

Acetone Extract of *Tricholoma scalpturatum* (Fr.) Quél Induced Apoptosis in Colon (Caco-2) Cancer Cell Lines**Authors:** Cansel Çakır¹, Kübra Tuna¹, Dilaycan Çam¹, Amine Hafis Abdelsalam², Fatma Aydoğmuş-Öztürk², Şevki Arslan³ and Mehmet Öztürk¹**Affiliation:** ¹Muğla Sıtkı Koçman University, Faculty of Science, Department of Chemistry, Muğla, Türkiye; ²Muğla Sıtkı Koçman University, Köyceğiz Vocational School, Köyceğiz-Muğla, Türkiye; ³Pamukkale University, Faculty of Science, Department of Biology, Denizli, Türkiye
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Numerous investigations have reported that mushrooms possess anticancer activities. For several decades, mushrooms have been approved as food additives against cancer particularly in Japan and China [1]. Since cancer incidence increases yearly, researchers have increased searching for effective anticancer compounds from mushrooms. This research explores the cytotoxic potential and apoptosis mechanisms of the acetone extract of edible *Tricholoma scalpturatum* (Fr.) Quél. mushroom against colorectal cancer (Caco-2) cell lines [2,3]. The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to assess the cytotoxic activity to find the effective concentration (EC₅₀) value. The apoptotic effect was evaluated through three methodologies: image cytometry using the Annexin V-FITC/PI apoptosis detection kit, Western Blot analysis targeting apoptosis-associated proteins, and Real-Time PCR (qPCR) to determine mRNA levels of apoptosis-related genes. The extract from *T. scalpturatum* exhibited a dose-dependent suppression of Caco-2 cell lines proliferation, with an EC₅₀ value of 46.86±2.01 µg/mL. The image cytometry results showed that *Tricholoma scalpturatum* acetone extract stimulated 6.75-fold apoptotic cells compared to the control group. According to the real-time PCR analysis, the acetone extract increased the expression level of the BAX gene 2.71-fold while it decreased the expression of the BCL-2 gene 1.90-fold ($p<0.05$) compared to the control group. Moreover, the extract also notably increased mRNA expressions of Caspase 3, 8, and 9 (5.46, 4.46, and 3.01-fold, respectively). Results were normalized using GAPDH (glyceraldehyde 3-phosphate dehydrogenase). In addition, the anti-apoptotic BCL-2 protein level was significantly decreased. However, BAX, Caspase 3, 8, and 9 protein levels were significantly induced as a result of extract treatment. Apoptosis involves both intrinsic and extrinsic pathways regulated by various genes. Since the acetone extract appears to induce apoptosis by affecting both pathways, isolation of bioactive compounds should be performed as further investigations.

Keywords: *Tricholoma scalpturatum*; apoptosis; cytotoxicity; colon cancer (Caco-2) cell lines.**Acknowledgements:** This study was supported by TUBITAK (The Scientific and Technological Research Council of Türkiye), Project number TUBITAK1001-121Z551.**References**

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