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Fatty acid profile and biological activity of supercritical fluid extract of *Rosa canina L.* seeds

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Abstract

The present study concerns the extraction by means of supercritical carbon dioxide, SFE-CO_2, from the seeds of *Rosa canina* L. Extraction experiments were carried out at a pressure of 300 bar and a temperature of 40 °C, SFE[300:40]. Analyses by GC-FID and HPLC-DAD of the extract showed elevated levels of linoleic acid, 18:2 n-6 (54.7 %) and linolenic acid 18:3 n-3 (19.8 %). The sample SFE[300:40] was compared with that obtained using *n*-hexane in a Soxhlet apparatus, (Sx). The antioxidant properties determined by the ABTS test, showed that SFE[300:40] had EC_{50} = (0.34 ± 0.01) mg/mL and Sx, had no antioxidant activity. The total phenolic content, expressed as the concentration of Gallic Acid Equivalents (GAE), in the SFE[300:40] was (16 ± 2 mg/g) and a concentration below the detection limit of the method for the Sx sample. The inhibition percentages against xanthine oxidase were 5 % for SFE[300:40] and 12 % for Sx extract.

Keywords: *Rosa canina L.*; supercritical fluid extraction; fatty acid composition; total phenol; antioxidant property; xanthine oxidase.

1. Introduction

Rosa canina L., commonly known as rosehip, is a species of plant belonging to the Rosaceae family. This plant is a perennial shrub distributed in different geographical regions of Europe, Asia, the Middle East, and North America (Negrean et al. 2024). Throughout history, it has been used as a source of food and medicine in different cultures (Khelfi et al. 2024; Çürük et al. 2023). *R. canina* fruits are used in many traditional medicines for the prevention of colds, gastrointestinal disorders, urinary tract diseases, respiratory problems, hypertension, diabetes, osteoarthritis, and other infections (Manzione et al. 2024; Yalçinkaya et al. 2024). This fruit possesses several biological properties such as antioxidant, anti-inflammatory, antitumor, and antimicrobial activity, which have been confirmed by *in vitro*, *in vivo*, and human pharmacological studies (Miljkovi´c et al. 2024). Most probably, the antioxidant properties could be mainly due to the high content of ascorbic acid, although the presence of flavonoid glycosides would exert a synergistic effect, and the ascertained presence of some carotenoids (mainly β-carotene, lycopene, cryptoxanthin) cannot be underestimated (Sanjust et al. 2008).

Methanol extracts of dog rose fruits have the potential to be used against citrus green mould and even for the improvement of postharvest quality of orange fruits (Jafari et al. 2014).

The biological potential of this plant is related to its phytochemical composition, which includes high content of ascorbic acid, phenolic compounds, fatty acids, carotenoids, various vitamins, such as B1, B2, K, A, minerals (calcium and potassium), pectin, etc. (Sanz-Lamora et al. 2024; Mármol et al. 2017). The fruits are used in the food, pharmaceutical, cosmetic and perfume industries (Sabahi et al. 2022). In several European countries, they are commonly used in the preparation of herbal teas, jellies, jams, yoghurts etc. (Teodorescu et al. 2023). Especially, seeds serve as an excellent source of vitamin E, unsaturated fatty acids (UFA) and polyunsaturated fatty acids (PUFA) such as α-linolenic and linoleic acids (Saini et al. 2024; Çürük et al. 2023). Linoleic and linolenic acids are essential fatty acids (EFA) and must be obtained from the diet as humans cannot synthesise them (Kulaitiene et al. 2020). Recent literature reports the importance of EFA in regulating several biological functions such as blood pressure and blood coagulation (Firoozabad et al. 2022).

From a nutritional point of view, the consumption of seed oils has been associated with the reduction of the risks of various chronic and degenerative diseases, such as cancer,

diabetes, and cardiovascular disorders, related to the content of UFA and bioactive compounds in seeds oils (Rigano et al. 2025).

Recent studies evaluated the potential of incorporating various seed oils into in bakery products. Sanfilippo et al. (2025) used rosehip seed flour for the production of bakery products with good technological properties.

The incorporation of rosehip seed oils into cracker formulations has proven to be a promising approach for enhancing both the nutritional and sensory properties of the product (Barut et al. 2025).

Yasar et al. 2025 created biodegradable and edible films using a combination of alginate and rosehip extract, that could be used as a suitable alternative for edible food packaging.

This study is related to our previous research being published as an international patent, in which we highlighted the antibacterial properties of a rosehip extract (Piras et al. 2024).

In this work, we examined the fatty acid composition, total phenolic content, antioxidant property, and xanthine oxidase inhibitory activity of rosehip seed extracts obtained by supercritical fluid extraction. SFE-CO_2 is a promising alternative to conventional methods for obtaining bioactive compounds (Wrona et al. 2017). This extraction method is attracting attention due to the chemical and physical properties of the solvent used: non-flammable, non-toxic, non-corrosive etc. (Han et al. 2009). The oils obtained by SFE-CO_2 are considered to be of superior quality as compared to those obtained by organic solvent extraction methods and rarely require refining (Piras et al. 2021; Wrona et al. 2017). Generally, they are recognized as safe for use in food products (Ndayishimiye et al. 2017). Xanthine oxidase (XO) is an enzyme that catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid (UA) with the production of superoxide anion (Di Petrillo et al. 2024; Fais et al. 2023). High levels of UA in the body leads to hyperuricemia, which is also closely associated with gout (Era et al. 2020). This important enzyme is required for normal purine metabolism in humans and has been implicated in several human diseases due to its ability to generate UA and reactive oxygen species (ROS) (Kushiyama et al. 2016).

2. Results and discussion

2.1. Preparation of extracts

Supercritical CO2 extraction was conducted at 300 bar, with the temperature maintained at 40 °C throughout the extraction protocol, SFE[300:40]. CO2 was employed as the supercritical solvent, with no co-solvents introduced. The SFE-derived extract was benchmarked against oil obtained *via* conventional Soxhlet extraction using *n*-hexane (Sx oil extract).

The obtained extracts were subjected to chemical and biological analyses, all performed in triplicate to ensure reproducibility and statistical reliability. Results are reported in the corresponding tables as mean values accompanied by their respective standard deviations.

2.2. Chemical profile of fatty acids

FA analysis of the oils by *R. canina* seeds was carried out by HPLC-DAD and GC-FID. The oil obtained by supercritical carbon dioxide extraction, SFE[300:40], showed a concentration of about 7.07% saturated FA (SFA), mainly palmitic acid 16:0 (3.73%) and stearic acid 18:0 (2.39%), 17.61% monounsaturated FA (MUFA) (mainly oleic acid 18:1 n-9, 17.00%), and 74.5% of PUFA, mainly consisting of linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3), which represented 54.66% and 19.83% of the FA content, respectively (**Table 1**). Lauric (12:0), myristic (14:0), palmitoleic (16:1 n-7), arachidic (20:0) and gondoic (20:1) acids were present in seed oil at lower levels compared to the other fatty acids. The most abundant FA in seed fixed oil was 18:2 n-6 (400 mg/g of oil), followed by 18:3 n-3 (177 mg/g of oil), and 18:1 n-9 (113 mg/g of oil) (**Table 2**).

Chemical composition of SFE[300:40] was compared with Sx. This sample showed a chemical profile similar to that obtained with SFE-CO2 (**Tables 1 and 2**). Both *R. canina* seed oils were characterised by a high proportion of PUFA (577 and 696 mg/g of oil in SFE[300:40] and Sx, respectively, **Figure 1**) and UFA; the UFA:SFA ratio value was 13.02 in SFE[300:40] versus 12.15 in Sx.

Our results are in agreement with other published data (Illes et al. 1997; Barros et al. 2010; Javanmard et al. 2018; Saini et al. 2024; Sharopov et al. 2025).

Illes et al. (1997) performed an extraction from the *R. canina* seeds with supercritical CO2 at 35 °C and 250 bar and obtained similar FA profiles for linoleic (52.4%), linolenic (23.4%), and oleic (16.2%) acids. Linoleic (39.5%), linolenic (26.3%), oleic

(14.4%), and palmitic (6.7%) acids were previously identified as the major components in the oil obtained by petroleum ether extraction, using a Soxhlet apparatus, of *R. canina* fruits collected in Portugal (Barros et al. 2010). As reported in Saini et al. 2024 total FA, UFA, MUFA and PUFA were found in similar proportions in extracts of rosehip seeds.

According to Javanmard et al. (2018), linoleic acid (43.1-55.0%), linolenic acid (18.3-28.4%), and oleic acid (17.2-22.6%) were present in the seed extract obtained with the Soxhlet apparatus. Palmitic acid, the most important saturated fatty acid, was present in concentrations of 3.7-5.0%.

2.3. Total phenol content

The extracts obtained from *R. canina* seeds were evaluated for polyphenolic content and antioxidant activity. Phenolic content – expressed as concentration of Gallic Acid Equivalents (GAE) – in the SFE[300:40] sample was 16.0 ± 2 mg GAE/g, instead a concentration below the detection limit was determined in the Sx sample (**Figure 1**).

2.4. Antioxidant properties and xanthine oxidase inhibition

The EC50 values of *R. canina* seed extracts were calculated (ABTS assay) and presented in Figure 1. The results indicated that SFE[300:40] possessed antioxidant activity, EC50 = (0.34 ± 0.01) mg/mL, and the Sx extract had no antioxidant activity. An EC50 = (0.0013 ± 0.0004) mg/mL was obtained for Trolox, the positive control.. The percentages of inhibition against XO(Ixo %) were 5 ± 7% for SFE[300:40] and 12 ± 4% for the Sx extract (**Figure 1**).

3. Experimental. See supplementary material

4. Conclusions

In this work we have presented our results concerning the extraction of seeds of *R. canina* L. by means of CO2 in the supercritical state at high pressure. The extraction experiments were carried out at a pressure of 300 bar and a temperature of 40 °C. It is interesting to note that SFE[300:40] seed oil was characterised by a high concentration of PUFA and UFA. In addition, SFE[300:40] showed a high content of EFA (18:2 n-6 and 18:3 n-3). The chemical profile of SFE[300:40] was similar to that of Sx extract, however, SFE[300:40] extract exhibited a higher phenolic content and antioxidant activity determined by ABTS assay, probably ascribable to the high temperature-induced impact on antioxidant components/lipid peroxidation process in Sx extract.

Considering the two extraction techniques used, if compared to Soxhlet extraction with *n*-hexane, SFE offers notable benefits, including the use of a non-toxic, food-grade solvent (CO2) that leaves no harmful residues, an essential factor for pharmaceutical and food applications. Additionally, SFE is characterised by a significantly lower environmental impact due to the absence of hazardous organic solvents and the recyclability of CO2. The mild operational conditions also contribute to the preservation of thermolabile bioactive compounds, thereby improving the nutritional and functional quality of the extracts. These advantages underscore the broader applicability and sustainability of SFE in high-value product development.

In conclusion, the results of this study demonstrated the nutritional properties of the oils obtained by SFE-CO2 from *R. canina* seed due to their high content of essential fatty acids important for health. These aspects qualify these extracts as a potential, environmentally friendly, natural source for pharmaceutical, food and nutraceutical applications.

Conflicts of Interest:

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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