SHORT COMMUNICATION

Antimicrobial and cytotoxic activity to human colon adenocarcinoma cell lines (HT-29) potential of olive oil extraction residue

Vanessa Ferreira do Amaral, Angela Cristina Mello dos Santos, Josué Guilherme Lisboa Moura, Juliana de Castilhos, Tanise Gemelli, Jessica Fernanda Hoffmann, Valmor Ziegler and Cristiano Dietrich Ferreira

Technological Institute in Food for Health, University of Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil

ABSTRACT
In the Olive drupe (Olea europaea L.) oil extraction process, 80% of the volume generated is waste (bagasse). Advancing the expansion of the olive oil market, it is necessary to develop alternatives that, in addition to adding value to industrial waste, also reduce possible environmental damage. Our study aimed to understand the antimicrobial and Cytotoxic activity potential of the residues from the extraction of olive oil from the blend of the varieties Arbequina and Arbosana. The extract shows cytotoxic activity, inhibiting about 75% of cancer cells in the human colon at a concentration of 0.15 mg of Gallic Acid equivalent (GAE)/mL. The effectiveness of the extract against microorganisms often associated with foodborne diseases and food decomposition has also been discovered, without compromising the microorganisms responsible for fermentation. Thus, this study provides future perspectives for the use of active ingredients extracted from the residue from the extraction of olive oil.

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1. Introduction

The International Olive Council estimates that world oil production for 2021 will reach 3,197,000 tonnes, which means a 2.5% growth compared to 2019/2020 (IOC 2021). In proportion to the increase in production, the residue increases, which roughly represents 80% of the initial mass. The bagasse from the extraction of olive oil is composed of the pulp, crushed fruit core, leaves and a residual oil content. These residues have a
high organic content and phytotoxicity due to the high concentration of phenolic compounds, which are characterized by resistance to biological deterioration and by the antimicrobial effect, affecting the anaerobic digestion processes, which makes their degradation harmful to the environment (Böhmer-Maas et al. 2020; Mohammadnejad et al. 2021).

Bagasse retains 98 to 99.5% of the total phenolic compounds in whole olives, while olive oil retains an average of 2%, demonstrating a greater antioxidant potential in the residue compared to that found in olive oil (Cecchi et al. 2018; Böhmer-Maas et al. 2020; Russo et al. 2020). Gorzynik-Debicka et al. (2018) points out that the antioxidant properties present in olives can prevent the proliferation of cancer cells in the body. The occurrence of cancer in developing countries is currently around 56% to the world total, with an increase forecast to 60% by 2030. Cancer is the second leading cause of death in the world and in Brazil colon cancer is the second most common cancer among men and women, with a worldwide incidence rate of about 9.1% (Jemal et al. 2010). Food-borne diseases affect 600 million individuals worldwide each year, and of these, 420,000 die. Natural antimicrobials are compounds capable of inhibiting the growth of pathogenic and/or deteriorating microorganisms, such as bacteria and fungi (Aliakbarian et al. 2012; Pannucci et al. 2021). Research with extracts obtained from olive pomace has shown beneficial effects for its use in different areas. According to Liu et al. (2011) supplementation of olive pomace extract at a dosage of (200 mg kg\(^{-1}\) body wt day\(^{-1}\) for 15 days helped in the prevention and treatment of hyperlipidaemia in rats. However, Alesci et al. (2014) reported that the beneficial activity of polyphenols reduced liver fat into goldfish submitted to a hypercholesterolemic diet. Averna et al. (2019) suggested that bioactive compounds extracted from olive bagasse have the ability to protect cells against intracellular Ca\(^{2+}\) overload.

The present study aims to evaluate the cytotoxic activity and antimicrobial potential of the free phenolic extract obtained from the residue from the extraction of olive oil from the blend of the varieties Arbequina and Arbosana.

2. Results and discussion

In the extract, 24 compounds were identified, namely Isocitric acid, Hydroxytyrosol glucoside isomer 1, Hydroxytyrosol glucoside isomer 2, Hydroxytyrosol, Vanillic acid 4-beta-D-glucoside, Oleoside, 7-\(\beta\)-1-D-Glucopyranosyl- 11-methyl oleoside (isomer1), Oleuropein aglycone related compound, Elcenolic acid glucoside, 7-\(\beta\)-1-D-Glucopyranosyl- 11-methyl oleoside (isomer 2), Loganic acid glucoside, Calceolarioside, Demethyl oleuropein isomer 1, Demethyl oleuropein isomer 2, Rutin, \(\beta\)-methoxyverbasoside, Verbasoside, Luteolin rutinoside, Dihydro oleuropein, Isoverbasoside, Luteolin-7-glucoside, Oleuropein-diglucoside, p-Coumaroyl-6-secologanoside, and Luteolin (Table S1).

An increase in the potential for inhibition of phenolic extracts was observed up to a concentration of 0.15 mg GAE/mL, which inhibited around 75% of the growth. From this concentration, there was a decrease in the potential for inhibition of cell proliferation. It was possible to observe that extracts of free phenolics have cytotoxic activity in vitro against the HT-29 cell line of colon cancer (Figure S1).
It is observed that for Gram-Positive bacteria *Staphylococcus aureus* and *Bacillus cereus* the MIC was 6.25% (0.38 mg GAE/mL) and the MBC was 12.5% (0.76 mg GAE/mL), while for the bacteria *Enterococcus faecalis* the MIC was 25% (1.53 mg GAE/mL) and the MBC was 50% (3.07 mg GAE/mL). For Gram-Negative *Escherichia coli* bacteria, MIC was 12.5% (0.76 mg GAE/mL) and MBC was 25% (1.53 mg GAE/mL) and for *Pseudomonas aeruginosa* and *Salmonella Typhimurium* a MIC was 25% (1.53 mg GAE/mL) and MBC was 50% (3.07 mg GAE/mL). For *Candida albicans* yeasts the MIC was 50% (3.07 mg GAE/mL) and for MFC, there was no fungicidal action in the 50% dilution, for Saccharomyces cerevisiae, there was no growth inhibition and fungicide of this yeast in the dilution of 50% (Table S2).

In a study by Böhmer-Maas et al. (2020) that optimized the extraction of phenolic compounds from olives and identified the main compounds such as Hydroxybenzoic Acid, Coumaric Acid, Vanillic Acid, Gallic Acid, Caffeic Acid, Ferulic Acid, Syringic Acid, Chlorogenic Acid, Rutin, Catechin, Oleuropein, Hydroxytyrosol, and Tyrosol. Aludatt et al. (2011) carried out a study with the optimization of the extraction of phenolic compounds in fresh and defatted olives by varying the extraction temperature. The authors reported a higher extraction yield when performed with methanol at 60°C, when compared to the extraction with methanol at 25°C, the main molecules found being phenolic acids (Gallic, Protocatechuic, Hydroxybenzoic, Vanillic, Caffeic, Syringic, Sinapic, Ferulic, Cinnamic and p-Coumaric) and the flavonoids (Rutin, Hesperidin, and Quercetin). Gorzynik-Debicka et al. (2018) carried out a review and reported several benefits of phenolic compounds present in olives, such as antihypertensive, antioxidant, and antiseptics effect. These authors also reported anti-cancer properties of the hydroxytyrosol and tyrosol molecules, and their secairidoid derivatives (oleuropein aglycon, ligstroside aglycon, oleocanthal, and oleuropein). Studies suggest that the cytotoxic activity of phenolic compounds in colon cells is associated with the action of these substances as selective estrogen receptor modulators (ERα). Pampaloni et al. (2014) analyzed the cytotoxicity of 0.01 mg/mL total phenolic extracts of extra virgin olive oil against the HCT 8-8 colon cancer cell line. A reduction in cell proliferation was observed, which was explained by an increase in the expression of genes associated with tumor suppression, such as BRCA and SOX4, and a reduction in the expression of genes related to tumor proliferation, such as WNT10A and WNT6. The transcription of these genes was regulated by the action of ERα associated with phenolic compounds. An increase in the expression of genes responsible for apoptosis has also been observed. Oleuropein also had a protective effect against healthy cells. In a survey by Ruzzolini et al. (2018), an intensification of the cytotoxic effect of chemotherapeutic agents combined with 0.12 mg/mL of oleuropein was demonstrated against A375 melanoma cells.

Regarding the antimicrobial activity, the phenolic compounds present in the residues of the olive oil production, in dilutions of up to 50%, showed efficacy against all microorganisms, except for *Saccharomyces cerevisiae*. This yeast is widely used in fermentation processes, so its resistance is of great importance since it allows the association of compounds with products that go through these processes. Caporaso et al. (2018) reported that the antimicrobial activity of phenolic compounds present in olive pomace. The inhibition of *S. aureus* and *E. coli* by isolated oleuropein, a component
present in the residues of olive oil production was reported by Belaqziz et al. (2017). Amini et al. (2017) described the isolated and combined antimicrobial action of phenolics present in olives against *E. coli* and mutations of the bacterium, they obtained results between 50% and 99% of ATP synthase inhibition of the microorganism, varying according to the species mutation and combination of phenolic compounds. These compounds have hydroxyl groups (OH), which provide inhibitory activity to bacteria (Bouarab-Chibane et al. 2019), they generate the unblocking of the membrane of these microorganisms and cause the overflow of their components (Xue et al. 2013). Phenolic compounds also cause the inhibition of ATP synthesis leading to cell death (Sharifi-Rad et al. 2018).

3. Experimental

More details of the experimental section is available online in supplementary material.

4. Conclusions

The phenolic compounds of the free fraction extracted showing potential for use in the enrichment of food and the pharmaceutical industry in the development of chemotherapy assisting blocking the development of human colon adenocarcinoma cells, inhibiting about 75% of cancer cells in the human colon at a concentration of 0.15 mg of GAE/mL. For Gram-Positive bacteria, MIC varies between 0.38 and 1.53 mg GAE/mL; and MBC varies between 0.76 and 3.07 mg GAE/mL. For Gram-Negative, MIC varies between 0.76 and 1.53 mg GAE/mL; and MBC varies between 1.53 and 3.07 mg GAE/mL. Antimicrobial activity against pathogens and not against commercial yeasts shows the value of the results found. The advances in these researches may be related to the application of these compounds and extracts in food products, in an attempt to glimpse new preservatives and/or natural antioxidants that may replace some synthetic additives.

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