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Antioxidant Evaluation of Essential oils extracted from *Juniperus phoenicea* Linn.

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Abstract

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids; tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants. The aim of this study was to evaluate antioxidant activity of the *Juniperus phoenicea* L.

Key words: Anti-oxidant Activity, Essential oil, *Juniperus phoenicea* L.

Introduction

Juniper (*Juniperus phoenicea*) (Rank: Pinales, family: Cupressaceae, genus: *Juniperus*) [1], includes six juniper (Juniperus) 60 species. These are all classified within the plant kingdom where the trace section and Taif (pinopsida) in the Northern Hemisphere Ground with a (J. procera) in the Southern Hemisphere [2] and because of foot and presence on the ground is considered by some that juniper is the fossil of living [3], and *Juniper* is trees and shrubs conical sustained single-evergreen or bi-dwelling fruit is a cones small spherical includes a number of seeds varies according to type [4], and *Juniper* mountainous tree reaches a height often from 30 to 35 m and is the largest in the sex as Qatar up root of 2 to 3 meters and color trunk black pale to auburn and leaves a gray or green pale at puberty, and in some cases there are juniper shrubs with scaly

leaves and brown cones and it happens in Southern Europe and South-West Asia and North Africa and this type occupies the western part of the Mediterranean dunes coastal and marine and to some extent the internal slopes and enters in the mountainous areas and up to 2400 m altitude in Morocco in 1800 Spain [5]

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Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical. The major antioxidant capacities of plants such as fruits and vegetables are vitamins C and E and phenolic compounds, especially flavonoids. Phenolic compounds possess different biological activities, but most important are antioxidant activities. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties [6].

Medicinal plants have possess good antioxidant activity as reported and reveled by several scholars, keeping the in view the present investigation was undertaken to evaluate anti-oxidant activitv of *Juniperus phoenicea*.

Material and Methods

Plant material

The samples of *Juniperus phoenicea* collected from the EL-Gabal EL-Akhdar area (Asulntah area) and then choose three random plants were next to some includes ((Sample1= plant intact naturally grow, sample2= plant grow almost normal and sample 3= No plant grows naturally)). All samples were collected from the same location

Chemicals

1,1-Diphenylpicrylhydrazyl (DPPH˙) , methanol, Ethanol and acetone were supplied by Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of Chemistry department-Benghazi University.

Methods

Extraction of essential oil from leaves of *Juniperus phoenicea* (sample 1, sample 2 and sample 3):

The dry powdered leaves of *Juniperus phoenicea* (500g) were subjected to hydro distillation using Clevenger apparatus. The isolation of volatile oils was completed within 6 hours [7].

Store essential oils

The oil samples were stored at 7°C in dark air-tight containers after drying over anhydrous sodium sulfate and filtered before injecting to GC-MS analysis.

Oil analysis

The oil samples extracted from leaves of *Juniperus phoenicea* were subjected to the following tests:

Antioxidant activities assays and quantitative analysis

All of these experimental assays have been conducted in biochemistry laboratory at Benghazi University.

Total phenolic content (TPC)

Total concentration of phenolic compound in all oil extracts obtained from leaves of *J. phoenicea* was estimated using the colorimetric method based on Folin-Ciocalteu reagent [8]. 0.05 ml of the oils at different concentrations "100,200,300,400,500 µg/ml" were mixed separately with 0.05 ml of Folin-Ciocalteu reagent. Then 0.5 ml of 15% sodium carbonate solution was added to the mixture and then the adjusted to 1 ml with 0.4 ml of distilled water. The reaction was allowed to stand for 10 min, after which the absorbance were recorded at 725 nm by UV-visible spectrophotometer. Quantification was done with respect to standard calibration curve of Pyrogallol The results were expressed as pyrogallol "µg/ml" Fig 1. Estimation of the phenolic compounds was carried out in triplicates. The results were mean values (standard deviations).

Table 1: Total phenolic content (TPC) of Pyrogallol

Concentration of Pyrogallol " µg/ml "	*Mean (Standard Deviation)*
100	0.410 (0.032)
200	0.799 (0.0220)
300	1.333 (0.0045)
400	1.828 (0.0117)
500	2.105 (0.0225)

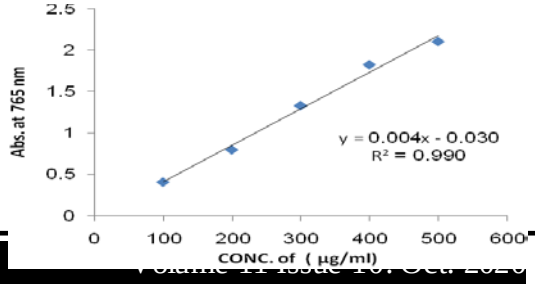

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Fig. 1: Total phenolic content of pyrogallol

Total flavonoids content (TFC)

Aluminum chloride colorimetric method was used for determination of total flavonoids [9]. 2 ml of different concentration "100, 200, 300, 400, 500 µg/ml " of oil extracts mixed with 0.1ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was obtained by preparing different quercetin solutions in methanol at concentrations "100 to 500 µg/ml" Fig 2.

Table 2: Total flavonoids content of qurecetin

Concentration of qurecetin " µg/ml "	*Mean (Standard Deviation)*
100	0.279(0.092)
200	0.560 (0.035)
300	0.834 (0.003)
400	1.066 (0.009)
500	1.300 (0.006)

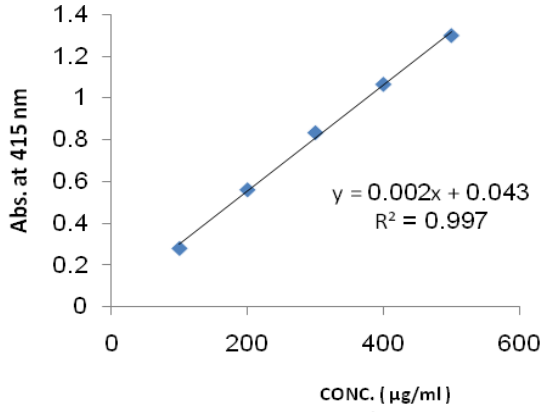

Fig. 2
Total flavonoids content of qurecetin

Reducing power assay (RPA)

The reducing power was determined according to [10]. 2ml of the all oil extracts with different concentrations "100,200,300,400,500µg/ml" were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide then mixture was incubated in water bath at 50 C^0 for 20 minutes and 2.5 ml of trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 1 ml $Fecl_3$, substances, which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm by UV-Visible spectrophotometer. Quantification was done with respect to standard calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml" Fig 3.

Potassium ferricyanide + ferric chloride $\xrightarrow{\text{antioxidant}}$ potassium ferricyanide + ferrous chloride.

Table 3: Reducing power assay of vitamin C

Concentration of vitamin C " µg/ml "	*Mean (Standard Deviation)*
100	0.201 (0.0280)
200	0.495 (0.0350)
300	0.697 (0.0087)
400	0.992 (0.0727)
500	1.201 (0.0305)

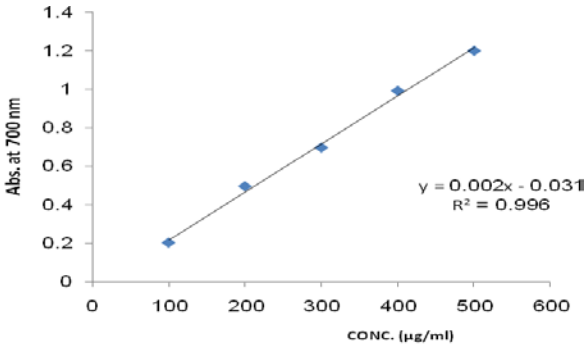

Fig. 3
Reducing power assay of vitamin C

DPPH free radical scavenging activity (RSA)

The antioxidant activity of all oil extracts were measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH ˙method as modified by[11]. The reaction mixture

containing 2 ml of all extracts at different concentrations "100,200,300,400,500µg/ml" and 2ml of DPPH· (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. When the DPPH· reacted with an antioxidant compound in oil that can donate hydrogen, it was reduced and resulting in decrease of absorbance at 517nm using UV-visible spectrophotometer, and the mean values were obtained from triplicate experiments. The percentage of the remaining DPPH· was plotted against the sample concentration. A lower value indicates greater antioxidant activity. Radical scavenging activity was expressed as percent ages of inhibition and was calculated using the following formula:-

% DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

Results and Discussion

Antioxidant evaluation of essential oils extracted from leaves of *Juniperus phoenicea* L.

The antioxidant activities of essential oils extracted of three samples from leaves of *Juniperus* phoenicea growing in Al-Jabal Al Akhdar were evaluated by:

Total phenolic content (TPC)

Figure (9) show the total phenolic content found in essential oils where the essential oil of sample number (1) contained high total phenolic content, the results expressed according to pyrogallol as phenolic compound in figure (1).

Total flavonoids content (TFC)

The results obtained in this study as shown in figure indicated that the essential oil of healthy sample contain high amounts of flavonoids compounds as compared with the qurecetin in figure (2) which was used as standard.

Reducing power assay (RPA)

As shown in figure the reducing power assay of essential oil of sample 1and sample 2 exhibit higher reducing activity than the ascorbic acid.

The DPPH· radical scavenging activity

The result of the DPPH· radical scavenging activity of essential oil are shown in figure, this result compared with the well-known antioxidant ascorbic acid were the percent of the inhibition is 97% at 500 µg/ml of the essential oil of sample 1 and 92% at 500 µg/ml of the essential oil of sample 2 , while the percent of the inhibition is 38% at 500 µg/ml of the essential oil sample 3 .

Table 4: Total phenolic content (TPC) of essential oils extracted of three samples from leaves *Juniperus* (Sample1= plant intact naturally grow, sample2= plant growth almost normal , sample 3= No plant grows naturally) and and % variation from the corresponding control (pyrogallol as standard)

concentration " µg/ml"	*Mean phenolic content (Standard Deviation)*						
	Pyrogallol	*Sample1*	% var.	*Sample2*	% var.	*Sample3*	% var.
100 (1)	0.410 (0.032)	0.711(0.03)*	73.4↑	0.699 (0.05)*	70.5↑	0.025 (0.0162)***	97.5↓
200	0.799 (0.0220)	0.799 (0. 01)†	Zero	0.782 (0.011)†	2.13↓	0.085 (0.020)**	89.4↓
300	1.333 (0.0045)	1.435 (0.02)†	7.65↑	0.988 (0.002)*	31.2↓	0.222 (0.022)**	84.5↓
400	1.828 (0.0117)	1.78 (0.01)†	2.63↓	1.56 (0.016)†	12.4↓	0.754 (0.036)*	57.6↓
500	2.105 (0.0225)	2.69(0.07)†	27.8↑	2.21 (0.009)†	17.8↓	1.061 (0.037)**	60.6↓

† Insignificant difference from the corresponding control at $P > 0.1$ * Significant difference from the corresponding control at $P < 0.05$ ** Highly sig. difference from the corresponding control at $P < 0.01$ *** Very highly sig. difference from the corresponding control at $P < 0.001$ ↓ Decrease ↑ Increase

Table 5: Total flavonoids content essential oils extracted of three samples from leaves *Juniperus* (Sample1= plant intact naturally grow, sample2= plant growth almost normal , sample 3= No plant grows naturally) and % variation from the corresponding control(qurecetin as standard)

concentration " µg/ml"	Mean phenolic content (Standard Deviation)						
	qurecetin	Sample1	% var.	Sample2	% var.	Sample3	% var.
100	0.279 (0.092)	0.297 (0.032)†	6.45↑	0.276 (0.002)†	1.07 ↓	0.0845 (0.055)**	69.7 ↓
200	0.560 (0.035)	0.497 (0.011)†	11.25 ↓	0.375 (0.001)*	33.0 ↓	0.095 (0.045)**	83.0 ↓
300	0.834 (0.003)	0.664 (0.002)†	20.4 ↓	0.609 (0.008)†	27.0 ↓	0.141 (0.075)**	83.1 ↓
400	1.066 (0.009)	0.820 (0.090)†	18 ↓	0.869 (0.003)†	18.4 ↓	0.423 (0.071)**	60.3 ↓
500	*1.300 (0.006)*	*1.690 (0.011)**	30 ↑	*1.340 (0.001)*†	3.08 ↑	0.866 (0.085)*	33.4 ↓

† Insignificant difference from the corresponding control at P > 0.1 * Significant difference from the corresponding control at P < 0.05 ** Highly sig. difference from the corresponding control at P < 0.01 *** Very highly sig. difference from the corresponding control at P < 0.001↓ Decrease↑ Increase

Table 6: Reducing power assay essential oils extracted of three samples from leaves *Juniperus* (Sample1= plant intact naturally grow, sample2= plant growth almost normal , sample 3= No plant grows naturally) and % variation from the corresponding control(vitamin C)

concentration " µg/ml"	Mean phenolic content (Standard Deviation)						
	vitamin C	Sample1	% var.	Sample2	% var.	Sample3	% var.
100	0.201 (0.0280)	0.503 (0.0448)***	150.3↑	0.564(0.07)***	180.6↑	0.044(0.006)**	78.1↓
200	0.495 (0.0350)	0.875(0.0965)**	76.8↑	0.871 (0.01)**	75.9↑	0.104 (0.002)**	79↓
300	0.697 (0.0087)	1.293 (0.0471)**	85.5↑	1.321 (0.003)**	89.5↑	0.432 (0.005)*	38.0↓
400	0.992 (0.0727)	1.563 (0.0266)*	57.6↑	1.899(0.01)***	91.4↑	0.902(0.016)†	9.07↓
500	1.201 (0.0305)	2.339 (0.0401)***	94.8↑	2.486(0.01)***	107.0↑	1.052(2.965)†	12.41↓

† Insignificant difference from the corresponding control at P > 0.1 * Significant difference from the corresponding control at P < 0.05 ** Highly sig. difference from the corresponding control at P < 0.01 *** Very highly sig. difference from the corresponding control at P < 0.001↓ Decrease↑ Increase

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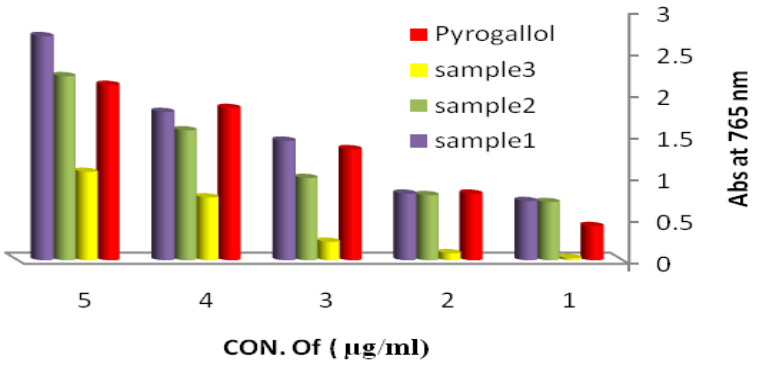

Fig. 4: Total phenolic content (TPC) of essential oils extracted from leaves *Juniperus* and Pyrogallol as phenolic compound.

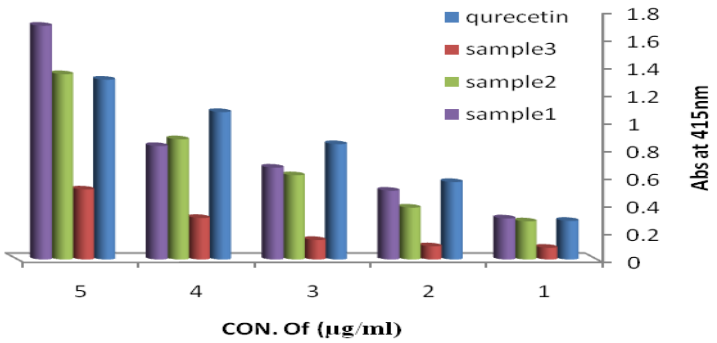

Fig. 5: Total flavonoids content of essential oils extracted from leaves *Juniperus* and qurecetin as flavonoid compound

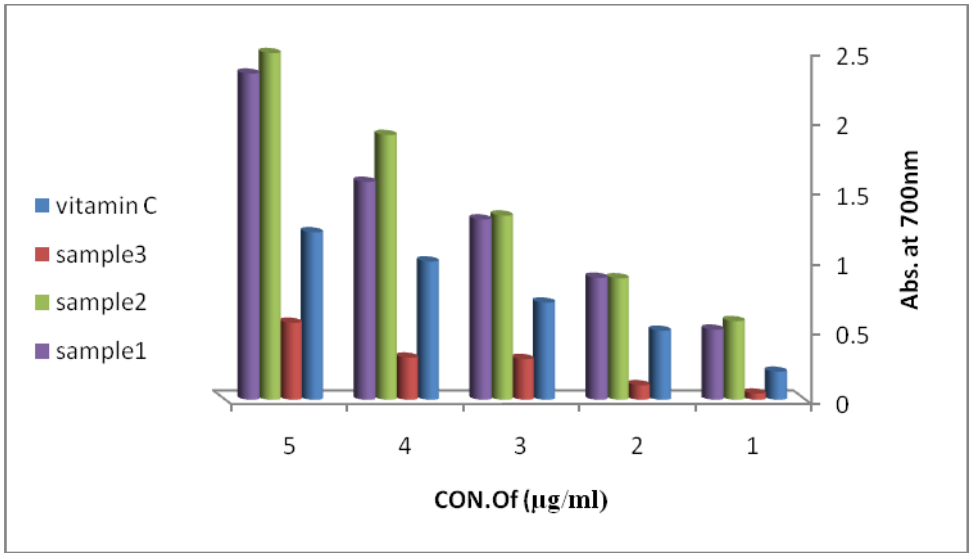

Fig. 6: Reducing power assay of methanol , ethanol and acetone extracted from leaves *Juniperus* and vitamin C

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Table 7: DPPH radical scavenging activity of vitamine C, and essential oils extracted of three samples from leaves *Juniperus* according to % inhibition

concentration " µg/ml"	Percent of inhibition (%)						
	vitamin C	Sample1	% var.	Sample2	% var.	Sample3	% var.
100	92.3%	57% *	38.2↓	39% *	57.7↓	11% **	88.1↓
200	93.8%	79% †	15.8↓	59% *	37.1↓	19% **	79.7↓
300	95.1%	85% †	10.6↓	75% †	21.1↓	27% **	71.6↓
400	95.8%	89% †	7.1↓	87% †	9.19↓	33% **	65.6↓
500	96.7%	97% †	0.31↑	90% †	6.93↓	38% **	60.7↓

† Insignificant difference from the corresponding control at $P > 0.1$ * Significant difference from the corresponding control at $P < 0.05$ ** Highly sig. difference from the corresponding control at $P < 0.01$ *** Very highly sig. difference from the corresponding control at $P < 0.001$ ↓ Decrease ↑ Increase

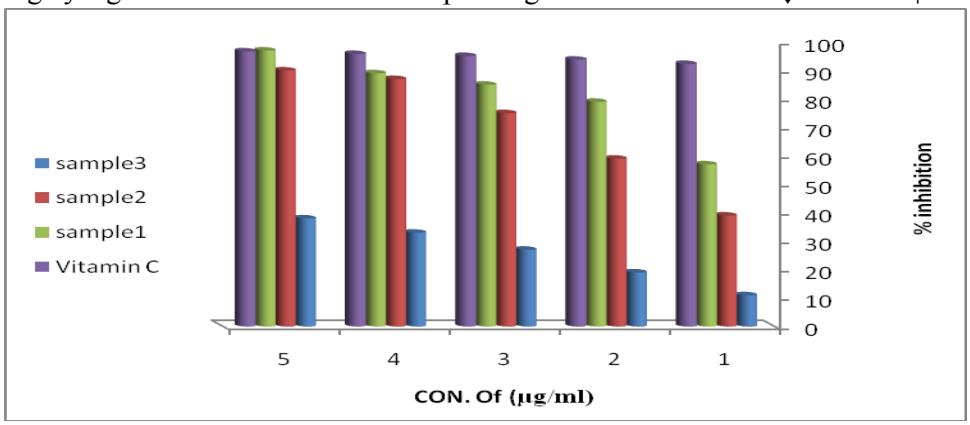

Fig. 7: DPPH radical scavenging activity of vitamine C and essential oils extracted from leaves *Juniperus* according to % inhibition

Conclusion

The antioxidant activities of essential oils extracted from leaves of *Juniperus* wereevaluated by Total phenolic content (TPC) and total flavonoids content (TFC): Data in figure (9) showed that total phenolic content found in essential oils , were the essential oil of sample number 1(plant intact naturally grow) contain

high total phenolic content, the results expressed according to pyrogallol as phenolic compound in figure (1). The total flavonoid obtained in this study as shown in figure indicate that the essential oil of sample number 1contain high amount of flavonoids compounds as compared with the qurecetin in figure (2) which was used as standard. In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe+3 to Fe+2 by donating an electron. The amount of Fe+2 complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. As shown in figure the reducing power assay of essential oil of sample 1and sample 2 exhibit higher reducing activity than the ascorbic acid.

This results obtained from the in vitro antioxidant screened showed that essential oil of sample number 1 and 2 from leaves *juniperus* has considerable amounts of polyphenols and flavonoids compounds which are responsible for the antioxidant properties. And also they gave the reductive potential due to reducing capacity and

DPPH free radical scavenging activity which serves as strong indicator of antioxidant activities (Figure**).**

The phenolic and the flavonoids compounds are groups of secondary metabolites with broad range of biological properties such as: antioxidant, antibacterial ,anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function, it has been reported that antioxidant activity of the phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors play an important role by adsorbing and neutralizing reactive free radicals, and chelating ferric ions which catalyses lipid peroxidation, and regarded as promising therapeutic agent for free radical-linked pathologies [12].

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