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Polyphenols, Flavonoids, Carotenoids and Antioxidant Activity of Lupine (*Lupinus termis* L.) Seeds Affected by Vitamin C, vitamin B_3 and Turmeric Rhizomes Extract

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ABSTRACT

Field experiment was carried out at the Applied Research Center for Medicinal Plants, National Organization for Drug Control and Research, Egypt to examination the effect of foliar application with ascorbic acid (A_{100}& A_{200}), nicotinic acid (N_{100}& N_{200}) and turmeric rhizomes extract(C_5& C_{10}) as well as control on total polyphenols, total flavonoids, total carotenoids and antioxidant activity of lupine (*Lupinustermis* L.) seeds.The treatments were applied and arranged in a randomized complete block design with three replicates. Theresults indicated that, foliar application treatments had a significant effect on all ingredients. The maximum values of total polyphenols (37.30, 36.82 and 35.65) were recorded with A_{200}, N_{200} and A_{100}, respectively. The highest value of total flavonoids (16.60 µg/g) was recorded with A_{100}. The treatment of A_{200} gave the highest value of total carotenoids, but both the nicotinic acid and Curcuma glabra extract under two concentrations (low and high) had a negative effect on total carotenoids. Both the treatments of A_{200} and C_{10} gave the highest values of antioxidant activity that were 97.97 and 97.50 % respectively. The antioxidant activity is not well correlated with total phenolic contents.

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INTRODUCTION

About 80% of the world population is essentially dependent on herbal traditional drugs which came from the virtue of pragmatic knowledge [38]. Lupine (*Lupinustermis*L.) is a member of the genus *Lupinus* in the family Fabaceae or Leguminosae. The seeds of *Lupinustermis* have long been utilized as integral food in developing countries prior to being recognized as a hypoglycemic agent and vitamin C is known as a potent antioxidant [43],[21].Lupins are good source of protein and lipids and have no lectins and very low content of protease inhibitors [9]. Its seeds are employed not only for their nutritional value, but also for their adaptability to marginal soils and climates. Humanconsumption of lupins has increased in recent years [28].Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. Reactive oxygen species generated in tissues and cells can damage DNA, proteins, carbohydrates and lipids [19]. Naturally occurring antioxidants include a range of enzymes (for example superoxide dismutase, gluthatione peroxidase), coenzyme Q, melatonin, iron binding proteins (for example transferrin, lactoferrin), vitamins C and E as well as carotenoids, flavonoids and other plant phenolic[22].Most essential physiological processes such as photosynthesis, building of all organic foods and enzymes, nutrient and water uptake and cell division depended more or less on the availability of vitamins [32]. Application of vitamins C, B, K, and E, in ascending order was significantly very effect in enhancing both physical and chemical characteristics of the fruits [32].It is indicated that Vitamins treatments had a positive effect on fruit chemical properties namely, total soluble solids percentage, total acidity percentage (as malic acid), total and reducing sugars percentage, non-reducing sugars percentage and total tannins percentage of Amhat date palms [6].

Ascorbic acid is synthesized in the higher plants and has been associated with several types of biological activities in plants such as in enzyme co factors, antioxidant, and as a donor / acceptor in electron transport at the plasma membrane or in the chloroplast [12],[15]. It is stated that ascorbic acid is currently considered to be a regulator on cell division and differentiation and added that ascorbic acid is involved in a wide range of

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Advances in Environmental Biology, 7(14) December 2013, Pages: 4914-4924

important functions as photoprotection and regulation of photosynthesis and growth [10]. Ascorbic acid also, increased chlorophyll content and prevented of abscisic acid accumulation [14]. Antioxidant application (vitamin C) reduced the hydrogen peroxide accumulation, lipid peroxidation, membrane permeability, sodium and chloride content over control plants. In addition, ascorbic acid revealed an effect on the metabolism of gibberellic acid [27].

The major function of niacin (nicotinic acid) in nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP) is hydrogen transport in intermediary metabolism. Most of these enzyme systems function by alternating between the oxidized and reduced state of the coenzymes NAD-NADH and NADP-NADPH[34], [33].NAD is also required for non-redox adenosine diphosphate-ribose transfer reactions involved in DNA repair and calcium mobilisation. NAD functions in intracellular respiration and with enzymes involved in the oxidation of fuel substrates such as glyceraldehyde 3-phosphate, lactate, alcohol, 3-hydroxybutyrate, and pyruvate. NADP functions in reductive biosyntheses such as fatty acid and steroid syntheses and in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway. Both NAD and NADP are involved in the synthesis of high energy phosphate bonds (ATP) which furnish energy for certain steps in glycolysis, in pyruvate metabolism, amino acid and protein metabolism, and in photosynthesis[16].

Turmeric (Curcuma longa L.) powder, curcumin and its derivatives and many other extracts from the rhizomes were found to be bioactive. A water extract of Curcuma longa L. (Zingiberaceae), having O2-scavenging activity [29]. Turmeric contains curcumin along with other chemical constituents known as the curcuminoids that accumulated in rhizomes (2-6%) [46], which is responsible for the yellow color and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%) [45].Curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H2O2 and nitrite radical generation by activated macrophages [42].

Carotenoids are colorful antioxidant plant pigments that are found mostly in carrots, tomatoes, spinach and blueberries. They can be converted to vitamin A in the body when needed, ensuring that an optimum level of vitamin A is maintained. Beta-carotene, the most popular member of the carotenoid family, is important for immune function, as it can increase and activate our white blood cells and also improve immune cell communication[40]. Carotenoids play a major role in the protection of plants against photo oxidative processes. They are efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals. In the human organism, carotenoids are part of the antioxidant defense system. They interact synergistically with other antioxidants; mixtures of carotenoids are more effective than single compounds. According to their structure most carotenoids exhibit absorption maxima at around 450 nm. Filtering of blue light has been proposed as a mechanism protecting the macula lutea against photo oxidative damage. There is increasing evidence from human studies that the carotenoids protect the skin against photo oxidative damage [52]. Carotenoids have many biological activities, including antioxidant activity, influences on the immune system, control of cell growth and differentiation, and stimulant effects on gap junction communication.Carotenoids play important roles in human nutrition through their provitaminA activity, but also by acting as antioxidants, for prevention of age-related macular degeneration or skin protection against UV radiation[30].

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine [36].Flavonoids (or bioflavonoids), also collectively known as Vitamin P and citrin, are a class of plant secondary metabolites.Flavonoids arepowerful antioxidants by giving protection versus oxidative and free radical damage andincrease intracellular vitamin C levels[44].

Phenolic compounds have a role in the visual appearance (peel and flesh pigmentation, browning), taste (astringency and bitterness), and health-promoting properties (free radical scavengers).Phenolic compounds having one or more aromatic rings bearing one or more hydroxyl groups can potentially quench free radicals by forming resonance-stabilized phenoxyl radicals and therefore have redox properties [11].

There are relatively few studies with regard to improving the content of lupine antioxidant contents. So the goal of this study was to examine the effect of Vitamin C, vitamin B_3 and turmeric rhizomes extract on total polyphenols, total flavonoids, total carotenoids and Antioxidant Activity of Lupine (*Lupinustermis* L.) Seeds.

MATERIALS AND METHODS

Field experimentwas carried out at the Experimental Farm,Applied Research Center for Medicinal Plants, National Organization for Drug Control and Research,Egypt during agricultural season (2010–2011). Lupine seeds were obtained from Applied Research Center for Medicinal Plants. Seeds were sown in the prepared nursery on 18thOctober. The seedlings were thinned one month after sowing to leave two plants per hill.The soil was prepared and divided into plots of 2 m×1.5 m, with three rows at 60 cm apart and 30 cm between hills.All the recommended cultural practices for lupine production were applied according to the Egyptian Ministry of Agriculture. Foliar application of vitamin C, nicotinic acid and *Curcuma longa* extract, were sprayed twice with freshly prepared solutions (water extract) at 45 and 60 days from planting with low and high concentration of all

foliar application ingredients, as well as untreated plants (control; distilled water). The spraying was done manually using a spraying bottle, on both sides of the leaves evenly. The experimental treatments were laid out in a randomized complete block design (RCBD) with 3 replicates. Mechanical analysis [41] and chemical analysis [25] of the soil were carried out before sowing and presented in Table 1.
Seven treatments were applied as the following:
Control: (distilled water).
A_{100}: Low concentration of vitamin C, 100 ppm.
A_{200}: High concentration of vitamin C, 200 ppm.
N_{100}: Low concentration of nicotinic acid, 100 ppm.
N_{200}: High concentration of nicotinic acid, 200 ppm.
C_5: Low concentration of *Curcuma longa* extract, (5 g/l) 5000 ppm.
C_{10}: High concentration of *Curcuma longa* extract, (10 g/l) 10000 ppm.

Table 1: Mechanical and chemical analysis of experimental soil.

Characteristics												
Mechanical analysis		Chemical analysis										
		Soluble cations (m.equ/L)		Soluble anion (m.equ/L)		Macro element (ppm)		Micro elements (ppm)		pH	EC m.mohs/cm	$CaCO_3$
Coarse sand%	18.7	Ca^{+}	1.4	HCO^{-3}	0.8	Total N	10	Fe	3	7.8	0.55	3.2
Fine sand%	69.5	Mg^{++}	0.8	Cl^{-}	1.5	P_2O_5	5	Cu	0.25			
Silt %	3.1	Na^{+}	1.8	SO^{-4}	3.12	K_2O	388	Za	0.98			
Clay %	8.7	K^{+}	1.41					Mn	4.4			
Soil texture	Loamy sand											

Prepared lupine seed extract:

Seed samples were taken randomly from each experimental plot, oven dried at 70°C until constant weights and then dried samples were ground to fine powder. Each seed sample 10g was transferred to dark-colored flasks and was dissolved in 250 ml of 95% methanol and stored at room temperature. After 24 hours, infusions were filtered through Whatman No. 1 filter paper. The combined supernatants were evaporated to dryness under vacuum at 40 ^{o}C using Rotary evaporator. The obtained extracts were stored in a refrigerator at 4 ^{o}C.

Determination of Total Polyphenols Content:

The total polyphenol content was determined by spectrophotometry, using gallic acid as standard, according to the method described by [8]. The dried plant material was weighed, and extracted. Stock solutions of the prepared extracts were made so as to be 50mg extract per 100ml methanolic solution. One milliliter of each extract (equivalent to 500ug extract) was mixed with the reagents above and after 30 min the absorbance was measured at 765 nm against methanol blank to determine the total phenolic contents. The polyphenols content was expressed as µg gallic acid equivalent/g dry weight by reference to the gallic acid standard calibration curve. Standard calibration curve was prepared using serial dilutions of gallic acid (400, 300, 200,100, 50, and 25 µg/ml), by mixing 1ml methanol solution of gallic acid with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and 4 ml sodium carbonate (0.7 M). Absorbance values were measured at 765 nm against methanol blank and the standard curve was drawn (Fig.1).

Determination of Total Flavonoids Content:

The total flavonoid contents were measured by a colorimetric assay according to [31], using quercetin as a standard for the calibration curve. The dried plant material was weighed, and extracted. Stock solutions of the prepared extracts were made so as to be 50 mg extract per 100ml methanolic solution. One milliliter of each extract (equivalent to 500ug extract) was evaporated till dryness, and then treated with 5ml of 2% $AlCl_3$. Absorbance values were measured at 415 nm using methanol as blank. Flavonoids content were calculated from the calibration curve of quercetin standard solutions, and expressed as µg quercetin equivalent/g dry weight. Calibration curve was prepared using serial dilutions of Quercetin (100, 80, 60, 40, 20, 10 µg/ml), transfer different aliquots corresponding to these dilutions to test tubes and evaporate to dryness. To the residue add 5ml of 2% $AlCl_3$. Absorbance values were measured at 415 nm using, methanol as blank and the standard curve was drawn (Fig. 2).

Determination of total Carotenoids content:

Total Carotenoid contents were determined according to [47]. Powder sample of lupine seeds (0.5gm each) were extracted with 85% acetone in the presence of little amounts of Na_2CO_3 and silica quartz, then filtered through centered glass funnel (G4). The residue was washed several times with acetone until the filtrate become colorless. The combined extract was completed to a known volume (50 ml). An aliquot (50 ml) of this extract

was taken for the colorimetric determination of pigments. The determination was conducted using a spectrophotometer at different wave length of 660, 640 and 440 nm and using acetone 85% as a blank.
Chl.a (mg/l) = 9.784 A_{660}- 0.99 A_{640}
Chl.b (mg/l) = 21.426 A_{640}- 4.65 A_{660}
Total carotenoids (mg/l) = 4.695 A_{440}- 0.268 (Chl.a + Chl.b)

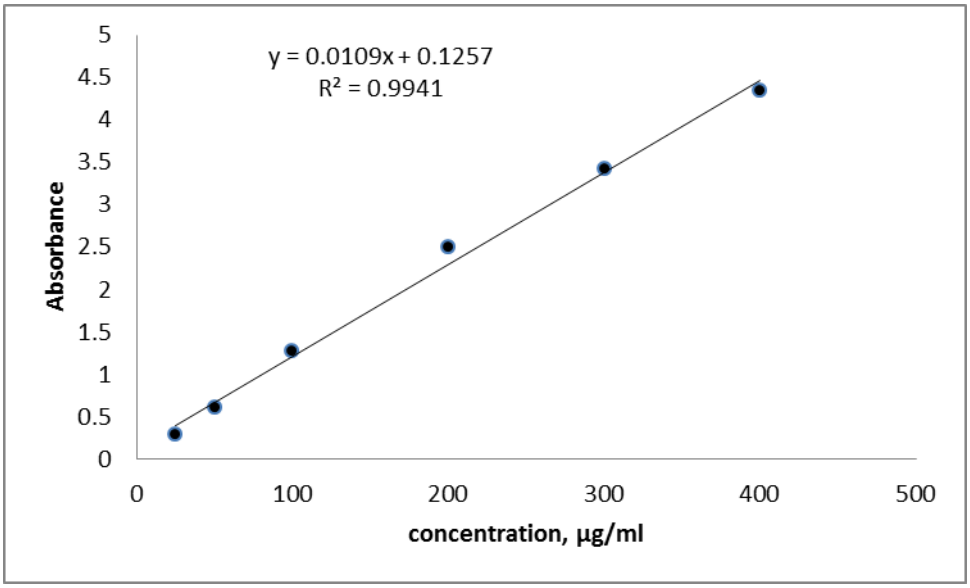

Fig. 1: Standard calibration curve of gallic acid.

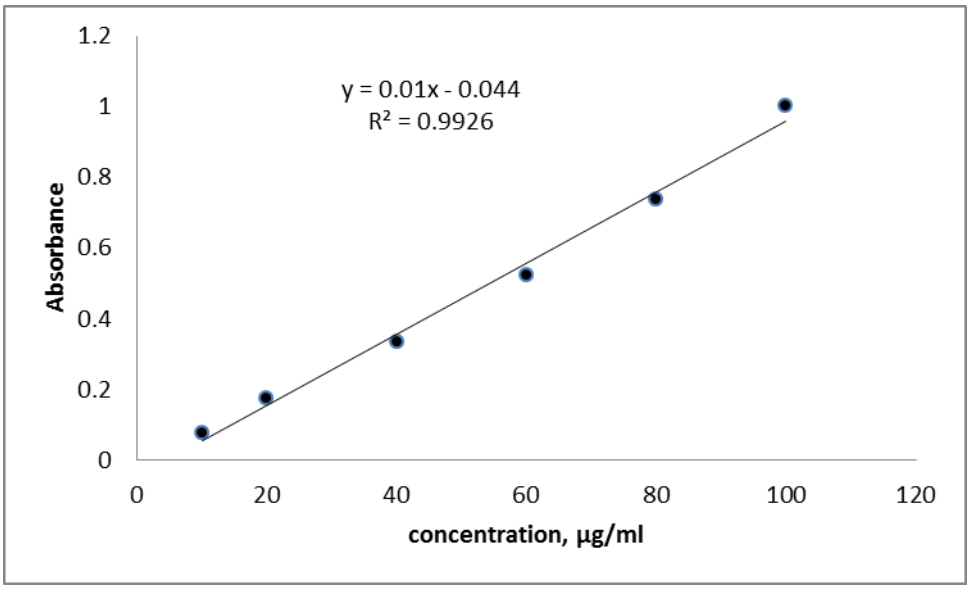

Fig. 2: Standard calibration curve of quercetin.

Determination of antioxidant activity (AA %):

The ability of the extract to scavenge DPPH radical was determined according to the method described by [35]. Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µg/ml in methanol. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solutions of the extracts or standard and allowed to react at room temperature in dark for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %) using the formula:
$AA\% = 100 - [(Abs_{Sample} - Abs_{Blank}) \times 100]/Abs_{Control}$
Where:
Methanol (1.0ml) plus extract solution (2.5ml) was used as a blank.
1ml of 0.3 mM DPPH plus methanol (2.5ml) was used as a negative control.
Solution of gallic acid served as positive control.

Advances in Environmental Biology, 7(14) December 2013, Pages: 4914-4924

Statistical analysis:

The obtained results were statistically analyzed according to the Technique of Analysis of Variance (ANOVA) of a randomized Complete Blocks design. The content of total phenols, flavonoids and carotenoids were presented as mean ± SD. Least significant difference (LSD) method was used to test the differences between treatment means at 5% level probability as published by [20].

Results:

Analysis of variance (Table 2) showed that foliar application of ascorbic acid, nicotinic acid and turmeric rhizome extract had significant effect on total polyphenols, total flavonoids, total carotenoids and antioxidant activity.

Table 2: Analysis of variance of total polyphenols, total flavonoids, total carotenoids and antioxidant activity.

Source of variation	df	Mean Squares (MS)			
		Total Polyphenoles	Total Flavonoids	Total carotenoids	Antioxidant activity at 10µg/ml
Foliar application Treatments	6	202.4*	8.421*	314.6*	8.756*
Error	14	4.012	0.379	9.277	0.428
Total	20	----	----	----	----

* Significant at 0.05 level

Total polyphenols:

The total polyphenols in lupine seed (µg/ g) in methanol extracts were determined from regression equation of calibration curve (R^2 = 0.99), expressed as µg GAE (µg gallic acid eq)/ g of seed, as summarized in Table 3 and Fig 3. The total polyphenolic content greatly varied with foliar application treatments, ranging from 14.23 to 37.30 µg GAE/g. Supplying the lupine plant with ascorbic acid (vitamin C), nicotinic acid (vitamin B3) and turmeric rhizome (*Curcuma longa* L.) extract were significantly very effective in enhancing the total polyphenoles in relative to the control treatment. The promotion was significantly depended on using turmeric extract, nicotinic acid and ascorbic acid, in ascending order. The maximum values of total polyphenols(37.30, 36.82 and 35.65) were recorded with A_{200}, N_{200} and A_{100}, respectively. On the other hand, it is clear that there were no significant differences between these treatments. The minimum values were recorded at untreated plants (control). From Fig. 3, it is clear that there is no significant difference between N_{100} and C_{10}. Treatments can be arranged in descending order according to the values of total polyphenols as follows: A200 > N200 > A100 > N100 > C10 > C5 > control.

Table 3**:** Effect of ascorbic acid, nicotinic acid and turmeric rhizomes extract on total polyphenols, total flavonoids, total carotenoids of *Lupinustermis* seeds.

Foliar application Treatments	Total polyphenols content, µg/g	Total Flavonoids content, µg/g	Total carotenoids content, µg/g
Control	14.23 ±1.38	12.40 ±1.56	26.41 ±3.43
A100	35.65 ±2.96	16.60 ±0.20	29.84 ±5.33
A200	37.30 ±3.4	15.20 ±0.20	34.56 ±3.03
N100	31.43 ±1.91	14.60 ±0.20	24.01 ±2.94
N200	36.82 ±0.24	13.67 ±0.12	12.33 ±1.49
C 5	27.17 ±1.27	11.80 ±0.20	4.93 ±0.99
C10	30.41 ±0.75	15.00 ±0.02	20.58 ±1.96
LSD	3.51	1.07	5.33

Total flavonoids:

As shown in Table 3 and Fig.4 all applied treatments led to significant increase of total flavonoids compared with control. Also, it could be noticed that the application of vitamin C (ascorbic acid) was more effective than vitamin B_3 (nicotinic acid) or turmeric extract. The highest value of total flavonoids(16.60 µg/g) was recorded with A_{100}. From Fig.4, it is clear that, there were no significant differences between A_{200}, C_{10} and N_{100}. It is also shown that, there were no significant differences between N_{100} and N_{200}. The minimum values were recorded on untreated plants (control) and C_5. However, at low level of *Curcuma glabra* (5 g/l), there was no significant effect on total flavonoids of lupine seed extract compared with control. Treatments can be arranged in descending order according to the values of total flavonoids as follows: A100 > A200 > C10 > N100 > N200 >control >C5.

Total carotenoids:

Data in Table 3 and Fig. 5 show that, the concentration of total carotenoids in plant extracts from *Lupinustermis* seeds ranged from 4.93 to 34.56 µg/g. The treatment of A_{200} gave the highest value of total carotenoids, but the lowest value was obtained with C_5 treatment. From Fig. 5, it is clear that, there were no

significant differences between A_{200} and A_{100}. In addition, both the nicotinic acid and *Curcuma glabra*extract under two concentrations (low and high) had a negative effecton total carotenoids of lupine seed extract.

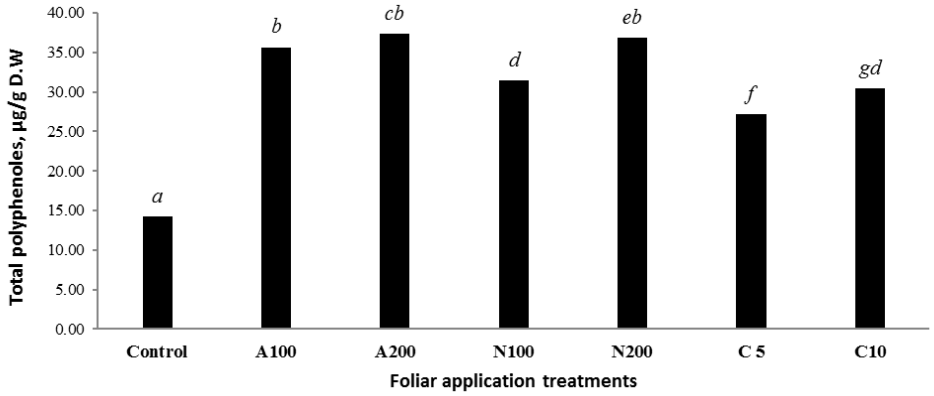

Fig. 3: Effect of ascorbic acid, nicotinic acid and turmeric rhizomes extract on total polyphenols of *Lupinustermis* seeds.

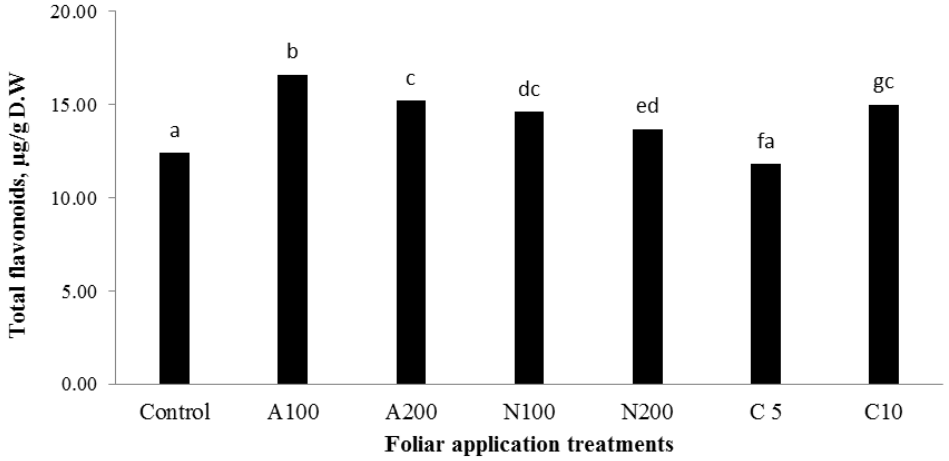

Fig. 4: Effect of ascorbic acid, nicotinic acid and turmeric rhizomes extract on total flavonoids of *Lupinustermis* seeds.

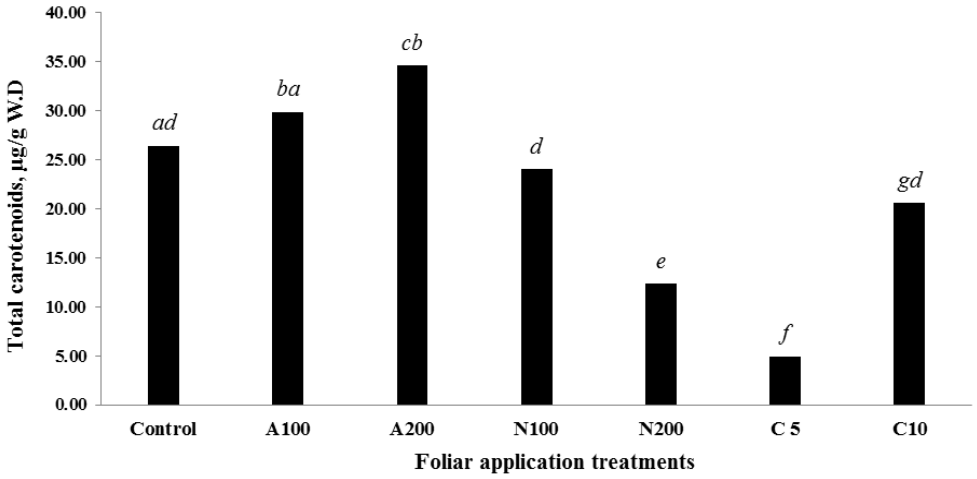

Fig. 5: Effect of ascorbic acid, nicotinic acid and turmeric rhizomes extract on total carotenoids of *Lupinustermis* seeds.

Advances in Environmental Biology, 7(14) December 2013, Pages: 4914-4924

Antioxidant activity:

Data in Table 4 and Fig. 6 show that, the antioxidant activity of Lupinus*termis* seeds ranged from 93.79 to 97.97 % under the different studied treatments. Both the treatments of $A_{200}$and C_{10}gave the highest values of antioxidant activity that were 97.97 and 97.50 % respectively. However, there were no significant differences between them. The lowest values of antioxidant were 93.79 and 93.87withboth the treatments of N100 and control,respectively. In addition, there were no significant differences between control, N100 and N200. Also, it is noted that there were no significant differences between A100, N200 and C5. Treatments can be arranged in descending order according to the values of total antioxidant as follows: A200 > C10 > A100 > C5 > N200 >N100 >control.

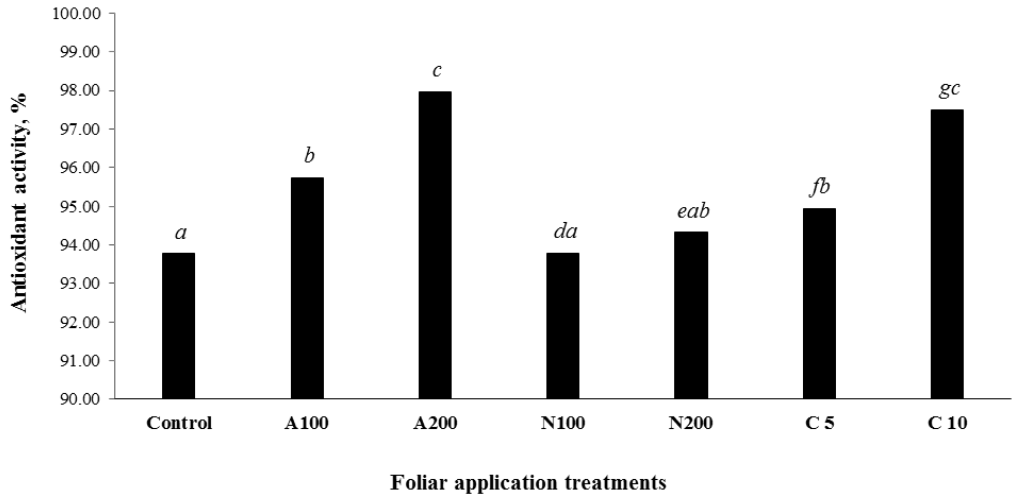

Fig. 6: Effect of ascorbic acid, nicotinic acid and turmeric rhizomes extract on antioxidant activity of *Lupinustermis* seeds.

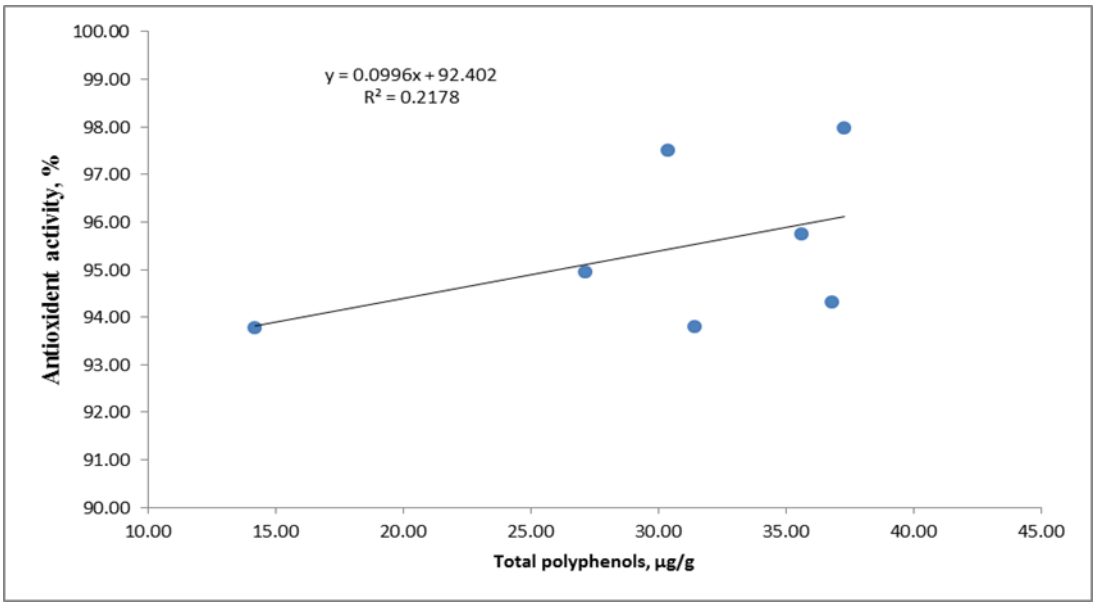

Fig. 7: Relationship between total phenolic content and antioxidant activity of *Lupinustermis* seeds.

The antioxidant activity is not well correlated with total phenolic contents (R^2 = 0.218) Fig. 7 although it showed a trend that the higher phenolic contents have a stronger antioxidant activity. This result suggests that 21.8% of the antioxidant activity of Lupinustermis accessions results from the contribution of phenolic compounds. These results are harmony with the results of literature [54]. It was also found that antioxidant activity was not correlated with total phenolic contents of lupin genotypes seeds [39] and also of some plants [37]. It is suggested that the antioxidant activities can be ascribed to the different mechanisms exerted by different compounds as well as phenolic compounds. On the other words, it can be mentioned that antioxidant

Advances in Environmental Biology, 7(14) December 2013, Pages: 4914-4924

activity of plant extracts is not limited to phenolics. Antioxidant activity may also come from the presence of other antioxidant secondary metabolites, such as carotenoids and flavonoids [26]. Figs8 and 9 show the correlation between antioxidant activity and both flavonoids and carotenoids with the correlation coefficient R^2= 0.274 and 0.143, respectively. These results display that 14.3 and 27.4% of antioxidant activity of Lupinustermis result from the contribution of flavonoids and carotenoids, respectively.

Table 4: Effect of ascorbic acid, nicotinic acid and turmeric rhizomes extract on antioxidant activity by DPPH (%) and EC_{50} (µg/ml) of *Lupinustermis* seeds.

Treatment	Antioxidant activity by DPPH (Inhibition%)		
	2.5 µg/ml	5µg/ml	10 µg/ml
Control	19.21 ±0.34	34.09 ±0.14	93.87 ±0.11
A100	15.48± 0.48	71.20 ±1.31	95.74 ±0.37
A200	22.14 ±0.27	75.67 ±0.67	97.97 ±0.24
N100	24.90 ±0.21	61.56 ±0.32	93.79 ±0.53
N200	36.73 ±0.52	66.79 ±1.2	94.32 ±0.55
C5	26.02 ±1.30	67.50 ±1.72	94.94 ±0.29
C10	50.00 ±0.38	96.44 ±0.47	97.50 ±1.44
Ascorbic acid	……..	…….	…….
LSD	……..	……..	1.15

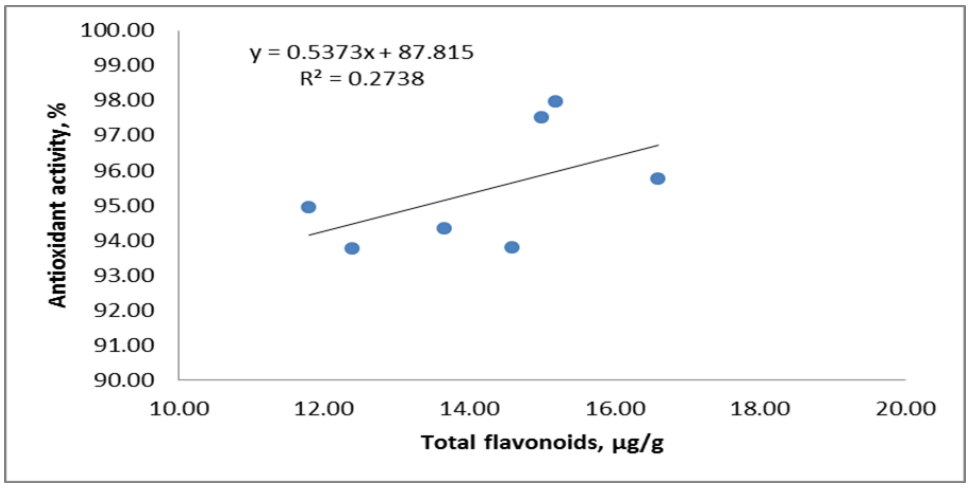

Fig. 8: Relationship between total flavonoids content and antioxidant activity of*Lupinustermis* seeds.

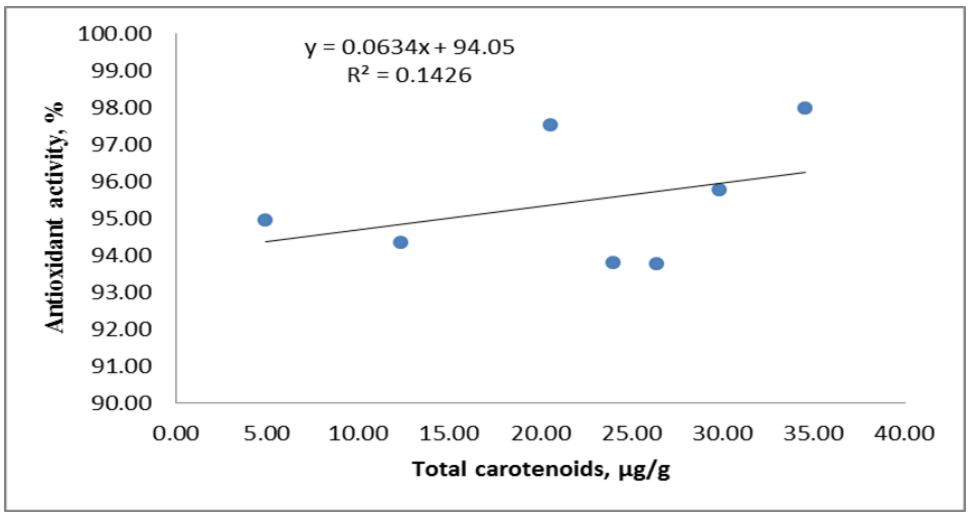

Fig. 9: Relationship between total carotenoids content and antioxidant activity of*Lupinustermis* seeds.

Discussion:

Result showed that,the effect of ascorbic acid was superior to both nicotinic acid and turmeric extractwith regard to total carotenoids, especially at a concentration of 200 ppm, and on total flavonoids at the concentration of 100 ppm. In addition,both ascorbic acid and nicotinic acid treatments achieved higher total polyphenols than

Curcuma longa extracttreatments especially the concentration of 200 ppm. These results clarified the positiveeffect of vitamin Con enhancingthe total polyphenols, total flavonoids and total carotenoids content.These results are in agreement with that reported in literature that studied the effect of ascorbic acid as foliar application on total phenol content and found that increasing ascorbic acid concentration from 50 to 200 ppm increased total phenol contents compared with untreated plants [2]. In addition, these results are in agreement with that reported in literature, whichconcluded that ascorbic acid at 100 ppm gave a significantly increased chlorophyll a, b, a+b and carotenoids, but total soluble sugar content significantly increased when plants were treated with ascorbic acid at 200 ppm [5]. The present data also, are in agreement with that reported in literature on rosemary plants [55], on Khyasenegalensis plants [1], on Syngoniumpodyphyllum L. plants [2], and on Cupressussempetrirens L. [17].They found that foliar application of Asc. caused an increase in photosynthetic pigments and total soluble sugars content. The present data are in harmony with the literature that the enhanced accumulation of carotenoids was seen in antioxidants sprayed plants compared with control plants at 65 days after sowing of wheat plant[18], with that reported in literatures on rosemary plants[55], on Khyasenegalensis plants [1], on Syngoniumpodyphyllum L. plants [2], and on *Cupressussempetrirens* L.[17].They found that foliar application of ascorbic acid caused an increase in photosynthetic pigments.Ascorbic acid is synthesized in the higher plants and has been associated with several types of biological activities in plants such as in enzyme co factors, antioxidant, and as a donor / acceptor in electron transport at the plasma membrane or in the chloroplast [12], [15]. This positive effect of vitamin C may be due tothe beneficial effect of vitamins on promoting the biosynthesis of carbohydrates and uptake of most nutrients [32] especially; N, P and K and soluble sugars [53], [4].It is stated that Asc.has a wide range of important functions as antioxidant defense, photo protection, regulation of photosynthesis and growth [10]. In this manner, it is reported that ascorbic acid has a central role in photosynthesis, as high content ration in chloroplast would imply [49]. Application of ascorbic acid or tocopherol increased total chlorophyll either through the stimulation of its biosynthesis and/or delay of its degradation and enhanced accumulation of ascorbate, phenol, carotenoids, calcium, potassium and magnesium was seen in antioxidants-sprayed plants compared with control plants [18].

The term “antioxidant” refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Antioxidant activities of lupine seeds are due to the presence of antioxidant components such as flavonoids, phenolic acids and carotenoids. These compounds can remove free radicals, chelating metal catalysts and inhibit oxidases [23]. Antioxidants stimulated the activities of catalase and peroxidase enzymes besides leading to an accumulation of ascorbic acid, total carotenoids and total soluble phenol in flag leaves compared to untreated plants under the corresponding salinity levels [18].In this respect, we found that the treatment of ascorbic acid (A200) gave the highest antioxidant activity (97.97%) compared with the other treatments. It is due to that this treatment (A200) gave the highest total polyphenols (37.30 µg/g) and total carotenoids (34.56 µg/g) as well as high value of flavonoids (15.30 µg/g). These results were agreement with that reportedin literature that the antioxidants enzyme activities were significantly increased by antioxidant spray of wheat plant[18].

The positive effect of nicotinic acid on total polyphenols and total flavonoids may be due to the major function of nicotinic acid in the structure of nicotinamide adenine dinucleotide (NAD) and its phosphate; NADP [34], [33]. Both NAD and NADP are involved in the synthesis of high energy phosphate bonds (ATP) which furnish energy for certain steps in glycolysis, in pyruvate metabolism, amino acid and protein metabolism, and in photosynthesis [16].

The positive effect of *Curcuma longa* extract on total polyphenols and total flavonoids may be due to yellow color in turmeric rhizomes that are mainly due to the presence of 3 major pigments; curcumin 1,7-bis(4-hydroxy-3-methoxyfenil) -1,6-heptadiene-3,5- dione), demethoxy-curcumin and bisdemethoxy- curcumin. These curcuminoidshave a free radical scavenger activity namely hydroxyl radical that is responsible to protect DNA from damage and inhibit lipid peroxidation [50],[51],[24],[48] and[13]. A water extract of *Curcuma longa* L. (Zingiberaceae), having superoxide anion (O2*-) scavenging activity[29]. On the other hand, turmeric extract is rich in carbohydrates, (50 % starch), arabinogalacton, potassium salt and pigments. It is also, known for its anti- oxidant and anti- microbial properties [7].

Conclusions:

It can be concluded that ascorbic acid enhance the total polyphenols, flavonoids and carotenoids and then antioxidant activity of Lupine *(Lupinustermis L.)*Seeds.

REFERENCES

[1] Abd El-Aziz, Nahed G., A.M. AzzaMazher and E. El-Habba, 2006. Effect of foliar spraying with ascorbic acid on growth and chemical constituents of Khyasenegalensis grown under salt condition. American-Eurasian J. Agric. & Environ. Sci., 1(3): 207-214.

[2] Abd El-Aziz, Nahed, G., E.M. Fatma El-Quesni and M.M. Farahat, 2007. Response of vegetative growth and some chemical constituents of Syngoniumpodophyllum L. to foliar application of thiamine, ascorbic acid and kinetin at Nubaria. World J.Agric.Sci., 3(3): 301-305.
[3] Abd El-Aziz, Nahed, G., E.M. Fatma El-Quesni and M.M. Farahat, 2007. Response of vegetative growth and some chemical constituents of Syngoniumpodophyllum L. to foliar application of thiamine, ascorbic acid and kinetin at Nubaria. World J.Agric.Sci., 3(3): 301-305.
[4] Abdel Aziz, Nahed G., S. Lobna, Taha and Soad M. Ibrahim, 2009. Some studies on the effect of putrescine, Ascorbic acid and thiamine on growth, flowering and some chemical constituents, of gladiolus plants at Nubaria. Ozean J. of Applied Sciences, 2(2): 169-179.
[5] Abdel Aziz, G. Nahed, S. LobnaTaha and Soad M.M. Ibrahim, 2013. Some Studies on the Effect of Putrescine, Ascorbic Acid and Thiamine on Growth, Flowering and Some Chemical Constituents of Gladiolus Plants at Nubaria. Ozean Journal of Applied Sciences, 2(2): 169-179.
[6] Ahmed, Dorria, M., N.E. Ashour, E.A.M. Mostafa, M.M.S. Saleh and H.S.A. Hassan, 2012. Yield and fruit quality of Amhat date palms as affected by spraying some vitamins. J. Appl. Sci. Res., 8(10): 4922-4926.
[7] Ahmed, F.F., A.E.M. Mansour, M.A.A. Montasser, M.A. Merwad and E.A.M. Mostafa, 2013. Response of Valencia Orange Trees to Foliar Application Of Roselle, Turmeric and Seaweed Extracts. Journal of Applied Sciences Research, 9(1): 960-964.
[8] Aliyu, A.B., M.A. Ibrahim, A.M. Musa, H. Ibrahim, I.E. Abdulkadir and A.O. Oyewale, 2009. Evaluation of antioxidant activity of leave extract of bauhinia rufescens lam. (caesalpiniaceae). Journal of Medicinal Plants Research, 3(8): 563-567.
[9] Australia New Zealand Food Authority, 2001. Lupin alkaloids in food. A toxicological review and risk assessment. Technical Report Series No.3, pp 1-21.
[10] Blokhina, O., E. Violainen and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation stress. a review. Ann. Bot., 91: 179-194.
[11] Choi, H.Y., 2009. Antioxidant Activity of Curcuma Longa L. Novel Foodstuff. Mol. Cell. Toxicol., 5(3): 237-242.
[12] Conklin, P.L., 2001. Recent advances in the role and biosynthesis of ascorbic acid in plants. Plant, Cell and Environment, 24: 383-394.
[13] Cousins, M., J. Adelberg, F. Chen and J. Rieck, 2007. Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric (Curcuma longa L.) grown in vitro. Industrial Crops and Products, 25: 129-135.
[14] Dolatabadian, A., R.S. Jouneghani, 2009. Impact of exogenous Ascorbic Acid on antioxidant activity and some physiological traits of common bean subjected to salinity stress. Not Bot HortiAgrobot, 37(2): 165-172.
[15] El-Kobisy, D.S., K.A. Kady, R.A. Hedani and R.A. Agamy, 2005. Response of pea plant (Pisumsativum) to treatment with ascorbic acid. Egypt. J. Appl. Sci., 20: 36-50.
[16] FAO, 1980. Fish Feed Technology, Chapter 6. The Vitamins, Water-Soluble Vitamins.
[17] Farahat, M.M., M.M. Soud Ibrahim, S.L. Lobna Taha and E.M. Fatma El-Quesni, 2007. Response of vegetative growth and some chemical constituents of Cupressussempervirens L. to foliar application of ascorbic acid and zinc at Nubaria. World J. of Agric.Sci., 3(3): 282-288.
[18] Farouk, S., 2011. Ascorbic Acid and α-Tocopherol Minimize Salt-Induced Wheat Leaf Senescence. Journal of Stress Physiology & Biochemistry, 7(3): 58-79.
[19] Gill, S.S., N. Tuteja, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48: 909-930.
[20] Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research, 2nd Edition. John Wiley and Sons, New York.
[21] Hady, Maha, M., 2013. Potentiality of Vitamin C and / or Lupinustermis to Modulate Blood Glucose level and Oxidative Stress Status of Alloxan-induced Diabetic Rats. Int. Res. J. Biological Sci., 2(11): 41-46.
[22] Halliwell, B., J.M.C. Gutteridge, 2007. Free Radicals in Biology and Medicine 4th ed. Halliwell B., Gutteridge J.M.C., editors. Oxford University Press; New York, pp: 440-487.
[23] Heim, K.E., A.R. Tagliaferro and D.J. Bobilya, 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationship. J. Nutr. Biochem., 13: 572-84.
[24] Ishita, C., B. Kaushik, B. Uday and K.B. Ranjit, 2004. Turmeric and curcumin: biological actions and medicinal applications. Current Science, 87: 44-53.
[25] Jackson, M.L., 1960. Soil Chemical Analysis. Printice Hall, Englewood Cliffs, N.J., U.S.A.
[26] Javanmardi, J., C. Stushnoff, E. Locke and J.M. Vivanco, 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chemistry, 83: 547-550.
[27] Kamiya, Y., M. Takashi, N. Takahashi and J.E. Graebe, 1984. Conversion of gibberellin A20 to gibberellins A and A5 in cell free system from Phaselus vulgaris. Planta, 162(2): 152-158.

[28] Kohajdová, Z., J. Karovičová and Š. Schmidt, 2011. Lupin composition and possible use in bakery a review. Czech J. Food Sci., 29(3): 203-211.
[29] Koo, B.S., W.C. Lee, K.H. Chung, J.H. Ko and C.H. Kim, 2004. A water extract of Curcuma longa L. (Zingiberaceae) rescues C12 cell death caused by pyrogallol or hypoxia/reoxygenation and attenuates hydrogen peroxide induced injury in C12 cells. Life Sci., 75(19): 2363-2375.
[30] Krinsky, N.I., E.J. Johnson, 2005. Carotenoid actions and their relation to health and disease. Mol Aspects Med., 26: 459-516.
[31] Makky, E.A., M.Y. Mashitah and M.M. Ibrahim, 2012. Impact of medicinal plants phytocomponents against antibiotic resistant bacteria. Journal of Chemical and Pharmaceutical Research, 4(1): 881-893.
[32] Masoud, A.A.B. and O.A.M. El-Sahrawy, 2012. Effect Of Some Vitamins And Salicylic Acid On Fruiting Of Washington Navel Orange. Trees. J. Appl. Sci. Res., 8(4): 1936-1943.
[33] McCormick, D.B., 1997. Co-enzymes, Biochemistry. In: Encyclopedia of Human Biology 2nd edition. Dulbecco, R., ed.-in-chief. San Diego: Academic Press, pp: 847-64.
[34] McCormick, D.B., 1996. Co-enzymes, Biochemistry of. In: Encyclopedia of Molecular Biology and Molecular Medicine, Vol. 1. Meyers, R.A., ed. Weinheim: VCH, pp: 396-406.
[35] Mensor, L.L, F.S. Meneze, G.G. Leitao, A.S. Reis J.C. Dos santor, C.S. Coube and S.G. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127-130.
[36] Middleton, E.J., 1998. Effect of plant flavonoids on immune and inflammatory cell function. Adv. Exp. Med. Biol., 439: 175-182.
[37] Miliauskas, G., P.R. venskutonis and T.A. van Bekk, 2003. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry, 24(10).
[38] Muller, M.S. and E. Mechler, 2005. Medicinal plants in tropical countries: Traditional use-experience-facts (6th ed.) Thieme; Stuttgart, Germany.
[39] Oomah, B.D., N. Tiger, M. Olson and P. Balasubramanian, 2006. Phenolics and antioxidative activities in narrow- leafed lupins (Lupinusangustifolius L.). Plant Foods Hum Nutr., 61(2): 91-97.
[40] Osiecki, H., 2000. Bio Concepts The nutrient bible Edition 7th ed. Published Eagle Farm, Qld. 259 pages The Nutrient Bible. Bioconcepts Publishing, Australia.
[41] Piper, C.S., 1950. Soil and Plant Analysis. Inter. Science Publishers, New York, 213- 217.
[42] Prasad, S.A.J.V.R.M., G. Trimurtulu, K.N. Reddy and M.L. Naidu, 2013. Analytical Evaluation of Aqueous Extract of Haridra. International Ayurvedic Medical Journal, 1(3): 1-7.
[43] Quiles, M., I. Oquendo-Jiménez, D. Herreño-Saénz and M.D. Antoun, 2010. Genotoxicity of Alkaloid-Rich Extract from Lupinustermis Seeds. Pharm Crops, 1: 18-23.
[44] Robert, J.N., E.V. Nood, D.E.C.V. Hoorn, P.G. Boelens, K.V. Norren and P.A.M.V. Leeuwen, 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am J ClinNutr., 74: 418-425.
[45] Ruby, A.J., G. Kuttan, B.K. Dinesh, 1995. Cancer Lett., 94: 79-83.
[46] Shishodia, S., G. Sethi and B.B. Aggarwal, 2005. Curcumin: Getting Back to the Roots. Ann. N.Y. Acad. Sci., 1056: 206-217.
[47] Saric, M., R. Kastrori, R. Curic, T. Cupina and L. Geric, 1967. Chlorophyll determination. Univerzitet M Novon Sadu. Praktikumlz Piziologize Bilijaka Beograd Haucna Ajiga, pp: 215.
[48] Sharma, R.A., A.J. Gescher and W.P. Steward, 2005. Curcumin: The story so far. European Journal of Cancer, 41: 1955-1968.
[49] Smirnoff, N.,1996. The function and metabolism of ascorbic acid in plant. Annals of Botany, 78: 661-669.
[50] Srimal, R.C., 1997. Turmeric a brief review of medicinal properties. Fitoterapia, 68(6): 483-494.
[51] Alonso, J., 2004. Tratado de Fitofarmocos y Nutraceutices. Barcelona Corpus., p: 395-403.
[52] Stahl, W. and H. Sies, 2003. Antioxidant activity of carotenoids. Molecular Aspects of Medicine, 24: 345-351.
[53] Talaat, N.B., 2003. Physiological studies on the effect of salinity, ascorbic acid and putrescine of sweet pepper plant. Ph.D. Thesis , Fac. Agric., Cairo Univ., Egypt.
[54] Wang, S. and J. Clements, 2008. Antioxidant Activities of Lupin Seeds. IN J.A. Palta and J.B. Berger (eds). 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept., remantle, Western Australia. International Lupin Association, Canterbury, New Zealand. ISBN 0-86476- 153-8.
[55] Youssef, A.A. and Iman, M. Talaat, 2003. Physiological response of rosemary plants to some vitamins. Egypt. Pharm. J., 1: 81-93.