Introduction

Plants produce a wide range of secondary metabolites that exhibit antioxidant activities such as phenolic acids, flavonoids, quinines and coumarins, nitrogen compounds (alkaloids and amines), vitamins, terpenoids and others. Free radicals (FR) and reactive oxygen species (ROS) are produced through physiological and biochemical processes in the human body. ROS includes a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻), hydroxyl radical (OH⁻), etc. Overproduction of such free radicals might lead to oxidative damage of biomolecules in the body (e.g., lipids, proteins and DNA), that can initiate diseases such as atherosclerosis, diabetes mellitus, cancer and heart and neurodegenerative diseases.

As polyphenols were found to be beneficial as strong antioxidants, the evaluation of polyphenols and antioxidant activity has become important to understanding the healing property of medicinal plants that provide opportunities for new drugs.

Among secondary metabolites produced by vegetables, flavonoids constitute an important group of natural polyphenols among which flavonoids and anthocyanins are recognized to exhibit a vast range of biological effects.

A number of plants have therapeutic potential, such as *Illicium verum* (badiane or star anise), *Crataegus oxyacantha* ssp monogyna (hawthorn) and *Allium cepa* (onion). *I. verum* (*Schisandraceae*) is a little tree that grows in the south of China and north of Vietnam. It is an aromatic plant which produces oils such as anethol and contains some polyphenols, including flavonols (quercetin and kaempferol), anthocyanins, tannins and phenolics acids like shikimic and gallic acid. Antimicrobial, antifungal and antioxidant activities have been reported for *I. verum*.

The seeds of *C. oxyacantha* ssp monogyna (*Rosaceae*) are rich in flavonoids, and the plant is considered as a cardiotonic, diuretic and antispasmodic. A. cepa L (*Amaryllidaceae*), contains many flavonoids, with quercetin as the most abundant flavonol, oils, organosulfuric compounds and saponins. It has been attributed with anti-inflammatory, anticancer, antimicrobial, antiparasitic and antioxidant activities.

The aim of this study was to determine antioxidant and antibacterial effects of aglycones (flavones/flavonols) and anthocyanins extracts from *I. verum*, *C. oxyacantha* ssp monogyna and *A. cepa*.
Results

Total flavonols and anthocyanins content. Quantitative spectrophotometric study of the extracts of I. verum (badiane), C. oxyacantha ssp monogyna (hawthorn) and A. cepa (onion), showed total flavonols contents ranging from 103 μg/g to 1.65 μg/g. Inner layers extract of the red onion had the highest content (173 ± 0.69 μg/g), followed by outer layers of the white onion (143 ± 0.21 μg/g) and the leaves of hawthorn (143 ± 0.61 μg/g). The berry extract of the C. oxyacantha ssp monogyna (hawthorn) (1.65 ± 0.61 μg/g), outer layers of the red onion (2.37 ± 0.10 μg/g) and the inner layers of the white onion (negligible) exhibited the lowest flavonol contents (Fig. 1).

The anthocyanins content varied between 5.31 ± 0.27 and 0.045 ± 0.003 mg/g. C. oxyacantha ssp monogyna (hawthorn) berry extract presented the highest content (5.31 ± 0.266 mg/g), followed by hawthorn leaves (2.96 ± 0.9 mg/g) and the outer layer of red onion (1.283 ± 0.569 mg/g). The white onion had the lowest content with 0.045 ± 0.0335 mg/g for the inner layer and 0.077 ± 0.001 mg/g for the outer layer (Fig. 2). The outer layer of white and red onion had the highest proportions in flavonols with 27.76% and 7.73%.

The respective proportions in anthocyanins were 72.23% and 92.56%.

Evaluation of anti-oxidant capacity. The antioxidant activity of plant extracts was expressed as IC_{50} (half inhibitory concentration). The results were compared with those of quercetin.

| Plants      | IC_{50} (mg/mL) | Quercetin IC_{50} |
|-------------|-----------------|-------------------|
| Hawthorn    | 2.36 × 10^{-5}  | 1.33 × 10^{-4}    |
| Red onion   | 7.53 × 10^{-4}  | 5.89 × 10^{-3}    |
| Ascorbic acid| 2.75 × 10^{-4}  |                   |

Table 1. Free radical scavenging activity and reducing power in tested plants on dry weight basis.

Evaluation of anti-bacterial activity. The antibacterial activity of the plant extracts was evaluated in vitro against four bacterial test species known to cause human diseases. Extracts of A. cepa L. and C. oxyacantha ssp monogyna were the most effective (Table 2). The largest inhibition zone was observed with flavonols of the inner layer of A. cepa L. “Red Onion” (40 mm), inhibiting the Gram-negative E. coli. Flavonol extracts from I. verum and C. oxyacantha ssp monogyna were slightly effective against E. coli with an inhibition zone of 18 mm and 12 mm respectively.

Figure 1. Flavonols content in micrograms per gram “quercetin” expressed as (DW). 1, Illicium verum; 2, C. oxyacantha ssp monogyna berries; 3, C. oxyacantha ssp monogyna leaves; 4, inner layers of red onion; 5, outer layers of red onion; 6, inner layers of white onion; 7, outer layers of white onion.

Figure 2. Anthocyanins content (mg/g−1) “Cyanidin-3-glucoside” (DW). 1, Illicium verum; 2, C. oxyacantha ssp monogyna berries; 3, C. oxyacantha ssp monogyna leaves; 4, inner layers of red onion; 5, outer layers of red onion; 6, inner layers of white onion; 7, outer layers of white onion.
harmful effects of UVB radiation in the 280–315 nm range.15 Cells of leaves where they protect the parenchyma against the noids than the berries as aglycones accumulate in epidermal monogyna which explains the richness of ssp C. oxyacantha Anthocyanins are responsible for the color of flowers and fruits, extracts of the three plants exhibited very high antioxidant properties as compared with standard antioxidants (quercetin and kaempherol). Flavonols of the outer layer of the red onion had the highest antioxidant activity as compared with the white variety. This result could be linked to its richness in quercetin. Overall, aglycones had a higher antioxidant activity as compared with anthocyanins, which could be due to the structure of flavonols. In fact, the ability to scavenge free radicals depends on many structural factors.20 Flavonols are considered powerful compounds, particularly quercetin which contains all criteria previously described (antioxidant and anti-inflammatory). Anthocyanins differ from other flavonols by the absence of the double bond C2-C3 and the function 4-oxo, which explains the richness of C. oxyacantha ssp monogyna berries in anthocyanin.50

Leaves of C. oxyacantha ssp monogyna were richer in flavonoids than the berries as aglycones accumulate in epidermal cells of leaves where they protect the parenchyma against the harmful effects of UVB radiation in the 280–315 nm range.53 Anthocyanins are responsible for the color of flowers and fruits, which explains the richness of C. oxyacantha ssp monogyna berries in anthocyanin.50

Our results confirm data from the literature reporting that onion contains more aglycones than hawthorn.54,55 Flavonoid extracts of the three plants exhibited very high antioxidant properties as compared with standard antioxidants (quercetin and ascorbic acid). But flavonoid contents were not correlated with the anti-reducing power for the different species. Flavonoids seem to be effective donors of hydrogen to DPPH radical, because of their ideal chemical structure. Flavonols (e.g., quercetin), are considered as an antioxidant model due to their ability to scavenge free radicals.15 The extracts showed a higher antioxidant activity than quercetin that could be explained by the presence of other phenolic compounds (phenolic acids, flavonols and flavones), also endowed with a significant antioxidant activity. However, the highest antioxidant activity of C. oxyacantha ssp monogyna extracts could be attributed to the presence of epicatechine (flavan-3-ol) which is present in very high amounts as compared with quercetin.18

For I. verum, anti-oxidant activity could be attributed to gallic acid present in higher amount than the other phenolic com­ounds such as quercetin and kaempherol.50 Flavonols of the outer layer of the red onion had the highest antioxidant activity as compared with the white variety. This could be linked to its richness in quercetin. Overall, aglycones had a higher antioxidant activity as compared with anthocyanins, which could be due to the structure of flavonols. In fact, the ability to scavenge free radicals depends on many structural factors.20 Flavonols are considered powerful compounds, particularly quercetin which contains all criteria previously described (antioxidant and anti-inflammatory). Anthocyanins differ from other flavonols by the absence of the double bond C2-C3 and the function 4-oxo, which could explain their lower antioxidant activity. Therefore, the antioxidant activity depends on the concentration, the structure and the nature of the flavonol compounds. The antibacterial activity study showed that extracts of A. cepa L. and C. oxyacantha ssp monogyna were the most effective. However, flavonols of the inner layer of red onion had a larger inhibiting activity on the growth of Escherichia coli than those of I. verum and C. oxyacantha ssp monogyna. Flavonols and anthocyanins of leaves and berries of C. oxyacantha ssp monogyna were active on the growth of Pseudomonas aeruginosa. Their strong antibacterial activity against Gram-negative bacteria could be explained by the effect of these molecules (flavonols and anthocyanins) on the parietal cells and the cytoplasmic membrane activity. They inhibit the activity of some extracellular and intracellular enzymes such as dehydrogenase important for the bacterial metabolism and the synthesis of compounds essential for their growth. They can also chelate some heavy metal necessary for the enzymatic reactions such as iron.57,58 The outer layers of red onion were slightly effective against Staphylococcus aureus, but none of the extracts obtained from C. oxyacantha ssp monogyna and I. verum had an inhibitory effect against these species. This resistance could be related to the organization and structure of

**Table 2.** Diameters of inhibition zones (mm)  

| Bacteria species | Allium cepa “Red Onion” | Allium cepa “White Onion” | Castanea oxyacantha Monogyna | Ilicium verum |   |
|------------------|-------------------------|---------------------------|----------------------------|--------------|---|
|                   | Inner layers            | Outer layers              | Inner layers               | Outer layers | Leaves Berries |
| *Escherichia coli* | 1                       | 2                         | 1                          | 2            | 1            |
| ATCC 25922        | 12                      | -                         | -                          | -            | -            |
| *P. aeruginosa*    | 9                       | -                         | -                          | -            | -            |
| ATCC 27852        | 10                      | -                         | -                          | -            | -            |
| *S. aureus*        | 18                      | 12                        | 16                         | 11           | 12           |
| ATCC 25923        | 18                      | -                         | -                          | -            | -            |
| *S. aureus*        | 12                      | 16                        | 13                         | -            | -            |
| ATCC 43100        | 13                      | -                         | -                          | -            | -            |

1 Flavonols, 2 Anthocyanins, – no activity.

**Discussion**

It is generally expected that, when antimicrobial activity is measured, most of the materials tested would be active against Gram-positive bacteria.59,60 In this study, extracts inhibited especially Gram-negative bacteria. Plants contain a wide range of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids. All have drawn considerable attention because of their physiological functions including free radical scavenging. Our results showed that white and red onions had the highest contents of aglycones, while the berries and leaves of C. oxyacantha ssp monogyna had the highest anthocyanin contents. Red onion contained more aglycones than the white variety and was rich in anthocyanin. Flavonoid content was higher in the outer layer than in the inner layer. This difference could be explained partly by the age of the tissue (the outer layer is older than the inner layer) and partly by the protective role of the outer layer against radiation.15,16

Leaves of C. oxyacantha ssp monogyna were richer in flavonoids than the berries as aglycones accumulate in epidermal cells of leaves where they protect the parenchyma against the harmful effects of UVB radiation in the 280–315 nm range.53 Anthocyanins are responsible for the color of flowers and fruits, which explains the richness of C. oxyacantha ssp monogyna berries in anthocyanin.50

For *I. verum*, anti-oxidant activity could be attributed to gallic acid present in higher amount than the other phenolic compounds such as quercetin and kaempherol.50 Flavonols of the outer layer of the red onion had the highest antioxidant activity as compared with the white variety. This result could be linked to its richness in quercetin. Overall, aglycones had a higher antioxidant activity as compared with anthocyanins, which could be due to the structure of flavonols. In fact, the ability to scavenge free radicals depends on many structural factors.20 Flavonols are considered powerful compounds, particularly quercetin which contains all criteria previously described (antioxidant and anti-inflammatory). Anthocyanins differ from other flavonols by the absence of the double bond C2-C3 and the function 4-oxo, which could explain their lower antioxidant activity. Therefore, the antioxidant activity depends on the concentration, the structure and the nature of the flavonol compounds. The antibacterial activity study showed that extracts of *A. cepa* L. and *C. oxyacantha* ssp monogyna were the most effective. However, flavonols of the inner layer of red onion had a larger inhibiting activity on the growth of *Escherichia coli* than those of *I. verum* and *C. oxyacantha* ssp monogyna. Flavonols and anthocyanins of leaves and berries of *C. oxyacantha* ssp monogyna were active on the growth of *Pseudomonas aeruginosa*. Their strong antibacterial activity against Gram-negative bacteria could be explained by the effect of these molecules (flavonols and anthocyanins) on the parietal cells and the cytoplasmic membrane activity. They inhibit the activity of some extracellular and intracellular enzymes such as dehydrogenase important for the bacterial metabolism and the synthesis of compounds essential for their growth. They can also chelate some heavy metal necessary for the enzymatic reactions such as iron.57,58 The outer layers of red onion were slightly effective against *Staphylococcus aureus*, but none of the extracts obtained from *C. oxyacantha* ssp monogyna and *I. verum* had an inhibitory effect against these species. This resistance could be related to the organization and structure of
the cell wall of Gram-negative bacteria, which have a lipopoly-
saccharide-rich membrane forming a barrier against the penetra-
tion of active molecules, whereas Gram-positive bacteria do not possess such a structure.12

Materials and Methods

Chemicals compound. 2,2-diphenyl-1-picrylhydrazyl, (DPPH) ascorbic acid (AA) and quercetin.

Plant materials. We used three species considered as healing plants: Allium cepa (onion) with two varieties (red and white), including the inner and the outer layers, Crataegus oxyacantha sap marmoyctis (hawthorn), which is encountered in the north of Algeria, and Illicium verum (star anise or badiane), whose used part is the fruit (commercial variety).

Flavonoids determination. We used the protocol of Koundan and Anupam10 that relies on acid hydrolysis of glycosides of plant material. The flavonoids are extracted by ethyl ether, evaporated and taken up in methanol. A differential spectrophotometric assay allowed the flavonoid estimation at 420 nm in the presence of AlCl3. The amount is expressed as equivalent mg of quercetin.

Antihelminthics determination. Total anthocyanin contents in the extracts were determined according to Lehmann et al.12 After extraction with methanol (1%) and centrifugation, the absorbance was measured at A = 517 nm and A = 657 nm. The formula

\[ A = (A_{517} - 0.25 A_{657}) \]

was used to compensate for absorption of chlorophyll and its degradation products at 530 nm. The anthocy-

The flavonols are extracted by ethyl ether, evaporated and taken up in methanol. A differential spectrophotometric assay allowed the flavonoid estimation at 420 nm in the presence of AlCl3. The amount is expressed as equivalent mg of cyanidin-3-glucoside per gram.

Evaluation of antioxidant capacity. DPPH radical scavenging activity was followed by monitoring the decrease in absorbance at 517 nm. The activity was expressed as percentage of inhibition. Inhibition (%) = (Acontrol - Asample) / Acontrol × 100.

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