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Determination of Essential Oil Composition and Investigation of Phenolic Substance and Antioxidant Effect of "*Helichrysum stoechas"* from Hatay

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ABSTRACT: This study was carried out to determine the essential oil composition, antioxidant capacity and total phenolic substance of *Helichrysum stoechas* which was gathered from Hatay region. *H. stoechas* had a moisture content of 13.55 weight%, while the essential oil content of 0.3 wt% and ten volatile compounds were determined and identified by GC. Predominantly volatile components are rosifoliol 4.89%, 3-methoxy-γ-asarone 34.69%, elemicin 3.24%, myristicin 23.35%, apiol 27.38%. Methanol soluble fraction was found to be 15.3%. Total phenol concentration was found 256.55 ± 3.7 mg gallic acid equivalent/g of extract powder. DPPH free radical-scavenging activity IC_{50} was found as 2.76 ± 44x10^{-2} mg mL^{-1}. The inhibition value of methanolic extract was compared with BHA and BHT. The volatile oil is rich in hallucinogenic elemicin and myristicin. Obtained results showed that the methanolic extract was rich in phenolic content and has a close antioxidant activity to the synthetic antioxidants BHT and BHA.

Keywords: *Helichrysum stoechas*, essential oil, antioxidant activity, total phenolic content.

Hatay'da yetişen "*Helichrysum stoechas*" ın Fenolik Madde ve Antioksidan Etkilerinin incelenmesi ve Esansiyel Yağ Bileşiminin Belirlenmesi

ÖZET: Bu çalışma, Hatay bölgesinden toplanan *H. stoechas*'ın esansiyel yağ bileşimi, antioksidan kapasitesi ve toplam fenolik içeriğini belirlemek amacıyla yapılmıştır. *H. stoechas* ağırlıkça % 13,55 nem içeriğine sahipken, esansiyel yağ içeriği ağırlıkça % 0,3 oranında olup içerdiği on uçucu bileşik GC ile belirlenmiştir. Ağırlıklı olarak uçucu bileşenler rosifoliol % 4,89, 3-metoksi-y-asaron % 34,69, elemisin % 3,24, miristisin % 23,35, apiol % 27,38'dir. Metanolde çözünen madde miktarı % 15,3 olarak bulundu. Toplam fenolik madde konsantrasyonu 256,55 ± 3,7mg Gallik asit eşdeğeri/g olarak bulunmuştur. DPPH serbest radikal süpürme aktivitesi IC_{50} değeri 2,76 ± 44x10^{-2} mg mL^{-1} olarak bulundu. Metanolik ekstrenin inhibisyon değeri BHA ve BHT ile karşılaştırıldı. Uçucu yağ, halüsinojenik özelliğe sahip elemisin ve miristisin açısından zengindir. Elde edilen sonuçlar, metanolik ekstraktının fenolik madde bakımından zengin olduğunu ve sentetik antioksidanlar BHT ve BHA'ya yakın bir antioksidan etkinliğe sahip olduğunu gösterdi.

Anahtar kelimeler: *Helichrysum stoechas*, uçucu yağ, antioksidant aktivite, toplam fenolik madde

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INTRODUCTION

Helichrysum stoechas (*Helichrysum stoechas* (L.) MOENCH) is a species of Helichrysum Gaertner in the family Asteraceae, in Europe and in the Mediterranean region (Italy, the Balkans, Cyprus, Lebanon, Cyrenaica and northwest Africa) (http://www.pfaf.org/User/Plant.aspx?LatinName=Helichrysum+stoechas).

Helichrysum species have traditionally been used to treat injuries, infections, and respiratory conditions (Hutchings et al., 1996). *Helichrysum* genus in Turkey is widely used as a public struggle in the treatment of diuretics, lithography and stomach pain in folk medicine (Sezik et al., 2001). *H. Stoechas* is an expectorant and is used in the treatment of colds (Grieve, 1984).

People traditionally benefit from medicinal herbs to protect themselves from diseases. Essential oils have been used since ancient times for medical and health purposes, perfumes and cosmetics, as well as in some types of household cleaner (Manniche, 1999; Krishna et al., 2000). In terms of alternative medicine, essential oils are most frequently used today in aromatherapy. There is a huge amount of useful plant extracts which can be used or aromatherapy (Esposito et al., 2014).

The composition of the essential oil may vary in quality, quantity and composition depending on the climate, soil composition, plant organ, age and vegetative cycle stage (Masotti et al., 2003; Angioni et al., 2006).

Essential oil composed mainly of rosifoliol, 3-methoxy-γ-asarone, elemicin, myristicin and apiol are accountable for the distinct odor and flavor of *H. Stoechas*. The essential oil is a pale yellow color and has a more bitter scent compared to the fresh plant. Oil contains in excess amount of elemicin and myristicin.

However, elemicin and myristicin are responsible for most of the pharmacological effects (Latha et al., 2005). Elemicin is partly responsible for the hallucinogenic properties of medical herbs. Myristicin and elemicin rich essential oils have been reported to be reproduced many of the psychotropic characteristics of plant (Truitt, 1967).

Myristicin and elemicin can be converted to chemicals similar to hallucinogens, such as TMA (3,4,5-trimethoxyamphetamine) (Weil, 1965) or MMDA (3-methoxy-4,5-methylene dioxyamphetamine) (Kalbhen, 1971) which both known as hallucinogens. The structure of MMDA is similar to MDMA also known as Ecstasy (Shulgin, 1966).

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are synthetic antioxidants that are often added to foods to prolong the storage stability (Suh et al., 2005; Race, 2009).

Because of their carcinogenicity and some side effects they have restrictions on use. So in the last few years people have preferred to use natural products instead of customary preservatives (Ksouri et al., 2009). Phenolic compounds in natural products are the major active groups that function as radical scavengers and antioxidants (Halliwel et al., 1992). The harmful effect of free radicals can be prevented by antioxidant substances which cleanse free radicals and detoxify the organism (Wang et al., 1996; Zheng and Wang, 2001).

As a result, consumers are interested in natural products including spices, herbs, especially plant extracts and their essential oils to enhance the flavor, color and aroma of the dishes (Bannach et al., 2012).

Today, many people prefer to use herbal remedies prepared from medicinal plants instead of chemical medicines when they are sick.

In this study, essential oil composition with the total phenolic substance and antioxidant activity of methanol extract of *H. Stoechas* which locally known as kudama, growing in the region of HATAY Antakya Castle was determined.

MATERIAL AND METHODS

Essential oil extraction and GC analysis

The air dried and milled plant was subjected to steam distillation using a Clevenger apparatus for 3 hours. The resulting volatile oil was dried over anhydrous sodium sulfate and after filtration it was stored at +4 °C until analysis.

GC-MS analysis was performed with an HP6890 gas chromatography device combined with a mass detector. The analytes were separated using a HP-5MS column (30m × 250 µm, 0.25 µm film thickness) using Helium (99.99%) as the carrier gas at a flow rate of 0.9 mL min^{-1}. The oven temperature was kept at 45 °C for

5 minutes and then heated to 220 °C at 3 °C min^{-1} and held at 250 °C for 10 minutes. One microliter ether solution of the essential oil was injected.

Methanolic extract

Firstly, *H. stoechas* was ground in an electric blender and for removing its fatty oil, it was extracted with a Soxhlet apparatus by using petroleum ether. Then the defatted plant material was extracted with methanol. Approximately 100 g of plant was soaked for 2 hours at 40 °C in 400 mL methanol. This process has been repeated ten times. The solution was filtered and the filtrate was concentrated at 40 °C using a rotary evaporator. The extract was stored at -4 °C until analysis.

Total phenolic substance

The total phenolic substance of methanol extract was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965; Zheleva-Dimitrova et al., 2010 All analyzes were performed in triplicate. For the calibration curve Gallic acid was used as a standard. The amount of phenolic substance was calculated from the calibration curve. The total phenolic concentration was expressed as the Gallic acid equivalent (mg GAE/g of extract powder). Phenolic contents of the plant extracts were determined by using 0.25mL of sample (0.4 mg/mL), 1.25 mL of 10 % dilution of Folin- Ciocalteu reagent and 3.75 mL of Na2CO3 (10%, w/v). The resulting mixture was incubated at room temperature for 15 minutes and the absorbances were measured at 765 nm.

DPPH radical-scavenging activity

Using the DPPH radical method, the free radical scavenging activity of the methanolic extract of Helichrysum Stoechas was measured in terms of radical scavenging ability. (Ozgen et al., 2010). 3.75 ml methanol solution of the DPPH (6×10-5 mol/L) was added to 1.25 ml methanol solution of extract (or standard) at different concentrations (0.1-0.00625mg/mL). Incubated at 40°C in a thermostatic bath 30 minutes and then decreasing of the absorbance (n=3) was measured at 517nm. For comparison with synthetic antioxidants BHT and BHA as positive control the same procedure was repeated. DPPH scavenging ability was calculated as Inhibition level (I %) of the free radical.

Inhibition level (I %) was calculated as follows:

$$I\ \% = (Ablank - Asample/Ablank) \times 100 \quad (1)$$

Where, the absorbance of the control reaction is Ablank (containing all reagents except the test compound) and the absorbance of the test compound is Asample. 50% inhibition value (IC50) was calculated from by plotting the inhibition percentage against extract concentration.

Total antioxidant capacity (CUPRAC method)

The total antioxidant capacity of methanolic extract was determined by the CUPRAC method (Apak et. al., 2006). Measurements were made at 450 nm and calculations were carried out with a calibration curve obtained with TROLOX. Total antioxidant concentrations were expressed as trolox equivalent mmol TR/g dry matter.

1 ml CuCl2 solution (1.0x10-2 M), 1 ml neocuproine alcoholic solution (7.5x10-3 M) and 1 ml NH4Ac buffer solution were added to a test tube and then by mixing; (x) ml extract followed by (1.1-x) ml water were then added (total volume, 4.1 ml) and mixed. Absorbance (at 450 nm) was measured after 30 min against a reagent blank. In the CUPRAC method, the molar absorptivity of trolox is ε=1.67x104 L/mol.cm, and the calibration curve for trolox is a line passing through the origin, the trolox equivalent molar concentration of the methanol extract sample in the final solution may be found by dividing the observed absorbance by ε for trolox.

Calculation of trolox equivalent antioxidant capacity is as follows:

$$\text{Capacity (mmol TR/g)} = (Af/\varepsilon TR) \times (Vf/Vs) \times r \times (Vcup/m) \quad (2)$$

Af; final absorbance of sample

ε TR: molar absorptivity of trolox

Vf : final volume

Vs: sample volume

r : dilution factor

Vcup: initial volume of sample or trolox

m: (g) dry matter

RESULTS AND DISCUSSION

H. stoechas essential oil was obtained using Clevenger apparatus by using all portions the plant (flower, leaf and body). The color of the volatile oil is light yellow and the total yield of dry matter was found to be 0.3% (w w^{-1}). In GC analysis, the volatile oil constituents and their percentages were identified on HP 5 capillary column and listed according to their elution order as in Table 1.

Table 1. Volatile constituents of *H. stoechas*

Peak no	Time	Components	Cas No.	%
1	27.17	D-Fenchone	004695-62-9	0.77
2	38.90	Caryophyllene	000087-44-5	1.10
3	63.48	Rosifoliol	063891-61-2	4.89
4	64.44	3-methoxy-γ-asarone	015361-99-6	34.69
5	64.98	Elemicin	000487-11-6	3.24
6	65.63	Myristicin	000607-91-0	23.35
7	69.02	Lauric acid	000143-07-7	1.17
8	69.32	Apiol	000523-80-8	27.38
9	73.42	Myristic acid	000544-63-8	1.38
10	80.18	Pentadecanoic acid	001002-84-2	2.03

In the essential oil, ten components were found (Fig.1). Major components of the volatile oil are rosifoliol, 3-methoxy-γ-asarone, elemicin, myristicin and apiol.

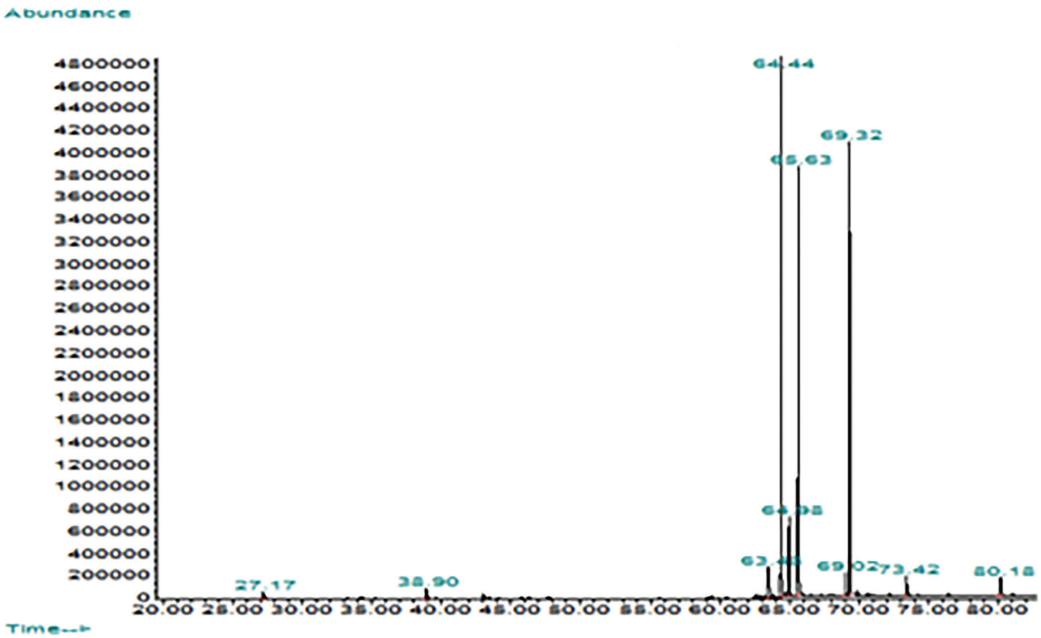

Figure 1. GC spectrum of *H. Stoechas* essential oil.

Total phenolic subtance of the methanol extracts of *H. stoechas* was determined according to the Folin-Ciocalteu method. Total phenolic content was 256.55 ± 3.7 mg Gallic acid equivalent/g of extract powder in reference to the standard curve, y=1,9409x+0,0032, r^2 =0,9996 (Fig. 2).

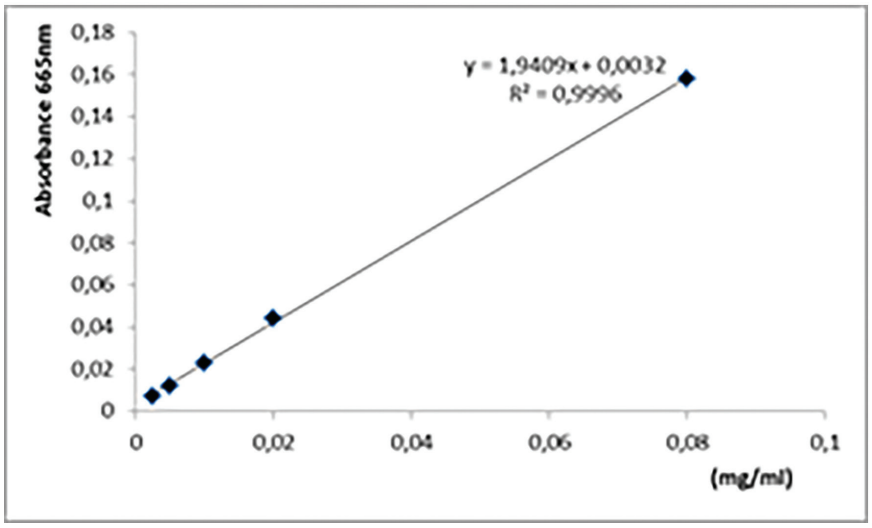

Figure 2. Calibration curve of Gallic acid.

The free radical scavenging effect was determined according to the DPPH method and the result was compared with BHA and BHT.

Inhibition values of solutions at the same concentration (0.1000-0.00625 mg mL^{-1}) were calculated by using equation 1.

Inhibition value of *H. stoechas* methanol extract; 90.40%, 65.1%, 48.32%, 22.56%, 8.76%, BHA; 93.33%, 92.67%, 92.00%, 91.61%, 86.28% and BHT; 93.20%, 92.31%, 90.92%, 80.71%, 63.14% respectively (Fig. 3).

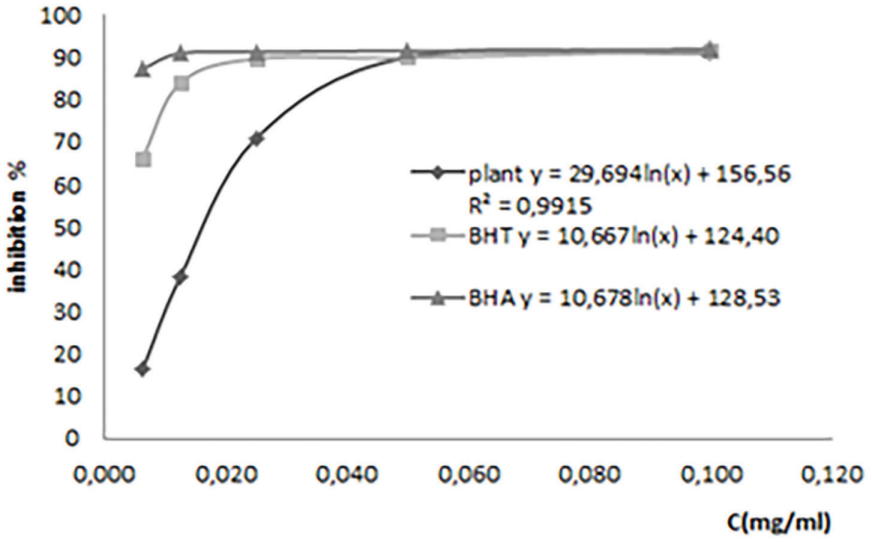

Figure 3. Comparison of inhibition value of *H. stoechas*, BHT and BHA

DPPH radical-scavenging activity IC_{50} values required for the 50% inhibition of the DPPH radical in the reaction medium were calculated from equations in Fig. 3 and found to be BHA: 6.40±0.19x10^{-4}, *H. Stoechas*: 2.76±0.44x10^{-2} and BHT: 9.35±0.37x10^{-4} mg mL^{-1}, respectively. The low IC_{50} value is indicative of the high radical scavenging activity.

The DPPH removal activity of the standards and plant extract was found to decrease as plant extract< BHA< BHT. Reductive Antioxidant Capacity was determined according to CUPRAC method. The CUPRAC antioxidant assay for Trolox at different dilutions, depicted in Fig. 4 showed agreement (r=0.985) CUPRAC values was decreased proportional to dilution ratio.

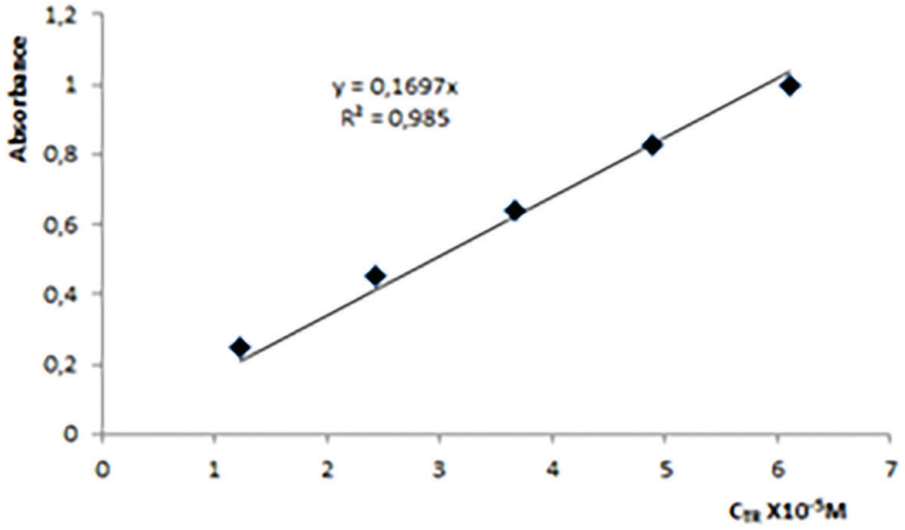

Figure 4. Calibration curve of Trolox (TR)

$TEAC_{CUPRAC}$ value was calculated by using equation 2 and Trolox calibration curve. It was seen that both values were close to each other.

Cupric reducing antioxidant capacity (From the Trolox calibration curve) $TEAC_{CUPRAC}$: 8.31 mmol TR/g and from the formula, calculated $TEAC_{CUPRAC}$: 8.44 mmol TR/g was found as. Calculated and measured CUPRAC values are in an agreement.

CONCLUSION

As a conclusion; for better pharmacognostical knowledge of *H. stoechas* which grown in Hatay region, the essential oil composition, antioxidant capacity and total phenolic substance of the plant were determined. It has been found that volatile oil is rich in hallucinogenic elemicin and myristicin. Obtained results showed that the methanolic extract of *H. Stoechas* was rich in phenolic content and has a close antioxidant activity to the synthetic antioxidants BHT and BHA.

ABBREVIATION USED

GC: Gas chromatography; **%**: Percentage; **GAE**: Gallic acid equivalents; **TR**: Trolox; **mL**:milliliter; **BHA**:Butylated hydroxyanisole; **BHT**: Butylated hydroxytoluene; **DPPH**: 2,2-diphenyl-1-picrylhydrazyl; **µm**: Micrometer

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