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In-Vitro Nitric Oxide Scavenging and Anti-Tyrosinase Activity of Two *Convolvulus* L. Species

İki Convolvulus L. Türünün In-Vitro Nitrik Oksit Süpürme ve Anti-Tirozinaz Aktivitesi

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Abstract
Convolvulus L. is a member of Convolvulaceae family which have been utilized for their biological activities in folk medicine. The aim of this study is to investigate the inhibitory effect on tyrosinase activity of two *Convolvulus* species as well as their nitric oxide (NO) scavenging activity potentials. Up to now, no reports have been found on their anti-tyrosinase and NO scavenging activity properties in the literature. *C. galaticus* and *C. phrygius* aerial parts were extracted with methanol and then NO scavenging activity assay was utilized for the assurance of radical scavenging activity of the extracts. Anti-tyrosinase activity of the extracts was determined using the modified 96-well microplate method. The highest NO scavenging and anti-tyrosinase activity were found in *C. galaticus* as 55.20% and 53.16%, respectively.

Keywords: *Convolvulus* L.,nitric oxide scavenging activity, plant extract, tyrosinase inhibition

Öz
Convolvulus L., Convolvulaceae familyasının bir üyesi olup, biyolojik aktivitelerinden dolayı halk arasında tıbbi amaçlı kullanılmaktadır. Bu çalışmanın amacı, iki Convolvulus türünün tirozinaz aktivite üzerindeki inhibitör etkisi ve nitrik oksit (NO) süpürme aktivite potansiyellerini belirlemektir. Şimdiye kadar yapılan yayınlarda bu iki bitkinin anti-tirozinaz ve NO süpürme aktivite özelliklerine ilişkin bir rapor bulunamamıştır. C. galaticus ve C. phrygius toprak üstü kısımları metanolde ekstrakte edildikten sonra ekstraktların radikal süpürme aktivitelerinin belirlenmesi için NO süpürme aktivite deneyi kullanıldı. Ekstraktların anti-tirozinaz aktivitesi, 96-kuyucuklu mikroplaka metodunun modifiye edilmesiyle belirlendi. En yüksek NO süpürme ve anti-tirozinaz aktivitesi C. galaticus'ta sırasıyla % 55.20 ve % 53.16 olarak bulundu.

***Anahtar kelimeler**: Convolvulus L.,nitrik oksit süpürme aktivitesi, bitki ekstraktı, tirozinaz inhibisyonu*

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1. Introduction

Nitric oxide (NO), which is produced by macrophages, endothelial cells and neurons, is a free radical showing significant reactivity with other free radicals and certain types of proteins (Amaeze et al., 2011). NO has an important role for the regulation of different physiological events, such as vasodilatation (Nagmoti et al., 2011). Low level of NO is adequate in most cases to impact the physiological functions (Bhaskar and Balakrishnan, 2009). Prolonged exposure to NO radical is related to the emergence of lots of serious illness including cancer, arthritis and multiple sclerosis. NO radical can be scavenged by phenolics and removal of free radicals can be possible with the antioxidant activity of phenolics (Lakhanpal and Rai, 2007). *In vitro* scavenging of NO radical is one of the assays that may be used to figure out the antioxidant activity (Nagmoti et al., 2011).

Tyrosinase (monophenol monooxygenase, EC 1.14.18.1) is a copper-containing enzyme, which is taken part in the first two steps of melanin biosynthesis (Karioti et al., 2007). Melanin formation results in enzymatic browning in human skin and plants. Also, it is known that tyrosinase causes to unwanted changes in taste, scent and nutritious rates of plant-originated foods (Friedman, 1996). Thus, tyrosinase inhibitors have become progressively significant in food, cosmetic and pharmaceutical products in terms of hyperpigmentation (Lim et al., 2009). Some tyrosinase inhibitors have been identified in natural sources from particular plants (No et al., 1999).

Convolvulus is a genus of the Convolvulaceae family comprising about 250 species worldwide (Cronquist, 1981). In Turkey, this genus is represented with about 37 taxa (Aykurt and Sümbül, 2010). Certain *Convolvulus* species are known for their medicinal utilization and exhibit some biological properties such as antidepressant, antioxidant, antinociceptive, anticancer and neuroprotective activities (Dhingra and Valecha, 2007; Nacef et al., 2010; Atta and El-Sooud, 2004; Sadeghi-Aliabadiet al., 2008; Bihaqi et al., 2009). Previously, the NO scavenging activity of some Convolvulaceae members were reported (Gomathi et al., 2015; Baruah et al., 2014) but there hasn't been found any research on NO scavenging and anti-tyrosinase activity properties of the *Convolvulus* species in the literature.

The goal of this study was to investigate the NO scavenging activity of two *Convolvulus* methanolic extracts and to determine their inhibitory effect on tyrosinase activity.

2. Material and Method

2.1. Collection of Plant Material and Extraction

C. galaticus Rotsan ex Choisy and *C. phrygius* Bornm. were collected from Eskişehir-Turkey (900 m) and Antalya-Turkey (1100 m), respectively. The aerial parts of plants were air-dried over shadow at 25°C, powdered to as a fine grain. Then methanolic extracts were obtained by following the method of Ozay et al (2015). The extracts were lyophilized and stored at -20°C until use.

2.2. Nitric Oxide Scavenging Activity

Nitric oxide (NO) was produced from sodium nitroprusside (SNP) which measured as described by Balakrishnan et al (2009) by using the Griess reaction. The mixture containing SNP (5mM) in PBS (pH 7.3), with the plant extracts were prepared in PBS at different concentrations (125-1000 µg/mL) and incubated for 3 hours at 25°C. The absorbance value was determined at 546 nm wavelenght. The positive control was ascorbic acid. The NO scavenging activity was calculated:

NO scavenging activity (%)= [(Ac-At) / Ac] x 100

where, Ac is the absorbance value of the control, At is the absorbance value of the *Convolvulus* extracts.

2.3. Tyrosinase Inhibitory Activity

Extract solution was mixed with phosphate buffer (pH 6.8) and tyrosinase solution (33.3 U/mL) in a 96-well microplate and incubated at 23ºC for 10 min. Then, L-DOPA was added to start the reaction. Likewise, all reaction reagents were added to the extract solution without tyrosinase (blank). After 15 min incubation at 23ºC, the blank and extract absorbances were measured at 475nm using ELISA microplate reader (Masuda et al., 2005). The percentage inhibition of the tyrosinase activity was calculated:

$I\ (\%) = (A_0 - A_1) / A_0 \times 100$

where, A_0 is the absorbance value of the control, A_1 is the absorbance value of the *Convolvulus* extracts.

3. Statistical analysis

All the assays were performed in triplicate and all the results were presented as mean values and standard deviation (SD). Statistical analysis was conducted by using software SPSS17.0. Duncan test was used to determine the significance of experimental results and *p*-value < 0.05 was considered to be statistically significant.

4. Results and Discussion

4.1. NO Scavenging Activity

The free radical scavenging activity of the *C. galaticus* and *C. phrygius* extracts was determined by NO radical scavenging assay, which was examined by using ascorbic acid as standard. NO created from SNP at appropriate pH in aqueous solution at physiological interacts with oxygen to generate nitrite ions, which were detected by Griess reagent. Scavengers of NO compete with oxygen leading to decreased formation of NO (Marcocci et al., 1994). The NO scavenging activity of *C. galaticus* extract was 17.98% at the minimum concentration of 125 µg/mL, whereas the maximum activity was 55.20% at 1000 µg/mL. Figure 1 shows the percentage of inhibition was increased with rising concentration of the extracts. Nevertheless, the activity of ascorbic acid was more pronounced than that of the extracts of two *Convolvulus* L. species.

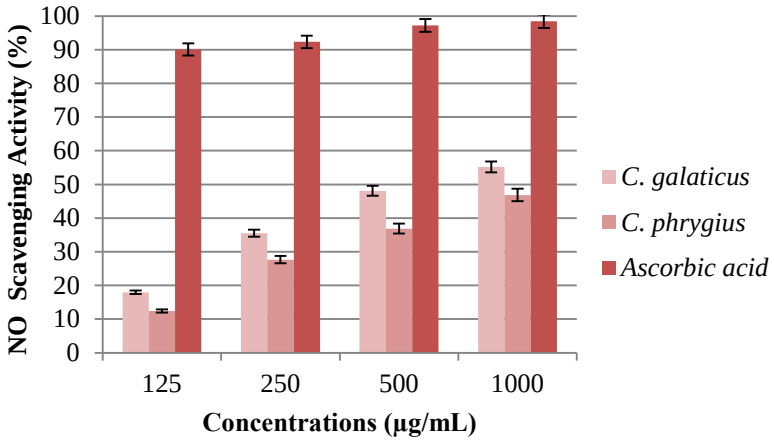

Figure 1. NO scavenging activity of *Convolvulus* extracts. Data are presented as the mean value ± SD

It can be concluded that *C. galaticus* extract has higher antioxidant activity than *C. phrygius.* Extracts of several members of *Convolvulus*, such as *C. althaeoides* (Tawaha et al., 2007), *C. fatmensis* (Atta et al., 2007) have been reported to exhibit antioxidant activity.

4.2. Anti-tyrosinase Activity

Tyrosinase inhibitory activity was determined by dopachrome method with L-DOPA as substrate. The inhibitory effect of *C. galaticus* and *C. phrygius* extracts and ascorbic acid (positive control) on biological activity of tyrosinase were shown in Table 1. It can be clearly seen that tyrosinase inhibitory activity of two *Convolvulus* L. species increased by rising extract concentration, but this increase was not statistically significant (P < 0.05).

C. galaticus extract has higher anti-tyrosinase activity than *C. phrygius.* The highest tyrosinase inhibitory activity was observed in *C. galaticus* as 53.16%, while the lowest activity was observed in *C. phrygius* as 26.18% at the maximum concentration of 4 mg/mL.

Plants active secondary metabolites are not definitely required for the normal plant growth but responsible for a lot of bioactive compounds used in health of organisms. The most important of secondary metabolites can be classified as alkaloids, phenols, terpenoids, saponins and glicosides (Taiz and Zeiger, 1998). Alkaloids are a class of secondary metabolites which have been accepted as the most characteristic and important chemical compounds in *Convolvulus* species. Some *Convolvulus* species contain tropane and pyrrolidine alkaloids (Todd et al., 1995; El-Shazlya and Wink, 2008).

Table 1: Tyrosinase inhibitory activity of methanol extracts from two *Convolvulus* L. species

Concentration (mg/mL)	Tyrosinase inhibition%		
	C. galaticus	***C. phrygius***	**Ascorbic acid**
1	$26.73^{a} \pm 0.22$	$23.46^{a} \pm 0.42$	$98.00^{a} \pm 0.34$
2	$28.12^{a} \pm 0.27$	$24.15^{a} \pm 0.34$	$98.53^{a} \pm 0.53$
3	$52.45^{b} \pm 0.35$	$26.00^{a} \pm 0.41$	$99.90^{a} \pm 0.25$
4	$53.16^{b} \pm 0.41$	$26.18^{a} \pm 0.45$	$100.68^{a} \pm 0.40$

Data are expressed as mean ±SD.
Different letters within the given column are significantly different at $P < 0.05$.

5. Conclusion

From the above results it can be concluded that *C. galaticus* possesses more NO scavenging and anti-tyrosinase activity than *C. phrygius.* These activities may be due to alkaloids probably found in the extracts but further studies should be carried out on the identification of responsible active components.

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