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A powder X-ray diffraction method for detection of polyprenylated benzophenones in plant extracts associated with HPLC for quantitative analysis

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ABSTRACT

A robust, direct, rapid and non-destructive X-ray diffraction crystallography method to detect the polyprenylated benzophenones 7-*epi*-clusianone (**1**) and guttiferone A (**2**) in extracts from *Garcinia brasiliensis* is presented. Powder samples of benzophenones **1** and **2**, dried *hexane* extracts from *G. brasiliensis* seeds and fruit's pericarp, and the dried ethanolic extract from *G. brasiliensis* seeds were unambiguously characterized by powder X-ray diffractometry. The calculated X-ray diffraction peaks from crystal structures of analytes **1** and **2**, previously determined by single-crystal X-ray diffraction technique, were overlaid to those of the experimental powder diffractograms, providing a practical identification of these compounds in the analyzed material and confirming the pure contents of the powder samples. Using the X-ray diffraction crystallography method, the studied polyprenylated benzophenones were selectively and simultaneously detected in the extracts which were mounted directly on sample holder. In addition, reference materials of the analytes were not required for analyses since the crystal structures of the compounds are known. High performance liquid chromatography analyses also were comparatively carried out to quantify the analytes in the same plant extracts showing to be in agreement with X-ray diffraction crystallography method.

1. Introduction

The phytotherapeutic medicines are plant preparations presenting complex mixtures from one or more plants which exhibit bioactive properties [1]. Certain pharmacologically active chemicals from plant biosynthetic pathways are embedded in such preparations and they should be at optimal concentrations since either inefficacy or toxicity can be noticed otherwise. Even so, data certifying quality, efficacy and safety do not exist for most plant medicines [2]. Some methods have been developed to analyze raw phytotherapeutic preparations. For instance, protocols describing the use of high performance liquid chromatography (HPLC) [3], gas chromatography (GC) [4], mass spectrometry (MS) [4,5], nuclear magnetic resonance spectroscopy (NMR) [3] and infrared spectroscopy (IR) [6] techniques are commonly reported in the literature.

Although crystallographic techniques are primarily employed for structure determinations, they are further useful to characterize and quantify substances in solid mixtures [7,8]. In addition, previous sample preparation and destructive effects on constituents are not experienced with powder X-ray and neutron diffraction analyses. However, diffraction techniques are not explored for analytical purposes of featuring phytotherapeutic products.

Few examples of studies involving crystallographic techniques to characterize complex mixtures from natural sources can be found in literature. Interestingly, certain lipids of lichens, as atranorin, are largely secreted out of the fungal cells in association with algae (or cyanobacteria), crystallizing in the thallus of the symbiotic organism [9]. In this case, the lipid crystals deposited in the thallus were analyzed by PXRD. Crystalline sugars have been also detected by PXRD procedures in honey products, being this technique one of the most used to feature honeys [10].

Using powder X-ray diffraction (PXRD) techniques, it is possible that the quality of a phytomedical material could be proved comparing the powder diffraction profile of raw plant preparation with that of either an active or marker compound. For this pur-

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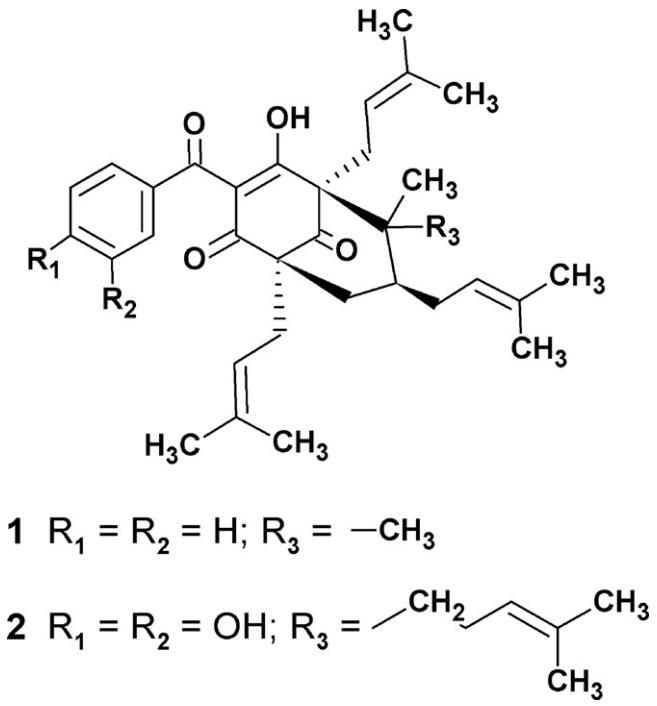

Fig. 1. Chemical diagram of the polyprenylated benzophenones **1** and **2**.

pose, it is important to know the crystal structure of the active compound by single-crystal XRD experiment or at least to know its certified experimental powder diffraction profile previously performed in a pure and crystalline active compound. Both situations would be appropriate for matching to PXRD fingerprint of whole products. Equally, a certified PXRD profile of the whole phytotherapeutic medicines could be used to analyze commercial samples.

Parts and extracts of *Garcinia brasiliensis* (Mart.) Planch. & Triana (Syn. *Rheedia brasiliensis*), which is traditionally named 'Bacupari', are used in Brazil as phytomedicines for the treatment of urinary pathologies and several kinds of tumors [11]. Two polyprenylated benzophenones were obtained from hexane and ethanol extracts from fruits and seeds of *G. brasiliensis* as major secondary metabolites: 7-*epi*-clusianone [*rel*-(1*R*,5*R*,7*R*)-3-benzoyl-4-hydroxy-8,8-dimethyl-1,5,7-tris(3-methylbut-2-en-1-yl)bicyclo[3.3.1]non-3-ene-2,9-dione; **1**, Fig. 1], which exhibits vascular activity on the rat aorta [12] and anti-HIV property [13]; and guttiferone A [*rel*-(1*R*,5*R*,7*R*,8*S*)-3-(3,4-dihydroxybenzoyl)-4-hydroxy-8-methyl-1,5,7-tris(3-methylbut-2-en-1-yl)-8-(4-methylpent-3-en-1-yl)bicyclo[3.3.1]non-3-ene-2,9-dione; **2**, Fig. 1], a HIV-inhibitory metabolite [14]. Other pharmacological effects on biological systems are also attributed to polyprenylated benzophenones, as for instance the radical-scavenging property, iNOS and COX-2 expression inhibition ability in colon carcinoma, apoptosis inducting, antiulcer, antioxidant, trypanocidal [15–19], anti-inflammatory [20], and antitumoral activities [21].

The polyprenylated benzophenones **1** and **2** have been isolated from *G. brasiliensis* extracts by column and preparative thin layer chromatography (TLC) [22,23]. Compound **1** is the major compound extracted from the fruits and it is also found in the seeds of this species [22,23]. On the other hand, phytochemical trials reveal that compound **2** to be present only in the seeds [24]. Both natural benzophenones **1** and **2** were previously characterized by single-crystal XRD technique [24–26].

In our research groups we have been studying the chemical constituents from several plant species in the search for biologically active compounds [27–33]. More recently we have concentrated some effort in the investigation of species belonging to the *Garcinia* (or *Rheedia*) genus from the Guttiferae family [22–24]. In the present investigation our effort is concentrated in developing a new method based on PXRD to characterize qualitatively the chemical composition of extracts and raw materials derived from *G. brasiliensis* which are potential phytomedicines. The materials prepared from *G. brasiliensis* have their biological activities associated to the presence of polyprenylated benzophenones. In addition, a HPLC method was used to gauge the polyprenylated benzophenones concentrations in the extracts, since PXRD technique was useful only to detect the natural compounds in the samples.

2. Experimental part

2.1. Extracts

Fruits of *G. brasiliensis* were obtained from a specimen grown in nature on the campus of the Federal University of Viçosa (UFV), Viçosa-MG, Brazil. A botanist of UFV has confirmed the identity of the fruits and a voucher specimen was deposited in the Botanical Garden of UFV under registry code VIC2604. Fruits and seeds were separated and both plant parts were air-dried at room temperature for two weeks. The dried materials were then powdered and the resulting fruit's pericarp and seeds weighted *ca.* 1 kg and 0.7 kg, respectively.

Whole air-dried fruit material content was left to stand for 7 days under maceration with 3 L of *hexane*, at room temperature. After the maceration procedure, the liquid phase was separated from the solid residue by filtration and the solvent evaporated at 45 °C under reduced pressure in a rotary evaporator. On the same batch of fruit material, the overall procedure was repeated five times to afford approximately 80 g of *G. brasiliensis* fruit *hexane* extract (**GBFHE**).

Two air-dried seed material fractions of *ca.* 0.35 kg each were left to stand for 7 days under maceration either with 2 L of *hexane* or with 2 L of ethanol–water (95:5 v/v), at room temperature. All procedures described for **GBFHE** preparation were performed after extractions on the two seed material batches with *hexane* and hydrated ethanol, yielding total amounts of *ca.* 40 g and 35 g of *G. brasiliensis* seed *hexane* (**GBSHE**) and ethanol (**GBSEE**) extracts, respectively.

2.2. Isolation and characterization of bioactive polyprenylated benzophenones *1* and *2*

Compound **1** was isolated by silica gel column chromatography eluting the **GBFHE** extract with *n*-hexane/ethyl acetate and ethyl acetate/ethanol mixtures of increasing polarity. Compound **2** was isolated from the **GBSEE** extract by silica gel column chromatography purification eluting the column with *n*-hexane/ethyl acetate and ethyl acetate/ethanol mixtures of increasing polarity. After isolation, compounds **1** (2.0 g) and **2** (0.3 g) were submitted to repeated processes of crystallization in methanol to afford pure analytical samples.

Our research group has already described crystallographic and spectroscopic characterizations of the polyprenylated benzophenones **1** and **2** [22–24]. We have presented the complete descriptions for the isolation of compounds **1** and **2** from **GBFHE** and **GBSEE**, respectively, *e.g.*, solvent systems used as eluent for chromatographic runs, stationary phase, supports, volume and numbers of eluated fractions, grouping of related fractions, subdivisions of fraction groups, wash steps, likewise analytical condition sets and data for UV, IR, MS, ^{1}H and ^{13}C NMR, and single-crystal XRD experiments are detailed.

2.3. HPLC analyses

To compare with the PXRD results, the natural compounds **1** and **2** were quantified in **GBFHE**, **GBSHE** and **GBSEE** by HPLC. Before extract analyses, calibration curves for analytes **1** and **2** were generated. For this purpose, solutions of benzophenones **1** and **2** at 1.2 and 1.5 g L^{-1}, respectively, were prepared by dissolving some crystals of each compound in methanol. In sequence, diluted solutions at 40.0, 20.0, 10.0, 5.0 and 0.5 mg L^{-1} were prepared in the initial mobile phase for HPLC runs [methanol–acetic acid (pH 3.84; 0.001 M) (40:60 v/v)]. From each previous standard dilution, three samples of 20 μL were injected into HPLC device (*Shimadzu Corporation*, Kyoto, Japan) equipped with two pumps (*LC-10ATvp*) and diode array detector (*SPD-M10Avp*) at 254 nm. The analytical column was a *C18* (150 mm × 4.6 mm) with 5 μm particle size protected by a compatible guard column. An optimal gradient for benzophenone analysis was achieved using the initial mobile phase above described to methanol 100% within 10 min with a solvent flow rate of 1.2 mL min^{-1} at 30 °C. A Millipore filter of 0.45 μm (*HAWP 01300*) was used to filter all mobile phase solvents which were also degassed separately prior to use (direct He sparging). The *ClassVP-LC10* software was used as computer interface setting the analysis conditions on the equipment and acquiring the chromatograms. Except for internal standard signal (*cf.* below), just a single high chromatographic peak was detected for analyzed samples of each benzophenone solution used to construct the calibration curve, and these peaks corresponded to the analytes **1** and **2**. The peak areas were also integrated with the *ClassVP-LC10* computer program, and the percent area values calculated with peaks from all diluted solution analyses for compounds **1** and **2** were 99.8 ± 0.2% and 98.3 ± 1.5 (mean ± S.D.), respectively.

The internal standard (IS) procedure was adopted by adding a methanol solution of (2,4-dihydroxyphenyl)(2-hydroxyphenyl)methanone at 10 g L^{-1} to each final 10 mL of diluted calibration solutions and extract samples (final IS concentration of 100 mg L^{-1}) [34]. Linear regression analysis of ratios between peak areas of the analytes and those of the internal standard *vs.* concentrations of the diluted solution has generated the calibration equations.

Benzophenones **1** and **2** were determined in **GBFHE**, **GBSHE** and **GBSEE** by HPLC runs, in triplicate, using the same procedures formerly described for analytical standardization. However, previous sample treatment has been carried out prior to the HPLC runs, as follows: three samples of each extract (0.727, 0.689 and 0.750 g of **GBFHE**; 0.505, 0.519 and 0.499 g of **GBSHE**; 0.551, 0.569 and 0.575 g of **GBSEE**) were added to 5 mL of methanol and the resulting mixtures were vigorously shaken during 10 min at 40 °C only to facilitate the dissolution of the extract components in the solvent. In this step, the internal standard was introduced accordingly. After this stirring process, quantitative filtrations of the hot mixtures (*Whatman®-41* filter paper) yielded clear solutions which were made up to 10 mL with methanol. A volume of 25 μL of methanol solutions prepared with extract filtrates was diluted to a final volume of 10 mL in methanol–acetic acid (pH 3.84; 0.001 M) (40:60 v/v) (initial mobile phase for HPLC analysis). From this last solution, three samples of 20 μL were taken out and individually injected into HPLC equipment. The ratios between areas of peaks corresponding to the polyprenylated benzophenones **1** and **2** at the **GBFHE**, **GBSHE** and **GBSEE** samples and those of the internal standard were interpolated in the calibration curves in order to quantify the analytes. The quantification of **1** in **GBFHE** by HPLC was described previously by us [23].

2.4. PXRD fingerprints

Crystals of compounds **1** and **2** were finely ground up to obtain its respective pure and monophase powder (polycrystalline) samples. The powder of **1** and **2** and **GBFHE**, **GBSHE** and **GBSEE** were individually mounted on a grooved glass slides used as sample holders. The PXRD patterns were recorded at room temperature on a *Rigaku Denki* diffractometer (CuKα beam, λ = 1.5418 Å, beam generator at 50 kV and 100 mA, *RINT2000* wide angle goniometer, continuous scan mode, scan axis θ–2θ, scan speed 1.000°/min, data acquisition width and range of 0.020° and 5–35° 2θ, respectively). Each sample was fast scanned within a time of 30 min. For setting analytical parameters on our diffractometer and for monitoring PXRD data collection, the DataScan software (version 3.2) was employed [35].

The calculated PXRD patterns from the determined crystal structures of **1** and **2** were used in the search/match procedures to identify the benzophenones in the experimental PXRD patterns of the powder samples of **1** and **2** [36]. It is expected that the respective experimental and calculated PRXD patterns of **1** and **2** have matched to each other. Therefore, the calculated PRXD patterns of **1** and **2** can be used to identify them in matrixes containing **1** and **2** in the crystalline solid by search/match procedures comparing the experimental and theoretical PRXD patterns. To test this expectation the experimental pattern of **GBFHE**, **GBSHE** and **GBSEE** were compared with the calculated and experimental ones of **1** and **2**. To the search/match procedures of the PXRD profiles, it was used the software MERCURY [37].

3. Results and discussion

Retention times (t_R) of 17.94 ± 0.06 and 16.93 ± 0.05 min were reported for benzophenones **1** and **2** through the HPLC analysis [23,24]. As expected to occur as a consequence of at least two major keto–enol tautomers of each benzophenone [23,38], a low asymmetric shoulder on the peak tails was observed. Regarding the standard solutions for analytical calibration, the concentration range of 0.5–40 mg L^{-1} showed a satisfactory linear relationship with the peak-area ratios (Table 1). The linear equations obtained with working calibration solutions of benzophenones **1** and **2** are also displayed in Table 1.

The HPLC analysis of **GBFHE**, **GBSHE** and **GBSEE** has revealed few peaks in the chromatograms (Fig. 2), with those associated with compounds **1** and **2** been well separated. The concentration values of the analytes in the extracts were determined by interpolating the peak-area ratios observed therein in the calibration curves corresponding to each benzophenone (Table 1). In *G. brasiliensis* fruits, the tetraprenylated benzophenone **1** is a major secondary metabolite [23], whereas compound **2** is not detected in the **GBFHE** chromatogram. On the other hand, the pentaprenylated benzophenone **2** is a major compound in *G. brasiliensis* seeds, and it is present

Table 1
Analytical calibration and quantification of benzophenones **1** and **2** (mg per g of extract) in the *G. brasiliensis* seed and fruit extracts by HPLC technique.

	Linear equation[a]	Correlation coefficient	GBFHE[a]	GBSHE	GBSEE
1	$y = (0.012 \pm 0.002)x \pm 0.016 \pm 0.011$	0.998 ± 0.008	263 ± 40[b]	70 ± 2	38 ± 2
2	$y = (0.010 \pm 0.002)x \pm 0.022 \pm 0.010$	0.998 ± 0.012	–	101 ± 4	170 ± 14

[a] The HPLC analysis conditions are given in experimental part.
[b] To express the analyte concentrations in percentage of extract weight, the given values should be divided by factor 10.

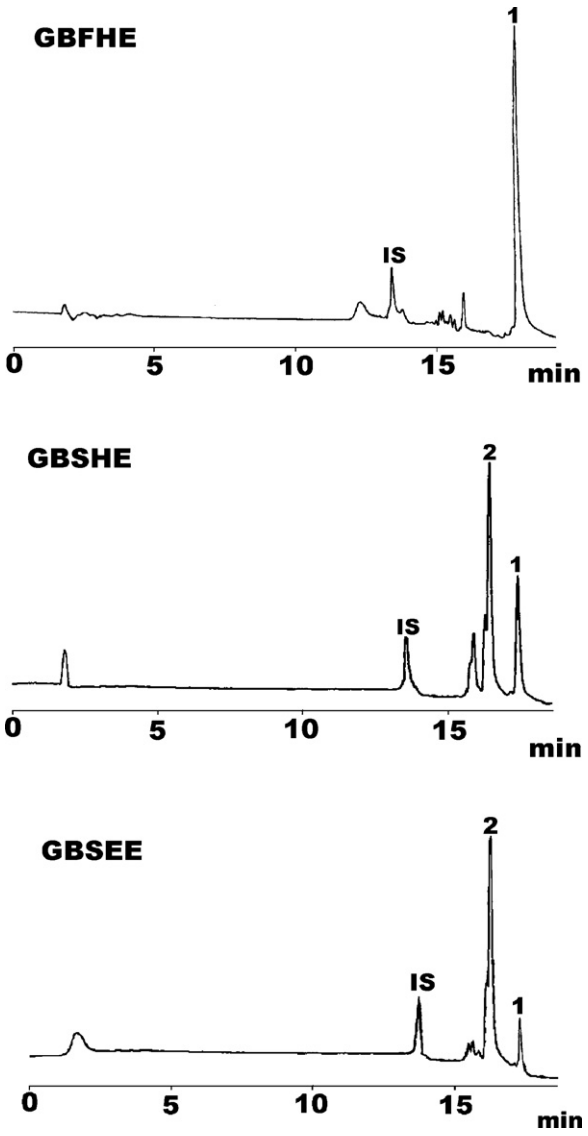

Fig. 2. HPLC chromatograms of **GBFHE**, **GBSHE** and **GBSEE**. The peaks of the polyprenylated benzophenones **1** and **2** and of the internal standard (IS) are labeled, whereas the analytical conditions are found in experimental part.

at a higher amount in the ethanolic extract compared with the hexane extract. In *G. brasiliensis* seeds, compound **1** content was lower than that of **2**. In addition, compound **1** appears in the hexane extract of seeds at an amount larger than that one in the ethanol extract of the same plant part.

Once the HPLC methodology for the analysis of compounds **1** and **2** was established, the next step was to investigate the use of PXRD technique to analyze the same constituents occurring in *G. brasiliensis* fruits and seeds.

Initially the simulated PXRD peak positions [36] were compared with the measured diffractograms of **1** and **2**. It is observed a good agreement between the calculated and experimental PXDR pattern of **1** and **2** (Fig. 3). In other words, the PXRD technique has permitted, as expected, to identify qualitatively the benzophenones. It is important to emphasize that for theses samples the grinding procedure was good enough to avoid a significant preferred orientation, since the experimental normalized reflection intensities were in good agreement with the calculated ones. Moreover, due to their high crystalline no broad hump indicating amorphous material was observed in these experimental diffractograms.

Since the purposed crystallographic method could be applied for detection of benzophenones **1** and **2** in purified powder samples, the possibility of identification of these analytes in extracts was tested. The calculated PXRD patterns of compounds **1** and **2** obtained from their respective single-crystal XRD structures were compared to the PXRD fingerprints of **GBFHE**, **GBSHE** and **GBSEE**. The PXRD analysis of **GBFHE** reveals that several calculated Bragg peaks of benzophenone **1** as those at 9.38° (hkl = 0 1 2) and 14.36° (hkl = 0 2 1) in 2θ match to the experimental ones at 9.46° and 14.40° in 2θ, respectively (Fig. 4a). If simulated and measured diffraction peak positions of the most intense Bragg reflections are in agreement, the analyte, as well as its specific crystalline phase (polymorph), can be confirmed by PXRD [8]. Thus, the polyprenylated benzophenone **1** was selectively detected in **GBFHE** using the crystallographic method. Furthermore, the experimental PXRD pattern of **GBFHE** has no peak from benzophenone **2** phase, showing that it is absent in this extract. The HPLC analysis of **GBFHE** is in good agreement with the crystallographic one, since compound **1** was determined as a major metabolite in this extract and compound **2** was not detected herein by both techniques. In addition, the broad hump, called commonly amorphous halo, presented low intensity in the **GBFHE** PXRD pattern. Therefore, the crystalline material amount can be considered significant in this extract.

The **GBSHE** diffractogram is relatively more complex than that from **GBFHE** due to the more pronounced amorphous halo (Fig. 4b). Nevertheless, some peaks in the experimental PXRD overlay to those ones observed in the simulated X-ray diffractogram from the single-crystal structure of benzophenone **1**. Another important feature observed is that the remaining crystalline peaks have matched well to that one expected to benzophenone **2**. Therefore, the PXRD analysis of **GBSHE** selectively allows for detection of both analytes in this extract. Observed diffraction peaks at 9.38° and 14.38° in 2θ are overlaid to those from the compound **1** crystal structure at 9.38° (hkl = 0 1 2) and 14.36° (hkl = 0 2 1) in 2θ (Fig. 4b), whereas the simulated diffraction lines from the crystal structure of benzophenone **2** which are at 7.96° (hkl = 0 1 1), 9.08° (hkl = 0 1 2), 10.16° (hkl = 1 0 1), 10.70° (hkl = 0 1 3), 12.72° (hkl = 1 1 1), 15.24° (hkl = 0 0 6) and 18.34° (hkl = 1 2 1) in 2θ also appear in **GBSHE** diffractogram as diffraction peaks at 7.98°, 9.10°, 10.14°, 10.68°, 12.78°, 15.24° and 18.30° in 2θ (Fig. 4c). Those peaks at 9.10° and 15.24° in 2θ were the most intense ones of the compound **2** in the **GBSHE** PXRD pattern**.** In **GBSHE**, neither **1** nor **2** had been previously detected/isolated [22–24]. Furthermore, the simultaneous identification of **1** and **2** in **GBSHE** also agrees well with the HPLC analysis results presented here. Thus, such extract was a trial sample for which the obtained results have allowed us to state that the developed method in fact is useful for selective and simultaneous analyses of natural compounds in the evaluated complex matrices. As an exercise in attempt to approach semi-quantitatively the amount of **1** and **2** in the matrices, the area ratio of the most intense experimental PXRD peaks of benzophenones **1** (at 9.38° in 2θ) and **2** (at 9.10° in 2θ) was measured in **GBSHE**. For this purpose, these peaks were deconvoluted in the **GBSHE** diffractogram and their areas were integrated after peak profile fitting from the analytical peak-shape Lorentzian function and background subtraction [39]. This area ratio value is 1.69, which is comparable to the proportion between the amounts of these analytes determined by HPLC in the same extract, 1.44.

In **GBSEE** analyses, both benzophenones **1** and **2** have been quantified by HPLC procedure, whereas the presence of analyte **2** was confirmed by PXRD technique since only their Bragg reflections at 7.90°, 9.04°, 10.12°, 10.70°, 12.64°, 15.24° and 18.20° (2θ) were observed in the **GBSEE** extract (Fig. 4d). Surprisingly, the chromatographic and crystallographic data are strongly correlated. By HPLC analysis, the concentration of benzophenone **1** in **GBSEE** was determined to be 3.8 ± 0.2% (Table 1), a value that is lower than

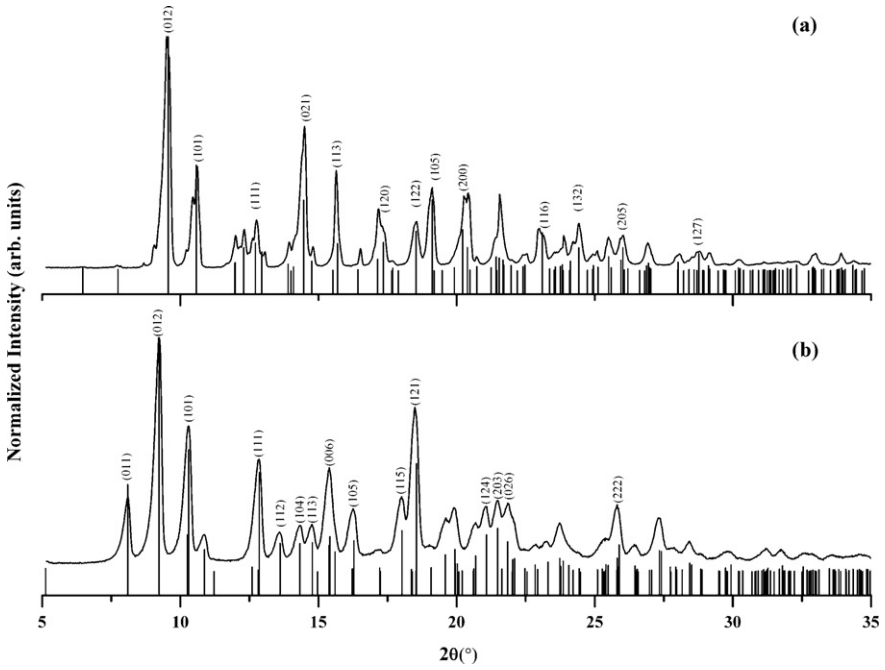

Fig. 3. The experimental X-ray powder diffractograms of benzophenones (a) **1** and (b) **2** (continuous lines) are overlaid to the simulated Bragg reflections from the corresponding crystal structures (vertical lines).

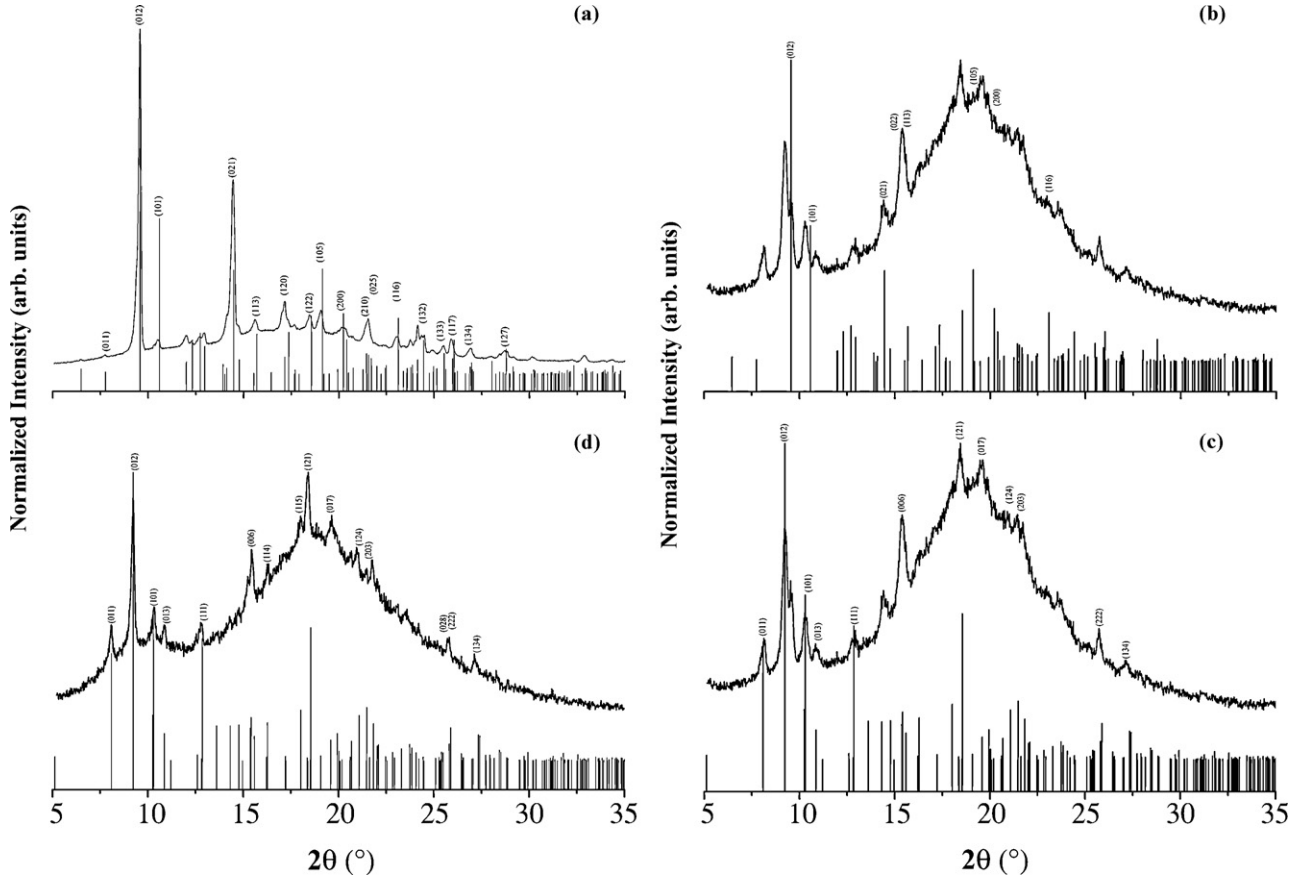

Fig. 4. (a) The PXRD fingerprint of **GBFHE** (continuous line) overlaid to the simulated PXRD lines of benzophenone **1** (vertical bars). (b) Matching the PXRD fingerprint of **GBSHE** (continuous line) to the simulated PXRD lines of benzophenone **1** (vertical bars). (c) Overlaying of PXRD fingerprint of **GBSHE** (continuous line) to the simulated PXRD lines of benzophenone **2** (vertical bars). (d) The simulated Bragg reflections of benzophenone **2** (vertical lines) are present in the **GBSEE** powder X-ray diffractogram (continuous line) as diffraction peaks. The PXRD peaks of benzophenone **1** are not detected in this diffractogram due to concentration of this analyte to be lesser than 5% herein.

5%, the lowest concentration of either the analyte or a phase of a compound that can be surely detected in powder mixtures through PXRD investigations [8,40]. For that reason, the diffraction peaks of the analyte **1** are not observed in **GBSEE** diffractogram, and this compound could be not recognized in this extract. Only diffraction peaks of the analyte **2** are observed over the GBSEE diffractogram halo, since its concentration in this extract was determined by HPLC as being $17.0 \pm 1.4\%$. To strength such observation, benzophenone **1** was found by HPLC assay to be at a concentration value of $7.0 \pm 0.2\%$ in **GBSHE**, and, therefore, it was doubtless detected with the crystallographic treatment for characterization of this hexane extract. Indeed, the commonly adopted detection limit of 5% for phase quantifications using PXRD techniques should be also considered when analyzing the polyprenylated benzophenones **1** and **2** in *G. brasiliensis* extracts.

4. Conclusions

A crystallographic method for direct detection of bioactive compounds in raw polycrystalline materials and plant extracts is reported. It is based on simple and rapid overlays of simulated PXRD patterns from reported single-crystal structures of target analytes to experimentally acquired diffractograms of analyzed samples. The polyprenylated benzophenones **1** and **2** were the pharmacologically active compounds analyzed in extracts from *G. brasiliensis* seeds and fruit's pericarp. However, the results obtained demonstrate the use of PXRD method to analyze these natural compounds in phytopreparations. In fact, this study encourages exploring the XRD crystallography as a phytochemical analysis tool for identification and quantification of compounds in complex plant products.

To evaluate the efficiency of the method developed, a HPLC study was carried out with the same samples and analytes, showing good agreement with the results obtained by XRD technique. The crystallographic procedure requires a small amount of sample and it is robust, direct, rapid and non-destructive. Indeed, the analytical parameters are easily set on a conventional powder X-ray diffractometer, the data collection is completed within approximately 30 min and the results are interpreted by comparing simulated and experimental PXRD patterns.

The main contribution of this study is the development of a new method based on PXRD for characterization and quantification of polyprenylated benzophenones in *G. brasiliensis* seed and fruit extracts and those in raw materials to be incorporated into phytotherapeutic compositions. The reported advance was possible due to the spontaneous crystallization of the benzophenones in the prepared extracts. In case of extracts, microsized crystals were formed herein, which was responsible by XRD phenomenon through honey-like consistency samples even no experiencing undesirable effects due to preferred orientation of particles. Really, crystalline materials are expected to occur in plant extracts and other phytopreparations so that analytical methods with XRD techniques can be used for phytochemical characterizations, being such need a challenge therein.

As a consequence of this study, we believe that the crystallographic methods will be better explored for quality control analyses and selection trials of raw materials and extracts from plants.

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