

## Variability of Antioxidant Properties and Identification of Phenolic Contents by HPLC-DAD in Different Organs of *Acacia albida* and *Acacia raddiana*

Karoune S<sup>1,2\*</sup>, Kechebar M S A<sup>1</sup>, Djellouli A<sup>1</sup>, Belhamra M<sup>4</sup>, Rahmoune C<sup>2</sup>, Ksouri R<sup>3</sup>

<sup>1</sup>Centre de Recherche Scientifique et Technique sur les Régions Arides, Campus Universitaire, BP 1682 R.P., Biskra 07000, Algeria.

<sup>2</sup>Laboratoire d'Ecotoxicologie et stress abiotiques, Dépt Biologie et Ecologie, Faculté SNV, Université des Frères Mentouri Constantine 1, Algeria.

<sup>3</sup>Laboratoire des Plantes aromatiques et médicinales, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisia.

<sup>4</sup>Laboratoire de Diversité des Ecosystèmes et Dynamiques des Systèmes de Production Agricoles en Zones Arides, Département d'agronomie, Université Mohamed Khider, Biskra 07000, Algérie.

Available Online: 1<sup>st</sup> May, 2016

### ABSTRACT

The mainly aims of this work is to study the interspecific variability of polyphenol contents and anti-radical activity of two species, *Acacia albida* and *Acacia raddiana* in order to select the best species in both respects, quantitative (phenolic content) and qualitative (antioxidant activity). Initially we performed a sequential extraction with four solvents: Hexane, Chloroform, Ethanol and Water on three organs leaves, fruit and bark, collected on field in the Tindouf region. The eighteen extracts obtained were assayed for total polyphenols by the *Folin-Ciocalteu* method and an evaluation of the antioxidant activity by testing the free radical DPPH. Results showed significant differences between organs of both species for the three extracting solvents as well as for the polyphenol contents and antioxidant properties. The ethanol extracts of *Acacia albida* exhibit the best results of total polyphenol concentration with 100.94 and 59.50 mg GAE.g<sup>-1</sup>DW, respectively for the leaves and bark. The IC<sub>50</sub> values of the DPPH test is in favor of leaves and bark of *Acacia albida* for both aqueous and ethanolic extracts. For the ethanolic extract we recorded IC<sub>50</sub> values of 28 and 26 µg.ml<sup>-1</sup> for leaves and bark, while the aqueous extract exhibited the values of 22.5 and 29 µg.ml<sup>-1</sup> for leaves and bark, respectively. Both extracts were analyzed by high performance liquid chromatography (HPLC-DAD). These results allow us to conclude that the *Acacia albida* is the most active species in comparison to *Acacia raddiana* and the ethanol and aqueous extracts of leaves and bark have achieved the best values.

**Keywords:** *Acacia albida*, *Acacia raddiana*, polyphenol, DPPH, leaves, bark.

### INTRODUCTION

Since independence, the Algerian government deployed an important efforts in order to remedy the restoration, conservation and development of natural resources through numerous programs namely; the privatization of farm management and also the emergence and application of the concept of sustainable development, which places a particular importance to the balanced development of natural resources of the territories and consequently to rural development, the launch of the NPR (National Programme for Reforestation) in 1999 and the NADP and PNDAR, the implementation of the lute convention against desertification and the one on Biological Diversity; and other policies related to heritage conservation and enhancement of the living standards of populations, including the rural population. In the same view, Algeria gives more importance to the maintenance and development of indigenous plant species. GDF (General

Directorate of Forestry) is among the organizations aimed at protecting, conserving and enhancing native species while remaining within a context of respect the environment. Some threatened species as *Acacia albida* and *Acacia raddiana* have the high priority to be protected given their endemism and interest ecologically and economically for arid regions. Arid and semi-arid zones are practical habitat for a number of herbaceous, shrub and tree also known as the rustic plants. These plants can be potentially useful for business applications such as new sources of natural antioxidants<sup>1</sup>. These habitats are exposed to various abiotic stresses (salinity, drought, heat and cold, light and other difficult environmental conditions), which induce oxidative stress in plants generating reactive oxygen species (ROS). These plants are able to resist and to staunch the toxics ROS because they are endowed with powerful antioxidant, enzymatic

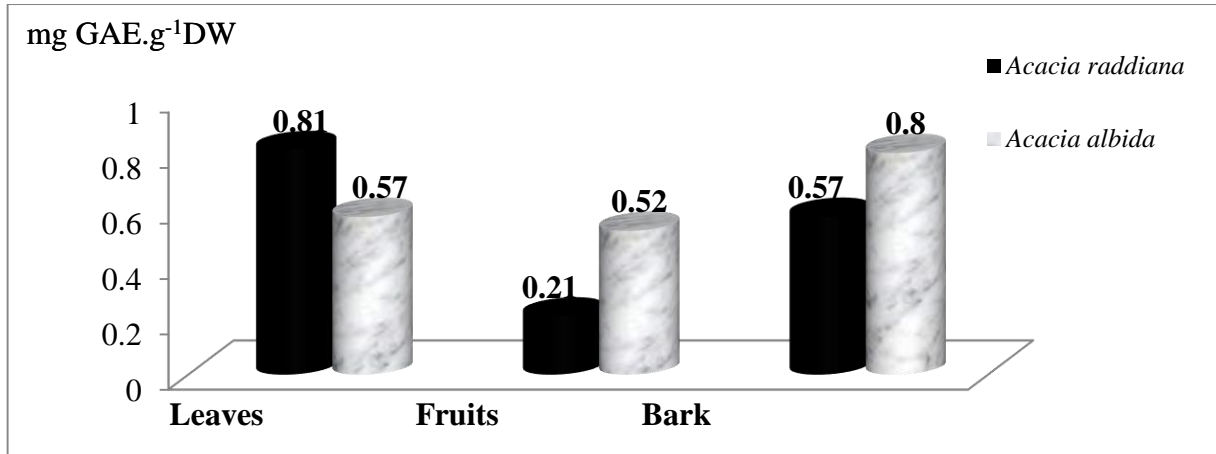


Figure 1: Total polyphenol content of the chloroform extract expressed in mg GAE.g<sup>-1</sup>DW of the three organs in *Acacia albida* and *Acacia raddiana*

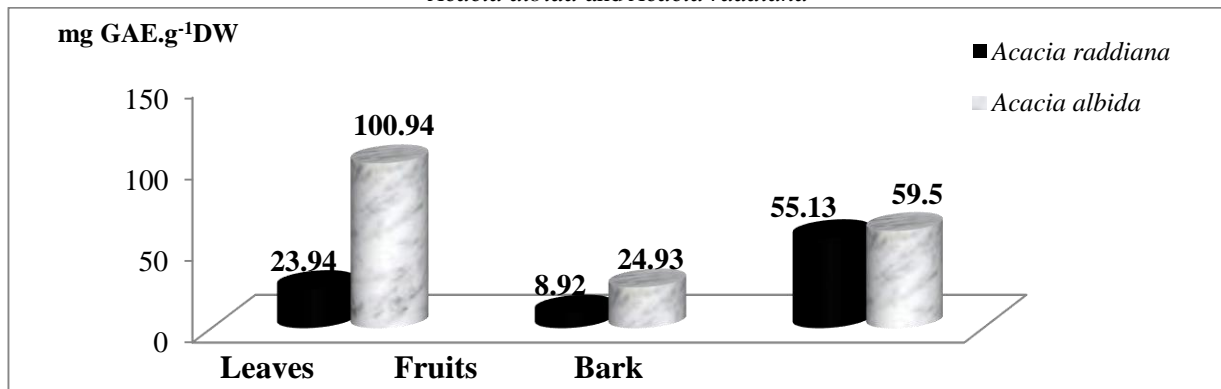


Figure 2: Total polyphenol contents of the ethanol extract EAG expressed in mg GAE.g<sup>-1</sup>DW of the three organs in *Acacia albida* and *Acacia raddiana*

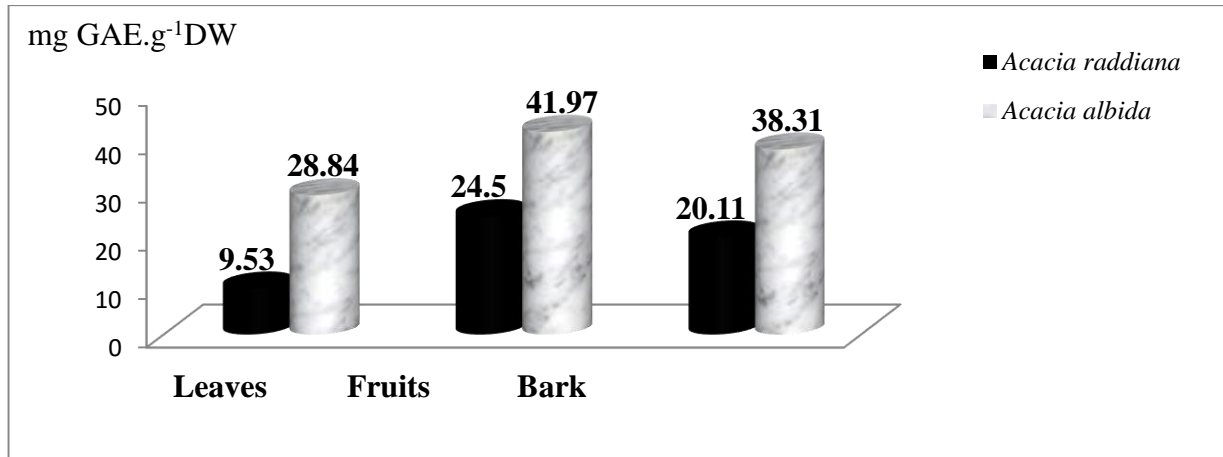


Figure 3: Total polyphenol contents of the aqueous extract expressed in mg GAE.g<sup>-1</sup>DW of the three organs in *Acacia albida* and *Acacia raddiana*

and non-enzymatic systems to overcome the harsh environmental conditions<sup>2,3</sup>. Among the various types of natural antioxidants, the polyphenols present a great importance, because of their multiple applications in the food industry, cosmetic, pharmaceutical and medicinal materials<sup>4</sup>. The Acacias have a multifunctional role as the food of the ruminants, provide wood for cooking and is also used as a source of drug to treat illnesses of livestock and other human diseases by most communities living in arid and semiarid regions of Africa<sup>5,6</sup>.

Indeed, the leaves of *Acacia raddiana*, with crushed beans, are used in the treatment of allergic contact dermatitis. They are also used in hair care and the treatment for ringworm. The fruits are used against inflammations and toothache. The bark of the *Acacia raddiana* has deworming properties and heal skin diseases, the powder of the dried bark is sprinkled on wounds to disinfect and heal them<sup>7</sup>. As the same for *Acacia albida*, that is a multipurpose tree with leaves and fruits fodder, wood, medicinal properties, and potentially nitrogen fixer. This tree was noted for its ability to improve soil fertility and

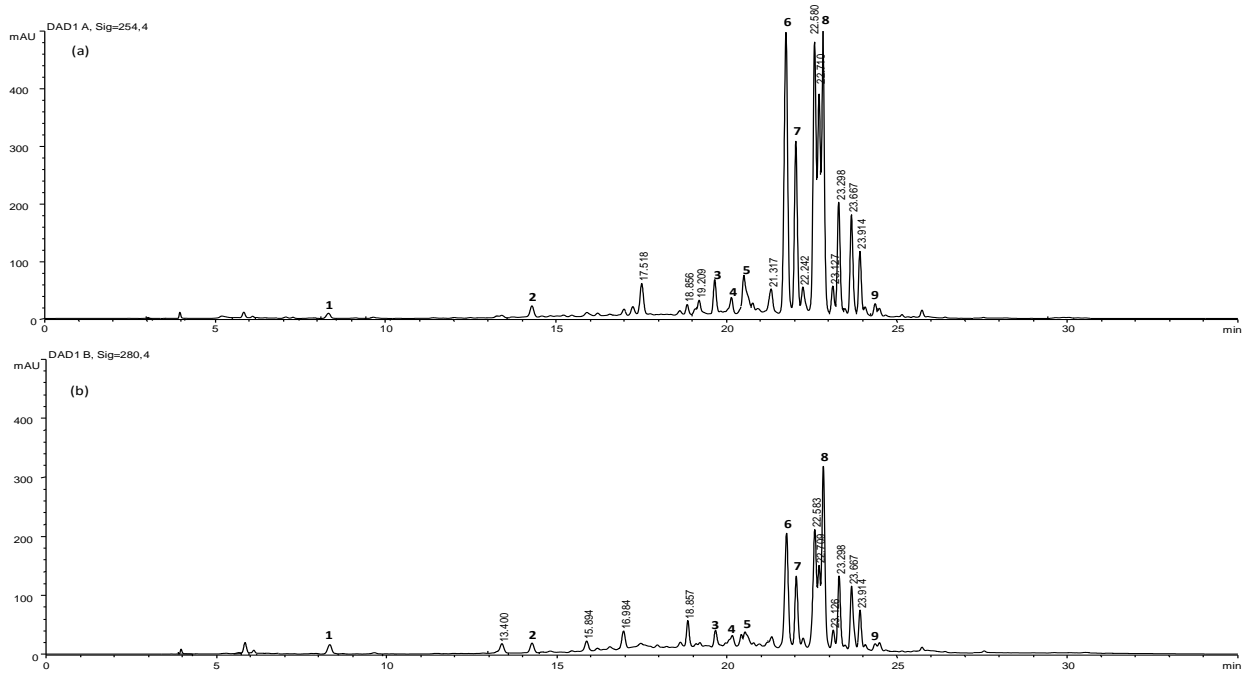


Figure 4: Chromatographic profiles of the ethanol extract of leaves recorded in UV: (a) UV chromatogram recorded at 254 nm, (b) UV chromatogram recorded at 280 nm. The peaks correspond to: (1) gallic acid, (2) - (-) Epigallocatechin (3) Resveratrol 3-O- glucoside, (4) P-coumaric acid, (5) ferulic acid, (6) Rutin (7) Oleuropein, (8) kaempferol-3-O rutinoside, (9) Quercetin.

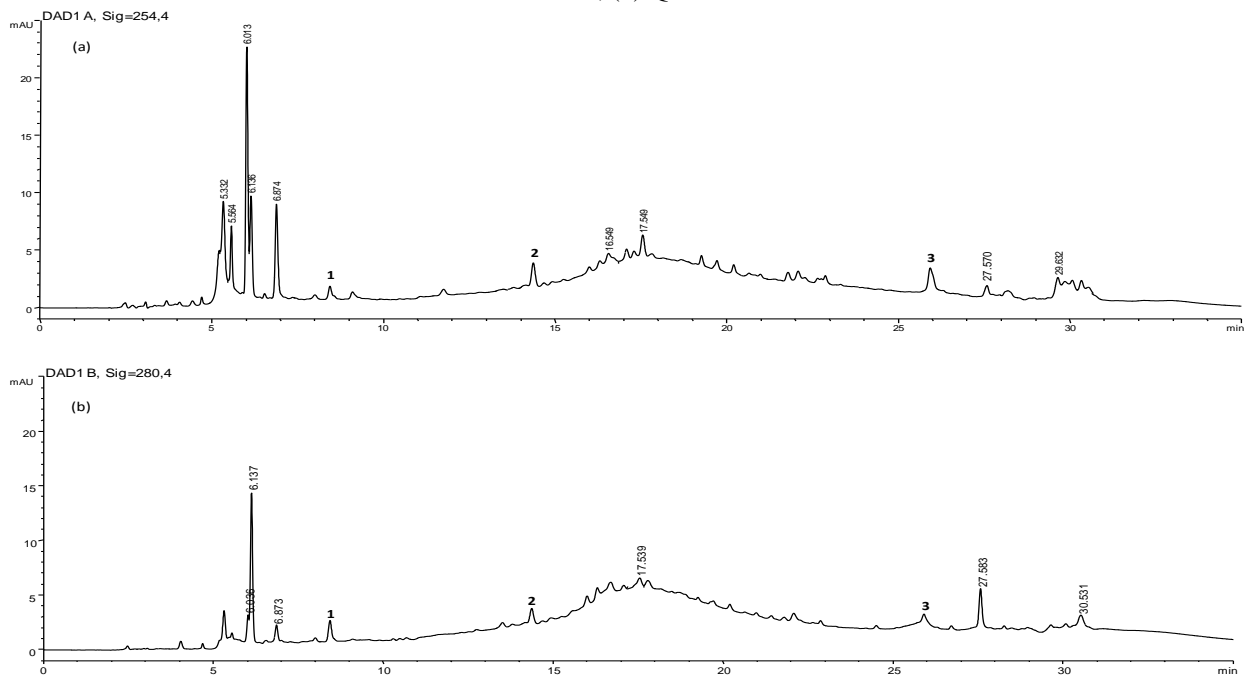


Figure 5: Chromatographic profiles of the aqueous extract of the leaves recorded in UV: (a) UV chromatogram recorded at 254 nm. (b) UV chromatogram recorded at 280 nm. The peaks correspond to: (1) Gallic acid, (2) - (-) Epigallocatechin, (3) Apigenin.

increase crop yields planted in the area of its shadow<sup>8</sup>. Phenolic composition is related to the genetic of the species<sup>9</sup>, and their quantification also depends on the solvent used for extraction. For example, absolute methanol was used for the extraction of tea polyphenols<sup>10</sup> and 50% of acetone for the extraction of total polyphenols

in wheat<sup>11</sup>, that proved to be more efficient than water. Furthermore, <sup>12</sup>reported that water and organic solvents used alone or in a mixture affect significantly the total polyphenol content of *Quercus coccifera* L. and *Juniperus phoenicea* L. It also appeared that the extracts of the same plant material depending on solvent, can vary widely on

Table 1: IC<sub>50</sub> values (expressed in µg. ml<sup>-1</sup>) in chloroform extract for DPPH test of the three organs of *Acacia albida* and *Acacia raddiana*.

	<i>Acacia raddiana</i>	<i>Acacia albida</i>
Leaves	190	165
Fruits	59	>200
Bark	61	100

Table 2: IC<sub>50</sub> values (expressed in µg. ml<sup>-1</sup>) of the ethanolic extract for DPPH test of the three organs of the *Acacia albida* and *Acacia raddiana*.

	<i>Acacia raddiana</i>	<i>Acacia albida</i>
Leaves	125	28
Fruits	165	138
Bark	175	26

the polyphenol concentration and antioxidant properties. In this context, <sup>13</sup>report that the water is found to be the best solvent for extracting tea catechins compare to methanol 80% and ethanol 70%. Therefore, the solubility of the phenolic contents is in fact determined by the type of solvent used, the degree of polymerization of phenolic contents, as well as by the interaction of the phenolic contents with other components<sup>14</sup>. The purpose of the present work is to establish a comparison between two species of *Acacia*, *Acacia albida* and *Acacia raddiana* using three solvents of increasing polarity (chloroform, ethanol and water) for three types of organs collected on land (leaves, fruit and bark). The comparison will be made at the basis by a quantitative study of total polyphenol and a qualitative study of the antioxidant properties while applying the test of DPPH free radical quenching. These two parameters allow us to choose the best species for possible recovery.

## MATERIALS AND METHODS

### Sample preparation

*Acacia albida* and *Acacia raddiana* species were selected based on the total lack of studies on this species in Algeria and their beneficial uses in traditional medicine. Samples of *Acacia albida* and *Acacia raddiana* (leaves, fruits and bark) were collected from the region of Tindouf in the extreme southwestern Algeria (28°28'15.6" N and 08°08'25.7" W Saharan climate). The harvested organs were rinsed with distilled water, left at room temperature for 6 days in the dark, oven-dried for one hour at 60 °C, and grinded to fine powder. A voucher specimen was deposited under number Ac 1324 at the herbarium of the Laboratory of Biochemistry, Scientific and Technical Research Center for Arid Areas (CRSTRA), Biskra, Algeria.

### Sample extraction

The extraction was performed by Soxhlet system where we opted for a successive extraction or by exhaustion by passing hexane in the first in order to eliminate all that is inactive (pigments and lipids) and then we made three successive solvents with increasing polarity, namely, ethanol, chloroform and finally water. The cycle of every solvent lasted 24 hours. The extract was filtered through a Whatman filter paper (N° 4); then recovered and stored in the darkness at 4 °C until analysis.

### Determination of total polyphenol content

Total polyphenol content (TPC) was determined according to the method of<sup>15</sup> with some modifications. An aliquot of diluted sample fraction was added to 0.5 ml distilled water and 0.125 ml Folin–Ciocalteu reagent. The mixture was shaken and incubated for 6 min before adding 1.25 ml Na<sub>2</sub>CO<sub>3</sub> (7%). The solution was then adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark, the absorbance was read at 760 nm versus a prepared blank. Total phenolic contents were expressed as milligrams gallic acid equivalents per gram dry residue (mg GAE.g<sup>-1</sup>DW) through the calibration curve with gallic acid. All samples were analyzed in triplicates

### Determination of 1,1-diphenyl-2-picryl hydrazyl radical scavenging activity (DPPH)

The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity was determined by the method of<sup>16</sup> with slight modifications. One milliliter of the extract at known concentrations was added to 0.5 ml of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature in the dark for 30 min. The absorbance was then measured at 517 nm and corresponds to the extract ability to reduce the radical DPPH to the yellow-coloured diphenylpicrylhydrazine. BHT was a synthetic phenolic used as positive standard. The antiradical activity was expressed as IC<sub>50</sub> (µg.ml<sup>-1</sup>), the antiradical dose required to cause a 50% inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] * 100 \quad (1)$$

Where A<sub>0</sub> is the absorbance of the control at 30 min, and A<sub>1</sub> is the absorbance of the sample at 30 min. All samples were analyzed in triplicates.

### Analysis of polyphenols using high performance liquid chromatography (HPLC)

The identification of phenolic compounds in *Acacia albida* and *Acacia raddiana* organs was done using an HPLC system (consisting of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 600 bar; Agilent 1260, Agilent technologies, Germany) equipped with a reversed phase C18 analytical column of 4.6 x 100 mm and 3.5µm particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200-400 nm. Column temperature was maintained at 25°C. For two extracts (ethanol and water), the injected sample volume was 2 µl and the flow-rate of mobile phase was 0.4 ml.min<sup>-1</sup>. Mobile phase B was milli-Q water consisted of 0.1% formic acid and mobile phase A was methanol. The following linear gradient was applied: 10% A; 0-10 min, 20% A; 5-10 min, 30% A; 10-15; 50% A; 15-20 min, 70% A; 20-25 min, 90% A; 25-30 min; 50% A 30-35 min; and finally 10% A. Identification of phenolic compounds was performed by comparing the retention times of peaks obtained for those of the standard phenolic compounds injected in the same chromatographic conditions.

### Statistical analysis

All analyses were done in triplicates. Results were expressed as means ± standard deviations. The data were

statistically analysed using the Minitab. 2000 statistical software. An independent *t*-test was used for comparison of means between groups. One-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference test were used to compare means among groups. A Pearson correlation test was used to study the relationship between the antioxidant components and the antioxidant activities. The level of significance was set at  $p < 0.05$ .

## RESULTS

Total polyphenol contents in different organs of the *Acacia albida* and *Acacia raddiana*

### Chloroformic extract

The assessment of the total polyphenol content using the Folin Ciocalteu reagent indicates that the *Acacia albida* is richer in phenolic compound relative to *Acacia raddiana*. (Figure 1). The results in Figure 1 indicates that the total polyphenol content of the chloroform extract is very low that is located between 0.21 and 0.81 mg GAE.g<sup>-1</sup>DW m, however, by comparing the organs of these two species we can conclude that there is a difference for fruit and bark in the favor of the *Acacia albida*, while this difference for leaves is more marked in *Acacia raddiana*.

### Ethanol extract

The results of total polyphenol contents of the ethanol extract expressed in mg GAE.g<sup>-1</sup>DW of the three organs in *Acacia raddiana* and *Acacia albida* are shown in Figure 2. The results of the Figure 2. show a very important intraspecific variability based on organ and interspecific depending on the species. This variability is more significant in the leaves where we noted a total polyphenol concentration 100.94 mg GAE.g<sup>-1</sup>DW for *Acacia albida* against a value of 23.94 for mg GAE.g<sup>-1</sup>DW *Acacia raddiana*. For the bark, the values are close where we noted 59.5 and 55.13 mg GAE.g<sup>-1</sup>DW for the *Acacia albida* and *Acacia raddiana* respectively. The lowest values are observed for the fruit with 24.93 mg GAE.g<sup>-1</sup>DW for *Acacia albida* and 8.92 mg GAE.g<sup>-1</sup>DW for *Acacia raddiana*.

### Aqueous extract

The results obtained from the assays of total polyphenols performed on leaves, fruit and bark of *Acacia albida* and *Acacia raddiana* are summarized in Figure 3. Figure 3 shows that there are differences in the total polyphenol capacity from one species to another for three organs with a balance in favor of *Acacia albida*. For the aqueous extract, the fruits that are exhibit the best result as we have noted 41.97 and 24.5 mg GAE.g<sup>-1</sup>DW for *Acacia albida* and *Acacia raddiana* respectively. For bark, total polyphenol amount is almost double in *A. albida* compared to *A. raddiana* with 38.31 mg GAE.g<sup>-1</sup>DW. The leaves of the *Acacia albida* contain 28.84 mg GAE.g<sup>-1</sup>DW which is a trip le value in comparison with that of the *Acacia raddiana*.

### Quenching capacity of DPPH radical in different organs of the *Acacia albida* and *Acacia raddiana*

As the phenolic compound, the antioxidant properties for quenching free radical DPPH showed significant variability between the two investigated species. The use of this test showed that the antioxidant capacity is very

different between *Acacia albida* and *Acacia raddiana*, usually higher for *Acacia albida*. The differences between the IC<sub>50</sub> values are usually very meaningful for the three extracting solvents used.

### Chloroform extract

The chloroform extract exhibits an inter- and intra-specific variability where the IC<sub>50</sub> values ranged from 59 to over 200 µg. ml<sup>-1</sup> (Tableau.1). Comparing the ability to trap the radical DPPH between the two species, we find that the results are in favor of fruit and bark of *Acacia raddiana* and their values are two and four times higher than those recorded for *Acacia albida*. While for leaves, the IC<sub>50</sub> is more or less better in *Acacia albida*.

### Ethanol extract

The result of the IC<sub>50</sub> values are recorded in the table.9. According to this table, we see that all the results are in favor of the *Acacia albida* where the extract of the bark is in the first place with IC<sub>50</sub> equal to 26 µg. ml<sup>-1</sup> followed by the extract leaves with 28µg.ml<sup>-1</sup>. These values are five to six times higher than those recorded for *Acacia raddiana*. The values recorded for fruit extracts are similar.

### Aqueous extract

The result of the quenching of the radical DPPH for the aqueous extract is shown in tableau.3. As for the ethanol extract, all results on the ability to trap the DPPH radical are in favor of the *Acacia albida*, we noted IC<sub>50</sub> values of 22.5, 29 and 55 µg. ml<sup>-1</sup> for bark leaves, and fruits, respectively.

### Identification of phenolic content by HPLC

For the HPLC analysis, we chose to compare the two extracts, ethanol and aqueous of leaves of *Acacia albida* for their important capacity in polyphenols and because their good antioxidant activity against the free radical DPPH. Identification of phenolic contents was performed by comparing the retention times of peaks obtained for those phenolic content standards injected in the same chromatographic conditions.

### Ethanol extract

The analysis of the ethanol extract of leaves of *Acacia albida* by HPLC revealed that this plant contains phenolic contents. Nine compound were identified in comparison with standards injected in the same chromatographic conditions. The chromatogram of the ethanol extract of leaves recorded in UV at 254 nm and 280 nm is represented by the Figure 4. According to Figure4, analysis of the chromatogram of the ethanol extract revealed the presence of three kinds of phenolic contents: phenolic acids, four types of flavonoids and one from the class of stilbenes. Phenolic acids identified in this extract is gallic acid, p-coumaric acid and ferulic acid (eluted at 8.323, 20.159 and 20.526 min respectively). The detected flavonoids are Epigallocatechin, Rutin, kaempferol-3-O rutinose and quercetin (eluted 14.284, 21.749, 22.836 and 24.362 min respectively). Regarding the Resveratrol 3- O- glucoside, which is part of the class of stilbenes, was eluted at 19.669 min.

### Aqueous extract

Analysis of the aqueous extract of the leaves of *Acacia albida* by HPLC revealed that this fraction contains three phenolics contents. These were identified by comparison

Table 3: IC<sub>50</sub> values (expressed in µg. ml<sup>-1</sup>) of the aqueous extract for the DPPH test of the three organs of *Acacia albida* and *Acacia raddiana*.

	<i>Acacia raddiana</i>	<i>Acacia albida</i>
Leaves	199	22.5
Fruits	80	55
Bark	175	29

with standards injected in the same chromatographic conditions. The ion chromatogram of the aqueous extract of the leaves recorded in UV at 254 nm and 280 nm is shown in figure 5. Regarding the aqueous extract of the leaves of *Acacia albida*, we observed a different phenolic profile of the ethanolic extract. Indeed, this chromatogram (Figure 5.) is composed of three phenolic contents; gallic acid which is a phenolic acid eluted at 8444 min and two flavonoids that are Epigallocatechin and Apigenin eluted at 14 365 and 25.927 min, respectively.

## DISCUSSION

In this study, the protective effect of antioxidant of *Acacia raddiana* and *Acacia albida* was measured in vitro by a method of quenching free radicals. The total polyphenol content of the different organs of the two species was also assessed. The organs of the two species were collected in the Tindouf area belonging to the Saharan bioclimatic stage. Our data show that the two studied species present a significant intra-specific variability in their polyphenolic content and in their anti-radical activity. Based on the absorbance values after reaction with the Folin-Ciocalteu reagent, the results of the colorimetric analysis are given by the figures. 1, 2 and 3. These results show a large variability of phenolic contents according to the species, organ and the extraction solvent under ANOVA statistical analysis. In terms of absorbance values after reaction with the Folin-Ciocalteu reagent, the results of the colorimetric analysis are given by the figures.1, 2 and 3. These results show a large variability of phenolic compounds according to the species, the organs and the extraction solvent depending on ANOVA statistical analysis. Regarding the total content of phenolic compounds, ethanolic extracts are those which gave the highest total phenol contents, reaching a value of 100.94 mg GAE.g<sup>-1</sup>DW, followed by aqueous extracts with a maximum value of 41.97 mg GAE.g<sup>-1</sup>DW and finally chloroform extracts with a value that not exceeding 0.81 mg GAE.g<sup>-1</sup>DW. In this sense, several studies have shown that the polyphenol content differs with the polarity of the extraction solvent<sup>17</sup>. First, intraspecific variability is also pronounced where we recorded a significant amount of total polyphenol in favor of *Acacia albida* where the maximum values reached 100.94 mg GAE.g<sup>-1</sup>DW and while the highest value recorded in *Acacia raddiana* is nearly half corresponding to 55.13 mg GAE.g<sup>-1</sup>DW. This difference is probably linked to the genetic of variety. Earlier works by<sup>18,9</sup> have previously reported that the phenolic content in plants is bound to the genetic of the species. Furthermore, we also noted an interspecific variability between the different organs of the same species. For example in *Acacia albida* for the ethanol extract, the leaves contain 100.94 mg

GAE.g<sup>-1</sup>DW of total polyphenols, followed by the bark with 59.5 mg GAE.g<sup>-1</sup>DW and finally the fruit with 24.93 mg GAE.g<sup>-1</sup>DW. Comparing our results with previous works, we can say that the total content of phenols of *Acacia albida* is significantly higher compared to other halophytes medicinal plants such as *Tamarix gallica* with (34.44 mg GAE.g<sup>-1</sup>DW) and other species like glycophytes *Nigella sativa* L. with (10.04 mg GAE.g<sup>-1</sup>DW)<sup>19,20</sup>. For the evaluation of the antioxidant activity of different extracts of *Acacia albida* and *Acacia raddiana*, the fast and reliable test of free radical quenching DPPH, was realized in vitro. DPPH is a free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule<sup>21</sup>. The capacity of reduction of the radical DPPH has been determined by the reduction of the absorbance induced by the plant antioxidants. The BHT is the reagent used like standard. As the capacity of polyphenols, the results of the quenching capacity of the DPPH radical followed the same trend as the ethanol and aqueous extracts and they give the best IC<sub>50</sub> compared to the chloroform extract. This is probably due to the very low concentration of phenolic compounds of this latter extract. Our findings join those found by<sup>22</sup> who compared the effect of three extraction solvents containing ethanol, ethyl acetate and hexane, respecting quenching the free radical DPPH. His results showed that the best IC<sub>50</sub> (116 µg.ml<sup>-1</sup>) is recorded by the ethanol extract which confirms partially our results those we have obtained on the Acacia. It is extremely important to note that some studies have reported that there is a positive correlation between the antioxidant potential and phenolic content estimated by the Folin-Ciocalteu method<sup>23</sup>. Therefore, the high content of total phenols in the ethanolic extracts may explain the strong antioxidant property of *Acacia albida*, mainly in the leaves and in the bark. In fact, the meaningful relationships between phenolic concentration and antioxidant efficiency have been reported in *Suaeda maritima*<sup>24</sup> and *Cakile maritima*<sup>23</sup>. However, although the aqueous extract showed a lower richness than the ethanol extract, it exhibits an anti-radical activity similar to the latter. Studies have demonstrated that the antioxidant activity against free radical DPPH depends not only on the high degree of polyphenols but also the phenolic nature, structure and synergistic interactions<sup>14</sup>. Comparing our results with previous studies indicate that *A. albida* is more active compared to *Argania spinosa* in which fruit extracts<sup>25</sup> exhibited IC<sub>50</sub> equal to 32.3 µg.ml<sup>-1</sup>. In addition, the anti-radical activity of the extracts of Acacia is better in comparison with other halophytes medicinal plants such as *Salicornia herbacea* (IC<sub>50</sub> = 55.3 µg.ml<sup>-1</sup>)<sup>26</sup> and other glycophyte species as *Pisonia alba* and *Centella asiatica* (IC<sub>50</sub> = 175 µg.ml<sup>-1</sup> and 200 µg.ml<sup>-1</sup>, respectively)<sup>27</sup>. The analysis of phenolic compounds by HPLC confirmed the results gotten of the dosage of the total polyphenols by spectrophotométrie, where we could identify nine molecules in the ethanol extract of leaves against only three molecules in the aqueous extract. Both extracts exhibited a strong antioxidant activity to neutralize the free radical DPPH, this high activity of *Acacia albida* leaves could be attributed to the presence of phytochemicals

compounds as phenolic contents<sup>28,29</sup>. In this sense, many studies have shown that a few polyphenols contribute significantly to the antioxidant activity of many fruits and vegetables<sup>30,31</sup> and for medicinal plants<sup>19</sup>. Many phenolic contents identified in the leaves of *Acacia albida* are known for their antioxidant power.

Gallic acid is a phenolic acid which has been reported as a compound with numerous biological activities as the quenching of the free radical<sup>32,33</sup> and disruption of the signal paths that generates the reactive species of the oxygen<sup>34-36</sup>. It was demonstrated that p-coumaric acid is an antioxidant which acts against the free radicals<sup>37</sup>. Ferulic acid is also a phenolic acid which the specificity is to eliminate reactive species of the oxygen and the free radicals. These antioxidant properties were confirmed by many studies as<sup>38-43</sup>. Epigallocatechin is a flavonoid known to neutralize excessive amounts of reactive species of the oxygen such as superoxide anion, hydroxyl radicals and singlet oxygen<sup>44,45</sup>. The rutin is also a flavonoid known as Vitamin P, it has the ability to strengthen blood capillary vessels which is the result of its high antioxidant activity and its ability to trap free radicals<sup>46</sup>. The quercetin as the kaempferol-3-O rutinolide are flavonoids known for their high antioxidant activity and for increasing the stress resistance<sup>38,47,48</sup>. demonstrated that apigenin is a flavonoid with a significant antioxidant activity and inhibits the generation of reactive species of the oxygen. Several studies have reported that there is a significant and positive correlation between the levels of phenolic contents and anti-radical activity<sup>49,17</sup>. However, this is not the case of our results which we obtained a significant DPPH anti-radical activity of the aqueous extract that contains fewer polyphenols in comparison with the ethanol extract. This can be explained by the results obtained by<sup>16</sup> who estimates that the existence of a synergy between the various phenolic contents can be decisive in the antioxidant capacity from a given plant. So this activity does not only depend on the polyphenol content but also on the structure and the interaction between different compounds. In addition, phenolic contents of an extract may act synergistically or antagonistically or whether which influences the final antioxidant activity of the extract<sup>50-52</sup>. As a conclusion to this work, it should be said that the quantitative study of phenolic compounds and evaluation of antioxidant activity throughout the test of quenching the free radical DPPH of these three extracts of *Acacia albida* and *Acacia raddiana* reveals significant variability between both species and between extraction solvents. In general, *Acacia albida* has distinguished of *Acacia raddiana* by its richness in phenolic compounds as well as its antioxidant property. The ethanol and aqueous extracts of leaves and bark are those that gave the most significant results. This first selection allowed to retain these two organs extracts of *Acacia albida* on which will be conducted other analysis in the continuation of our work.

#### ACKNOWLEDGEMENTS

The authors wish to thank Algerian Ministry of Higher Education and Scientific Research for financial support.

#### REFERENCES

1. Meot-Duros L, Le Floch G, Magné C. Radical scavenging, antioxidant and antimicrobial activities of halophytic species. *J. Ethnopharmacol* 2008 ; 116 : 258-262.
2. Ben Hamed K, Ben Youssef N, Ranieri A, Zarrouk M, Abdelly C. Changes in content and fatty acid profiles of total lipids and sulfolipids in the halophyte *Crithmum maritimum* under salt stress. *J. Plant. Physiol* 2005 ; 162 : 599-602.
3. Jithesh MN, Prashanth SR, Sivaprakash KR, Parida AK. Antioxidative response mechanisms in halophytes : their role in stress defence. *J. Genetics* 2006 ; 85 : 237-254.
4. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chem* 2007 ; 4 : 1409-1418.
5. New TR. *A biology of acacias*, Oxford University Press Melbourne 193.
6. Grade JT, Tabuti JRS, Van Damme P. Ethnoveterinary knowledge in pastoral Karamoja, Uganda. *Journal of Ethnopharmacology* 2009 ; 122 : 273-293.
7. Bellakhdar J. *La pharmacopée marocaine traditionnelle* (Paris, Ibis Press), 1997 ; 764 p.
8. Vandenberg R.J. 1992. *Faidherbia albida* in the West African Semi-arid Tropics Proceedings of a Workshop, 22-26 April 1991, Niamey, Niger. ICRISAT & ICRAF, Patancheru, A.P. 502324, India. 206 pp.
9. De Abreu N, Mazzafera P. Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiol. Biochem* 2005 ; 43 : 241-248.
10. Yao LH, Jiang YM, Caffin N, D'Arcy B, Datta N, Liu X, Singanusong R, Xu Y. Phenolic compounds in tea from Australian supermarkets. *Food. Chem* 2006 ; 96 : 614-620.
11. Zhou K, Yu LL. Antioxidant properties of bran extracts from trego wheat grown at different locations. *J. Agric. Food. Chem* 2004 ; 52 : 1112-1117.
12. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chemistry* 2007 ; 105 : 1126-1134.
13. Khokhar S, Magnusdottir SGM. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *J Agric Food Chem* 2002 ; 50 : 565-570.
14. Djeridane M, Yousfi B, Nadjemi D, Boutassouna P, Stocker N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *J. Agric. Food Chem* 2006 ; 97 : 654-660.
15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Amer. J. Enol. Viticult* 1965 ; 16 :144-58.
16. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technology* 1995 ; 28 :25-30.

17. Trabelsi N, Megdiche W, Ksouri R, Falleh H, Oueslati S, Bourgou S, Hajlaoui H, Abdelly C. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. *LWT - Food Science and Technology* 2010 ; 43 : 632–639.
18. Fratianni F, Tucci M, De Palma M, Pepe R, Nazzaro F. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). *Food Chem* 2007 ; 104 : 1282-1286.
19. Bourgou S, Ksouri R, Skandrani I, Chekir-ghedira L, Marzouk B. Antioxidant and antimutagenic activities of the essential oil and methanol extract from Tunisian *Nigella sativa* L. (Ranunculaceae). *Italian Journal of Food Science* 2008 ; 2 : 191-202.
20. Ksouri R, Falleh H, Megdiche W, Trabelsi N, Hamdi B, Chaieb K, Bakhruf A, Magné C, Abdelly C. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L and related polyphenolic constituents. *Food Chem. Toxicol* 2009 ; 47 : 2083-2091.
21. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant Activities of some Extracts of *Thymus zygis*. *Free Radical Research* 1997 ; 26 : 469-478.
22. Srikanth S, Pragathi BH. Pulmonary function tests in type ii diabetics in correlation with fasting blood glucose. *Int J Med Res Health Sci* 2013 ; 2 (4) :756-761.
23. Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritime*. *Plant Physiology and Biochemistry* 2007; 45: 244-249.
24. Gazala S, Pelletier JS, Storie D, Johnson J, Demetrios J, Kutsogiannis, Bédard E. A Systematic Review and Meta-Analysis to Assess Patient-Reported Outcomes after Lung Cancer Surgery, 2013 ; <http://dx.doi.org/10.1155/2013/789625>
25. El Babili F, Bouajila J, Fouraste I, Valentin A, Mauret S, Moulis C. Chemical study, antimalarial and antioxidant activities, and cytotoxicity to human breast cancer cells (MCF7) of *Argania spinosa*. *Phytomedicine* 2010; 17: 157-160.
26. Essaidi I, Brahmi Z, Snoussi A, Ben Haj Koubaier H, Casabianca H, Abe N, El Omri A, Chaabouni MM, Bouzouita N. Phytochemical investigation of Tunisian *Salicornia herbacea* L., antioxidant, antimicrobial and cytochrome P450 (CYPs) inhibitory activities of its methanol extract. *Food Control* 2013; 32: 125-133.
27. Subhasree B, Baskar R, Keerthana RL, Susan RL, Raja sekaran P. Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chem* 2009 ; 115 : 1213 1220.
28. Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdelly C. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Compt. Rend. Biol* 2008 ; 331 : 372-379.
29. Oueslati S, Ksouri R, Falleh H, Pichette A, Abdelly C, Legault J. Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. *Food Chemistry* 2012 ; 132 : 943–947.
30. Negro C, Tommasi L, Miceli A. Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology* 2003 ; 87 : 41-44.
31. Luo ZX, Kielan-Jaworowska Z, Cifelli RL. In quest for a phylogeny of *Mesozoic mammals*. *Acta Palaeontologica Polonica* 2002 ; 47 (1) : 1–78.
32. Kanai S, Okano H, Abe H. Efficacy of toki-shigyaku-ka-gosyuyu-syokyo-to on peripheral circulation in autonomic disorders. *Am J Chin Med* 1998 ; 25 : 69–78.
33. Dwibedy P, Dey GR, Naik DB, Kishore K, Moorthy PN. Pulse radiolysis studies on redox reaction of gallic acid: one electron oxidation of gallic acid by hallic acid OH adduct. *Phys. Chem. Chem. Phys* 1999 ; 1 : 1915–1918.
34. Sakaguchi S, Nishiyama Y, Ishii Y. Selective oxidation of monoterpenes with hydrogen peroxide catalyzed by peroxotungstophosphate (PCWP). *J. Org Chem* 1996 ; 61 : 5307-5311.
35. Inoue YH, Do Carmo Avides M, Shiraki M, Deak P, Yamaguchi M, Nishimoto Y, Matsukage A, Glover DM. Orbit, a novel microtubule-associated protein essential for mitosis in *Drosophila melanogaster*. *J. Cell Biol* 2000 ; 149(1): 153-166.
36. Sohi KK, Mittal N, Hundal MK, Khanduja KL. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes : a Bcl-2 independent mechanism. *J. Nutr. Sci. Vitaminol* 2003 ; 49 : 221–227.
37. Laranjinha J, Almeida A, Madeira V. Reactivity of dietary phenolic acids with peroxy radicals : antioxidant activity upon low density lipoprotein peroxidation. *Biochem.Pharmacol* 1994 ; 48 : 487-494.
38. Graf E. Antioxidant potential of ferulic acid. *Free Radic Biol Med* 1992 ; 13(4) : 435-48.
39. Nabi G, Liu ZQ. Ferrocenyl chalcones: antioxidants or prooxidants in radical-induced oxidation of DNA?. *Med Chem Res* 2012 ; 21 : 3015–3020.
40. Niwa Y, Akamatsu H, Niwa H, Sumi H, Ozaki Y, Abe A. Correlation of tissue and plasma RANTES levels with disease course in patients with breast or cervical cancer. *Clin Cancer Res* 2001 ; 7(2) : 285-9.
41. Ogiwara T, Satoh K, Kadoma Y. Radical scavenging activity and cytotoxicity of ferulic acid. *Anticancer Res* 2002 ; 22 : 2711–2717.
42. Rice-Evans CA, Miller JM, Paganga G. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic. Biol. Med* 1996 ; 20 : 933-956.
43. Xiaokun S, Antoine R, Michael ZL, Todd AA, Varda L, Paul A, Roger Y. Mammalian Expression of Infrared Fluorescent Proteins Engineered from a Bacterial Phytochrome. *Science* 2009 ; 324 : DOI :10.1126/science.1168683.
44. Yang H, Pfister S, Bhaduri A. Accounting for a scarce resource : virtual water and water footprint in the global



- water system. *Current Opinion in Environmental Sustainability* 2013 ; 5(6) : 599-606.
45. Lambert JD, Elias RJ. The antioxidant and pro-oxidant activities of green tea polyphenols : a role in cancer prevention. *Arch Biochem Biophys* 2010 ; 501(1) : 65-72.
46. Guo Q, Johnson CA, Unger JB, Lee L, Xie B, Chou CP, Pentz M. Utility of the theory of reasoned action and theory of planned behavior for predicting Chinese adolescent smoking. *Addictive Behaviors* 2007 ; 32(5) : 1066-1081.
47. Kampkötter A, Timpel C, Zurawski RF, Ruhl S, Chovolou Y, Proksch P, Wätjen W, Kampkötter A, Timpel RF, Zurawski. Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. *Comp Biochem Physiol B Biochem Mol Biol* 2008 ; 149(2) : 314-23.
48. Sreemanti D, Das J, Samadder A, Paul A, Khuda-Bukhsh A. Strategic formulation of apigenin-loaded PLGA nanoparticles for intracellular trafficking, DNA targeting and improved therapeutic effects in skin melanoma *in vitro*. *Toxicology letters* 2013 ; 223 (2) : 124-138.
49. Hanson GT, Aggeler R, Oglesbee D, Cannon M, Capaldi RA, Tsien RY. Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators. *J. Biol. Chem* 2004 ; 279 : 13044–13053.
50. Troszyńska A, Ciska E. Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum L.*) and their total antioxidant activity. *Czech J. Food Sci* 2002 ; 20 : 15–22.
51. Subba Rao MV, Muralikrishna G. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (ragi, *Eleusine coracana* Indaf 15). *J Agric Food Chem* 2002 ; 50(4) : 889-92.
52. Lim YY, Quah EPL. Antioxidant Properties of Different Cultivars of *Portulaca oleracea*. *Food Chemistry* 2007 ; 103 : 734-740.