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Cytoprotective, antioxidant, and anti-migratory activity of *Pistacia lentiscus L.* supercritical carbon dioxide extract on primary human endothelial cells

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ABSTRACT

Green chemistry is a useful tool for producing valuable chemicals from biomass. However, extracted compounds need to be tested for safety and efficacy before their use in humans. Here we investigate the chemical composition and biological effects of a leaves *Pistacia lentiscus L.* supercritical carbon dioxide ($SCCO_2$) extract. Terpenes represented the main extract fraction, with Germacrene D (11.18%), delta-cadinene (10.54%), and alpha-pinene (8.7%) the most abundant molecules. Challenged with endothelial cells (ECs), increasing extract concentrations failed to affect cell proliferation or promote cell toxicity. ROS assessment in unstressed and H_2O_2-treated ECs revealed an extract dose-dependent antioxidant activity. Exposition of H_2O_2-treated ECs to increasing extract concentrations dose-dependently counteracted H_2O_2-induced cell impairments. The extract significantly counteracted fetal calf serum-induced ECs migration. For the first time, we report that a $SCCO_2$ extract obtained from PL leaves is safe on ECs and may be a useful source of valuable compounds with vasculoprotective properties.

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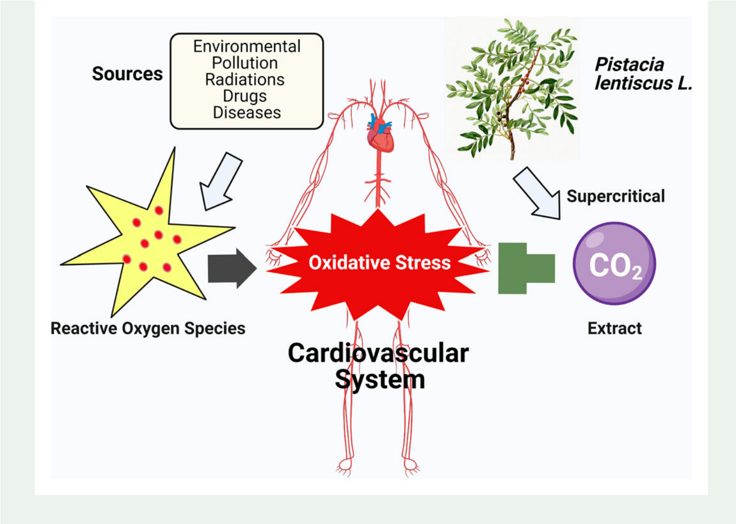

1. Introduction

Biomass exploitation is emerging as an important component in producing chemicals from renewable sources (Posadino et al. 2012, 2018; Cho et al. 2020; Posadino et al. 2021). In this regard, wild aromatic plants are invaluable sources of new potential drugs. Indeed, plant essential oils (EO) and their constituents have been widely used in the pharmaceutical, cosmetic, food, and beverage industries (Fabian et al. 2006). Different beneficial properties, such as antioxidant, anti-inflammatory, antiviral, antibacterial, antidiabetic and anticancer have been reported for EO, which are increasingly employed in complementary therapies (Ali et al. 2008). Among aromatic plants, *Pistacia lentiscus L* (PL) has been extensively used in folk medicine for several therapeutic uses, including anti-hypertensive, anti-inflammatory and antiseptic (Bozorgi et al. 2013). PL is an aromatic bush indigenous to Mediterranean and Middle East countries (Milia et al. 2021). PL extracts and EO have shown several properties, including antioxidant, antimicrobial, anticancer and cytoprotective (Loutrari et al. 2006; Benhammou et al. 2008; Xanthis et al. 2021). Among the different extraction processes, supercritical CO_2 ($SCCO_2$) is emerging as a superior extraction technology since it can be performed at low temperatures, thus protecting matrix components from thermal degradation (Abbas et al. 2008). $SCCO_2$ emerged as the most desirable technique for separating natural products used in foods and medicines because of its inertness, non-toxicity, critical temperature and low pressure (Starmans and Nijhuis 1996; Santos et al. 2013). Reactive oxygen species (ROS) are recognized as by-products of the aerobic metabolism and essential second messengers regulating vital cellular functions. ROS's physiological levels are tuned by the orchestrated action of ROS-generating enzymes and cellular antioxidant mechanisms (Costa et al. 2021). However, dysregulation of the above-mentioned homeostasis can generate oxidative stress, a phenomenon linked to several pathological conditions (Vono et al. 2016; Thuan et al. 2018; Giordo, Ahmed, et al. 2021; Giordo, Thuan, et al. 2021). This linkage suggests that counteracting oxidative stress with antioxidants might prevent disease occurrence or ameliorate its associated complications, and for this reason, natural antioxidants are now seen as potential therapeutic candidates (Ceriello et al. 2016). However, although plant-derived compounds are recognized as valuable adjuvants or therapeutic tools (Phu et al. 2020; Shaito et al. 2020; Giordo, Nasrallah, et al. 2021; Giordo, Zinellu, et al. 2021), their efficacy and safety in humans are still significant concerns (Posadino et al. 2013; Goszcz et al. 2015; Shaito et al. 2020). Moreover, the extracted compounds' quality may be affected by the technological process employed; therefore, it is essential to test their safety, efficacy, and quality before therapeutic utilization. To our knowledge, no previous reports have investigated the effects of PL leaves $SCCO_2$ extract on human ECs. In this light, the present work aims to examine the safety and potential cytoprotective, antioxidant and anti-migration properties of a $SCCO_2$ extract obtained from PL on human ECs.

2. Results and discussion

2.1. Essential oil composition

Percentages of individual components of PL extract were calculated based on gas chromatography (GC) peak areas without flame ionization detection (FID) response factor correction. Chromatogram and main chemical components of PL-EO are reported, respectively, in Figure S1 and Table S1. The analytical results were consistent with those reported in the literature concerning terpene compounds, representing the more important fraction with antioxidant activity (Said et al. 2011). The total EO yield, after 4 h of extraction was 0.14% and the main abundant constituents included germacrene D (11.18%), delta-cadinene (10.54%), alpha-pinene (8.7%), beta-caryophyllene (5.74%), myrcene (4.56%), beta-phellandrene (4.33%), terpin-4-ol (4.306%), epi-alpha-muurolol (3.262%) and beta-pinene (3.02%). The complete profiling of the EO components is reported in Table S1.

2.2. PL extract does not affect ECs viability and proliferation

Using primary human ECs (Zinellu et al. 2009), we investigated the extract's ability to modulate different biological cell functions. Compounds safety is of paramount importance for their therapeutic employment; therefore, we first examined the potential toxicity of the obtained extract by assessing its effects on cell viability, cell proliferation, and intracellular ROS production. Indeed, intracellular ROS generation is closely related to EC survival, proliferation and apoptosis (Shaito et al. 2022). Based on previously reported data (Catalani et al. 2017), we evaluated the possible harmful effects of three extract concentrations [50, 150 and 600 μg/mL] on ECs viability and proliferation. To this end, cells were treated for 24 h with the PL extract, then cell viability and proliferation were assessed as previously reported (Posadino et al. 2019). As depicted in Figure S2A, the PL extract had no toxic effect at any of the tested concentrations indicating the safety of both the extract and the extraction process applied. Likewise, the data in Figure S2B indicate that the tested extract concentrations failed to induce detrimental effects on ECs proliferation, further confirming the extract's safety.

2.3. PL extract showed an antioxidant effect against H_2O_2-induced oxidative stress

Whether PL $SCCO_2$ extract can exert antioxidant effects in biological models remains to be elucidated. Therefore, we sought to investigate whether the obtained extract could counteract oxidative stress in H_2O_2-treated ECs. For this purpose, H_2DCFDA-loaded cells were pre-treated for 3 h with the indicated extract concentrations and then incubated for 6 h in the presence or absence of H_2O_2 (75 μM). The data derived from five pooled measurements were expressed as percentages of untreated control cells (Boin et al. 2014). As depicted in Figure S3A, exposure of H_2O_2-treated cells to increasing concentrations of PL extract showed a significant dose-dependent antioxidant effect compared to cells treated with only H_2O_2. We next wondered whether the PL extract per se could exert any antioxidant or prooxidant effect in the absence of oxidative insults. In this regard, exposure of unstressed cells to increasing extract concentrations induced a significant antioxidant effect at 150 and 600 μg/mL while failing to affect the intracellular redox state at 50 μg/mL. (Figure S3B). These findings agree with the cell viability and proliferation results showing no extract toxicity up to 24 h of cell treatment (Figure S2A, B).

2.4. PL extract showed a protective effect against H_2O_2-induced oxidative stress

Oxidative-induced endothelial damage triggers and sustains cardiovascular diseases (CVD) (Shaito et al. 2022). Therefore, much research is now focused on finding natural antioxidants capable of preventing or countering CVD-associated ROS increases. To determine whether the observed extract antioxidant effect could be protective against the H_2O_2-induced oxidative damage, we measured the ECs viability. To this end, cells were exposed for 3 h to increasing concentrations of PL extract and then incubated for 24 h in the absence or presence of H_2O_2 (75 μM). As indicated in Figure S4, cell exposition to increasing doses of PL extract provided a significant dose-dependent cytoprotective effect with respect to the H_2O_2-induced cell damage. Indeed, all the tested PL concentrations were able to significantly counteract H_2O_2-induced cell damage. Consonant with these findings is the data in Figure S3B, reporting the extract's ability to dose-dependently prevent the detrimental effect on HUVEC proliferation elicited by H_2O_2.

2.5. PL extract showed an antimigratory effect against serum-induced migration

EC migration is an essential step of the angiogenic process; indeed, ECs, which generally are maintained in a quiescent state, are stimulated to degrade the basement membrane and migrate into the perivascular stroma in response to either proangiogenic factors or by the downregulation of antiangiogenic factors (Lamalice et al. 2007). For this reason, we investigated the ability of PL extract to modulate ECs migration using the matrigel transfilter cell invasion assays (Pintus et al. 2018). Figure S5 demonstrated that the PL extract dose-dependently counteracted the serum-induced cell migration, eliciting a significant reduction at the doses of 150 and 600 μg/mL.

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Ethics statement

This research does not involve Human Participants and/or Animals.

Authors' contributions

RG, GP, AMP: Conceptualization. RG, AC, MCP, RC, GB, JSR, AMP: Methodology. RG, AC, MCP, RC, GB, AMP: Investigation. GKN, LP, GP: writing—original draft preparation. RG, AC, MCP, RC, GB, JSR, GKN, LP, GP, AMP: writing—review and editing. GP: supervision. GKN, LP, GP: funding acquisition.

Disclosure statement

No potential conflict of interest was reported by the authors..

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Data availability statement

The data presented in this study are available in this article.

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