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Identification of antioxidant and antimicrobial compounds from the oilseed crop *Ricinus communis* using a multiplatform metabolite profiling approach

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ABSTRACT

Ricinus communis is an important oilseed crop, which is widely used by traditional communities due to its medicinal properties. The extracts obtained from this plant are used to treat rhinitis, chest inflammation, bronchitis, dental caries, scabies, skin diseases, and infections in the digestive apparatus. Despite all its medicinal properties, there is a lack of studies that apply advanced chemical profiling techniques to characterize the chemical diversity of these extracts. The objective of this study was to apply a detailed multiplatform-based metabolite profiling approach to identify antioxidant and antimicrobial compounds in *R. communis* extracts. Leaf, stem and root extracts were obtained by maceration in hexane, ethyl acetate and ethanol. Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay and total phenolic compounds were quantified by Folin-Ciocalteu method. Antimicrobial activity was assessed against *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonela choleraesuis*, *Candida albicans* and *C. glabrata*. Nuclear magnetic resonance, liquid chromatography–mass spectrometry, and gas chromatography–mass spectrometry were used to characterize the chemical profile of the extracts. The antioxidant activity (IC_{50}) of the extracts ranged from 31.73 ± 2.59 to 571.74 ± 45.92 μg mL^{-1}, whereas total phenolic content varied from 16.96 ± 0.30 to 135.06 ± 1.69 mg GAE/g of extract. Extracts obtained with ethanol showed the greatest antioxidant activity, the highest total phenolic content, along with the most promising antimicrobial properties. For example, the ethanolic extract of the leaves showed antimicrobial activity against *P. aeruginosa*, *S. choleraesius*, and *C. albicans*, whereas the ethanol extract of the roots showed antimicrobial activity against *P. aeruginosa* and *C. albicans*. Three metabolites were annotated and quantified by NMR, 54 by LC–MS, and 36 by GC–MS, which included alkaloids, fatty acids, terpenes, phenolic compounds, steroids and carotenoid derivatives. The ethanol extract of the leaves showed high levels of the alkaloid ricinine, which seems to contribute for the activities of this extract. This approach allowed us to identify antioxidant and antimicrobial present in *R. communis* extracts. Therefore, providing important leads into the discovery of new active compounds and possibly to the development of new pharmaceuticals.

1. Introduction

R. communis is an important oilseed crop and it is used by traditional communities due to its medicinal properties (Ribeiro et al., 2016; Scarpa and Guerci, 1982b). Ethnobotanical studies describe the leaves as one of the most used parts of the plant in the traditional medicine (Abe and Ohtani, 2013; Rashid et al., 2015). Nevertheless, since Scarpa and Guerci (1982a) described the medicinal use of *R. communis*, several

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authors have further investigated the phytochemical and pharmacological activities of *R. communis* extracts (Abbas et al., 2018; Araújo et al., 2017; Kaingu et al., 2012; Rajeshkumar et al., 2013; Ramos-López et al., 2012, 2010; Rana et al., 2013; Ribeiro et al., 2016).

Pharmacological activities of *R. communis* extracts and isolated compounds have been recently reviewed. Eighty-three compounds have been identified in *R. communis* extracts, which includes alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, tocopherols, terpenoids and fatty acids (Ribeiro et al., 2016). Although the antimicrobial activity was the most extensively studied pharmacological activity, many others have been investigated, such as acaricidal (Ghosh et al., 2013), antidiabetic (Mann et al., 2013), antioxidant (Abbas et al., 2018), antiasthmatic (Taur and Patil, 2011), anthelmintic (Rana et al., 2013), antihistamine and anti-inflammatory (Lomash et al., 2010), immunomodulatory (Kumar et al., 2011), contraceptive (Nath et al., 2013), cytotoxic (Vandita et al., 2013), and anticonvulsant activities (Tripathi et al., 2011). Nonetheless, very few studies have investigated the chemical composition of the extracts in association with their biological activities. For example, antibacterial activity of *R. communis* extracts was assessed against different species of Bacillus (Oyewole et al., 2010; Rampadarath et al., 2014). Phytochemical screening assays detected the presence of alkaloids, cardiac glycosides, coumarins, flavonoids, phenolic compounds, tannins, and terpenoids in the extracts. Phytochemical screening consists of a superficial attempt to characterize the chemical profile of a given extract. They encompass preliminary assays based on color intensity or the precipitate formation, which are used as analytical responses to infer the presence or absence of a given class of molecules (Farnsworth, 1966; Saraswati et al., 2016). Therefore, more advanced chemical profiling techniques are required to characterize the chemical diversity of these extracts.

Metabolite profiling techniques provide vital information for the understanding of biological processes. Nuclear magnetic resonance (NMR), liquid chromatography–mass spectrometry, and gas chromatography–mass spectrometry have been applied to identify potential bioactive compounds in different biological samples (Bittencourt et al., 2015; Chaves-López et al., 2018; D'Urso et al., 2018; Farag et al., 2017; French et al., 2018; Ribeiro et al., 2014a, b; Ribeiro et al., 2015a). The greatest challenge for metabolite profiling studies is to provide a comprehensive picture of the extract composition. Therefore it is advisable to use chromatographic separation techniques coupled to detection techniques to obtain a complete response of the studied biological system (Canuto et al., 2018). Liquid and gas chromatography are commonly used for metabolite separation in complex matrices, whereas nuclear magnetic resonance and mass spectrometry are generally for metabolite detection (Canuto et al., 2018; Khoomrung et al., 2017; Liu and Wang, 2017; Wilson, 2017). Notably, there is a lack of studies correlating the biological activities of *R. communis* extracts with their metabolite profile. Therefore, we present a detailed multiplatform-based metabolite profiling approach aiming at identifying antioxidant and antimicrobial compounds present in hexane, ethyl acetate, and ethanol extracts obtained from leaf, stem, and roots of the oilseed crop *Ricinus communis*.

2. Materials and methods

2.1. Sample preparation and extraction

Ricinus communis (genotype MPA34) used in this study was developed by the breeding program of the Empresa Baiana de Desenvolvimento Agrícola S.A (EBDA-Brazil). Plants were grown at the experimental farm Gameleirinha located at Iraquara city (12°15′03″S and 41°37′11″W). Leaf (0.71 Kg), stem (1.77 Kg) and roots (1.84 Kg) were dried at room temperature (25 °C) and ground to a fine powder. The dried and ground samples were extracted by maceration with hexane, followed by extraction with ethyl acetate and ethanol. Solvents were removed under reduced pressure at 40 °C using a rotary evaporator (4000 Laborota echo, Germany). Samples remained in the exhaust hood at room temperature (25 °C) until all residual solvent evaporated and the extracts were completely dried.

2.2. DPPH radical scavenging assay

Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by Bittencourt et al. (2015). Initially, 1 mL of DPPH (120 mmol L^{-1}; methanol) was added to 1 mL of each extract to provide 2 mL of the reaction mixture with a final concentration of the extracts ranging from 10 to 1000 μg mL^{-1}. Absorbance was measured at 517 nm after 30 min of reaction. Ethanol was used as a blank and DPPH solution (1.0 mL; 120 mmol L^{-1}) plus ethanol (1.0 mL) was used as negative control (no consumption of DPPH expected). Experiments were performed in triplicate and the results were expressed as IC_{50}.

2.3. Total phenolic content

Total phenolic content was assessed as described by D'Sousa Costa et al. (2015) with minor modifications. Initially, 100 μL of the extract (1 mg mL^{-1} in methanol) were mixed with 50 μL of Folin-Ciocalteu's phenol reagent and 750 μL of water. After one minute, 100 μL of sodium carbonate solution (Na_2CO_3, 15% w/v) were added to the mixture reaching a final volume of 1 mL. The reaction mixture was kept in the dark for 120 min. After this time, the absorbance of each reaction mixture was read at 725 nm (VersaMax™ Microplate Reader, USA). Gallic acid standard curve was used to calculate total phenolic content in the extracts and the results were expressed as mg GAE/g of dry extract.

2.4. Antimicrobial activity

Antimicrobial activity of the extracts was assessed as minimum inhibitory concentration (MIC) by using the successive microdilution assay in 96-well plates as described by Ribeiro et al. (2011) and Araújo et al. (2017). Nutrient broth (Acumedia, USA) and malt extract (Acumedia, USA) were used as culture media for bacteria and fungi growth, respectively. Chloramphenicol (0.19–25 μg mL^{-1}), gentamicin (0.039–5 μg mL^{-1}) and ciclopirox olamine (0.39–50 μg mL^{-1}) were used as positive control. Extracts were dissolved in either 20% DMSO (v/v) or in 20% Tween (v/v). For this reason, both tween and DMSO were used as negative controls. After the serial dilution, the concentration of the extracts ranged from 3.90 to 500 μg mL^{-1}. The 96-well plates were incubated at 36 °C (24 h) and 26 °C (72 h) for bacterial and fungi growth, respectively. The MIC was determined through the emergence of turbidity in the wells. Antimicrobial activity of the extracts was assessed against *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (CCT 0096), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 94863), *Pseudomonas aeruginosa* (CCT 0090), *Salmonela choleraesuis* (ATCC 14028), *Candida albicans* (ATCC 18804) and *C. glabrata* (CCT 0728). All analyses were performed in triplicate. The MIC was determined through the absence of turbidity in the wells and extracts were considered active when they inhibited microbial growth at concentrations below or equal to 500 μg mL^{-1}. From those wells that showed the absence of turbidity, 10 μL of the content of were inoculated in solid nutrient broth or malt extract to evaluate whether the observed activity was microbiostatic or microbiocide. All samples were tested in triplicate.

2.5. Metabolite profiling analyses

2.5.1. Nuclear magnetic resonance (NMR) analysis

Extracts (20 mg) were dissolved in a mixture of $CDCl_3$ (400 μL) and DMSO-d_6 (200 μL), filtered and transferred to 5-mm NMR tubes. ^{1}H-NMR spectra were measured at 20 °C using a Varian 500 spectrometer