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Comparison of Soxhlet and Cold Extraction Methods for Tasköprü (Kastamonu-Türkiye) Garlic Extracts against Colon Cancer Cells and Bone-Marrow Mesenchymal Stem Cells

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Abstract

Garlic organosulfur compounds exhibit anticarcinogenic potential, but the extraction method strongly shapes the composition and bioactivity of the extracts. This study compared Soxhlet (hot) extraction with cold (ethanol maceration) extraction of white, geographically registered Taskopru garlic, evaluating anticancer activity against HCT116 human colon cancer cells and effects on healthy rat bone marrow mesenchymal stem cells (MSCs). For the Soxhlet method, sliced cloves were boiled for 1 h in 50% ethanol, and the extract was concentrated by evaporation. For the cold method, crushed cloves were macerated in absolute ethanol at room temperature for 3 days, then the solvent was removed by evaporation. The extracts' composition was characterized by ATR-FTIR (4000-400 cm-1) and LC-MS/MS. Cytotoxicity was assessed by the MTT assay in HCT116 (5000 cells/well) and MSCs (1000 cells/well) at 1-200 ppt for 1, 3, and 5 days, with cisplatin as a positive control; data were analyzed using Student's t-test in SPSS. LC-MS/MS confirmed organosulfur markers: the cold extract retained alliin and gamma-glutamyl-S-allyl-L-cysteine, while seventeen garlic-derived compounds were identified in the Soxhlet extract. FTIR of the Soxhlet extract revealed hydroxyl, aromatic C-H, ester, carbonyl, carboxylic O-H, and characteristic organosulfur S=O and primary-amine bands consistent with alliin, allicin, and diallyl disulfide. Both extracts reduced HCT116 viability in a dose- and time-dependent manner. The Soxhlet extract was more potent than the cold extract (IC50 about 60 versus 120 ppt), with the cold extract showing a transient day-3 attenuation followed by stronger day-5 cytotoxicity. In MSCs, both extracts were non-cytotoxic at low doses, but the Soxhlet extract was cytotoxic at high doses, whereas the cold extract generally promoted MSC viability. Extraction method markedly influenced potency and selectivity, indicating that high-dose hot extracts warrant caution.

Keywords: *garlic extract, Soxhlet extraction, cold extraction, colon cancer, mesenchymal stem cells*