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Phytochemical analysis and *in vitro* antioxidant activities of marjoram oil

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There is a growing interest in the addition of natural antioxidants, especially herbs and spices in food technology. Medicinal plants are sources of bioactive compounds with recognized beneficial effects on human health. An example is Origanum majorana, which is known for its traditional therapeutic properties in some countries. The aim of the present study was to analyze the phytochemical content and evaluate the *in vitro* antioxidant properties of marjoram oil. Chemical composition was analyzed using chromatography–mass spectrometry (GC-MS) method. *In vitro* antioxidant activity was determined by assessing free radicals scavenging activity and reducing power. The total phenolic and flavonoid contents were quantified by spectrophotometry using Folin−Ciocalteu and aluminum chloride colorimetric methods, respectively. GC-MS analysis revealed the presence of 4-terpineol, alpha-pinene, sabinene, o cymene, alpha-terpinolene, terpinolene, alpha and gamma terpinene and sabinene as main constituents. Total phenolic content and total flavonoid content in marjoram oil were found to be 0.399 ± 0.038; 1.022 ± 0.007 mg/10 µl oil, respectively. Marjoram oil exhibited concentration-dependent inhibitory effects on 2,2`diphenylpicrylhydrazyl (DPPH) and reducing power with IC50 values of 0.509 ± 1.36 ; 0.008 ± 0.01 mg/ml, respectively. We can conclude that the marjoram oil has a significant potential to be used as a natural antioxidant.

Keywords: Origanum majorana; GC-MS; antioxidants; polyphenols; flavonoid.

INTRODUCTION

Many medicinal plants have been shown to contain high levels of antioxidants, which can help to scavenge free radicals and reactive oxygen species. During the time of stress created by drugs, toxic substances, or diseases, the production of active oxygen species increases, which has the potential to cause oxidative damage (Frei et al. 1996). Antioxidants play a critical role in avoiding the oxidative pathways that cause degenerative illnesses (Cardador-Martinez et al. 2002). Nowadays, the world has given special attention to the medicinal and aromatic plants as an excellent source for bioactive agents.

Species in the genus *Origanum* have been utilized as medicinal, aromatic, and ornamental plants since antiquity (Meyers 2005). *In vitro* pharmacological investigations showed their antibacterial, antifungal, antioxidant, anti-spasmodic, anti-mutagenic, anti-tumoral, analgesic, anti-thrombin and anti-hyperglycaemic activities (Chihti et al. 2013).

Origanum majorana is an herb from the family Lamiaceae herb that commonly grows in Mediterranean regions and is widely used in traditional medicine as well as the food and

cosmetic industries. It's been used for centuries as a traditional treatment for asthma, indigestion, headaches, and rheumatism (Baranauskiene et al. 2006; Banchio et al. 2008). Marjoram is a medicinal plant with a wide range of pharmacological activities, including antioxidant, antibacterial, and anti-inflammatory actions, which are attributed to its high phenolic acid and flavonoid content (Banchio et al. 2008; Qari, 2008; Mossa et al. 2011; Erenler et al. 2016).

To date, many phytochemical researches have been undertaken to examine the chemical composition of sweet marjoram essential oil. The findings revealed that the oils exist in two forms: one with terpinene-4-ol and sabinene hydrate as main components, and the other with terpinene-4-ol and sabinene hydrate as major components (Banchio et al. 2008) and the other with thymol and/or carvacrol as predominant compounds (Baser et al. 1993). In the first chemotype, terpinene-4-ol together with *cis*-sabinene hydrate is responsible for the characteristic flavour and fragrance of marjoram oil (Vagi et al. 2005). Other compounds could be present in the essential oil in appreciable amounts such as p-cymene and γ-present in the essential oil in appreciable amounts such as p-cymene and γ-terpinene (Vera et al. 1999).

Commercial *O. majorana* oil is utilized as a spice and condiment. The fresh ordried highly aromatic leaves and flowering tops of marjoram (*O. majorana* L.) are widely used to flavour many common food products. Moreover, the oil is used in perfumery for its spicy herbaceous notes (Vera et al. 1999).

With the increased interest in natural bioactive compounds, health professionals interested in holistic practices and research scientists are carrying out experimental trials to confirm the *in vitro* results obtained with phytochemicals in the prevention of many diseases (Tiwari, 2001; Halliwell, 2006). Meanwhile, it is important to explore the medicinal plants in order to find alternative sources of antioxidant molecules that could be used in chemoprevention of inflammatory processes and chronic diseases. The aim of this study was to determine the chemical composition as well as the *in vitro* antioxidant activity of marjoram oil.

MATERIALS AND METHODS

Chemicals

Marjoram oil was obtained from commercial CAP PHARM. 2, 2-diphenyl 1-picrylhydrazyl, Folin–Ciocalteu, gallic acid, Butylated hydroxytoluene, catechin, ascorbic acid were purchased from Sigma Chemicals Co., St. Louis, MO (USA).

Analysis of marjoram oil composition

Composition of marjoram oil was analyzed using Perkin Elmer Clarus 500 gas chromatograph capillary column HP-5 (30m×0.25mmID, 0.25-µm film thickness), coupled to Clarus 500 mass spectrometer. The marjoram oil was diluted with methanol and was injected to this gas chromatography–mass spectrometry (GC-MS) system. The GC operating conditions were as follows: helium as carrier gas with a flow rate 2.0 ml/min; the column temperature was programmed to rise from 60 to 280°C at 5°C/min; injector temperatures 250°C. The following were the MS operating parameters: ionization potential, 70 ev; ion source. The components were identified by comparing GC retention indices and mass spectral fragmentation patterns to those of authentic samples.

Total phenolic content

The total phenolic content of marjoram oil was determined using Folin-Ciocalteu reagent according to the method of Li et al. (2007). An aliquot of the marjoram oil was mixed with 1 ml of Folin–Ciocalteu reagent during 1 min then 800 µl of 7.5% sodium carbonate was added. The controls contained all the reaction reagents except the oil. After incubation during 2 h, the absorbance was measured at 760 nm. Various amounts of gallic acid were used to create a standard curve. Total phenolic content was expressed as mg gallic acid/10 µl of marjoram oil/ml methanol. Analyses were done in triplicate.

Total flavonoid content

The method of Dewanto et al. (2002) was used for the estimation of total flavonoid content of the oil. An aliquot of 400 µl of the marjoram oil was added to 1.2 ml of distilled water, followed by immediate addition of 120 µl of 5% (w/v) $NaNO_2$, 120 µl of $AlCl_3$ 10% (w/v) after 5 min and 400 µl of 1 M NaOH after 6 min; contents of each reaction flask were diluted with 2.5 ml of distilled water and mixed immediately. The absorbance of the pink-colored solution was measured at 510 nm in comparison to a blank (distilled water). Samples were analysed in triplicate. Flavonoid content was expressed as mg catechin equivalents (CE)/10 µl marjoram oil/ml methanol. The calibration curve range was 50 to 500 mg/ml.

In vitro antioxidant activity

The antioxidant activity of marjoram oil was assessed by DPPH free radical scavenging assay and reducing power.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

The free radical scavenging ability of the oil was evaluated as described by method of Brand-Williams et al. (1995). 100 µl of different concentrations (10-100 µg/ml) of marjoram oil in methanol were mixed with 2ml of methanol solution of DPPH. The absorbance was measured against a blank at 517 nm after a 30-minute incubation period at room temperature. From the graph of percentage inhibition against oil concentrations, the concentration providing 50% inhibition (IC50) was derived. The following formula was used to calculate DPPH scavenging activity:

DPPH scavenging effect (%) = [(A blank – A sample)/A blank] × 100

Where, A blank is the absorbance of the control reaction and A sample is the absorbance in the presence of marjoram oil. Samples were analyzed in triplicate. Butylated hydroxytoluene (BHT) was used as standards.

Reducing power

The reducing power of the marjoram oil was determined according to the method of Oyaizu (1986). 100 µl of various concentrations of the oil (10–100 µg/ml) in methanol were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and potassium hexacyanoferrate [K3Fe(CN)6] (2.5 ml, 1% w/v). After 30 min incubation at 50ºC, 2.5 ml of 10% trichloroacetic acid were added. The mixture was centrifuged for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous FeCl3. The absorbance was measured at 700 nm. The mean absorbance values were plotted against concentration, and a linear regression analysis was carried out. The data are presented as ascorbic acid equivalents (AscAE) in mmol ascorbic acid per gram of oil.

Statistical analysis

All values are expressed as means ± SEM. Results were analyzed by one-way ANOVA followed by an LSD Fisher test (Statistica Stat Soft, Maisons-Alfort, France). Statistical significance was defined as a value of $p < 0.05$.

RESULTS

Composition analysis of marjoram oil

Marjoram oil was analysed using GC-MS and its chromatographic pattern is showed in Table 1. GC-MS analysis was allowed for the identification of monoterpene hydrocarbons, including alpha-pinene, sabinene, o-cymene, alpha-terpinolene, terpinolene, alpha and gamma terpinene, alpha thujene, and beta myrcene. Terpinene 4-ol is main oxygenated monoterpenes. Caryophylene is oxygenated sesquiterpene; as major components of marjoram oil.

Table 1: Marjoram oil analysis by GC/MS. Rt, retention time in min.

Compounds	Approximate Rt (min)
Sabinene	4.909
O- Cymene	5.599
2 Alpha terpinene	5.489
2 Alpha terpilonelene	6.555
2 Bergamotene	11.367
Beta myrcene	8.821
Caryophylene	11.252
Gama terpinene	5.67
Megastigma	12.212
4 Thujanolstereoisomer	7.9
Alpha pinene	8.301
Alpha thujene	8.301
Estragole	8.15
Gama terpinene	8.896
Terpilonelene	6.505
Terpinen4-ol	7.825

Determination of total phenolic and flavonoid content

Total phenolic content (TPC) and total flavonoid content (TFC) in marjoram oil were found to be 0.399±0.038 mg of Gallic acid (GAE)/ 10µl of marjoram oil/ml methanol and 1.022±0.007 mg catechin/10µl oil/ml methanol, respectively (Table 2).

Table 2: Total phenolic, flavonoids content of Origanum majorana oil

	Total phenolic	Total flavonoids
Marjoram oil	**0.399±0.038**	**1.022±0.007**

Total phenolic expressed as mg Gallic acid (GAE)/10µl of marjoram oil/ml methanol,
Total flavonoids expressed as mg Quatechin/10µl of oil/ml methanol.

In vitro antioxidant activity of marjoram oil

DPPH radical scavenging assay

Marjoram oil was capable of scavenging DPPH in a concentration-dependent manner. BHT, used as standard, exhibited an inhibitory activity of 164.52 µg/ml, whereas the concentration of the oil resulting in 50% inhibition of the free radical scavenging (IC50) was 509.8 µg/ml which was comparatively higher than that of the standard (Table 3). This result suggested that the marjoram oil was capable of scavenging free radicals.

Table 3: IC50-inhibitory concentration, radical scavenging and reducing power (Concentration - mg/ml)

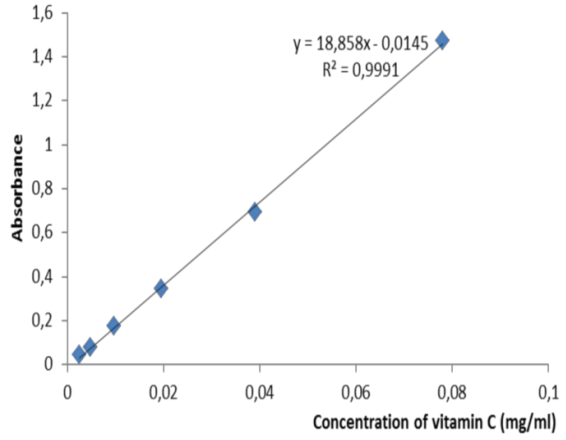
	Marjoram oil	BHT	Ascorbic acid
DDPH (IC50 mg.ml-1)	0.509 ±1.36	0.164±1.69	----
Reducing power (EC50 mg.ml-1)	0.008±0.01	---	0.0272±0.02

All the values were expressed as Mean ± SE (n = 3).

Reducing power

Figure 1 showed the reducing potential of the marjoram oil and vitamin C taken as the positive control.

A



B

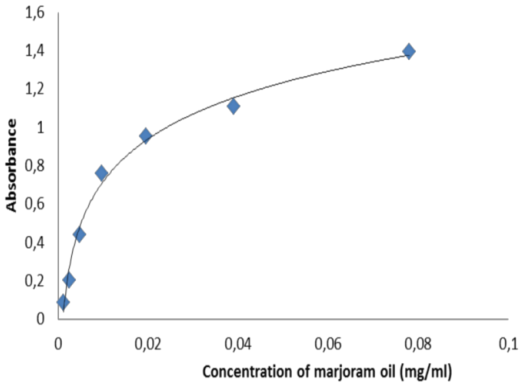


Figure 1: Reducing power (A) of vitamin C (synthetic antioxidants) and (B) marjoram oil.

The marjoram oil possessed the ability to reduce iron (III) and showed good linear relationship in both standard and marjoram oil. This property suggests that the Origanum majorana may act as free radical chain terminators, transforming reactive free radical species into more stable non-radical products.

DISCUSSION

Marjoram oil showed a chemical composition rich in phenolic compounds. Phenolic compounds act as free radical scavengers and reactive oxygen species (ROS) quenchers, being also able of decomposing peroxides, chelating transition metals and inhibiting enzymes involved in the production of reactive species (Pokorny et al. 2001; Embuscado et al. 2015).

The GC-MS analysis indicated the presence of monoterpenic constituents. The most abundant compounds included α-terpinene, δ-terpinene, terpinen-4-ol and sabinene. This chemical profile is in accordance with that reported in the literature, with some quantitative variations. Rodrigues et al. (2002) and V´agi et al. (2005) have reported the presence of terpenes as the main components of Origanum majorana essential oil. The most abundant components in Origanum majorana essential oil are usually classified as terpinen-4-ol and -terpinene. Sabinene and δ- terpinene are also observed (Busatta et al. 2008; Alizadeh et al. 2011;Ramos et al. 2011).

In our study, MO showed a good reducing power capacity, which was concentration-dependent. Essential oils (EO) are mixtures of several components which canreact with free radicals to stabilize and terminate radical chain reactions. However, it is difficult to assign the antioxidant activity of the total EO to one or a few

active molecules because both minor and major constituents must be taken into consideration to account for their biological activity (Wang et al. 2008).

Some of the compounds present in MO have previously been reported to exhibit antioxidant activity. While γ-terpinene sabinene (Ruberto et al. 2000; Singh et al. 2005) terpinene and terpinolene (Choi et al. 2000; Kim et al. 2004) have been shown to exhibit high antioxidant activity (Lis et al. 2007), α-pinene (Kim et al. 2004; Sellami et al. 2009), p-cymene (Soares et al. 1997), terpenes and terpinen-4-ol were described to display low activity. Indeed, a high positive association between phenolic content and antioxidant capacity has been found in numerous investigations (Abidille et al. 2005). The high potential of phenolic components to scavenge radicals might be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Sawa et al., 1999; Ruberto et al. 2000). In addition, the number and the position of hydroxyl group of phenolic compounds govern their antioxidant activity (Lee et al. 2001).

CONCLUSION

Based on the obtained results, we can conclude that MO contained a considerable amount of phenols (phenolic and flavonoid contents), exhibited a strong antioxidant power and a potential free radical scavenger ability. These results indicated that marjoram could represent a significant source of naturel antioxidants with might be helpful in the pharmaceutical and food industries.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Research design: IBD and HK; Writing original draft preparation: IBD and HG; Writing review and editing: OK and DS.

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