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Research Article

Identification and Infrared Spectroscopic Study of Lapachol, β-Lapachone and Hydroxy-hydrolapachol

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Abstract

Metabolites of Brazilian Cerrado species are considered an immense font of biologically active compounds The diversity of organic compounds generated by the secondary metabolism of various Cerrado plants draws attention especially because many of these compounds have the capacity to be structurally modified and, consequently, produce other very interesting derivatives for pharmacological purposes. Despite this, little is described in the literature about fast, easy, and accessible identification methods for any laboratory, such as infrared spectroscopy. In this sense, this work demonstrates the synthesis and elucidation through spectroscopic techniques of lapachol and its synthetic derivatives. Through quick and simple extractions or reactions, lapachol, β-lapachone, and hydroxy-hydrolapachol were obtained with adequate yields. From this, the main FTIR absorptions of the mentioned naphthoquinones are described, which facilitates the identification of these metabolites with high biological potential. The present work contributes could become a simpler source of data for extraction, synthesis, and spectroscopic characterization by FTIR of the compounds.

More Information

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Introduction

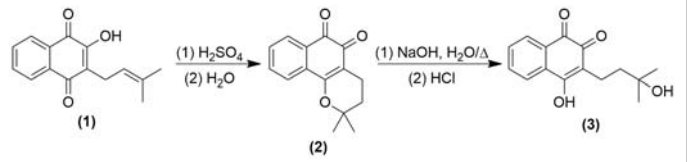
Brazilian Cerrado is the second largest Brazilian biome, occupying about 21% of the country's area in the plateau of the South American continent [1,2]. The biome presents an interesting diversity in both fauna and flora, showing an uncountable number of native species [3]. The biome is widespread in the Brazilian territory, occupying the largest areas in the states Federal District, Goiás, Tocantins, Maranhão, Mato Grosso do Sul and Minas Gerais. However, the prevention of deforestation or even government inactions to decrease the devastation of the biome, mainly in the past four years have been impacting subsequently the preservation of native species [3,4].

Particularly, some species of plants are native to the biome Cerrado, being considered a natural marker of it, such as the presence of *Tabebuia heptaphylla* (Veloso) Toledo, called popularly in Brazil as "Ipê-roxo". The specie from the Biognoniaceae family is characterized by a crooked trunk, measuring up to 35 meters tall, with opposite typed leaves usually five-seven leaflets, and tubular showy flowers lilac to pink colored [5]. In addition, some *Tabebuia* spp. have been described in the literature, differing visually by the color of flowers, such as white (*Tabebuia roseoalba*), yellow (*Tabebuia alba*), pink (*Tabebuia impetiginosa*) and others [5,6]. As commonly observed in Cerrado plants, *T. heptaphylla* is known for its uses in ethnopharmacology, being considered a "wonder drug" for some natives in Brazil and other South American countries, including Argentina, Paraguay, Bolivia, and Peru for some preparations [7-9]. The presence of special metabolites such as flavonoids, napthoquinones, furanonapthoquinones, quinines, benzoic acids, and others can be pointed out as one of the reasons why the use in traditional medicine is commonly related in native populations on the American continent [10].

Due to the presence of remarkable substances made by secondary metabolism, *T. heptaphylla* is described in the literature with a great range of biological responses. Some of that biological properties have been described for *Tabebuia avellanedae* Lorentz ex Grisebach or *Tecoma impetiginosa* Martius, being both botanical sinonimum of *T. heptaphylla* [5]. El-Hawary and coworkers reviewed the biological responses as well as the presence of substances catalogued in *Tabebuia* spp [11]. Some parts of the plant, extracts, oils, resins and other preparations have been used, exhibiting anti-inflammatory [12-14], anti-ulcer [15,16], wound healing activity [17], antinociceptive [18], anti-obesity [19,20], antidepressant [21], antimicrobial [22-24], antileishmanial [25], antiviral [26,27], insecticidal [28], anti-oxidant [29], cytotoxic [30,31] and among others.

In major of *Tabebuia* spp. we observed the presence of naphthoquinones, special metabolites class of organic compounds that have important antioxidant and electrolytic effects, especially by the electrophilic, oxidant, and acid-base properties of a conjugated benzoquinolinic moiety present in them [32,33]. The main naphthoquinone considered a chemical marker of *Tabebuia* spp. is Lapachol (Scheme 1, (1)), a 1,4 naphthoquinone, which has been described in the literature in at least fifteen species [11]. Lapachol is commonly obtained by extraction from parts of the wood or bark [34,35], and gained prominence after several studies of the substance and the semi-synthetic derivatives obtained over the years against tumor and cancer cell lines in both in vitro and in vivo investigations [36]. In a recent study conducted by Oliveira and coworkers [37], the authors prepared Lapachol-Ru(II)/diphosphine complexes and tested with success the substances against breast cancer, becoming Lapachol's complex an interesting drug candidate for cancer treatments [37]. Similar results had described for the main derivative, β-Lapachone (Scheme 1, (2)), which displays similar or better actions against cancer cell lines, being used in anticancer agents [38,39].

Considering the remarkable importance of Lapachol and its metabolites, a low-cost, fast, and robust analytical method is necessary to identify the substances. In this sense, Fourier transformed infrared (FTIR) technique has been employed in the analysis of natural products, pharmaceutical substances, industrials, and research laboratories as protocol, mainly due to the fast simpler analysis, with a small amount of sample, being non-destructive technique [40,41]. In addition, the use of FTIR as a technique to identify functional groups and moieties or bonding groups in natural products is done by quantized energy absorption in natural vibrations for each molecular arrangement, becoming especially Mid-Infrared (MIR) an important tool that can be used as a fingerprint for identification of organic compounds [41,42]. Although MIR data had been used as a tool for Lapachol derivatives identification, is observed a clear difficulty to corroborate the experimental information of the structures with data in the literature, becoming the simple analysis by FTIR of its derivatives a tortuous and difficult mission. In this sense, we proposed here a standardization and study of Lapachol and derivatives by MIR as a way to help the rapid identification of these molecules.



Scheme 1: Synthesis of β-lapachone (2) and hydroxy-hydrolapachol (3) from lapachol (1).

Materials and methods

Extraction and synthesis of lapachol derivatives

The tree heartwood sawdust of *T. heptaphylla* (Veloso) Toledo was collected in the city of Ipameri, state of Goiás, Brazil (48° 9' 36.0''S 17°44'20.7'' W), identified and deposited in the Herbarium of Núcleo de Biologia, at Instituto Federal de Educação, Ciência e Tecnologia Goiano Campus Urutaí, Urutaí, Goiás, Brazil. The extraction of lapachol (1) (Scheme 1) occurred as a modified methodology proposed in previous studies of the literature [43]. The bark collected was dried in the stove, and 456 grams of the powder was suspended in a 2 L beaker flask with 1 L of sodium carbonate solution (1% m/v, Sigma-Aldrich, St. Louis, MO, USA). The mixture was stirred for 45 minutes, obtaining after filtration a red-colored solution. To the crude mixture was added HCl 6.0 mol L^{-1} (Neon, Suzano, SP, Brazil) up to the solution became yellow-colored and started the precipitation process (pH 2.0). The precipitate was filtered off under vacuum filtration and recrystallized in dichloromethane P.A. (Dinâmica Química, São Paulo, SP, Brazil) and dry under the stove (80 °C, 1 h), obtaining 33.5 g of yellow crystals (7.3 % yield). The structure (1) was confirmed by NMR analysis.

Compound (2) (Scheme 1) was synthesized as previously described by Alves and coworkers [44], where β-lapachone was obtained by reacting 484 mg of (1) with 5.0 mL of sulphuric acid P.A. (Sigma-Aldrich, St. Louis, MO, USA) in a roam-bottomed flask under stirring for 15 minutes. The mixture was vested in 100 mL of cold distilled water (4 °C), obtained by vacuum filtration, and dried in a stove (60 °C) with 376 mg of an orange solid (77.7% yield). Synthetical obtainment of hydroxy-hydrolapachol (3) was performed as described by Petit & Houghton, [45] by a base-catalyzed Michael-type reaction where 250 mg of (2) reacts with 18.0 mL of sodium hydroxide solution 5% (m/V) (Sigma-Aldrich, St. Louis, MO, USA) in a roam-bottomed flask under heating (85 °C to 120 °C) and stirring for 10 minutes. The mixture was cooled and neutralized with HCl 6 mol L^{-1} up to color change from red-brown to a yellow-colored mixture. The interest compound was obtained by a partition with ethyl acetate P.A. (3 x 15 mL) (Dinâmica Química, São Paulo, SP,

Brazil), the organic layer was dried with magnesium sulfate, rotavap under reduced pressure, giving 174 mg of a yellow solid hydroxy-hydrolapachol (64.7% yield). All structures (2) and (3) were confirmed by NMR analysis.

NMR and infrared analysis

All ^{1}H and ^{13}C NMR analyses were performed on a Bruker DRX400 spectrometer (Karlsruhe, Germany, 400 MHz for ^{1}H and 100 MHz for ^{13}C). The ^{1}H and ^{13}C chemical shifts (δ) were described in terms of parts per million (ppm), using tetramethylsilane (TMS, 0.03% v/v) as an internal reference. The experiments were performed at 300 K, in sample concentrations of 10 mL mL^{-1}, in $CDCl_3$ (99.8 atom % D, Sigma-Aldrich, St. Louis, MO, USA). FT-IR analysis was performed in the mid-infrared region in solid state KBr (Shimadzu Scientific, spectroscopic grade, Kioto, Japan) pellet using an infrared spectrometer Shimadzu Affinity-1 model (Shimadzu Corporation, Kioto, Japan). We achieved the region of 4000 to 400 cm^{-1} with 16 scans per experiment, as previous methodologies reported in the literature [46,47]. All FTIR data were analyzed on Origin program version 6.1 (OriginLab Corporation, Northampton, MA, USA) [48].

Results and discussion

NMR analysis

The characterization of the synthesized compounds was achieved by ^{1}H and ^{13}C NMR experiments, described above, and the spectra of (**1-3**) are in agreement with the chemical shifts as previously published in the literature [49,50].

2-hydroxy-3-(3-methylbut-2-en-1-yl) naphthalene-1,4-dione (lapachol, (1)), yellow powder, mp 137-139 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (s, 3H), 1.71 (s, 3H), 3.23 (d, 2H J = 7.4 Hz), 5.13 (tt, 1H J = 7.4, J = 1.4 Hz), 7.62 (m, 2H), 7.62 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 22.6, 25.7, 119.7, 123.5, 126.0, 126.7, 129.4, 132.8, 132.9, 133.8, 134.8, 152.7, 181.7, 184.5.

2,2-dimethyl-3,4-dihydro-2H-benzo[H]chromene-5,6-dione (β-lapachone, (2)), orange solid, mp 153-155 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 6H),1.84 (t, J = 6.7 Hz, 2H), 2.59 (t, J = 6.7 Hz, 2H), 7.50 (dt, J = 1.1, 7.4 Hz, 1H), 7.66 (dt, J = 1.3, 7.4 Hz, 1H), 7.81 (dd, J = 1.1, 7.7 Hz, 1H), 8.00 (dd, J = 1.4, 6.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 22.6, 25.7, 119.6, 123.5, 126.0, 126.8, 129.4, 132.8, 132.9, 133.8, 134.8, 154.7, 179.7, 184.5.

4-hydroxy-3-(3-hydroxy-3-methylbutyl) naphthalene-1,2-dione (hydroxy-hydrolapachol, (**3**)), yellow solid, mp 126-128 °C, ^{1}H: δ 1.41 (s, 6H), 1.85 (t, J = 6.7 Hz, 2H), 2.58 (t, J = 6.7 Hz, 2H), 7.53 (dt, J = 1.1, 7.4 Hz, 1H), 7.61 (dt, J = 1.3, 7.4 Hz, 1H), 7.82 (dd, J = 1.1, 7.7 Hz, 1H), 8.01 (dd, J = 1.4, 6.9 Hz, 1H); ^{13}C: δ 16.8, 26.8, 31.7, 79.4, 112.9, 124.1, 128.7, 130.3, 130.7, 132.8, 134.8, 154.7, 162.1, 178.7, 180.1, 184.5.

Mid-infrared study of lapachol, β-lapachone and hydroxy-hydrolapachol

FTIR analysis using specific frequencies on the infrared region to promote absorptions in quantized energy content, promoting stretching and bending of the bonds in befitting natural vibrations, bond types, functional groups, and special molecular arrangements, promoting proven structural elucidation by spectra analysis [51]. Lapachol is characterized by an intense and broadband in 3352 cm^{-1}, common in O-H bonds by symmetrical stretching. Hydrogen-oxygen bonds are observed particularly in regions between 3500 cm^{-1} and 3100 cm^{-1}, present in some natural products which exhibit an α,β-unsaturated O-H bond, such as flavonoids and naphthoquinones [52,53]. In addition, in a similar region, the symmetrical and asymmetrical stretching in the region between 3100 - 2700 cm^{-1} are diagnostic of the C–H bond in sp^2 and sp^3 carbon atoms. In lapachol, we observed depicted in Figure 1.1 the bands referred to C-H bonds in an aliphatic moiety and in the benzenic structure of naphthoquinone, like other natural products which exhibit C-H bonds in structure [54].

We still emphasize the presence of two characteristic bands in 1658 and 1638 cm^{-1}, which distinguishes the presence of double carbonyl groups in the C2 and C9 positions (Figure 1.1). The presence of each band is in consonance with the C = O bond between 1655 - 1635 cm^{-1}, which exhibits an α,β-unsaturated ketones of quinolinic moiety [55]. The extended conjugation increases the effects by a keto-enolic effect with a hydroxyl group of enolic moiety in C1 [55,56]. The harmonic bands in the region between 2000 and 1667 cm^{-1}, deserve to be highlighted due to the presence of a double substitution at the aromatic ring (C3 and C8), which corroborates with the *ortho*-evidenced band of angular stretching observed in 720 cm^{-1} [55,57]. The aliphatic moiety was still evidenced by the axial stretching in 1589 cm^{-1}, which characters an is methyl propene ramification, in consonance with the literature [51]. In addition, the methyl group can be confirmed by a double band in 1369 and 1350 cm^{-1} (Figure 1.1), common in geminal moieties as observed in the lapachol structure [55,58]. The presence of geminal groups is common in some isomethylated compounds, such as sesquiterpene hydrocarbons and other natural products, as reported in the literature [58]. In addition, the additional bands as well the characteristic bands discussed above of (1) are in accordance with computational proposes in the literature [59], and are listed in Table 1.

The FTIR of β-lapachone (2), depicted in Figure 1.2 presents a band between 3430 and 3261 cm^{-1}, which proceeds by enolization of carbonyl groups and was fully cited in the literature in both synthetic and isolated molecules [60-62]. The signals at 2933 cm^{-1} and 2973 cm^{-1} are characteristic of the stretching of the C sp^2–H bond of the naphthoquinone aromatic ring [63]. Aromatic ring resonance confirmation

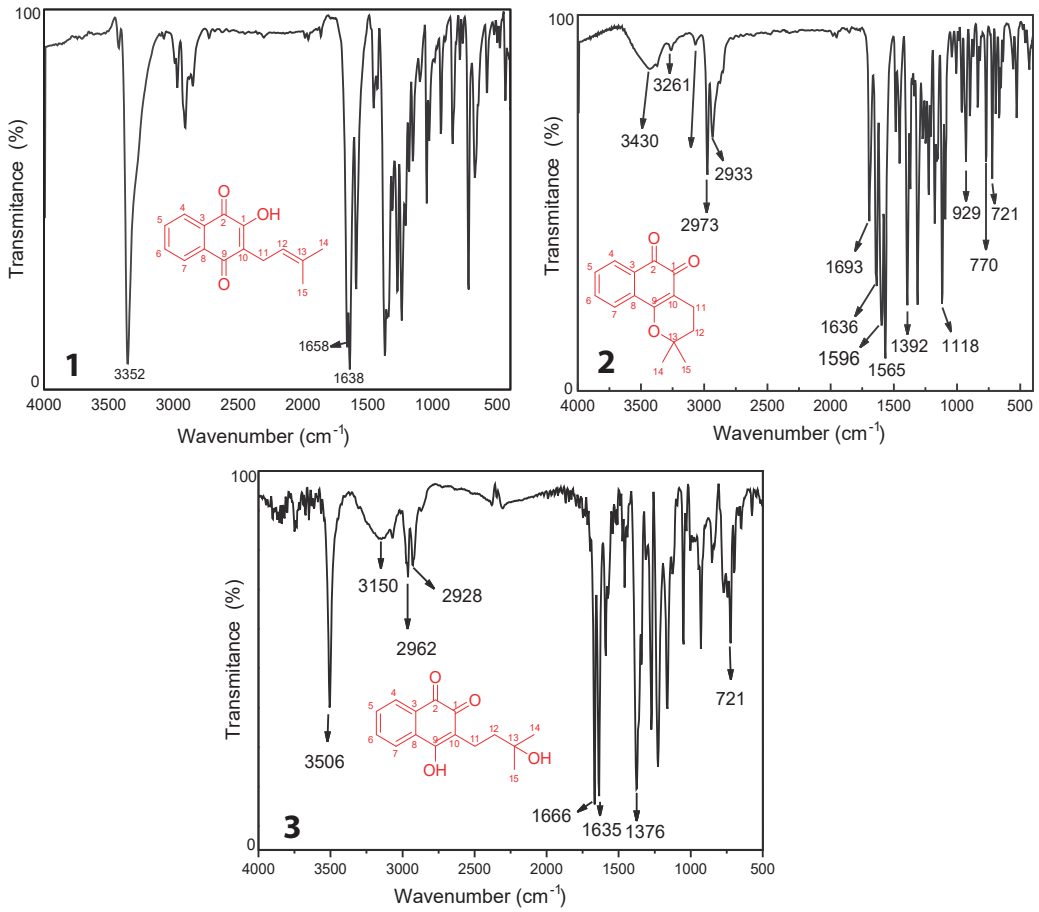


Figure 1: FTIR spectra of lapachol (1), β-lapachone (2) and hydroxy-hydrolapachol (3) in KBr pellet.

Table 1: Experimental wavenumber (cm^{-1}) data of lapachol (1), β-lapachone (2) and hydroxy-hydrolapachol (3) (KBr pellet).

Lapachol		β-lapachone		Hydroxy-hydrolapachol	
ν (cm^{-1})	Assignment	ν (cm^{-1})	Assignment	ν (cm^{-1})	Assignment
3352	ν_{OH}	3430	$\nu_{C=O}$	3506	$\nu_{C=O}$
2000-1667	$\nu_{C=CArom}$	3261	$\nu_{C=O}$	3150	$\nu_{C=O}$
1658	$\nu_{C=O}$	2973	ν_{C-C}	2962	ν_{C-C}
1638	$\nu_{C=O}$	2933	ν_{C-C}	2928	ν_{C-C}
1589	$\nu_{C=Ctrisubt.}$	1689	$\nu_{C=O}$	1666	$\nu_{C=O}$
1369	$\nu_{C-CH3\ gem}$	1636	$\nu_{C=O}$	1635	$\nu_{C=O}$
1350	$\nu_{C-CH3\ gem}$	1596	$\nu_{C=C}$	1376	ω_{C-H}
720	Ω_{Arom}	1565	$\nu_{C=C}$	721	ω_{Arom}
		1392	i_{ce-O-C}		
		1118	ν_{C-O-C}		
		929 770 721	ω_{Arom} ω_{Arom} ω_{Arom}		

Note: V = Stretching, ω = Wagging, Arom = Aromatic ring, Gen = Geminal

bands in the characteristic ranges similar to lapachol (1), which show signals in the range of 2000 to 1667 cm^{-1} confirming substitutions in *ortho* and *para* positions of the aromatic ring [59]. The bands at 1596 and 1565 cm^{-1} are characteristic of C = C aromatic bond stretching, common in some classes of natural products, such as flavonoids [52]. In addition, the region between 1400 – 1000 cm^{-1} is characteristic of asymmetric and symmetric axial stretching of ether, so bands in 1392 and 1118 cm^{-1} (Figure 1.2) are C-O-C stretchings [64]. The preferred region of IR spectra also shows signals for simple alkanes to be in this same range, according to the literature [59,65]. Table 1 summarizes the bands observed in the spectrum of β–lapachone observed in Figure 1.2.

The FTIR spectrum of hydroxy-hydrolapachol (Figure 1.3) shows an intense band at 3506 cm^{-1} characteristic of the O–H bond free of intermolecular interaction from the hydrogen in the aliphatic chain of the molecule, followed by a broad band in low intensity at 3150 cm^{-1} which could be assigned to O–H bond with intermolecular hydrogen bond interaction [66]. In the range between 2962 – 2968 cm^{-1}, we observed stretch bands of Csp^2–H and C sp^3–H, as proposed for (1), as well as a C–H folding absorptions at 1376 cm^{-1} corresponding to the methyl group (CH_3) of the hydroxy-hydrolapachol [49,51], as detailed described on Table 1.

The similarity of hydroxy-hydrolapachol (3) and lapachol (1) undoubtedly makes difficult the distinction between those structures on FTIR spectra, however, some important and interesting details can be used to distinguish the main characteristics of that spectra, as depicted in Figure 2 which shows a comparison between compounds (1 - 3). The confirmation bands of free O–H are present in both, but the hydroxy-hydrolapachol spectra show an O–H intermolecular

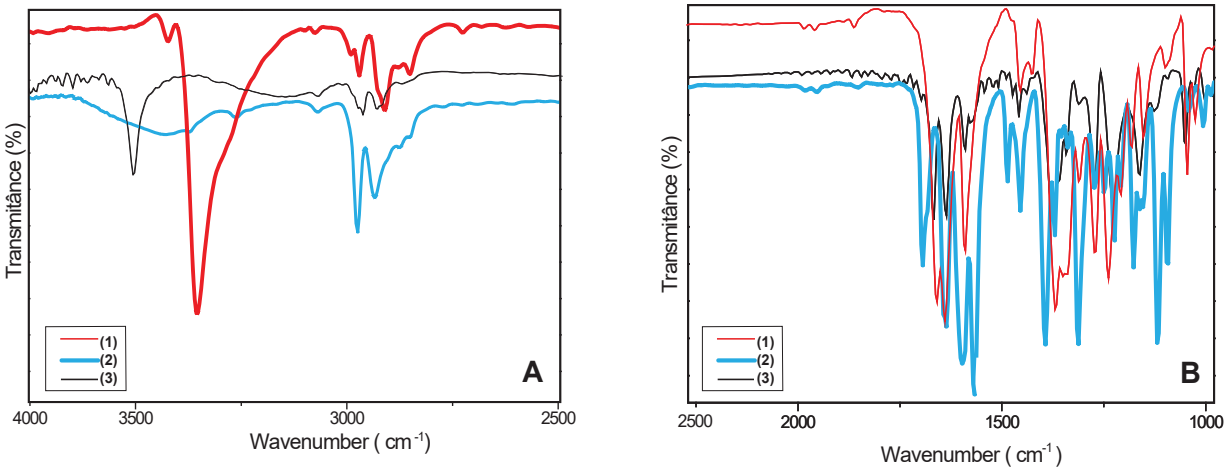


Figure 2: Comparison of FT-IR spectra ranging between 4000 and 2500 cm^{-1} (A) and ranging between 2500 and 1000 cm^{-1} (B), of lapachol (1), β-lapachone (2), and hydroxy-hydrolapachol (3) (KBr pellet).

interaction band between 3500 and 3150 cm^{-1}, which are characteristics of the aliphatic hydroxyl in the structure, as shown on Figure 2A. β-lapachone (2) presents a confirmation band of O–H intermolecular interaction, that can also be influenced by the C = O group band at 3150 cm^{-1}. Another remarkable characteristic that can be highlighted is the less defined bands for Csp^3–H by cyclized carbons of the aliphatic radical compared to naphthoquinone compounds (1) and (3) (Figure 2A).

In the region between 2500 to 1000 $cm^{-1,}$ the bands observed for the spectra of (1) and (3) between 2000 and 1666 cm^{-1} showed similar harmonic signals, characteristic of aromatics substituents, observed in Figure 2B. Considering the substitutions in the aromatic ring being in the same positions for (1) and (3), compound (2) differs in harmonic signal due to the heterocyclic structure linked to the quinone ring. In the regions where carbonyl groups are firmed, the literature predicts higher values for ketones and lower values for others, proving that the energy for this vibration occurs higher for C = O bonds than for C-O-C [55]. Therefore, this is a fundamental point of differentiation between the ether signal in the region of 1390-1100 cm^{-1} in β-lapachone ((2)), in relation to the ketone vibrations present in the compounds (1) and (3) (Figure 2B). Due to the higher number of signals that occur in the same range, the present work sought to focus on those the main distinction of the compounds, because the structures studied present structural similarities resulting in similar signals with subtle displacements, making this region difficult to analyze.

Conclusion

The extraction of lapachol from sawdust of the ipê tree heartwood was carried out an in according to the literature. From the extracted lapachol, β-lapachone, and hydroxy-hydrolapachol were synthesized with good yield. The main characteristics of the FTIR and ^1H and ^{13}C NMR spectra of these compounds were presented and discussed. Lapachol, β-lapachone, and hydroxy-hydrolapachol are substances of great pharmacological potential, and this maintains the growing interest in research and new applications. Ipê-roxo metabolites, as well as several metabolites from plants from the Brazilian Cerrado, have attracted attention due to the wide range of applicability regarding their pharmacological potential. FTIR technique, which is easy, cheap, and fast in its acquisition, does not degrade the sample and allows, through this study, an unequivocal identification of the characteristic bands of the compounds listed above. Thus, this work contributes by being a source of literature that brings together the extraction, synthesis, and spectroscopic characterization of these compounds.

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ZDSS and GRA worked in obtaining the compounds, in the synthesis of derivatives, and in the analysis of FTIR data. RMFS acted in obtaining the NMR spectra. MFG acted in the conception, writing, and discussion of results arising from this work. HJD worked on the conception, discussion of results, writing of the manuscript, revision, and approval of the final version of the manuscript. All authors agree with the submission of the manuscript and declare that they have not submitted it to another journal during the review process.

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