

Cytotoxic Activity Against Cancer Cells of *Pistacia vera*

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Abstract: *Pistacia vera* fruit, one of the leading products of today's sweet and snack sector, which has been seen in the history of the kingdom, are able to support daily nutritional needs and treatment of many diseases due to the phenolic and flavonoid content in the literature. Especially the studies on colon cancer and breast cancer are remarkable potential antitumoral activity of pistachio examined. The aim of this study is to examine in more detail the *P. vera* plant, in which we have previously tested the methanol-hexane extracts of the seed and the test and obtained positive results. As well as observing the cytotoxic activities of the plant extracts and active ingredients obtained from different parts on the lung cancer cells. After counting A549 and HUVEC cell lines which replicated in the medium, were added on 96 well plate. Then, different part of plants extract and 2 major active ingredients also added. Then MTT dye was applied and measured spectrophotometrically. *P. vera* extracts and active ingredients which studied cytotoxic activity research were found to be effective in all cell lines in general. Particularly *P. vera*'s remaining after the fruit parts, methanol extract which obtained from waste containing leaf and stem parts and PVK-1 active agent showed selective activity on normal cells and cancer cells, therefore we consider that it has a high therapeutic index. We believe that this plant should be imparted to sciences and health sector with further studies.

Keywords: *Pistacia vera*, Lung cancer, Cytotoxic activity, Phytotherapy

Introduction

Pistacia vera L. (Pistachio) also known as “the king of fruit” is a member of Anacardiaceae family and it grows on the Pistachio tree (Maskan & Karataş, 1999). The production of pistachio, which is the mainland Middle East, is mostly performed in Iran, USA, Turkey and Syria. *P. vera* fruit, one of the leading products in today's sweet and snack industry. It is also used to increase the nutrition, color and taste in the making of pasta, chocolate, baklava, ice cream and meat products (Tous & Ferguson, 1996; Yahia, 2011). For this reason, it is of great commercial importance.

In our country, *P. vera* is produced and consumed especially in the Southeast Anatolia and due to having numerous biological activities it helps to protect (Surh, 2003) and therapy (Kalkanci et al., 2007) a large variety diseases.

Pistachio nut plays an important role in healthy nutrition and reducing the risk of disease due to its rich protein, fat and fatty acids, vitamins, minerals and fiber content. It also contains important phytochemicals that can provide antioxidant support for cardiovascular and autoimmune diseases including carotenoids (β -carotene and lutein), γ -tocopherol and phenolic compounds such as phenolic acids, flavonoids, lignans, anthocyanins and proanthocyanidins (Bolling, Chen, McKay, & Blumberg, 2011).

Pistachio nut has rich content and because of this it is a potent source for therapeutic biological activator. For instance, studies by Sari and colleagues (2012) and Hosseinzadeh and colleagues (2010) have shown to have antioxidant activities of seed, testa, leaf and resin of *P. vera*. Also Halvorsen and colleagues (2006) indicated that *P. vera* is included among the top 50 foods with high antioxidant potential. In addition, studies on the anticancer activity of this plant have come to the forefront in recent years. In a study, phytosterols in pistachio have been reported to prevent the development of prostate cancer (Kashaninejad & Tabil, 2011) It has also been reported that Pistachio is a good source of gamma-tocopherol and may reduce the risk of lung cancer (Anonim, 2009). In the other studies, researches indicated that *P. vera* has been shown to play a therapeutic role on HepG2 hepatoma cell lines, MCF-7 human breast cancer cell line and colon carcinogenesis (Fathalizadeh et al., 2015; Gleis et al., 2017; Seifaddini pour, Farghadani, Namvar, Mohamad, & Abdul Kadir, 2018). (Kashaninejad & Tabil, 2011)

In this study, we investigated that some biological properties of *P. vera*. For this purpose, cytotoxic activities of seven extracts and two major metabolites from *P. vera* were studied by MTT method.

Method

Extracts and Major Metabolites of *P. vera*

We collected the plant samples in Campus of Gaziantep University. Seven extracts from different parts (hull, peduncle, leaf, leaf and peduncle together, seed coat) of *P. vera* by soxhlet apparatus. Then alcohols were removed by evaporation in the rotary evaporator. The extracts obtained were stored at + 4 °C until beginning of the experiment (Fakili, 2010).

In addition, two major metabolites from *P. vera* were isolated by Prof. Dr. Ahmet CAKIR from Kilis 7 Aralık University, Kilis, Turkey. Silica column chromatography was used and chloroform-ethyl acetate as mobile phase for the extraction. After this, obtained fractions were checked by thin layer chromatography (TLC). Their chemical characterizations were confirmed by FTIR, 1H-NMR, 13C-NMR, 1D-NMR and 2D-NMR.

Cell Lines

A549 (non-small cell lung cancer) and HUVEC (Human umbilical vein endothelial cell) cell lines was cultured at complete culture medium (DMEM) (Gibco company USA) as supplemented with 10% fetal bovine serum (FBS), 1% antibiotic solution (100 U/ml penicillin and 100 μ g/ml streptomycin) and then incubated at 37 °C in 5% CO² and humidity of 80% for cells attachment (Jin, Zhang, Kang, Wang, & Zhao, 2010). Prior to the cytotoxic activity study, cells were counted with hemacytometer.

Cytotoxic Activity Assay

Cell growth inhibitory assay was performed by MTT (3–4,5- dimethylthiazol-2,5 biphenyl tetrazolium bromide) method on A549 (non-small cell lung cancer) and HUVEC (Human umbilical vein endothelial cell) cell lines with various concentrations (6,25 –100 μ g/ml) for 48 h (Jin et al., 2010) After counting cell lines which replicated in the DMEM (Dulbecco's Modified Eagle Medium), were added on 96 well plate to be 5×10^3 in each well. Different part of plants extract and 2 major active ingredients also added in 5 different doses (6.25-100 μ g/ml) and then MTT dye was applied. After dissolving the formazan crystals formed, the color intensity was measured with a spectrophotometer at a wavelength of 570 nm. Viability rates of cells were determined using absorbance readings (Berridge, Herst, & Tan, 2005).

Viable cells were calculated by following formula:

$$\text{Viable cells (\%)} = (A_s / A_c) * 100$$

As: Absorbance of extraction sample treated cells
 Ac: Absorbance of control group (max viability)

Statistical Analysis

Data collected for assays and measurements were statistically analyzed as factorial experiments in a completely randomized design with at least three replications.

Results and Discussion

The % viability values obtained by colorimetric measurements of the MTT assay are shown on the graph (Figure1 and Figure 2). According to the graph of survival results of A549 lung cancer cell line (Figure 1), it was observed that leaf methanol, hull methanol extracts and PVK-X major component showed low cytotoxic activity, whereas peduncle methanol extract showed no effect. Peduncle acetone, leaf and peduncle together methanol, hull ethanol and hull ethyl acetate extracts were found to be effective in reducing the viability of cancer cells. In addition, PVK-I major component showed strong cytotoxic activity on the cells and completely killed the cancer cells.

According to % viability graph of HUVEC cell line (Figure 2), leaf methanol and hull ethanol did not show cytotoxic effect, also leaf + peduncle methanol extract and PVK-X compound showed low efficacy. Hull methanol, seed coat methanol and peduncle methanol extracts were reduced cell viability to half. As well as hull ethyl acetate, peduncle acetone extracts and PVK-I showed strong cytotoxic activity.

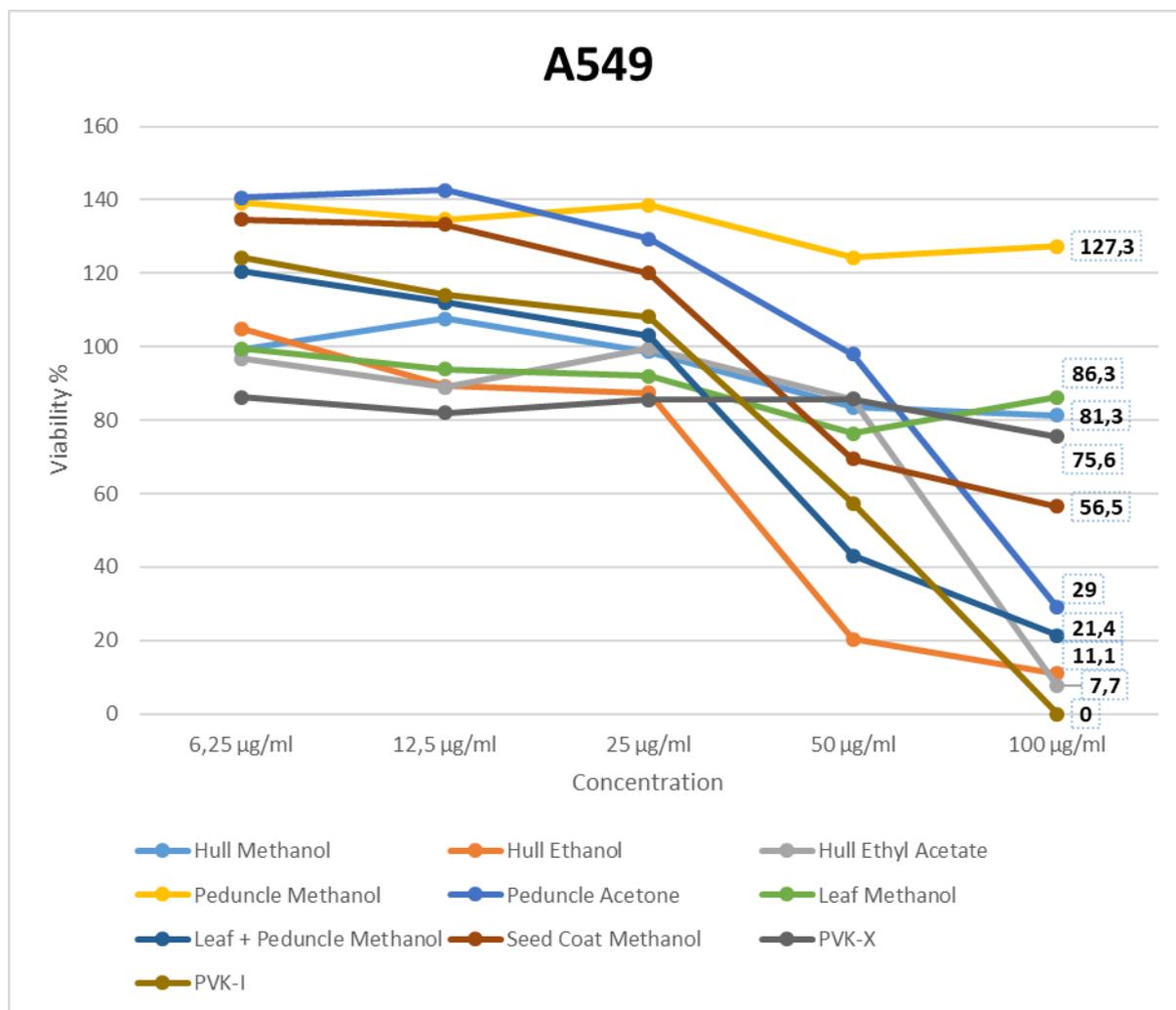


Figure 1. Cell Viability of A549

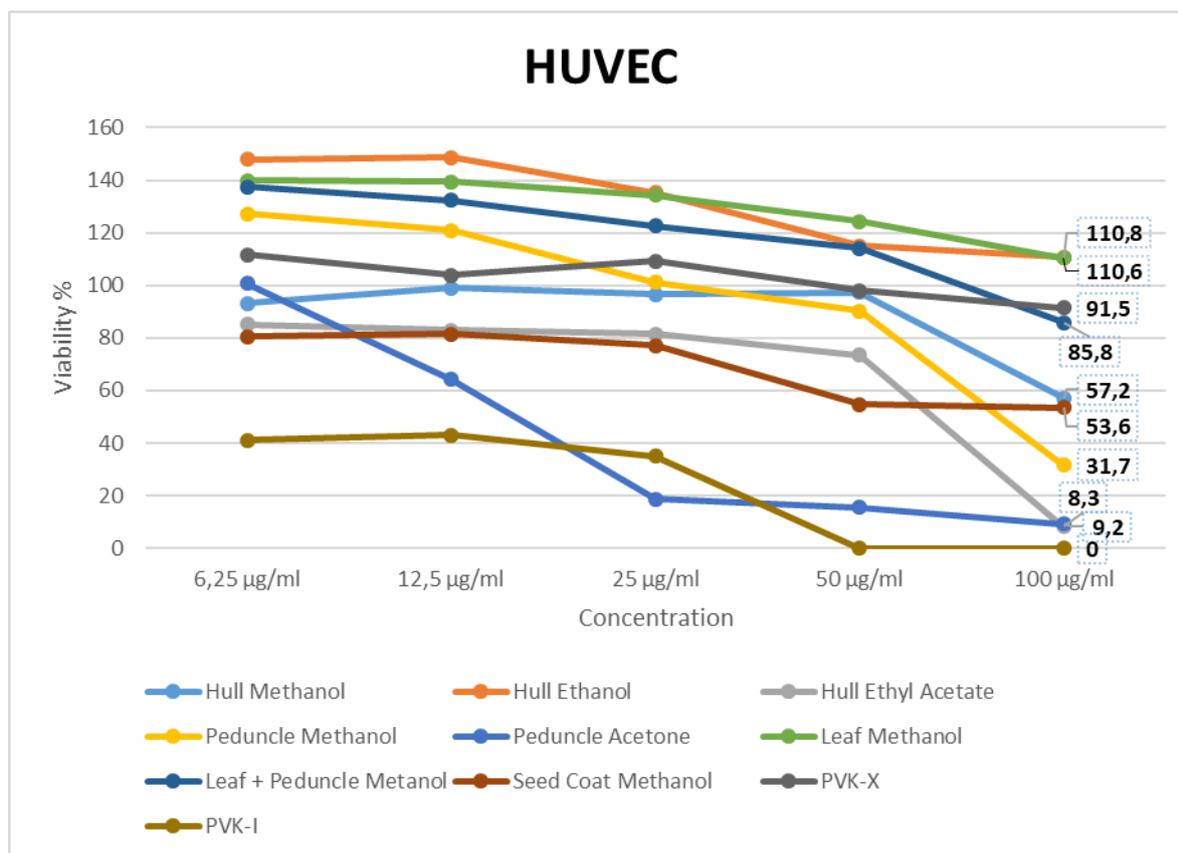


Figure 2. Cell Viability of HUVEC

Conclusion

Particularly, *P. vera*'s remaining after the fruit parts, methanol extracts which obtained from waste containing leaf, peduncle and hull parts showed selective activity on normal and cancer cells. The results of our study are similar to Fathalizadeh et al. (2015), Seifaddinipour et al. (2017) and Gleis et al. (2018)'s received. It also confirms the Anonim's hypothesis (Anonim, 2009).

Therefore, we consider that it has a high therapeutic index. In the direction of the results, we think that *P. vera* plant will contribute to the scientific world by foreground in the pharmacological sense. For this reason, we suggested that *P. vera* plant may be potential therapeutic herbaceous plant and it would be useful for the scientific world to evaluate it in with more detailed studies.

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