity assays for the HeLa cells were performed with adaptations according to the colorimetric method with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich®) as described by Mosmann (1983) and Hansen *et al*. (1989). This assay was conducted to determine the maximum doses of the herbal medicines to be used in the gene report assays . Then, the herbal medicine concentrations allowed for ≥ 70.0% of cell viability were used in the gene report assays.

A cell density of 3.0×10^4 cells per well was used in 96 wells plates. The cells were treated for 24 h with increasing concentrations of the herbal medicines. IC_{50} values were determined by averaging at least three independent assays.

Human pregnane X receptor (hPXR) activation assay

After seeding for 24 h, the HeLa cells (5.0×10^4 cells/well) were co-transfected using the Lipofectamine 2000 reagent (Thermo Fisher®) according to the manufacturer's protocol. Two plasmids were used: 60.0 ng of Gal4-PXR-LBD [hPXR ligand-binding domain (LBD) fused to the DNA-binding domain (DBD) of the transcription factor of yeast Gal-4] and 240.0 ng of Gal4 luciferase reporter per well in 48 well plate. Transfected cells were then treated with the known receptor agonists (positive controls), rifampicin 1.0 µM (Sigma®) and *Hypericum perforatum* extract 2.5 µg/mL (Sigma®) or herbal medicines. The following concentrations of the herbal medicines used were: *C. scolymus* L. capsule, 1.5 mg/mL; *M. glomerata* Spreng oral solution, 5.5 mg/mL; *R. purshiana* DC. Capsule, 1.5 mg/mL; and *U. tomentosa* (Willd. Ex Roem. & Schult.) capsule, 2.0 mg/mL. The treatment was maintained for 24 h in an incubator at 37ºC and 5.0% CO_2. Luciferase activity was measured according to the manufacturer's protocol (Luciferase Assay Systems, Promega®) in a luminometer (Turner Designs Inst. Mod TD-20/20), and reported as reporter activity (fold increase over control). The control group consisted of cells treated only with the vehicle [DMSO: ethanol (2: 1)].

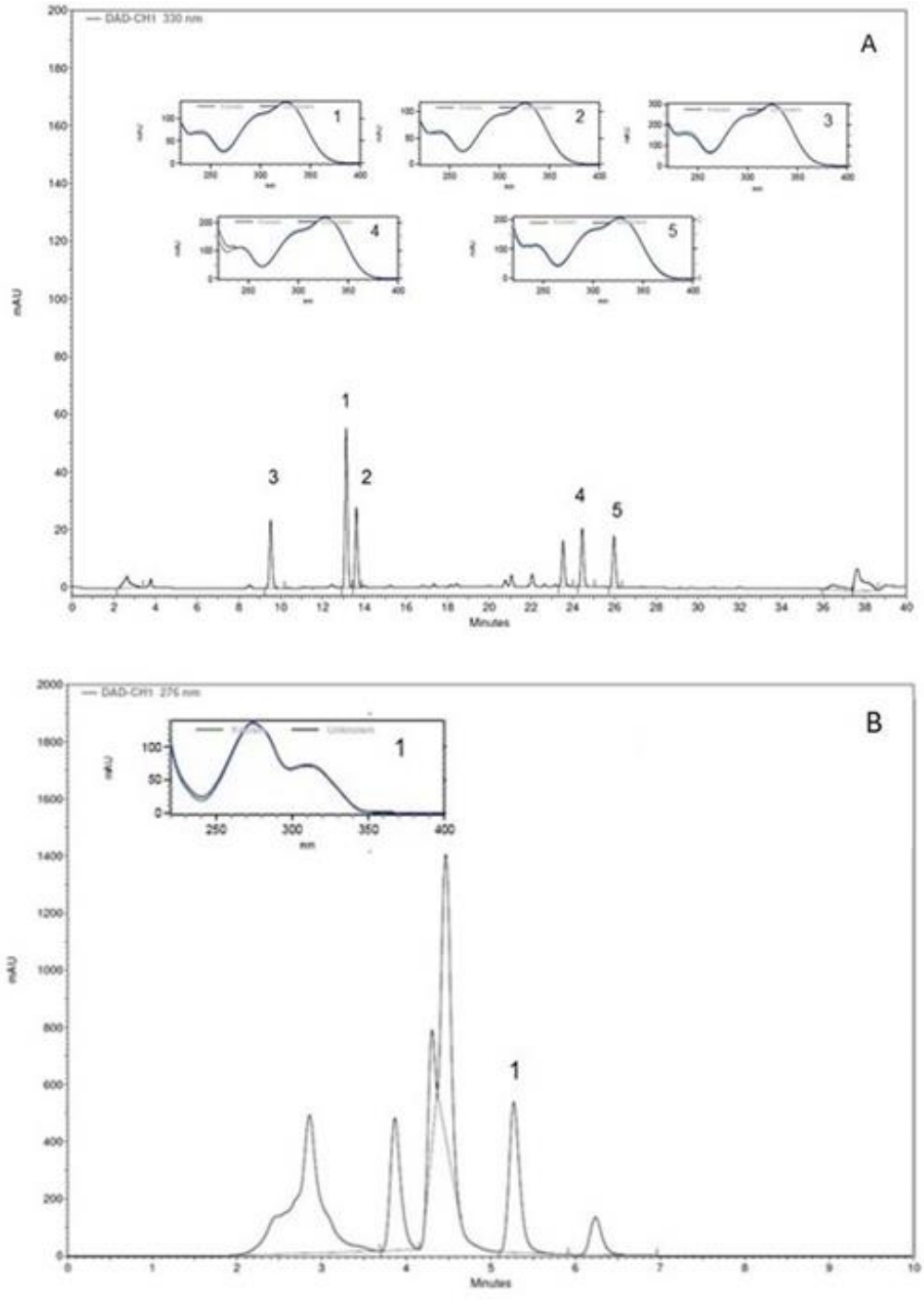
Statistical analysis

Results are expressed as mean ± standard error with at least three independent experiments in triplicate. Statistical analysis was performed using one-way analysis of variance (one-way ANOVA) with Tukey's post-test for results with a parametric distribution, or Kruskal-Wallis with Dunn´s post-test for results with a nonparametric distribution, using the GraphPad Prism 5.0® program. The confidence level was 95.0%.

RESULTS

Phytochemical marker analysis

In the HPLC analysis of *C. scolymus* L. capsules, five caffeoylquinic derivatives were identified: chlorogenic acid (1), cryptochlorogenic acid (2), neochlorogenic acid (3), isochlorogenic acid A (4), and isochlorogenic acid C (5) (Figure No. 1A). The chlorogenic acid content found in these capsules was 675.0 µg/capsule (or 2.3 µg/mg of dry extract) (Table No. 2).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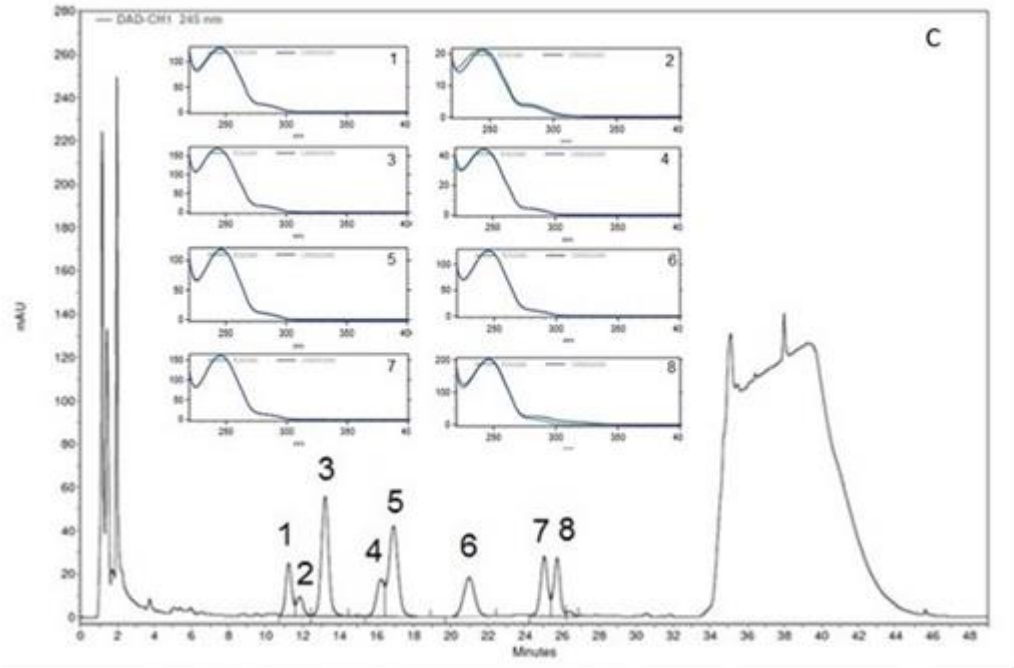

Figure No. 1
HPLC-DAD analysis of herbal medicines. A: *C. scolymus* L. capsule, (1) chlorogenic acid, (2) cryptochlorogenic acid, (3) neochlorogenic acid, (4) isochlorogenic acid A and (5) isochlorogenic acid C. B: *M. glomerata* Spreng oral solution, (1) coumarin. C: *U. tomentosa* (Willd. Ex Roem. & Schult.) capsule, (1) speciophylline, (2) uncarine F, (3) mitraphylline, (4) isomitraphylline, (5) pteropodine, (6) rhynchophylline, (7) isorhynchophylline and (8) isopteropodine

Table No. 2
Phytochemical marker content found in Herbal medicines

Herbal medicine	Dry extract	Phytochemical marker content
C. scolymus L. capsule	300.0 mg	675.0 µg chlorogenic acid
M. glomerata Spreng. oral solution	81.5 mg/mL	0.3 mg coumarin
R. purshiana DC capsule.	75.0 mg	TLC profiles positive for cascarosides
U. tomentosa (Willd. Ex Roem. & Schult.) capsule	100.0 mg	0.7 mg alkaloids (isopteropodine)

The chromatographic profile of the *M. glomerata* Spreng oral solution presented a peak similar to the coumarin (1) standard in relation to retention time and UV/VIS spectral characteristics (Figure No. 1B). HPLC analysis showed the solution to contain coumarin at 0.3 mg/mL of total solution (or 3.9 µg/mg dry extract) (Table No. 2).

The oxindole alkaloid content of the *U. tomentosa* (Willd. Ex Roem. & Schult.) capsule was 0.7 mg/capsule (or 6.5 µg/mg dry extract), with the total content of pentacyclic oxindole alkaloids and tetracyclic oxindole alkaloids corresponding to 0.6 mg and 0.1 mg, respectively, present in 100.0 mg of dry extract. The total alkaloid content was calculated by comparing the peak area of the isopteropodine standard with the peak area corresponding to each marker (Figure No. 1C).

The chromatographic profile of the *R. Purshiana* DC. Capsule (B), determined by the TLC method, was similar to that of the standardized extract Casantranol (A) (Sigma-Aldrich®) (Figure No. 2). In addition, the chromatographic profiles observed were also similar to those presented by Wagner & Bladt (1996). According to these authors, the first high-intensity band, Rf 0.09, suggests the presence of cascarosides A and B (1). The second band of lower intensity, Rf 0.19, is suggestive of cascarosides C and D (2). The presence of bands around Rf 0.38 and 0.46 (bluish streaks with yellow) could indicate naphthalide derivatives (3 e 4) (Wagner & Bladt, 1996).

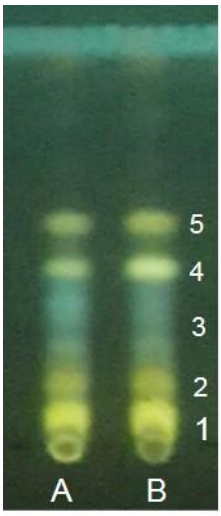

Figure No. 2
Chromatographic profile by TLC method of *R. Purshiana* DC. capsule. A: standardized extract (Casantranol - Sigma-Aldrich®). B: herbal medicine. 1: cascaroside A/B; 2: cascaroside C/D; 3 and 4: naphthalide derivative; 5: not identified. Eluent: ethyl acetate/methanol/water (39:7:4). Stationary phase: aluminum plates covered with silica gel. Detection: NP/PEG reagent

Cytotoxicity assay

After 24 h of treatment, concentrations greater than 2 mg/mL of *C. scolymus* significantly affected the viability of the HeLa cells (66.39 ± 3.44%). The concentration used in the hPXR activation assay, 1.5 mg/mL, showed 73.24 ± 3.90% cell viability, and the chlorogenic acid content was determined to be 3.45 µg (or 9.7 µM).

The *M. glomerata* Spreng oral solution showed low cytotoxicity. The concentration used in the hPXR activation assay, 5.5 mg/mL, showed 72.12 ± 1.79% cell viability. The coumarin content was measured at 21.5 µg (or ≅147 µM).

After 24 h of treatment with *R. purshiana*, the viability of HeLa cells was significantly affected at concentrations of 1.75 mg/mL (68.27 ± 2.48%), 2.0

mg/mL (65.92 ± 2.28%), 2.25 mg/mL (54.21 ± 2.97%), and 2.5 mg/mL (37.56 ± 1.68%). However, the concentration used in the hPXR activation assay, 1.5 mg/mL, showed 99.28 ± 6.78% cell viability.

The cell viability of HeLa cells after treatment with *U. tomentosa*, at a concentration of 2.0 mg/mL (containing 13 µg oxindole alkaloids), corresponded to 76.03 ± 1.60%. This was the concentration used in the hPXR activation assay. The highest concentration studied, 3.0 mg/mL, impacted cell viability by 64.59 ± 2.67%.

Pregnane X receptor activation assay

In the present study, rifampicin (1 µM) presented the highest hPXR activation rate at 5.49 ± 0.59, without causing cell damage (Figure No. 3). *H. perforatum* (2.5 µg/mL) showed a maximum activation rate of 4.41 ± 0.28 (Figure No. 3).

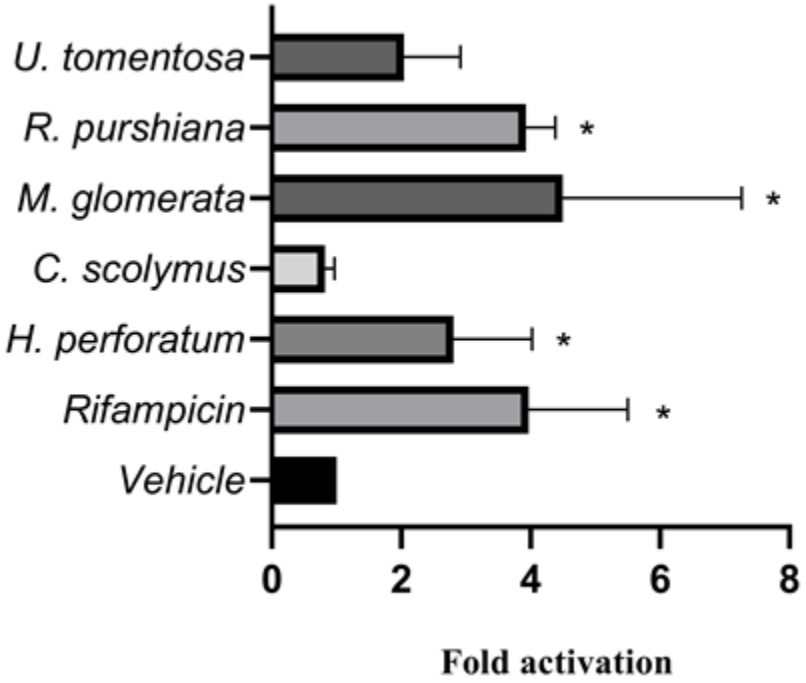

Figure No. 3

Pregnane X receptor (PXR) reporter gene assay. *R. purshiana* and M. glomerata is shown to be a hPXR agonist. Concentrations of substances used were: Rifampicin 1.0 µM, *H. perforatum* extract 2.5 µg/mL, *C. scolymus* L. capsule 1.5 mg/mL, *M. glomerata* Spreng. oral solution 5.5 mg/mL, *R. purshiana* DC. capsule 1.5 mg/mL and *U. tomentosa* (Willd. Ex Roem. & Schult.) capsule 2.0 mg/mL. Data was expressed by the mean ± standard error of transcription activation in relation to the vehicle (n = 9). One-way ANOVA with Tukey post-test. * $p<0.05$ vs vehicle

Cynara scolymus L. capsule (1.5 mg/mL) presented an activation rate of 0.82 ± 0.04 and showed no statistically significant difference compared to the vehicle (Figure No. 3).

The *M. glomerata* Spreng oral solution, at a concentration of 5.5 mg/mL, revealed a significant activation rate of 4.49 ± 0.92 (Figure No. 3). This herbal medicine conferred agonistic action to the hPXR. The high activation of this plant species when compared to rifampicin provides evidence of strong induction of the nuclear receptor.

Rhamnus purshiana DC. capsule (1.5 mg/mL), revealed a significant activation rate of 3.92 ± 0.15 (Figure No. 3).

Uncaria tomentosa (Willd. Ex Roem. & Schult.) capsule (2.0 mg/mL) showed a hPXR activation rate of 2.00 ± 0.29 (Figure No. 3).

DISCUSSION

Four herbal medicines [*Cynara scolymus* L., *Mikania glomerata*, Spreng. *Rhamnus purshiana* DC., and *Uncaria tomentosa* (Willd. Ex Roem. & Schult.)] used in Brazil were selected for use in this study. The interaction potential of these four herbal medicines in the activation *in vitro* model of the hPXR was evaluated.

Rifampicin and *Hypericum perforatum* were selected from various hPXR activating compounds as positive controls for the luciferase reporter gene assay (Chang & Waxman, 2006). Rifampicin, a synthetic macrolide antibiotic, induces receptor activation in a dose-dependent manner at low concentrations (Chang & Waxman, 2006). *H. perforatum*, a medicinal plant popularly known as St. John's wort, is one of the most reported plant species that interacts with the hPXR. This action is mainly due to the hyperforin bioactive compound (Moore *et al*., 2000; Markowitz *et al*., 2003; Chang & Waxman, 2006).

In this study, *Cynara scolymus* L. did not show statistically significant activation of hPXR. In the HPLC analysis, five caffeoylquinic derivatives were identified (chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, isochlorogenic acid A, and isochlorogenic acid C). Our results corroborate with the findings in others studies, *C. scolymus* showed no action on the PXR receptor, one of the regulatory keys of CYP3A4, one CYP450 isoform.

Teel & Huynh (1998), were one of the first authors to study the relationship between isolated chlorogenic acid, a compound that is present in *C. scolymus*, and its effects on alkoxyresorufin O-dealkylase activity, a measure of cytochrome P450 activity. In other study, Jin *et al*. (2016), evaluated the direct inhibitory effects of isochlorogenic acid A on CYP450 isoforms. In human liver microsomes, the compound did not show any relevant inhibition in the 3A4, 2C19, 2D6, and 2E1 isoforms; however, the compound promoted weak inhibition in the 2C9 isoform. Moore & Kliever (2000), evaluated the induction of CYP3A4 by chlorogenic acid mediated by the pregnane X receptor. The authors initially investigated the action of the compound on the expression of CYP3A4 mRNA in human colon adenocarcinoma cells (LS174T) by real-time PCR (RT-PCR), considering that transcriptional regulation is of paramount importance to establish the mechanism and modulation of gene expression. Chlorogenic acid at 0.1 µM positively induced mRNA levels, a fact also observed by the authors in the activation of CYP3A4 in cells LS174T with activation of 2 times compared to the negative control. Despite activation at levels of gene expression, chlorogenic acid did not induce significant transcriptional activation of CYP3A4 behind the transcriptional pathway mediated by the pregnane X receptor. Moore & Kliever (2000), also showed a possible activation of CYP3A4 by the transcriptional route mediated by the constitutive androstane receptor, another relevant receptor as a mediator of xenobiotic metabolism. Other studies related to the expression of CYP3A4 mRNA without the mediation of nuclear receptors have been reported for the *C. scolymus* extract and isolated compounds derived from caffeylquinic acid (Teel & Huynh, 1998; Löhr *et al*., 2009). Lohr *et al*. (2009), investigated the action of the standardized extract of the leaves of *C. scolymus* on HepG2 cells, an extract composed of 27% caffeoylquinic acids and 7% flavonoids, among which 10.3% was 3-O-caffeoylquinic and 5.8% was luteolin-7-O-glycoside. After treatment for 24 h with 100 µg/mL of extract, RT-PCR showed significant inhibition of the expression of mRNA of the cytochrome P450 isoform 3A4 and the enzymes involved in the second phase of biotransformation of xenobiotics, including glutathione transferase, glutamyl transferase, and glutathione peroxidase.

The *M. glomerata* Spreng oral solution conferred agonistic action to the hPXR and presence of 0.3 mg/mL of coumarin in the present study. Corroborating with our results, some authors have showed effects of this compound on enzymes regulated by hPXR receptor, suggesting that this effect could be mediated by coumarin.

Zhuo *et al*. (1999), and Born *et al*. (2002), observed by cDNA expression that the human isoenzymes CYP1A1, CYP1A2, CYP2B6, CYP2E1, and CYP3A4 could catalyze the metabolism of coumarin in o-hydroxyphenylacetaldehyde, a toxic secondary metabolite of excretion. Born *et al*. (2002), observed that in addition to this coumarin metabolite, the compound can be metabolized to 3-hydroxycoumarin, an inactive metabolite, predominantly by CYP3A4, and to a lesser extent by CYP2A6 and other isoforms. In other study, Lewis *et al*. (2006), showed probable interactions between coumarin and human enzymes belonging to the P450

superfamily, among which coumarin seems to be able to bind to a phenylalanine residue present in CYP3A4. In general, the authors suggested that coumarin interacts with at least one amino acid residue by hydrogen bonds present in CYP1A1, CYP1A2, CYP2A6, and CYP3A4, which determines the substrate metabolism site and the type of metabolite formed.

The agonistic action observed in this study revealed possible important interactions with conventional drugs metabolized by the CYP3A4 metabolic route, such as antibiotics, anti-inflammatory drugs, oral contraceptives, antidepressants (amitriptyline), anxiolytics (benzodiazepines), antiepileptics (carbamazepine), and antiretrovirals (Rosenkranz *et al*., 2012; Mazzari & Prieto, 2014).

Therefore, the results obtained in this work through the activation of the hPXR and the evidence found for the coumarin compound suggests an action to regulate the metabolism of xenobiotics and the self-induction of its metabolism. This also occurs with rifampicin, a well-known activator of hPXR receptors and there are reports of the induction of its own metabolism in humans (Immanuel *et al*., 1985; Chang & Waxman, 2006).

Rhamnus purshiana also showed agonistic action on hPXR nuclear receptor, suggesting possible important interactions with conventional drugs metabolized by the CYP3A4 metabolic route (Rosenkranz *et al*., 2012; Mazzari & Prieto, 2014). However, the main compounds responsible for their laxative activity are aglycone of the glycoside derivatives of anthracene, as anthraquinones (Cascarosides). These glycosides functionate as pro-drugs, which after metabolism in the colon by bacterial glycosidases create poorly absorbed compounds that have a laxative effect (Cirillo & Capasso, 2015; Bartnik & Facey, 2017). Although anthraquinones are poorly absorbed in the colon, some of these compounds are absorbed, and have potential pharmacological action (de Witte, 1993; Bartnik & Facey, 2017). Then, the results from this study alert of the potential of *R. purshiana* to interact with drugs metabolized by the CYP3A4 metabolic route.

In this study, *U. tomentosa* showed an agonistic effect on the human pregnane X receptor and presence of oxindole alkaloids. Some authors have showed effects of this plant on enzymes regulated by hPXR receptor. Thus, Budzinski *et al*. (2000), were the first authors to performed an analysis of the action of *U. tomentosa* on CYP3A4. Among the 21 plant species investigated by the authors, the ethanol extract of *U. tomentosa* showed inhibitory activity in the formation of metabolites of the substrate 7-benzyloxyethorphine by human CYP3A4 in the fluorescence microtiter assay. Foster *et al*. (2003), showed that the methanol extract of *U. tomentosa* (25 mg/mL) inhibited approximately 56% of isoform 3A4 and, to a lesser extent, isoforms 2C9 (11%), 2C19 (5%), and 2D6 (13%).

These studies reported that *U. tomentosa* showed a negative interaction with CYP3A4. However, in the present study, it was observed for *U. tomentosa* significantly activated the hPXR. The results of this study suggest that although the compounds present in the species may provide activation of the hPXR, the final biological action in the physiological system inhibits the drug metabolism by CYP3A4. This final inhibiting effect may have occurred due to the presence of other possible routes of metabolism.

CONCLUSION

Mikania glomerata, *R. purshiana*, and *U. tomentosa* showed an agonistic effect on the human pregnane X receptor. The results suggest a potential interaction with the conventional drugs metabolized by the same isoforms of the CYP superfamily. In addition, they may guide future studies, and health professionals can use them as a reference to be able to prevent adverse effects with the concomitant use of conventional drugs, making the use of these herbal medicines safer and more effective.

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