Selective extraction of antimicrobial agents from *Jodina rhombifolia* by supercritical fluid carbon dioxide: phytochemical profile

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**ABSTRACT**

The goals of this study were to determine the phytochemical profile of *Jodina rhombifolia* and to evaluate the ability of supercritical fluids (ScFCO₂) to selectively extract the metabolites responsible for the bioactivity. This species has simple aromatic compounds and lignan monomers, as well as glycerides containing epoxidized saturated fatty acids. Regarding the extraction by ScFCO₂, the extracts showed a higher antimicrobial activity against human pathogenic strains, with respect to the ethanolic extracts obtained from plant residues after extraction by ScFCO₂. Furthermore, the bioactive compounds were concentrated in just 1% P/P of the weight of the dry plant material. Extraction by ScFCO₂ was carried out under different conditions of pressure and temperature, with the best results being obtained at 30°C and 30 MPa. The results obtained demonstrate the advantages of ScFCO₂ extractions over classical solvent extractions, in terms of improved safety and the ability to selectively extract the compounds of interest.

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1. Introduction

The monotypic genus *Jodina* is endemic to central and south-eastern South America, where it grows from southern Bolivia and Brazil, Uruguay and Paraguay to northern and central Argentina. The only species included in the genus is *Jodina rhombifolia* (Hook. & Arn.) Reissek (Santalaceae, Cervantesiaceae), which is a small hemiparasitic tree of about 4–8 m high and characteristic of the Chaco, an environment of the Neotropical region and Monte of South America (Amuchástegui et al. 2003).

Regarding relevant phytochemical and ethnopharmacological studies, the information available was mainly obtained from the study of aqueous or hydroalcoholic polar fractions. The presence of C-glycosylflavonoids vicenin-2, vitexin, orientin, and swertisin from hydroethanolic extract (Montanha et al. 2009), as well as of acetylenic, hydroxylated, and epoxy fatty acids from the seed oil (Spitzar et al. 1994), has been reported. This species has been widely used in folk medicine for a variety of health problems.

Several ethnopharmacological studies of aqueous and hydroalcoholic extracts of *J. rhombifolia* have been carried out, including for the treatment of digestive problems (Spitzer et al. 1994), and for the treatment of diseases of the renal system (Teves et al. 2018). In addition, some preliminary studies have indicated that extracts of *J. rhombifolia* possess antibacterial activity against Gram-positive and Gram-negative strains (Soberón et al. 2007).

Supercritical extraction is often used for the separation of valuable products, through their fractionation or refining using a solvent under near critical or supercritical conditions. The extraction performance depends on the pressure and temperature conditions, which determine the solvent power of the fluid and the vapor pressure of the solutes. Moreover, as mass transfer effects have a great influence on the kinetic aspects of the process, parameters such as solvent flow, particle size and matrix pretreatment are important for process control (Ahmad et al. 2019). The use of non-toxic and environmentally safe solvents has stimulated an increased interest in this process (Manjare and Dhingra 2019). Near and supercritical fluid extraction has a great potential for different applications (Rostamian and Lotfollahi 2020; Rostamian et al. 2021). In the case of natural products in particular, the application of supercritical fluids has
permitted the selective extraction of numerous secondary bioactive metabolites (Khaw et al. 2017; Gallego et al. 2019).

Continuing the search for bioactive compounds, the objectives of this investigation were: to analyze the phytochemical profile of the non-polar extract of *J. rhombifolia*, in which there are no previous studies; to evaluate the antibacterial activity of this species against important human pathogenic strains; and to explore the use of CO₂ in a quasi-critical condition as a potential technique for the extraction of bioactive metabolites from this plant resource. In this sense, an experimental design was carried out to explore different temperature and pressure conditions, with the aim of to determine optimum extraction conditions.

2. Results and discussion

2.1. Extraction with organic solvents

The ethanolic extract of the aerial parts of *J. rhombifolia* was partitioned with hexane to remove fat and pigments, and was then subsequently partitioned with dichloromethane. The dichloromethane extract was fractionated by several chromatographic techniques, finally obtaining simple aromatic compounds such as piceol, a phenolic compound found in the needles and mycorrhizal roots of Norweigan spruces (*Picea abies*) (Løkke 1990; Münzenberger et al. 1990), as well as 4-hydroxybenzaldehyde, which can be found in the orchids *Gastrodia elata* and *Galeola faberi* (Ha et al. 2000), and also vanillin (Figure 1). During the purification process, several complex mixtures were obtained, which could not be solved using either normal phase chromatography or reversed phase chromatography. However, the ^1^H NMR spectra of these mixtures showed typical signals of aromatic protons between δ 6.2 and δ 7.0, and also of methoxy groups at δ 3.9 and at δ 3.6, corresponding to methoxy groups linked to the sp² and sp³ carbons, respectively (See Figure S3, Supplementary Material).

The occurrence of pyrrolizidine and quinolizidine alkaloids has been reported in the *Osyris* genus (Shyaula 2012), which is very close to the *Jodina* genus in the Santalaceae systematic (Der and Nickrent 2008). For this reason, 46 g of the initial ethanolic extract were processed in the search for alkaloids, using a specific methodology for their extraction, both with and without the use of Zn/ClH. This extract was fractionated by TLC and the fractions were analyzed by ^1^H NMR, but no alkaloids...
could be observed. However, from both extracts, the typical syringyl lignan lyoniresinol (previously isolated from *Lyonia ovalifolia*) (Rahman et al. 2007) and an appreciable amount of glycerides containing epoxy saturated acids were obtained (Figure 1), (See structural elucidation in Supplementary Material).

### 2.2. Bioactivity of the extract obtained with ethanol

Given the background of the antimicrobial activity of *J. rhombifolia*, the ethanolic extract was tested against several Gram-positive *Staphylococcus aureus* strains (*S. aureus* 271, *S. aureus* 239, *S. aureus* 172, and *S. aureus* 298), and was found to be active against all these strains. This test was performed by bioautography, with punctual seeding of 500 μg of the sample on chromatographic paper.

### 2.3. Extraction using ScFCO₂

Because ethanolic extracts of this plant exhibit antibacterial properties, we tested whether ScFCO₂ could specifically extract the metabolites that contribute to the plant’s antibacterial properties. The aerial parts of this species were extracted with ScFCO₂ at different pressures and temperatures, and then each vegetable residue was extracted with ethanol to evaluate if the bioactive compounds were appreciably extracted using supercritical fluids. Table S1 and Figure S9 of the Supplementary Material show the operating conditions and yield (mg extract/g substrate). Typical overall extraction curves were obtained under all experimental conditions (Sousa et al. 2002). Although it was possible to distinguish constant extraction rate periods, there was a predominance of falling and diffusion-controlled rate periods. In addition, higher performance operating conditions were evident.

Screening at relatively high pressures (20–40 MPa) increased the solvent power, although it may have decreased the selectivity. The temperature was set between 40 °C and 60 °C, which is in the vicinity of the critical point and low enough to avoid degradation of the thermolabile compounds. The experimental design included a multiple-stage processing at the minimum and maximum working pressure and at the highest temperature in order to verify the possibility of obtaining selective extractions, as recommended in the literature (Reverchon and Marco 2006).

The highest yield using ScFCO₂ was obtained at a medium pressure (30 MPa) and a medium temperature (50 °C). The opposite effect of increasing solubility with decreasing density, due to an increase in temperature, was observed despite the low number of experiments. At low pressure (20 MPa), the yield decreased with increasing temperature, because the density of the solvent decreased (See Figure S9, Supplementary Material). However, at 30 MPa and 40 MPa, the yield increased with increasing temperature, with an increase in solubility due to higher temperature dominating, because under these conditions there is a low coefficient of expansion of the supercritical mixture. Thus, high pressures and high temperatures produced little change in the density of the solvent, and therefore an increase in solubility resulted.
2.4. Bioactivity of the extracts obtained by ScFCO₂

The antibacterial activity of the extracts obtained using ScFCO₂, and also of the ethanolic extracts obtained from the vegetable residues after being extracted by ScFCO₂, was tested against several strains of *Staphylococcus aureus* (*S. aureus* 271, *S. aureus* 239, *S. aureus* 172, and *S. aureus* 298). This test was performed by bioautography on chromatographic paper, with punctual seeding at saturation. All the extracts obtained using ScFCO₂ showed an inhibitory effect on the strains tested whereas most of the ethanolic extracts were inactive (Table S2). The same test was repeated by sowing 500 μg of the sample on chromatographic paper, with an inhibitory effect only being observed with samples extracted by ScFCO₂ III and VIII. The metabolites responsible for the antimicrobial activity were more efficiently extracted at medium pressures and variable temperatures. However, the small number of experiments carried out did not permit a thorough analysis. These results demonstrated that extraction using ScFCO₂ allowed the bioactive compounds to be selectively extracted, with the bioactive compounds being concentrated in the range 10.4% to 21.9% of the total extract (Table S1).

2.5. Phytochemical profile of the extracts obtained using ScFCO₂

The extracts obtained using ScFCO₂ were partitioned by CC, and the fractions were analyzed by ¹H NMR. For all extracts (I–VIII), fractions rich in methoxy-substituted aromatic compounds and a high fat content were obtained (See Figure S10, Supplementary Material). For extract III, obtained by ScFCO₂, it was possible to purify and characterize a small amount of methyl 2,3,4-trimethoxybenzoate (PubMed). The structures of the isolated compounds were established based on their NMR data and by comparison of these compounds with those previously reported.

3. Experimental section

See Supplementary Material.

4. Conclusions

The phytochemical study of the low polarity extract of *J. rhombofolia* species allowed the isolation of mainly complex mixtures of methoxylated phenolic and aliphatic compounds, such as simple aromatic compounds, lignan monomers and epoxidized glycerides. Based on the antimicrobial activity observed against important human pathogenic strains such as *Staphylococcus aureus* for the whole ethanolic extract, the extraction was carried out using ScFCO₂ under different pressure and temperature conditions (40°C–60°C and 20 MPa–40 MPa). This methodology was shown to be efficient for concentrating metabolites with a particular bioactivity profile, even in the case of difficult-to-resolve mixtures, with the best results being obtained at 30°C and 30 MPa. The ¹H NMR spectra of all the extracts obtained by ScFCO₂ showed characteristic signals of methoxylated phenolic groups. For the extraction using ethanol, the bioactive compounds represented approximately 10% of the dry weight of the plant,
while for the extraction using ScFCO₂ the bioactive compounds were concentrated in 1% P/P with respect to the dry weight of the plant. This efficient and low toxicity methodology could be applied to other plant matrices with pharmacological and/or nutraceutical potential.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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