PKP
PUBLIC KNOWLEDGE PROJECT

Journal of Biological Studies
ISSN 2209-2560

JBS
An Open Access Peer Reviewed Journal

The effects of medicinal plants: "*Viscum album*, *Juniperus communis* and *Xanthium strumarium*" leaf extracts on fibroblastic L929 and BHK21 immune cells

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Received: October 15, 2021; **Accepted:** November 16, 2021; **Published online:** December 22, 2021.

Abstract: Although reports indicate that *Viscum album* and *Juniperus communis* extracts affect immune system function, their impact on fibroblastic cells proliferation remains unclear. The aim of this study was to determine the antioxidant capacity of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf aqueous and ethanol extracts and their cytotoxic effects on L929 and BHK21 cells. Antioxidant capacity of the extracts was measured using DPPH method. L929 and BHK21 cells were treated with 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of the extracts for 24 h in a complete growth medium. Cell viability was evaluated by MTT assay and the data were analyzed using one-way ANOVA. Lower concentrations (0.001, 0.01 and 0.1) mg/ml of *Viscum album* and *Junipeurs communis* leaf extracts exhibited higher antioxidant capacity and had no significant cytotoxic effects on L929 and BHK21 cells, however, higher concentrations (1 and 10) mg/ml had lower antioxidant activity and exhibited cytotoxic effects on the cells. Lower and higher concentrations of *Xanthium strumarium* leaf extracts significantly increased L929 and BHK21 cells viability. Despite higher concentrations, lower concentrations of ethanol and aqueous *Viscum album* and *Junipeurs communis* have positive effects on fibroblastic L929 and BHK21 cells viability; however, ethanol and aqueous leaf extracts of *Xanthium strumarium* have no cytotoxic effects on L929 and BHK21 cells, which therefore, can improve immune system function safer than *Viscum album* and *Juniperus communis*.

Keywords: *Viscum album*; *Juniperus communis*; *Xanthium strumar*; Antioxidant capacity; Cytotoxicity; L929; BHK21.

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1 Introduction

Fibroblasts are the most common type of cells distributed through connective tissue and play a significant role in immune responses. These cells produce and secrete collagen fibers necessary for tissue construction and maintenance (Helary et al., 2006)). Fibroblast has key role in healing wounds and repairing damaged tissues by responding to cytokines and other chemicals, fibroblasts may trigger inflammatory responses in the body. Among fibroblastic cell lines, L929 cells (human fibroblastic cells) and Baby Hamster Kidney fibroblasts (BHK21 cells) are used widely in many investigations in vitro (Stojkovic et al., 2015; Prahasanti et al., 2018).

Experimental data of past studies showed that herbal extracts including *Viscum*, *Juniper* and *Xanthium* species have been used for the treatment of various diseases by their positive effects on immune system. Among *Viscum* species, *Viscum album* L. (Loranthaceae) has long been used in traditional medicine for its immunomodulatory, hypotensive, antidiabetic, anticancer, and anti-obesity properties. Lectins, viscotoxins, flavonoids, cyclitoles, phytosterols and triterpenes are major bioactive components in *Viscum album* extract (Prendecka et al., 2016; Khan et al., 2016). *Viscum album* also contains a variety of immunoactive compounds (Gamooshi et al., 2008) and showed antioxidant activities (von Schoen-Angerer et al., 2015).

Juniperus communis is a small tree widely distributed in South Asia, and North America and has been used (and even still is used) in folk medicine as antifungal, antidiabetic, antidiuretic and anti-inflammatory medicinal plant. It has also protecting effects on liver and nervous system. The anti-inflammatory effect of *Juniperus communis* has also been reported, indicating that the plant has modulatory effects on immune system (Büssing and Schietzel, 1999). *Juniperus communis* extract has been revealed to have a significant antioxidant activity by enhancing the activity level of enzymes involved in antioxidant reactions (Modnicki and Łabędzka, 2009). Monoterpene hydrocarbons, coumarins, ascorbic acid, -pinene, camphor, flavonoids and bicyclic diterpenes are major components existing in *Juniperus communis* extract (Nanjwade et al., 2010; Höferl et al., 2014; Han and Parker, 2017).

Xanthium strumarium is a well-known medicinal plant applied for rhinitis, nasal sinusitis, headache, gastric ulcer, rheumatism, bacterial and fungal infections, and arthritis treatment Anti-tumor, anti-inflammatory, and anti-diabetic effects of *Xanthium strumarium* have also been reported, demonstrating the immunomodulatory effects of the plant (Bogolitsyn et al., 2019). Certain chemical components in *Xanthium strumarium* extract have shown a significant high level of free radical scavenging activity indicating the high anti-oxidant activity of the plant extract (Lin et al., 2014). More than 170 chemical compounds have been isolated from *Xanthium strumarium* extract including triterpenoids, phenylpropanoids, coumarins, steroids, flavonoids and thiazides (Fan et al., 2019).

Previous studies were somehow contradictory about the effects of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* aqueous and ethanolic extracts on cell proliferation. Clinical studies have shown that *Viscum album* aqueous extract induces the proliferation of immune system cells. However, in vitro studies indicated that *Viscum album* extracts may have anti-proliferative effects on certain cells. Recent observations also have demonstrated that high concentration of *Viscum album* extract can reduce cell viability. *Xanthium strumarium* aqueous and ethanolic extracts have proliferative or cytotoxic effects on cells in vitro. Stimulatory or inhibitory effects

of *Juniperus communis* on cell proliferation are also associated with the solvent used to produce the extract (Khil et al., 2007; Kan et al., 2011; Kuonen et al., 2013; Stan et al., 2013; Francisco Fernandez et al., 2019).

Although medicinal plants extracts are now widely considered as safe herbal medicines, they can have serious cytotoxic side effects on body cells including immune fibroblastic cells. Indeed, fibroblasts in immune system are the most common cells affected by cytotoxic effects of the herbal extracts. In addition, the safety of the plant extracts applied for diseases treatment is strongly affected by preparing methods of the extracts and their concentrations which is still challenging issue. Given the pivotal place of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* as well-known herbal plants in folk medicine, and highly contradictory reports on the cytotoxic effects of these plants on body cells, the present study aimed to investigate the anti-oxidant capacity of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* aqueous and hydroalcoholic extracts and also cytotoxic effects of the extracts on fibroblastic L929 and BHK21 cells in vitro to conclude whether the extracts have cytotoxic effects on fibroblast cells.

2 Materials and Methods

2.1. Plant preparation:

The fresh leaves of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* were collected from the forest located in Mazandaran province in the north of Iran. The scientific names of the plants were diagnosed by Iranian academic centre for education, culture and research. The fresh leaves of the plants were sorted out to remove extraneous material and rinsed with water to remove debris and dust particles and were air deride. Then warm distilled water and ethanol were added to powdered leaves as solvent to prepare aqueous and hydroalcoholic extract, respectively. It was then filtered using distiller and stored in refrigerator (Van Slambrouck et al., 2007).

2.2. Antioxidant assay:

Free radical scavenging potential of the extracts was measured based on their capability to scavenge the stable DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) according to a previously described method. Briefly, 1 µL DPPH solution was prepared in 999 µL methanol. Different concentration of each extract was added to 100 L of DPPH. The mixtures were shaken thoroughly and allowed to stand in dark at room temperature for 30 minutes. A color change from violet to yellow occurred during the reaction time. Sample absorbance was read at 517 nm using UV-VIS spectrophotometer and the percentage of free radical scavenging potential of the different extracts against DPPH was determined using the following equation:

$$\text{DPPH radical scavenging } \% = [(A0 - A1)/A0] \times 100$$

DPPH solution was used as a control and ascorbic acid (Vitamin C) was used as a reference standard. The fibroblastic L929 and BHK21 cell lines were obtained from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran) (Tian et al., 2017).

2.3. Cell culture:

The L929 and BHK21 cells were maintained in complete growth medium (CGM) supplemented with 10 % FBS and 1 % antibiotics (penicillin/streptomycin). The cells (1 × 106 cells/ml) were plated in T-25 flasks containing 5 ml of CGM and grown in a humidified incubator under an atmosphere of 95 % air and 5 % CO2 at 37°C to sub confluence (90-95) %. The culture medium was replaced every 48 hours. Once the cells reached 90-95 % confluences, the medium was aspirated, and the cells monolayer was washed three times with sterile phosphate buffered saline.

The cell monolayer was treated with 1 ml of 0.25 % (w/v) trypsin-EDTA and incubated briefly at 37°C and visualized microscopically to ensure complete cell detachment. Cells were re-suspended in complete growth medium. Cells were also stained with trypan blue (100 l of cell suspension and 100 l of 0.4 % trypan blue), incubated for 2 minutes at room temperature, and counted using a hemacytometer. The cells were seeded at a density of 1 × 104 cells/well in 96-well microliter tissue culture plates prior to treatment.

2.4. Cytotoxicity assay:

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed for assessing cell proliferation activity and cytotoxicity in L929 cells exposed to 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml and 1 mg/ml of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* aqueous and hydro alcoholic extracts. Cell viability was determined using the MTT assay 24 hours after incubation. The MTT assays were performed according to standard protocols. L929 cells were seeded in 96-well plates with 1 × 104 cells/well and placed at 37 °C in a 5 % CO2 humidified incubator until 60 % confluence (Sirivibulkovit et al., 2018).

The complete growth medium was removed and the cells were serum-starved for 24 h prior to treatment. Cells incubated in culture medium alone served as a control for cell viability (untreated cells). The cells were treated with 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml and 1 mg/ml of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf aqueous and hydro alcoholic extracts for 24 h in complete growth medium. The medium was then removed and 100 l of MTT solution (5 mg/mL in sterile H2O) was added to each well. The plates were incubated under 95 % atmosphere air and 5 % CO2 at 37°C for 4 h. The MTT solution was removed and 200 l aliquots of DMSO were added to each well to dissolve the formazan crystals followed by incubation for 10 min at 37 °C. Treatments were performed in triplicates, and optical densities were read at 531 nm by spectrophotometric method.

2.5. Data analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA method) followed by post hoc Tukey's multiple comparisons test in SPSS 18 software. Differences were considered significant at the $P < 0.05$ level.

3 Results and Discussions

3.1. Evaluation of antioxidant capacity of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf ethanol and aqueous extracts:

Antioxidant capacity aqueous and ethanol *Viscum album* leaf extracts (0.001, 0.01, 0.1 and 1) mg/ml significantly increased compared to vitamin C, however, antioxidant capacity of higher concentration of aqueous and ethanol extracts (10 mg/ml) significantly decreased and non-significantly increased compared with vitamin C, respectively. There was no significant difference in antioxidant capacity between 0.001 mg/ml, 0.01 mg/ml and 0.1 mg/ml of aqueous or ethanol *Viscum album* leaf extracts (Table 1). Although antioxidant capacity of 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml and 1 mg/ml of aqueous and ethanol *Juniperus communis* and *Xanthium strumarium* leaf extracts significantly increased compared to vitamin C, it did not significantly change in 10 mg/ml of aqueous or ethanal extracts compared with vitamin C. Significant difference was not observed in antioxidant capacity between 0.001, 0.01 and 0.1 mg/ml of aqueous or ethanol *Juniperus communis* and *Xanthium strumarium* leaf extracts (Tables: 2 and 3). There was not also significant difference in antioxidant capacity of 0.001, 0.01 and 0.1 mg/ml concentrations between aqueous and ethanol *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf extracts and antioxidant capacity of 1 and 10 mg/ml of aqueous and ethanol extracts was significantly lower than other concentrations (Tables: 1, 2 and 3).

Table 1. Antioxidant capacity of vitamin C, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of ethanol and aqueous *Viscum album* leaf extract.

Viscum album/Vitamin C	Mean±SEM (ethanol extract)	*P*	Mean±SEM (aqueous extract)	*P*
Vitamin C	0.26±0.01	-	0.26±0.01	-
-	-	$P< 0.001$ ***	-	$P< 0.001$ ***
0.001 mg/mL	1.42±0.05		1.11±0.04	
-	-	$P< 0.001$ ***	-	$P< 0.001$ ***
0.01 mg/ml	1.48±0.04		1.18±0.07	
-	-	$P< 0.001$ ***	-	$P< 0.001$ ***
0.1 mg/ml	1.54±0.04		1.21±0.06	
-	-	$P< 0.001$ ***	-	$P< 0.001$ ***
1 mg/ml	0.64±0.02		0.82±0.04	
-	-	$P< 0.05$ *	-	-
10 mg/ml	0.1±0.03		0.50±0.02	N.S

Table 2. Antioxidant capacity of vitamin C, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of ethanol and aqueous *Junipers communis* leaf extract.

Junipers communis/Vitamin C	Mean±SEM (ethanol extract)	*P*	Mean±SEM (aqueous extract)	*P*
Vitamin C	0.26±0.01	-	0.26±0.01	-
- 0.001 mg/ml	- 1.43±0.05	$P< 0.001$ ***	- 1.13±0.04	$P< 0.001$ ***
- 0.01 mg/ml	- 1.50±0.03	$P< 0.001$ ***	- 1.20±0.02	$P< 0.001$ ***
- 0.1 mg/ml	- 1.54±0.04	$P< 0.001$ ***	- 1.22±0.05	$P< 0.001$ ***
- 1 mg/ml	- 0.68±0.07	$P< 0.001$ ***	- 0.85±0.05	$P< 0.001$ ***
10 mg/ml	0.29±0.04	N.S	0.50±0.03	N.S

Table 3. Antioxidant capacity of vitamin C, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of ethanol and aqueous *Xanthium strumarium* leaf extract.

Xanthium strumarium/Vitamin C	Mean±SEM (ethanol extract)	*P*	Mean±SEM (aqueous extract)	*P*
Vitamin C	0.26±0.01	-	0.26±0.01	-
- 0.001 mg/ml	- 1.38±0.07	$P< 0.001$ ***	- 1.05±0.06	$P< 0.001$ ***
- 0.01 mg/ml	- 1.46±0.07	$P< 0.001$ ***	- 1.10±0.04	$P< 0.001$ ***
- 0.1 mg/ml	- 1.52±0.05	$P< 0.001$ ***	- 1.16±0.07	$P< 0.001$ ***
- 1 mg/ml	- 0.60±0.02	$P< 0.001$ ***	- 0.75±0.02	$P< 0.001$ ***
10 mg/ml	0.10±0.02	N.S	0.40±0.04	N.S

3.2. The cytotoxic effects of ethanol and aqueous *Viscum album* leaf extract on L929 and BHK21 cells:

Lower concentrations (0.001, 0.01 and 0.1) mg/ml of *Viscum album* ethanol and aqueous leaf extract did not exhibit significant cytotoxic effects on L929 and BHK cells compared to control (untreated) groups. 1 mg/ml of ethanol and aqueous extract had only significant cytotoxic effects on L929 cells. However, higher concentration (10 mg/ml) of *Viscum album* ethanol and aqueous leaf extract significantly decreased viability of both L929 and BHK21 cells (Figure 1: 1a and 1b).

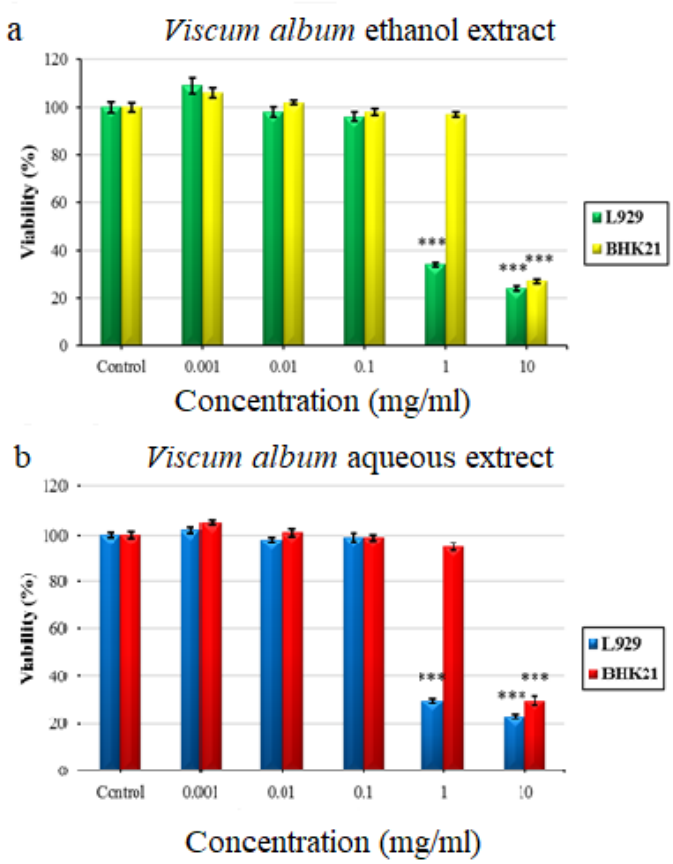

Figure 1: (a) Viability of L929 and BHK21 cells exposed to control, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of ethanol *Viscum album* leaf extract in cell culture. (b) Viability of L929 and BHK21 cells exposed to control, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of aqueous *Viscum album* leaf extract in cell culture (b). *** indicates significant difference compared with control group at $P<$ 0.001.

3.3. The cytotoxic effects of ethanol and aqueous *Juniperus communis* leaf extract on L929 and BHK 21 cells:

L929 cells viability significantly increased when exposed to 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml and 1 mg/ml of *Juniperus communis* ethanol and aqueous leaf extracts. Although viability of L929 cells significantly increased, BHK21 viability significantly decreased when exposed to 1 mg/ml of aqueous and ethanol extracts. Higher concentration (10 mg/ml) of *Juniperus communis* ethanol and aqueous leaf extracts had significantly severe cytotoxic effects on both L929 and BHK21 cells (Figure 2: 2a and 2b).

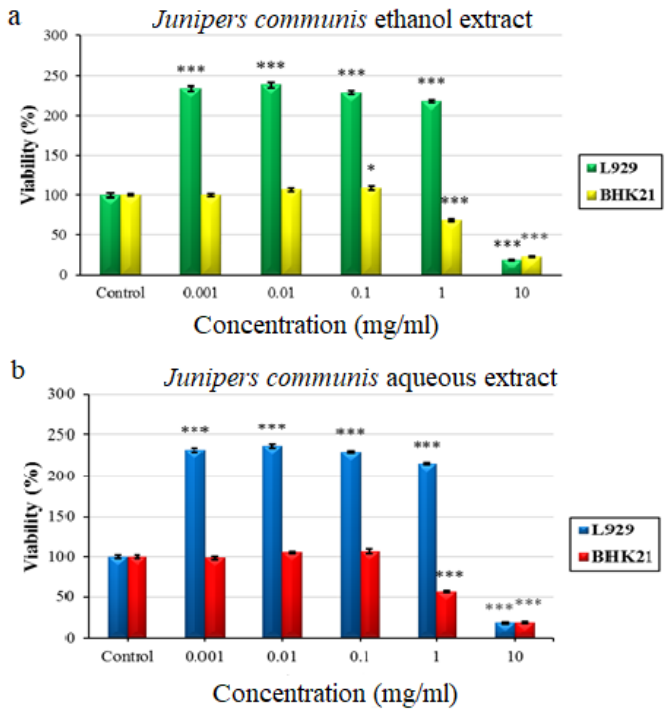

Figure 2: (a) Viability of L929 and BHK21 cells exposed to control, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of ethanol *Junipers communis* leaf extract in cell culture. *** and * indicate significant difference compared with control group at $P < 0.001$ and $P < 0.05$. (b) Viability of L929 and BHK21 cells exposed to control, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of aqueous *Junipers communis* leaf extract in cell culture. * indicates significant difference compared with control group at (***: $P < 0.001$; *: $P < 0.01$).

3.4. The cytotoxic effects of ethanol and aqueous *Xanthium strumarium* leaf extract on L929 and BHK21 cells:

BHK21 and L929 cells viability significantly increased when exposed to lower (0.001, 0.01, 0.1)mg/ml or higher (1 and 10)mg/ml concentrations of *Xanthium strumarium* ethanol and aqueous leaf extracts (Figure 3: 3a and 3b).

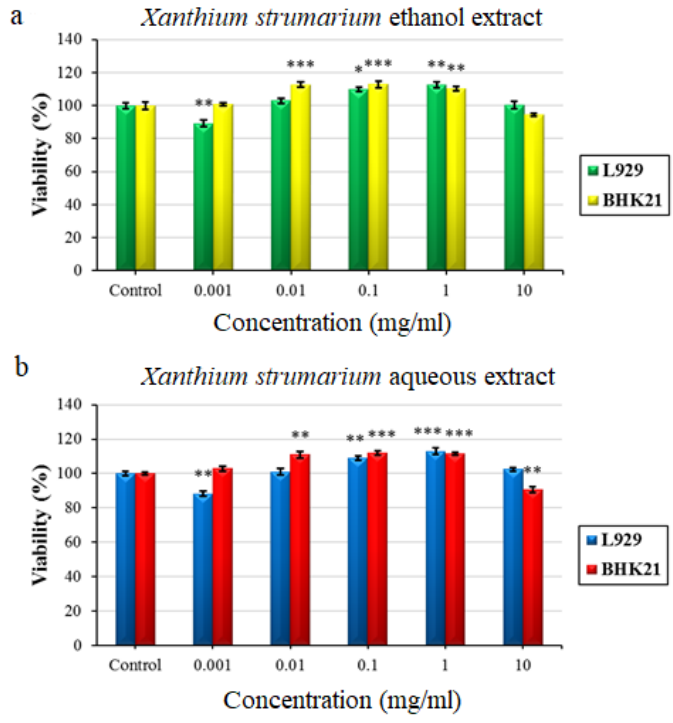

Figure 3: (a) Viability of L929 and BHK21 cells exposed to control, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of ethanol *Xanthium Strumarium* leaf extract in cell culture. ***, ** and * indicates significant difference compared with control group at $P < 0.001$, $P < 0.01$ and $P < 0.05$. (b) Viability of L929 and BHK21 cells exposed to control, 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml of aqueous *Xanthium Strumarium* leaf extract in cell culture. *** and ** indicates significant difference compared with control group at $P < 0.001$ and $P < 0.01$, respectively.

Challenges on applying plant extracts in medicine and their safety continues to be a controversial issue among scientists all over the world. Although practitioners in traditional medicine strongly believe in safety of medicinal plants for treatment of the diseases in patients, recently it has been strongly argued by researchers that medicinal plants can have serious cytotoxic effects on body if are not prepared or used properly and with safe treatment dosage. In this context, we evaluated the anti-oxidant capacity and cytotoxic effects of well-known medicinal plants (*Viscum album*, *Juniperus communis* and *Xanthium strumarium*) aqueous and ethanol extracts on fibroblastic L929 and BHK21 cells in vitro to determine the safe dosages of the extracts having higher anti-oxidant capacity and lower cytotoxic effects on immune cells.

3.5. The antioxidant capacity of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf extracts:

Our findings indicated that lower concentrations of ethanol and aqueous *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf extracts had higher or similar antioxidant capacity compared to vitamin C. However, higher concentrations of ethanol and aqueous leaf extracts had lower antioxidant capacity than vitamin C. In line with our findings, previous studies also have reported the antioxidant capacity of *Viscum*, *Juniperus* and *Xanthium* species. It has been found that methanolic extracts of *Viscum album* ssp. grown on different host trees exhibit a wide range of antioxidant activity. However, the antioxidant capacity of the plant differed according to the harvesting time as well as the host tree (Van Meerloo et al., 2011). Years of scientific research show that *Viscum album* extract, which contained the highest amount of flavonoid compounds, showed strong anti-oxidant capacity. The antioxidant capacity of *Viscum album* extract is attributable to its components particularly caffeic acid and lyoniresinol, which have been shown to exhibit strong anti-oxidative activity (ÖnayUçar et al., 2006). *Juniperus communis* aqueous and hydroalcoholic extracts have also been shown to have in vitro antioxidant properties (Kim et al., 2016). In vitro antioxidant effect of Juniperus extract is exerted by increasing the radical scavenging activity and prevention of radical formation (Elmastaş et al., 2006). In vivo studies also demonstrated the antioxidant property of *Juniperus communis* extract which was modulated by increasing activity of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase (Nanjwade et al., 2010). Antioxidant capacity of *Xanthium strumarium* extracts has been reported in studies carried out in vitro and in vivo. Recently it has been shown that *Xanthium strumarium* extract has radical scavenging activity comparable to ascorbic acid (Ben Mrid et al., 2019). *Xanthium strumarium* is a potential source of antioxidants and the antioxidant activity may also be due to the presence of flavonoid and phenolic compounds. In contrast to our findings, there is a report indicating that *Xanthium strumarium* is poisonous to some mammals (El-Gawad et al., 2019; Jiang et al., 2021). In our study, higher concentrations of ethanol and aqueous extracts of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf extracts showed lower antioxidant capacity compared to vitamin C. This finding suggests that higher concentrations of the extracts not only do not increase the antioxidant capacity but also decreases the antioxidant capacity. Therefore, it is of significant importance to use precisely determined doses of *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf extracts for treatment of the diseases.

3.6. The cytotoxic effects of *Viscum album* and *Juniperus communis* leaf extracts on fibroblastic L929 and BHK21 cells:

We have shown that lower doses of *Viscum album* and *Juniperus communis* leaf extracts exhibited higher antioxidant capacity, and therefore have no cytotoxic effects on fibroblastic L929 and BHK21 cells in vitro, however, higher doses, which possess lower antioxidant activity, have significant cytotoxic effects on L929 and BHK21 cells. Recently, the association between antioxidant capacity and cell viability has been demonstrated in different types of cell lines (Witte et al., 1990). The previous studies have suggested that antioxidant activity may be necessary to support the viability of various cells including immune cells and therefore, when the antioxidant capacity reduces, the cytotoxic effect increases (e.g., Elluru et al., 2008; Hawk et al., 2016; Medina et al., 2017). Also, some research that were aimed to determine the effects of *Viscum album* extracts on immune system and immune cells, have revealed that *Viscum album* extracts can influence immune cells function. A research have reported that *Viscum album* extract has immune-stimulatory effect and can induce innate immune response. The findings also indicate that *Viscum album* can enhance humoral and cellular immune responses (Park et al., 2012). In addition, association between *Viscum album* extract application and better immune cells function and quality of life has been observed, indicating that *Viscum album* extract can induce immune cells to strengthen immune system function in human being (Harikrishnan et al., 2011).

Fully comparable to our findings, the results of previous experiments have shown that different concentrations of *Viscum album* extracts have contradictory effects on immune cells. It has been reported that despite higher concentrations, lower doses of *Viscum album* can positively enhance immune cells function (Buessing et al., 2008). Fibroblasts viability also increases in response to appropriate concentrations of *Viscum album*, however, the toxic range of concentrations have suppressive effects on migration of fibroblasts (Höferl et al., 2014). Differential effects of *Viscum album* preparations have been reported on the maturation and activation of immune cells (Lyu and Park, 2007). *Juniperus communis* compounds have been found to affect signaling pathways involved in inflammation and immune response by acting on fibroblasts. However, higher concentrations of *Juniperus communis* extracts had cytotoxic effects on cells in vitro. In a study, it was shown that higher concentration (0.3mg mL1 of post-distillation waste) of *Juniperus communis* had genotoxic effects on lung fibroblast cells38. However, anti-inflammatory effects of *Juniperus communis* extract indicate that the extract can have positive effects on fibroblast viability and function when applied with safe dosage (Saha et al., 2016).

Bioactive compounds in *Viscum album*, *Juniperus communis* and *Xanthium strumarium* leaf extracts mediate the proliferative or cytotoxic effects of the extracts on cells. Lectin in *Visum album* extract is a component referred to as a strong inducer of immune cells. Viscotoxin is a compound in *Visum album* extract that possesses significant cytotoxic effects (Vasilijević et al., 2018). Phytochemical studies have shown that phenolic compounds, such as quercetin, rutin, naringin, catechins, caffeic acid, gallic acid, and chlorogenic acid, and flavonoids found in *Visum album* ethanol extract have high level of antioxidant activities and can induce proliferative pathways in immune cells such as fibroblasts (Nazaruk and Orlikowski, 2016; Alkhedaide, 2019).

Juniperus communis leaves contain the cupressuflavone, hinokiflavone, biflavones, isocryptomerin amentoflavone, and sciadopitysin. These compounds are active chemical constituents which contribute to viability of immune cells. Indeed, *Viscum album* and *Juniperus communis* leaf extracts contain both cytotoxic and cytoprotective constituents. In our study, due to higher antioxidant activity in lower concentrations of the extracts, cytoprotective effects of lower concentrations were significant on fibroblastic cells, resulting in enhanced or normal viability. However,

lower antioxidant activity at higher concentrations caused cytotoxic effects on fibroblastic cells (Kang, 2016).

3.7. The cytotoxic effects of *Xanthium strumarium* leaf extracts on fibroblastic L929 and BHK21 cells:

It was the most interesting finding of the present study that lower and higher concentrations of *Xanthium strumarium* aqueous and ethanol leaf extracts did not show cytotoxic effects and caused an increase in viability of L929 and BHK21 cells at a very significant range. *Xanthium strumarium* has been found to have stimulatory effects on immune cells which is in part modulated by acting on fibroblasts. It has been shown that *Xanthium strumarium* extract can inhibit the production of interleukin-17, which may be a beneficial result for the treatment of inflammatory disorders (Bais et al., 2014). Non-toxic concentrations of *Xanthium strumarium* ethanol extract has also shown anti-inflammatory effects, indicating that the extract can have modulatory effect on immune cells including fibroblasts (Kim and Choe, 2009).

Although the aerial parts of *Xanthium strumarium* contain alkaloids, xanthatin and carboxyatractyloside which may act as toxic compounds, the *Xanthium strumarium* extracts are rich in flavonoids including ononin, quercetin, allopatuletin, patuletin-3-glucuronide, quercetin-3-O-glucuronide and formononetin which have a significant antioxidant activity and can increase cell viability (Jung, 2018).

Further phytochemical analysis and molecular studies are required to determine the exact mechanisms by which *Viscum album*, *Junipeurs communis* and *Xanthium strumarium* leaf extracts act on fibroblastic L929 and BHK21 cell in vitro. Although higher concentrations of *Xanthium strumarium* leaf extract had lower antioxidant capacity, it seems that bioactive compounds in *Xanthium strumarium* leaf extract follow mechanisms other than antioxidant activity process to increase the viability of fibroblastic cells.

Conclusion

Despite higher concentrations, lower concentrations of ethanol and aqueous *Viscum album* and *Juniperus communis* leaf extracts possess higher antioxidant capacity than vitamin C and increase the viability of fibroblastic L929 and BHK21 cells which can have positive effects on immune system. However, higher concentrations of *Viscum album* and *Juniperus communis* leaf extracts has cytotoxic effects on fibroblastic cells which in turn can weaken immune system function. Unlike *Viscum album* and *Junipeurs communis*, ethanol and aqueous leaf extracts of *Xanthium strumarium* enhances viability and has no cytotoxic effects on of fibroblastic L929 and BHK21 cells indicating that this medicinal plant can improve immune system function safer than *Viscum album* and *Juniperus communis*.

Acknowledgments

This research was financially supported by Global Research, Education, and Event Network (GREEN). The authors would like to thank Javid Biotechnology Laboratory staff for their assistance.

Conflict of interests

The authors declare that there are no competing interests.

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