Journal of Food and Nutrition Research, 2014, Vol. 2, No. 5, 221-227
Available online at http://pubs.sciepub.com/jfnr/2/5/3

DOI:10.12691/jfnr-2-5-3

Chemical Composition and Antioxidant Activity of the Essential Oil of *Pulicaria Inuloides*

Nabil Qaid M. Al-Hajj[1,2], Hongxin Wang[1,*], Mohammed A.A. Gasmalla[1], Chaoyang Ma[1], Riyadh Thabit[1], Md Ramim Tanver Rahman[1], Yuxia Tang[1]

[1]State key laboratory of Food science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, P.R. China
[2]Marine Science and Biological Research Authority, Aden, Yemen
*Corresponding author: cnwhx1964@163.com

Received April 18, 2014; Revised May 09, 2014; Accepted May 19, 2014

Abstract In order to evaluate the potential of medicinal effects of the popular edible *Pulicaria inuloides* Yemen, the methanol, ethanol and diethyl ether extracts from leaves of *Pulicaria inuloides* were analyzed for their antioxidant properties. The major components of the oil of *P. inuloides* were 2-Cyclohexen-1-one, 2-methyl-5-(1-methyl (56.8%), Benzene, methyl- (21.7%), Z.citol 2.00. The total phenol, flavonoid, contents of sample were measured by Folin Ciocalteu and $Alcl_3$ assays. Results showed that methanol extract from leaves of *Pulicaria inuloides* has more contents of total phenols and total flavonoid (91.2 ± 09.5, 89.9± 0.61 and 64.9±07. mg(GAE)/g respectively) and the level of total tannin and total anthocyanins contents were not identical in the extracts. Results from antioxidant activity assays showed that methanol in DPPH radical scavenging, B-carotene bleaching assays and metal-chelation is more active than diethyl ether extract. This study suggested that the differences of the potency of the antioxidant activity may be explained by the differences in the polyphenol and flavonoid levels.

Keywords: *Pulicaria inuloides, essential oil, Total phenol, Total flavonoid, Total Tannin, Total Anthocyanin, Antioxidant activity*

Cite This Article: Nabil Qaid M. Al-Hajj, Hongxin Wang, Mohammed A.A. Gasmalla, Chaoyang Ma, Riyadh Thabit, Md Ramim Tanver Rahman and Yuxia Tang, "Chemical Composition and Antioxidant Activity of the Essential Oil of *Pulicaria Inuloides*." *Journal of Food and Nutrition Research*, vol. 2, no. 5 (2014): 221-227. doi: 10.12691/jfnr-2-5-3.

1. Introduction

The genus *Pulicaria*, family Asteraceae, is represented 100 species widespread all around the world, more than 10 of which grow wild in Yemen. Chemically, this genus is not homogenous. As pointed out previously some species contain monoterpenes, diterpenes, sesquiterpene lactones [1] and caryophyllane derivatives [2]. Also the literature reports that Pulicaria species afforded different flavonoid profiles [3]. The Pulicaria species proved various activities such as antiinflammatory, antilukemic, Potential cancer chemopreventive and cytotoxic agents [4]. The Pulicaria crisps indigenous toYemen, locally known as Anssif, is traditionally used as diuretic, pyritic conditions in urogenetic organs, and to cure fever. The flowers of *Pulicaria inuloides* was also used as spice and to make various delicious foods. Some investigation reported that this species reveal antimicrobial, antifungal, antimalaria and insecticides properties [5]. *P. arabica* is used medicinally to treat painful swellings and boils. The whole plant is strongly aromatic and sprigs were often plucked and rubbed between the hands and then over the body as a deodorant. It grows abundantly in drier areas, where the water levels lie close to the surface, and is a reliable indicator for the proximity of water [6]. The biological activities of medicinal plants are mainly due to the presence of their active constituents or secondary metabolites, which are present almost in higher plants, usually in a high structural diversity. As a rule, a single group of secondary metabolites dominates within a given taxon. A few major compounds are often accompanied by several derivatives and minor components [7]. In this respect, since up to the present, there is no study on the chemical or the antioxidative activity of *Pulicaria inuloides.* The aim of this study was to investigate the antioxidative activity of different extracts (methanol, ethanol and diethyl ether extracts) of the leaves of *Pulicaria inuloides* as new potential source of natural antioxidants.

2. Materials and Methods

2.1. Experimental Plant

The leaves of *P. inuloides* were collected from natural sources in August, 2013 from Sana'a area in Yemen. Plant was from natural sources in Yemen: *Pulicaria inuloides* Identification was carried out by Agricultural Research Corporation (Dammar).

2.2. Extraction of Essential Oils

The aerial part essential oils of *P. inuloides* (3kg) were ground in blender. The essential oils were obtained by hydro distillation at 100°C with a clevenger type apparatus (Full description of model with the city and province (Sana'a) for 4 h with 3 L of distilled water. Started oils were dried extract with sodium sulphate (Na_2O_4S), and stored at 4°C until use.

2.2.1. Analysis of the Essential Oil

The components of the essential oils were identified by GC-MS analysis [8]. Gas chromatography-mass spectrometry (Varian 1200L) was incorporated with a relatively no polar capillary column (DB-5, 30 m length, 0.25 mm film thickness, 0.25 internal diameters). The injection port and interface were held at 220 and 260°C, respectively. The temperature was programmed from 50°C to 220°C at 15°C per min and a hold at 220°C for 25 min with helium as the carrier gas. Mass spectra: electronic impact, ionisation potential 70 e V, ion source temperature 200°C and mass range 35 – 500 Da. Most of the compounds were identified according Toes Kovats indexes in reference to n –alkanes(NIST, 2010) and mass spectra (authentic chemicals and Wiley spectral library collection).

2.3. Preparation of the Extracts

Twenty grams leaves from plant was individually extracted with 600 ml of 90% methanol, Ethanol and diethyl ether (separately) in an ultrasonic device at room temperature, The extracts were filtered and the residues were re-percolated for three times and was eliminated from the solvent using a rotary evaporator and sample was preserved at 4°C until used.

2.3.1. Determination of Total Phenolic Contents (TPC)

Total phenolics of *P. inuloides* extracts were determined using the method of Singleton [10] with some modification. 200 µl portions of diluted extracts were introduced into test tubes followed by addition of 1000 µl of Folin-Cio- calteu reagent (1:10). After Thirty seconds and just prior to 8 min, 800 µl of Na_2CO_3 (7.5%) was added to extracts in tubes. The reaction mixtures were incubated at 24°C for 1 h prior to recording the absorbance at 765 nm against blank. Total phenolic were calculated from standard gallic acid solutions used under the same conditions, and concentrations were expressed as mg gallic acid equivalents (GAE) per g extract.

2.3.2. Determination of Total Flavonoid Contents (TFC)

Total flavonoid content was determined by the $AlCl_3$ [10], with slight modifications. Avolume of 0.5 ml of 2 % AlCl3 ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 430 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.2 mg/ml. Total flavonoids were calculated as quercetin (mg/g) using the calibration curve. Results were expressed as mg quercetin equivalents (QE) / g extract.

2.3.3. Determination of Tannins

The tannins were determined by Folin and Ciocalteu method [10]. 0.1 ml of the sample extract was added with 7.5 ml of distilled water and adds 0.5 ml of Folin Phenol reagent, 1 ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of gallic acid is treated in the same manner as described earlier and read against a blank.

2.3.4. Determination of Total Anthocyanin Content

Total anthocyanin content of diluted *P. inuloides* extracts were estimated by the pH differential method [11]. Absorbance was measured with a UV visible spectrophotometer at 510 nm and 700 nm at pH 1 and 4.5 where A = (A510 - A700) pH 1 - (A510 - A700) pH 4.5. Data were calculated with the extinction coefficient for cyanidin-3-glucoside (29 600) and expressed as mg cyanidin 100 g-1 fresh weight. All analyzes were performed in triplicate.

2.3.5. Determination of DPPH Radical Scavenging Activity

The ability to scavenge the stable free radical 1 ,1-diphenyl-2-picrylhydrazyl (DPPH) was determined based on the method [12]. A solution of 0.2 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of extract in methanol (5 to 150 µg/ml).The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min .A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and quercetin were used as references. Results were expressed as percentage of inhibition of the DPPH radical according to the following equation:

$$1-\frac{absorbance\ of\ sa,\ ple\ at\ 517\ nm\times 100}{absorbance\ of\ control\ at\ 517nm}$$

2.3.6. β-Carotene Bleaching Test

Antioxidant activity was determined also using the B-carotene bleaching test with some modifications 14. 1 ml of β-carotene (0.2mg/ml chloroform) was mixed with linoleic acid. Following 90 min, the absorbance was read spectrophotometrically at 725 nm .The standard calibration (0.01 – 0.05 mg/ml) curve of Gallic acid in 80% methanol curve was plotted .Results were expressed as small cap gallic acid equivalents GAE (in mg per 100 g sample extracts.

2.4. Metal Chelating Activity

The chelation of ferrous ions by extracts was estimated by the method of [13]. The reaction mixture contained 0.5 mL of various concentrations of the extracts (0.2, 0.4, 0.6, 0.8, and 1 mg/mL), 1.6 mL of deionized water and 0.05 mL of 2 mM of $FeCl^{2+}$ solution. After 30 Second, 0.1 mL of 5 mM ferrozine solution was added. $Fe2^{+}$-Ferrozine magenta complex was very soluble and stable in water. After 10 min at room temperature, the absorbance at 562 nm was measured. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as:

$$\% \text{ Metal chelating activity} = \left(A_{control} - A_{sample}\right) / A_{control} \times 100$$

Ascorbic acid and TBHQ were used as positive control.

2.5. Statistical Analysis

Data are means of triplicate experiments, each in duplicate. The analysis of variance (ANOVA, one-way) was used to separate means at a 0.05 significant level (SPSS Version 13.0 for Windows, SPSS Inc., Chicago, IL).

3. Results

3.1. Chemical Composition of the Essential Oil

The composition of the essential oils obtained by hydrodistillation from the flowers and leaves of *P. inuloides* was analyzed by GC/MS, and show in Table 1 and Forty-two components were identified accounting for 98.955% of leave essential oil of *P. inuloides* included 2-Cyclohexen-1-one, 2-methyl-5-(1-methyl (56.8 %), Benzene, methyl- (21.7%), Z-Citral (2.00 %), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)- (1.734 %), alpha.-Farnesene (1.68 %), 7 Oxabicyclo [4.1.0] heptane, 1-methyl- (C (1.538 %), Thymohydroquinone dimethyl ether 1.511 %), Eugenol (1.16%) and Zingiberene (1.094 %) (Table 1).

Table 1. The main compounds of the essential oil of *Pulicaria Inuloides*

Peak	RT (min)	Area (%)	Compounds	Percentage
1	5.785	3.68	Benzene, methyl- (CAS)	21.7
2	7.634	6.21	Benzene, ethyl- (CAS)	0.348
3	7.813	6.44	p-Xylene	0.361
4	7.973	8.31	p-Xylene	0.466
5	9.02	1.38	Pyridine (CAS)	0.772
6	14.825	5.91	FILIFOLONE	0.331
7	14.904	1.19	LINALOOL OXIDE CIS	0.066
8	15.995	9.01	chrysanthenone	0.505
9	16.144	3.57	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimet	0.2
10	16.201	4.65	Benzaldehyde (CAS)	0.261
11	16.596	5.42	LINALOOL L	0.304
12	16.704	1.21	Cyclohexanone, 2-methyl-5-(1-methylethyl	0.679
13	16.871	1.72	2-Cyclohexen-1-ol, 1-methyl-4-(1-methyle	0.097
14	17.299	6.42	METHYL THYMYLETHER	0.36
15	18.966	9.83	2-Cyclohexen-1-one, 2-methyl-5-(1-methyl	56.8
16	19.081	1.95	Zingiberene	1.094
17	19.182	5.20	Z-Citral	2.915
18	19.284	6.36	Naphthalene, decahydro-4a-methyl-1-methy	0.036
19	19.355	2.09	.alpha.-Farnesene	1.168
20	19.43	1.29	NERYL ACETATE	0.725
21	19.484	1.06	[No name]	0.592
22	19.538	2.00	delta.-Cadinene	0.112
23	19.684	3.09	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-	1.734
24	19.961	2.74	7-Oxabicyclo[4.1.0]heptane, 1-methyl- (C	1.538
25	20.04	2.41	Carvotanacetol, cis-	0.135
26	20.093	3.69	Bicyclo[3.1.1]heptane-2,3-diol, 2,6,6-tr	0.207
27	20.478	5.81	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl	0.326
29	20.7	2.70	Thymohydroquinone dimethyl ether	0.176
28	20.609	3.15	Geranyl propionate	1.511
30	20.818	9.67	Benzenemethanol (CAS)	0.542
31	21.037	2.56	Thymyl acetate	0.143
32	21.122	5.54	A-PHELLANDRENE EPOXIDE	0.311
33	21.532	2.69	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cycl	0.151
34	21.82	1.34	No Match	0.075
35	22.097	1.76	(-)-Caryophyllene oxide	0.985
36	22.249	2.26	2,5,6,6-tetramethylcyclohex-2-en-1-one	0.127
37	22.437	1.33	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethy	0.075
38	22.661	1.90	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-c	0.106
39	22.914	2.50	NEROLIDOL-EPOXYACETATE	0.14
40	23.305	1.45	2-Pentadecanone, 6,10,14-trimethyl-	0.081
41	23.734	1.16	Eugenol	0.065
42	23.837	6.32	Thymyl acetate	0.354
43	23.999	6.74	No Match	0.378
44	24.128	1.44	Phenol, 5-methyl-2-(1-methylethyl)- (CAS	0.805
45	24.414	4.87	alpha.-Cadinol	0.100
Total				100%

3.2. Total Phenolic, Total Flavonoid, Total Anthocyanins and Total Tannin Contents Activity

In Table 2, the total phenolic content of methanolic, ethanolic and diethyl ether extracts of *P. inuloides* measured by Foline Ciocalteau reagent in terms of gallic acid equivalent was 91.2, 75, 89.9 ± 0.61 and 64.9 ± 07 mg/g respectively. The flavonoid content of the plant sample calculated as catechol equivalent was 75 ± 85.7, 56.4 ± 2.7 and 37.9 ± 0.67 mg/g respectively. The tannin content was 0.66 ± 0.07, 0.51 ± 0.01, and 08.5 ± 11.5 mg/g respectively and total anthocyanins was 0.44 ± 0.03, 0.63 ± 0.02 and 0.02 ± 0.01 mg/ g respectively. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The results strongly suggest that the phenolics are important components of this plant, and some of the pharmacological effect could be attributed to the presence of this invaluable component.

Table 2. Total phenol, flavonoid, tannin and anthocyanin contents of the *P. inuloides* extrects

Extracts	Total phenols (mg(GAE) /g)	Total flavonoids (mg (quercetin) / g)	Total tannins (mg(GAE) /g)	Total anthocyanins (mg/100 g)
Methanol	91.2 ± 09.5^{b}	75 ± 85.7^{b}	0.66 ± 0.07^{b}	0.44 ± 0.03^{c}
Ethanol	89.9 ± 0.61^{b}	56.4 ± 2.7^{c}	0.51 ± 0.01^{c}	0.63 ± 0.02^{b}
diethyl ether	64.9 ± 07.1^{c}	37.9 ± 0.67^{d}	08.5 ± 11.5^{d}	0.02 ± 0.01^{d}

Experiment was s performed in triplicate and expressed as mean± SD. Values in columns with different superscripts are significantly different (P < 0.05).

3.3. DPPH Radical- Scavenging Activity

The reaction capability of DPPH radical was determined by the decrease in its absorbance at 515 nm induced by antioxidants. At 1- 0.2 mg, the antioxidant activities of methanolic extract of *P. inuloides* and the standard ascorbic acid were 81- 43.55% and 70.91-50 and 50.44-10 %, respectively. In addition, the standard TBHQ were 84.60 ± 2.25-57.00 ± 3.32.

The extract exhibited concentration-dependent radical scavenging activity, that is, the higher the concentration, the scavenging potential. The DPPH radical scavenging activity of methanolic extract of *P. inuloides* is shown in the Figure 1.

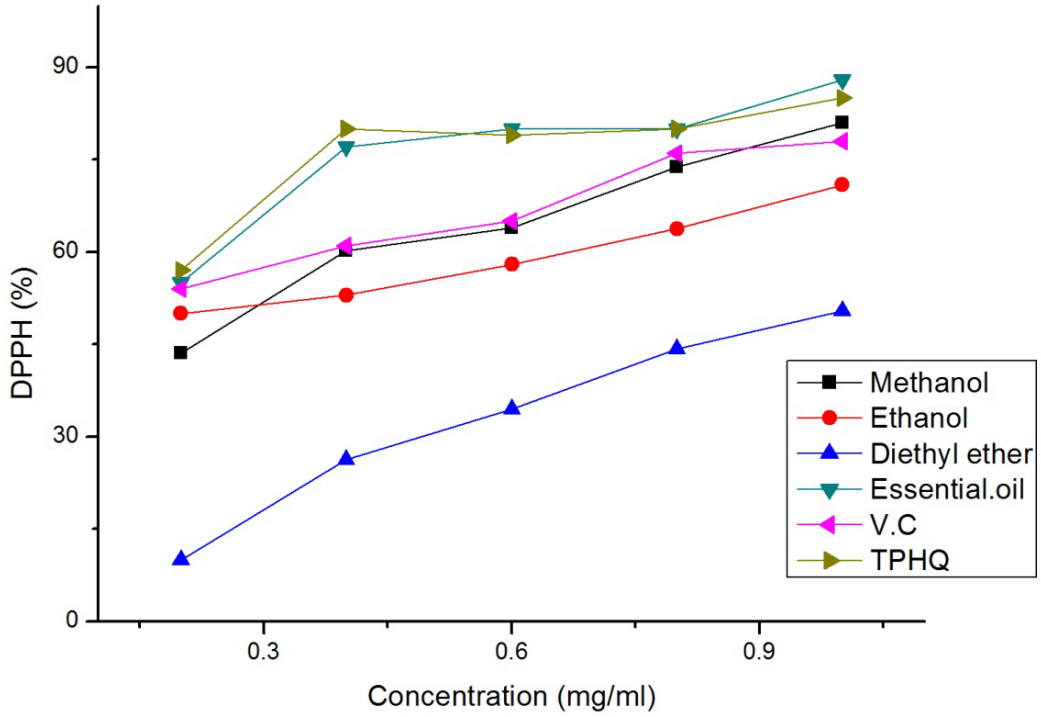

Figure 1. DPPH radical scavenging activity of *P. inuloides* extracts. Data are expressed as mean ± SD (standard deviation) (n = 3). Ascorbic acid and TBHQ were used as positive control

Table 3. Means of antioxidant activity of selected extracts for plant and standard assayed by β-carotene linoleate bleaching

Extracts	β –carotene linoleate bleaching (%)
Methanol	72.23 ± 0.86
Ethanol	55.30 ± 0.73
Diethyl ether	22.02 ± 0.82
Ascorbic acid	90.01 ± 0.44
TBHQ	96.73 ± 0.08

3.4. β-Carotene Bleaching Test

The antioxidant activity of *P. inuloides* was also studied using the β -carotene bleaching method, which is based on oxidative decomposition of b-carotene in the presence of linoleic acid. As seen in Table 3.

3.5. Metal Chelating Activity

The antioxidant activity of *P. inuloides* was also studied using the Metal chelating method. As seen in Figure 2.

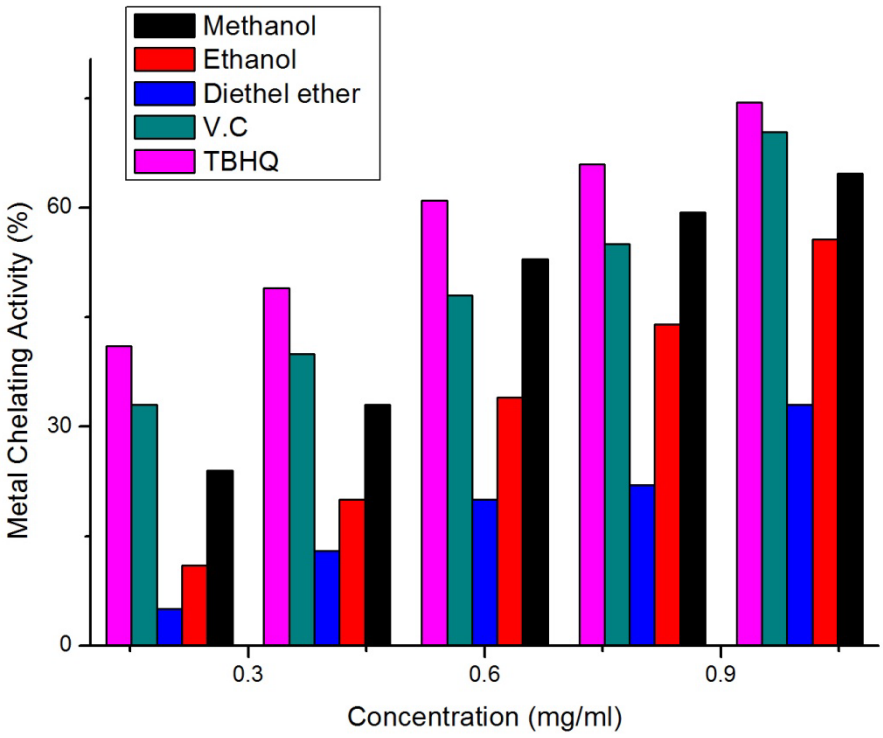

Figure 2. Metal chelating effect on ferrous ions in extracts of *Pulicaria inuloides*

4. Discussion

The percentage and retention time of components analysed by GC/MS are shown in Table 1, in *P. inuloides* oil, 52 components represented about 100% of the total oil, were identified. 2-Cyclohexen-1-one, 2-methyl-5-(1-methyl (55.1%), Benzene, methyl- (20.6%) and eugenol (1.16%) were the major component in *P. inuloides* essential oil. As it is shown in Table 2, the largest part of *P. inuloides* essential oil was formed by Bicyclo [3.1.1] hept-2-ene, 2, (22.369%) and Naphthalene, 1,2,3,4,4a,5,6 (19.327%). Essential oil extracted from *P. inuloides* was light yellow in colour and with a perfumery odor.

To the best of our knowledge, there is no any report on the chemical composition of *P. inuloides* essential oil in the literature. However, there are few reports on the chemical composition of the oils from the other plants belonging to the genu of *P. inuloides* previous studied on the composition of *P. dysenterica and O. basilicum* oils show that there are some qualitative and quantitative differences which, can be attributed to growth conditions, genetic factors, geographical variations and analytical procedures [14].

The plant is important source of potential compounds for the development of new therapeutic agents. Plants phenolics are widely distributed in the tissues of plants as well as play a vital role in the highly effective free radical scavengers and antioxidant activity. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [15]. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acid and flavonoids. These compounds possess diverse biological activities, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These activities might be related to their antioxidant activity [16]. The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. Phenolic compounds are important plant antioxidants which exhibited reduce free radical formation and to scavenge free radicals. Thus antioxidant capacity of a sample can be attributed mainly to its phenolic compounds [17].

4.1. Beta-Carotene Bleaching Test

The result showed the control had a substantial and rapid oxidation of β-carotene. Accordingly, the absorbance decreased rapidly in sample without antioxidant, while the sample extracts with the presence of antioxidant retained their color and also absorbance for a longer time. Table 3 show the mean antioxidant activity based on the β-carotene bleaching rate of the extracts of different plant of the *P. inuloides*. The extract with the lowest β-carotene degradation rate exhibit the highest antioxidant activity. All extracts had lower antioxidant activities than had standards (V.C and TBHQ). The highest antioxidant activity among the samples was observed in methanol extract whereas diethyl ether had the lowest antioxidant activity. The highest antioxidant activity among the samples was observed in methanol extract whereas diethyl ether had the lowest antioxidant activity. Result showed that there was considerably variation in the antioxidant activities where it ranges from the lowest of 58% to the highest of 96% where the orders of the antioxidant activity are as follow: TBHQ, Essential oil, Ascorbic acid, Methanol, Ethanol and Diethyl ether.

4.2. Reactive Scavenging Activity

In this study, radical scavenging activity was determined for the selected extracts of *P. inuloides* plant. Being a stable free radical, the DPPH assay is a simple

and rapid method frequently used to evaluate the ability of antioxidants to scavenge free radicals. As shown in Figure 1, all the sample extracts exhibited significant dose dependent inhibition of DPPH activity that rapidly increase from 1 to 0.2 mg/ml. Scavenging effect increases as the concentration of the sample increased until reached a plateau at 0.2 mg/ml. This showed that the young leaves exhibit a strong scavenging activity and it has been reported that phytochemicals especially plant phenolics constitute a major group of compounds that act as primary antioxidant [13]. Their protection mechanisms are through the reaction with the oxygen radicals, superoxide anion radicals and lipid peroxyl radicals.

4.3. Total Phenolic Content and Total Flavonoid Content

This study showed that the selected extracts of *Pulicaria inuloides* plant varied significantly. The phenolics ranged from 91.2 ± 09.5 to 64.9 ± 07.1 mg GAE/ 100 respectively (Table 2). The result also indicates that the methanol extract of plant contained high phenolic content that may provide good sources of dietary antioxidant**.** For this reason, it is obvious that TPC present in the sample have strong effects against the scavenging activity rather than discoloration of β-carotene. However, [18] found that the radicals scavenging activity is not only due to the phenolic content itself, but with other various antioxidant compounds. They respond differently depending on the number of phenolic groups that they have [19]. More to the point, TPC does not incorporate necessarily to all the antioxidants that may present in the extracts. Therefore, sometimes there is a vague correlation between TPC and antioxidant activity of several plant species [20]. Other than that, TFC of the extracts in terms of rutin equivalent/ 100 g dry weight (standard curve equation: $y = 3.021x + 0.0831$, $R^2 = 0.9975$) were between 64.9 and 91.2. mg rutin equivalent/ 100 g dry weight as shown in Table 2.

In recent years, studies have shown that papaya fruit contains not only vitamins and other nutrients but also contains biologically flavonoids [21]**.**

4.4. Total Tannin and Total Anthocyanins Content

From the data presented in Table (2) it was apparent that tannin content varied significantly in the some plants. Extract where Tannin was low in *Z. vulgaris* (0.48 mg/g) and high in *C. canadensis* (0.99 mg/g) which also had the highest level of total phenolics. the results of *P. granatum* extract agreed with the CSMD study [22] Anthocyanins are water-soluble pigments responsible for the red and blue colors in some plants.In the present study Found that there were significant differences evident between study samples at ($P \leq 0.05$). The highest Anthocyanins content ranged between 0.97 and 0.63 mg/100 g for *C. canadensis* and *P. granatum* respectively, where the smallest values of total anthocyanins 0.02 mg/100 g in *R. graveolens* due to their low total and colored anthocyanins content, have weakly expressed in color.

4.5. DPPH Radical-Scavenging Activity

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and biological systems [18]. DPPH molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidant. The free radical scavenging activities of the three extracts, methanol, ethanol and diethyl ether were also assayed by using DPPH. In this study results showed that DPPH radical scavenging abilities of the extracts were low than those of ascorbic acid and TBHQ (Figure 1). According to [23], methanolic extracts may include phenolic and hydrox- phenolic compounds with acid, alcohol, sugar or glycoside and part of the antioxidative activity may be due to these components or flavonoids. In other studies on the DPPH scavenging abilities of extracts were that are different from each other and also from ours. These differences might have been derived from local, climatic and seasonal factors [24].

4.6. Metal Chelating Activity

Iron is the most important lipid oxidation Pro-oxidant among the transition metals. Lipid oxidation is accelerated by breaking down of hydrogen peroxide and lipid peroxides by ferrous state of iron to reactive free radicals through the reaction: $Fe^{2+} + H_2O_2 \rightarrow F^{3+} + {}^{-}OH + {}^{-}OH$, known as the Fenton reaction. Radicals from peroxides are also produced by F^{3+} ion. However the rate is one tenth of Fe^{2+} [25]. In the metal chelating assay, ferrozine can quantitatively form complexes with Fe^{2+} In the presence of other chelating agents, the complex formation is disrupted with consequent decrease in the intensity of the red color of the complex [25]. As shown in results (Figure 2), the formation of the red colored complex was inhabited in the presence of methanol and ethanol extracts, indicating chelating activity. However, the color did not change much in the presence of ethanol suggesting weak chelating activity. The reducing power results of various extracts of leaves of *P. inuloides* and standard references which are presented in Figure 2, followed the order: TBHQ, ascorbic acid, methanol, ethanol and diethyl ether. The results indicated that both methanol and ethanol extracts of *P. inuloides* interfered with the formation of activity and captured ferrous ions before ferrozine. Asit can be deduced from Figure 2, these two extracts, methanol (64.67 %) and ethanol extract (55.65 %) had the highest ferrous ion chelating activity at a concentration 1 mg/ml. Although the diethyl ether extract did not show ion chelator activity even at the highest concentration, the antioxidant compounds contained in methanol and ethanol extracts of *P. inuloides* probably inhibited interaction between metal and lipid through formation of insoluble complexes with ferrous ion [23].

5. Conclusion

The results obtained in this study clearly showed that both methanol and ethanol extracts from the leaves of *P. inuloides* possess antioxidant activity. The methanol extract exhibited a strong antioxidant activity and had the most potent scavenging abilities of various radicals which may be caused by the high content of polyphenols and flavonoids. The very strong antioxidant activity of the *P.*

inuloides suggests that the extracts obtained by polar solvents from the leaves could be used as an effective natural source of antioxidant and food additives.

Acknowledgment

This work is supported by National Science and Technology program (no. 2014BAD33B05). The authors express their thanks to the students at the Nutrition Laboratory of School of Food Science and Technology at Jiangnan University for their technical assistance.

References

[1] Dubaie, A.S., A.A. El-Khulaidi. Medicinal and aromatic plants in Yemen, deployment-components of effective-uses. Ebadi Center for studies and Publishing Sana'a- Yemen, 2005. 127.

[2] Dendougui, H., S. Benayache, F. Benayache and G.D. Connoly. Sesquiterpene lactone from Pulicaria crispa. Fitoterapia, 71. 373-378. 2000.

[3] Rizk, A.M., F.M. Hammouda, S. I.Ismail, H.A. Hussiney. Constituents of plants growing in Qatar XXIII. Flavonoids of Francoeuria crispa, Qatar Univ, Sci. J., 13(1): 51-52.1993.

[4] Al-Yahaya, A. Moharnmed, Khafagy, Saad, Shihata, Amir, Koziowski, John, F. Antoun, Mikhail D. Cassady M. John, 1984.

[5] Dubaie, A.S., A.A. El-Khulaidi. Studies on the flora of Yemen, on the flora of Tihama plain. Feddes Repertorium 104: 3-4: 259-265, 1993 Berlin-Germany.

[6] Miller, A. G. & Morris, M. Plants of Dhofar. The southern region of Oman traditional, economic and medicinal uses. Muscat, 1988.

[7] Wink, M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry, 64: 3-19, 2003.

[8] Kumar KA, Patel J, Choudhary R K. Chemical composition and antimicrobial activity of the essential oil of Desmostachya bipinnata linn. International Journal of Phytomedicine 2 (4), 2010.

[9] SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTOS, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152-178, 1999.

[10] AVANMARDI, J., et al. Antioxidant activity and total phenolic content of Iranian i Ocimum I accessions. *Food chemistry*, 83.4: 547-550, 2003.

[11] Zheng, Y., Ch. Wang, Sh. Wang, and W. Zheng. Effect of high oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. Journal of Agricultural and Food Chemistry 51:7162-7169, 2003.

[12] F. Nanjo, K. Goto, R. Seto, M. Suzuki, M. Sakai, Y. Hara Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical Free Radic. Biol. Med., 21 pp. 895-902, (1996).

[13] Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 315: 161-169, 1994.

[14] Elkamali HH, Ahmed AH, Mohammed A S, Yahia A A M, EltayebI H, Ali AA. Antibacterial properties of essential oil from *Nigella citrates* leaves and *Pulicaria Undulata* Aerial Parts. *Fitoterapia* (69): 77-78, 1998.

[15] Hatano, T., R. Edamatsu, M. Hiramatsu, A. Mori and Y. Fujita. Effects of the interaction of tannins with co-existing substances. VI: Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2-picrylhydrazyl radical. Chem. Pharmaceutical Bull., 37: 2016-2021, 1989.

[16] Khamsah, S. M., Akowah, G. and Zhari, I. Antioxidant activity and phenolic content of Orthosiphon stamineus benth from different geographical origin. Journal of Sustainability Science and Management, 1:14-20, 2006.

[17] V. Singleton and J. Rossi, "Colorimetry of Total Pheno- lics with Phospho-Molybdicphosphotungstic Acid Re- agents," *American Journal of Enology and Viticulture*, Vol. 16, pp. 144-158, 1965.

[18] Tawaha, K., Alali, F.Q., Gharaibeh, M., Mohammad, M. and El-Elimat, T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry 104: 1372–1378, 2007.

[19] Wang, J., Wu, F. A., Zhao, H., Liu, L. and Wu, Q. S. Isolation of flavonoids from mulberry (Morus alba L.) leaves with macroporous resins. African Journal of Biotechnology 7: 2147-2155, 2008.

[20] He, J. Isolation of anthocyanin mixtures from fruits and vegetables and evaluation of their stability, availability and biotransformation in the gastrointestinal tract. PhD dissertation, Food Science and Nutrition. The Ohio State University, 2008.

[21] Kikuzaki, H., Nakatani, N. Antioxidant effects of some ginger constituents. Journal of Food Sciences. 58:1407-1410, 1993.

[22] CSMD (Climate System Modeling Division), 2005: An introduction to the First general operational climate model at the National Climate Center. Advances in Climate System Modeling, 1, National Climate Center, China Meteorological Administration, 14 pp (in English and Chinese).

[23] Miller HE. A simplified method for the evaluation of antioxidants. J Am Oil Chem Soc. 48: 91-105, 1971.

[24] Yamaguchi F, Ariga T, Yoshimura Y and Nakazawa H. Antioxidative and antiglycation activity of garcinol from Garcinia indica fruit rind. J Agric Food Chem. 5: 48-180, 2000.

[25] Heijnen, C G, Haenen G R.M.M, Oostveen R M, E.M. Stalpers and Bast A. Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. Free Radic. 36: 575-581, 2002.