

In Vitro Antioxidant Property of *Convolvulus aucheri* and Its NO Inhibitory Potential in LPS-activated NSCLC Cells

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ABSTRACT: Latterly, scientists have turned into progressively curious about therapeutic herbs. A lot of *Convolvulus* taxa display different biologic activities. In this study, we aimed to evaluate nitric oxide (NO) inhibitive effect of *Convolvulus aucheri* in LPS-induced non-small cell lung cancer (NSCLC) cells, such as HCC78 and H1975 and determine its antioxidant capacity. While Griess assay was applied to determine the nitrite level as an index of NO production, β -carotene/linoleic acid test was used for the determining antioxidant effect. Cytotoxic activity of *C. aucheri* extract was detected by using CellTiter-Glo assay. The extract induced a bigger cytotoxic activity on H1975 cells than the HCC78 cells and decreased NO production in concentration-dependent manner. The highest inhibitory potential against NO formation was observed to be 113.06 μ M nitrite at a concentration of 40 μ g/mL in HCC78 cells. As for the antioxidant activity, *C. aucheri* showed the highest antioxidant activity of 70.89%. These data bring to mind that *C. aucheri* may be a useful source for exploration new anticancer compounds.

Keywords: Convolvulaceae, *C. aucheri* extract, lipopolysaccharide, lung cancer, NO assay

Convolvulus aucheri'nin In vitro Antioksidan Özelliği ve LPS-aktif NSCLC Hücrelerinde NO Engelleyici Potansiyeli

ÖZET: Son zamanlarda, bilim adamları tedavi edici bitkiler hakkında giderek daha fazla ilgili hale geldiler. Birçok *Convolvulus* taksonu farklı biyolojik aktiviteler göstermektedir. Bu çalışmada, *Convolvulus aucheri*'nin LPS ile uyarılmış HCC78 ve H1975 gibi küçük hücre dışı akciğer kanseri (KHDAK) hücrelerinde nitrik oksidi (NO) inhibe edici etkisini değerlendirmeyi ve antioksidan kapasitesini belirlemeyi amaçladık. NO üretiminin bir göstergesi olan nitrit düzeyini belirlemek için Griess testi uygulanırken, antioksidan etkinin belirlenmesi için ise β -karoten/linoleik asit testi kullanıldı. *C. aucheri* ekstraktının sitotoksik aktivitesi, CellTiter-Glo deneyi kullanılarak tespit edildi. Ekstrakt H1975 hücrelerinde HCC78 hücrelerinden daha büyük bir sitotoksik aktiviteye sebep olmuştur ve NO üretimini konsantrasyona bağlı olarak azaltmıştır. NO oluşumuna karşı en yüksek inhibitör potansiyelin, HCC78 hücrelerinde 40 μ g/mL konsantrasyonda 113.06 μ M nitrit olduğu gözlenmiştir. Antioksidan aktiviteye gelince, *C. aucheri* en yüksek antioksidan aktivitesini %70.89 ile göstermiştir. Bu veriler, *C. aucheri*'nin yeni antikanser bileşiklerinin araştırılması için yararlı bir kaynak olabileceğini akla getirmektedir.

Anahtar Kelimeler: Convolvulaceae, *C. aucheri* ekstraktı, lipopolisakkarit, akciğer kanseri, NO testi

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INTRODUCTION

Since ancient times, plants have been utilized by humans for remedial goals and have shaped the source of a lot of medications used in modern eras. The bioactive compounds obtained from plants have been used in medicine for different curative cares since old times (Schmidt et al., 2008; Mammadov, 2014). A wide range of significant plants from Convolvulaceae family have been utilized for curing lots of disorders with regard to their secondary metabolites (Jacobs and NRCS, 2007). Alkaloids, flavonoids, coumarins, sterols, saponins, resin glycosides, tannins and stilbene derivatives have been isolated from plants of the genus *Convolvulus* L. (Noda et al., 1990; Todd et al., 1995; Menemen et al., 2002).

The genus *Convolvulus* L. belongs to Convolvulaceae family, including 250 taxa, generally recognized as bindweeds. With respect to recent researches, this genus is represented with 35 species in Turkey (Güner et al., 2012). Extracts of various members of *Convolvulus* have been demonstrated to have antioxidant, anticancer and antinociceptive activities (Rachitha et al., 2018; Atta and El-Sooud, 2004; Sadeghi-Aliabadi et al., 2008).

The free radical nitric oxide is included in numerous physiological mechanisms such as antimicrobial, anticonvulsant and anticancer activities (Nakagawa and Yokozawa, 2002). However, excessive and unregulated NO synthesis possibly facilitates tumour growth and dissemination in many human cancers (Choudhari et al., 2013). For this reason, there is a need for the improving of novel medications as powerful inhibitors of NO generation in connection with the cure for cancer.

Antioxidants convert reactive oxygen species to non-toxic products and stop or eliminate the side effects of reactive oxygen species, prevent some disorders, such as cancer, cardiovascular diseases, diabetes, infections and ischemia (Al-Dabbas, 2017). As it is known,

several biological activity tests such as antioxidant, antimicrobial and anticancer assays reveal the biological potential of various plants. For that reason, we determined the antioxidant and antiproliferative activity of *Convolvulus aucheri* Choisy and also its NO inhibitory potential in lipopolysaccharide (LPS) stimulated NSCLC cells.

MATERIAL AND METHODS

Plant material and extraction

The individuals of *Convolvulus aucheri* Choisy were collected from Hatay: NATO Radar Station in Kisecik, serpentine slopes, Turkey, ca 880 m, June 2009. The voucher specimen was deposited at the Akdeniz University Herbarium (Voucher no: C. Aykurt 2665). The plants were air-dried and their aerial parts were powdered. Methanol, ethanol, acetone and petroleum benzene were used for the extraction in a shaker water bath for 6 hours at 55°C (Ozay et al., 2015). The extracts were filtered and vaporized by using rotary evaporator and then lyophilized. The crude extracts were kept at +4°C until needed.

β-carotene/linoleic acid test

Antioxidant activity of the extracts was detected according to the method of inhibiting linoleic acid oxidation (Sokmen et al., 2004). BHT (an artificial antioxidant) was utilized as positive control.

Cytotoxicity assay

H1975 and HCC78 cells were used as human non-small cell lung cancer cell lines and cultured in RPMI 1640 medium in a CO₂ incubator. 24 hours incubation after seeding into 96-well plates (2×10³ cells/well), the medium was removed from the well leaving the adherent cells and cells were applied with extracts for 72 hours in the range of 0.625-40 µg/mL. After time was up cytotoxicity was determined by using CellTiter-Glo assay. Viability was

calculated using the background-corrected absorbance as follows:

$$\text{Viability (\%)} = \frac{\text{Abs of experiment well}}{\text{Abs of control well}} \times 100$$

Nitric oxide assay

After 24 hours preincubation of H1975 and HCC78 cells with lipopolysaccharide (1µg/mL), the extracts (0.625-40 µg/mL) were put in and incubated for 48hours (Yang et al., 2009). Nitrite, as an indicator of NO production in the medium, was determined via Griess reagent. After mixing the supernatant with the reagent in equal amounts, it was allowed to incubate for 10 minutes. And then using a microplate reader, absorbances were measured at 560 nanometre.

Statistical analysis

Statistical analysis was performed using the software SPSS version 22.0 program. Statistical significance was determined using the one-way ANOVA. Multiple group comparisons were analyzed with Tukey's multiple comparison test. Data were expressed as a mean \pm SD. p value of < 0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

Antioxidant activity

Different solvent extracts of *C. aucheri* were used so as to analysed antioxidant activity via β -carotene-linoleic acid test. Linoleic acid oxidation was prevented excellently by antioxidants (Tepe et al., 2007). The outcomes of

the antioxidant activity of *C. aucheri* were tabulated in Table 1. Among all the extracts, ethanolic extract showed the highest antioxidant activity (70.89 \pm 0.09%) while petroleum benzine extract showed the lowest activity (42.43 \pm 0.04%) ($p < 0.05$). The reason of the same plant's extracts showing different antioxidant activity may be due to the polarities of the solvents. None of the tested extracts exceed BHT antioxidant efficiency. Extracts of various members of this genus, such as *C. arvensis* (Krzaczek et al., 2004), *C. pluricaulis* (Vijayakumar et al., 2005), *C. hystrix* (El-Askary et al., 2006), *C. althaeoides* (Tawaha et al., 2007) and *C. fatmensis* (Atta et al., 2007) have been reported to show antioxidant activity.

The use of only one method does not reflect the antioxidant activity of plant extracts due to complicated structure of bioactive secondary metabolites (Du et al., 2009). In a previous study, DPPH free radical cleaning power of *C. aucheri* was reported as 59.50% (Cengiz et al., 2015). Several literatures also informed that DPPH scavenging power of *Convolvulus* species such as *C. arvensis* (Elzaawely and Tawata, 2012) and *C. dorycnium* (Nacef et al., 2010). In a preliminary study, Thrakal et al. (2010) reported the antioxidant activity of *C. arvensis* extract using the DPPH method, nitric oxide scavenging activity and the reducing power assay.

Table 1: Antioxidant power of *C. aucheri*

Extracts	β -carotene linoleic acid assay (%)
Ethanol	70.89 \pm 0.09 ^a
Methanol	66.43 \pm 0.07 ^a
Acetone	45.43 \pm 0.05 ^b
Petroleum benzine	42.43 \pm 0.04 ^b
BHT	95.64 \pm 0.12 ^c

Values expressed are means \pm SD, Different letters indicate significant difference ($p < 0.05$).

Cytotoxic activity

Because of ethanol extract has higher antioxidant power than others, ethanol extracts were used in CellTiter-Glo assay for cell viability and nitric oxide assay. To evaluate the antiproliferative activity of the *C. aucheri* ethanol extract against H1975 and HCC78 cells, CellTiter-Glo assay was carried out. Decrease in viability in H1975 and HCC78 were observed in a concentration-dependent manner ($p < 0.05$) (Fig. 1). Cytotoxic potential of *C. aucheri* on H1975 cells was observed to be more effective than HCC78. The percent of cell viability of H1975 and HCC78 cells was determined as 56.92% and 61.23% respectively at a concentration of 40 $\mu\text{g/mL}$.

Lung cancer is one of the deadliest cancers for human being. Non-small cell lung cancer (NSCLC) accounts for the majority of (85%) whole lung cancers (Zappa and Mousa, 2016). Plant origin products show hopeful resources of antitumor substances with lower adverse effect as compared to synthetic medications. In a previous study, where researchers demonstrated that different solvent extracts of *C. arvensis* had superior cytotoxic potential on HeLa cells (Sadeghi-Aliabadi et al., 2008). In another study researchers showed that the ubiquitous *C. arvensis* extract could be considered as a promising anti-cancer agent, with over 50% inhibition of tumor growth activity at non-toxic doses (Meng et al., 2002).

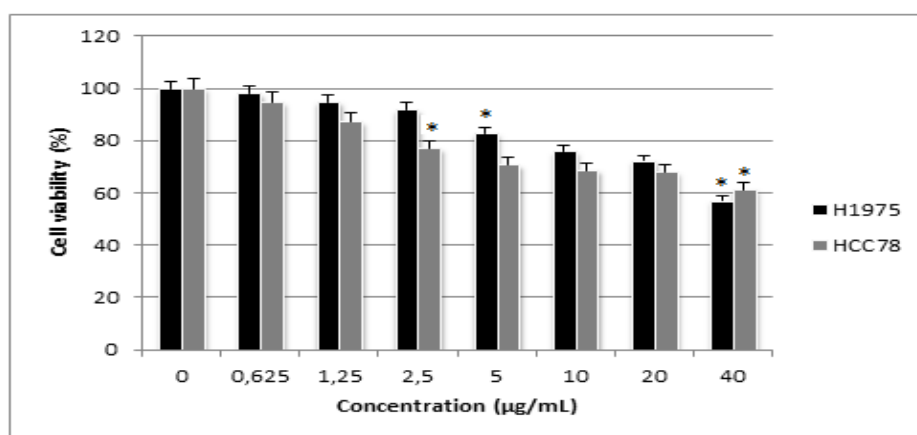


Figure 1: Cell viability of *C. aucheri* on H1975 and HCC78 cells proliferation. Data are presented as mean \pm SD. * $p < 0.05$.

Nitric oxide inhibitory activity

It is indicated that diverse regulating reactions to different NO levels have been viewed in various types of cancers. In general, at low concentrations of nitric oxide are considered to stimulate the growth of tumors. Nitric oxide acts as a powerful anticancer agent by stimulating apoptosis and necrosis in too high concentrations. While increase in nitric oxide synthase production is anticipated to indicate anticancer effect, it has been showed that nitric oxide may rise the proliferation and progress of cancer because of the impacts on metastasis process (Derici and Demirel-Yılmaz, 2017).

In this study, ethanolic extract obtained from *C. aucheri* was investigated for its effects on the LPS-activated NO production in H1975 and HCC78 cells. Nitric oxide generation was determined as nitrite concentration in the supernatant. The nitrite accumulation in the cells decreased due to the rising extract concentration ($p < 0.05$). Nitrite levels of H1975 and HCC78 cells ranged from 145.10 to 240.55 and 113.06 to 250.32 μM , respectively. Nitric oxide inhibitory activity of *C. aucheri* extract in LPS-stimulated cells was shown in Figure 2 and 3.

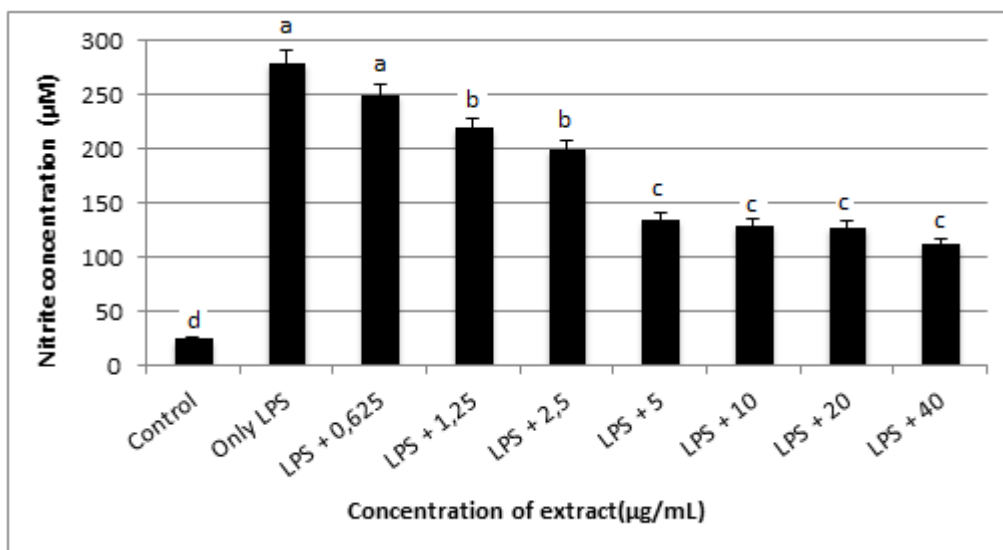


Figure 2: Nitric oxide inhibitory activity of *C. aucheri* in LPS-stimulated HCC78 cells. Data are presented as mean±SD. Different letters indicate significant difference ($p < 0.05$).

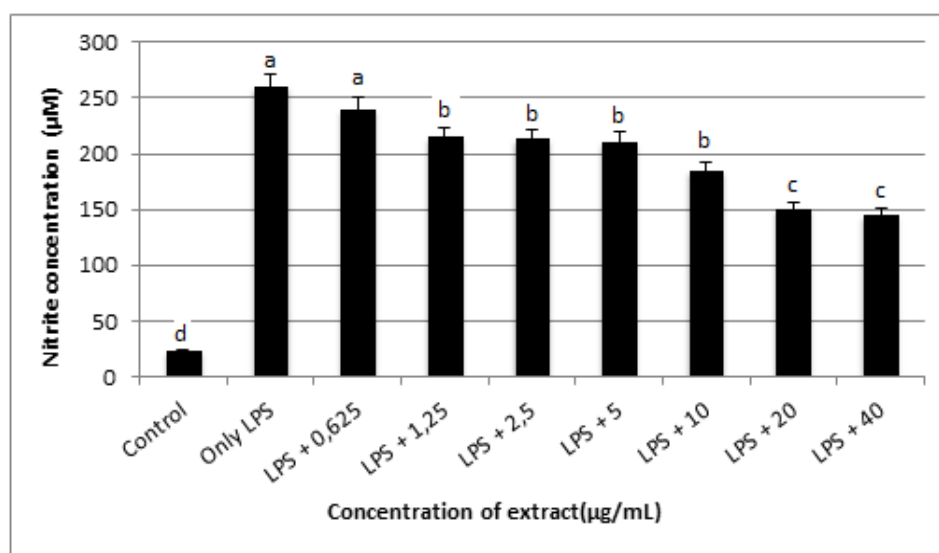


Figure 3: Nitric oxide inhibitory activity of *C. aucheri* in LPS-stimulated H1975 cells. Data are presented as mean±SD. Different letters indicate significant difference ($p < 0.05$).

Recent researches have pointed out the working principles in back of the action of natural antioxidants on the prevention of nitric oxide generation (Al Dhaheri et al., 2013). Plants are rich in bioactive molecules acted as natural antioxidants, such as phenolic compounds, alkaloids, terpenes, saponins and glycosides. Hence, prominent interest has been concentrated on the use of natural antioxidants to prevent nitric oxide generation (Yen et al., 2008; Mohsen and Ammar, 2009).

CONCLUSION

In conclusion, we demonstrated that *C. aucheri* ethanolic extract, which has antioxidant activity, reduces nitric oxide production in H1975 and HCC78 cells. These findings imply that ethanol extracts obtained from *C. aucheri* deserve better examination so as to identify its phytochemicals with NO inhibitory potentials.

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