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Assessment of Total Antioxidant Capacity and Antiradical Scavenging Activity of Three Egyptian Wild Plants

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The present study was undertaken to evaluate *in vitro* antioxidant and antiradical activities of the hydro-ethanolic (50%) extract of the above and underground parts of three wild geophytic species, *Asparagus stipularis*, *Cyperus capitatus* and *Stipagrostis lanata.* Total Antioxidant Capacity (TAC) using phosphomolybdenum method and antiradical scavenging activity using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) and hydrogen peroxide methods and Total Phenolics (TP), Total Flavonoids (TF) contents of plant extracts were assessed. Results revealed that, the extract of *C. capitatus* (underground part) had the highest values of TP (163.35 mg tannic acid equivalent/g extract) and TF (12.31 mg catechol equivalent/g extract) while; the lowest values of TP (14.33 mg TAE g^{-1}) and TF (0.53 mg CE g^{-1}) were recorded in the extract of *A. stipularis* (underground part). The same trend was found in case of Total Antioxidant Capacity (TAC) and antiradical scavenging activity, where *C. capitatus* (underground part) exhibited the highest TAC (21.21 mg TAE g^{-1} extract) and antiradical activity with IC_{50} = 0.061 and 0.167 mg mL^{-1} for DPPH and H_2O_2 radicals, respectively. However, all plant extracts displayed strong scavenging activity against DPPH except the underground part of *A. stipularis* extract, exhibited moderate activity. Furthermore, the highest H_2O_2 scavenging activity was detected in the extracts of *C. capitatus* followed by *A. stipularis* and then *S. lanata.* This study concluded that, the investigated geophytic plants could be utilized as good sources of natural antioxidants for medicinal and commercial uses especially the underground parts of *C. capitatus*.

Key words: Geophytic species, phenolics, flavonoids, antioxidant, antiradical

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INTRODUCTION

In recent years, research efforts are concerned with the possibilities of utilization of plants as natural source of bioactive compounds for their biological activities. Reactive Oxygen Species (ROS) which inactivate enzymes and damage the important cellular components can initiate or propagate the development of many diseases, such as cancer, liver injury, diabetes, cardiovascular diseases, aging, etc. (Bandyopadhyay *et al*., 1999). Plant antioxidants that play an important role in converting free radicals to less reactive species have health-promoting effects in the prevention of degenerative diseases (Biglari *et al*., 2008; Fang *et al*., 2002).

Because of the health risk due to the use of synthetic antioxidants, attention is being focused on the protective biochemical functions of naturally occurring antioxidants. Plants are considered a good source of safe natural antioxidants that protect the human body from free radicals, prevent oxidative stress and associated diseases (Steer *et al*., 2002; Yao *et al*., 2004b). Phenolic compounds derived from plant material are considered one of the important natural antioxidants that act as reducing agents and activator of antioxidative defense enzyme systems to suppress radical damage in biological system (Anderson *et al*., 2001; Proestos *et al*., 2006).

Monocot geophytes, *A. stipularis* (Asparagaceae), *C. capitatus* (Cyperaceae) and *S. lanata* (Poaceae) are distributed in the Deltaic Mediterranean coast of Egypt (Boulos, 2009). Ecologically, these species inhabited in harsh environments, where *C. capitatus* and *S. lanata* are growing in non-saline sandy soils and can tolerate drought stress, while, *A. stipularis* is growing in saline and non- saline sandy and calcareous clay soils and can tolerate drought and salt stress (Maswada and Elzaawely, 2013a).

Biologically, these species are rich in bioactive compounds (Hassan and Maswada, 2012; Maswada and Elzaawely, 2013b) and have antifungal activity (Maswada and Abd-Allah, 2013). In addition, *A. stipularis* has been used in folklore medicine to remove renal stones and as a diaphoretic, appetizer, stomachic, diuretic and others (Boulos, 1983). The purpose of this study was to evaluate *A. stipularis*, *C. capitatus* and *S. lanata* as new potential sources of natural antioxidants and phenolic compounds.

MATERIALS AND METHODS

Plant material: Three geophytic species namely *Asparagus stipularis* Forssk., *Cyperus capitatus* Vend. and *Stipagrostis lanata* (Forssk.) De Winter were collected at flowering stage (during spring and summer seasons, 2011) from their natural habitats in the Deltaic Mediterranean coast of Egypt. The underground and aerial parts of each plant were separately cut and air dried. The dried materials were powdered and kept in the refrigerator till use.

Preparation of plant extracts: Forty gram air-dried powdered samples were extracted with 400 mL of 50% ethanol for a week at room temperature. The extracts were separately collected, filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum and concentrated.

Estimation of total phenolics content (TP): According the method of Lister and Wilson (2001), TP contents in different plant extracts were assessed using Folin-Ciocalteau's Reagent (FCR). The amount of TP in different plant extracts were assessed using Folin-Ciocalteu reagent procedure using the method of Lister and Wilson (2001). To 50 μL of each ethanolic extract (three replicates), 2.5 mL 1/10 dilution of Folin-ciocalteau's Reagent (FCR) and 2 mL of Na_2CO_3 (7.5%, w/v) were added and incubated at 45°C for 15 min. Absorption was measured spectrophotometrically at 765 nm versus blank. Calibration curve was established using varying concentrations of tannic acid. Total phenolic content was expressed as mg Tannic Acid Equivalent (TAE)/g crude extract.

Estimation of total flavonoids content (TF): Total flavonoids of various extracts expressed as Catechol Equivalent (CE) was determined by the method of Zhishen *et al*. (1999) with slight modification. Briefly, 0.2 mL of $NaNO_2$ (5%) was mixed with 0.4 mL of plant extract and then, 0.2 mL of $AlCl_3$. $6H_2O$ (10%) was added after 5 min. Afterwards, 2 mL of 1 M NaOH was added and the mixture was immediately diluted with 4 mL distilled water and thoroughly mixed and its absorbance was measured at 510 nm versus blank. Calibration curve was prepared using Catechol as standard. Total flavonoids content was expressed as mg CE/g crude extract.

Total antioxidant capacity assay (TAC): The total antioxidant capacity of the extracts was assessed spectrophotometrically by the phosphomolybdenum method according to the procedure described by Prieto *et al*. (1999). One milliliter of each sample extract (0.5 mg mL^{-1}) was mixed with 3 mL reagent solution (0.6 M H_2SO_4, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The blank solution contained 4 mL reagent solution only. The mixtures were incubated at 95°C for 150 min. After the mixture had cooled to room

temperature, absorbance was measured at 695 nm. Total antioxidant capacity (TAC) was expressed as tannic acid equivalent (TAE).

Measurement of free radical scavenging activity

DPPH scavenging assay: As described by Lim and Quah (2007), the ethanolic extract (1 mL) of studied plants in different concentrations (20-1200 ppm) was added to 2 mL of 0.15 mM DPPH. The Blank was prepared by adding 2 mL of DPPH to 1 mL ethanol 50%. After shaking, the mixture was incubated for 30 min. and the absorbance was measured spectrophotometrically at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = 1-\frac{A_{sample}}{A_{blank}}\times 100$$

where, A is absorbance at 517 nm. IC_{50} (mg mL^{-1}) which denotes the amount of plant extract required to reduce initial concentration of DPPH radicals by 50% was also calculated.

Hydrogen peroxide scavenging assay: H_2O_2 scavenging activity of the plant extracts was determined by replacement titration method (Zhang, 2000) with slight modification. Aliquot of 2 mL of 1 mM H_2O_2 and 1 mL of various concentrations of plant extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 mL of 0.2 M H_2SO_4, 7 mL of 1.8 mM potassium iodide and 2 drops of 1% starch indicator. The mixed solution was titrated with 0.5 mM sodium thiosulphate until blue color disappeared. Percentage of scavenging of H_2O_2 was calculated as:

$$\text{Inhibition (\%)} = \frac{V_0-V_1}{V_0}\times 100$$

where, V_0 and V_1 were the volume of sodium thiosulphate used to titrate blank and sample extract, respectively. IC_{50} (mg mL^{-1}) which denotes effective concentration yielding 50% inhibition of H_2O_2 radicals was also calculated.

Statistical analysis: All experiments were run in triplicate. Data were analyzed by one way ANOVA and the differences between means were evaluated by Duncan's Multiple Range Test (DMRT) at 1% probability (Gomez and Gomez, 1984). Data analysis was performed using MSTAT-C Statistical Software Package (Michigan State University, 1983).

RESULTS

Total phenolics and total flavonoids contents: The yield of crude plant extracts as well as Total Phenolics (TP) as Tannic Acid Equivalent (TAE) and Total Flavonoids (TF) as Catechol Equivalent (CE) that tested in the hydro-alcoholic extract (ethanol 50%) of underground and aerial parts of investigated geophytes are summarized in Table 1. The results showed that, there are significantly differences ($p\leq 0.01$) among yield of crude extracts, total phenolics and flavonoids contents of investigated plants. The underground part (root tubers) of *A. stipularis* showed the highest yield (60.34%). In spite of relatively high crude extract yield (60.34%) of the underground part (root tubers) of *A. stipularis*, its extract recorded the lowest values of TP (14.33 mg TAE g^{-1} extract) and TF (0.53 mg CE g^{-1} extract). The highest values of TP (163.35 mg TAE g^{-1}), TF (12.31 mg CE g^{-1}) and TF/TP ratio (7.53%) were detected in *C. capitatus* (underground part) extract. The extract of underground part of *S. lanata* was rich in its contents of TP (131.30 mg TAE g^{-1} extract) and TF (5.18 mg CE g^{-1} extract), while it had the lowest crude extract yield (4.08%). Total phenolic contents in the aerial parts of *S. lanata* extract (84.21 mg TAE g^{-1}) were higher than those of the aerial parts of *C. capitatus* extract (68.51 mg TAE g^{-1}). While, TF contents in the extracts of *S. lanata*, *C. capitatus* (aerial parts) showed versus trend. However, the contents of TP and TP were high in the investigated plant extracts.

Table 1: Extraction yield (%) and contents of total phenolics (TP) and flavonoids (TF) in the ethanolic extract of underground (U) and aerial (A) parts of investigated geophytes

Species	Part	Extraction yield (%)	TP (mg TAE g^{-1} extract)	TF (mg CE g^{-1} extract)	TF/TP ratio
A. stipularis	U	60.34±1.65[a]	14.33±0.31[f]	0.53±0.02[f]	3.72
	A	20.77±0.92[b]	56.49±1.37[e]	2.67±0.05[e]	4.72
C. capitatus	U	20.14±1.12[b]	163.35±3.91[a]	12.31±0.08[a]	7.53
	A	19.07±0.88[b]	68.51±1.98[d]	4.24±0.33[c]	6.18
S. lanata	U	4.08±0.30[d]	131.30±3.59[b]	5.18±0.35[b]	3.94
	A	6.51±0.21[c]	84.21±2.59[c]	3.76±0.06[d]	4.46
LSD (p = 0.01)		1.787	7.188	0.463	-

Values are means of 3 replications±SD. TAE and CE represent tannic acid and catechol equivalents, respectively. Within the same column, means followed by different letters are significantly different at $p\leq 0.01$

Total antioxidant capacity (TAC): Results in Fig. 1 indicate that, the extract of the underground part of *C. capitatus* showed the highest TAC among other plant extracts (21.21 mg TAE g^{-1} extract) followed by the extract of the underground part of *S. lanata* (TAC = 17.21 mg TAE g^{-1} extract). Significantly ($p \le 0.01$), there are no differences between total antioxidant capacity of the underground and arerial parts extracts of *A. stipularis* and *S. lanata*, respectively. Also, there are no significant differences between the extracts of *A. stipularis* and *C. capitatus* (aerial parts).

DPPH free radical scavenging activity: The DPPH radical scavenging activity and the concentration of the extract required to inhibit 50% of the initial DPPH free radicals (IC_{50}) are shown in Fig. 2. IC_{50} value is inversely related to the activity. Among all plant extracts, *C. capitatus*

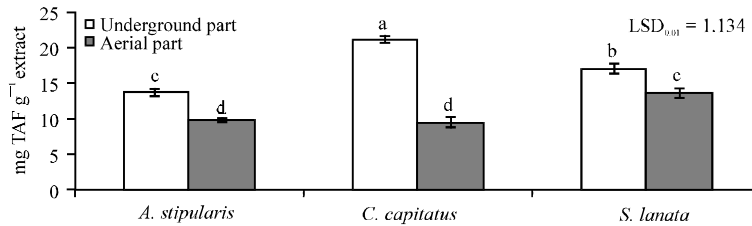

Fig. 1: Total antioxidant capacity (mean of 3 replications±SD) of the underground and aerial parts of ethanolic extract of the investigated plants at incubation period (150 min.) as tannic acid equivalents. Different letters are significantly different at $p \le 0.01$

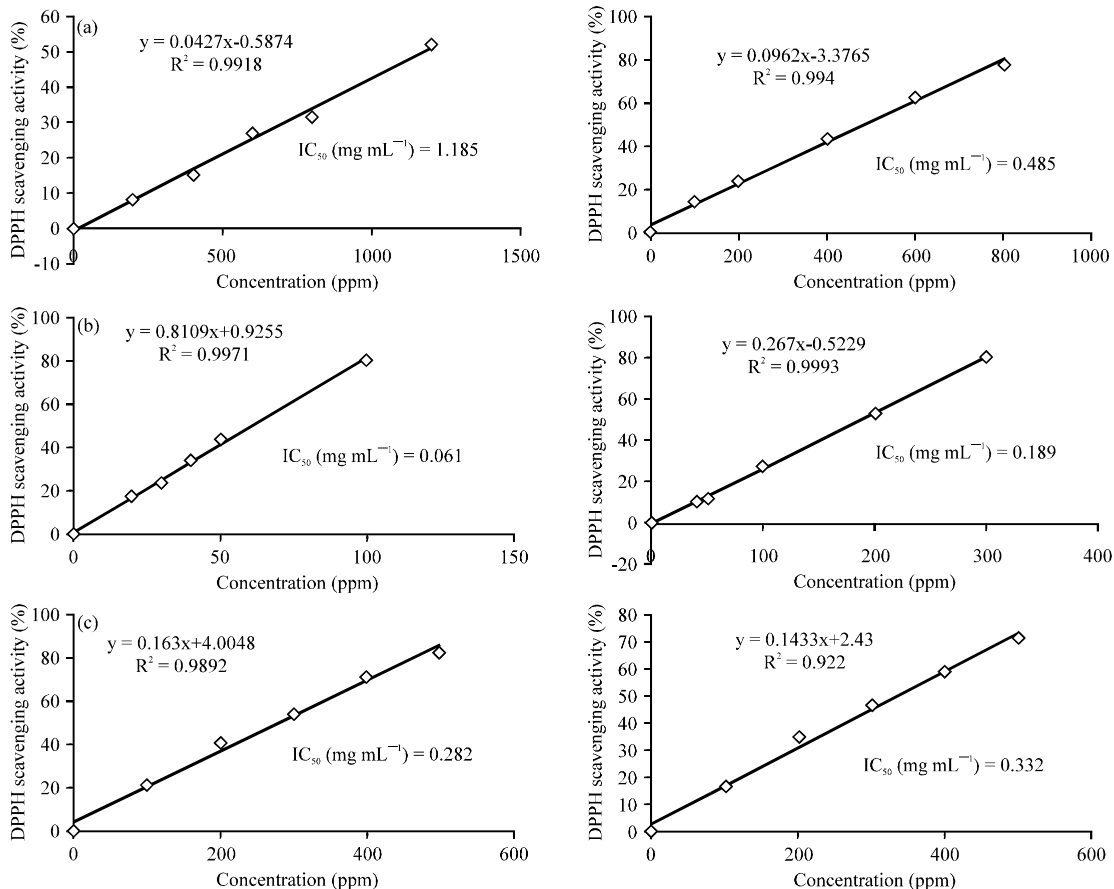

Fig. 2(a-c): DPPH radical scavenging activity (%) and IC_{50} values of ethanolic extracts of underground (left) and aerial parts (right) of the investigated plants, (a) *A. stipularis*, (b) *C. capitatus* and (c) *S. lanata*

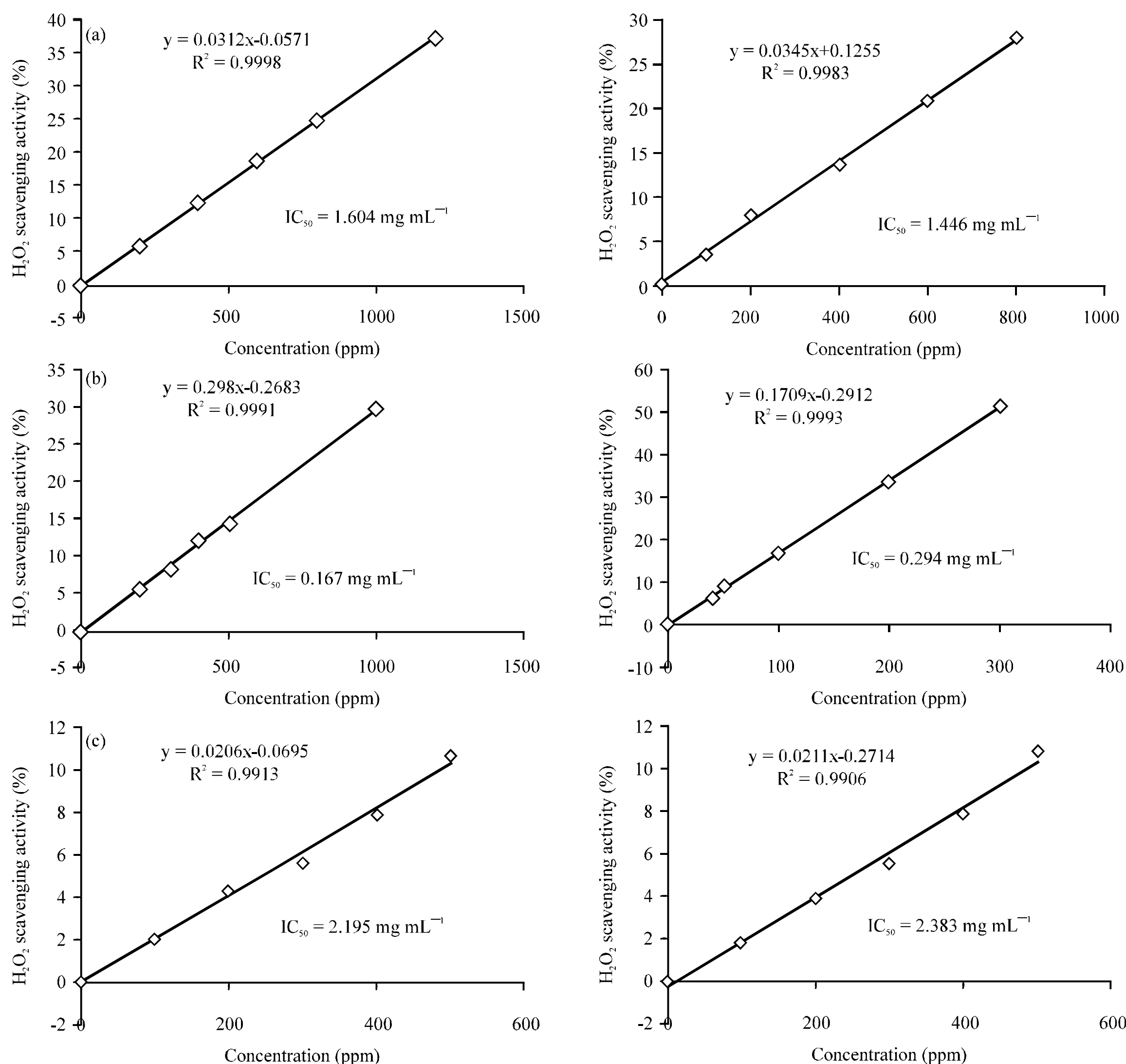

Fig. 3(a-c): H_2O_2 radical scavenging activity (%) and IC_{50} values of the underground (left) and aerial parts (right) ethanolic extracts of the investigated plants, (a) *A. stipularis*, (b) *C. capitatus* and (c) *S. lanata*

extracts possessed the highest DPPH activity with the lowest IC_{50} value of 0.061 and 0.189 mg mL^{-1} for underground and aerial parts, respectively. In addition, the extracts of the underground and aerial parts of *S. lanata* exhibited strong scavenging activity (IC_{50} = 0.282 and 0.332 mg mL^{-1}, respectively). The lowest DPPH scavenging activity (IC_{50} = 1.185 mg mL^{-1}) was recorded in the extract of the underground part of *A. stipularis*.

H_2O_2 free radical scavenging activity: Results of Fig. 3 display that, ethanolic extract of the underground part of *C. capitatus* showed the highest H_2O_2 scavenging activity with the lowest IC_{50} value (0.167 mg mL^{-1}). However, the scavenging activity of the other plant extracts against H_2O_2 radicals arranged as follows: *C. capitatus* (aerial parts)>*A. stipularis* (aerial parts)>*A. stipularis* (underground parts)>*S. lanata* (underground parts)>*S. lanata* (aerial parts) with IC_{50} values, 0.294, 1.446, 1.604, 2.195 and 2.383 mg mL^{-1}.

DISCUSSION

The societal demand and increasing interest for practical applications of biological and epidemiological studies has been stimulated to characterize the health promoting properties of specific phenolic compounds with antioxidant activities (Boudet, 2007). In recent years, the medicinal properties of plants have been investigated

in a wide range of the world, due to their potent antioxidant activities, no side effects and economic viability (Auddy *et al*., 2003). Velioglu *et al*. (1998) stated that, interest in finding naturally occurring antioxidants has increased considerably to replace synthetic antioxidants, which are being restricted due to their toxicity and carcinogenicity. It is well known that phenolic compounds contribute directly to the antioxidant activity of plant extracts (Elzaawely *et al*., 2007). Therefore, the contents of total phenolics and flavonoids were estimated in the studied plant extracts. The Folin-Ciocalteu's Reagent (FCR) procedure is a widely used method and provides a rapid and useful estimation of the total phenolic content of plant extracts (Luximon-Ramma *et al*., 2003).

Results indicated that, in spite of relatively high crude extract yield of the underground part of *A. stipularis*, its ethanolic extract recorded the lowest values of total phenolics and flavonoids. This may be due to the presence of many chemical compounds in the root tubers of *A. stipularis* other than phenolic compounds. Among of these compounds, saponin (40.92%) and alkaloids (1.92%) as mentioned by Hassan and Maswada (2012). In addition, the *C. capitatus* (underground part) extract exhibited the highest values of TP and TF as well as TF/TP ratio. The contents of TF in the ethanolic extract of *A. stipularis* and *C. capitatus* were less than TF contents in the methanolic extracts of the same plants estimated by Hassan and Maswada (2012). Opposite trend was observed in case of TP content. This is may be due to the difference in estimation methods in case of TP and the extraction solvent in case of TF. Accordingly, methanol was suitable solvent in the extraction of polyphenolic compounds such as flavonoids from plants tissue, due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols and its ease of evaporation compared to water (Yao *et al*., 2004a). The results also revealed that, TP value (56.59 mg/TAE g^{-1}) of *A. stipularis* aerial parts was higher than those of *A. officinalis* stem (3.17 mg g^{-1}) as reported by Aberoumand and Deokule (2008). In contrast, TF content in the root tubers of *A. stipularis* (0.53 mg CAE g^{-1}) was less than those of *A. officinalis* root (0.47%) as reported by Visavadiya and Narasimhacharya (2007).

Total Antioxidant Capacity (TAC) assay by phosphomolybdenum method that based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/Mo (V) complex at acidic pH, usually detects antioxidants such as some phenolics, ascorbic acid, α-tocopherol and carotenoids (Prieto *et al*., 1999). The extract of the underground part of *C. capitatus* showed the highest TAC among other plant extracts followed by the extract of the underground part of *S. lanata*. This could due to the high contents of total phenolics and flavonoids in these extracts. Due to the redox properties of phenolic compounds, they can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Therefore, phenolic compounds are considered as good antioxidants (Rice-Evans *et al*., 1995). Although the lowest values of TP and TF contents recorded in the extract of *A. stipularis* underground part; this extract exhibited relatively high antioxidant capacity. This observation can be explained by the phenolic structure and presence of antioxidant compounds unlike phenolics and flavonoids. Javanmardi *et al*. (2003) mentioned that, antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant compounds such as carotenoids, vitamins and others.

The DPPH radical scavenging is a rapid and most widely method employed to characterize antioxidant activity of plant materials (Chung *et al*., 2006). DPPH which produces a violet solution in ethanol is a free radical stable at room temperature (Mensor *et al*., 2001). The DPPH radical scavenging activity was expressed as IC_{50} which denote the concentration of each sample required to scavenge 50% of DPPH free radicals. Lower IC_{50} value reflects higher DPPH radical scavenging activity.

According to Al-Ismail *et al*. (2007), all ethanolic plant extracts exhibited strong antiradical activity against DPPH radicals (IC_{50}<1 mg mL^{-1}), except the extract of the underground part of *A. stipularis* which showed moderate scavenging activity (IC_{50}>1 and <2 mg mL^{-1}). The high scavenging activity of DPPH radical with the lowest IC_{50} values of *C. capitatus* (underground part) extract is attributed to its high contents of phenolic compounds especially flavonoids. Alves *et al*. (1992) and Seabra *et al*. (1998) isolated numerous of 1-4 benzoquinon and methyl-aurone derivatives from *C. capitatus*. Despite the relatively high content of the ethanolic extract of *S. lanata* (underground part) than those in the extract of *C. capitatus* aerial parts; the DPPH scavenging activity in *C. capitatus* were higher than those in *S. lanata* extracts. The possible reasons may be able to account for this, are the reversible reaction of DPPH with certain phenols such as eugenol and its derivatives and the slow rate of the reaction between DPPH and the substrate molecules (Lim *et al*., 2007).

Hydrogen peroxide (H_2O_2) is considered one of free radicals and can be injurious for the cells when present in excess (Halliwell and Gutteridge, 1999). Hydrogen peroxide radicals can be scavenged by antioxidant

compounds such as phenolics and phenolic acids (Sroka and Cisowski, 2003). The underground and aerial parts extracts of *C. capitatus* showed the similar trend in their scavenging activity against H_2O_2 and DPPH radicals. While, the extracts of *A. stipularis* and *S. lanata* exhibited the opposite trend, where the antiradical activity of *A. stipularis* was lower than those of *S. lanata*. Accordingly, the antioxidant compounds that inhibit H_2O_2 radicals differ from the antioxidants that scavenge DPPH radicals. Sroka and Cisowski (2003) reported that, the ability to scavenge H_2O_2 radicals by phenolic acids is positively correlated with the number and position of hydroxyl groups bonded to the aromatic ring. In addition, the character of constituents (carboxyl or acetyl group) and their position in relation to the hydroxyl groups seem to influence also the antiradical activities of phenolic compounds.

The present work indicated that, the antioxidant capacity and antiradical activity of plant extracts against DPPH and H_2O_2 free radicals were relatively correlated with their contents of total phenolics and flavonoids. However, some authors (Djeridane *et al*., 2006; Katalinic *et al*., 2006; Moussa *et al*., 2011; Javanmardi *et al*., 2003; Wojdylo *et al*., 2007) have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity, while others (Capecka *et al*., 2005; Wong *et al*., 2006) show poor linear correlation or report total antioxidant activity and phenolic content with no comment.

CONCLUSION

Based on these results, it is suggested that the investigated plant extracts can be utilized in food and pharmaceutical manufactures as an effective and safe source of natural antioxidants especially the extract of underground parts of *C. capitatus*. However, further studies should be performed for isolation and identification of the antioxidant compounds of these extracts and evaluate their antioxidant potential in an *in vivo* system.

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