INTERNATIONAL JOURNAL OF SECONDARY METABOLITE

Int. J. Sec. Metabolite, Vol. 3, Issue 2 (2016) pp. 74-81

Research Article

ISSN: 2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

Phenolic Contents and Antioxidant Properties of *Echinops ritro* L. and *E. tournefortii* Jaup. Et. Spach Extract

Çiğdem Aydın [1, 2*], Gülten Taşdelen Özcan[1], Murat Turan[1], Ramazan Mammadov[1]

[1]Department of Biology, Faculty of Science and Arts, Pamukkale University, 20100 Denizli, Turkey

Received: 01 February 2016 - Revised: 30 March 2016 - Accepted: 26 April 2016

Abstract: Aim of the study was to evaluate antioxidant activity and total phenolic content of *Echinops ritro* L. and *E. tournefortii* (Asteraceae). The dried leaves and seeds of *E. ritro* and *E. tournefortii* were extracted separately with ethanol, methanol, chloroform and dH_2O. Total phenolic content was measured by Folin-Ciocalteu method. Antioxidant activities of the extracts were determined by two test systems namely, radical scavenging on DPPH and β-carotene bleaching methods. dH_2O extracts has the highest phenolic content (92.24 GAE mg/100g). The results were compared to those of BHT as synthetic antioxidant. dH_2O extracts were found to be rich as a source of phenolics. According to the results of antioxidant activity, dH_2O extracts exhibited higher antioxidant activity than all types of solvent. The strongest antioxidant properties were obtained by dH_2O extract. Radical scavenging activities (%) were found to be in the following order: Chloroform<Ethanol<Methanol<dH_2O<BHT. These results of the present study demonstrated that the extracts of *E. ritro* and *E. tournefortii* may be used as a source of natural antioxidant in the food and pharmaceutical industries.

Keywords: *Echinops ritro*, *E. tournefortii*, antioxidant activity, phenolic content,

1. Introduction

Plants are good source of biologically active secondary metabolites which have many therapeutic potential in many diseases and even in free radical associated disorders [1]. Among secondary metabolites synthesized, plant polyphenols are the aromatic hydroxylated compounds which have the most potent and therapeutically useful bioactive substances. Promising radical scavenging ability of the phenolic compounds produced in higher plants is studied extensively [2]. Oxidation stress is one of the major concerns of health in modern era and antioxidants have been reported to prevent oxidative damage caused by free radical, via interfering with the oxidation process by reacting with free radicals, by chelating with catalytic metals, and also by acting as oxygen scavengers [3]. In the presence of antioxidants, the oxidative rates decrease due to an increased activation energy for reaction, thus increasing the "lifetime" of the substrate, serving as a parameter for the evaluation of the antioxidant activity [4]. Although several synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, are available because of their toxicity problems; there is an upsurge of interest in the therapeutic potentials of plants as antioxidants. In addition to the natural antioxidants like vegetables, fruits, spices and tea, scientific evaluation of plant's properties

*Corresponding Author Tel.: +90 505 5712960. E-mail: cdem.86@hotmail.com

2148-6905 /© 2016 DOI: 10.21448/http-ijate-net-index-php-ijsm.243309

through potent pharmacological activities, toxicity profiling and economic viability are needed for growing recognition for medicinal plants and herbal products as novel antioxidants in recent decades. Therefore, significant consideration has been directed toward the detection of antioxidant properties in plant species.

The genus *Echinops* L. (Asteraceae) consists of approximately 120 species [5], distributed in Africa and the Mediterranean basin [6]. In Turkey, the genus comprises 19 species, including 2 subspecies and 3 varieties [7,8]. *Echinops* plant was reported to possess variety of compounds belonging to various classes like: alkaloids, flavonoids, terpenoids, lipids, steroids and polyacetylenes [9]. Echinopsine was quinoline alkaloid isolated in 1900 by M. Greshoff from seeds of the blue globe thistle, *E. ritro* and its presence was also demonstrated in 14 other species of *Echinops* like *E. latifolius*, *E. setifer* [10]. *Echinops* species have been used as traditional medicine for treatment of migraine, diuretic, heart diseases, urinary infection, as well as worm and hemorrhoid in Ethiopia [11]. In the present study, total phenolic of the ethanol, methanol, chloroform and dH_2O extracts prepared from *E. ritro* and *E. tournefortii* the were determined as mg/g GAE. These extracts were tested for their antioxidant activity by using two methods namely β- carotene-linoleic acid test system and DPPH free radical scavenging assay. This study examined the antioxidant activities of these species for the first time.

2. Material and Methods

2.1. Plant materials

Echinops ritro and *E. tournefortii* was collected from Denizli Kınıklı field during the period of investigation and in July 2015. The voucher specimen of *E. ritro* was confirmed and deposited in Herbarium at the Department of Biology. The collected plant material was air-dried in darkness at room temperature (20^{o}C). Dried plant parts were cut up and stored in tight-seal dark containers until needed.

2.2. Preparation of plant extracts

Echinops ritro and *E. tournefortii* species cut into small pieces with a blender. Extractions were prepared using different solvents (methanol, ethanol, chloroform and dH_2O). For extractions 10 g of the plant and 100 mL of solvent (Merck) were used for each sample. The mixture was extracted after being heated in a shaker water bath at 55ºC for 6 h. The extract obtained was filtered through filter paper (Whatman No: 1), and the solvents were evaporated in a rotary evaporator (IKA, RV 10 basic V-C, Germany) at 48 – 49°C. The water in each extract was frozen in Freeze-drying (Thermo, savant) machine and then drawn out (stored at -20^{o}C).

2.3. Determination of total phenolic content

The total phenolic content of extracts was determined using to the Folin-Ciocalteu method [12]. Briefly, 0.75 mL of Folin-Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and 100 mL of sample (5 mg/mL) were put into a test tube. The mixture was mixed and allowed to stand for 5 min at room temperature. The mixture was allowed to stand at room temperature for 5 min. 0.75 mL of 6 % (w/v) Na_2CO_3 was added to the mixture and then mixed gently. The mixture was homogenized and allowed to stand at room temperature for 90 min. Total polyphenol content was determined using a spectrophotometer at 750 nm. The standard calibration (0.01-0.05 mg/mL) curve was plotted using gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g plant extract.

2.4. Determination of total antioxidant activity

The antioxidant activity of the crude extracts was evaluated using the β-carotene-linoleic acid test system with slight modifications [13]. β-Carotene (0.2 mg) in 1mL of chloroform was added to 20 μL of linoleic acid, and 200 mg of Tween-20 emulsifier mixture. The mixture was then evaporated at 40^{o}C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation of chloroform, 100 mL of distilled water saturated with oxygen, 4.8 mL of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 of the extract in them. For control, 0.2 mL of solvent (methanol, ethanol, chloroform and dH_2O) was placed in test tubes instead of the extract. As soon as the emulsion was added into the test tubes, initial absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Japanese) to be at 470 nm. The measurement was carried out at 0.5 h intervals for 2 h. All samples were assayed in triplicate. BHT was used as standards. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation. The measurements were made using the equation below:

$$AA: [\ 1- (A0-At\ /\ A0o - Ato) \times 100$$

Where AA is the total antioxidant activity, A0 is the initial absorbance of the sample, At is the initial absorbance of the control, A0o is the sample's absorbance after 120 min, and Ato is the control's absorbance after 120 min.

2.5. Determination of DPPH free radical scavenging activity

Free radical scavenging activity of the extracts was determined using the free radical DPPH [14]. 4 ml of the DPPH's 0.004% metanolic solution was mixed with 1 ml (0.2 - 1.0 mg/mL) of the extracts, and their absorbances were measured to be at 517 nm after incubation for 30 min at room temperature the absorbance value of the samples were evaluated against empty control group (where all determinants except the test compound were present). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Every test was treated three times and the averages as determined. Free radical scavenging activity was measured using the equation below:

$$\text{Scavenging activity} = [(A_0 - A_1 / A_0) \times 1\ 00\ )]$$

where A0 is the absorbance of the control (blank, without extract) and A1 is the absorbance in the presence of the extract.

3. Results and Discussion

Polyphenols are known for their antioxidant activity as radical scavengers and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular disease, other pathologies [15]. Plants containing high phenolic compounds can be a good source of antioxidants. For this reason, this information has led to the determination of the total phenolic content of the sample under study. The amounts of total phenolic contents ranged from 31.54-92.24 (GAE mg/100g) for extracts respectively (Table 1.). In our investigation, the dH_2O extract of *E. ritro* (92.24 GAE mg/100g) exhibited the highest total phenol content.

Table 1. Total phenolic content of extracts

Extract	Total phenols (GAE mg/100 g)	
	E. ritro	*E. tournefortii*
Ethanol	58.21	49.32
Methanol	83.45	76.81
Chloroform	47.34	31.54
dH_2O	92.24	83.48

According to β-carotene-linoleic acid bleaching assay, β-carotene undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time. This is due to the oxidation of linoleic acid that generates free radicals that attack the highly unsaturated β-carotene molecules in an effort to reacquire a hydrogen atom. When this reaction occurs the β-carotene molecule loses its conjugation and, as a consequence, the characteristic orange color disappears. The presence of antioxidant avoids the destruction of the β-carotene conjugate system and the orange color is maintained

Inhibition of linoleic acid was affected by different solvents [16]. BHT showed maximum value of inhibition (94.07 %). The antioxidant activity efficiency were also calculated and given in Fig. 1 and Fig 2. As it can be seen from this figure, the highest antioxidant activity efficiency is determined in dH_2O extract of *E.ritro* (76.79 %) and the least efficiency in chloroform extract of *E. tournefortii* (13.30 %).

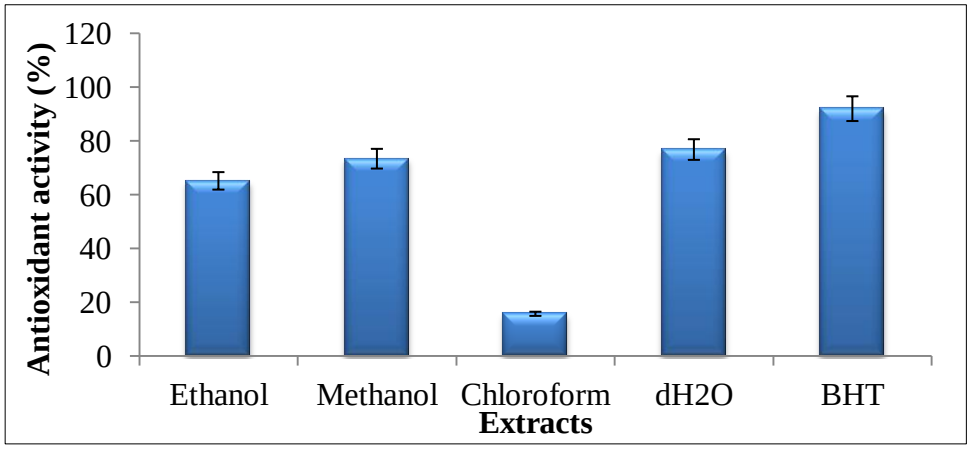

Fig. 1. Antioxidant activities efficiency in the extracts of *E.ritro* and BHT

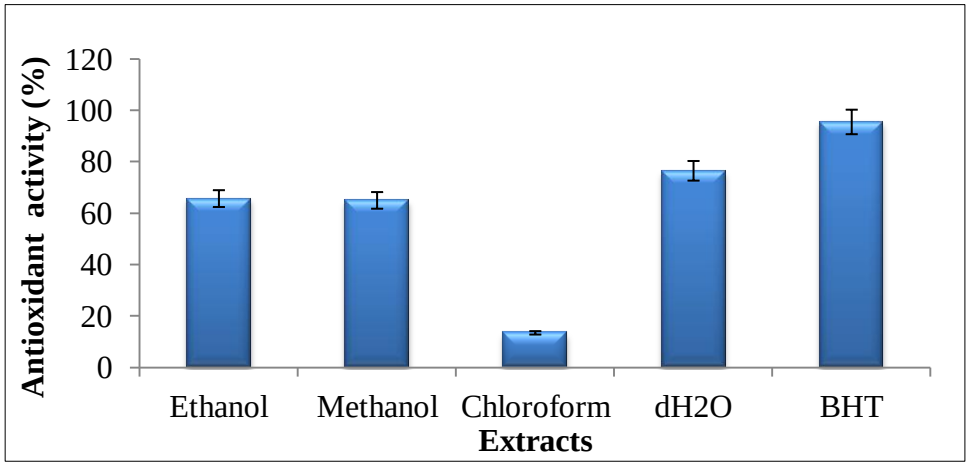

Fig. 2. Antioxidant activities efficiency in the extracts of *E. tournefortii* and BHT

DPPH radical scavenging activity assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of many plant extracts or compounds [17]. DPPH is a stable free radical which exhibits a deep purple color with maximum absorption at 517 nm. Antioxidant molecules react with the free radical by hydrogen or electron donation, resulting in discoloration of DPPH because of their conversion into yellow colored diphenylpicryl hydrazine [18]. As shown in Fig.3 and 4, the

DPPH radical scavenging activities of four extracts of *E. ritro* and *E. tournefortii* were concentration-dependent. DPPH radical scavenging activities (%) were found to be in the following order: Chloroform<Ethanol <Methanol <dH_2O < BHT (Fig.3 and 4).

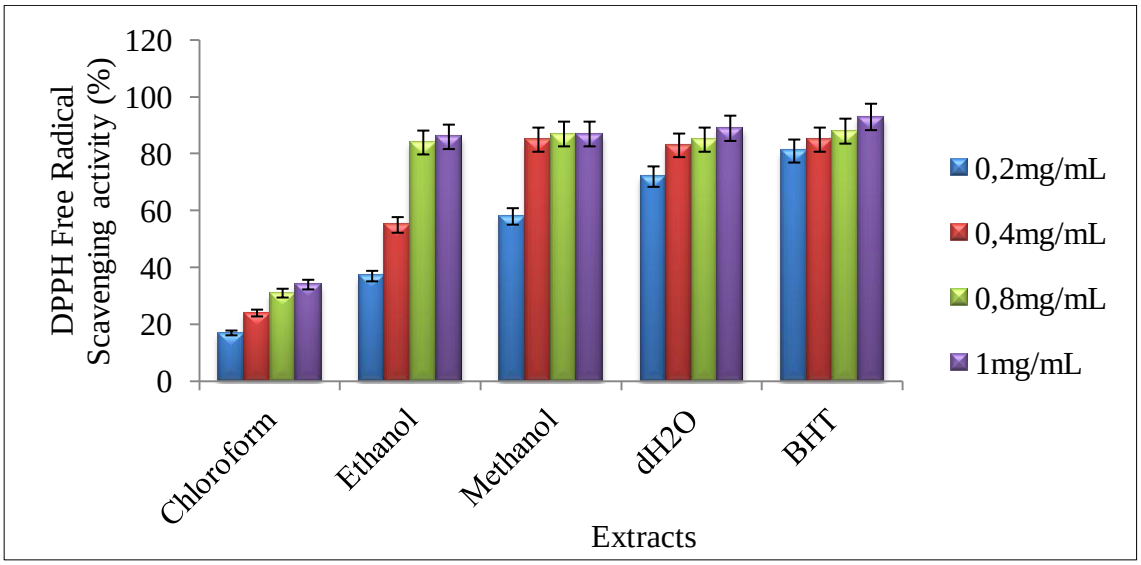

Fig. 3. The DPPH radical scavenging activities of *E. ritro* extracts and BHT

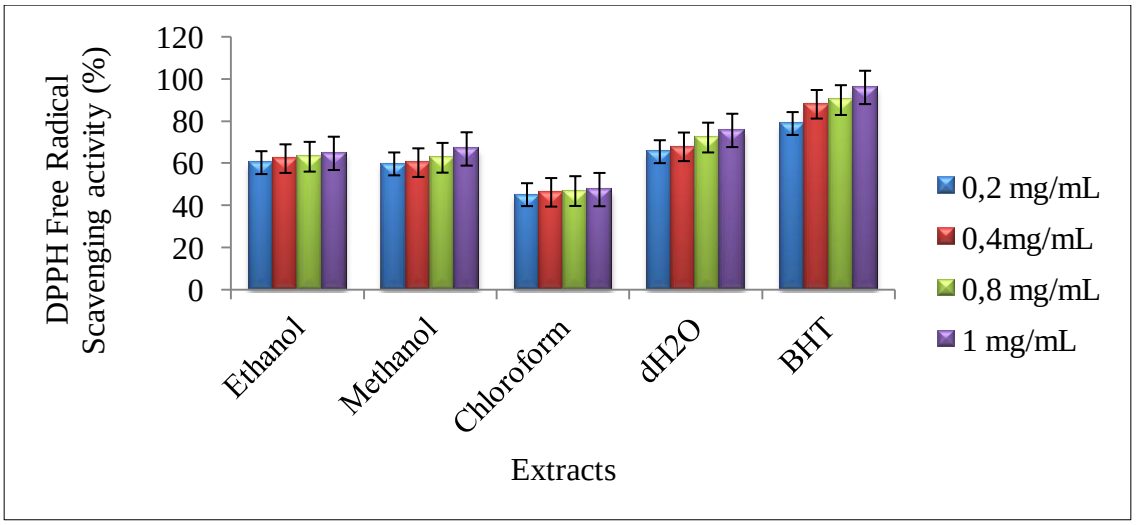

Fig. 4. The DPPH radical scavenging activities of *E. tournefortii* extracts and BHT

Fokialakis et al. (2006) reported that the root extracts of *E. ritro* were evaluated for their antifungal activity using direct-bioautography assays with three *Colletotrichum* species that cause strawberry anthracnose. Among the bioactive extracts, the dichloromethane extract of the radix of *E. ritro* was the most potent [19]. It was indicated that aerial parts of *E. ritro* L. and *E.spinosissimus* from the Greek island of Crete could be extracted, and the extracts obtained have been investigated for in-vitro anti-protozoal activity. The activity against chloroquinesensitive (D6) and resistant (W2) strains of *Plasmodium falciparum* and *Leishmania donovani promastigotes* was determined as well as the cytotoxicity on a mammalian kidney fibroblast (Vero) cell line was tested. Dichloromethane of aerial part

extract *of E. ritro* and *E. spinosissimus* had moderate activity against *L. donovani* with no significant anti-malarial activity or cytotoxicity [20]. Apigenin-glucopyranoside 1, Apigenin-glucoside 2, methoxycarbonylindole 3 and beta-sitositerol 4 were isolated from of *E. orientalis* dried leaves and seeds. Isolated compounds and extracts were applied to antioxidant activity tests. While seeds and leaves extracts have high DPPH and moderate ABTS radical scavenging activities, the isolated flavones exhibited high cation radical scavenging activities [21].

The antioxidant activities of methanolic extracts of the *E. kotschyi* were determined via 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and also screened for cytotoxic activity against three human cancerous cell lines (MOLT-4, K562 and MCF7) using the MTT assay (3-[4,5-dimethylthiazol-2-yl]- 2,5 diphenyl tetrazolium bromide) assay. The methanolic extract of *E. Kotschyi* exhibited potent cytotoxic activity against MOLT-4 and K562 cell lines among all extracts tested in this study [22]. On the other hand, the ethanolic extract of *E. spinosus* has efficient action on muscular fibers; anti-inflammatory activity; The ethanolic extract of *E. Spinosus* (100 mg/kg, intraperitoneal) exhibited a very good anti-inflammatory activity against carrageenan-induced paw edema in mice and rats, and it selectively inhibited prostaglandin E2 (PGE2) -induced inflammation [23].

In the present study, the antioxidant capacities and phenolic content of various extracts (methanol, dH_2O, ethanol and chloroform) from *E. ritro* were determined. The dH_2O extract had higher antioxidant capacity and free radical scavenging activity than other extract at the same concentrations. Results of the study indicated that dH_2O extract derived from *E. ritro* possessed remarkable antioxidant activities. Because the synthetic antioxidants (BHA and BHT) have some toxic effects, such as the promotion of carcinogenesis, *E. ritro* can be considered as a source of both natural antioxidants and lauric acid in the food industry and pharmacological applications.

4. Conclusion

According present the study, it may be concluded that the extracts of *Echinops ritro* and *E. tournefortii* demonstrated *in vitro* antioxidant activities. Higher levels of total phenolics of plant are probably responsible from the biological activities observed. This finding candidates the plant as a good case for more in-depth studies and we wish our future research lead to the identification of biologically active molecules present in its extracts

5. References

[1]. Lee, Y.M., Kim, H., Hong, E.K., Kang, BH., Kim, S.J. (2000). Water extract of 1:1 mixture of *Phellodendron cortex* and *Aralia cortex* has inhibitory effects on oxidative stress in kidney of diabetic rats. *J. Ethnopharmacol*., *73,* 429-36.

[2]. Pai, S.R., Nimbalkar, M.S., Pawar, N.V., Patil, R.P., Dixit, GB. (2010). Seasonal discrepancy in phenolic content and antioxidant properties from bark of *Nothopodytes nimmoniana* (Grah.) Mabb. *Int J Pharm Biosci*., 1, 1-17.

[3]. Buyukokurouglu, ME., Gulcin, I., Oktay, M., Kufrevioglu, O.I. (2001). *In vitro* antioxidant properties of dantrolene sodium. *Pharmacol Res. 44,* 491.

[4]. Carvalho, R.N., Moura, L.S, Rosa, P.T.V, Meireles, M.A.A. (2005). Supercritical fluid extraction from rosemary (Rosmarinus officinalis): kinetic data, extract's global yield, composition, and antioxidant activity. *J. Supercrit Fluids*, *35,* 197-204.

[5]. Susanna, A., Garcia-Jacas, N. The tribe Cardueae In: Kadereit J & Kubitzki K (eds.). Compositae. *The Families and Genera of Vascular Plants*. Heidelberg: Springer-Verlag, 2007, pp. 135-158.

[6]. Garnatje, T., Valles, J., Garcia, S., Hidalgo, O., Sanz, M., Canela, MA., Siljak-Yakovlev, S. (2004). Genome size in *Echinops* L. and related genera (Asteraceae, Cardueae): karyological, ecological and phylogenetic implications. *Biol Cell. 96*, 117-124.

[7]. Özhatay, N., Kültür, Ş., Aslan, S. (2009). Check-list of additional taxa to the Supplement Flora of Turkey IV. *Turk J Bot. 33*, 191-226.

[8]. Vural, C., Şapcı, H. (2012). Five new records of the genus *Echinops* (Asteraceae) from Turkey. *Turk. J. Bot. 36*: 151-160.

[9]. Shukla, Y.N. (2003). Chemical, botanical and pharmacological studies on the genus *Echinops* : A review. *J. Med Arom Pl Sci.*, *25*, 720-32.

[10]. Suarez, C., Barrera, C., Caballero, A. (2011). Quinolone alkaloids and friedelanetype triterpenes isolated from leaves and wood of *Esenbeckia alata* kunt Rutaceae. *Quim. Nova, 34(6)*, 984-86.

[11]. Desta, B. (1993). Ethiopian Traditional Herbal Drugs Antimicrobial activity of 63 medicinal-plants. *J. Ethnopharmacol., 39*, 129-139.

[12]. Slinkard, K., Singleton, V.L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American J. Enology and Viticulture*., *28(1)*, 49-55.

[13]. Amin, I., Tan, S.H. (2002). Antioxidant activity of selected commercial seaweeds. *Malaysian Journal of Nutrition., 8*, 167-177.

[14]. Wu, C., Chen, F., Wang, X., Kim, H., He, G., Haley-Zitlin, V., Huang, G. (2006). Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. *Food Chem*., *96*, 220–227.

[15]. Yesıloglu, Y., Sıt, L., Kılıc, I. (2013). I*n vitro* Antioxidant Activity and Total Phenolic Content of Various Extracts of *Satureja hortensis* L. Collected from Turkey. *Asian Journal of Chemistry*, *25(15)*, 8311-8316.

[16]. Khadri, A., Serralheiro, M.L.M., Nogueira, J.M.F., Neffati, M., Smiti, S., Araújo, M.E.M. (2008). Antioxidant and antiacetylcholinesterase activities of essential oils from *Cymbopogon schoenanthus* L. Spreng. Determination of chemical composition by GC-mass spectrometry and ^{13}C NMR. *Food Chemistry*, *109(3*), 630–637.

[17]. Kamatou, G.P.P., Viljoen, A.M., Steenkamp, P. (2010). Antioxidant, anti-inflammatory activities and HPLC analysis of South African *Salvia* species. *Food Chem., 119*, 684-688.

[18]. Krimat, S., Dob, T., Toumi, M., Kesouri, A., Noasri, A. (2015). Assessment of phytochemicals, antioxidant, antimicrobial and cytotoxic properties of *Salvia chudaei* Batt. et Trab. endemic medicinal plant from Algeria. *J. Mater. Environ. Sci., 6(1*), 70-78.

[19]. Fokialakis, N., Cantrell, CL., Duke, SO., Skaltsounis, AL., Wedge, DE. Antifungal activity of thiophenes from *Echinops ritro*. J. *Agric. Food Chem.* 2006, *54(5)*, 1651-5.

[20]. Fokialakis, N., Kalpoutzakis, E., Tekwani, B.L., Khan, S.I., Kobaisy, M., Skaltsounis, A.L., Duke, S.O. (2007). Evaluation of the antimalarial and antileishmanial activity of plants from the Greek island of Crete. *J. Nat. Med., 61*, 38–45.

[21]. Erenler, R., Yilmaz, S., Aksit, H., Sen, O., Genc, N. Elmastas, M., Demirtas, I. (2014). Antioxidant Activities of Chemical Constituents Isolated from *Echinops orientalis* Trauv. *Rec. Nat. Prod., 8(1*), 32-36.

[22]. Afshaki, S., Jafari, A., Javidnia, K., Firuzi, O. (2012). Antioxidant and cytotoxic activities of four plant extracts from Dena region of Iran. *Research in Pharmaceutical Sciences., 7(5),* 853-9.

[23]. Rimbau, V., Cerdan, C., Vila, R., Iglesias, J. (1999). Antiinflammatory activity of Some extracts from plants used in the traditional medicine of North-African Countries. *Phytother Res., 13(2*), 128-32.