Screening of Antioxidant Activity of Selected Traditionally Used Libyan Medicinal Plant Extracts

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Abstract

In different parts of the world, the use of medicinal plants has always been important in the therapeutic armory of mankind and remains an important source for the discovery of new bio-active compounds. Libya constitutes an apt example where medicinal plants are widely used. While some individual plant species such as *Ginkgo biloba* have been investigated in some detail, there is relatively little information available concerning the antioxidant potential of plant species in general and Libyan plants in particular. In this study twenty three Libyan medicinal plants were chosen for the study of antioxidant capacity and phenolic content. Aqueous plant extracts were screened for their antioxidant activity using the FRAP, TEAC and DPPH methods. These methods enable high-throughput screening of potential antioxidant capacity. Results show that of these twenty three plants, hot and cold extracts of *Myrtus communis*, *Quercus robur* and *Syzygium aromaticum* exhibited the strongest antioxidant activity in all tests and this is higher than that of the green tea control. It is suggested that the efficacy of these plants could be explained, at least in part, by their antioxidant activity.

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Introduction

Reactive Oxygen Species (ROS) are highly reactive and potentially damaging transient chemical species created in all cells through various physiological and biochemical process (such as activation of phagocytes, mitochondrial respiration, and biosynthesis of endoperoxide) as undesirable metabolic by-products of normal aerobic metabolism (Rice-Evans and Miller, 1994, Parke et al., 1991, Rice-Evans, 2000). Most ROS such as superoxide radical ($O^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), and nitric oxide radical ($NO^{\cdot}$), attack biological molecules such as proteins, lipids, DNA and RNA leading to cell or tissue damage and injury associated with many diseases, from malignancy to cardiovascular disease and dementia (Hashim et al., 2005). ROS are responsible in part for the ageing process finally leading to death. Parke (1999) has summarised the molecular mechanisms of ROS toxicity as: oxidation of vital thio-compounds to disulphate; loss of tissue GSH (glutathione); impairment of energy generation (ATP, NADH, and NADPH); oxidation of cytoplasm; inhibition of Ca^{2+} transport and electrolyte homeostasis; DNA cleavage and the initiation and promotion of mutations and carcinogenesis.

There are, however, many naturally occurring substances which function to protect against the potentially harmful effects of pro-oxidants. These substances, termed antioxidants, are simply defined as “chemical compounds or substances that inhibit oxidation”(Balcerczyk and Bartosz, 2003). Antioxidant compounds must be present in biological systems in sufficient concentrations to prevent an accumulation of pro-oxidant molecules, a state known as oxidative stress (Buettner and Schafer, 2000). Antioxidants can interfere with the production of free radicals and/or inactivate them once they are formed. In other words, these

antioxidants can act by either interfering with the propagation stage of free radical generation itself or act directly as free radical scavengers. For example, vitamins E and C act as free radical scvengers which can quench free radicals as well as singlet oxygen (Rice-Evans and Miller, 1996).

Currently used synthetic antioxidants have been suspected to cause or promote negative health effects (Amarowicz et al., 2004); hence stronger restrictions have been placed on their application. Therefore, there is a trend to substitute synthetic antioxidants with naturally occurring antioxidants. Some natural antioxidants such as rosemary, sage (Ollanketo et al., 2002) and *Ginkgo biloba* (Arredondo et al., 2004) are exploited commercially either as antioxidant additives or as nutritional supplements.

During the last 20 years many publications have appeared on the measurement of antioxidants and a large number of different methods and strategies have been proposed and developed for the evaluation of the total antioxidant capacity in diverse samples such as biological samples, plant tissue and foodstuffs (Prior and Cao, 1999). Although there is a great multiplicity of methods used for antioxidant testing, none of them provides an ideal individual, approved, standardised reference method. This is simply because within biological systems, there are various sources of antioxidants including enzymes (such as superoxide dimutase, glutathione peroxidase and catalase), large molecules (like albumin and ferritin), small molecules (uric acid and polyphenols), some hormones (melatonin and estrogen) and dietary origin molecules such as vitamin C, carotenoids and flavonoids. On the other hand, both oxidants and antioxidants may have different chemical and physical features.

Furthermore, antioxidants may respond in different manners to different radical or oxidant sources (Prior et al., 2005).

Consequently, in order to give an overall picture to describe the total antioxidant activity in any sample, more than one analytical assay must be applied (Frankel and Meyer, 2000). However, the most widely and frequently used assays among these are ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996, Benzie and Strain, 1999) (µmol ferrous ion equivalents); Trolox equivalent antioxidant capacity, (TEAC) (Re et al., 1999) (mmol Trolox equivalents) and scavenging activity of 1,1′-diphenyl-2-picryl-hydrazyl (DPPH) radicals (Brand-Williams et al., 1995, Blois, 1958, Koleva et al., 2002) (mmol Trolox equivalents).

Recently, there has been an upsurge of interest in the therapeutic potential of traditional medicinal plants as antioxidants in reducing such free radicals induced by tissue injury. However, Mantle et al. (2000b) have determined the relative levels of endogenous antioxidant activity in a range of British medicinal plants selected on the basis of their widespread use in traditional herbal medicine including rosemary, sage, and mint.

Traditional medicine is widely practiced in Arabic countries in general, and particularly in Libya. Libya constitutes an apt example where medicinal plants are widely used in everyday life as part of folk medicinal remedies. Ethnopharmacological surveys conducted among herbal practitioners of traditional Arab medicine in these countries revealed a large number of indigenous plants are used as sources of their herbal therapies (Ali-Shtayeh et al., 2000). Some of these herbal therapies are used to treat diabetes, heart disease, high blood pressure, and liver disease, conditions in which oxidative stress is prominent (Kotb, 1983).

At present, no laboratory data on the bioactivity of herbal medicines used to treat these diseases in traditional Arab medicine in Libya exist.

Moreover, there is relatively little knowledge and information available concerning the antioxidant potential of plant species in general and in Libyan plants in particular. We hypothesized that the beneficial effects of these plants might be due to their antioxidant properties. Considering the importance of this area, the objective of this study is, therefore, to evaluate the relative level of antioxidant activity of selected medicinal plants which are being used traditionally in Libya for various disorders where free radicals are thought to be involved using the three different assays (FRAP, TEAC and DPPH). In the long term plants identified as having high levels of antioxidant activity *in vitro* may prove of value in the design of clinical trials of novel treatment in which free radical induced tissue damage has been implicated.

Table 1 A list of Libyan medicinal plants as used in this study
(adapted from El Gadi, 1992 and Kotb, 1983)

Local name	English name	Scientific name	Family name	Locations	part used	Uses
شاي أخضر	Green tea	*Camellia sinensis*	Theaceae	-	Leaves	Stimulant to the CNS, diuretic
الدفلة, دفلي, ورد الحمار	Oleander, Rose bay	*Nerium oleander* (LINN.)	Apocyanaceae	Many places all over Libya.	The whole plant.	Treatments of heart diseases, dried leaves are rubbed upon afflicted parts for headache or neuralgia.
زعتر, دوس, تومس	Thyme, common thyme	*Thymus vulgaris* (LINN.)	Labiatae	Tripoli, Garian and Benghazi	Leaves and flowering tops	Digestive, stimulant, carminative intestinal antiseptic, antifungal, used for whooping cough and bronchitis.
بردقوش, ريحان داوود	Sweet marjoram	*Origanum majorana* (LINN.)	Labiatae	Derna	the flowering plant without the root	Carminative, condiment, antispasmodic, very mild laxative.
مرسين, جدره	Myrtle, common myrtle	*Myrtus communis* (LINN.)	Myrtaceae	common in Tripoli and Benghazi areas	Leaves, berries and the volatile oil	Antidiabetic, astringent, in eczema epilepsy, wound and ulcers.
عاقول, شوك الجمال	Manna tree, camel thorn, prickly alhagi	*Alhagi maurorum* (MEDIK)	Leguminosae	Sebha, Wadi el Ajial, Chat and Ghadames	Leaves and the exudates of branches	Diuretic and expectorant, treatment of rheumatism, mild laxative.
جعدة, حشيشة الريح	Hulwort, cat thyme	*Teucrium polium* (LINN.)	Labiatae	Tripoli , Benghazi and Fezzan	Leaves	Antidiabetic, antiintestinal inflammation and antimalarial, bitter tonic.
حريق, شعر العجوز	Stinging nettle, small nettle	*Urtica urens* (LINN.)	Urticaceae	different areas in Libya	The whole plant.	Antianemic, haemostatic, antidiabetic, diuretic.
عشبة الارنب, طعام الارنب	African fleabane	*Phagnalon rupestre* (LINN.)	Compositae	Tripoli , Benghazi, Derna,Trhuna and Tobruk	The whole herb	Effective in cases of urinary calculi, reduce the renal colic pain.

ورق الزيتون	Common olive	*Olea europaea* (LINN.)	Oleaceae	everywhere in Libya	Leaves	show hypoglycemic activity, increases blood circulation and urine secretion and hypotensive
اكليل الجبل, حصالبان	Common rosemary	*Rosmarinus officinalis* (LINN.)	Labiatae	Many places all over Libya	Leaves and flowering tops	Antirhematic, antiseptic, antispasmodic, carminative, cholgogue, respiratory antiseptic.
فجل بري, عيش وجبن	Wild radish, runch	*Raphanus raphanistrum* (LINN)	Cruciferae	cultivated areas	seeds	Rubfacient, stimulant, emetic, antihaemorrhagic.
دمسيسة, افسنتين	Absinthe, worm-wood	*Artemisia absinthium*(LINN)	Compositae	grows wildly in waste areas	Dried herbs especially leaves and flowering tops	An excellent bitter tonic, antiseptic and diuretic, increase the hepatic secretion, affect the CNS.
اقحوان, صفيرة	Marygold flower, garden marygold	*Calendula officinalis* (LINN.)	Compositae	common in the gardens	Flowers and leaves	Diuretic, diaphoretic, assist antiemetic, antianemic, healing of ulcers, oxytocic
مرمية, شاي درنه, تفاح, تيه	Sage	*Salvia officinalis* (LINN.)	Labiatae	Many places all over Libya	The volatile oil and shade-dried leaves	In cases of nervous disorders, dizziness and trembling
روبيه, فرسيون ابيض	White horehound, horehound	*Marrubium vulgare* (LINN.)	Labiatae	Tripoli, Benghazi, Shahat and many places in Libya.	The flowering plant without the root	To cure cough, sore throat and cold. Hypoglycaemic, cholgogue.
فيجل, سذب	Herb of grace, rue	*Ruta graveolens* (LINN.)	Rutaceae	Many places all over Libya	Leaves	Relieve teeth and ear pains, to ease delivery, emmenagogue and ecbolic
قميلة, فلية, بابونج	Chamomile, German chamomile	*Matricaria chamomilla* (LINN.)	Compositae	Many places all over Libya	The flower heads and their volatile oil	Tonic, mild laxative, diuretic, antispasmodic, diaphoretic, carminative, urinary and respiratory antiseptic.

كركديه	Roselle, karkade	*Hibiscus sabdariffa* (LINN.)	Malvaceae	Many places in Libya	Calyx, leaves and seeds	As a source of vit. C, laxative, diuretic, reduce blood pressure, mild laxative and intestinal antiseptic.
بلوط	Chestnut- oak	*Quercus robur* (LINN.)	Fagaceae	Between Garian and Yefern	Fruits	Very astringent, used to treat haemorrhoids
القزاح		*Pituranthos tortousus*	Apiaceae	Many places in Libya	The whole plant	Reduce blood pressure
قرنفل	Clove	*Syzygium aromaticum*	Myrtaceae	-	Dried flower buds	Headaches, respiratory disorders
زنجبيل	Ginger root	*Zingiber officinale*	Zingiberaceae	-	Rhizomes	Cardiotonic, pain relief

Materials and methods

Plant materials:

Plant collection and identification:

The medicinal plant species used in this study (Table 1) were collected fresh from different areas of Libya, during the period from 12th April to 5th May 2005. The green tea, clove and ginger roots were purchased from a local market for herbs in Benghazi. The botanical identification of the plant species was determined with the aid of descriptions given by Kotb (1983) and confirmed by Dr Osama Rahoma. The plant parts used were allowed to dry in air and then ground into a powder state using a commercial miller and finally used for the preparation of extracts within approximately one month of collection.

Plant extract:

Plant extracts with either hot or cold water (hot means freshly boiled water and cold means room temperature water) were prepared as described by Koleva et al., 2002; Mantle et al., 2000b in triplicate in ways that mimic the traditionally used methods in folk medicine.

Chemicals:

The chemicals used in these experiments, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) powder ($ABTS^{+}$), potassium persulphate, (+/-)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), ferrous sulphate, ferric chloride, 2,4,6 tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu reagent, gallic acid and 1,1′-diphenyl-2-picryl-hydrazyl (DPPH) were supplied by Sigma Chemical Company, UK. All other solvents, salts and reagents were obtained from VWR International, Country Durham, UK.

Antioxidant analysis:

FRAP assay:

The FRAP assay followed in this study was originally developed by Benzie and Strain (1996) to measure reducing power in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in botanical tissues.

TEAC assay:

The standard TEAC assay described by Van den Berg et al. (1999) and Re et al. (1999) was used with minor modifications for the determination of TEAC values. This assay assesses the total radical scavenging capacity based on the ability of compounds to scavenge the stable ABTS radical ($ABTS^{\bullet}$).

DPPH˙ assay:

The original DPPH˙ (1,1′-diphenyl-2-picrylhydrazyl) method of Brand-William et al. (1995) was modified by Fukumoto and Mazza (2000). This method of Fukumoto and Mazza was further modified as follows: DPPH˙ (150 µM), a stable free radical, was dissolved in 80% (v/v) methanol. Using 80% (v/v) methanol had the advantage of a faster reaction rate for some compounds such BHA and lower µl of plant extract or standard solution and 30 µl de-ionized water as a diluter were mixed with 300 µl of DPPH˙ solution and incubated for 25 min at 30°C. The change evaporation losses.

Determination of total phenolic content:

The total phenolic content of the plant extracts was determined according to the Folin- Ciocalteu method (Duan et al., 2006) with a slight modification. Instead of reading samples spectrophotometrically the assay was performed in a Multiskan Ascent micro plate reader (Thermo Labsystem, Helsinki, Finland). In each well of a 96-well flat-bottom polystyrene micro plate a 10 µl aliquot of plant extract or calibration standard was added to 130 µl of Folin-Ciocalteu reagent (the concentrated commercial 2 N reagent was diluted 1:10 (v/v) with de-ionized water). After 5 min 100 µl of 7.5% (w/v) sodium carbonate solution was added. The plates were shaken in the automated micro plate reader for 1minute and incubated for 30 minutes at 37°C. The absorbance was measured at 750 nm, and then compared to a gallic acid calibration standard curve (0 to 1500 mg/L in de-ionized water) (figure 2.1). The total phenolic content was expressed as gallic acid equivalents (GAE) (figure.1).

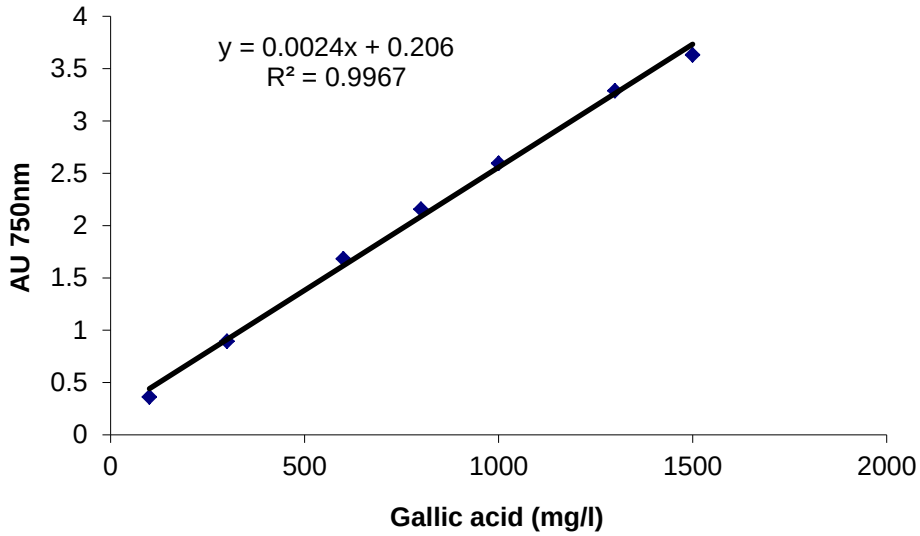

Figure.1 Phenolic Content Standard Calibration Curve.

The absorbances of the standard curves were plotted versus reference concentrations (ferrous sulphate conc. for FRAP and Trolox conc. for TEAC and DPPH). The absorbances of the test samples were read from the standard curve to give antioxidant activity as ferrous ion equivalent (FIE) for FRAP assay and as mmol Trolox equivalent for TEAC and DPPH assays (the data were corrected for any initial dilution of samples if required). Samples with high antioxidant activity need to be diluted for example, typically by 1:5, 1:10, or 1:20 before pipetting the sample into the assay tube.

Statistical analysis:

The experiments were carried out in triplicate extracts. The results are given as mean ± standard deviation (SD). The data for antioxidant activity for each assay were analysed by one-way analysis of variance (ANOVA), and for comparison with green tea extract (standard antioxidant reference), the Dunnett's post–test was used which is

designed to compare several treatments with one control treatment (Ljubuncic et al., 2005, Arredondo et al., 2004). A difference was considered statistically significant when $p < 0.05$. All statistical tests were completed using Minitab version 14.0 and Microsoft Excel. Linear regressions between the content of phenolics and data for the antioxidant assays were assessed.

Results

Antioxidant analysis:

FRAP assay:

In this study, the water phase antioxidant activity of plant extracts (hot and cold) produced from twenty three different Libyan plant species in comparison with the well established antioxidant properties of green tea have been investigated. As shown in Table 2 and Table 3 there were big differences in total antioxidant capacity for Ferric Reducing Antioxidant Power between the studied plants. The FRAP values varied from 748 to 40263 and 864 to 71010 (μmol Fe^{2+} E/g dried weight) for hot and cold water extracts, respectively. According to the comparison of reducing ability/antioxidant power of the green tea (standard antioxidant reference) with FRAP of the twenty three selected plant extracts, these plant extracts were divided into three groups (Table 4). Group (a) represented the plant extracts that shown high FRAP values as compared with the green tea (highly significant, p values ranged from less than 0.0001 to 0.05), group (b) moderate FRAP values (lower than the green tea), and group (c) low FRAP (lower than 1mmol Fe^{2+} E/g). Out of the twenty three selected plant extracts which were assayed for FRAP antioxidant activity, nine of the hot extracts and three of the cold extracts were found in the group of

high antioxidant (group a) (Table 4). The group with moderate activity was represented by *Teucrium polium> Hibiscus sabdariffa> Matricaria chamomilla> Marrubium vulgare> Artemisia absinthium> Phagnalon rupestre> Pituranthos tortousus> Ruta graveolens> Urtica urens> Zingiber officinale> Raphanus raphanistrum* for the hot extracts and *Hibiscus sabdariffa> Calendula officinalis> Salvia officinalis>Rosmarinus officinalis> Teucrium polium> Thymus vulgaris> Olea europaea> Origanum majorana> Marrubium vulgare> Phagnalon rupestre> Nerium oleander> Ruta graveolens> Artemisia absinthium> Pituranthos tortousus> Raphanus raphanistrum> Matricaria chamomilla> Zingiber officiale> Urtica urens* for the cold extracts. *Urginea maritima* and *Alhagi maurorum* represented the group with low antioxidant activity of both hot and cold water extracts (Table 4). Figure 2 and Table 5 illustrate the comparison of antioxidant activity for the hot and the cold water extracts assayed by the FRAP method.

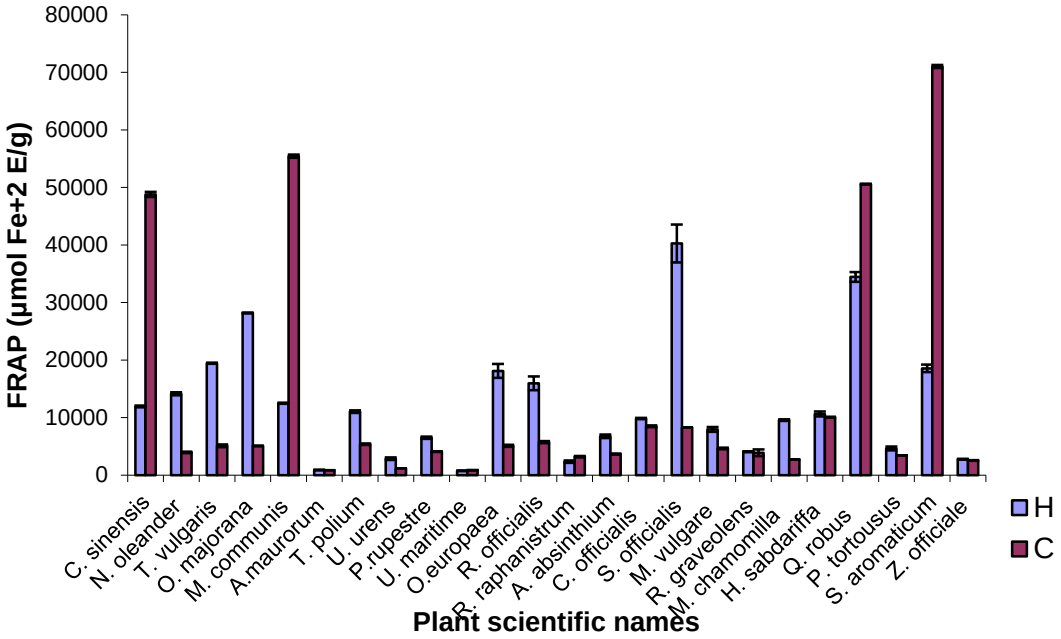

Figure 2 FRAP assay of hot and cold water extracts

Table 2. Water soluble antioxidant capacity and phenolic content of hot water extracts.

Scientific name	FRAP(µmol Fe^{2+} /g dried weigh	TEAC(mmolTroloxE/g dried weight)	DPPH(mmolTrolox E/g dried weight)	Phenolic content(mgGAE/ g dried weight)
Camellia sinensis	11933 ± 153	39.467 ± 0.208	19.433 ± 0.321	1385.9 ± 21.0
Nerium oleander (LINN.)	14150 ± 265[b]	21.133 ± 0.208	7.767 ± 0.208	1022.3 ± 30.5
Thymus vulgaris (LINN.)	19450 ± 100[a]	23.267 ± 0.058	10.267 ± 0.153	1101.5 ± 52.9
Origanum majorana (LINN.)	28183 ± 76[a]	21.500 ± 0.100	0.515 ± 0.005	1125.8 ± 3.8
Myrtus communis (LINN.)	12500 ± 100[d]	43.333 ± 0.252[a]	20.433 ± 0.058[d]	4456.0 ± 51.5[a]
Alhagi maurorum (MEDIK)	899 ± 35	1.100 ± 0.006	0.504 ± 0.007	303.2 ± 21.5
Teucrium polium (LINN.)	11050 ± 218	21.367 ± 0.252	5.867 ± 0.153	895.9 ± 30.7
Urtica urens (LINN.)	2867 ± 232	11.767 ± 0.058	0.399 ± 0.017	382.3 ± 14.9
Phagnalon rupestre (LINN.)	6500 ± 200	21.700 ± 0.004	0.510 ± 0.009	760.9 ± 41.8
Urginea maritima (LINN.)	748 ± 20	0.900 ± 0.002	0.464 ± 0.005	267.0 ± 10.4
Olea europaea	18117 ± 206[a]	22.200 ± 0.100	13.267 ± 0.115	1252.1 ± 8.9
Rosmarinus officinalis (LINN.)	15967 ± 126[a]	20.767 ± 0.153	12.900 ± 0.173	1123.9 ± 31.5
Raphanus raphanistrum (LINN.)	2350 ± 200	25.467 ± 0.058	0.360 ± 0.009	332.3 ± 17.6
Artemisia absinthium(LINN.)	6750 ± 304	23.333 ± 0.115	5.267 ± 0.115	711.8 ± 42.7
Calendula officinalis (LINN.)	9847 ± 104	18.300 ± 0.100	4.867 ± 0.058	996.6 ± 13.8
Salvia officinalis (LINN.)	40263 ± 3287	19.833 ± 0.115	19.533 ± 0.153	1327.9 ± 10.9
Marrubium vulgare (LINN.)	7947 ± 419	22.167 ± 0.115	3.833 ± 0.115	682.5 ± 14.2
Ruta graveolens (LINN.)	4073 ± 76	11.067 ± 0.058	0.704 ± 0.007	527.5 ± 28.8
Matricaria chamomilla (LINN.)	9563 ± 144	20.967 ± 0.115	4.967 ± 0.115	833.2 ± 21.8
Hibiscus sabdariffa (LINN.)	10630 ± 436	21.800 ± 0.100	5.200 ± 0.100	856.0 ± 26.2
Quercus robur	34447 ± 852[a]	22.167± .351	23.600 ± 0.520[c]	2472.7 ± 17.0[a]
Pituranthos tortousus	4640± 331	11.400 ± 0.006	0.902 ± 0.021	439.3 ± 17.7
Syzygium aromaticum	18560 ± 656[a]	25.167 ± 0.850	29.867 ± 0.153[a]	6937.5 ± 75.4[a]
Zingiber officinale	2773 ± 76	11.933 ± 0.058	0.367 ± 0.058	271.4 ± 2.9

Data expressed as mean ± SD, (n = 3)

[a] Extremely statistically Significantly higher than the green tea (the standardantioxidant), $P<0.0001$

[b] $P<0.001$

[c] $P<0.005$

[d] $P<0.01$

Scientific name	FRAP(µmol Fe^{2+}E/g dried weight)	TEAC(mmolTroloxE/g dried weight)	DPPH(mmolTrolx E/g dried weight)	Phenolic content(mgGAE/ g dried weight)
Camellia sinensis	48783 ± 439	35.852 ± 0.104	17.044 ± 0.069	3039.8 ± 8.9
Nerium oleander (LINN.)	3964 ± 126	10.1167 ± 0.058	3.156 ± 0.026	593.3 ± 32.1
Thymus vulgaris (LINN.)	5106 ± 238	10.833 ± 0.153	0.949 ± 0.021	704.2 ± 8.8
Origanum majorana (LINN.)	5078 ± 25	9.215 ± 0.072	0.859 ± 0.0199	960.5 ± 28.1
Myrtus communis (LINN.)	55433 ± 270 [a]	36.443 ± 0.060 [b]	19.047 ± 0.0398 [a]	3829.2 ± 38.2 [a]
Alhagi maurorum (MEDIK)	864 ± 8	1.085 ± 0.007	0.472 ± 0.007	312.4 ± 7.7
Teucrium polium (LINN.)	5389 ± 126	10.533 ± 0.058	0.227 ± 0.005	620.0 ± 5.1
Urtica urens (LINN.)	1186 ± 1	1.130 ± 0.004	0.365 ± 0.017	262.6 ± 29.1
Phagnalon rupestre (LINN.)	4086 ± 63	5.657 ± 0. 125	3.881 ± 0.117	678.3 ± 18.9
Urginea maritima (LINN.)	866 ± 14	1.000 ± 0.002	0.724 ± 0.004	270.9 ± 3.7
Olea europaea	5101 ± 176	6.133 ± 0.208	4.088 ± 0.052	765.7 ± 42.1
Rosmarinus officinalis (LINN.)	5751 ± 176	5.800 ± 0.200	0.517 ± 0.003	673.3 ± 21.4
Raphanus raphanistrum (LINN.)	3226 ± 126	2.100 ± 0.100	0.472 ± 0.007	353.1 ± 6.4
Artemisia absinthium(LINN.)	3659 ± 104	3.567 ± 0.058	0.897 ± 0.017	426.3 ± 26.0
Calendula officinalis (LINN.)	8502 ± 153	7.833 ± 2.08	0.573 ± 0.007	916.3 ± 11.9
Salvia officinalis (LINN.)	8286 ± 29	7.060 ± 0.053	0.811 ± 0.025	771.5 ± 29.6
Marrubium vulgare (LINN.)	4651 ± 144	3.467 ± 0.115	0.434 ± 0.009	494.1 ± 10.2
Ruta graveolens (LINN.)	3893 ± 588	4.500 ± 0.700	0.600 ± 0.009	519.9 ± 58.3
Matricaria chamomilla (LINN.)	2719 ± 14	3.397 ± 0.060	2.155 ± 0.052	610.3 ± 37.5
Hibiscus sabdariffa (LINN.)	10072 ± 76	8.486 ± 0.419	5.622 ± 0.150	806.3 ± 10.9
Quercus robur	50558 ± 75 [c]	36.443 ± 0.060 [b]	20.635 ± 0.242 [a]	3018.0 ± 38.4
Pituranthos tortousus	3448± 14	3.667 ± 0.231	0.462 ± 0.009	355.8 ± 3.4
Syzygium aromaticum	71010 ± 265 [a]	37.165 ± 0.104 [d]	20.497 ± 0.210 [a]	6361.1 ± 20.5 [a]

Zingiber officinale	2561 ± 52	2.053 ± 0.040	1.080 ± 0.007	248.0 ± 6.0

Table 3 Water soluble antioxidant capacity and phenolic content of cold water extracts.

Data expressed as mean ± SD, (n = 3)

[a] Extremely statistically Significantly higher than the green tea (the standard antioxidant), $P<0.0001$

[b] $P<0.001$

[c] $P<0.005$

[d] $P<0.05$

Table 4 Comparative Antioxidant Activities by various methods of selected Libyan medicinal plants**.**

Plant Scientific Name	Hot Water Extracts				Clod Water Extracts			
	FRAP	TEAC	DPPH	PC	FRAP	TEAC	DPPH	PC
Nerium oleander (LINN.)	+ + +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Thymus vulgaris (LINN.)	+ + +	+ +	+ +	+ +	+ +	+ +	+	+ +
Origanum majorana (LINN.)	+ + +	+ +	+	+ +	+ +	+ +	+	+ +
Myrtus communis (LINN.)	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Alhagi maurorum (MEDIK)	+	+	+	+	+	+	+	+
Teucrium polium (LINN.)	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +
Urtica urens (LINN.)	+ +	+ +	+	+	+ +	+	+	+
Phagnalon rupestre (LINN.)	+ +	+ +	+	+ +	+ +	+ +	+ +	+ +
Urginea maritima (LINN.)	+	+	+	+	+	+	+	+
Olea europaea	+ + +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Rosmarinus officinalis (LINN.)	+ + +	+ +	+ +	+ +	+ +	+ +	+	+ +
Raphanus raphanistrum (LINN.)	+ +	+ +	+	+	+ +	+ +	+	+
Artemisia absinthium(LINN.)	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +
Calendula officinalis (LINN.)	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +

Salvia officinalis (LINN.)	+ + +	+ +	+ + +	+ +	+ +	+ +	+	+ +
Marrubium vulgare (LINN.)	+ +	+ +	+ +	+ +	+ +	+ +	+	+ +
Ruta graveolens (LINN.)	+ +	+ +	+	+ +	+ +	+ +	+	+ +
Matricaria chamomilla (LINN.)	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Hibiscus sabdariffa (LINN.)	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Quercus robur	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
Pituranthos tortousus	+ +	+ +	+	+ +	+ +	+ +	+	+
Syzygium aromaticum	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Zingiber officinale	+ +	+ +	+	+	+ +	+ +	+	+

P C = Phenolic content.

+ + + = group a (significantly higher than the green tea)

+ + = group b (moderate group, lower than the green tea)

+ = group c (low antioxidant activity or phenolic content)

Table 5 Antioxidant activities shown by hot and cold water extracts selected Libyan medicinal plants.

Plant Scientific Name	FRAP	TEAC	DPPH	Phenolic Content
Camellia sinensis	C	H/C	H/C	C
Nerium oleander (LINN.)	H	H	H	H
Thymus vulgaris (LINN.)	H	H	H	H
Origanum majorana (LINN.)	H	H	C	H/C
Myrtus communis (LINN.)	C	H	H/C	H/C
Alhagi maurorum (MEDIK)	H/C	H/C	H/C	H/C
Teucrium polium (LINN.)	H	H	H	H
Urtica urens (LINN.)	H	H	H/C	H
Phagnalon rupestre (LINN.)	H	H	C	H/C
Urginea maritima (LINN.)	H/C	H/C	C	H/C
Olea europaea	H	H	H	H
Rosmarinus officinalis (LINN.)	H	H	H	H
Raphanus raphanistrum (LINN.)	C	H	C	H/C
Artemisia absinthium(LINN.)	H	H	H	H
Calendula officinalis (LINN.)	H	H	H	H/C

Salvia officinalis (LINN.)	H	H	H	H
Marrubium vulgare (LINN.)	H	H	H	H
Ruta graveolens (LINN.)	H/C	H	H/C	H/C
Matricaria chamomilla (LINN.)	H	H	H	H
Hibiscus sabdariffa (LINN.)	H/C	H	H/C	H/C
Quercus robur	C	C	H/C	C
Pituranthos tortousus	H	H	H	H
Syzygium aromaticum	C	C	H/C	H/C
Zingiber officinale	H/C	H	C	H/C

H = Hot water extract value is higher than the cold extract value.

C = Cold water extract value is higher than the hot extract value.

H/C = No differences between hot extract values and cold extract values

TEAC assay:

The water phase antioxidant activities of hot and cold extracts produced from 23 selected Libyan plants were studied in comparison with green tea, the established antioxidant reference. Measurement of antioxidant activity of these plant extracts by the TEAC assay showed a significant variation between studied species (Tables 2, 3 and 4). The low antioxidant activity included *Urginea maritima* and *Alhagi maurorum* of both hot and cold plant extracts and *Urtica urens* of cold plant extract. Interestingly, *Myrtus communis* (hot and cold extracts) and *Quercus*

robur and *Syzygium aromaticum* (cold plant extracts) exhibited high antioxidant potential compared to that of green tea (p value ranged between < 0.001 and < 0.05). Out of twenty three studied plant extracts a group of twenty (hot extracts) and seventeen (cold extracts) species were identified, with considerable (above 2 mmol) TEAC values ranging between 25.467 and 11.067 mmol T E, and between 10.833 and 2.053 mmol T E of hot and cold extracts respectively. In general, the TEAC values of cold plant extracts were lower than those of hot extracts (figure 3 and Table 5).

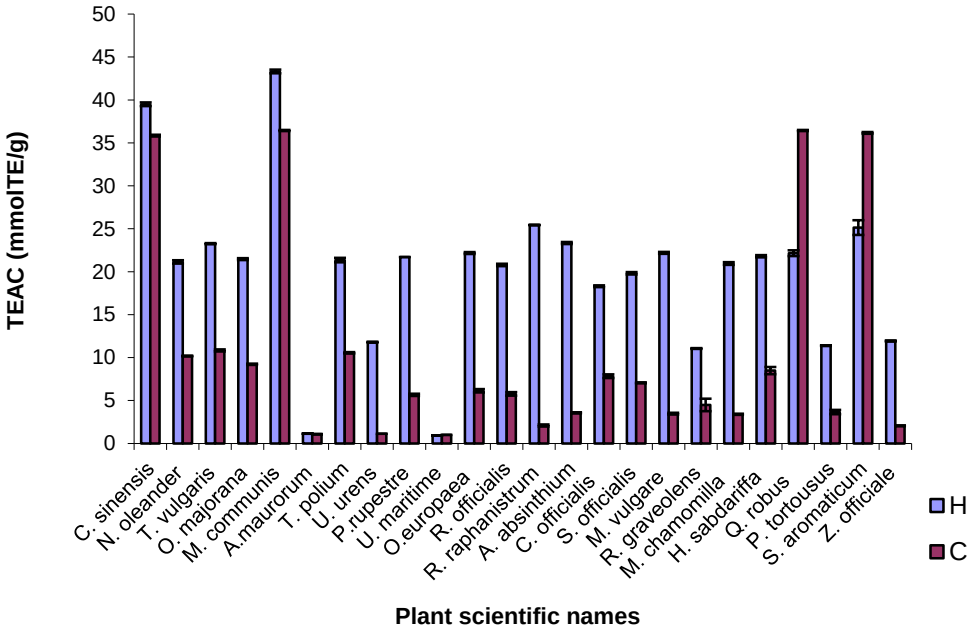

Figure 3 TEAC assay of hot and cold water extracts

DPPH˙ assay:

Results of DPPH reduction by hot and cold plant extracts are summarized in Tables 2 , 3 and Table 4. The results obtained show that the hot and cold extracts of *Myrtus communis*, *Quercus robur* and *Syzygium aromaticum* had a significant higher capacity of scavenging

activity against the DPPH free radical ($p < 0.01$, 0.005, and 0.0001 of hot extracts respectively, and < 0.0001 of cold extracts) than the green tea extract used as a standard (positive control). The hot extract of *Salvia officinalis* also showed activity against DPPH (19.533 mmol TE/ g) which was almost the same as those obtained from the green tea extract (19.433 mmol TE/ g). Nine hot and fifteen cold extracts exhibited low DPPH values below 2 mmol T E which is considered as the group with a slight scavenging activity against the DPPH free radical. The remaining plant extracts showed a moderate scavenging activity with values ranging from 13.267 to 3.833 mmol T E /g and 5.156 to 2.155 mmol T E /g for hot and cold extracts respectively, in the following decreasing order: *Olea europaea> Rosmarinus officinalis> Thymus vulgaris> Nerium oleander> Teucrium polium> Artemisia absinthium> Hibiscus sabdariffa> Matricaria chamomilla> Calendula officinalis> Marrubium vulgareh* (hot extracts) and *Hibiscus sabdariffa> Olea europaea> Phagnalon rupestre> Nerium oleander> Matricaria chamomilla* (cold extracts). Generally, in this assay, the antioxidant values of hot extracts exhibit higher capacity of scavenging activity against the DPPH free radical as compared with the cold extract capacity (figure 4 and Table 5).

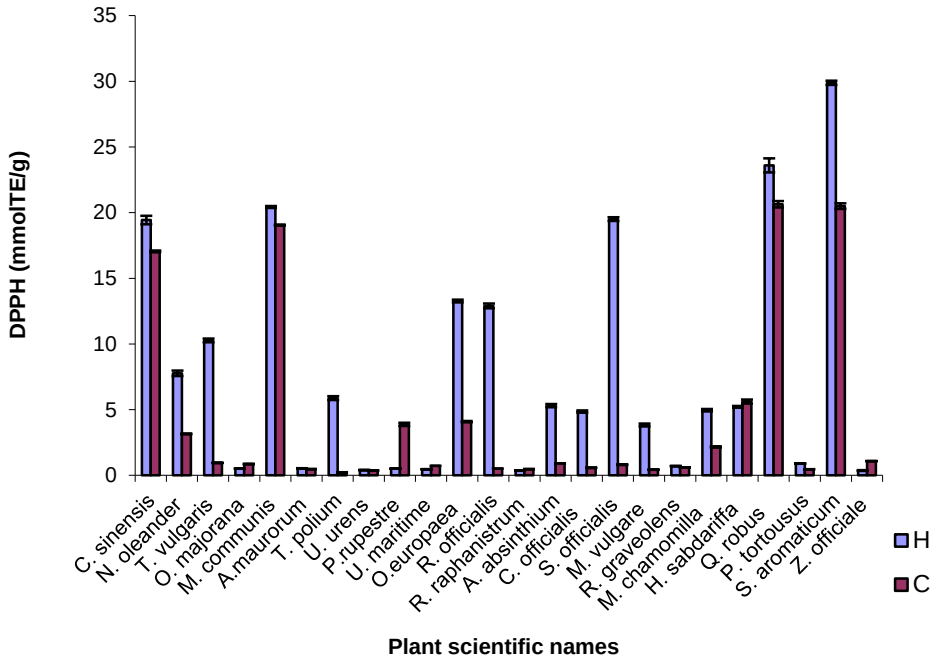

Figure 4 DPPH assay of hot and cold water extracts.

Phenolic content:

The total phenolic content of the hot and cold plant extracts investigated in this study varied widely from 6937.5 to 267.0 mg GAE/ g of dry material and 6361.1 to 248.0 mg GAE/ g of dry material for hot and cold extracts respectively (Table 2, 3, and 4). *Myrtus communis* and *Syzygium aromaticum* of hot and cold extracts and *Quercus robur* hot extract presented the highest total phenol content as compared with the green tea ($p < 0.0001$; figure 5 and Table 5). Correlation between the content of phenolic compounds and antioxidant activity was examined. Strong correlation between the total phenolic content and antioxidant capacity by FRAP assay was found in cold water extracts with correlation coefficient equal to 0.972 ($r^2 = 0.95$) (figure 6). As shown in figure 7 a positive relationship was also observed between total phenolic content and the TEAC assay result with correlation coefficient equal to 0.901 ($r^2 = 0.837$) for cold extracts. Weak correlation was found between total phenolic content and the FRAP and TEAC assays for hot extracts with correlation coefficient equal to 0.376 and 0.505 ($r^2 = 0.143$, $r^2 = 0.255$)

for FRAP and TEAC assays respectively. A positive correlation was obtained between the DPPH assay results and the total phenolic content in hot extracts with correlation coefficient equal to 0.813 (r^2 = 0.661). Cold extracts showed a strong correlation between the content of phenolic compounds and DPPH with correlation coefficient equal to 0.915 (r^2 = 0.837) (figure 8). In general, cold water extracts showed a stronger correlation between the phenolic content and total antioxidants for the three methods (FRAP, TEAC and DPPH) than hot water extracts.

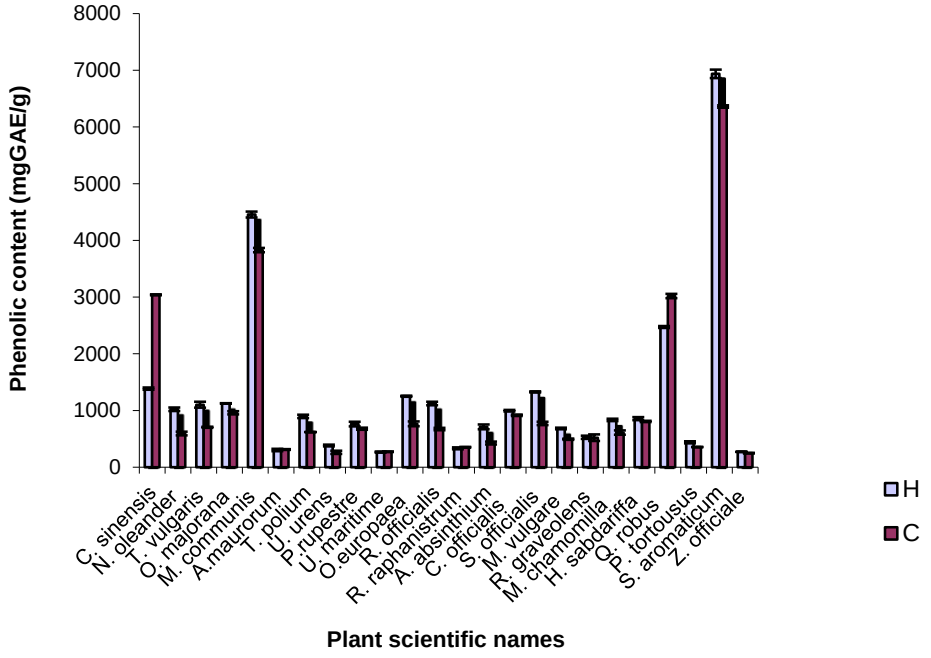

Figure 5 Phenolic content of hot and cold water extracts

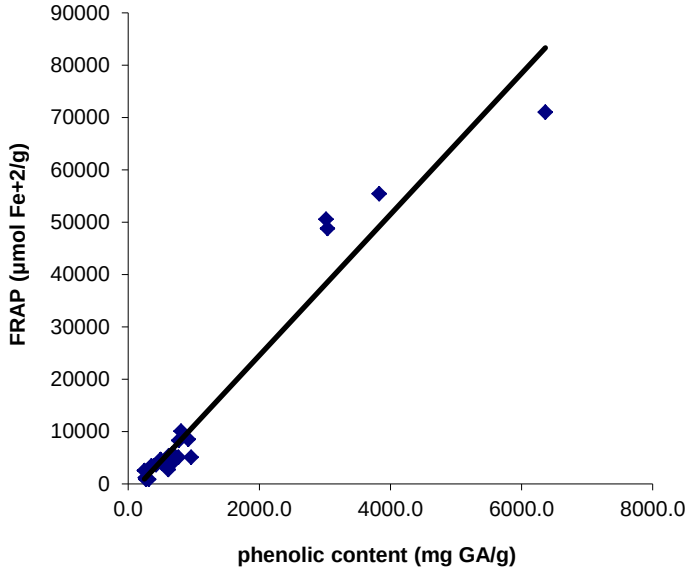

Figure 6 Correlation between phenolic content and FRAP for cold extracts.

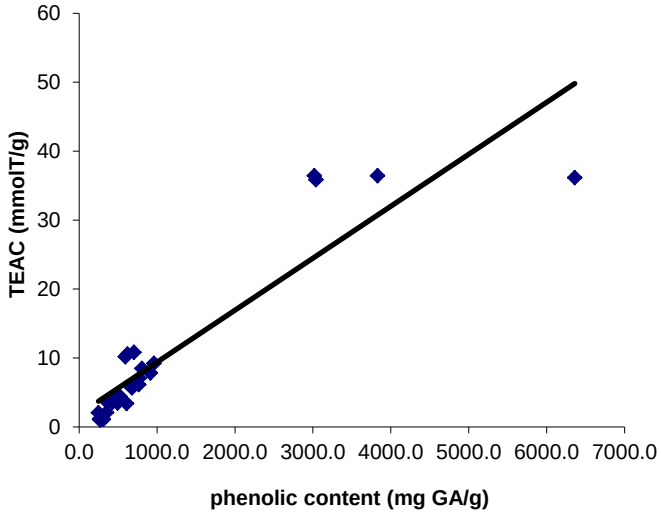

Figure 7 Correlation between phenolic content and TEAC for cold extracts

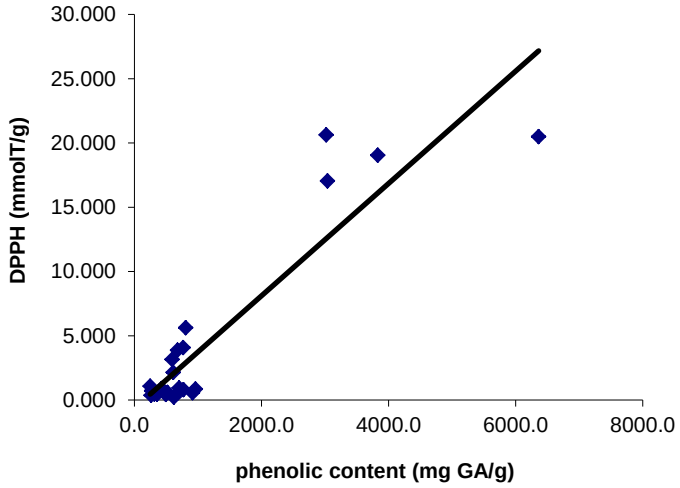

Figure 8 Correlation between phenolic content and DPPH for cold extracts

Discussion

In the present study twenty three medicinal plants were chosen native to the Libyan region traditionally used for the treatment of various disorders, where the free radicals are thought to be implicated. Based on the traditional way of ingestion of plant derived antioxidants it was appropriate to study the water-soluble antioxidant capacity and phenolic content of herbal teas prepared from Libyan medicinal plants. Plant extracts with

either hot (freshly boiled water) or cold (room temperature water) were prepared simply in ways that mimic the traditionally used methods in folk medicine (Triantaphyllou et al., 2001). The extracts from these plants were screened for their antioxidant activity using the three most popular methods (FRAP, TEAC and DPPH). The results show that out of these twenty three extracts, the crude extracts (hot and cold) of *M. communis*, *Q. robur* and *S. aromaticum* exhibited the strongest antioxidant activity, in all tests used in this study (FRAP, TEAC and DPPH), and were higher than that of the positive control, green tea. Indeed, this suggests that for

these extracts any of these methods will offer a reliable measurement of antioxidant status. Similar activities of *M. communis* extracts have been previously assessed by the linoleic acid assay (Rosa et al., 2003, Souri et al., 2004), the β-carotene/ linoleic acid system and the DPPH free radical scavenging assay (Yadegarinia et al., 2006, Hayder et al., 2004). The results presented here were consistent with these reports. To the best of the knowledge of the author, no previous reports have dealt with *M. communis* antioxidant activity by FRAP and TEAC assays. The results obtained from *S. aromaticum* extracts in this study exhibited a high antioxidant activity which confirmed the existing literature using different antioxidant assays. *S. aromaticum* demonstrated a good degree of antioxidant capacity tested using the thiobarbituric acid reactive substance (TBARS) and the β-carotene agar assays (Lean and Mohamed, 1999, Dorman et al., 2000), the lipid / MA and aldehyde/ carboxylic assays (Lee, 2001); reductive potential, superoxide anion radical scavenging (Gulcin et al., 2004), TEAC (Juliani et al., 2004) and DPPH free radical scavenging (Owen and Johns, 2002, Gulcin et al., 2004, Nassar, 2006). No data have been found in the literature for the FRAP assay. Despite few reports in the literature regarding antioxidant activity of *Q. robur* bark (Andrensek et al., 2004), only one recent report has dealt with *Q. robur* fruit as a convenient nutritional source with antioxidant effects (Rakic et al., 2006).

The results show that, in the experimental conditions described, most of the extracts possess moderate antioxidant capacity (especially the cold extracts). Their behaviour differed according to the type of substrate in the assay and the temperature used for extraction. Accordingly, the differences in the activity of various extracts (the cold and the hot) can be explained by the loss of certain phytoconstituents (mainly polyphenols),

probably because of the difference in temperature of the extraction procedures (Velioglu et al., 1998, Gazzani et al., 1998, Sun and Ho, 2005). In contrast, Zhou et al. (2000) and Katalinic et al. (2006) have suggested that a higher temperature promotes extraction of polyphenols and consequently free radical scavenging ability. On the other hand, at a higher temperature certain amounts of polyphenols may react with other components to produce an insoluble complex or may be oxidized (Vijayakumari et al., 1995). Preparation of plant inffusions with hot (100^{o}C) and cold (25^{o}C) water revealed that although antioxidants were liberated from leaves into the water at both the temperatures studied, infusions of hot water had higher antioxidant capacities as determined with FRAP. This notion was proposed when *Melissae folium* inffusions were tested for their antioxidant capacity at different effusion temperatures by FRAP, TEAC and DPPH free radical scavenging (Katalinic et al., 2006).

In this study, there was good agreement between the antioxidant activity (especially for FRAP assay) and the extraction method used in folklore for most plant species. For instance, *N. oleander, M. chamomilla, T. vulgaris, S. officinalis* and *O. europaea* were suggested to be used as a hot infusion only in the traditional uses (El Gadi, 1992). Our results showed a higher antioxidant activity for those plants as hot extracts. Similarly, for those suggested to be used as cold infusions, such as *Q. robur* and *S. aromaticum*, the highest activities have been found in the cold extracts.

Plant phenolics comprise one of the major groups of compounds of primary antioxidant or free radical terminators (Gao et al., 2000). In addition, numerous studies have suggested that the potent antioxidant

activities of green tea (Rechner et al., 2002, Campanella et al., 2003) are due largely to its polyphenolic content. There are no publications on phenolic content and related antioxidant properties of the medicinal plants traditionally used in Libya; therefore, it was reasonable to determine their total phenolic content in the selected plant extracts. Phenolic content for these plant extracts were assayed by the Folin-Ciocalteu (F-C) method (Duan et al., 2006). The F-C method is actually not an antioxidant test but instead an assay for the quantity of oxidizable substances, i.e. phenolic compounds (Wangensteen et al., 2004). Again, *M. communis*, *Q. robur* and *S. aromaticum* showed the highest phenolic content among the examined extracts. The report by Romani et al. (2004) has offered quantitative and qualitative details of *M. communis* polyphenol composition. Interestingly, they found that *M. communis* had the same main polyphenols that exist in green tea, such as galloyl derivatives, and this explained the high antioxidant capacity of *M. communis.* In addition, Abdel-Wahhab and Aly (2005) have demonstrated that the antioxidant capacity of *S. aromaticum* is due in part to the contribution of aromatic chemicals such as eugenol and eugenol acetate.

The values of FRAP, TEAC and DPPH in the crude extracts (the cold) were highly correlated with the content of total phenolics. In the results shown here the higher radical scavenging activity of the polar extracts confirms that the phenolic compounds are likely to contribute to the radical scavenging activity of these plants. According to Tepe et al. (2004) the presence of polar phenolics in the extracts is considered as an important factor in the free radical scavenging activity. These results from this experiment are in agreement with the literature (Katalinic et al., 2006, Duan et al., 2006, Sun and Ho, 2005), which found a good correlation between antioxidant capacity (by FRAP, TEAC and DPPH)

and phenolic contents. Furthermore, the correlation between total phenolic and ORAC values was previously reported by Velioglu et al (1998). In contrast, the previous report by Kahkonen et al. (1999) and Velioglu et al. (1998) did not show any correlation between total phenolic content and oxidation with the Mello assay. The difference between the results shown in this study and reports in the literatures could be explained by the difference in the procedure of the assays used. There are many investigations of antioxidant activity of
medicinal plant extracts (Mantle et al., 1998, Mantle et al., 2000b, Souri et al., 2004, Chanwitheesuk et al., 2005, Ivanova et al., 2005, Katalinic et al., 2006, Bouzouta et al., 2003, Navarro et al., 2003, Yingming et al., 2004, Tepe et al., 2005b, Tepe et al., 2005a, Tepe et al., 2006, Kaur and Kapoor, 2002, Dapkevicius et al., 1998, Miliauskas et al., 2004a, Miliauskas et al., 2004b). However, it is difficult to compare the results of these studies unless they are based on the same assay and extraction procedures.

Antioxidant capacity estimation is assay dependent. The specificity and sensitivity of one method does not lead to complete examination of all phenolic compounds and antioxidants in the extracts. Therefore, a combination of several methods *in vitro* could provide a more reliable assessment of antioxidant activity. The FRAP assay is a method of antioxidant activity evaluation based on redox- reactions. It is quick and simple to perform and it is a reasonable screen for the ability to maintain redox status in cells or tissues. Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols (Zaporozhets et al., 2004). The FRAP mechanism measures electron transfer. However, it cannot detect compounds that act by radical quenching (hydrogen transfer), such as thiols and proteins (Prior et al.,

2005). Thus it is helpful to use FRAP in combination with other methods. The TEAC assay gained popularity because it enables high-throughput screening on potential antioxidant capacity (Van den Berg et al., 1999, Re et al., 1999). This assay assesses the total radical scavenging capacity, based on the ability of a compound to scavenge the stable ABTS radical (Arts et al., 2004a, Arts et al., 2003, Arts et al., 2004b). The extracts that showed high TEAC values could contain substances that have a redox potential lower than that of ABTS˙. Indeed, many phenolic compounds have low redox potential and can thus react with ABTS˙ (Frankel et al 2000; Prior et al 2005). The DPPH˙ system is a stable radical generating procedure. It can be used to assay a large number of samples in a short period of time, and is sensitive enough to detect active compounds even at low concentrations and was used in the present study. In the DPPH˙ assay hydrogen –donating ability is an index of the primary chain-breaking antioxidant. These antioxidants donate hydrogen to free radicals which are then converted to non-radical species and thus inhibit the propagation phase of lipid peroxidation (Koleva et al., 2002, Apati et al., 2003). Consequently, the extracts that showed strong or remarkable activity against DPPH˙ free radical scavenging could have substances rich in available hydroxyl groups such as flavonoids.

The results of the present work suggested that the efficacy of these plants could be explained, at least in part, by their antioxidant activity. These plants, rich in polar phenols, could be a good source of compounds that would help to increase overall antioxidant capacity of an organism and protect it against lipid peroxidation induced by oxidative stress.

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الخلاصة

إن استخدام النباتات الطبية كان علي مدي العصور المختلفة ذو أهمية كبيرة في علاج العديد من أمراض الإنسان في مختلف أنحاء العالم ولازال يعد من أهم المصادر للحصول علي العديد من المركبات الحيوية المهمة. و تعد ليبيا من بين الدول التي يسود فيها استخدام هذه النباتات بشكل واسع . وفى حين أن بعض النباتات كنبات *G.biloba* قد تم دراسته بشكل وافى إلا أن هناك العديد من النباتات الأخرى التي تحتاج إلى الدراسات لمعرفة ما تحتويه من مواد نشطة.

وقد تم فى هذا البحث دراسة ثلاثة وعشرون نوعاً من النباتات الطبية الليبية لإيجاد محتواها من المركبات المضادة للأكسدة والفينول . المستخلصات المائية لهذه النباتات تم فحصها لتحيد نشاطها المضاد للأكسدة وذلك باستخدام ثلاثة طرق وهى DPPH , TEAC , FRAP .وتعد هذه الطرق ذات كفاءة عالية لتحيد مضادات الأكسدة وقد دلت النتائج على أن من بين الثلاثة والعشرون نباتاً أن المستخلص البارد والساخن لنباتات

Myrtus communis, Quercus robur و*Syzygium aromaticum* أظهرت الأكثر نشاطاً فى محتواها المضاد للأكسدة فى جميع الاختبارات كما أثبتت تفوقها على الشاي الأخضر المستخدم كمعامل للسيطرة للاختبار . ويعتقد أن فعالية هذه النباتات يمكن إرجاعها جزئياً على الأقل الى نشاطها المضاد للأكسدة .