

## Anticancer, Antiproliferative, Lysosomal and Lactate Dehydrogenase Inhibitory Effects of Fruit Extracts from Sumac (*Rhus coriaria* L.) on Human Lung Cancer Cells

### Sumak (*Rhus coriaria* L.) Meyve Özütlerinin İnsan Akciğer Kansere Hücre Hatları Üzerinde Antikanser, Antiproliferatif, Lizozomal ve Laktat Dehidrojenaz İnhibitör Etkileri

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#### ÖZET

**GİRİŞ ve AMAÇ:** Sumak (*Rhus coriaria* L., Anacardiaceae), Anadolu'da geleneksel olarak yenilebilir bitkidir. Sumak, pek çok biyoaktif fitokimyasal ihtiva etmesinden dolayı, alternatif tedavi yaklaşımlarında tercih edilmesine rağmen; literatür araştırmaları sumak özütlerinin antikanser ve antiproliferatif aktivitelerini analiz etmek amacıyla sınırlı sayıda çalışmalar yapıldığını göstermiştir.

**YÖNTEM ve GEREÇLER:** Bu nedenle, sumak bitkisinin tıbbi ve geleneksel kullanımlarına katkıda bulunmak amacıyla, *R. coriaria* L. meyvelerinden elde edilen özütlerin kanseri önleyici potansiyellerinin araştırılması amaçlanmıştır. Bu bağlamda, A549, H1299 ve H460 insan akciğer kanseri hücre hatlarına karşı antikanser ve antiproliferatif potansiyeller, lizozomal ve laktat dehidrojenaz inhibitör etkileri belirlenmiştir.

**BULGULAR:** Sonuçlar, sumak ekstraktlarının zamana ve doza bağımlı olarak güçlü antikanser ve sitotoksik aktivite gösterdiğini ortaya koymuştur. Sumak özütleri arasında, sulu ve metanolik ekstraktların, test edilen tüm insan akciğer kanseri hücrelerine karşı 5,08 - 6,49 µg / mL IC50 değer aralığıyla yüksek derecede sitotoksik olduğu bulunmuştur. Kanser hücre hatlarında, hücre büyümesinin ve hücre canlılığının sumak özütleri tarafından engellendiği gözlemlenmiştir. Ayrıca, hücre hatlarının uzun süre ve artan dozlarda özütlerle maruz kalması, hücrelerde lizozomal fonksiyonları ve membran geçirgenliğini artırmıştır.

**TARTIŞMA ve SONUÇ:** Sonuç olarak, çalışma bulguları sumak bitkisinin gıda ve baharat olarak kullanımının yanı sıra; alternatif bitkisel terapötik ajan olarak kanser tedavisinde kullanılma potansiyelini ortaya koymuştur.

**Anahtar Kelimeler:** Antiproliferatif, laktat dehidrojenaz, lizozomal, akciğer kanseri, *Rhus coriaria* L

#### ABSTRACT

**INTRODUCTION:** Sumac (*Rhus coriaria* L., Anacardiaceae) is traditionally used edible-plant in the Anatolia. Although, it has been preferred in alternative therapeutic approaches due to the presence of many bioactive phytochemicals, the literature survey demonstrated limited works have been undertaken for analyzing the anticancer and antiproliferative activities of the extracts.

**METHODS:** Therefore, the current research was aimed to evaluate totally anticancer potentials of extracts from *R. coriaria* L. fruits for contribution to medicinal and traditional uses of sumac. In this respect, anticancer and antiproliferative potentials, lysosomal and lactate dehydrogenase inhibitory effects were determined against A549, H1299 and H460 human lung cancer cell lines.

**RESULTS:** The results revealed almost all the extracts of sumac exhibited powerful anticancer and cytotoxic activities in a time and dose dependent manner. Among the extracts, aqueous and methanolic extracts were found to be highly cytotoxic with IC50 values in range of 5,08 – 6,49 µg/mL against all tested human lung cancer cells. It was observed that cell growth and cell viability in cancer cell lines were inhibited by the extracts. Additionally, increasing extracts exposure time and dose resulted in increasing the lysosomal functions and membrane permeability in the cell lines.

**DISCUSSION AND CONCLUSION:** Consequently, these findings suggest that sumac has potential uses in cancer therapy as an alternative herbal therapeutic agent as well as their uses as food and spice.

**Keywords:** Antiproliferative, lactate dehydrogenase, lysosomal, lung cancer, *Rhus coriaria* L.

## INTRODUCTION

From ancient times to present, there has been considerable interest to use medicinal and aromatic plants almost in all industries such as food, cosmetic, textile, leather, pharmaceutical, medicine etc. It is known that they have been not only used for nutrition, but also preferred for traditional medicine and cure for various diseases and/or disorders including cancer, diabetes, ulcer, urinary system disorders, viral eye infections, cardiovascular diseases, neurodegenerative diseases, liver and heart diseases etc. (1,2). The active ingredients of many drugs currently in use have been isolated from medicinal plants. Therefore, they have a wide range of uses for prevention and treatment of many diseases around the world, due to presence of many natural bioactive products and secondary metabolites (3-5).

Within the traditional medicinal plants *Rhus coriaria* L., also known as sumac, is a wild-edible plant that belonging to the family of *Anacardiaceae*. Sumac is one of the most commonly used spice almost all over the world, it grows in temperate and subtropical areas, and widespread especially all Mediterranean and Middle East regions, including Turkey, in where it grows natively in the Mediterranean and Southeastern Anatolia (5). In the Anatolian traditional herbal medicine, sumac is also believed as being useful for prevention of diarrhea, diabetes, hemorrhoids, obesity, colitis, fever, dermatitis and hyperglycemia (1,6). Besides these, it has been used traditionally in the treatment of sore throat, reduction of stomach pains and cholesterol as an herbal remedy (7).

Previously both *in vivo* and *in vitro* several researches demonstrated that sumac possesses wide range of pharmacological properties e.g. anti-fibrogenic, anti-inflammatory, antimutagenic, hypoglycaemic, anti-ischemic, antimalarial, antiviral, antimicrobial, antifungal, antioxidant, antiulcer, hypouricaemic, hepatoprotective, and atheroprotective, owing to its rich bioactive substances and phytochemicals, particularly rich in terms of tannins, phenolic acids, flavones and flavonoids, gallic acids, anthocyanins, terpenoids quercetin, isoquercetin, myricitrin, kaempferol etc (8-11). Besides its rich bioactive phytochemical compositions, *R. coriaria* L. has

rich mineral contents such as Aluminum, Barium, Bromine, Calcium, Chlorine, Chrome, Copper, Iron, Magnesium, Manganese, Potassium, Lithium, Nitrogen, Phosphorus, Zinc, Silisium, Strontium, Lead, Titanium and Vanadium, of which, Calcium, Magnesium, Phosphorous and Potassium are predominant elements found in sumac fruits (12,13).

In view of the comprehensive pharmacological and phytochemical properties of sumac, sumac consumption has a great importance in sectors of nutrition and medicine. Although, the literature survey demonstrated valuable biological activities of the extracts of *R. coriaria* L., limited works have been undertaken for analyzing the anticancer and antiproliferative activities of the extracts using different assays, additionally, limited information have been available on research that has been analyzed biological activities of the different extracts of sumac on cancer proliferation. Therefore, in the current research, the fruits of *R. coriaria* L. were extracted using four different solvents as water, methanol, hexane, dichloromethane, and aimed to investigate the anticancer and cytotoxic activities using different assays; MTT assay for measurement of the cell proliferation, trypan blue exclusion assay for determine the antiproliferative properties, neutral red uptake assay for evaluation lysosomal activities of the cells, and lactate dehydrogenase activity assay for detection of necrosis in the cultured cells.

## Materials and Methods

### Collection of Plant Material

The fruits of *R. coriaria* L. were collected during the month of July 2016 from Kilis and Gaziantep, located in South-eastern part of Turkey. A specimen identified by N. Sekeroglu, and a voucher specimen was deposited in the Biology Department at University of Kilis 7 Aralik, Turkey. The fruits samples were dried at laboratory conditions at room temperature (25°C) with no direct light.

### Preparation of Crude Extracts

Air-dried and powdered fruits of *R. coriaria* L. (50 g) were extracted with water, methanol, n-hexane and dichloromethane as solvents. Extraction was carried out using Soxhlet apparatus (Sigma Aldrich, USA) during 6 h according to Waksmundzka-Hajnos et al. (14)

with some modifications. Extracts were filtered and concentrated under vacuum at 40°C using a Rotary Evaporator (Sigma Aldrich, USA), and then the extracts were kept at 4°C until analysis and used freshly.

### **Cell Lines and Culture**

Human lung cancer cell lines: A549 (human lung carcinoma), H1299 (non-small cell lung cancer), H460 (human lung adenocarcinoma) and non-tumorous HUVEC (human umbilical vein endothelial cell), obtained from the American Type Culture Collection (ATCC, USA) were used for evaluation the potential anticancer and antiproliferative effects of fruit extracts of *R. coriaria* L. The cells were cultured in Dulbecco's modified Eagle medium (DMEM, ThermoFisher Scientific), supplemented with 10% (v/v) fetal bovine serum (FBS), 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) in the flasks at 37°C in a humidified CO<sub>2</sub> (5%) incubator. All the experiments were performed in triplicates using A549, H1299, H460 and HUVEC cells from passage 24 or less than.

### **Measurement of Cell Proliferation by MTT Assay**

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed for evaluation cytotoxic properties of the sumac as reported by Mosmann (15) and Gezici et al. (16) with minor modifications. Briefly, densities of 5 x 10<sup>3</sup> cells were seeded in 200 µl medium into 96-well plates for 24 h and after seeding, the fruits extracts of *R. coriaria* L. with concentrations from 1 to 100 µg/mL were added all wells, and doxorubicin used as a standard cytotoxic agent with different concentrations 0,01-1 µg/mL, and then all the wells incubated at 37°C for 48 h. After incubation, the medium was discarded and 50 µL/well of MTT solution (5 mg/mL) was added into each well and incubated for 4 h at 37°C. The medium-containing MTT was discarded and 200 µL of DMSO (dimethyl sulphoxide) was added for both lysis the cells and solubilization formazone. Then, the absorbance was measured at 570 nm with a Thermo Lab systems 408 Multiskan multiplate spectrophotometer and the inhibition (%) was calculated as;

$$\left[ \frac{\text{Mean OD without test agent (negative control)} - \text{mean OD with test agent}}{\text{mean OD without test agent (negative control)}} \right] \times 100$$

The assay was done using three replicates. The dose response curve was used to generate the IC<sub>50</sub> (µg/mL) values for each cell line as the concentration of drug needed to produce 50% inhibition of cell growth.

### **Trypan Blue Assay**

Antiproliferative activities of the extracts were evaluated against the A549, H1299 and H460 cell lines using trypan blue exclusion method described by Strober (17) previously. According to this method, firstly the cell suspensions at densities of 1 x 10<sup>4</sup> cells/well were plated into a 96 well plates and incubated for 24 h in a humidified 37°C incubator equilibrated with 5% CO<sub>2</sub> for cell attachment. Then, the stock concentration (10 mg/mL) of all the *R. coriaria* L. extracts were prepared in DMSO and used at 5, 10, 50 and 100 µg/mL concentrations from each extract, and incubated at 37°C for 24, 48, and 72 h. At the end of the every 24 h incubation period, 100 µL of cells was collected for counting and added 1mL of 0.05% trypsin-EDTA (ethylenediaminetetraacetic acid), and then washed with phosphate buffer saline (PBS, Sigma-Aldrich, USA). Finally, the collected cells were harvested for 10 min at 1000 rpm using a centrifuge (ThermoFisher Scientific). After removing the supernatant, 20 µL of 0.4% trypan blue solution was added to cell pellet (1:1 dilution of the cell suspension). The cell viability was determined microscopically (Nicon, Japan), and the viable cells were counted an automated cell counter Cedex XS Analyzer.

### **Lysosomal Activity Assay (neutral red uptake assay)**

In order to determine lysosomal activity of the cells treated with the extracts of *R. coriaria* L., neutral red uptake assay (NR) was used as previously reported with minor modifications (18). Briefly, the cells were seeded into 96-well culture plates and incubated for 24 h at 37°C in a humidified CO<sub>2</sub> (5%) incubator. When the cells reached at least 50% confluence, they treated with 0, 5, 10, 50 and 100 µg/mL concentrations of the extracts, before incubation at 37°C for 24, 48, and 72 h. At the end of the incubation periods, they washed with cold PBS

at three times, they were then re-incubated with the medium containing 200  $\mu$ L NR solutions at least 2 h at 25°C. After NR incubation, the cells were subsequently washed with acetic acid and ethanol (1:50) solution, and then fixed with  $\text{CaCl}_2$  and formaldehyde (2:1) solution for 2 min to remove the NR dye in each well. Finally, the plates were read at 540 nm using a microplate spectrophotometer system (Thermo Lab systems 408 Multiskan).

### Lactate dehydrogenase (LDH) assay

The release of the enzyme lactate dehydrogenase (LDH) activity assay was performed for detection of necrosis in the cultured cells described by Al-Qubaisi et al. (19) previously. The cells (a density of  $2 \times 10^4$  cells/well) were seeded in 96-well plates with 100  $\mu$ L fresh culture medium and incubated at 37°C for 18 h. And then, they were treated with 0, 5, 10, 50 and 100  $\mu$ g/mL concentrations of the *R. coriaria* L. extracts as used in the MTT assay and incubated for 24, 48 and 72 hours, respectively. For determination of the potential LDH concentration, 40  $\mu$ L of culture supernatant was collected from each well and transferred to a new plate, and then 40  $\mu$ L of 6% triton X-100 was added to all well. Before

plates were incubated at room temperature about 30 min in the dark, afterwards they were read at wavelength 340 nm using an ELISA microplate spectrophotometer system (Thermo Lab systems 408 Multiskan). The percentage of LDH release in medium was calculated with compare to total LDH in cell lysate in the same well, as followed equation:

$$[(\text{Mean OD from treated LDH activity} - \text{mean OD from untreated LDH activity}) / (\text{mean OD from maximum LDH release activity} - \text{mean OD from untreated LDH activity})] \times 100$$

The assay was done using three replicates. The dose response curve was used to generate the  $\text{IC}_{50}$  ( $\mu$ g/mL) values for each cell line as the concentration of drug needed to produce 50% inhibition of cell growth.

### Statistics

All the assays were conducted in triplicate, and three different microplate wells were used for the each concentration. A linear regression analysis was performed to calculate  $\text{IC}_{50}$  values. P value of <0.01 was considered statistically very significant.

## RESULTS

### MTT Assay Results

MTT assay was conducted to determine the cytotoxic activities of the *R. coriaria* L. fruit extracts to three human lung cancer cell lines (A549, H1299, and H460) by comparing the cytotoxic activity of non-tumorous HUVEC cell lines. Cytotoxic activity results of fruit extracts of *R. coriaria* L. are presented in Table 1 regarding of  $\text{IC}_{50}$  values after 48 h treatment period.

**Table 1.**  $\text{IC}_{50}$  values, determined by MTT assay, of fruit extracts of *R. coriaria* L.

Extracts	$\text{IC}_{50}$ values from MTT ( $\mu$ g/mL)			
	A549	H1299	H460	HUVEC
Water	5,81 $\pm$ 0,20**	6,04 $\pm$ 0,18*	5,27 $\pm$ 0,15**	35,07 $\pm$ 1,82
Methanol	5,08 $\pm$ 0,12**	6,49 $\pm$ 0,20**	5,63 $\pm$ 0,31*	38,63 $\pm$ 0,61
n-Hexane	29,04 $\pm$ 1,36*	30,63 $\pm$ 1,40**	36,14 $\pm$ 1,58*	60,41 $\pm$ 1,76
Dichloromethane	42,65 $\pm$ 1,67*	45,70 $\pm$ 1,38*	45,05 $\pm$ 0,90**	48,34 $\pm$ 0,80
Doxorubicin <sup>a</sup>	0,28 $\pm$ 0,01	0,22 $\pm$ 0,01	0,40 $\pm$ 0,15	0,72 $\pm$ 0,02
DMSO <sup>b</sup>	0	0	0	0

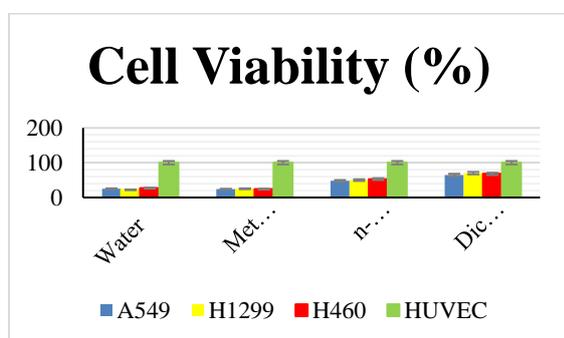
Data are shown as means  $\pm$ SD from three independent experiment (n=3).

\*\*P value of <0.01 and \*P value of <0.05 <sup>a</sup>Doxorubicin, positive control. <sup>b</sup>DMSO: negative control

As shown in MTT results among all the extracts from the *R. coriaria* L. fruits, the water and methanol extracts were found to be highly cytotoxic with IC<sub>50</sub> values in range of 5,08 – 6,49 µg/mL, while the dichloromethane extracts found low cytotoxic effect with IC<sub>50</sub> values in range of 42,65 – 45,70 µg/mL, and the n-hexane extracts found moderate cytotoxicity towards all lung cancer cell lines, comparing to HUVEC cell lines as untreated normal cell lines (IC<sub>50</sub>= 35,07 – 60,41 µg/mL), as well as, doxorubicin as positive control (IC<sub>50</sub>= 0,22 – 0,72 µg/mL), The IC<sub>50</sub> values of the water and methanol extracts of sumac were estimated ranged between 5,08 – 6,49 µg/mL and for cancer cell lines, whereas it was estimated at > 30 µg/mL for normal HUVEC cells after 48 h incubation period (Table 1). Based on the MTT test, these findings demonstrated that the sumac extracts had cytotoxicity against the cancer cells in a dose dependent manner, therefore sumac fruits could have significant potential as anti-lung cancer agent.

### Trypan Blue Assay Results

Trypan blue assay was assessed to determine antiproliferative activity of the extracts of sumac by cell counting for 24, 48, 72 h treatment with different doses (5, 10, 50 and 100 µg/mL) of the sumac extracts. Non-viable cells were visible as blue colored, while, viable cells were visible unstained under the microscope. Results of viability (%) percentage of A549, H1299, H460 lung cancer cell lines by comparing with non-tumorous HUVEC cells after 48 h treatment with the sumac extracts are shown in the Fig. 1.



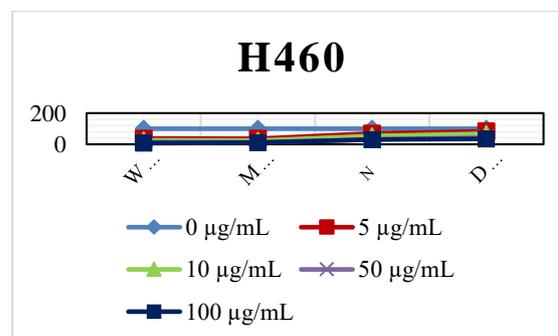
**Fig 1.** Cell Viability (%) of A549, H1299 and H460 cancer cells after 48 h treatment with 10 µg/mL concentration of the extracts.

\*Values are expressed as mean ± SD from three independent experiment (n=3). HUVEC cells were used as control and set as 100%.

As supported by MTT, results of trypan blue assay showed that cell growth and viability in cancer cell lines were inhibited by the extracts from *R. coriaria* L. fruits in a time and dose dependent manner, especially the aqueous and methanolic extracts showed significant reduction in cell growth of all the lung cancer cell lines with survival percentage of 22-27% against the cells even at the lowest concentration (5 µg/mL). However, dichloromethane extracts showed lower reduction in cell growth with 65-70% viability percentage to the treated cancer cells. This indicated that sumac extracts with water and methanol could have greater inhibitory effects on the cell growth in the treated lung cancer cell lines, and the increasing exposure time and dose resulted in decreasing the cell viability in all the cell lines.

### Lysosomal activity assay (neutral red uptake assay) Results

In this assay, lysosomal activities of the extracts from *R. coriaria* L. fruits were observed in a time and dose dependent manner against the A549, H1299 and H460 cancer cells treated with different doses (0, 5, 10, 50 and 100 µg/mL) of the extracts for 24, 48, 72 h treatment, and the results for 48 h treatment were shown in the Fig. 2 for each cell line.



**Fig 2.** Lysosomal activity (%) of A549, H1299 and H460 cancer cells after 48 h treatment.

\*Values are expressed from three independent experiment (n=3). The dose of 0 µg/mL was used as control and set as 100%.

As consistent with MTT and trypan blue assays, lysosomal activity results showed that all the extracts have significant anticancer activity against the tested cancer cells, of which the water and methanol extracts had stronger effects on lysosomal activity. Additionally, increasing extracts exposure time and dose resulted in increasing the lysosomal functions and membrane permeability in the cell lines.

**Lactate dehydrogenase (LDH) activity Results**

The release of LDH from the A549, H1299 and H460 human lung cancer cells was measured after treatment with 0, 5, 10, 50 and 100 µg/mL concentrations of sumac extracts, as well as in a time dependent manner for 24, 48 and 72 hours, respectively. LDH activity results of fruit

extracts of *R. coriaria* L. were shown in the Table 4 in regards of IC<sub>50</sub> values in a time dependent manner for 24, 48, and 72 h treatment period. Non-tumorous HUVEC cells were used as control and doxorubicin used as standard cytotoxic agent (Table 2).

**Table 2.** IC<sub>50</sub> (µg/mL) values obtained from LDH assay for A549, H1299, H460 lung cancer cell lines.  
**IC<sub>50</sub> value from LDH (µg/mL)**

Extracts	Hours	A549	H1299	H460	HUVEC
Water	24 h	7,57 ± 0,43	8,92 ± 0,50	7,64 ± 0,48	42,09 ± 1,96
	48 h	5,92 ± 0,25	6,34 ± 0,21	5,54 ± 0,24	37,01 ± 1,87
	72 h	4,07 ± 0,32	4,12 ± 0,14	4,05 ± 0,28	35,04 ± 1,90
Methanol	24 h	6,96 ± 0,35	7,43 ± 0,51	7,12 ± 0,22	43,62 ± 0,98
	48 h	5,45 ± 0,30	6,45 ± 0,23	5,57 ± 0,31	40,06 ± 1,74
	72 h	5,02 ± 0,20	5,26 ± 0,21	5,19 ± 0,12	38,38 ± 1,52
n-Hexane	24 h	19,86 ± 0,67	21,91 ± 0,88	24,10 ± 1,01	65,54 ± 2,02
	48 h	28,78 ± 1,64	31,25 ± 0,98	35,97 ± 1,29	62,01 ± 1,73
	72 h	35,01 ± 1,02	35,59 ± 0,98	36,26 ± 1,46	59,28 ± 1,60
Dichloromethane	24 h	40,61 ± 1,12	40,75 ± 1,69	39,96 ± 1,78	58,94 ± 1,86
	48 h	40,15 ± 2,03	43,09 ± 0,96	42,88 ± 1,76	55,68 ± 2,00
	72 h	48,14 ± 2,01	51,03 ± 2,05	52,86 ± 1,72	67,06 ± 2,50
Doxorubicin		0,28 ± 0,01	0,22 ± 0,01	0,40 ± 0,15	0,72 ± 0,02

\*Data are shown as means ± SD from three independent experiment (n=3).

As shown in the Table 2 the IC<sub>50</sub> values for lung cancer cells were calculated as comparing with non-tumor HUVEC cells in a time dependent manner, and a significant increasing was observed in the IC<sub>50</sub> value from LDH by depending the time for all the extracts, as supported by IC<sub>50</sub> value from MTT assay.

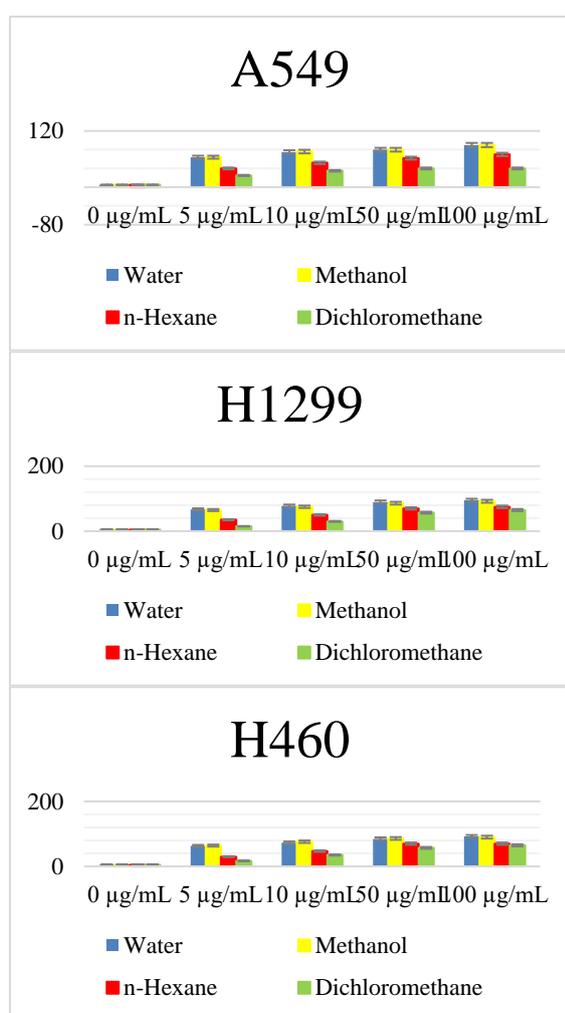
Among the extracts, the water and methanol extracts from the *R. coriaria* L. fruits were found to have high LDH activity with IC<sub>50</sub> values in range of 4,05 – 8,92 µg/mL, whereas, dichloromethane extracts found low LDH effect with IC<sub>50</sub> values in range of 39,96 – 52,86 µg/mL, and the n-hexane extracts found moderate LDH activity towards all lung cancer cell lines with IC<sub>50</sub> values in range of 19,86 – 36,26 µg/mL, comparing to HUVEC cell lines as untreated normal cell lines (IC<sub>50</sub>= 35,04 –

67,06 µg/mL), as well as, doxorubicin as positive control (IC<sub>50</sub>= 0,22 – 0,72 µg/mL) (Table 2).

Additionally, LDH release (% of total) results of the extracts were presented for each cancer cell line in a concentration dependent manner (Fig. 3). In the cancer cell lines, an increasing rate was observed in the percentage of LDH release in a dose dependent manner, as supported by MTT and lysosomal activity assays results.

In the Fig. 3, the water and methanol extracts showed greater LDH activity on all the lung cancer cell lines with LDH release percentage from 90% to 95% for treated with the concentration of 100 µg/mL extracts, comparing the other extracts. The dichloromethane extracts showed lower LDH

activity with 40-65% LDH release percentage to the treated cancer cells, and also had more effective on H1299 and H460 cells than A549 cells. LDH assay results indicated that sumac extracts especially with water and methanol could have greater effects on the cell death in the lung cancer cells, as consistent with the results of other anticancer assays.



**Fig 3.** LDH release (% of total) of A549, H1299 and H460 cancer cells after 48 h treatment.

\*Values are expressed as mean  $\pm$  SD from three independent experiment. The cells (a density of  $2 \times 10^4$  cells/well) were seeded for each treatment.

## DISCUSSION

According to global statistics on the prevalence of cancer, it has become the second most common diseases, behind cardiovascular diseases. Besides high rate of prevalence, it has become one of the top killer diseases almost all around the world, causing high rate of deaths. Therefore, it has been a major and critical public health problem in both developed and

developing countries, and it is estimated that it will probably continue to be more critical health problem in the following years (20). Among the cancer types, lung cancer is responsible for the second widespread cancer-related mortality in both men and women with increasing incidence over time (21,22).

However, there has been a variety of therapeutic approaches to treat cancer in the recent years, it seems necessary to develop more novel approaches with higher efficiency so that the disease intensity might be declined. Meanwhile, it has been reported that an increase in the incidences of drug resistant cancer and side effects has resulted in a need for new anticancer compounds with little to no side effects. In this regard, the researchers encouraged to discover novel and more effective plant-based bioactive compounds that involving in prevention and treatment various cancers, as natural anticancer agents (3,23). Thus, this research has been already examined anticancer and antiproliferative effects of the sumac extracts against the tested human lung cancer cell lines.

According to the results we obtained from this research, the sumac extracts inhibited the growth of lung cancer cells in a dose-dependent manner. It was clear that sumac fruits could have significant potential as anti-lung cancer agent. When the concentration of the sumac extract increased, the number of live cells decreased. Our results were consistent with the previously anticancer studies with sumac (1,9,24), the anticancer and growth inhibitory effects of the extracts may be due to the fact that rich secondary metabolites components of the *R. coriaria* L., as described previously (12, 25,26).

In case of trypan blue dye exclusion assay results, when the cells were exposed to sumac extracts, significantly inhibition were observed in the cell growth in a time and dose dependent manner. Whilst, the data produced from this assay points to the antiproliferation effects of the sumac extracts on exposed compared to cells that were not exposed to the extracts (1,5). This might be a positive factor for these extracts to contribute to their anticancer and antiproliferative activities. On this basis, it is suggested that sumac could offer additional benefit for its use in the management of decreasing the amount of cancer cells.

In addition to these assays, neutral red dye assay was performed which is used to analyze lysosomes activities of the cells that the dye can diffuse cellular membrane and accumulate in the lysosomes. NR penetrates the cell membrane and accumulates intracellularly in the lysosomes that leads to change in sensitivity of lysosomal membrane irreversibly. Therefore, it is possible to evaluate the number of viable cells response to the sumac extracts, which is the basis of NR assay. It can be clearly observed that the sumac extracts significantly accelerate reducing uptake of NR into the cell membrane, owing to its rich bioactive compounds such as tannins, gallic acid, pinene, flavones, anthocyanins etc. (2,9).

Furthermore, the extracts were also tested whether possess lactate dehydrogenase activity or not. LDH is a cytoplasmic enzyme that plays role in the conversion of pyruvate to lactate, this enzyme releases from the necrotic cell membranes when the cell membrane is damaged. Although, apoptosis and necrosis are two major metabolic processes observed in disease pathologies, necrosis is considered as a passive and accidental form of cell death causes the release of intracellular contents into extracellular milieu (27). Therefore, measuring the activity of LDH enzyme gives information about the percentage of dead and necrotic cells, as observed in our study.

To the best of our knowledge, there has been only a few studies so far examining anticancer activity of sumac extracts. On the other hand, this is the first evaluation of *in vitro* anticancer and antiproliferative activities of the fruit extracts from *R. coriaria* L. investigated by combining with lysosomes activities of the cells and LDH enzyme activities that releases from the necrotic cells.

## CONCLUSION

In the presented research, potential anticancer and antiproliferative activities of sumac extracts were analyzed in detail. Consequently, the extracts have been found to decrease the cell viability and increase the inhibitory effect on cell growth in a time and concentration dependent manner, even at lower concentration and minimum exposure time. These findings support the consumption of sumac has the potential in the prevention of cancer as natural sources. Although, there are some evidence in

support of anticancer activity of sumac, further researches should be conducted to identify which bioactive phytochemical(s) are responsible for these wide ranges of anticancer effects actually, and should be confirmed *in vivo* with animal models to be certain of the mechanisms underlying the potential effects of this plant part.

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## Conflict of interests

No conflicts of interest.

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