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Flavonoid profile and antioxidant activities of methanolic extract of *Hyparrhenia hirta* (L.) Stapf.

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In this study, we report isolation of flavonoids, *viz*., 3-O-methylquercetin, tangeritin, luteolin-7-O-glucoside, luteolin, apigenin-7-O-glucoside, apigenin-8-C-glucoside, luteolin-8-C-glucoside, luteolin-6-C-glucoside, diosmetin and catechin from the methanolic extract of *Hyparrhenia hirta* employing high performance liquid chromatography and liquid chromatography-electrospray ionization-tandem mass spectrometry. The total phenolic content of *H. hirta* extract was 105.58 ± 0.1 mg gallic acid equivalents/g of plant extract while the total flavonoid content was 45.20 ± 0.2 mg quercetin equivalents/g of plant extract and the total condensed tannin were 72.35 ± 0.7 mg catechin equivalents/g of plant extract by reference to standard curve. The antioxidant activity was assayed through the antioxidant capacity by phosphomolybdenum assay, the reducing power assay and the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl method. The extract showed dose dependant activity in all the three assays.

Keywords: Apigenin, Catechin, Diosmetin, HPLC, LC-ESI-MS, Luteolin, Methylquercetin, Tangeritin, Thatching grass

Herbal medicinal products are useful therapeutic options providing a safer form of therapy, and in many instances, clinically effective specific remedies[1]. There has been consistent interest among researchers in finding antioxidants from commonly available green leafy vegetables and fruits, which though underexploited in most cases, possess a tremendous potential to treat the deadly diseases of the modern world. Extracts *Coccinnia grandis* L. leaf and sorghum have been reported to exhibit antioxidant and radical-scavenging activities[2,3]. Phenolic compounds from plant extracs have been shown to protect cells against the oxidative damage caused by free radicals[4]. Such properties are attributed to the conjugated ring structures and hydroxyl groups present in them[5]. Increased production of oxygen species and a reduced level of antioxidant system results in cell damage, and thereby leads to a variety of diseases[6]. The role of exogenous antioxidants in the prevention and treatment of various diseases has considerably attracted the attention of researchers worldover[5-8].

Exogenous antioxidant compounds may exert beneficial action upon systems which have been deprived from sufficient amounts of endogenous antioxidants as in some cardiovascular diseases, tumors, inflammation, ulcer and aging[8]. Tocopherol, vitamin C, carotenoids and flavonoids found in many terrestrial plants are good sources of antioxidants[9]. Evaluation of antioxidant and antiradical activities of plant products cannot be carried out accurately by any single universal method or extraction solvent system due to the complex nature of the phytochemicals present in them[10,11]. Numerous tests have been proposed to evaluate and estimate different aspects of antioxidant potency of plant components[12]. They can be categorized into two groups: assays for radical-scavenging ability; and assays for their ability to inhibit lipid oxidation under accelerated aging conditions. In fact, the features of an oxidation process are a substrate, an oxidant and an initiator, as well as intermediates and final products[13]. Measurement of any of these parameters can be used to assess antioxidant activity[14].

Flavonoids are known to possess neuro-protective, anti-inflammatory effects, anticancer, anti-genotoxic and antiglycative activities, which are basically related to their antioxidant properties[15]. *Hyparrhenia hirta* (Thatching grass), a tufted perennial grass growing up to 1.5 m in height, is rich in natural antioxidants. It is distributed mainly along the

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Mediterranean and across the African continent[16]. *H. hirta* grows in Tunisia in higher densities in central and southern parts of the country[17]. Though isolation of triterpenes, a β-ketone and phenolic derivatives[18] from *H. hirta* have been reported flavonoids from them have not been studied in detail. Therefore, in the present work we analyzed the flavonoid composition of the methanolic extract of *H. hirta* using the HPLC and LC-ESI-MS techniques and evaluated its antioxidant properties by different *in vitro* test systems.

Materials and Methods

Chemicals and materials—All chemicals such as aluminium trichloride ($AlCl_3$), ascorbic acid, butylated hydroxytoluene (BHT), catechins, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu phenol reagent, gallic acid, quercetin, sodium carbonate (Na_2CO_3) and vanillin used in this study were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

Preparation of plant extract—Aerial parts of *H. hirta* plants growing near Sfax were collected in November 2012 and stored at room temperature in a dry place until use. A voucher specimen authenticated by Prof. Mohamed Chaieb (Faculty of Science, Sfax University), was deposited at the Faculty of Pharmacy (Monastir, Tunisia). The stored samples were washed in running water, dried in the shade to ambient temperature until total dehydration. The dried aerial parts of plants were blended into fine powder and stored in dark. For preparation of extract, the powdered aerial parts of plant were charged into Soxhlet extractor (hot extraction). The interfering chlorophyll was removed by pre-extraction with dichloromethane. The remaining was then extracted with methanol for 48 h. The extract was filtered with N°1 Whatmann Millipore filter paper (0.45 μm Ref HAWPO4700, MA, USA) and concentrated into a small volume to remove the entire methanol using rotary evaporation (Büchi Rotavapor, Büchi Laboratories, Switzerland) at 40 °C under vacuum. The yield of extraction was determined. The small volume was later freeze-dried. All the dried extracts were preserved in the refrigerator until further use.

Identification of flavonoid compounds using HPLC and LC-ESI-MS—For liquid chromatographic (LC) system, we used an Agilent 1100- series binary pump system installed with a G1322A degasser, a G1312A pump, a G1313A autosampler and a G1316A oven (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was carried out on a Zorbax StableBond Analytical SBC18 column (4.6 × 250 mm, 5 μm; Agilent Technologies). The binary solvent system was made up of 0.1 % aqueous formic acid and methanol/acetonitrile (1:1)[19]. The conditions for analyzing the methanolic extract of *H. hirta* were: 0–4 min, 20% acetonitrile (B); 4–50 min, 20–80 % B; 50–55 min, 80 % B; 55–65 min, 80–20 % B followed by 10 min of linear gradient 20 % B. The flow rate was 0.3 ml/min in 35 °C column temperature with an infusion volume, 10 μl in each experiment. The chromatographic data were collected and controlled using a ChemStation, Rev.B.0301. Spectral data were collected (200–400 nm, 2 nm resolution) for the entire progression and the flavonoids were separated by extracting each chromatogram at 350 nm and 320 nm. Tandem mass spectrometry (MS/MS) experiments were conducted using ion trap mass spectrometer (LC/MSD Trap XCT) equipped with an electrospray ionization source G1948A operating in positive ion mode (ESI+). The electron spray voltage was set at 5.2 kV with source temperature at 500 °C. The diode array detection (DAD) was set at 140 254, 280 and 350 nm to provide real time chromatograms and the UV/Vis spectra from 190 to 650 nm were recorded for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive (PI) and negative ionization (NI) modes, at low (70 V) and high fragmentation voltages (250 V) for both ionization modes. For brevity, the high and low fragmentation voltages of the PI and NI modes will be identified as PI250, PI70, NI250, and NI70 in the text. The mass spectra were recorded for the range of m/z 100–1000 with a step size of 0.06 amu as done by Lee *et al*.[19]. The LC system was directly connected with MSD without stream splitting.

Antioxidant activities in *H. hirta* extract

Determination of total phenolic content in H. hirta—The total phenolic content in methanolic extract was determined with Folin-Ciocalteau reagent using the method of Chen *et al.*[20]. Different concentrations of gallic acid were prepared in methanol, and their absorbance was recorded at 750 nm. The diluted sample of 100 μL was added to 2 mL of 2% Na_2CO_3 aqueous solution followed by 100 μL of 50% Folin-Ciocalteau reagent after 2 min. The resultant mixture was shaken and then incubated at room temperature for 30 min in dark. The

absorbance of all samples was measured at 750 nm. Gallic acid was used to prepare a standard curve (0.05–0.5 mg/ml; y=8.6286x; r^2=0.9976, where 'y' represented the absorbance, and 'x', the concentration). The results were expressed in milligrams gallic acid equivalents per gram of extract (mg GAE/g extract).

Determination of total flavonoid content in H. hirta—The total flavonoid content in methanolic extract was determined according to Djeridane *et al.*[21], that based on the formation of a complex flavonoid–aluminium, having the maximum absorbance at 430 nm. About one mL of diluted sample was mixed with one ml of 2% aluminium trichloride ($AlCl_3$) methanol solution, incubated at room temperature for 15 min, and the absorbance of the reaction mixture was measured at 430 nm. Quercetin was used for standard curve construction (0.05–0.5 mg/ml; y=13.365x; r^2=0.9819, where 'y' depicted absorbance and 'x', the standard concentration). The total flavonoid content was expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

Determination of condensed tannin content in H. hirta—The condensed tannins were determined according to the method of Julkunen-Titto[22]. An aliquot of extract (50 µL) or a standard solution was mixed with 1.5 ml of 4 % vanillin (prepared with MeOH), then 750 µL of HCl (12 M) was added. The well-mixed solution was incubated in dark at ambient temperature for 20 min. Absorbance against blank was determined at 500 nm. Catechin was used to make the standard curve (0.05–0.5 mg/ml; y = 6.346x; r^2 = 0.99, where 'y' was the absorbance, and 'x', the standard concentration). The results were expressed as milligrams of catechin equivalents by gram of extract (mg CE/g extract).

Determination of total antioxidant capacity by phosphomolybdenum method—The antioxidant activity of the extract was evaluated by the phosphomolybdenum method as described by Prieto *et al.*[23]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of a green phosphate/Mo (V) complex at acid pH. About 0.3 ml of methanolic extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The three tubes containing the reaction solution were incubated at 95 °C for 90 min, and the absorbance was measured at 700 nm using a spectrophotometer UV–Vis against blank after cooling to room temperature. Methanol (0.3 mL) was used as the blank. The experiment was performed in triplicates. A standard curve was prepared using various concentrations of vitamin E.

Ferric-reducing antioxidant power assay—The Oyaizu method (1986) was used to evaluate the reducing power of methanolic extracts[24]. Extract (1mg/mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH=6.6) and 2.5 mL of potassium ferricyanide, 1% ($K_3Fe(CN)_6$), and incubated in a water bath at 50 °C for 20 min. After cooling to room temperature, 2.5 mL of 10% trichloroacetic acid was added to the mixture, centrifuged at 650 × g for 10 min, and the supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 ml of ferric chloride solution 0.1%. The absorbance of the mixture was measured at 700 nm. Higher absorbance mixture indicates higher reducing power of the extract. A standard curve was prepared using various concentrations of vitamin E.

DPPH free radical scavenging activity—The scavenging effect for DPPH radical was evaluated according to Brand-Williams *et al*.[25]. Briefly, 2 mL of 0.1 mM DPPH methanol solution was added to a 1.0 mL of either methanol solution of extract (sample) or methanol (control). The mixtures were vortexed for 1 min and left to stand at room temperature in dark. After 30 min, absorbance was read at 517 nm. Radical-scavenging activity (RSA) for DPPH free radical is calculated by the equation:

$$1 - \frac{A_{sample}}{A_{Control}} \times 100 .$$

where the $A_{control}$ is the absorbance of the methanol control and the A_{sample} is the absorbance of the extract. Synthetic antioxidant, BHT, was used as a positive control. Bleached DPPH solution, prepared by adding 2 ml of 0.1 mM DPPH solution to 1 mL of BHT solution was used as blank. DPPH radical-scavenging activity was calculated as the concentration that scavenges 50% of DPPH free radical, and has thus, RSA = 50% (IC_{50}).

Results and Discussion

Identification of flavonoids in extract from H. hirta—The HPLC-DAD chromatogram at 350 nm (Fig. 1) shows flavonoids identified in *H. hirta*. The retention times (t_R), UV λ_{max} values, and the molecular ions of the flavonoids are listed in Table 1 for each

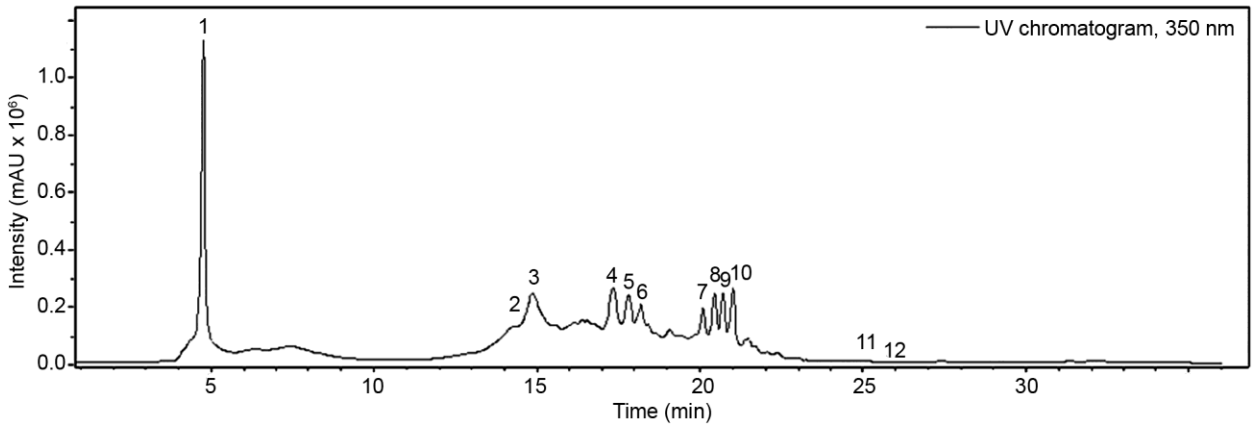

Fig. 1—HPLC-DAD chromatogram at 350 nm of methanolic extract of *Hyparrhenia hirta.* Details are given in table 1.

Table 1—Flavonoid compounds identified from *Hyparrhenia hirta*

Peak no.	t_R (min)	Mw (Da)	λ_{max} (nm)	Base peak *m/z*	Main fragment ions MS *m/z*	Main fragment ions MS^2 *m/z*	Compound identification
1	5.8	316.24	264; 356	317.8 $[M+H]^+$	317.8: 218.8; 155.8; 139.9	155.8: 137.9; 74.1	3-O-methylquercetin
2	14.9	372.32	270; 322	370.7$[M-H]^-$	370.7: 238.8	370.7: 220.8; 194.8; 176.9	Tangeritin
3	15.2	-	-	-	260.8; 239.9	239.9: 222.9	Unknown
4	16.7	448.38	270; 348	448.6$[M-H]^-$	448.6: 384.7; 242.8; 186.8	384.7: 286.8; 266.6; 241.9; 176.9; 151.9	Luteolin-7-O-glucoside (cynaroside)
5	17.8	286.24	270; 348	288.8$[M+H]^+$	288.8: 255.8; 240.8; 218.8; 196.9; 134.9	196.9: 178.9; 160.9; 146.8; 134.9; 108	Luteolin
6	18.2	432.10	270; 340	432.6$[M-H]^-$	432.6: 270.8	432.6: 270.8	Apigenin-7-O-glucoside (cosmosiin)
7	20.2	432.10	270; 338	430.7$[M-H]^-$	430.7: 394.8; 342.9; 270.8	394.8: 376.9	Apigenin-8-C-glucoside (vitexin)
8	20.4	448.38	270; 338	448.6$[M-H]^-$	448.6: 412.7; 394.8; 382.7; 326.9; 284.8	412.7: 368.6; 234.7; 218.7	Luteolin-8-C-glucoside (orientin)
9	20.9	448.38	270; 338	448.6$[M-H]^-$	448.6: 412.7; 382.7; 326.9; 284.8	326.9: 228.8	Luteolin-8-C-glucoside (orientin)
10	21.3	448.38	270; 348	448.6$[M-H]^-$	448.6: 412.7; 382.7; 326.9; 284.8; 238.8	326.9: 228.8	Luteolin-6-C-glucoside (isoorientin)
11	25.5	300.24	250; 348	300.8$[M+H]^+$	300.8: 278.9	300.8: 298.8; 282.8; 254.9; 222.9	Diosmetin (chrysoeriol)
12	26	290.27	276	291$[M+H]^+$	291: 248.9; 204.8	291: 258.9; 240.9; 176.9	Catechin

peak. By referring to the reported data of the chromatograms, [a full UV/V (190-600 nm) and mass spectra (counts *vs*. *m/z* for 100-1000 amu) for each peak], some flavonoids could be identified. For peak 1, the positive HPLC spectrum gave a molecular ion at *m/z* 317.8$[M+H]^+$. The UV spectrum showed characteristic flavones absorption at 264 and 356 nm. It was identified as 3-O-methylquercetin (Table 1).

In the mass spectrum at 14.9 min retention time, the corresponding ion at *m/z* 370.7 (peak 2) could be a de-protonated form of a compound with such molecular mass. The UV spectrum showed characteristic flavones absorption at 270 and 322 nm. These could be tangeritin with respect to the retention time and mass spectra standards[26]. The ions detected in the mass spectrum for 15.2 min chromatographic peak (peak 3) were: *m/z* 260.8 and 239.9. The MS^2 spectrum obtained by fragmentation of the ion *m/z* 239.9 presented the *m/z* value 222.9, however, the peak 3 could not be identified.

The UV absorption bands at 270 and 348 nm of peak 4 suggest the presence of luteolin. The molecular ion of peak 4 was 448.6[M-H]$^-$ and the *m/z* was 449.6, suggesting that it could be luteolin 7-O-glucoside[27]. Peak 5 resembles luteolin[28] as shown by the UV λ_{max} at 270 and 348 nm and molecular ions at *m/z* 288.8[M+H]$^+$. The [M+H]$^+$ ion at *m/z* 432.6 was detected and the fragmentation yielded the aglycone apigenin at *m/z* 270.8. However, an ion with equal fragmentation pattern was detected at 18.2 min retention time. The UV spectrum of peak 6 with maximum absorption at 270 and 340 nm, suggests that it could be related to apigenin as it matches with apigenin-7-O-glucoside. The peak 7 with maximum absorption at 270 and 338 nm was apigenin-8-C-glucoside[29] as revealed by the relative molecular ions at 430.7[M-H]$^-$ and the m/z 431.7.

Peaks 8 and 9 displayed identical UV spectra with maxima at 270 and 338 nm and gave [M-H]$^-$ ions at *m/z* 448.6. They were identified as luteolin-8-C-glucoside[29]. The ions detected in the mass spectrum for 21.3 min chromatographic peak (peak 10) were: *m/z* 448.6, 412.7, 382.7, 326.9, 284.8 and 238.8. The MS^2 spectrum obtained by fragmentation of the ion *m/z* 326.9 presented the following *m/z* values: 326.9 and 228.8 hinting that the peak 10 could be luteolin-6-C-glucoside[28]. Peak 11 was identified as diosmetin by comparison with an authentic sample. The peak 12 with molecular ion 291[M+H]$^+$ and the m/z, 290 and maximum absorption at 276 was catechin[30].

The identification of the isolated flavonoids was achieved using MS with fragmentation of the ions detected. The identified compounds were: 3-O-methylquercetin, tangeritin, luteolin-7-O-glucoside (cynaroside), luteolin, apigenin-7-O-glucoside (cosmosiin), apigenin-8-C-glucoside (vitexin), luteolin-8-C-glucoside (orientin), luteolin-6-C-glucoside (isoorientin), diosmetin (chrysoeriol) and catechin.

The antioxidant activity of putative antioxidants could be attributed to various mechanisms, including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[31].

Determination of total phenolic, flavonoid and condensed tannin contents in H. hirta— The extraction yield of *H. hirta* methanolic extract was 22.56 %. The total phenolic, flavonoid and condensed tannin contents of *H. hirta* methanolic extract were examined and are presented in Table 2.

The methanolic extract showed high amounts of phenolic and flavonoid compounds. The study revealed that the total phenolic content was 105.58 ± 0.1 mg GAE/g extract. The levels of total phenolics determined by this method were not absolute measurements of the amounts of phenolic compounds, but were based on their chemical reducing capacity relative to gallic acid. The phenol antioxidant index is a combined measure of the quality and quantity of antioxidants in vegetables[32]. Here, the total flavonoid content in methanolic extract was estimated at 45.20 ± 0.2 mg QE/g extract. These amounts were comparable with results described earlier for other extracts of plant products[33,34]. Sharififar *et al*. reported that the rich-flavonoids plants could be a good source of antioxidants that would help to increase the overall antioxidant capacity of an organism and protect it against lipid peroxidation[35]. The results of the present study suggest that phenolic acids and flavonoids could be the major contributors for the antioxidant activity to display characteristic inhibitory patterns toward the oxidative reaction *in vitro* and *in vivo*. Besides, the condensed tannin (proanthocyanidines) content of extract of *H. hirta* was 72.35 ± 0.7 mg of catechin equivalents/g of plant extracts. The phenolic and flavonoid compounds found in plants are the source of several biological activities including antioxidant and antimicrobial properties[36,37].

Table 2—Total phenolic, flavonoid and condensed tannin contents in *Hyparrhenia hirta* methanolic extract

[Values represent the means of three replicates±SE]

Samples	Extraction yield (%)[a]	Content		
		Total phenols (mg GAE/g extract)	Total flavonoids (mg QE/g extract)	Condensed tannins (mg CE/g extract)
Methanolic extract	22.56 % ± 0.01	105.58 ± 0.1	45.20 ± 0.2	72.35 ± 0.7

GAE, gallic acid equivalents; QE, quercetin equivalents; CE, catechin equivalents

[a]Extraction yield (in percent)=(sample extract weight/sample weight)×100

Antioxidant activities in *H. hirta* methanolic extract

Determination of total antioxidant capacity by phosphomolybdenum method—It is the routine method used to assess the total antioxidant capacity of extracts. In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green colored phosphomolybdenum V complex, which shows maximum absorbance at 700 nm. The total antioxidant activity of the extract was shown in Fig. 2A. The methanolic extract showed concentration dependent antioxidant activity. This high antioxidant activity of methanolic extract of *H. hirta* could be due to the presence of phytochemicals such as phenolic compounds. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants[35].

Reducing power assay of methanolic extract of H. hirta—The antioxidant potential of the methanolic extract was also estimated using the method of potassium ferric cyanide reduction. The yellow color of the solution changes during the test to various shades of green and blue depending on the reducing power of the extract.

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+}/ferric cyanide complex to ferrous form by donating an electron. Previous reports suggested that the reducing properties have been shown to exert antioxidant action by donating of a hydrogen atom to break the free radical chain[38]. Increasing absorbance at 700 nm indicates an increase in reducing ability. The reducing power of the extracts is shown in Fig. 2B. The extract exhibited a concentration dependent reducing power activity, comparable to that of vitamin E at all the concentrations. The data from this assay suggested that the extract was able to donate electrons, thereby reducing Fe^{3+} to Fe^{2+}, an indication of their antioxidant potential[39,40].

DPPH free radical scavenging activity—The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples[41]. DPPH is a stable free radical. Antioxidant or radical scavenger changes its color from violet to yellow when is reduced by either the process of hydrogen or electron donation. The DPPH radical has been widely used to assess the antioxidative activity of plant extracts and

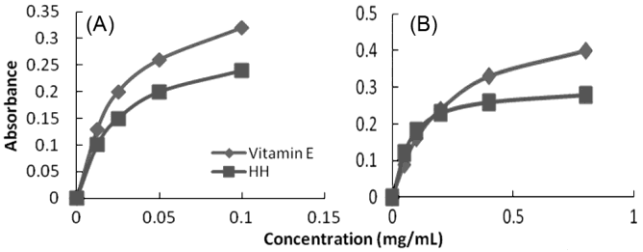

Fig. 2—(A) The total antioxidant capacity of vitamin E and the *Hyparrhenia hirta* methanolic extract; (B) The reducing power of vitamin E and the *Hyparrhenia hirta* methanolic extract.

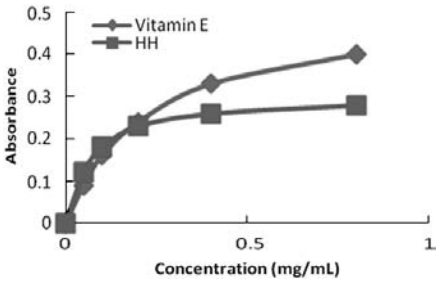

Fig. 3—The DPPH free radical scavenging potential of BHT and the *Hyparrhenia hirta* methanolic extract.

foods. It has been suggested that extracts which are rich in phenolics and flavonoids are involved in several biological activities including antioxidant ones. The free radical-scavenging activity of the extract was tested through DPPH-method and the results were compared with BHT (Fig. 3). The methanolic extract showed an important free radical-scavenging activity. The determination of the concentration corresponding to 50% of inhibition of radical DPPH showed that the IC_{50} of the methanolic extract of *H. hirta* was 150±2 µg/ml. In this study, the samples exhibited a concentration-dependent antiradical activity by inhibiting DPPH-radical (Fig. 3). Radical scavenging activity of extracts coould be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. The results obtained here in this study imply that the methanolic extract of *H. hirta* has notable antioxidant ability as compared to the reference (vitamin E) antioxidant and can serve as source of cost effective natural antioxidants.

In conclusion, the present investigation suggests that *H. hirta* possess a potential antioxidant activity, particularly with reference to its methanolic extract. This activity could be due to the presence of phenolic and flavonoid contents. Thus, the methanolic extract of the Thatching grass, *H. hirta* can be utilized as an effective and safe antioxidant source.

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References

1 Shirwaikar A, Verma R, Lobo R & Shirwaikar A, Phytotherapy-Safety aspects. *Nat Prod Rad*, 8 (2009) 55.
2 Umamaheswari M & Chatterjee TK, *In vitro* antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. *Afr J Trad Complement Altern Med*, 5 (2008) 61.
3 Kil HY, Seong ES, Ghimire BK, Chung IM, Kwon SS, Goh EJ, Hoe K, Kim MJ, Lim JD, Lee D & Yu CY, Antioxidant and antimicrobial activities of crude sorghum extract. *Food Chem*, 115 (2009) 1234.
4 Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS & Heinonen M, Antioxidant activity of plant extracts containing phenolic compounds. *J Agr Food Chem*, 47 (1999) 3954.
5 Shahidi F & Wanasusdara PD, Phenolic antioxidants. *Crit Rev Food Sci Nutr*, 32 (1992) 67.
6 Ashok Kumar K, Uma Maheswari M, Sivashanmugam AT, SubhadraDevi V, Subhashini N & Ravi TK, Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpetweed) using different *in vitro* assay systems. *Pharm Biol*, 47 (2009) 474.
7 Niki E, Assessment of antioxidant capacity *in vitro* and *in vivo*. *Free Rad Biol Med*, 49 (2010) 503.
8 Hasan SMR, Jamila M, Majumder MM, Akter R, Hossain M, Mazumder EH, Alam A, Jahangir R, Rana S & Arif Rahman S, Analgesic and antioxidant activity of the hydromethanolic extract of *Mikania scandens (*L.) Willd. Leaves. *Am J Pharm Toxicol*, 4 (2009) 1.
9 Larson RA, The antioxidants of higher plants. *Phytochemistry*, 27 (1988) 969.
10 Shahidi F & Naczk M, Antioxidant properties of food phenolics. *In*: *Phenolics in food and neutraceuticals*, (CRC Press, Boca Raton, FL) 2004, 401.
11 Prior RL, Wu X & Schaich K, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agr Food Chem*, 53 (2005) 4290.
12 Decker EA, Warner K, Richards MP & Shahidi F, Measuring antioxidant effectiveness in food. *J Agr Food Chem*, 53 (2005) 4303.
13 Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magné C & Abdelly C, Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food Chem Toxicol*, 47 (2009) 2083.
14 Antolovich M, Prenzler PD, Patsalides E, McDonald S & Robards K, Methods for testing antioxidant activity. *Analyst*, 127 (2002) 183.
15 Xiao JB & Kai GY, A review of dietary polyphenol-plasma protein interactions: characterization, influence on the bioactivity, and structure-affinity relationship. *Crit Rev Food Sci Nutr*, 52 (2012) 85.
16 Clayton WD, A revision of the genus *Hyparrhenia. Kew Bull*, 2 (1969) 1.
17 Chaieb M & Boukhris M, *Flore succinte et illustrée des zones arides et sahariennes de Tunisie. (edn) l'Or du temps*. Tunis A.P.N.E.S. ISBN, 99 (1998) 757.
18 Ben Salah H, Bouaziz M, Bahroun A, Damak M, McKillop A & Simmonds MSJ, Contribution à l'étude chimique d'*Hyparrhenia hirta* L. *J Soc Chim Tunis*, 4 (2000) 647.
19 Lee DH, Park KI, Park HS, Kang SR, Nagappan A, Kim JA, Kim EH, Lee WS, Hah YS, Chung HJ, An SJ & Kim GS, Flavonoids isolated from Korea *Citrus aurantium* L. Induce G2/M phase arrest and apoptosis in human gastric cancer AGS cells. *Evid Based Complement Alternat Med*, 2012 (2012) 515901.
20 Chen Z, Bertin R & Froldi G, EC_{50} estimation of antioxidant activity in DPPH assay using several statistical programs. *Food Chem*, 138 (2013) 414.
21 Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P & Vidal N, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*, 97 (2006) 654.
22 Julkunen-Titto R, Phenolic constituents in the leaves of northern willow: methods for the analysis of certain phenolics. *J Agr Food Chem*, 33 (1985) 213.
23 Prieto P, Pineda M & Aguliar M, Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem*, 269 (1999) 337.
24 Oyaizu M, Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Nippon Shokulin Kogyo Gakkaishi*, 35 (1986) 771.
25 Brand-Williams W, Cuvelier ME & Berset C, Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol*, 28 (1995) 25.
26 He XG, On-line identification of phytochemical constituents in botanical extracts by combined high-performance liquid chromatographic–diode array detection–mass spectrometric techniques. *J Chromatogr A*, 880 (2000) 203.
27 Plazonić A, Bucar F, Maleš Ž, Mornar A, Nigović B & Kujundžić N, Identification and quantification of flavonoids and phenolic acids in Burr Parsley (*Caucalis platycarpos* L.), using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. *Molecules*, 14 (2009) 2466.
28 Gordon A, Schadow B, Quijano CE & Marx F, Chemical characterization and antioxidant capacity of berries from Clidemia rubra (Aubl.) Mart. (Melastomataceae). *Food Res Inter*, 44 (2011) 2120.
29 Colombo R, Yariwake JH & Mc Cullagh M, Study of C-and O-glycosylflavones in Sugarcane extracts using liquid-chromatography-Exact Mass Measurement Mass Spectrometry. *J Braz Chem Soc*, 19 (2008) 483.
30 Zuo Y, Chen H & Deng Y, Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and puerh teas using HPLC with a photodiode array detector. *Talanta*, 57 (2002) 307.
31 Diplock AT, Will the "good fairies" please prove to us that vitamin E lessens human degenerative disease? *Free Rad Res*, 27 (1997) 511.
32 Elliott JG, Application of antioxidant vitamins in foods and beverages. *Food Technol*, 53 (1999) 46.
33 Ao C, Li A, Elzaawely AA, Xuan DT & Twata S, Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fill extract. *Food Control*, 19 (2008) 940.

34 Manian R, Anusuya N, Siddhuraju P & Manian S, The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* L., *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem*, 107 (2008) 1000.

35 Sharififar F, Dehghn-Nudeh G, Mirtajaldini M, Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem*, 112 (2009) 885.

36 Stevenson DE, Hurst RD, Polyphenolic phytochemicals-just antioxidants or much more? *Cell Mol Life Sci*, 64 (2007) 2900.

37 Ren W, Qiao Z, Wang H, Zhu L & Zhang L, Flavonoids: promising anticancer agents. *Med Res Rev*, 23 (2003) 519.

38 Gordon, MH, The mechanism of antioxidant action *in vitro*. In, *Food antioxidants*, edited by Hudson BJ, (Elsevier Applied Science, London), 1990, 1.

39 Pin-Der-Duh X, Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free radical and active oxygen. *J Am Oil Chem Soc*, 75 (1998) 455.

40 Dorman HJD, Peltoketo A, Hiltunen R & Tikkanen MJ, Characterization of the antioxidant properties of deodorized aqueous extracts from selected Lamiaceae herbs. *Food Chem*, 83 (2003) 255.

41 Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A & Bekhradnia AR, Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica Mey. *Pharmacologyonline*, 2 (2008) 560.