Ethanolic Extract of Origanum syriacum L. Leaves Exhibits Potent Anti-Breast Cancer Potential and Robust Antioxidant Properties

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Background: Breast cancer (BC) is the second most common cancer overall. In women, BC is the most prevalent cancer and the leading cause of cancer-related mortality. Triple negative BC (TNBC) is the most aggressive BC being resistant to hormonal and targeted therapies.

Hypothesis/Purpose: The medicinal plant Origanum syriacum is a shrubby plant rich in bioactive compounds and widely used in traditional medicine to treat various diseases. However, its therapeutic potential against BC remains poorly investigated. In the present study, we identified the bioactive compounds of the ethanolic extract of O. syriacum (OSEE) and investigated its anticancer effects and possible underlying mechanisms of action against the aggressive and highly metastatic human TNBC cell line MDA-MB-231.

Methods: Column chromatography and LC-MS were used to identify the phytochemical constituents of OSEE, MTT, trans-well migration, and scratch assays were used to assess cell viability, invasion, or migration, respectively. Antioxidant potential was evaluated in vitro using the DPPH radical scavenging assay and ROS levels were assessed in cells in culture using DHE staining. Aggregation assays were used to determine cell-cell adhesion. Flow cytometry was used to analyze cell cycle progression. Protein levels of markers of apoptosis (BCL-2, pro-Capase3, p53), proliferation (p21, Ki67), cell migration, invasion, or adhesion (FAK, E-cadherin), angiogenesis (iNOS), and cell signaling (STAT3, p38) were determined by immunoblotting. Chorio-allantoic Membrane (CAM) assay evaluated in ovo angiogenesis.

Results: We demonstrated that OSEE was rich in phytochemical constituents mainly carvacrol. We also found that the OSEE crude extract had potent radical scavenging activity in vitro and induced the generation of reactive oxygen species (ROS) in MDA-MB-231 cells, especially at higher OSEE concentrations. Non-cytotoxic concentrations of OSEE attenuated cell proliferation and induced G0/G1 cell cycle arrest, which was associated with phosphorylation of p38 MAPK, an increase in the levels of tumor suppressor protein p21, and a decrease of proliferation marker protein Ki67. Additionally, only higher concentrations of OSEE were able to attenuate inhibition of proliferation induced by the ROS scavenger N-acetyl cysteine (NAC), proposing that the anti-proliferative effects of OSEE can be ROS-dependent. OSEE stimulated apoptosis and its effector Caspase-3 in MDA-MB-231 cells, in correlation with activation of STAT3/p53 pathway. Furthermore, the extract reduced the migration and invasive properties of MDA-MB-231 cells through the deactivation of focal adhesion kinase (FAK). OSEE also reduced the production of inducible nitric oxide synthase (iNOS) and inhibited in ovo angiogenesis. Further studies have been carried out to assess the composition of various fractions from the OSEE crude extract, in an effort to identify the bioactive molecules. Interestingly, two fractions have shown a more potent activity against the proliferation of MDA-MB-231 cells when compared to the crude extract.

Conclusion: Our findings reveal that OSEE is a rich source of phytochemicals and has robust anti-breast cancer properties that significantly attenuate the malignant phenotype of MDA-MB-231 cells, suggesting that Origanum syriacum may act as a rich source of potential TNBC therapeutics and poising Origanum syriacum to offer novel avenues for the design novel TNBC drugs.