Total Phenolic Contents and Antioxidant Activity of some Herbs Traditionally used in Libya as Food Flavoring

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ABSTRACT

There is currently an upsurge of interest in phytochemicals as new sources of natural antioxidants to use them in foods and pharmaceutical preparations in order to replace synthetic antioxidants, which are being restricted due to their potential health risks and toxicity. Total equivalent antioxidant capacities (TEAC) and phenolic contents of four herbs extracts from tow botanical families (Lamiaceae and Caryophyllaceae) grown in Libya were investigated and compared to green tea. The total antioxidant capacity was estimated by the DPPH˙ (1, 1-diphenyl-2-picrylhydrazyl radical) and expressed as TEAC. Results revealed that the total phenolic content as measured using a Folin–Ciocalteu assay, ranged from 29.24to 79.34 mg of gallic acid equivalents (GAE)/ g (dw) while the green tea contained 116.33mg (GAE)/g (dw) as a control. The highest total phenolic content was observed in *Mentha spicata* L. followed by *R. officinalis L.*, *T. capitatus L.*, (79.34 , 51.67 and 49.70 mg (GAE)/ g (dw) respectively), while the lowest content was found in *P. arabica L.* (29.24 mg GAE/ g (dw). The highest TEAC value (µmol TE/g extract) was obtained from *M. spicata L.* (105 µmol trolox /g (dw).While the lowest value was observed from *P. arabica L.* (32.10). Data also revealed that the positive linear correlation between the antioxidant activity and total phenolic content in methanolic extract (R^2 = 0.9449) for all studied herbs. All extracts exhibited antioxidant activity descending in the following order *C. sinesis L.* > *M. spicata L.* > *R. officinalis L* > *T. capitatus L.* and *P. arabica L.* Based on these obtained results that two herbs, namely, *M. spicata L.* and *R. officinalis L.*, were identified as sources of free radical-scavenging compounds. As they contain good sources of phenolic compounds which used in different applications to preserve foods.

Keywords: Herbs, Total phenolic compounds, Antioxidant activity, DPPH˙ (1, 1-diphenyl-2-picrylhydrazyl radical).

1-INTRODUCTION

Free radicals and other reactive oxygen species (ROS) are generated in living organisms through many pathways. Accumulation of ROS in aerobic organisms is known as an exacerbating factor in cellular injury and the aging process. Free radicals can cause lipid peroxidation in foods which leads to the deterioration of the food. The oxidative deterioration of the lipid-containing food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the

nutritional quality and safety of foods, due to the formation of secondary, potentially toxic compounds. Recently, there is an increasing interest in finding natural antioxidants from plants because they can protect the human body from the attack of free radicals and retarding the lipid oxidative rancidity in foods (Chan *et al.*, 2007). Synthetic antioxidants such as *tert*-butylhydroxytoluene, *tert*-butylhydroxyanisole, and *tert*-butylhydroquinone (TBHQ) have been widely used to retard lipid oxidation in foods (Ahmad, 1996). However, such synthetic antioxidants are not preferred due to toxicological concerns. For this reason, there have been increasing interests in identifying plant extracts to minimize/retard lipid oxidation in lipid-based food products (Ahn, *et al.*, 1998). Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and ant carcinogens (Dillard and German, 2000). Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs (Chan *et al.*, 2007). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, antiinflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential (Aaby *et al.*, 2004; Luo *et al.*, 2004). Herbs and spices are used in many domains, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics (Djeridane *et al.*, 2006). The results show that rosemary, Thyme, Spearmint, sage and others belonging to the Labiatae family, exhibit antioxidant properties (Shan *et al.*, 2005). A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported (Ivanova *et al.*, 2005). Herode *et al.* (2003) indicted that methanol is a widely used and effective solvent for extraction of antioxidants and percentage of extraction yields will increase with the particle size of sample, temperature extraction and the ratio of solvent and sample extraction. On the other hand, methanol extracts showed the highest antioxidant and antimicrobial activities in seabuckthorn seeds which were extracted with chloroform, ethyl acetate, acetone and methanol (Negi *et al.*, 2005). Also, Mariya *et al.*, (2006) the percentage of methanol had a vital role in the extraction. It was observed that 80 to90% of the methanol gave high yield when compared to other mixture. Phenolic compounds dissolves in water by using the solvent in water and the extractability was also high.

The quality of natural extracts and their antioxidative performances depends not only on the quality of the original plant, the geographic origin, climatic condition, harvesting date and storage but also environ- mental and technological factors affect the activities of antioxidants from residual sources(Hagerman *et al.*, 1998). Green tea and black tea leaves are

obtained from dried leaves of *Camellia sinensis* Linn. belonging to the family Theaceae. Steaming or drying fresh tea leaves at elevated temperature makes the commercial green tea. Its chemical composition is similar to that of fresh tea leaves. Green tea contains polyphenols, which include flavonols, flavandiols, flavonoid and phenolic acids; these compounds may account for up to 30% of the dry weight (Khalaf *et al.*, 2008).

Several methods are available to evaluate antioxidant activities of natural compounds in foods or biological systems. Two methods commonly used in antioxidant activity assays are the DPPH and ABTS procedures, which use 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) as free radical generators, respectively. The mechanisms of both methods are similar, in that the absorption spectra of the stable, free radical changes when the molecule is reduced by an antioxidant or a free radical species. (Arnao, 2000).*Mentha spicata*, which is known locally in Libya as "Na'na'", is added to foods as a flavour; moreover, its decoction is used as a sedative, antirheumatic, and to relieve spasms and flatulence.*Paronychia arabica L.* plant grows naturally in the Al-jabal Alaktar which has a local name in Libya (Teamt alarnb) and belonging to Caryophyllaceae family and used in folk medicine. *Thymus capitatus L.*, which has a local name in Libya (Zaater) and used in folk medicine. *Rosmarinus officinalis* L. which has a local name in Libya (Al-jabal Alaktar) and used in flavouring foods and folk medicine. These plants grow naturally in the Al-jabal Alaktar in the east of Libya (El-Gadi, 1989; El-Darier and El-Mogaspi, 2009).

No earlier studies were carried out therefore; the aim of this study was to screen methanolic extracts of these plant species in Libya, with respect to their total phenolic content and their potential to scavenge the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical comparing with green tea as potential sources of natural antioxidants. And to evaluate the relationship between the antioxidative activity and total phenolic content in studied plants.

2-MATERIALS AND METHODS

2.1. Plant materials

Herbs used in this work were collected from their native land (El-Jabal El-Akhdar region, Libya) during the month of May 2009 as being the most herbs used in that region as folk medicine and is shown in Table(1).

Table (1): Scientific, family, common names, and the portions of herbs used

Scientific name of herbs	Family	Common name	Parts used
Rosmarinus officinalis L.	Lamiaceae (Labiatae)	Rosemary	leaf
Thymus capitatus L.	Lamiaceae (Labiatae)	Thyme	leaf and branch
Mentha spicata L.,	Lamiaceae (Labiatae)	Spearmint	leaf and branch
Paronychia arabica L.	Caryophyllaceae	Teamt alarnb	leaf and branch
Camellia sinensis L.	Theaceae	Green tea	leaf

Green tea was obtained from a local supermarket, Libya.
The samples were cleaned, grinded and sieved through 60-mesh sieve, and stored in refrigerator at 4C for further treatments and /or analysis.

2.2. Chemicals and reagents

1, 1-Diphenyl-2-picrylhydrazyl radical, Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-Carboxylic acid), methanol, Sodium carbonate anhydrous, Folin-Ciocalteus phenol reagent, Gallic acid (3, 4, 5 Trihydroxy Benzoic acid), were purchased from Sigma Chemical Co.

2.3. Yield extracts.

Phenolic compounds were extracted according to the method described by (Adegoke and Gopala, 1998).The powder of each dried samples (100g each) was extracted using 80% aqueous methanol 500 ml with constant stirring for 24hours at room temperature(25C°±2_). The extracts were filtered with whatmann No.1 filter paper. The filtered material was re-extracted to maximize the concentration of the extract. The filtrates were

evaporator under vacuum in a rotary evaporator at 45C and lyophilized and weighed to determine the extracted yield for each plant material.

2.4. Preparation of plant extracts for antioxidant activity and total polyphenol content.

Ground dry plant materials (1 g) were weighed into a test tube. A total of 10 ml of 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonicated twice for 15 min (Sonicator Jalabo, USR3) and then left at room temperature (20°C ±2_) for 24 h. The extract was centrifuged for 10 min (1500g), and supernatants were collected at 4 C° prior to use within 24 h.

2.5. Determination of total polyphenol content

Total polyphenol content was determined using Folin–Ciocalteu colorimetric method as described by Gao *et al.*, (2000). Plant extracts (100 µL) were mixed with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of H_2O, and incubated at room temperature for 3 min. 1 ml of 20% sodium carbonate was added to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with a UV–Visible spectrophotometer Milton Roy, Spectronic 601. The results were expressed as gallic acid equivalents (GAE), milligrammes per g of dry weight (DW). All determinations were performed in triplicate (n = 3).

2.6. Free radical-scavenging activity by using of a stable DPPH radical

The radical scavenging activity was measured using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox (2.5 mM in methanol) as reference substance according to (Chizzola *et al.*, 2008; Gao *et al.*, 2000). The presence of antioxidative active substances in the assay leads to the reductive decoloration of the DPPH radical. Depending on the content of antioxidative active substances, 50-400 µL of the methanolic extract sample was adjusted to 1 mL with 50% methanol and then added to 1 mL of DPPH reagent (7.5 mg in 50 mL of methanol). After 0.5 h in the dark at room temperature, the absorbance was measured against a blank at 515 nm with a UV–Visible spectrophotometer Milton Roy, Spectronic 601. The blank was a solution where 500 µL of Trolox and 500 µL of methanol reacted with 1 mL of DPPH reagent to obtain the complete decoloration of that radical. For the calibration curve 6-50 µg of Trolox in 1 mL of methanol was used.

2.7. Statistical analysis

All analyses were run in triplicates. Data were analyzed by an analysis of variance (ANOVA) ($P<0.05$) and the means separated by Duncan's multiple range test using Statistical Analysis System 6.21 package (SAS 1995).

3.RESULTS AND DISCUSSION

3.1. Extracted yield

Methanolic extracted yields (%) of the different herbs and the ratio of methanolic extracted yield of herbs to methanolic extract of green tea) is shown in Table (2). Data revealed that the methanolic extracted yields (%) for spearmint, rosemary, thyme and Teamt alarnb were 18.73%, 17.87%, 10.83%, 10.83%, respectively. Nevertheless the extracted yield from the green tea (21.97%) was higher than any extracted yields of the studied herbs. Green tea leaves contain a high level of catechins (flavanols and flavanols gallates). Catechins and other polyphenols in tea exhibit powerful antioxidant activities (Chan *et al.*, 2007). The polyphenols are the most biologically active group of the tea components, especially certain catechins.

Data of Table (2) also showed that the ratios of extracted yield for spearmint and rosemary to the extracted yield of green tea were higher than 80%, while the ratios of extracted yield for thyme and *Paronychia arabica* L. were lower than 50%. These results for both plant extracts are in accordance with literature data reported by (Shan *et al.*, 2005), except Teamt alarnb there are no previous studies. It has been reported that the extracted yields (%) of Rosemary was 16.4% (Tsai *et al.*, 2008); the extracted yields (%) of green tea ranged from 21.6 to 22.4% (Tsai *et al.*, 2008 and Khalaf, 2008); the extracted yields (%) of spearmint was 17.3% (Souri *et al.*, 2008). However, EL-Agbar *et al.* (2008) found that the extracted yield (%) of thyme was 5.11% and which was less than found in the present study (10.83%). This difference could be attributed to the extraction method employed and the genotypic differences herbs (Papageorgiou *et al.*, 2008). Taking into consideration the industrial requirements for extraction, both yields and economic parameters were primarily emphasized before detailed study of their antioxidant potential. Different solvent systems have been used for the extraction of polyphenols from plant material (Pinelo *et al.*, 2004). Extraction yield is dependent on the solvent and the method of extraction. The extraction method must allow

complete extraction of the compounds of interest, and it must avoid their chemical modification (Goli *et al.*, 2004).

Table (2): Extracted yield (%) of different herbs as compared to green tea

Common name of herbs	Methanolic extracted yield (%)	ratio extracted yield of herb : extracted yield of green tea
Rosemary.	17.87[c]	81%
Thyme.	10.83[d]	49%
spearmint	18.73[b]	85%
Teamt alarnb	10.83[d]	49%
Green tea.	21.97[a]	100%

Means with different letters within a column are significantly different ($P < 0.05$).

3.2. Total phenolic content

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans *et al.*, 1996). As shown in Table (3), there was a large and significant ($p > 0.05$) variation in the total phenolic content of the herbs ranging from 29.24 to 79.34 mg GAE/g dry weight as compared to the methanolic extract of green tea (116.33 mg of GAE/g). The highest levels of phenolics were found in spearmint, rosemary and thyme (79.34, 51.67, 49.70 mg of GAE/g, respectively. However, no significant differences were found among these herbs ($P < 0.05$). Nevertheless, the lowest content was found in Teamt alarnb (29.24mgGAE/g). Significant differences between the results were likely due to genotopic and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested, time of taking samples and determination methods (Kim *et al.*, 2005; Shan *et al.*, 2005). Green tea is known to have a high content of polyphenolics (Zheng, and Wang, 2001; Shahidi, 2000). Generally, the higher contents of total phenolic in green tea might be contributed to the

presence of catechins such as catechin, gallocatechin, gallocatechin gallate, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Yen and Chen, 1995). The value obtained in this work for green tea was similar to that reported for different brands commercialized in Malaysia. Chan *et al.*, (2007) they showed % GAE values of 11.37and 19.13 for green tea. The methanolic extracts for spearmint, rosemary and thyme contained high levels of phenolics (79.34, 51.67, 49.7 mgGAE/g, respectively) and no significant association between the total phenolic contents. Shan *et al.*, (2005) reported that spearmint, Rosemary and thyme Plants belonging to Lamiaceae (Labiatae) family. Among 12 families tested in that study, Myrtaceae (with only one tested species, i.e., clove), Lauraceae (three tested species), and Labiatae (six tested species, respectively) showed high mean antioxidant capacity (168.7, 67.9, and 48.6 mmol/100 g, respectively) and contained high levels of phenolics (14.38, 7.47, and 5.65 g of GAE/100 g, respectively).

The present study revealed that all herb extracts contained high amounts of phenolics with values comparable to those reported by Shan *et al.*, (2005) for methanolic extracts of rosemary and thyme as (5.07 ± 0.036, 4.52 ±0.006 g of GAE/ 100 g, respectively. For The methanolic extract content of spearmint was 84±7 mg GAE/g (Hosseinimehr *et al.*, 2007). Dorman *et al.* (2003) reported that the total phenolic content in water spearmint extract was 214.0 mgGAE/g. Tawaha *et al.*, (2007) evaluated the total phenolic from the Rosemary and spearmint and the results were 39.1, 39.1mg GAE/g dry weight), respectively. (Wojdylo *et al.*, 2007) found that total phenols in methanolic extracts of Rosemary and thyme 1.71± 0.02, 0.58± 0.02 of GAE/100 g of dry weight (dw). Different levels reported in these studies may be attributed to the choice of parts tested, drying and extraction technique employed, methods of analysis applied, time and location of sampling, and genotypic differences (Papageorgiou *et al.*, 2008) or may be due to the differences of the solvents used for extraction and the growing conditions of the plants (Ünver *et al.*, 2009).The methanolic extracts for Teamt alamb contained lowest level of phenolics (29.241mgGAE/g dw) when compared with other extracts in this study. Only (Tawaha *et al.*, 2007) noted that the total phenols in the *Paronychia argentea* Lam., which belongs to the same family, had a value (15.5 mg GAE/g dry weight).

Table (3): Total phenolic content (mg of GAE/g dw)of different herbs

common name of herbs	Total phenolic mg of GAE/g of dry weight (dw)
Rosemary	**51.67[b]**
Thyme	**49.7[b]**
spearmint	**79.34[b]**
Teamt alarnb	**29.24[c]**
Green tea	**116.33[a]**

[a–e] Data with different superscripts are significantly ($p < 0.05$) different.

3.3. Antioxidant activity of methanolic extracts.

The TEAC values for investigated methanolic extracts of herbs are shown in Figure. (1). the total antioxidant activity as measured by the DPPH method ranged from 32.1 to 105.0 (µmol trolox /g dw) for all herbs extracts. Methanolic extract of green tea, however, was 207 µmol trolox /g. The antioxidant activities of Teamt alarnb showed the lowest content 32.1 µmol trolox /g. There were significantly differences among results at $P<0.05$. All extracts exhibited antioxidant activity but were less than green tea and decreased in the following order: spearmint > rosemary > thyme and *P. arabica L.* which were not statistically significantly different. These differences may be due to their different phenolic contents in these extracts. These results basically coincided with those of total phenolic compounds of the herbs. In other words, the plant extract samples that had high phenolic content showed tendency to have high antioxidant activity as noted by the many references (Shan *et al.*, 2005; Cai *et al.*, 2004). On the other hand, data revealed that there was a relationship between total antioxidant capacity (Y) and phenolic content (X) as is evident in Figure(2) and was established as an equation ($Y=2.1138X - 50.63$) and showed a highly significant linear correlation ($R^2 = 0.9449$). Such high R^2 value suggested that the DPPH˙ radical scavenging activity could be credibly predicted on the basis of the Folin-Ciocalteu assay for total phenolic content and directly confirmed that the phenolic compounds in the 5

extracts were responsible for their antioxidant capacity. The results emphasized the importance of phenolic compounds in the antioxidant behavior of herbs extracts and also indicated that the phenolic compounds contributed significantly to the total antioxidant capacity. The relationships between total phenolic content and antioxidant properties of many plants (e.g., common vegetables, fruits, and medicinal herbs) were investigated in previous studies (Cai *et al.*, 2004; Dorman *et al.*, 2004; Dorman *et al.*, 2003). A high correlation between free radical scavenging and the phenolic content has also been reported for cereals (Peterson *et al.*, 2001; fruits Gao *et al.*, 2000; Jime´nez-Escrig *et al.*, 2001), beverages (Fogliano, *et al.*, 1999), and culinary herbs (Zheng *et al.*, 2001).The antioxidant activity of green tea (207uM trolox equivalents per gram dry weight) was high compared to other plants in this study. This is probably because green tea contains a high proportion of phenolic compounds suggesting that antioxidant activity of green tea leaf extract is due to its proton donating ability.

The antioxidant activity of herbs has been reported before (Anesini *et al.*, 2008; Khlaf *et al.*, 2008; Kumar *et al.*, 2008). The same results using different tests on plant materials were also observed in some other studies Aqil *et al.*, (2006) and Hajimahmoodi *et al.*, (2008). The antioxidant activity for the present studied herbs such as spearmint, rosemary, thyme and *Paronychia arabica L.* were less than that for green tea but moderate antioxidant activity against DPPH free radical. Methanolic extract of the spearmint had antioxidant activity (105uM trolox / g dw). They were also higher than that of rosemary and Thyme (59.63uM trolox/ g dw). Shan *et al.*, (2005) reported that the major type of phenolic compounds in mint were phenolic acids (caffeic acid, rosmarinic acid), volatile compounds (menthol), flavonoids. Kanatt *et al.*, (2007) reported that the extracts of *Mentha* species contained bound phenolic acids and flavonoids. The major phenolic acids reported in water-soluble spearmint, extract were eriocitrin, luteolin glucoside, rosmarinic acid and caffeic acid (Dorman *et al.*, 2003).

Rosemary contains a large number of compounds responsible for its antioxidant activity, such as rosmarinic acid, carnosic acid, and carnosol; the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of the rosemary extracts is related to the amount of rosmarinic acid (Celiktas *et al.*, 2007; Dorman *et al.*, 2003). The lowest levels of antioxidant activity were obtained from the methanolic extract of thyme (32.91 umol TE/g) and the methanolic extract of *P. arabica L.* (32.10umol TE/g dry weight) and the difference was not significantly at $P<0.05$. Literature review shows the presence of different phenolic compounds such as rosmarinic acid, apigenin, luteolin, caffeic acid, Ferulic acid, carnosic acid, and flavonoids in

the Thymus family Plants (Jordan *et al.*, 2009; Loziene *et al.*, 2007). Various compounds with antioxidant properties have also been described in thyme. They include components of the essential oil, phenolic acids and flavonoids. caffeic, syringic, and genistic acid were the main phenolic acids, and luteolin was the main flavonoid in thyme of Greek origin. Those flavonoids possessing *o*-dihydroxy groups displayed antioxidative activity (Chizzola *et al.*, 2008).For the Teamt alarnb no previous studies, however, the possible reason for the decline in antioxidant activity as a result low content of phenolic compounds in their methanolic extract. Especially as he noted the low (Tawaha *et al.*, 2007) total phenols in the *Paronychia argentea* Lam., which belongs to the same family and the values (15.5 mg GAE/g dry weight) for Total phenolic content and (85.7 ± 3.8 µmol TE/g dry weight) for antioxidant capacity measured by the improved $ABTS^{\cdot+}$ method.

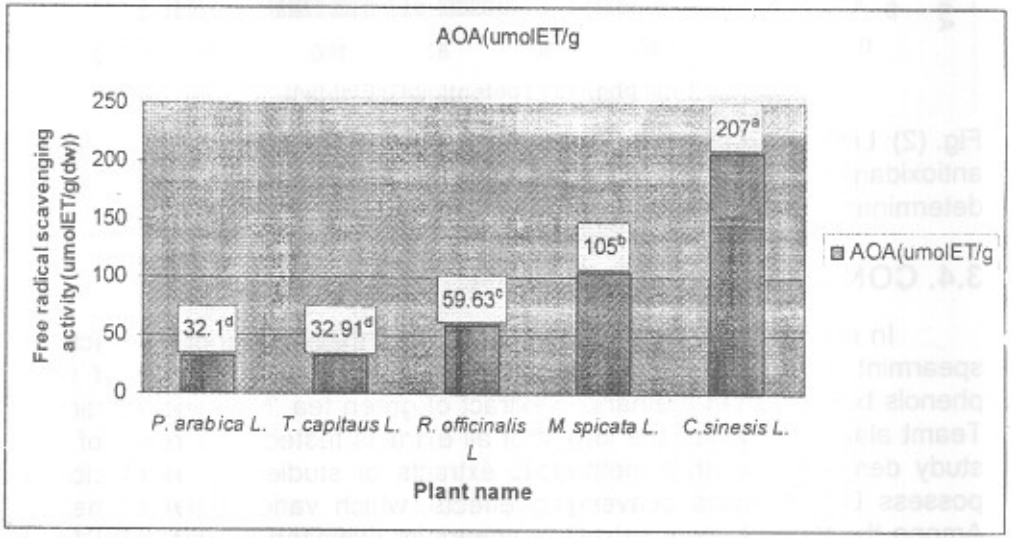

Fig. (1): The antioxidant potential (determined by TEAC assay) of rosemary, thyme, spearmint, *P. arabica* L., green tea expressed in Trolox equivalents.

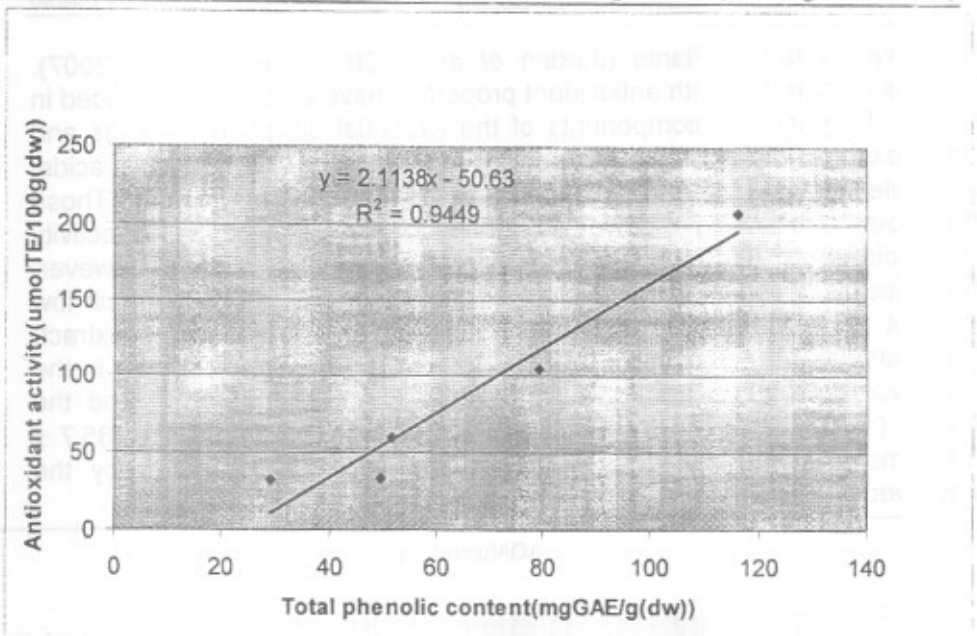

Fig. (2): Linear correlation between the total phenolic content and antioxidant activity (TEAC). For methanolic extracts. Coefficient of determination (r^2) = 0.9449.

3.4. CONCLUSION:-

In conclusion, the present study showed that methanolic extracts of spearmint, rosemary and thyme contained the highest amounts of total phenols but less than methanolic extract of green tea. Methanol extract of Teamt alarnb contained the lowest of all extracts tested. The result of this study demonstrates that methanolic extracts of studied herbs of Libyan possess DPPH radical-scavenging effects, which varies between herbs. Among the herbs tested, extract of spearmint exhibited the highest DPPH radicals scavenging activity less than green tea but more than other herbs. The other extracts in this study showed moderate activity against DPPH free radical. From the correlation analysis, it had been shown that total phenolic content had positive correlation with antioxidant capacity. Finely, the studied Libyan herbs could be used as potent sources for natural antioxidants.

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الملخص العربى

المحتوى الكلي من المركبات الفينولية والنشاط المضاد للأكسدة لبعض الأعشاب المستخدمة كمنكهات للأغذية في ليبيا

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يوجد في الوقت الحاضر اهتمام كبير بالمركبات الكيميائية النباتية كمصدر جديد لمضادات الأكسدة لاستخدامها في مجالي الأغذية والأدوية ولتحل محل مضادات الأكسدة الصناعية التي أصبح استخدامها مقيد بسبب سميتها والمخاطر الصحية المحتملة من استخدامها. وتم في هذه الدراسة تقدير المحتوى الكلي من المركبات الفينولية وتقدير النشاط المضاد للأكسدة لها في مستخلصات لأربع أعشاب من ليبيا تنتمي إلي عائلتين نباتيتين هما

(*Lamiaceae and Caryophyllaceae*) وتم مقارنتها مع الشاي الأخضر والمعروف بارتفاع محتواة من المركبات الفينولية وارتفاع نشاطها المضاد للأكسدة .وتم تقدير النشاط المضاد للأكسدة باستخدام طريقة

DPPH` (1, 1-diphenyl-2-picrylhydrazyl radical) وتم التعبير عنها بمجموع النشاط الكلي المضاد للأكسدة و المكافي للترولكس(µmol TE/g extract) . أما المحتوى الكلي من المركبات الفينولية فقد تم تقديره باستخدام طريقة (Folin–Ciocalteu assay) ومعبرا عنها بال مجم مكافي حمض الجاليك/جم من المادة الجافة حيث كان المحتوى الكلي في الأعشاب يتراوح بين 29.24 إلي 79.34 ،

بينما كان في الشاي الأخضر والمستخدم كعينة قياسية 116.33. أظهرت النتائج أن محتوى المركبات الفينولية في مستخلص النعناع و الروزماري و الزعتر 79.34 ،51.67،49.70 علي التوالي ، كما بينت النتائج أن اقل محتوى من المركبات الفينولية الكلية كان في مستخلص عشب (طعمه الأرنب) وهي 29 ، أيضا أوضحت النتائج أن أعلى قيمة للنشاط الكلي المضاد للأكسدة كان النعناع ، بينما اقل قيمة كانت في مستخلص طعمه الأرنب ، كما أوضحت النتائج أن هناك ارتباط وثيق في (علاقة خطية $R2 =$ 0.9449)بين النشاط المضاد للأكسدة والمحتوى الكلي من المركبات الفينولية في المستخلصات الميثانولية بين الأعشاب.

أظهرت كل المستخلصات الميثانولية للأعشاب المدروسة نشاط مضاد للأكسدة وقد ترتبت تنازليا علي النحو الأتي :

الشاي الأخضر > النعناع > الروزماري > الزعتر وطعمة الأرنب.