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Antioxidant properties of four Algerian medicinal and aromatic plants *Juniperus oxycedrus* L., *Juniperus phoenicea* L., *Marrubium vulgare* L. and *Cedrus atlantica* (Manetti ex Endl)

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ABSTRACT

This study was conducted to determine the total flavonoid and flavonol contents and the estimation of antioxidant activity by DPPH radical scavenging effect of crude ethanolic extracts of the aerial parts of four Algerian medicinal and aromatic plants : Juniperus oxycedrus L., Juniperus phoenicea L. (Cupressaceae), Cedrus atlantica Manetti (Pinaceae) and Marrubium vulgare L. (Lamiaceae). Total flavonoid and flavonol contents in these extracts were determined using $AlCl_3$ method and their amount calculated as quercetin µEQ/mg. Synthetic antioxidants such as rutin and BHA were used as standard. The results of antioxidant activity by DPPH radical scavenging indicated better activities for Cedrus atlantica Manetti and Marrubium vulgare L. (IC_{50} = 8.9 and 20.3 µg/ml respectively) than J. phoenicea L. and J. oxycedrus L (IC_{50} = 403.8 and 481.3 µg/ml respectively) by comparison with the standards rutin and BHA (IC_{50} = 1.38 and 1.87 µg/ml respectively). The results of the total flavonoid contents by $AlCl_3$ method showed that all these species contained relatively low amounts of flavonoids and that J. oxycedrus had a better content than C. atlantica, J. phoenicea and M. vulgare (23.1, 16.8, 13.9 and 5.0 µgEQ/mg respectively) . The results the total flavonols are in the following order J. oxycedrus > M. vulgare > J. phoenicea > C. atlantica. (32.1, 23.6, 18.1 and 14.1 µgEQ/mg respectively).

Keywords: *Juniperus oxycedrus* L., *Juniperus phoenicea* L. *Cedrus atlantica* Manetti, *Marrubium vulgare* L. phytochemical screening, antioxidant activity DPPH, total flavonoid and flavonol contents.

INTRODUCTION

The genus *Juniperus* comprising 67 species and 37 varieties, belongs to the family *Cupressaceae*, growing wild around the Mediterranean, Portugal, Israel, North Africa (Algeria and Morocco), the Canary and Madeira Islands [1,2]. Species of *Juniperus* are used in the form of decoction to treat diarrhea, rheumatism [3], diabetes [4], gastrointestinal disorders, common colds, analgesic and stomach disorders [5]. The mixture of leaves and berries of *J. Phoenicea* is used as an oral hypoglycemic agent, whereas the leaves are used against bronco-pulmonary disease and as a diuretic [6, 7]. It is commonly known as "arar lahmar" in Algeria [8] and is used in the Algerian folk medicine as a diuretic and a stimulating and stomachic tonic [9,10]. *Juniperus oxycedrus* L. is a shrub or tree with typical Mediterranean distribution [12]. It is used to prepare empyreumatic oil by dry distillation of the branches and wood of the plant, which is widely employed in human and veterinary dermatology to treat chronic eczema and other skin diseases [13].

Some studies revealed that *Juniperus phoenicea.*L contains a large variety of compounds, mainly diterpenoids [14], biflavonoids [15], lignans [16] phenylpropanoid glucosides [17], furanone glucosides, bis-furanone derivatives [18], norterpene and sesquiterpene glucosides [19].

The total phenolic contents and antioxidant activity of the extracts and essential oils of several *Juniperus* species, were evaluated showing variable results [20-24].

Marrubium vulgare L (Lamiaceae) is reported to possess vasorelaxant [26] hypoglycemic, antihypertensive [27] analgesic [28] anti-inflammatory [29] antispasmodic, antinociceptive, hypotensive, insecticidal, and antioxidant properties [30].

Several studies evaluated also the antioxidant activity of *M. vulgare* L. from different locations showing also variable results depending on the location and the constituents of the species. [31-33].

The genus *Cedrus* includes three extant species native to the Mediterranean mountains, distributed over Morocco and Algeria. *Cedrus atlantica* Manetti (Pinaceae), as a renewable source of natural products, is only distributed in Morocco and Algeria. The essential oil from this plant showed anti-inflammatory [32] antifungal [33] and antimicrobial [34] properties . It is also proved to be useful in the treatment of hair loss in a combination of aromatherapy oils [35]. It was little studied for its phenolic content or antioxidant activity [36].

This work aimed to compare the antioxidant activity by DPPH radical scavenging method and total flavonoid and flavonol content of four species from Aures area in Algeria : *Juniperus oxycedrus*, *Juniperus phoenicea*, *Cedrus atlantica* and *Marrubium vulgare*. The results were compared with the literature data.

MATERIALS AND METHODS

Chemicals

Hydrochloric acid, magnesium, ethanol, methanol, $FeCl_3$, sulfuric acid, Dragendorff reagent, DPPH (2, 2-diphenyl-1-picrylhydrazyl), quercetin, BHA, rutin, $AlCl_3$, sodium acetate were obtained from Merck Darmsdadt, Germany.

Collection of plant material and extraction procedure

Juniperus oxycedrus, *Juniperus phoenicea*, *Cedrus atlantica* and *Marrubium vulgare* were collected in February 2015 in the area of Aures (Arris-Batna, North East of Algeria).

The air-dried aerial parts of each plants (70 g) are cut into small pieces in ethanol/water (7:3) for three days, this is repeated three times with solvent renewal.

After concentration up to 37 °C, we obtained the results expressed in Table 1.

Table 1:Results of the extraction of the four species with EtOH : H_2O (7:3)

	Yield (%)
J.oxycedrus	9.81
J. phoenicea	14.88
M. vulgare	13.70
C. atlantica	9.80

Phytochemical screening

Identification of saponins

Crude extracts of aerial parts of the species are prepared in distilled water in a test tube. Stir for a few minutes. The appearance of persistent foam indicates the presence of saponins [36].

Identification of flavonoids

Crude extracts of aerial parts of the species are prepared in distilled water. After addition of HCl and then a few pieces of magnesium, appearance of redcolor indicates the presence of flavonoids [37].

Identification of tannins

Crude extracts of aerial parts of the species are prepared in distilled water. For detecting the presence or absence of tannins, iron trichloride ($FeCl_3$1%)was added. Changes of color to dark blue indicates the presence of gallictannins and to blue-green color, the presence of catechol tannins [38].

Identification of alkaloids

Crude extracts of aerial parts of the species are prepared, a few milliliters of sulfuric acid (10%) was added, and allowed to soak for 24 hours, after filtration, Dragendorff reagent is added. The appearance of a precipitate indicates the presence of alkaloids [39].

Determination of antioxidant activity

DPPH radical scavenging

Different dilutions (0.0625- 8 mg/ml) of crude ethanolic extract of the aerial parts of each species were prepared and a solution of DPPH was prepared by dissolving 6.0 mg of DPPH in 150 ml methanol. Then, 30 µl of each dilution have been added to test tubes containing 3 ml of the prepared DPPH solution. The negative control (sample) was prepared by adding 30 µl of methanol in 3 ml of the prepared DPPH solution. BHA and rutin were used as standards. The mixture was allowed to stand in the dark for 30 min. Absorbance was measured spectrophotometrically at 517 nm. The scavenging activity was calculated using the equation.

Scavenging activity (%) = (Absorbance of sample - Absorbance of extract) x 100 / Absorbance of sample

The sample and the reading were prepared and measured in couple. The radical scavenging activity was expressed as IC_{50} value, i.e. the concentration required to inhibit 50% of the DPPH radicals, and was calculated from the inhibition percentage graph drawn according to the concentration of the sample using Microsoft Office Excel [40].

Total flavonoid content

The total flavonoid content was determined by the method of aluminum trichloride using quercetin as a reference compound [41]. The method is based on the formation of a flavonoid-aluminum complex having an absorption maximum at 420 nm. 1 ml of the crude ethanolic extract of aerial parts of each species (1 mg/ml) was mixed with1 ml of 2% methanolicaluminum trichloride solution. The absorbance at 420 nm was read after 1 hour. All determinations were realized in couple. The absorption of quercetin standard solutions(0.195 to 125 µg/ml) was measured in the same conditions. The results are expressed as equivalent quercetin µQE/mg of extract.

Total flavonol content

The total flavonol content was determined using quercetin as a reference compound. This method is also based on complex formation with a maximum absorption at 415 nm [42]. 1 ml of the crude ethanolic extract of the aerial parts of each species (1 mg/ml) was mixed with 1 ml of 2% methanolic aluminum trichloride solution and 3 ml of sodium acetate (50 mg/ml).The absorbance at 415 nm was read after 2.5 hours. All determinations were realized in couple. The absorption of quercetin standard solution (0.195 to 500 µg/ml) was measured in the same conditions. The results are expressed in equivalent quercetin µQE/mg of extract.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening of the crude ethanolic extracts of the aerial parts of the four studied species showed the presence of saponins, flavonoids, catechin tannins, gallic tannins and alkaloids with variable amounts.

Table 1:Results of the phytochemical screening of the crude ethanolic extracts of the aerial parts of the four species

	J.phoenicea	*J.oxycedrus*	*C. atlantica*	*M. vulgare*
Saponins	+++	++	++	+++
flavonoids	++	++	+	+
Gallic tannins	+	+	+	+
Catechin tannins	+	+	+	++
Alkaloids	+++	+++	+++	+++

+ + +:very abundant, +: abundant, -: negative

DPPH radical scavenging activity assay

A lower IC_{50} value indicates a strong activity of the extract. The ethanolic extracts of the aerial parts of the studied species showed a strong activity for *C. atlantica* and *M. vulgare* L. extracts(IC_{50} = 8.919±0.353 and 20.379±2.186 µg/ml respectively) comparing to *J.oxycedrus* and *J.phoenicea* extracts (IC_{50} = 403.8±30.8 and 481.3± 1 µg/ml respectively).

Table 2: IC_{50} values of crude ethanolic extracts of aerial parts of the four species and standards

	J.phoenicea	*J.oxycedrus*	*C. atlantica*	*M. vulgare*	Rutin	BHA
IC_{50} (µg/ml)	403.89± 30.87	481.39±132.07	8.92± 0.35	20.379±2.186	1.38	1.87

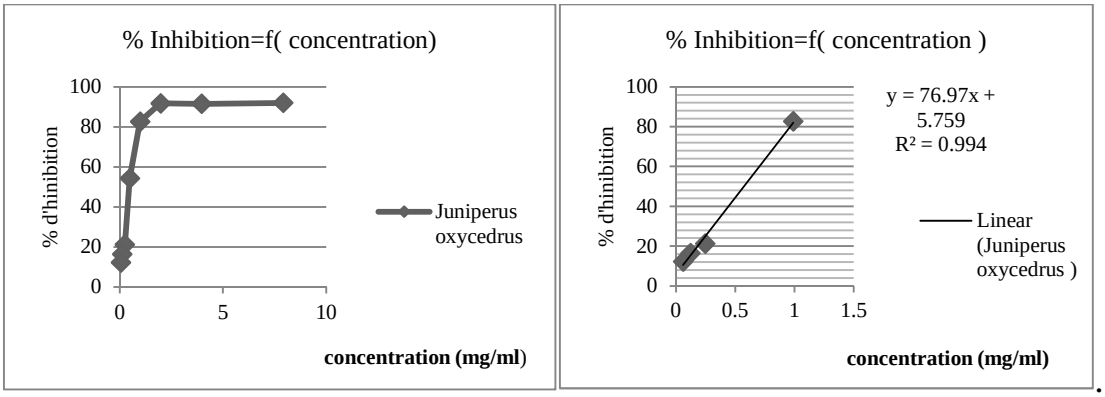

Figure 1: DPPH radical-scavenging activity of ethanolic extract of the aerial part of *J.oxycedrus* L.

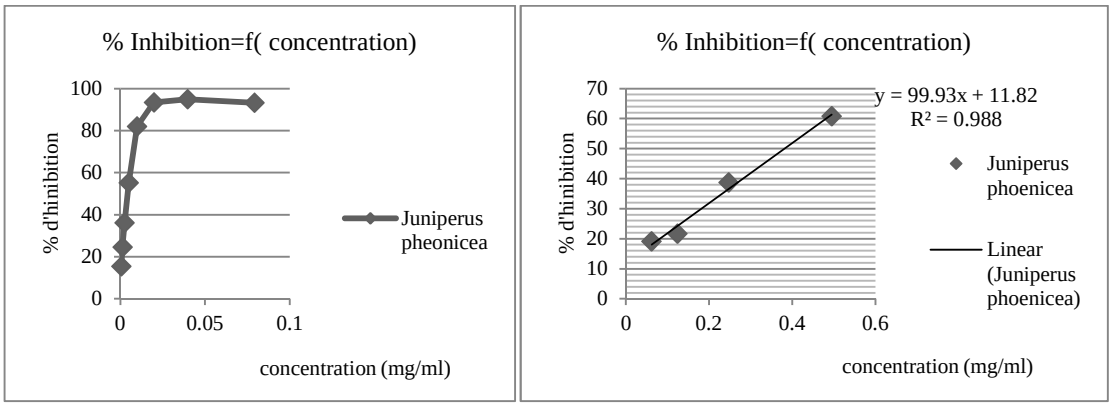

Figure 2 : DPPH radical-scavenging activity of ethanolic extract of the aerial part of *J.phoenicea* L.

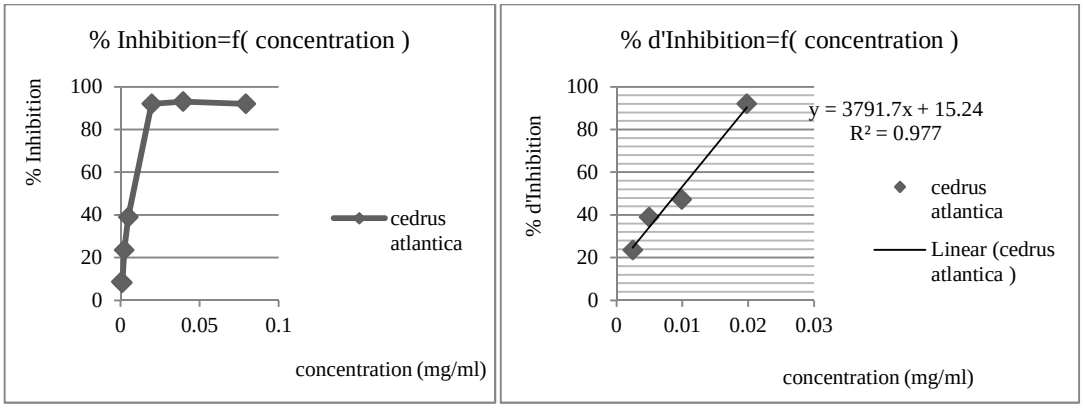

Figure 3: DPPH radical-scavenging activity of ethanolic extract of the aerial part of *C.atlantica*

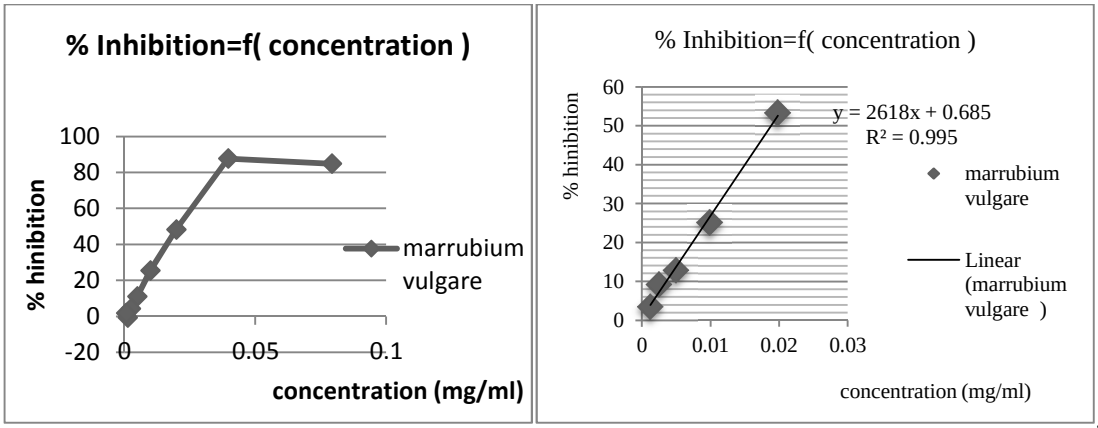

Figure 4 : DPPH radical-scavenging activity of ethanolic extract of the aerial part of *Marrubium vulgare* L.

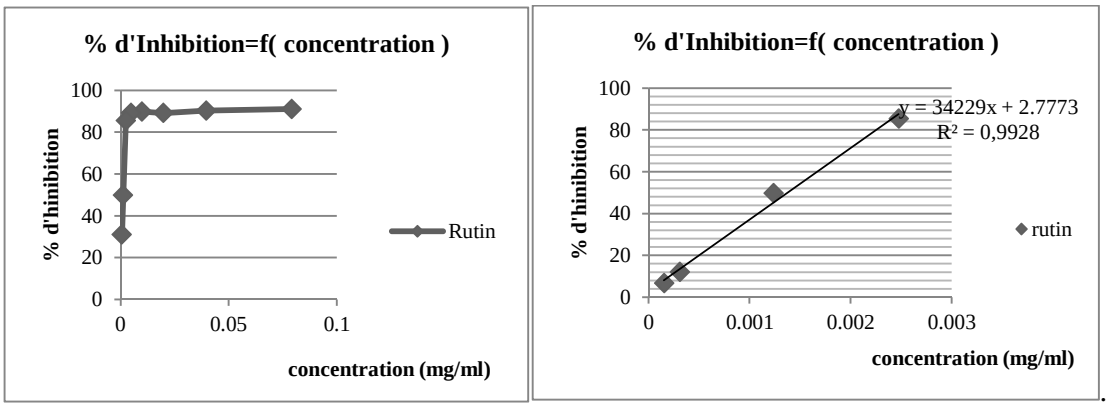

Fig.5:DPPH radical-scavenging activity of rutin standard

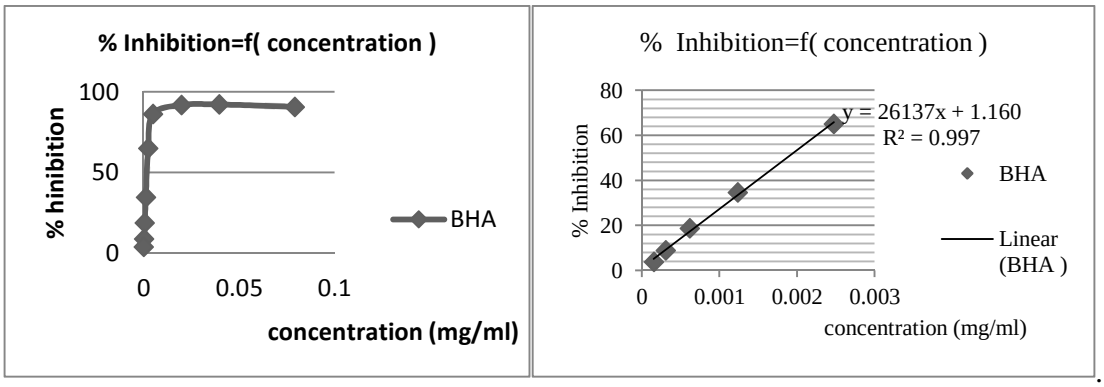

Figure 6: DPPH radical-scavenging activity of BHA standard

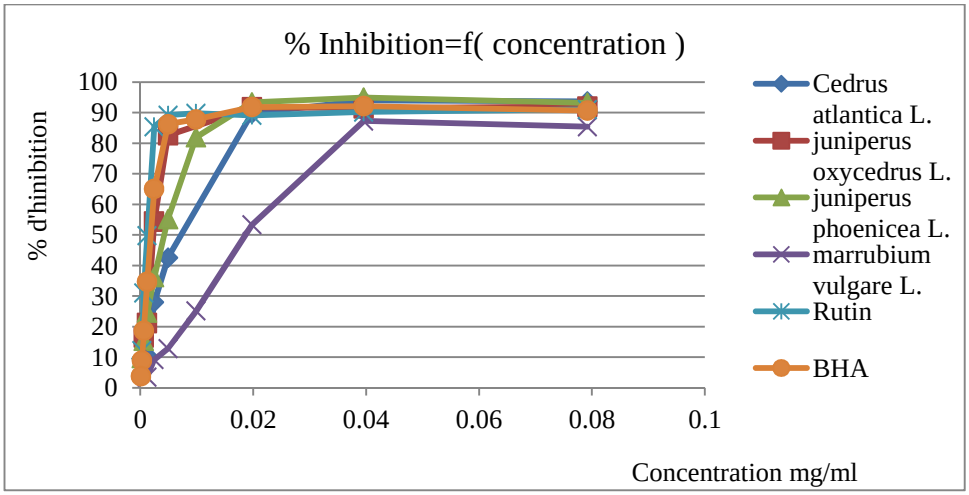

Figure 7: DPPH radical-scavenging activity of ethanolic crude extracts

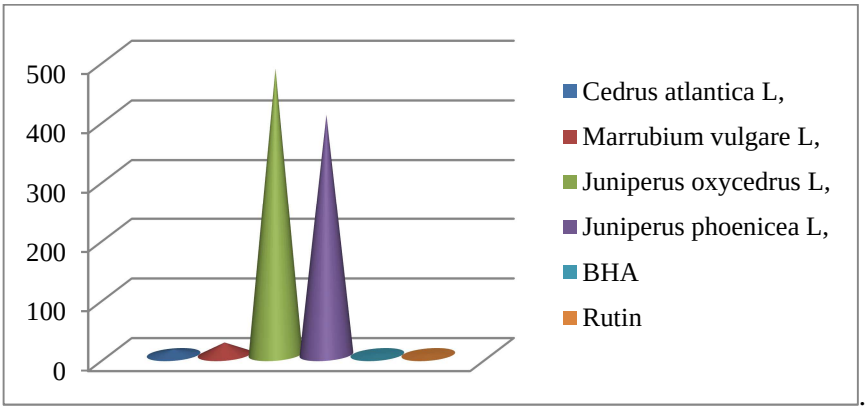

Fig.8:Comparison between IC_{50} values (µg/ml) of crude ethanolic extracts of aerial parts of the four studied species and standards

Total flavonoid content

The total flavonoid content was measured using the equation y= 0.024x + 0.025 withR^2 = 0.998, were; y = absorbance at 420 nm and x = total flavonoid compounds per mg of extract. The results are in the following order : *J.oxycedrus*>*C.atlantica*>*J.phoenicea*>*M.vulgare*(23.1±3.2>16.8±5.3>13.9±2.8>5.0±0.05 µgEQ/mg) respectively.

Table3 : Total flavonoid contentsin ethanolic crude extracts of the aerial parts of the four species

	J.oxycedrus	*C. atlantica*	*J. phoenicea*	*M. vulgare*
Total flavonoïd contents µgEQ/mg	23.119±3.226	16.860±5.318	13.949±2.180	5.008±0.058

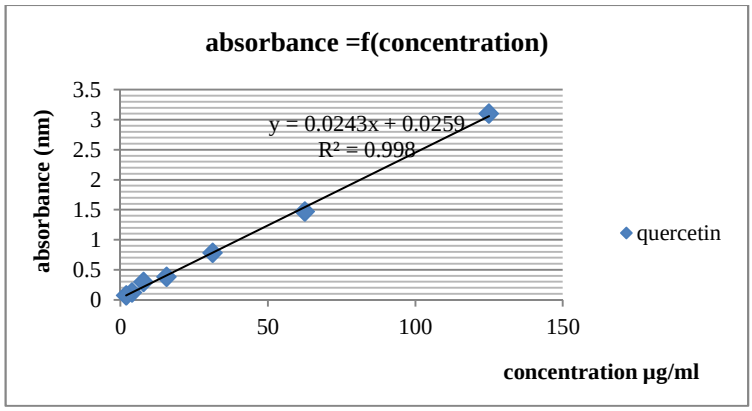

Fig.9: Determination of total flavonoids by quercetin standard

Total flavonol content

The total flavonol contents was measured using the equation y = 0.006x + 0.008 with R^2 = 0.999 ; y = absorbance at 415 nm and x = total flavonol compounds per mg of extract. The results indicated that the contents are in the following order:

J.oxycedrus>*M.vulgare*>*J.phoenicea*>*C.atlantica*.(32.1±4.7 >23.6±1.03>18.1±0.1>14.1±3.3µgQE/mg)respectively.

The fact that the flavonoid and flavonol contents didn't correlate directly with DPPH radical scavenging effect may be attributed tothe relatively low amounts of this type of compounds and the presence of higher amounts of other type of antioxidant compounds in the four extracts (essential oil, terpenoids and alkaloids).

Table4 :Total flavonol contents in ethanolic crude extracts of the aerial parts of the four species

	J.oxycedrus	*J.phoenicea*	*C. atlantica*	*M. vulgare*
Total flavonol content µgEQ/mg	32.116 ± 4.785	18.199 ± 0.165	14.116 ±3.299	23.633±1.036

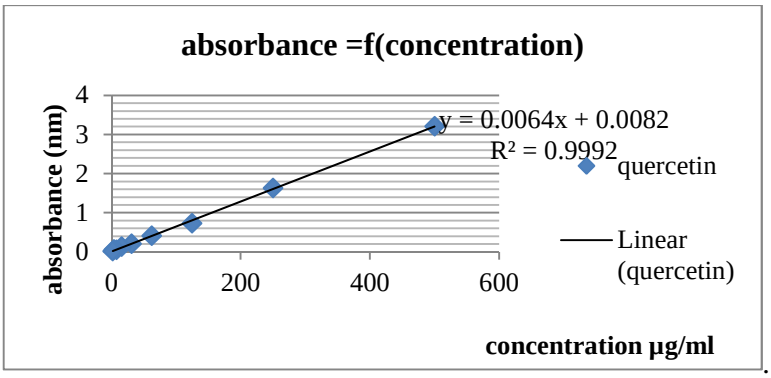

Fig.10:Determination of total flavonol contents using quercetin as standard

CONCLUSION

In the present work we report a comparative study of antioxidant activity by DPPH radical-scavenging method and the evaluation of the total flavonoid and flavonol contents of four Algerian medicinal and aromatic plants : *Juniperus oxycedrus*, *Juniperus phoenicea*, *Marrubium vulgare* and *Cedrus atlantica*. Our results showed good radical scavenging effect of *Cedrus atlantica* and *Marrubium vulgare* and weakest activity for the two *Juniperus* species (IC_{50} : 8.9, 20.3, 403.8 and 418.3 for *C. atlantica*, *M.vulgare*, *J. oxycedrus* and *J. phoenicea* respectively) comparing to BHA and rutin standards (1.38 and 1.87). These results agreed with the literature data which showed variable activities for *Juniperus* species and relatively potent activity for *Marrubium vulgare*, while there is only one report on the antioxidant activity of the seeds of *Cedrus atlantica* which showed the powerful actifity of this species. These results didn't correlate very well with the results of flavonoid and flavonol contents, which are in the following order : *J. oxycedrus* > *C. atlantica* > *J. phoenicea* > *M. vulgare* for flavonoid contents. This may be due to the fact that the values of flavonoid contents are not very high in the four species and that the antioxidant properties of *C. atlantica* and *M. vulgare* may be attributed to other components than flavonoids (essential oils, terpenoids, alkaloids …).

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