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Simultaneous Determination of Furanocoumarins in Infusions and Decoctions from "Carapiá" (*Dorstenia* Species) by High-Performance Liquid Chromatography

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The use of furanocoumarins, which are photosensitizing compounds, combined with exposure to UV-A radiation is a common treatment for vitiligo, psoriasis, and a number of other skin diseases. Although furanocoumarins plus UV-A treatment is highly effective, several studies have shown that exposure to high doses increases the risk to development of cutaneus carcinoma. Several *Dorstenia* species are used in folk medicine, mainly against skin diseases, because of the presence of biologically active compounds. We present here analysis of the chemical composition of furanocoumarins from infusion and decoction of "Carapiá" (*Dorstenia* species), which is used in Brazil against several diseases. We have employed high-performance liquid chromatography (HPLC) procedures for the quantitative determination of psoralen, bergapten, and isopimpinellin. The contents of furanocoumarins revealed an insignificant difference between infusion and decoction. *Dorstenia tubicina* and *D. asaroides* contained psoralen and bergapten only in the rhizomes, whereas *D. vitifolia* shows solely isopimpinellin in both rhizomes and aerial parts.

KEYWORDS: ***Dorstenia*; furanocoumarins; HPLC; Carapiá; psoralens**

INTRODUCTION

Linear furanocoumarins or psoralens such as psoralen, bergapten, and xanthotoxin are photosensitizing compounds commonly used for the treatment of psoriasis, vitiligo, and other skin diseases (*1*−*4*). Besides these compounds, isopimpinellin and 4,5,8-trimethylpsoralen are furanocoumarins, which in the presence of UV light, can intercalate into DNA (*4*), for both domestic animals and man (*5*). The furanocoumarin molecules absorb energy and then react mainly with epidermal DNA producing a covalent bond between the furanocoumarin and nucleic acid. Depending on the dose level, this bond can lead to either cell death or DNA repair, synthesis, and replication (*6*, *7*). Application or ingestion of these compounds can cause swelling, redness, and lesion formation on the epidermis or, in some cases, cutaneous carcinoma. The phototoxic reaction appears as erythemas which are usually manifested after a latent interval of several hours after UV-A exposure (*7*).

Within this group, 4,5,8-trimethylpsoralen is considered to be the most phototoxic followed by xanthotoxin, psoralen, and bergapten (*8*, *9*), whereas isopimpinellin is not considered a phototoxic furanocoumarin (*10*, *11*), and also is considered to be much less bioactive (*11*, *12*). Usually, the amount of the compounds psoralen and bergapten is used as an index of the phototoxic activity of the plant (*11*, *13*).

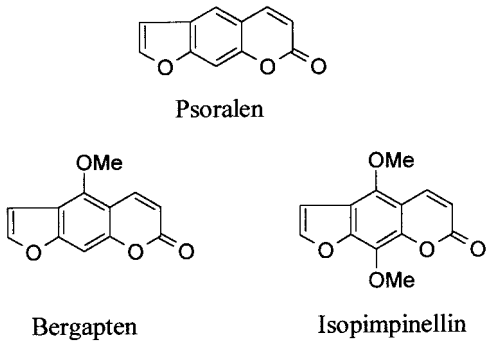

Figure 1. Chemical structure of the compounds investigated.

The main source of information about the phototoxicity of furanocoumarins is the fact that some derivatives are used as therapeutic agents in combination with UV-A irradiation in the treatment of psoriasis and vitiligo (PUVA therapy (psoralen plus UV-A)). In these treatments, typical doses of 0.4−0.7 mg psoralens/kg body weight are orally administered and followed by a 2-h exposure to UV-A (Dose: 0.5−7.0 J/cm^2) (*14*), where

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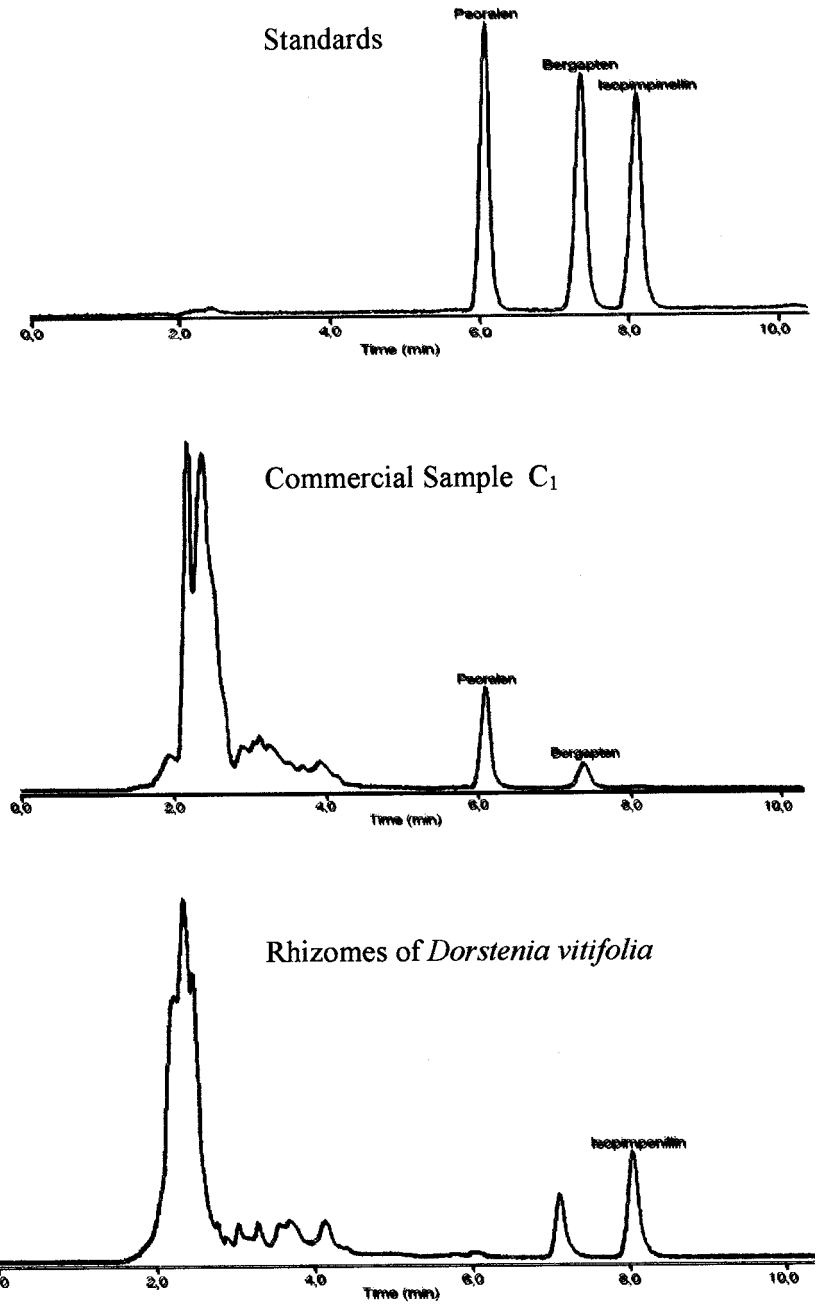

Figure 2. Chromatographic separations by HPLC of furanocoumarins standards and teas of commercial sample C_1 (decoction) and *Dorstenia vitifolia* rhizomes (decoction). For chromatographic conditions, see Materials and Methods section.

the severity of the response is governed by the duration of the UV radiation exposure (*4*). Quantities of psoralens as low as 18 ppm may cause lesions depending on the UV exposure conditions (*15*). For this reason, is very important to know precisely the levels of furanocoumarins in foods and teas, decoctions, and infusions consumed by humans.

Our studies have focused on *Dorstenia* species (Moraceae), which are assumed to contain furocoumarins with phototoxic effects (*16*). These plants are distributed in the subtropical regions of the world, and they are known by their ability to synthesize psoralen, bergapten, isobergapten, pimpinellin, and isopimpinellin (*17, 18*). The genus *Dorstenia* is used in the medicinal plant therapy in many countries in Africa and Central and South America as antiophidic and anti-rheumatic, and against infections and skin diseases (*19, 20*). The referred substances were previously isolated principally in Lathin America from species of *Dorstenia contrajerva* (*21, 22*), *D. brasiliensis* (*23*), *D. asaroides* (*18, 20*), *D. bryonifolia* (*24, 25*), *D. lindeniana* (*26*), *D. vitifolia,* and *D. tubcina* (*23, 27*).

In this work we report the quantification of psoralen, bergapten, and isopimpinellin (**Figure 1**) in infusions and decoctions of rhizomes and aerial parts of *Dorstenia* species and commercial samples known as "Carapiá" founded in small shops of Campo Grande/MS, Brazil. The analysis was performed by reversed-phase high-performance liquid chromatography (HPLC).

HPLC is the most used and efficient method for psoralens determinations (*28*−*30*), and in this work it allowed direct analysis of the teas, without any previous sample treatment such as cleanup or extraction. This is an important advantage because we can avoid losing material, and this contributes to minimization of experimental errors. However, gas chromatography (GC) has also been used in qualitative and quantitative furanocou-

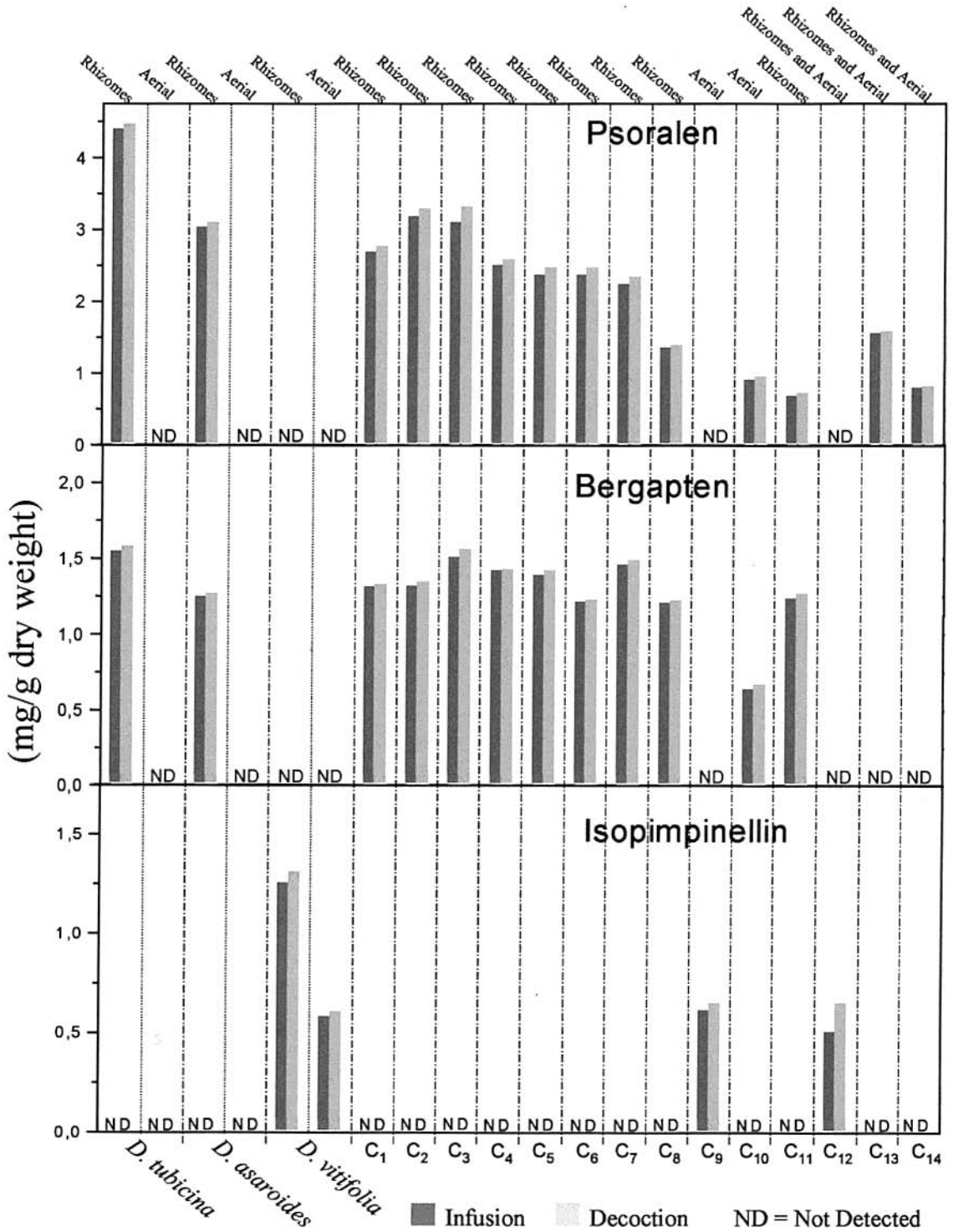

Figure 3. Psoralen, ergapten, and isopimpenillin contents in rhizomes and aerial parts of *Dorstenia* species and 14 commercial samples obtained by two extraction methods (infusion and decoction). Concentration of furanocoumarins is expressed in mg/g dry weight of plant.

marins analysis, although there are necessary sample pretreament steps.

MATERIALS AND METHODS

Chemicals. Spectroscopy-grade acetonitrile was purchased from Merck. Water purified by a Milli-Q system (Millipore) was used for sample preparation and HPLC analysis. Furanocoumarins standards were available from a collection purified by chromatography and recrystallization at our laboratory. The purity of the standards was confirmed by mass and hydrogen NMR spectra and with published values of HPLC retention times. Stock mixtures of furanocoumarins standards were made up in acetonitrile and used as external standards.

Apparatus. The analyses were performed on a Shimadzu liquid chromatograph with a ternary solvent delivery system (model LC-6AD), combined with a fixed wavelength UV−Vis detector (Shimadzu model SPD-6AV), and a Rheodyne loading valve fitted with a 100-μL sample loop. A microcomputer equipped with Microquimica-MQI8PCA software was used for recording chromatograms and measuring peak areas. For HPLC separation of the furanocoumarins we used a Shimadzu octadecyl Shim-pack CLC−ODS (4.6 mm i.d. × 25 cm long and 5 μm particle diameter) reversed-phase column, with a small precolumn (4.6 mm i.d. × 2.5 cm long) containing the same packing, used to protect the analytical column. Elution was carried out with acetronitrile/water (55:45; v/v) at a flow-rate of 1.0 mL/min. This mobile phase

was filtered through a 0.45-μm pore filter (Millipore) and then degassed ultrasonically prior to use. Samples of 10 μL were injected with a 25-μL Hamilton syringe, and the detection of the peaks was recorded at 223 nm (optimized wavelength). Chromatography was performed at ambient temperature (22 °C).

Sampling and Sample Preparation. The rhizomes and aerial parts of the plants *Dorstenia tubicina* Ruiz et Pavon, *D. asaroides* Gardn., and *D. vitifolia* Gardn., were collected in Aquidauana, Piraputanga and Rio Negro/Mato Grosso do Sul, Brazil, in December 1996, and identified by Dr. Pedro Carauta (Jardim Botânico do Rio de Janeiro, Brazil). The commercial samples were purchased from 14 different shops in the local commerce of Campo Grande/MS, Brazil during the same time period.

All samples were separately dried at 37 °C in a chamber with air stream for 24 h and then powdered. Two different procedures were performed: 20 mL of hot water was added to 1 g of the sample (infusion); or 1 g of sample was boiled in 20 mL of water for 5 min (decoction) using an Erlenmeyer flask heated by a Bunsen Burner, according to popular methods. After the solutions were cooled, they were filtered in a filter paper. Each solution (2 mL) was diluted in a 50-mL volumetric flask with acetronitrile/water (55:45, v/v) up to the mark, filtered through a Millex filter (0.45-μm diameter pore) and directly analyzed by HPLC−UV.

Determination of the HPLC Detection Limits. The HPLC detection limits were determined by injecting, in quadruplicate, solutions of psoralen, bergapten, and isopimpinellin of known concentrations (10 μL for each injection), and diluting the concentration of the sample until the peak was twice the height of the baseline noise. The corresponding concentration was take as the minimal amount detectable by HPLC−UV.

Calibration Curves. Determination of contents of psoralen, bergapten, and isopimpinellin in plant material was performed by construction of calibration curves with high linearity. The compounds were dissolved separately in spectroscopy-grade acetronitrile in order to obtain the stock solutions that were appropriately diluted to concentrations ranging from 1 to 20 μg/mL. Aliquots (10 μL) of 8 dilutions of each standard were analyzed by HPLC−UV. Each determination was carried out five times. For each standard compound, a corresponding chromatogram was obtained and a graphical plot of the average of the areas against the concentration for each furanocoumarin was constructed. Linear least squares regression of the peak areas ratio as a function of the concentrations was calculated to determine correlation coefficients. Furanocoumarins concentrations in the samples were calculated using results of the regression analysis. Specimens with an analyte concentration exceeding the calibration curve were repeated upon appropriate dilution of the samples.

RESULTS AND DISCUSSION

HPLC analysis was completed in about 9 min (**Figure 2**). The observed retention times were as follows: for psoralen, 6.05 min; bergapten, 7.33 min; and isopimpinellin, 8.08 min. The peaks were free of any interference coeluting in all of the decoctions and infusions of "Carapiá" samples tested. Representative chromatograms are shown in **Figure 2**.

Estimation of the content of furanocoumarins in the infusions and decoctions of plant materials was performed by external calibration. The coefficients determinations (r^2) for calibration curves were equal to or better than 0.9994 for all the furanocoumarins in the range 1−20 μg/mL. The limits of detection for psoralen, bergapten, and isopimpinellin were found to be 0.03, 0.07, and 0.09 μg/mL, respectively.Identification of the furanocoumarins in the samples was performed by comparison of sample peaks with those of standards, as well as by co-injection to confirm the presence of psoralen, bergapten, and isopimpinellin.

Infusions and decoctions of "Carapiá" are very often consumed by people in Campo Grande/MS, Brazil, and showed the presence of the furanocoumarins. The experiments demonstrated that rhizomes are the preferential accumulation sites of furanocoumarins in these plants (**Figure 3**). Infusion and decoction showed no statistically significant difference in the mean contents for both furanocoumarins obtained, analyzed by an analysis of variance (ANOVA) for a degree of confidence of 99% (**Figure 3**).

The furanocoumarins composition of *Dorstenia* species and commercial samples examined in this work were shown to be similar to those of other *Dorstenia* species investigated (*18, 20*−*27*).

Dorstenia tubicina and *D. asaroides* have qualitatively similar chemical compositions, revealing psoralen and bergapten only in the rhizomes (**Figure 3**). *D. vitifolia* shows only isopimpinellin, both in the rhizomes and aerial parts, and in folk medicine it is considered to be less toxic and not active for vitiligo treatment. The commercial samples C_9 and C_{12} were qualitatively similar to *Dorstenia vitifolia* (**Figure 3**).

The consumption of furanocoumarins in folk medicine without any qualitative or quantitative control is dangerous because the type and the quantity of these compounds can differ dramatically from plant to plant, and this can cause negative impacts to health. Nevertheless, there is no strict guideline for the best practice. The presented methods for identification and quantification of furanocoumarins can be helpful in the correct preparation and administration of phytotherapy medicaments. This study showed the precise furanocoumarins composition in *Dorstenia* species in infusion and decoction form. These data can be used for further optimal efficacy and safety in administration of furanocoumarins.

CONCLUSION

In conclusion, the data obtained in this study suggest that the rhizomes of *Dorstenia tubicina*, *D. asaroides*, and commercial samples C_1−C_8, C_{10}, C_{11}, C_{13}, and C_{14} are suitable (in both infusion and decoction forms) for alternative phytotherapeutic treatment of psoriasis and vitiligo. Besides this, the analysis can contribute to evaluation of the safety factor between relative benefits and toxic risks. The results of this investigation indicate that infusions and decoctions of "Carapiá" are sources of furanocoumarins and suggest that HPLC may be a very useful tool for the qualitative and quantitative analysis of these chemical constituents.

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