

**ANTIMICROBIAL, ANTIOXIDANT, ANTI-TYROSINASE AND ANTI-UREASE
ACTIVITIES AND FT-IR ANALYSES OF *HELICHRYSUM
GRAVEOLENS* (BIEB) SWEET**

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Abstract

Helichrysum graveolens (Bieb) Sweet possesses various medicinal applications such as diuretic, treatment of the sand and stone in kidney, regulation of digestion problems and strengthening of the immune system. The investigation was carried out to reveal ethanol extracts of flower and stem parts of *H. graveolens* for antimicrobial, antioxidant, anti-tyrosinase and anti-urease activities. Ethanol extract of stem part of *H. graveolens* exhibited more antibacterial activity against growth of bacteria except for *K. pneumoniae*. Stem part of *H. graveolens* has higher copper reducing antioxidant capacity (CUPRAC) and anti-urease activities than flower part of *H. graveolens*. Anti-tyrosinase activity (IC₅₀ values) of stem and flower parts of *H. graveolens* was found to be 0.01549 ± 0.0006 µg/ml and 0.01515 ± 0.0016 µg/ml, respectively. This survey suggests that ethanol extracts of different parts of *H. graveolens* is an alternative source for natural antioxidant and antimicrobial compounds, urease and tyrosinase inhibitors that could be utilized in drug and food industries.

Introduction

The *Helichrysum* species are xerophytes growing at altitudes from the sea level up to 1700 m preferably on sandy or loamy soils. *Helichrysum* species are commonly recognized as "olmez cicek or altinotu" in Turkish and are widely utilized as herbal tea in Turkey (Bağcı *et al.* 2013). *Helichrysum graveolens* is a medicinal plant which belongs to Asteraceae.

Plants play a substantial role in primary health care as therapeutic medicines in developing countries. Antibiotic resistance as well as appearance of undesirable side effects of some antibiotics (WHO, 2002) has caused to the investigate of alternative antibacterial agents from medicinal plants (Sharma *et al.* 2012).

Reactive oxygen species (ROS) are continually created inside the human body but excessive or inadequate production of ROS can easily affect antioxidant defense systems. This situation cause many illnesses like cancer, neurodegenerative diseases, arthritis, diabetes mellitus, and ageing process (Patel *et al.* 2010).

Tyrosinase is a the primary enzyme that catalyzes synthesis of melanin in mammalian, plants and microorganisms cells. Melanin biosynthesis inhibitory compounds are beneficial for skin whitening products utilized in cosmetics and also as a medicine for defects in pigmentation. Plants were acquired attention for cosmetic product improvement because they could have less toxicity than the synthetic ones (Rauniyar *et al.* 2007).

Antimicrobial, antioxidant, anti-tyrosinase and anti-urease activities and FT-IR analyses of ethanol extracts of flower and stem parts of *H. graveolens* were investigated.

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Materials and Methods

Helichrysum graveolens was collected from the local market of Giresun, Turkey in 2018 (the plant sample was collected from Eğribel passage, Şebinkarahisar) and it was identified by Associate Professor Doctor Zafer Türkmen.



Fig. 1. *Helichrysum graveolens*

Flower and stem parts of *H. graveolens* were separated, dried on laboratory and then they were grounded into powder, separately. Twenty gram flower and stem parts of *H. graveolens* were extracted with 200 ml ethanol with a Soxhlet extractor, separately (Kumar *et al.* 2012).

In antimicrobial activity assay, the extracts were added into Mueller-Hinton broth at concentration ranging from 0.009765 - 20 mg/ml. Whereas the mixtures were incubated for bacteria at 37°C, for fungi the mixtures were incubated for fungi 30°C (Yiğit *et al.* 2009).

Amount of total phenolic compounds (TPC) in the tested extracts was determined using the Folin-Ciocalteu solution according to the method described by Slinkard and Singleton (1977). TPC was expressed as microgram of gallic acid equivalent (GAE) per ml sample by using a calibration graph gallic acid. The tests were performed in triplicate.

Aluminium chloride colourimetric test was utilized to examine the total flavonoid content (TFC) in the extracts (Zhishen *et al.* 1999). TFC was expressed as microgram of catechin equivalent (CE) per ml sample by using a calibration graph catechin. TFC in the extracts were worked out in triplicates.

The total antioxidant capacity of the extracts was established by the phosphomolybdenum method (Prieto *et al.* 1999). Absorbance was measured at 695 nm using a Shimadzu 1240 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The total antioxidant capacity was expressed as microgram of ascorbic acid equivalent (AAE) per ml sample by using a calibration graph ascorbic acid. The data were presented as the average of triplicate analyses.

The DPPH scavenging activity of ethanol extracts of flower and stem parts of *H. graveolens* was calculated using the following formula (Brand-Williams *et al.* 1995):

$$\% \text{ Activity: } \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

A_0 = Absorbance of control, A_1 = Absorbance of sample.

CUPRAC test was employed by the method of Özyürek *et al.* (2009). Butylated hydroxytoluene (BHT) was used as standard antioxidant agent.

Tyrosinase-inhibitory activity of ethanol extracts of flower and stem parts of *H. graveolens* was determined according to Higuchi *et al.* (1993). Kojic acid was used as a standard tyrosinase-inhibitor. Urease inhibitory activity was determined according to Van Slyke and Archibald (1944). Thiourea used as standard urease inhibitor agent. The inhibition percentage of anti-tyrosinase and anti-urease activities were calculated from the following equation:

$$\% \text{ Activity: } \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

A_0 = Absorbance of control, A_1 = Absorbance of sample.

The IC_{50} describes as the concentration of inhibitor that decreased urease/tyrosinase activity by 50% and calculated by plotting the percent of inhibition. The experiments were done triplicate. Lower IC_{50} values indicate higher enzyme inhibitor activity.

Infra red (IR) analysis was done with the aid of infra red spectrophotometer (JASCO OFTIR 6600 with ATR attachment) at the Central Research Laboratory in Giresun University. A drop of extract was placed carefully on crystal surface. It was fixed on the infra red beam. The infra red data was compared to the table of IR frequencies.

Results and Discussion

Table 1 summarizes the Minimum Inhibition Concentration (MIC) of ethanol extracts of stem and flower parts of *H. graveolens*. MIC values were found to range from 0.019 to 5 mg/ml for flower and stem parts. Ethanol extract of stem part of *H. graveolens* exhibited more antibacterial activity against growth of bacteria except for *K. pneumoniae*. When antifungal activity was compared results were similar between stem and flowers parts of *H. graveolens*.

Table 1. MIC values of the extracts (mg/ml).

Microorganisms	EFH	ESH
<i>Bacillus subtilis</i> IMG 22	2.5	2.5
<i>Klebsiella pneumoniae</i> (lab isolate)	0.625	2.5
<i>Gordonia rubripertincta</i> (lab isolate)	2.5	1.25
<i>Enterococcus faecalis</i> ATCC 29212	2.5	1.25
<i>Proteus vulgaris</i> FMC 1	2.5	1.25
<i>Enterobacter aerogenes</i> CCM 2531	2.5	0.625
<i>Salmonella enterica</i> ATCC 14028	2.5	1.25
<i>Candida albicans</i> FMC 17	5	5
<i>Candida tropicalis</i> ATCC 13803	0.019	0.019
<i>Candida parapsilosis</i> ATCC 22019	2.5	1.25
<i>Saccharomyces cerevisiae</i> ATCC 9763	0.019	0.019

EFH: Ethanol extract of flower part of *H. graveolens*, ESH: Ethanol extract of stem part of *H. graveolens*.

Some studies were performed about antimicrobial activity of *H. graveolens*. For example, Aslan *et al.* (2007) searched antibacterial activity of petroleum ether and ethanol extracts from the capitula obtained from *H. graveolens* and it was found that ethanol extract of *H. graveolens* had activity against *Bacillus cereus*, *Bacillus megaterium*, *Streptococcus mutans*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Shigella sonnei* but petroleum extract had activity only against *S. sonnei*.

Öztürk (2004) demonstrated that methanol extract of *H. graveolens* had higher activity than standard antibiotic ceftazidime against *Staphylococcus aureus*, *E. faecalis* and *E. cloacae* but weak activity against *E. coli*, *P. aeruginosa* and *S. thyphimurium*. Moreover, it was revealed that methanol extract of *H. graveolens* had no activity against *C. albicans*. On the contrary of this findings, in the present study ethanol extracts of flower and stem parts of *H. graveolens* had activity against *C. albicans*. Smirnov *et al.* (1982) investigated also antimicrobial activity of *H. graveolens*.

Table 2 shows total phenolic and total flavonoid contents and total antioxidant capacity of stem and flower parts of *H. graveolens*. While total phenolic content and total antioxidant capacity of stem part was higher than flower part, total flavonoid content of flower part was higher than stem part.

The percentage of inhibition of DPPH radical at different concentrations is presented in Table 3. Flower part exhibited higher DPPH radical scavenging activity than stem part of *H. graveolens*. BHT and Rutin possess higher activity than flower and stem parts of *H. graveolens*.

There are studies related about antioxidant activity of *H. graveolens*. For example, according to a study performed by Aslan *et al.* (2007), total phenolic contents of ethanol and water extracts of *H. graveolens* was found as 91.4 ± 0.9 mg of GAE/g extract and 70.4 ± 0.9 mg of GAE/g extract, respectively. Moreover, total flavonoid contents of ethanol and water extracts of *H. graveolens* was found as 59.5 ± 3.9 mg of GAE/g extract and 32.7 ± 1.9 mg of GAE/g extract, respectively. Orhan *et al.* (2014) revealed that total phenolic contents of aqueous and ethanolic extracts of *H. graveolens* was 92.9 ± 2.0 mg GAE/ 1 g extract and 139.5 ± 6.5 mg GAE/1 g extract, respectively. In the present study, total phenolic and flavonoid contents of ethanol extracts of flower part of *H. graveolens* was detected as 28.85 ± 0.003 μ g GAE/ml and 59.26 ± 0.008 μ g CE/ml, respectively. The differences between three studies might be arised from collecting plants from different geographical locations.

Table 2. Total phenolic and flavonoid contents and total antioxidant capacity of the extracts.

Plant	μ g GAE/ml	μ g CE/ml	μ g AAE/ml
EFH	28.85 ± 0.003	59.26 ± 0.008	66.85 ± 0.024
ESH	41.41 ± 0.017	50.21 ± 0.005	128.65 ± 0.022

Value represents mean of three replicates.

Table 3. DPPH scavenging activity of the extracts (% inhibition).

Concentration (μ g/ml)	EFH	ESH	BHT	Rutin
50	14.77 ± 0.032	8.72 ± 0.037	80.22 ± 0.015	58.31 ± 0.025
100	32.25 ± 0.012	26.91 ± 0.014	84.49 ± 0.013	61.08 ± 0.003
150	48.20 ± 0.019	41.02 ± 0.018	88.09 ± 0.003	64.28 ± 0.002
200	61.36 ± 0.021	53.93 ± 0.012	94.06 ± 0.003	75.57 ± 0.013

Value represents mean of three replicates.

Table 4 presents CUPRAC activity of the extracts. Stem part of *H. graveolens* exhibited higher activity than flower part of *H. graveolens*. BHT which used as standard antioxidant showed considerably higher activity than *H. graveolens*.

Table 4. CUPRAC activity of the extracts (nm).

Concentration ($\mu\text{g/ml}$)	EFH	ESH	BHT
50	0.122 ± 0.001	0.133 ± 0.016	0.552 ± 0.028
100	0.152 ± 0.003	0.155 ± 0.007	0.920 ± 0.027
150	0.163 ± 0.005	0.170 ± 0.002	1.068 ± 0.014
200	0.173 ± 0.001	0.180 ± 0.002	1.130 ± 0.025

Value represents mean of three replicates.

Öztürk (2004) determined that DPPH scavenging activity of methanol extracts (with a range from 52.06 to 78.21%) of *H. graveolens* which were collected from different locations. Moreover, total antioxidant capacity of methanol extracts of *H. graveolens* which were collected from different locations was found to range from 7.8893 mmol α -tocopherolacetate/mg extract to 9.7666 mmol α -tocopherolacetate/mg extract. In the present study, it was found that DPPH scavenging activity of ethanol extract of flower part of *H. graveolens* ranged from 14.77 to 61.36% and DPPH scavenging activity of ethanol extract of stem part of *H. graveolens* ranged from 8.72 to 53.93%. Moreover, total antioxidant capacity was detected as 66.85 ± 0.024 μg AAE/ml for flower part and 128.65 ± 0.022 μg AAE/ml for stem part. The difference might be due to the use of different solvents and different plant parts in assays and collecting plant samples from different locations.

Table 5 shows anti-urease and anti-tyrosinase activities of *H. graveolens*. Flower part of *H. graveolens* showed slightly higher activity than stem part of *H. graveolens*. Moreover, kojic acid exhibited higher activity than tested plant parts. When anti-urease activity of the plant parts was compared, stem part showed higher activity than flower part. In addition to this, stem part demonstrated higher activity than thiourea which used standard anti-urease agent.

Table 5. Anti-urease and anti-tyrosinase activities of stem and flower parts of *H. graveolens* and standards.

Plant	Concentration ($\mu\text{g/ml}$)	IC ₅₀	Concentration ($\mu\text{g/ml}$)	IC ₅₀
Stem	0.00001	0.01549 ± 0.0006	0.00001	0.0082 ± 0.0008
	0.0001		0.0001	
	0.001		0.001	
	0.01		0.01	
Flower	0.00001	0.01515 ± 0.0016	0.00001	0.0108 ± 0.0012
	0.0001		0.0001	
	0.001		0.001	
	0.01		0.01	
Thiourea	0.00001	-	0.00001	0.00909 ± 0.00005
	0.0001		0.0001	
	0.001		0.001	
	0.01		0.01	
Kojic acid	0.00001	0.0088 ± 0.0002	0.00001	-
	0.0001		0.0001	
	0.001		0.001	
	0.01		0.01	

This is the first report about anti-urease activity of stem and flower parts of *H. graveolens*.

Table 6 presents results of FT-IR analyses of ethanolic extract of flower part of *H. graveolens*. (Table 6, Fig. 2). The absorptions in the frequencies 2800-3000 cm^{-1} were due to C-H stretching vibrations that are mainly generated by alcohol or phenols. The band at 2923.56/cm are assigned to aliphatic CH stretching which possibly due to the presence of alkanes. The vibration at frequencies 1500-1600/cm indicates the presence of aromatic ring compound so 1504.48/cm and 1508.06/cm. The absorptions in this region were due to the presence of aromatic C = C bond. The bands at 1446.35/cm, stretching assigned to C-H in-plane bending, which indicated the presence of alkanes. The presence these functional groups in *H. graveolens* make it a potential pharmacological source for new antibacterial, antioxidant and anti-tyrosinase agents (Rajiv *et al.* 2017, Oladunmoye *et al.* 2018).

Table 7 presents results of FT-IR analyses of ethanolic extract of stem part of *H. graveolens*. (Table 7, Fig. 3). 2915.04/cm and 2854.13/cm absorption bands corresponds the presence of alkanes and absorption band at 3324.68/cm revealed the presence of alcohols and phenols in the tested ethanolic extract. The band at 1052.94/cm are assigned to C-O stretching which possibly due to the presence of alcohol, carboxylic acid, ester and ether. The C-H stretching due to alkanes was observed at 1423.21/cm. The vibration at frequencies 1500-1600/cm represents aromatic ring compound so the bands at 1612.2/cm indicated aromatic C=C stretching (Rajiv *et al.* 2017, Oladunmoye *et al.* 2018).

Table 6. Results of FT-IR analysis of ethanol extract of flower parts of *H. graveolens*.

Wave numbers	Bond
3286.11	OH stretching
2923.56	Alifatic CH stretching
2352.73	C=N or C=C bond
1504.48	Aromatic C=C bond
1508.06	Aromatic C=C bond
1446.35	C-H in-plane bending
1349.93	OH stretching
1033.66	C-O stretching

Table 7. Results of FT-IR analysis of ethanol extract of stem part of *H. graveolens*.

Wave numbers	Bond
3324.68	OH stretching
2915.04	Alifatic C-H stretching
2854.13	Alifatic C-H stretching
2352.73	C=N or C=C bond
1612.2	Aromatic C=C stretching
1423.21	C-H in-plane bending
1261.22	O-H stretching
1052.94	C-O stretching

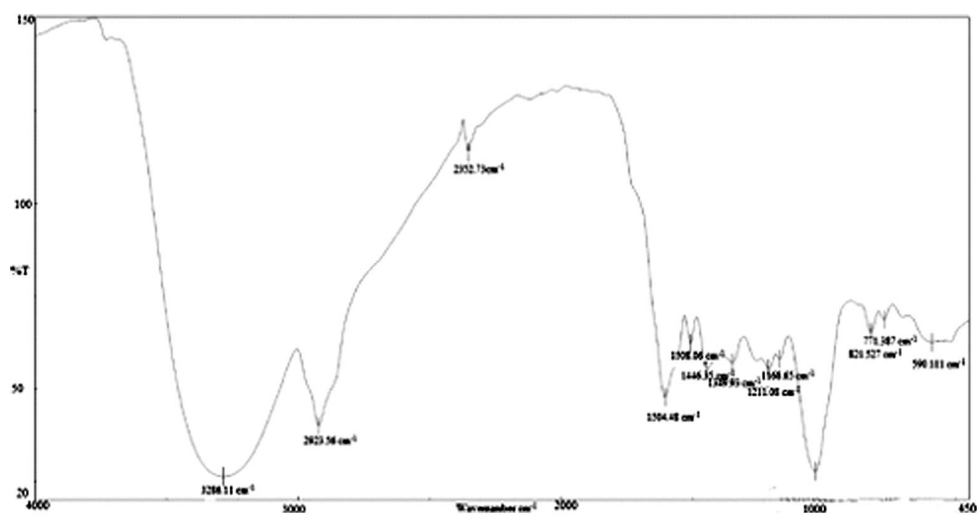


Fig. 2. FT-IR analysis of ethanol extract of flower part of *H. graveolens*.

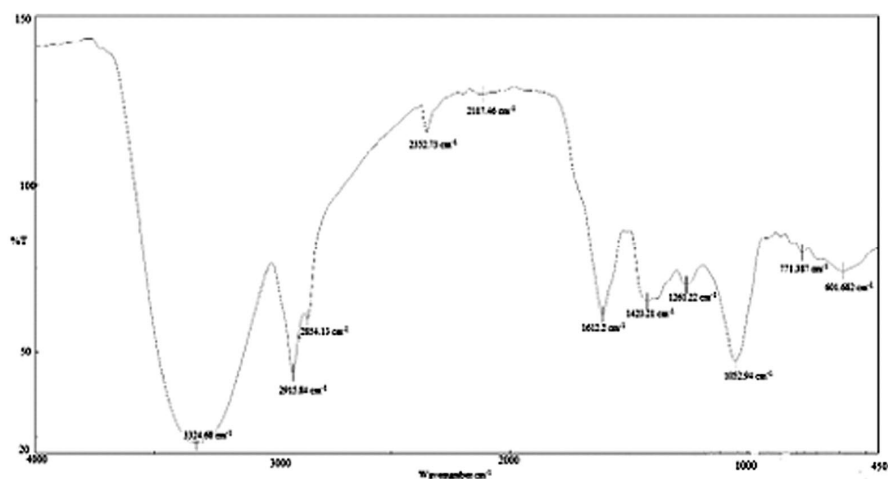


Fig. 3. FT-IR analysis of ethanol extract of stem parts of *H. graveolens*.

From the present it is revealed that ethanol extracts of flower and stem parts of *H. graveolens* possess important antimicrobial, antioxidant, anti-tyrosinase and anti-urease effects and this plant could be an alternative to synthetic antimicrobial, antioxidant, anti-tyrosinase and anti-urease agents but further investigations require to be carried out to detect active ingredients responsible for their antimicrobial, antioxidant, anti-tyrosinase and anti-urease effects which were found in *H. graveolens*.

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