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Evaluation of Pistacia vera Sap Waste Sections and Its Potential Role on Treatment

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Abstract: It is estimated that 1.3 billion waste is produced annually in the world and this amount is expected to increase to 38-67% by the end of 2025. Considering the waste production of pistachio, it is seen that around 132 165 thousand tons of waste products have been accumulated after the product processing phase due to excessive consumption and wide usage area. However, it has been reported that there are important secondary metabolites such as "masticadienonic acid", "masticadienolic acid", "tirucallol" and "pistasionic acid" in the extract content of unused waste stem parts. Masticadienonic acid has been shown in the literature to have anti-proliferative and apoptotic activity. It has been determined that masticadienolic acid component has cytotoxic activity on five different cancer cell lines (Leukemia, Breast, Prostate, Colon and CNS). Examining the studies on the Tirukallol component, it was determined that it inhibits adhesion molecules in human endothelial cells. Pistacionic acid is a newly purified and characterized compound and only anticholinesterase and antidiabetic activities have been studied. The aim of our study is to determine whether these components obtained from stem parts have any cytotoxic effect on Mcf7 cells. Within the scope of the study, the stems were obtained from pistachio processing plants and kept at room temperature, in a cool environment. In order to obtain the compounds from sap extracts, column chromatography, ion layer chromatography (TLC) and crystallization methods which are moving phase systems were used. Mcf7 cells were exposed to different concentrations of the purified components and their cytotoxic activity was evaluated using the MTT test. Mcf7 cells were exposed to different concentrations of the purified components and their cytotoxic activity was evaluated using the MTT test. The results of our study are important preliminary data in the literature and we believe that they may contribute to further studies.

Keywords: Waste Solid, Pistacia vera, Bioactive Metabolite, Antitumoral activity, Phytotherapy

Introduction

Pistacia vera L. is the only edible fruit species among 12 different tree species that is included in the Pistacia genus (Mannino vd., 2019). This fruit contains an edible long seed. This seed part consists of a lilac-colored skin and a greenish flesh with a pleasant taste and aroma (Fabani vd., 2013). Pistachio, when we look at the amount of production, equals to 551 307 tonnes in Iran, 447 700 tonnes in the US, it is reported that the production of Pistachio in Turkey equals to 240,000 tons and 74 828 tons in China (FAO, 2018). The latest data in our country show that its production has tripled in the last 50 years (Tüik, 2019). The reason why the

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production is so high is that it is used in various industrial areas such as cake, ice cream, chocolate, salami, tahini halva and baklava making. An average of 2 kg biomass waste accumulates for every 3 kg of pistachio with a wide range of usage areas. (Mehrnejad, 2001). Biomass wastes generally consist of shell, root, leaf or stem parts and it has been determined that these parts have valuable bioactive components. It has been determined that terpenes, essential oils, phenolics, fatty acids and sterols, which are among these bioactive components, play a role in various biological activities through ethnopharmacological studies (Bozorgi vd., 2013). Unfortunately, in phytochemical and pharmacological studies, mainly the shell, fruit, resin and leaf parts are used, while the stem parts are mostly ignored. However, it has been documented by several studies that these parts are also rich in phytochemical components and fulfill important pharmacological activities. Among these studies, Dambagı and her team determined that there are important secondary metabolites such as masticadienolic acid, masticadienolic acid, tirucallol and pistacionic acid in the waste stems. In addition, in the continuation of the study, it was also shown that pistacionic acid did not have any inhibitory effect on acetylcholinesterase, although these components had inhibitory effects on α-glucosidase, α-amylase, acetylcholinesterase and butyrylcholinesterase (Dambagı, 2019). It has also been determined that masticadienonic acid reduces blood sugar and serum fatty acid concentration by inhibiting the 11β-HSD1 enzyme and has anti-proliferative activity on prostate cancer cells (Vuorinen vd., 2015; Sânchez-Monroy vd., 2017). In the study with experimental mouse models, it has been proven that masticadienolic acid has an anti-inflammatory effect and this component has an antifungal effect (Giner-Larza vd., 2001; Johann vd., 2010). Although Tirukallol inhibits adhesion molecules such as VCAM-1 and ICAM-1 on Human Aortic Endothelial Cell (HAEC), it has been determined that it has no cytotoxic effect. In addition, it has been shown to inhibit critical events such as edema formation and migration of polymorph nuclear leukocytes caused by Tissue Plasminogen Activator (TPA) (Loizou vd., 2009; Fernandez-Arche vd., 2010). A new compound with an organic structure different from the existing phytochemical components was synthesized and characterized as pistacionic acid (Dambagı, 2019). There is no cytotoxic activity study on breast cancer cells in other components, including pistacionic acid, whose pharmacologically only anticholinesterase and antidiabetic effects have been determined.

Our aim in this study is to analyze the cytotoxic activities of major compounds to be purified from the waste stalks of Pistacia vera fruit on MCF-7 cell line by molecular approaches to determine which concentration is toxic and how it affects cell proliferation.

Method

Obtaining Pistachio Extracts

The stem part of the Pistachio (P. vera L.) we used as the study material was taken from the Pistachio processing facilities as waste material and the waste stem part was ground using a blender. Plant samples were macerated with n-Hexane, chloroform and ethyl alcohol and extracts were obtained. Solvents were removed by a rotary evaporator and the extracts were concentrated. Column Chromatography, Ion Layer Chromatography (TLC) and crystallization methods, which are moving phase systems, were used to obtain compounds from the extracts. Subsequently, masticadienonic acid, masticadienolic acid and triquallol compounds from chlorofome extracts, pistacionic acid compound from ethyl alcohol extract were purified. The organic structure of these components has been illuminated using FTIR, 1H-NMR, 13C-NMR, 1D- and 2D-NMR spectroscopic methods. (Dambagı, 2019).

Cell Culture

The Michigan Cancer Foundation-7 (Mcf-7) cell line was used as the experimental group and the human umbilical vein endothelial cell (HUVEC) cell line was used as the control. Cells were grown in DMEM (Dulbecco's Modified Eagle Medium) medium enriched with FBS (Fetal Bovine Serum), Penicillin-Streptomycin and L-glutamine. For the duplication of the cells, the cryovials were taken from -80 ^{o}C and dissolved quickly by using double boiler. Then, DMEM was taken into the medium and after centrifuging at 800 rpm for 5 minutes, the supernatant was removed and the medium was added to the remaining cell pellet. Finally, the cell suspension was taken into flasks of 75 cm^2 and left to incubation at 37 ^{o}C in an environment containing 5% CO^2, allowing it to duplicate (Cevatemre, 2012).

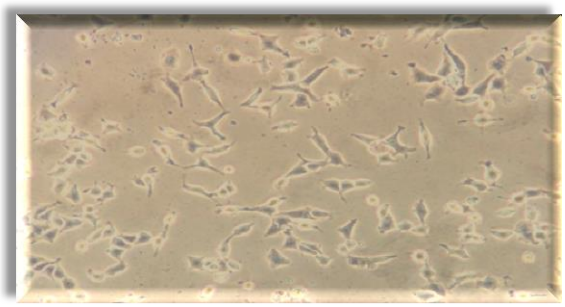

Figure 1. MCF-7 celss (X20)

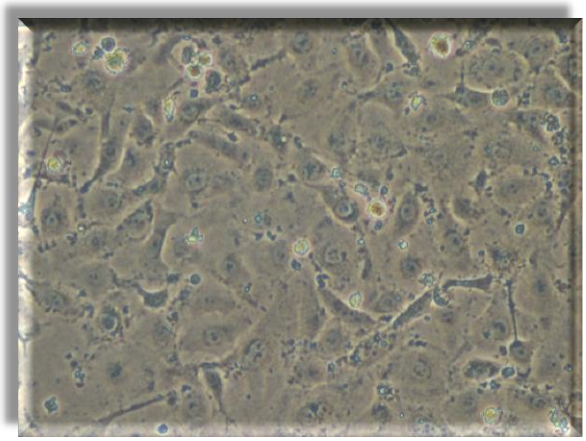

Figure 2. HUVEC cells (X40)

Cytotoxicity Test

Cytotoxic activity is determined by the reduction of "yellow tetrazolium (MTT)" compound of mitochondrial dehydrogenase enzyme activity in living cells. This method is based on measuring the color change colorimetrically. Cells were first planted in a 96-well plate with 5×10^3 cells per well and incubated for 24 hours at 37 °C, 5% CO^2. After incubation, pistacionic acid compound was added to the cells in 5 different concentrations (100 µm / ml, 50 µm / ml, 25 µm / ml, 12, 50 µm / ml and 6, 25 µm / ml) and incubated for 48 hours. In cells, the wells were completed by placing medium and cell as positive control (maximum viability, MO), medium and Triton X-100 as negative control, and cell-free medium for blank. After incubation, 40 µl of MTT dye was added to each well and incubated again at 37 ^oC for 4 hours. DMSO was added to each well to make the formazan crystals formed after incubation soluble. The resulting color intensity was measured in a spectrophotometer at a wavelength of 570 nm. (Cevatemre, 2012). The% viability rates of the cells were determined by using the absorbance values read in the calculation of the viability equation.In this equation, non-extracted control cell (MO) viability was assumed as 100%, and the viability rates of cells treated with extract were calculated using the formula.

% Viability = [100 × (mean of Compound-treated cell absorbance / Drug-treated control cell (MO) viability)
In the experiment, each concentration was repeated in three separate wells.

Findings

Findings of Cytotoxic Activity

Cytotoxicity of pistacionic acid on the MCF-7 breast cancer cell line was determined by the MTT (3- [4,5-dimethylthiazol-2-yl] -2,5 diphenyltetrazolium bromide) method and the absorbance and concentration graph is given in Figure 3.

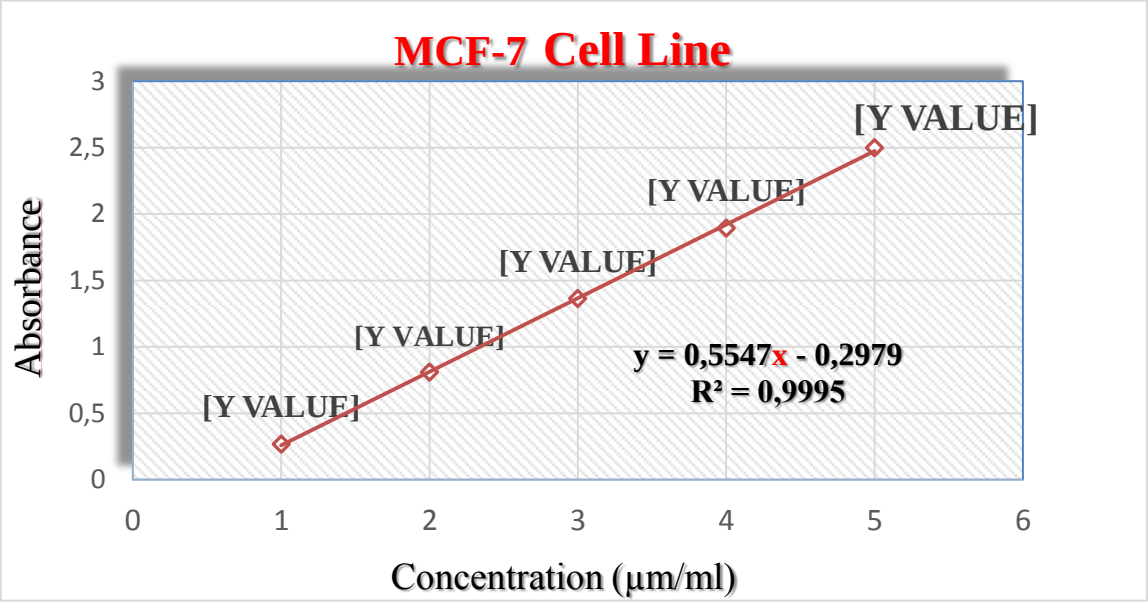

Figure 3. Absorbance and concentration graph of pistacionic acid compound in Mcf-7 cells line.

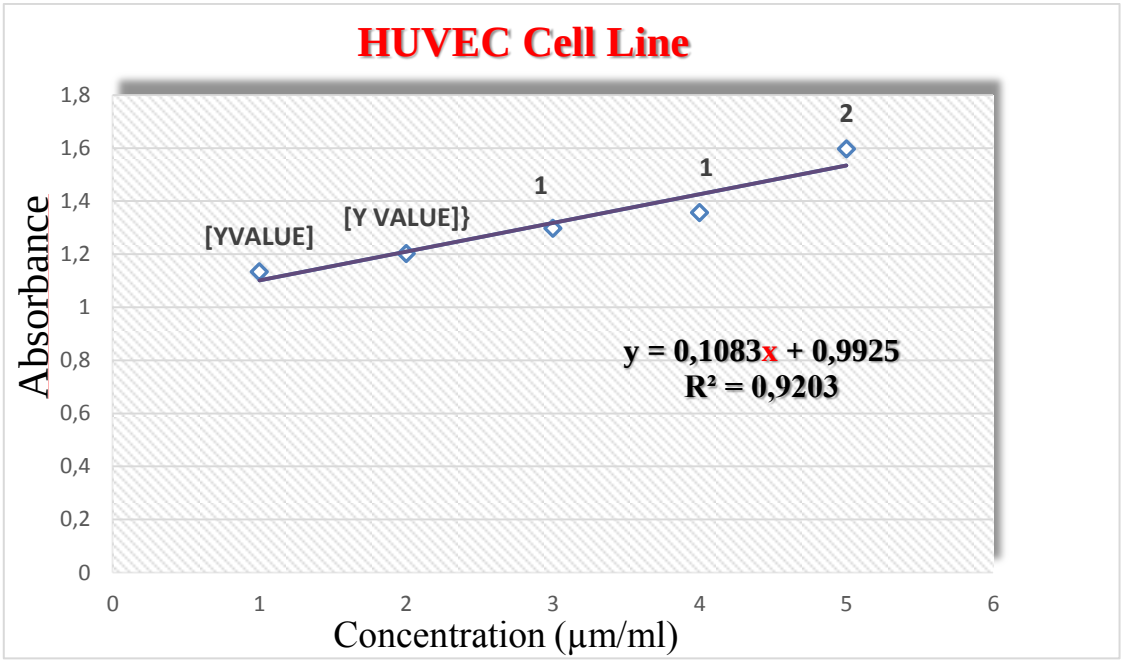

Figure 4. Absorbance and concentration graph of pistacionic acid Compound in HUVEC cells line.

Result and Discussion

Pistacionic acid is a newly synthesized and purified compound. For this reason, it has been found in the literature that it only inhibits α-glucosidase and α-amylase enzyme activity and shows antidiabetic activity. Anticholinesterase activity was determined by determining BchE (Butyrylcholine Esterase) enzyme activity and AchE (Acetylcholine Esterase) enzyme activity.

The results confirmed that Pistasionic acid exhibited a cytotoxic effect in Mcf-7 cells but was less toxic in HUVEC cells. In the remaining stages of our study, we think that determine the cytotoxic activities in other major compounds we purified. Then, how the compound affects apoptosis by determining the activity of the caspase 3 enzyme involved in the apoptosis process by immunocytochemical method. We anticipate that it has many biological activities waiting to be discovered in further studies and that its cytotoxic activity may be used for an advanced new drug design.

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