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TOTAL ANTIOXIDANT STATUS AND ANTIFUNGAL ACTIVITIES OF ENDEMIC GEOPHYTIC PLANTS COLLECTED FROM MUNZUR VALLEY IN TUNCELI, TURKEY

NURAN CIKCIKOGLU YILDIRIM[a*], MEHMET YAVUZ PAKSOY[a], EBRU YUCE[b], NUMAN YILDIRIM[a]
[a]*Tunceli University, Faculty of Engineering, Department of Environmental Engineering, 62000, Tunceli.*
[b]*Tunceli University, Tunceli Vocational High school, Department of Animal and Vegetable Production, 62000, Tunceli*

Total antioxidant status and antifungal activities of three endemic plants from the Munzur Valley in Tunceli, Turkey**;** *Bellevalia gracilis* Feinbrun, *Muscari aucheri* (Boiss.) Baker ve *Tulipa armena* Boiss. var. *lycica* (Baker) Marais were evaluated for the first time. Aqueous, hexanic, methanolic and ethanolic extracts were obtained from leaves and bulbs of *B. gracilis*, *M. aucheri* ve *T. armena* var. *lycica.* Total antioxidant status was determined by using TAS assay kit (Rel assay diagnostics). Antifungal assays were performed according to amended agar method against white rot fungus *Coriolus versicolor.*Among the plants tested, the leaves of *B. gracilis* exhibited highest total antioxidant status among all samples evaluated in this study. According to the results, *M. aucheri* plant exhibited the lowest antioxidant status. The results of this study showed that the aqueous leaf extract of *T. armena* var. *lycica* at a concentration 2.5% (w/v) suppressed significantly the radial growth of *C. versicolor* by 68.00%. It can be concluded that the plant species assayed possess antifungal and antioxidant properties.

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Keywords: Tunceli, Munzur Valley, Endemic geophyte, Total antioxidant status, Antifungal activity

1. Introduction

In all aerobic organisms, including human beings, production of reactive oxygen species (ROS) is balanced by antioxidant defence system [1]. A serious imbalance between the production of ROS and the antioxidant defence system is responsible for oxidative stress. Thus, ROS play an important role in the etiology of many diseases and ageing [2]. Antioxidants contribute to the protection of organism against oxidative stress and insufficient entry of antioxidants in organism can lead to the damages on DNA, lipids and proteins [3]. Antioxidants have been detected in a large number of food and agricultural products, including cereal grains, vegetables, fruits, and plant extracts [4,5].

Chemicals compounds are usually performed in the protection of wood. These products are based on metals such as copper chromated arsenate. Since, researchers have focused for developing new methods for preserving wood against fungi [6]. Chemical fungicides that are commonly used to control the fungal decay of wood are not suitable for indoor applications. The searches for natural sources that are user friendly and showing negligible toxicity to humans are increasingly sought [7,8].

Plants, in addition to their therapeutic use in herbal preparations, can serve as important sources of new drugs, new drug leads and new chemical entities [9]. Medicinal plants have been

*Corresponding author: nurancyildirim@tunceli.edu.tr

used for several purposes including its use as antimicrobial agents and they have been presented inhibition of fungi growth [10]. Plants produce a great deal of secondary metabolites, many of them with antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates [11].

Turkey is one of the richest countries in variability of flora. It has nearly 9000 plant species about 3000 of which are endemic [12]. There are about 600 species of flower bulbs in Anatolia [13] and many of them are known as ornamental and medicinal plants [14].

Muscaries are excellent bulbous plants which are generally called grape hyacinths and they deserve a greater breeding effort because of their excellent horticultural characteristic [15]. In addition, a number of polyphenolic compounds, which have pharmacological importance because of their antimutagenic effects, have been isolated from some Muscari species [16]. Muscari species are also an endemic and endangered species of Turkey, threatened by complete extinction. Irregular collection of the bulbs of *M. aucheri* from their habitat, erosion and the overgrazing of meadows of Anatolian, hamper the future cultivation of the species [17].

The genus Tulipa L. belongs to the family Liliaceae and is represented by 16 taxa (15 species), of which 2 are endemic in Turkey [18,19]. Tulipa armena Boiss. var. lycica (Baker) Marais is an endemic species [18]. 74 species of Bellevalia are presented in The World Checklist of Seed Plants [20]. The largest number of species in the genus is found in the Irano- Turanian phytogeographical region [21]. The total number of Bellevalia species has now reached to 21 in Turkey [22].

The aim of the study was to examine the in vitro antioxidant and antifungal activities of the ethanolic, methanolic, aqueous and hexanic extracts of *B. gracilis*, *M. aucheri* and *T. armena* var. *lycica*. In literature survey, there were not many studies about the antioxidant activity of this species for analyzing them. Therefore, we think that the results presented here will provide new information on the species examined.

2. Materials and methods

2.1 Plant Materials

B. gracilis, *M. aucheri* ve *T. armena* var. *lycica* were collected in the spring from Munzur Valley in Tunceli city. The fresh underground and above ground parts of the plant materials were cleaned and dried in the shadow for extraction. Extraction processes and antioxidant and antifungal studies have been done in Tunceli University, Department of Environmental Engineering.

2.2 Plant Extracts Preparations

Aqueous, hexanic, Methanolic and ethanolic extracts were obtained from leaves and bulbs of *B. gracilis*, *M. aucheri* ve *Tulipa armena* var. *lycica.*

Fresh plant material was washed with tap water, air dried and then chopped into small fragments, which was shade-dried and reduced to a coarse powder in a mortar and pestle. The aerial parts of the plant samples (2 g) were extracted with 20 ml methanol (MetOH), ethanol, water and hexane. The organic solvents were evaporated to dryness under vacuum at low temperature using a rotary evaporator. These extracts are; Muscari Bulb-Methanol, Muscari Bulb-Ethanol, Muscari Bulb-water, Muscari Bulb-hexane, Muscari Leaf-Methanol, Muscari Leaf-Ethanol, Muscari Leaf-Water, Muscari Leaf-hexane, *Bellevalia* Bulb-Methanol, *Bellevalia* Bulb-Ethanol, *Bellevalia* Bulb-water, *Bellevalia* Bulb-hexane, *Bellevalia* Leaf-Methanol, *Bellevalia* Leaf-Ethanol, *Bellevalia* Leaf-Water, *Bellevalia* Leaf-hexane, *Tulipa* Bulb-Methanol, *Tulipa* Bulb-Ethanol, *Tulipa* Bulb-water, *Tulipa* Bulb-hexane, *Tulipa* Leaf-Methanol, *Tulipa* Leaf-Ethanol, *Tulipa* Leaf-Water, *Tulipa* Leaf-hexane .

2.3 Total Antioxidant Status (TAS) assay

The dried extracts were dissolved in distillated water to a final concentration of 25 mg/ml and used as for antioxidant testing. Total antioxidant status was determined by using Rel assay

diagnostics TAS assay kit (Lot.RL024). The assay is performed in a microplate and is assessed with a 96-well multi-detection plate reader (Thermo Scientific Multiscan FC microplate photometer). Antioxidants in the sample reduce dark blue-green coloured ABTS radical to colourless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant levels of the sample. The assay is calibrated with a stable antioxidant Standard solution which is traditionally named as Trolox Equivalent that is a vitamine E analog.

2.4 Antifungal assays

In this study, white rot fungus *C. versicolor* was used in the antifungal assay. This fungal strain was provided from Dicle University, Department of Biology. Antifungal assays were performed according to Chang et al., 1999 [23]. The *in vitro* tests were carried out to measure the effects of the bulbous endemic plant extracts on radial growth of *C. versicolor*. Sabouroud dextrose agar (SDA) medium was used in the study. To every 13 ml of sterile SDA medium in Petri dishes, 5 ml either aqueous, hexanic, methanolic or ethanolic extracts of each plant were added at a concentration of 2.5% (w/v). The solution in each Petri dish was gently swirled and allowed to solidify. The extract-amended medium in the Petri dishes were inoculated each alone at the centre with 5 mm inoculum-disc of each test fungus and incubated at 27 °C for 15 days. The medium with inoculum-disc but without any extract served as control. When the mycelium of fungus reached the edges of the control plate (without added extract) the antifungal index was calculated as follows

Antifungal Index = DC - DT / DC x 100, where: DC = diameter of control, DT = diameter of test, each experiment was performed three times.

2.5 Statistical analysis

All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). Data were analyzed by analysis of variance (ANOVA) and presented are the averages of the results of three replicates with a Standard error.

3. Results and discussion

In Table 1, Total antioxidant status and antifungal activity of endemic plants collected from different region of Ovacik, Tunceli (Turkey) is illustrated.

Table 1. Total Antioxidant Status and antifungal activity of endemic plants collected from different region of Ovacik, Tunceli (Turkey).

Plant Species	Parts of plants	Solvents for extraction	TAS *	Antifungal Index (%)
M. aucheri	Leaf	Hexane	1.63±0.02	ND**
		Methanol	2.86±0.03	ND
		Ethanol	2.36±0.02	28.80±2.22
		Water	1.14±0.01	44.40±3.44
	Bulb	Hexane	3.16±0.03	ND
		Methanol	1.61±0.03	ND
		Ethanol	1.82±0.04	10.40±2.10
		Water	1.59±0.02	12.80±3.20
T. armena var. *lycica*	Leaf	Hexane	1.39±0.02	11.20±1.20
		Methanol	3.73±0.12	38.10±2.30

Plant Species	Parts of plants	Solvents for extraction	TAS *	Antifungal Index (%)
		Ethanol	3.28±0.04	41.10±4.50
		Water	2.32±0.05	68.00±4.42
	Bulb	Hexane	1.39±0.03	ND
		Methanol	1.34±0.07	ND
		Ethanol	1.24±0.05	11.20±3.20
		Water	3.78±0.08	16.10±3.10
B. gracilis	Leaf	Hexane	4.14±0.14	14.20±2.12
		Methanol	4.02±0.11	ND
		Ethanol	4.16±0.12	24.40±4.62
		Water	1.89±0.02	43.00±7.80
	Bulb	Hexane	1.30±0.02	ND
		Methanol	1.66±0.04	22.10±3.20
		Ethanol	1.38±0.02	12.40±4.12
		Water	1.35±0.01	18.80±2.14

***TAS:** Total Antioxidant Status; mmolTrolox Equiv./L., ****ND:** Not Deterined Inhibition, n: 3, Mean±SE.

The extracts produced different levels of antifungal activity against *C. versicolor*. The result revealed that highly significant percent inhibition (68%) of mycelial growth of *C. versicolor* was observed in SDA media amended with aqueous leaf extract of *T. armena* var. *lycica* and moderate or low activity was observed in aqueous leaf extract of *M. aucheri* (44%), aqueous leaf extract of *B. gracilis* (43%) and ethanolic bulb extract of *M. aucheri* (10.40%). No mycelial growth inhibition of *C. versicolor* was observed in SDA media amended with some extracts of parts of *M. aucheri*, *T. armena* var. *lycica* and *B. gracilis* (Table 1).

The leaves of *B. gracilis* exhibited highest total antioxidant status among all samples evaluated in this study (4.169 mmolTrolox Equiv./L.). This extract of *B. gracilis* parts can be utilized as an effective and safe source of antioxidants. Further, differences among the B. gracilis species showed some variability, ranging from 4,169 to 1,301 mmolTrolox Equiv./L. (Table-1). The total antioxidant status of *M. aucheri* ranged from 3,164 (bulb) to 1,145 (leaf) mmolTrolox Equiv./L. (Table 1). *M. aucheri* plant exhibited the lowest antioxidant status in this evaluation (1.14 mmolTrolox Equiv./L). *T. armena* var. *lycica* plant had the second highest levels of TAS, which ranged from 1.396 (leaf) to 3.786 (bulb) mmolTrolox Equiv./L.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents [24]. Many reports are available on the antiviral, antioxidant, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [25].

In present study, antifungal and antioxidant activities of three endemic plants; *B. gracilis*, *M. aucheri* ve *T. armena* var. *lycica*, from the Munzur Valley of Tunceli, Turkey evaluated in this study. According to our knowledge, there was no literature on antioxidant and antifungal properties of *B. gracilis*, *M. aucheri* ve *T. armena* var. *lycica* extracts.

Plants possess different antioxidant properties depending on their content of antioxidant molecules, as recently shown by studies demonstrating that strawberries have greater antioxidant capacity (2- to 11-fold) than apples, peaches, pears, grapes, tomatoes, oranges, or kiwifruit and This character is strongly affected by the type of fruit (species and variety within species), but it can be also affected by cultivation conditions of the plant (environmental and cultivation techniques) [26; 27]. In the present study, the leaves of the *B. gracilis* had the highest TAS values (4.169 mmolTrolox Equiv./L.), which were 3.5-fold higher than *M. aucheri*. This extract of *B. gracilis* parts can be utilized as an effective and safe source of antioxidants.

Variation in activity among different extracting solvents has earlier been reported [28,29]. In our study; hexane and methanol extract of *M. aucheri* did not showed activity against tested fungus. The ethanolic extract of *M. aucheri* had very little antibacterial activity against *C. versicolor*. Aqueous extract of *M. aucheri* leaves showed 44% antifungal activity aganist *C. versicolor* (Table. 1). Hexane and methanol extract of *T. armena* var. *lycica* bulbs had no antifungal activity aganist *C. versicolor*. The strongest effect was manifested by the aqueous extract of *T. armena* var. *lycica* leaves. This extract can be considered as one of the alternatives to chemical wood preservatives for controlling the wood decaying microorganisms. The lowest antifungal activity was observed in the ethanol extract of *T. armena* var. *lycica* aganist *C. versicolor* (Table-1). Methanol extract of *B. gracilis* leaves did not show antifungal activity against tested fungus. Hexane extract of *B. gracilis* bulbs had no antifungal activity. We found maximum antifungal activity of *M. aucheri in* aqueous extract of the leaves (Table 1).

4. Conclusion

To our knowledge, this is the first report showing the antifungal and antioxidant activities of endemic plants from the valley of Munzur in Tunceli, Turkey. The results of present investigation clearly indicate that the antioxidant and antifungal activity vary with the species of the plants and parts of the plants used. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. A further study is needed to isolate and identify the active compounds that are responsible for antifungal and antioxidant activity. Antifungal activities of this natural extracts wood decay fungi are user friendly and showing negligible toxicity to humans.

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