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ORIGINAL ARTICLE / ÖZGÜN MAKALE

CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF *ALCHEMILLA MOLLIS* (BUSER) ROTHM. AND ITS CONTITUENTS; HYPEROSIDE AND ISOQUERCETIN

ALCHEMILLA MOLLIS (BUSER) ROTHM. İLE BİLEŞENLERİ HİPEROZİT VE İZOKERSETİNİN SİTOTOKSİK VE ANTİOKSİDAN AKTİVİTELERİ

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ABSTRACT

Objective: *Alchemilla mollis (Buser) Rothm. (Rosaceae) is widely distributed in Europe, North Anatolia, Caucasus, North Iran. A commercial drug, "Herba Alchemillae" is obtained from aerial parts of A. mollis, is used for its astringent, diuretic, antispazmodic properties as well as for treatment of excessive menstruation and wounds in Bulgarian and Turkish folk medicine. Previous studies have reported that hyperoside, isoquercetin, miquelianin, cis-and trans-tiliroside, sinocrassoside D2 and rhodiolgin were detected in A. mollis. Present study is aimed to evaluate in vitro antioxidant effects and cytotoxic activities of A.mollis methanolic extract and its constituents, hyperoside and isoquercetin, on K562 leukemia cell line.*

Material and Method: *Spectrophotometric MTT assay and NO radical scavenging assay have been used to test the cytotoxic effects and antioxidant activities, respectively.*

Result and Discussion: *Results showed that A.mollis methanolic extract reduced cell viability of K562 cells at concentrations higher than 0.02 mg/ml whereas the compounds did not exhibit any cytotoxicity at same*

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concentration range. Additionally, significant inhibition on NO radical production was observed for all tested materials.

Keywords: *Alchemilla mollis, antileukemic activity, antioxidant activity, hyperoside, isoquercetin*

ÖZ

Amaç: *Alchemilla mollis (Buser) Rothm. (Rosaceae) Avrupa, Kuzey Anadolu, Kafkasya ve Kuzey İran'da geniş yayılış göstermektedir. A.mollis'in toprak üstü kısımlarından elde edilen ticari ilaç "Herba Alchemillae" Bulgar ve Türk halk tıbbında aşırı adet görme ve yara tedavisinin yanı sıra anstrenjan, diüretik, antispazmodik özellikleri için de kullanılmaktadır. Yapılan çalışmalarda A.mollis'te hiperozit, izokersetin, mikuelianin, cis- ve trans-tilirozit, sinokrassozit D2 ve rodiolgin tespit edildiği bildirilmiştir. Bu çalışmanın amacı A.mollis'in metanollü ektresi ile içerdiği bileşikler, hiperozit ve izokersetinin K562 lösemi hücre hattındaki sitotoksik aktiviteleri ile in vitro antioksidan aktivitelerini değerlendirmektir.*

Gereç ve Yöntem: *Sitotoksik aktiviteyi test etmek için MTT testi, antioksidan aktiviteyi değerlendirmek için NO radikali süpürücü test kullanılmıştır.*

Sonuç ve Tartışma: *Çalışma sonuçları A.mollis'in metanollü ekstresinin 0.02 mg/ml'den daha yüksek konsantrasyonlarda K562 hücrelerinin hücre canlılığını azaltırken, bileşiklerin aynı konsantrasyon aralığında herhangi bir sitotoksisite sergilemediğini göstermiştir. Ayrıca, test edilen tüm materyallerin NO radikali üretimini önemli ölçüde inhibe ettiği görülmüştür.*

Anahtar kelimeler: *Alchemilla mollis, antilösemik aktivite, antioksidan aktivite, hiperozit, izokersetin*

INTRODUCTION

Alchemilla genus is one of the largest genera of Rosaceae family with more than 1000 species over the World and 77 species in Turkey [1]. *Alchemilla mollis* (Buser) Rothm. is widely distributed in Europe, North Anatolia, Caucasus and North Iran and grows naturally in north and north-eastern Anatolia in Turkey [2, 3]. *Alchemilla* species are known as fındık otu, aslanpençesi, aslan ayağı, dokuztepe, yeditepe, locally [3, 4].

Alchemilla genus has been used against various diseases such as wounds, eczema, erythema, diabetes, gastrointestinal and gynecological disorders, inflammation, diarrhea, asthma, bronchitis and cough [5-13]. Aerial parts of *Alchemilla*, which is used for therapeutic purposes, have been reported to have rich phytochemical content including tannin, flavonoid, proatocyanidin, terpene and the other phenolic compounds [14-17]. Additionally, the angioprotective, antioxidant, antiviral, anticancer, wound healing and antimicrobial activities of *Alchemilla* species have been shown in different studies [14, 17].

A commercial drug, "Herba Alchemillae" is obtained from the aerial parts of *A. mollis*, is used for its astringent, diuretic and antispazmodic properties as well as for treatment of excessive menstruation and wounds in Bulgarian and Turkish folk medicine [15]. Previous studies have reported that hyperoside, isoquercetin, cis-and trans-tiliroside, sinocrassoside D2 and rhodiolgin were detected in *A. mollis* [17]. Our study aimed to test the cytotoxic and antioxidant activities of *A. mollis* and evaluate the role of its constituents; hyperoside and isoquercetin for the potential activities.

MATERIAL AND METHOD

Plant Material and Extraction

Aerial parts of *A.mollis* were collected from Sarıyar village, Sivas, Turkey and identified by Hayri Duman. Voucher specimens are deposited in Cumhuriyet University, Faculty of Science Herbarium (CUFH 1344). The plant parts were dried at room temperature then powdered. Powdered material was extracted by macerating with methanol for 24 hours followed by stirring in ultrasonic bath during 1 hour. Methanolic extract was evaporated under vacuum at 40-45°C to obtain crude extract after filtrating [18].

Cytotoxic Activity

Dried extract of *A. mollis*, hyperoside and isoquercetin were dissolved in DMSO. K562 cells were seeded to 24 well plates for incubation with different concentrations of *A. mollis* extract, hyperoside and isoquercetin dissolved in DMSO, cells without treatment were also incubated with the same concentration of DMSO. After 24 hours of incubation, cells were visualized under a light microscope and cellular morphology was examined. Besides examination of cell morphology, cell viability was determined with MTT assay. Briefly, cells were incubated with 5 mg/ml MTT solution. After 4 hours of incubation with MTT agent, formed formazan crystals were dissolved and absorbance at 550 nm was measured [19].

Antioxidant Activity

To analyse $NO^{\bullet}$ scavenging activity of extracts, a reaction mixture was prepared with SNP (5mM) in phosphate buffered saline (pH 7.3) with stock solutions (62.5 ug/ml, 125 ug/ml, 250 ug/ml, 500 ug/ml, 1000 ug/ml, 3000 ug/ml) of *A. mollis* extract, hyperoside and isoquercetin in DMSO. The mixture was incubated at 25^0C for 3 hours and then mixed with equal volume of Griess agent (1% sulphanilamide in 5% phosphoric acid and 0.1% N-naphthylethylenediamine dihydrochloride) before measuring absorbance at 550 nm [20]. Data obtained from NO scavenging assay were analyzed using Graph Pad Prism 7.00 software. Analysis of variance (ANOVA) and post-hoc Tukey test were used to compare means, and values were considered significant at $p<0.05$.

RESULT AND DISCUSSION

After incubation of K562 cells with extract, cells were visualized under the light microscope and it was found that the number of viable cells in *A. mollis* treated group was lower than non-treated group and there was significant difference in cell morphology (Figure 1). Cell viability and morphology was not significantly different between non-treated group and hyperoside treated cells.

Then we checked the antioxidant effects of *A. mollis* extract and its constituents. We found that *A. mollis* extract, isoquercetin and hyperoside inhibited SNP induced NO• production significantly ($p<0.05$) at all concentrations (62.5 ug/ml-3000 ug/ml) tested (Figure 3-5). Significant dose dependent inhibition of nitrite levels was also found between some of the doses tested. Briefly, differences between cells treated with *A. mollis* extract were significant except 62.5 ug/ml vs. 125 ug/ml treated, 125 ug/ml vs. 250 ug/ml treated, 250 ug/ml vs. 500 ug/ml treated, 500 ug/ml vs. 1000 ug/ml treated, 1000 ug/ml vs. 3000 ug/ml treated groups. For isoquercetin treated gropus, differences between groups were significant except 62.5 ug/ml vs. 125 ug/ml treated, 125 ug/ml vs. 250 ug/ml treated, 250 ug/ml vs. 500 ug/ml treated, 250 ug/ml vs. 1000 ug/ml treated and 1000 ug/ml vs. 3000 ug/ml treated groups. Finally for hyperoside, significant differences were found for all groups except 62.5 ug/ml vs. 125 ug/ml treated, 250 ug/ml vs. 500 ug/ml treated, 500 ug/ml vs. 1000 ug/ml treated, 500 ug/ml vs. 3000 ug/ml treated and 1000 ug/ml vs. 3000 ug/ml treated groups. Results of the MTT assay showed that *A. mollis* treatment (≥0.02 mg/ml) decreased viability of K562 cells significantly (Figure 2).

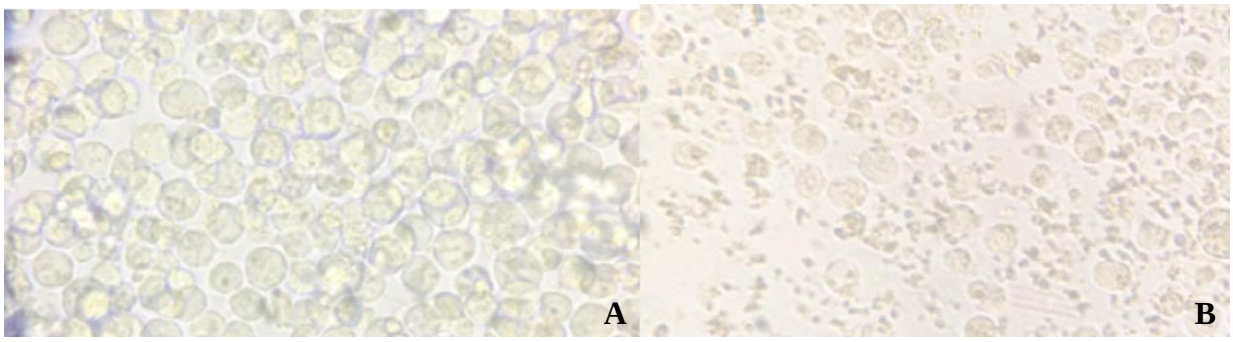

Figure 1. A: Cells without treatment, B: Cells treated with *A. mollis* (0.04 mg/ml)

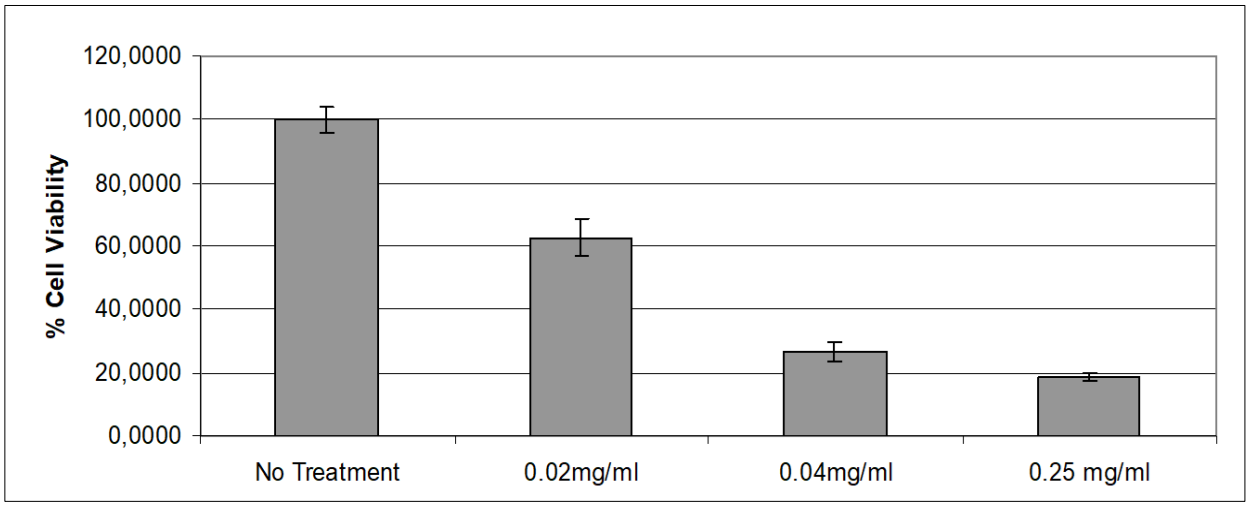

Figure 2. Cell viability of K562 cells treated with or without different concentrations of *A. mollis*

Cell viability decreased significantly ($p\leq 0.05$) at concentrations of *A. mollis* higher than 0.02 mg/ml.

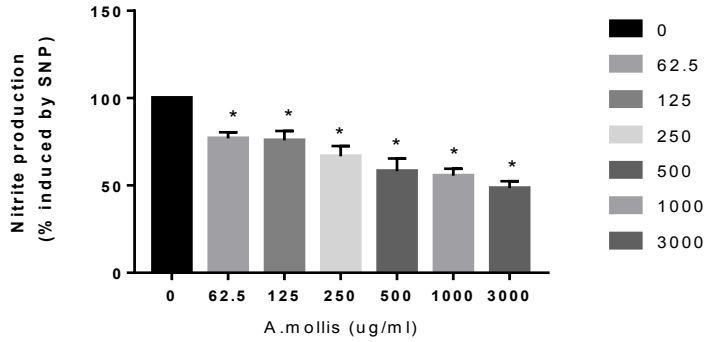

Figure 3. NO• scavenging activity of *A. mollis*

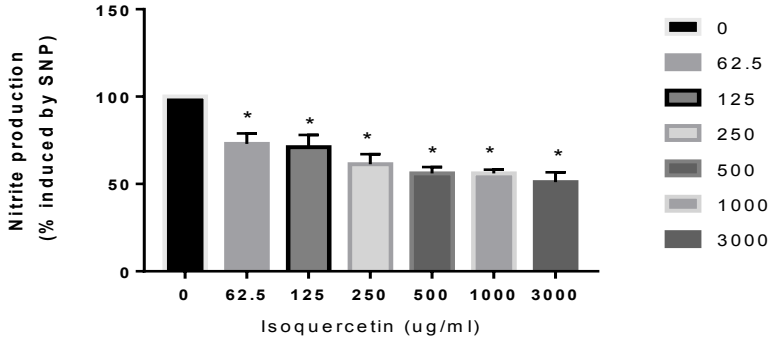

Figure 4. NO• scavenging activity of isoquercetin

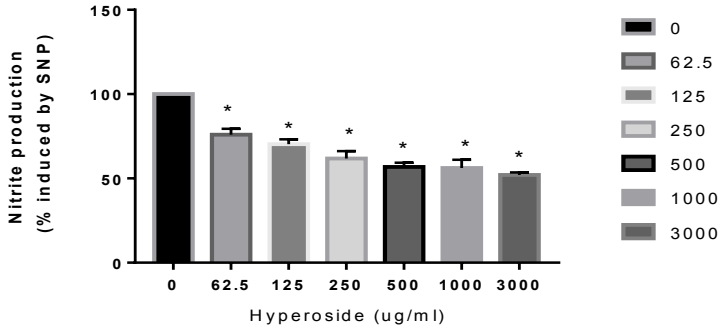

Figure 5. NO• scavenging activity of hyperoside

In a previous study, it was reported that the *A. mollis* water extract displayed significant cytotoxic effect between the 62.5-1000 µg/mL concentrations on MCF7 breast cancer cell line. When compared to deodorized water and methanol extracts. The IC_{50} value of the water, deodorized water and methanol extracts were determined as 59.34±3.41 µg/mL, 87.37±25.15 µg/mL and 68.18±6.12 µg/mL, respectively. Phenolic content of the extracts is considered as responsible for cytotoxicity by the reason of correlation was established between the extracts and the amount of phenolic contents and extract which contains phenolic compound in high amount exhibited the highest cytotoxic activity. It has been suggested that phenolic content is effective on the cytotoxic activity with the mechanisms of radical

scavenging properties. Phenolics display several different mechanisms against the free radicals such as scavenging the free radicals to terminate the radical chain reaction, absorbance of reactive oxygen species (ROS), chelating transition metals, interfering with ROS producing enzymes and stimulating the anti-oxidative enzyme activities. Furthermore, phenolic compounds may act as anti-proliferative agents due to their ability to induce cell cycle arrest, apoptosis, destruction of mitotic spindle formation and inhibit angiogenesis [21].

Phenolic compounds are considered as important secondary metabolites for their chemopreventive and chemotherapeutic effects in cancer. The antioxidant potential of phenolic compounds is almost bolded in the treatment and prevention of cancer [22].

Antioxidant capacity of compounds is associated with mechanisms such as scavenging reactive oxygen species, donating hydrogen atoms or electrons, chelating metal cations, and enhancing the production of antioxidant enzymes and thus acting at a prevention level [23].

Phenolic compounds which display antioxidant activity are among the most abundant bioactive phytochemicals found in our diet which act as natural cancer chemopreventive agents. Generally, their anticancer activities have been attributed to their antioxidant properties, antiproliferative effects, and the activation and/or inhibition of some subcellular signaling pathways, namely apoptosis and cell cycle progression [24].

In conclusion, our results exhibit significant cytotoxic effect for *A. mollis* and significant $NO^{\bullet}$ scavenging activity for *A. mollis* and its constituents isoquercetin and hyperoside. Further research may reveal which constituents of *A. mollis* are responsible for the inhibition of cell viability of K562 cells.

AUTHOR CONTRIBUTIONS

Concept: *Ö.B.A.*; Design: *Ö.B.A.*; Control: *M.T., Ö.B.A.*; Sources: *A.K., A.Z.K., M.T., Ö.B.A.*; Materials: *A.K., A.Z.K., M.T., Ö.B.A.*; Data collection and/or processing: *A.K., E.K., A.Z.K., Ö.B.A.*; Analysis and/or interpretation: *A.K., A.Z.K., Ö.B.A.*; Literature review: *E.K., Ö.B.A.*; Manuscript writing: *E.K., Ö.B.A*.; Critical review: *A.K., E.K., A.Z.K., M.T., Ö.B.A.*; Other: *M.T., Ö.B.A*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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