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Biochemical, quantitative and qualitative phenolic compounds, anti-free radical's activities of *Calligonum comosum* collected from different sites in the Algerian Desert

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Abstract. The aim of this study is to understand the effect on biochemical, quantitative and qualitative phenolic content and to estimate the anti-free radical's activities of the *Calligonum comosum* collected from various locations in Oued Souf region (South-eastern Algeria). Concerning the biochemical parameters, we registered pH values ranged between 4.8 and 4.2 and we observed oscillate in the amounts of proteins and the values of Catalase activity. The analysis of extracts by HPLC identified 43 compounds were counted as the maximum number of compounds, also we recorded the appearance of some phenolic compounds: Gallic acid, Chlorogenic acid, Vanillic acid, p-Comaric acid, Vanillin and Rutin in some extracts. Anti-free radical's activities which worked in two tests: DPPH• and TAC the results indicated that all extracts were highly anti-free radicals. Our results showed that there is a significant difference between the samples of *Calligonum comosum* collected in different sites.

Key words: *Calligonum comosum*; phenolic compounds; anti-free radical's activities; proteins; activity of Catalase; HPLC.

Introduction

Larta (*Calligonum comosum* L'her.) is a shrub leafless affiliate of Polygonaceae family (Quezel & Santa 1963), This bush distributed in the sandy soiled Arabian Sahara (Ozenda 1991) is used locally for treat stomach ailments, curing toothache (Liu et al. 2001) and as stimulant and astringent (Muschler 1912).

The Dehydrodicatechin, a compound isolated from this plant showed the best cytotoxicactivity (Badria et al. 2007) and high antioxidant and antimicrobial activities (Chouikh et al. 2015a, 2016). Two type compounds (beside Anthraquinones) were isolated in this plant by Zaki et al. (1984), the Flavonoids and Anthraquinones are the common compounds constituents in the aerial part of this plant **(**Ghazanfar 1994**).** The treatment of animals infected in Fasciolosis by ethanolic extract of *Calligonum comosum* improved their health conditions (Degheidy et al. 2013).

The aim of this study is to comprehend the effect of geoposition on biochemical, quantitative and qualitative phenolic compounds and to estimate the anti-free radical's activities of extracts of *Calligonum comosum* which is picked from various locations in Oued Souf region (South-east of Algerian Sahara).

Material and Methods

Vegetal Material: The plant leaves in the vegetative stage were harvested in October 2016 during the somatic stage from four sites of Oued Souf region (South-east of Algerian Sahara) confined between (6°89'06" E / 33°51'35" N); [S_{01}: Taghzout, S_{02}: Guemmar, S_{03}: Dokar, S_{04}: El Oued]. A part of the plant was reserved in the freezer and the rest was dried, crushed and stored.

Preparation of Extracts: In this study, for extraction we used the maceration method in methanol solvent (99%) (Chouikh et al. 2015b)

Measuring the pH: The pH is determined using a pH-meter (Meter Lab M210) by the method of (Mohammed et al. 2009).

Dosage of total polyphenol contents: Total Polyphenol content was estimated by method described at Chouikh et al. (2015b). A 0.2 ml of extracts was mixed with 01 ml of Folin-Ciocalteu reagent (10%). After 05 min, 0.8 ml of Na_2CO_3 (7.5%), after 30 minutes The absorbance is measured at 760 nm.

Dosage of flavonoids: For dosage the flavonoids, we adopted the method cited by Rebiai et al. (2015). 0.5 ml of extracts was added in 0.5 ml of $AlCl_3$ (2%). After 60 min the absorbance was measured at 420 nm.

Dosage of total proteins: A quantity of 100 mg of fresh vegetal material was ground with 5 ml distilled water, after filtration this solution was readjusted at 05 ml by adding distilled water; in tube add 0.2 ml of last solution and 1.6 ml distilled water and 0.2 ml of bleu Coomassie reagent and all were mixed, between 05 min and 01 hour, the absorbance was measured at 595 nm, the values of proteins are expressed in µg/g of fresh vegetal material (Bradford 1976).

Preparation of enzymatic extract: Before estimated enzymatic activities, we prepared enzymatic extract by the method described by Loggini et al. (1999), 0.5 g of leaves are crushed with 25ml of phosphate buffer solution (pH = 7). The recovered crushed material is centrifuged at 5000 rpm for 5 min. The supernatant is filtered to obtain the enzymatic extract which will be stored at 4°C until it is used.

Determination of catalase activity: The reaction mixture contains: 100 µl of the crude enzyme extract, 50µl of H_2O_2 (0.3%) and 2850µl of phosphate buffer (50 mM, pH = 7.2). Calibration of the device occurs in the absence of the enzyme extract. The reaction is triggered by the addition of hydrogen peroxide (Cakmak & Horst 1991). The result is expressed in µmol oxidized/min/g of fresh vegetal material.

Instrumentation and chromatographic conditions: In this study, we employed the device type Shimadzu LC 20 AL equipped with universal injector (Hamilton 25 µL), UV-VIS detector SPD 20A (Shimadzu).

The mobile phase was prepared of Acetonitrile and Acetic Acid 0.1%. The mixture was filtered through a 0.45 µm membrane filter, pumped from the solvent reservoir to the column at a flow rate of 1mL/min.

The volume of injection was 50 µL and the column temperature was maintained at room temperature level. Prior to injection of analysis, the column was equilibrated for 40-50 min with the mobile phase in the effluent of detected λ = 268 nm.

On this chromatography we can estimate the concentrations of four phenolic acids (Gallic acid, Chloroginic acid, Vanilic acid and p-coumaric acid) and two flavonoids compounds (Vanillin and Rutin).

Anti-free radical's tests: The anti-free radical's activities estimated in-vitro was performed by two tests: the free radical scavenging DPPH• and the total antioxidant capacity.

The scavenging of free radical DPPH• test: The anti-free radical activity of extracts was measured by the method described by Chouikh et al. (2016) with a slight modification: 0.5ml of different concentrations of extracts with 1ml DPPH (0.1mM), the tubes of mixtures were incubated for 30 min at laboratory temperature. The absorbance was measured in λ=515 nm. The percent inhibition is determined by this formula:

%DPPH radical scavenging = $[(A_c - A_s)/A_c]$ x100.

Where A_c is the abs of the control and A_s is the abs of the sample.

IC_{50} (The effective concentration having 50% radical inhibition activity) expressed as µg extract/ml, was determined from the graph of the free radical scavenging activity (%) against the extract concentrations (Chouikh et al. 2018).

Total antioxidant capacity: The antioxidant capacity of extracts collected in different sites was evaluated by the phosphore molybdenum method (Prieto et al. 1999). An aliquot of 0.1ml of extracts solution was combined with 1ml of phosphore molybdenum solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).The tubes were capped and incubated in water bath at 95°C for 90 min. The samples were cooled and the absorbance of each at 695nm was read. The antioxidant capacity was determined from the graph of Gallic acid (Y=12.103x+0.1139 R^2 = 0.9552), the results expressed in (mg Gallic acid equivalent /g dried extract).

Statistical analysis: Each value was the mean ± SD of three experiments. The correlation between these mean values was performed by Microsoft Excel 2010.

Results

Values of pH and the Dosage of total proteins: The values of pH in four samples are presented in Figure 1. The pH of all sites samples are acidic values confined between: 4.26 in S_{04} and 4.74 in S_{02}; But the high value of total protein was registered in site 03 (6.66µg/g FVM) followed by the values (5.32, 4.81, 4.08 µg/g FVM) for samples of sites 04, 02 and 01 respectively.

Estimate the catalase activity: According to Fig. 3, the high activity of catalase was gradually recorded as follows: S_{01} (2.21 µmol oxidized in min/g of FVM) > S_{02} (1.87 µmol oxidized in min/g of FVM) > S_{03} (1.58 µmol oxidized in min/g of FVM) and finally S_{04} (1.46 µmol oxidized in min/g of FVM).

In ours study, we was found significantly a correlation (Fig. 4) was established between Catalase activity and pH (R^2 = 0.6302), and between the values of Catalase activity and the values of total proteins (R^2 = 0.6025), that indicated the reaction of catalase is mainly due to pH and total proteins.

Total polyphenol and flavonoids contents: The contents of polyphenols and the flavonoids (Fig. 5) was determined from the equation of calibration line of Gallic acid [Abs= 1.9486 + 0.0369 (R^2 = 0.9765) and Quercetin: Abs= 11.74 + 0.0723 (R^2 = 0.9752)] respectively.

We observed variation in polyphenols contents ranging from 4.99mg AGE/g Ex in site 01to 2.36mg AGE/g Ex at site 04, but the flavonoids values were convergent and all sites within 1 mg QE/g Ex.

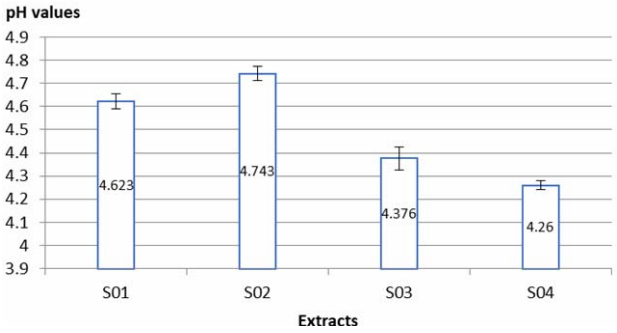

Figure 1. The values of pH of different samples collected in the four sites of the study area.

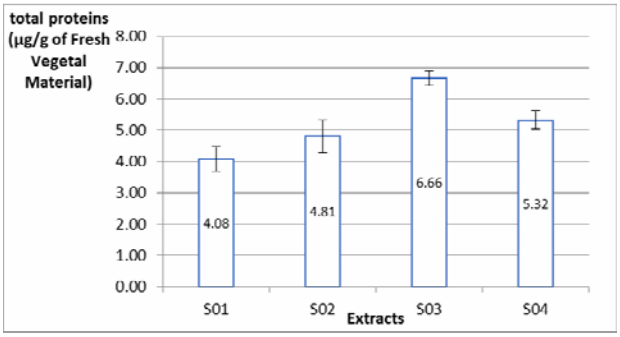

Figure 2. The values of total proteins (µg/g of Fresh Vegetal Material) of the four samples collected in the study area.

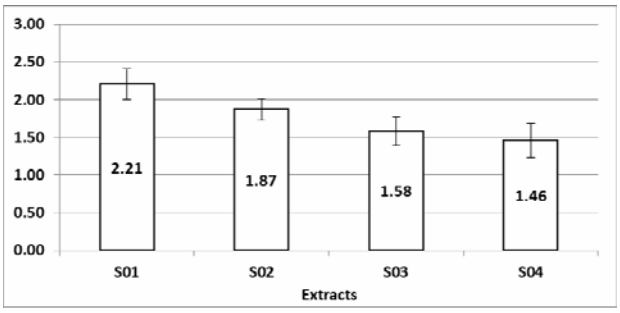

Figure 3. Catalase activity in (µmol oxidized in minute/g of Fresh Vegetal Material) of different samples collected in different sites.

HPLC Chromatography's of different extracts of samples collected in four sites: According to chromatography's set out in the Fig. 6. and Table 1, we were able to know the number of compounds for each extract (peaks) by comparing their retention times with standard compounds., resulted in a difference in the number of compounds per extract, about 43 compounds were counted as the maximum number in S_{03}. We also recorded the appearance of some phenolic compounds: Gallic acid, Chlorogenic acid, Vanillic acid, p-Comaric acid, Vanillin and Rutinin in some extracts and the absence in some other extracts.

The total numbers and the concentrations of phenolic compounds known were showed in Table 1.

Through the results listed in Table 1, we observed:

- Appearance of Vanillic acid in all extracts but in different concentrations.
- Gallic acid appeared in extract S_{03} only.

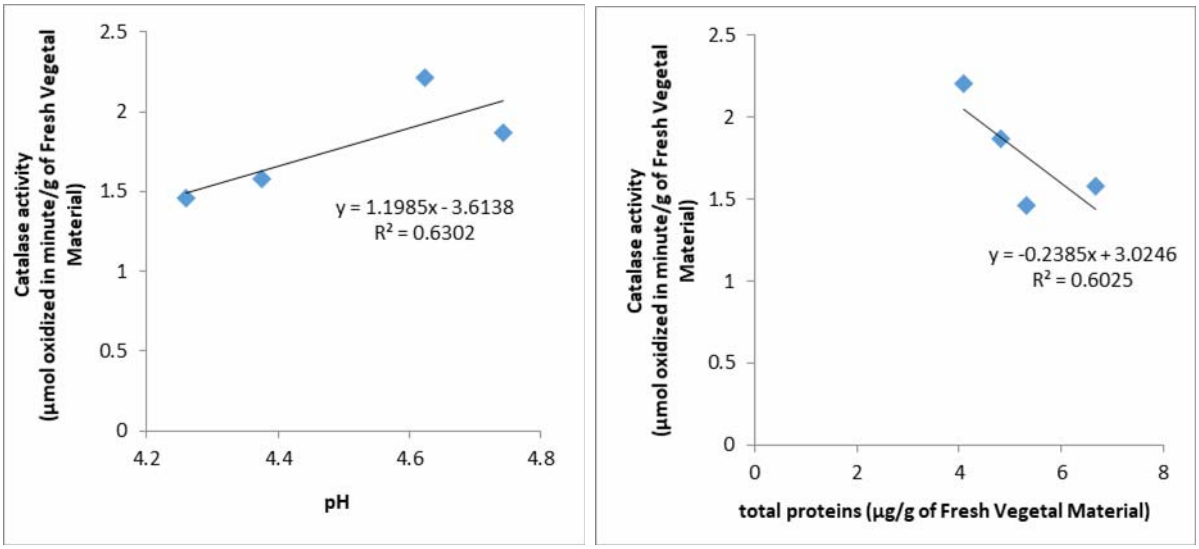

Figure 4. The correlation between Catalase activity and pH / Total proteins of the samples of *Calligonum comosum*.

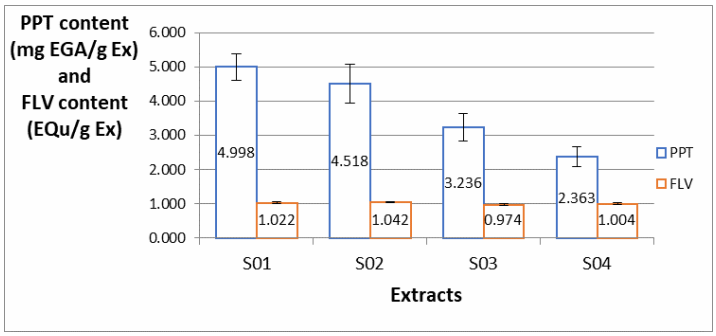

Figure 5. Polyphenols and Flavonoids contents in different extracts of *C. comosum* collected in different sites.

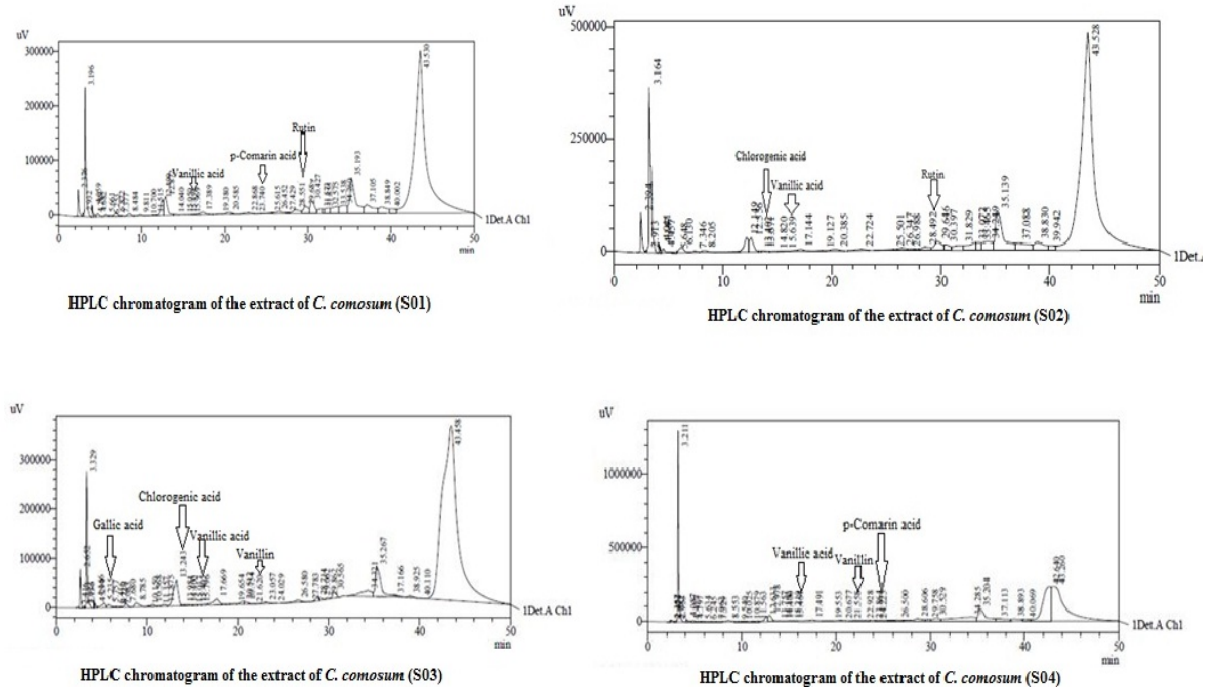

Figure 6. HPLC chromatogram'sof the extracts of *C. comosum* collected in four sites.

Table 1. Numbers total and concentrations of some phenolic compounds in the extracts of *C. comosum* collected in different sites.

Extracts of *C. comosum*	S_{01}	S_{02}	S_{03}	S_{04}
Numbers total of Compounds	41	35	43	42
Concentrations of some Phenolic Acids µg/mg extract				
Gallic acid	/	/	1.198	/
Chloroginic acid	/	0.379	17.152	/
Vanillic acid	0.067	0.148	0.143	0.612
p-Comaric acid	0.012	/	/	0.647
Concentrations of some Flavonoids µg/mg extract				
Vanillin	/	/	0.017	0.285
Rutin	2.937	2.993	/	/

- The Vanillin observed in two extracts (S_{03} and S_{04}), at variance that the Rutin appeared in other extracts (S_{01} and S_{02}).
- The high concentration of Chloroginic acid in S_{03} extract (17.152 µg/mg extract) compared to the concentration of other extract S_{02} (0.379 µg/mg extract).
- The significant concentration of p-Comarin acid in extract S_{04}compared to what it is at in extract of site 01.

The Tests of anti-free radical's activities: The results shown in Table 2 indicated that the all extracts of *C. comosum* had high antioxidant capacity with a best value in extract of sample of site 02(2.574 mg Gallic acid equivalent/g dried extract); for DPPH• radical scavenging test presented in Table 2 the all extracts have best values of IC_{50} which ranged between 11.6 and 14.6 µg/ml.

After examined the correlations between the PPT contents, FLV contents, quantitative and qualitative of phenolic compounds on one side and the tests anti-free radicals on other side we found five correlations with Total Antioxidant Capacity (TAC) (Fig. 7), four significant correlations were between TAC and (Vanillin, Vanillic acid, p-Comaric acid and PPT content) with the correlation values (R^2 = 9319; R^2 = 9061; R^2 = 8971 and R^2 = 8485 respectively); and one significant correlation between TAC and Rutin (R^2 = 0.6424).

Discussion

These results are in agreement with those found by Mohammed et al. (2009) based on their work on physicochemical and partial purification of crude alkaloids compounds in leaves and roots of (*Solanum nigrum*). The pH may be due to genetic factors (Tombesi 1994), also may be due to dirt and climatic factors (Yurtseven et al. 2005).

It has been shown by Sun et al. (2005), that the solubility of the phenolic compounds is a function of their degree of polymerization, the interaction with the other constituents and the type of solvent used.

As the chemical compositions of the plant play an important role in the extraction process, this may be due to the difference in geographical location and selective collection, or to the conditions in which the extraction was performed (Lee et al. 2003).

According to Seidel (2012), the variation in total polyphenol content between different extracts may be attributed to the solubility of the polyphenols, their degree of polymerization as well as their interaction with other constituents and the formation of insoluble complexes**.** In the other hand, the environmental factors such as light, temperature, UV radiation, Ozone and CO_2 have a direct or indirect influence on the formation of secondary metabolites (Sallas et al. 2001)**.**

In general, the content of polyphenols varies quantitatively from a plant to another; this can be related to several factors:

• Environmental factors: geographical area, drought, aggressions and diseases etc. (Ebrahimi et al. 2008).

• Genetic factors, time of harvest and stage of plant development (Miliauskas et al. 2004).

• The nature of the soil and the type of microclimate (Miliauskas et al. 2004).

According to Moure et al. (2001), is not necessarily the high content of polyphenols that exhibits potent antioxidant activity. The antioxidant activity depends not only on the concentration but also on the structure of these molecules. The low or high antioxidant capacity may be due to the polarity of solvents that change the ability to dissolve a selected group of antioxidant compounds, which influences the evaluation of antioxidant activity (Turkmen et al. 2007), and due to the structure and nature of phenolic compounds and their concentration in plant tissues (Rice-Evans et al. 1997).

Generally the antioxidant activity studied varies according to the composition of each extract and always reflects this variability which could be due to the different factors: the temperature, it strongly determines the level of activity of the enzymes involved in the synthesis of polyphenols. As a result, the various thermal conditions could lead to significant changes in the final concentration of polyphenols. Other environmental factors could probably play a role in polyphenol synthesis such as CO_2 content, (C / N interaction) or the level of oxidative stress (Sallas et al. 2001).

The accumulation of amino acids can indeed play a role in the osmo-regulation of cells in cases of water deficit and serve as an indicator of drought and / or a stress detector (Aspinall & Paleg 1981, Grote & Claussen 2001). According to Heckathorn et al. (2002), protein expression increases when the body is exposed to metals. The increase in protein content may also be due to an activation of a set of genes allowing the synthesis of specific proteins associated with stress, such as proteins that provide protection for the vital whole of cellular proteins (David & Grongnet 2001).

In the arid and semi-arid zones, are subject in particular

Table 2. The values of Total Antioxidant Capacity (TAC) and IC_{50} of different extracts of *C. comosum* collected in different sites.

Extracts of *C. comosum*	S_{01}	S_{02}	S_{03}	S_{04}
Total Antioxidant Capacity (TAC)mg Gallic acid equivalent/g dried extract	2.539±0.039	2.574±0.074	2.391±0.091	2.023±0.021
IC_{50} (µg/ml) DPPH• test	14.6±0.5	11.7±0.4	11.6±0.6	14.3±0.2

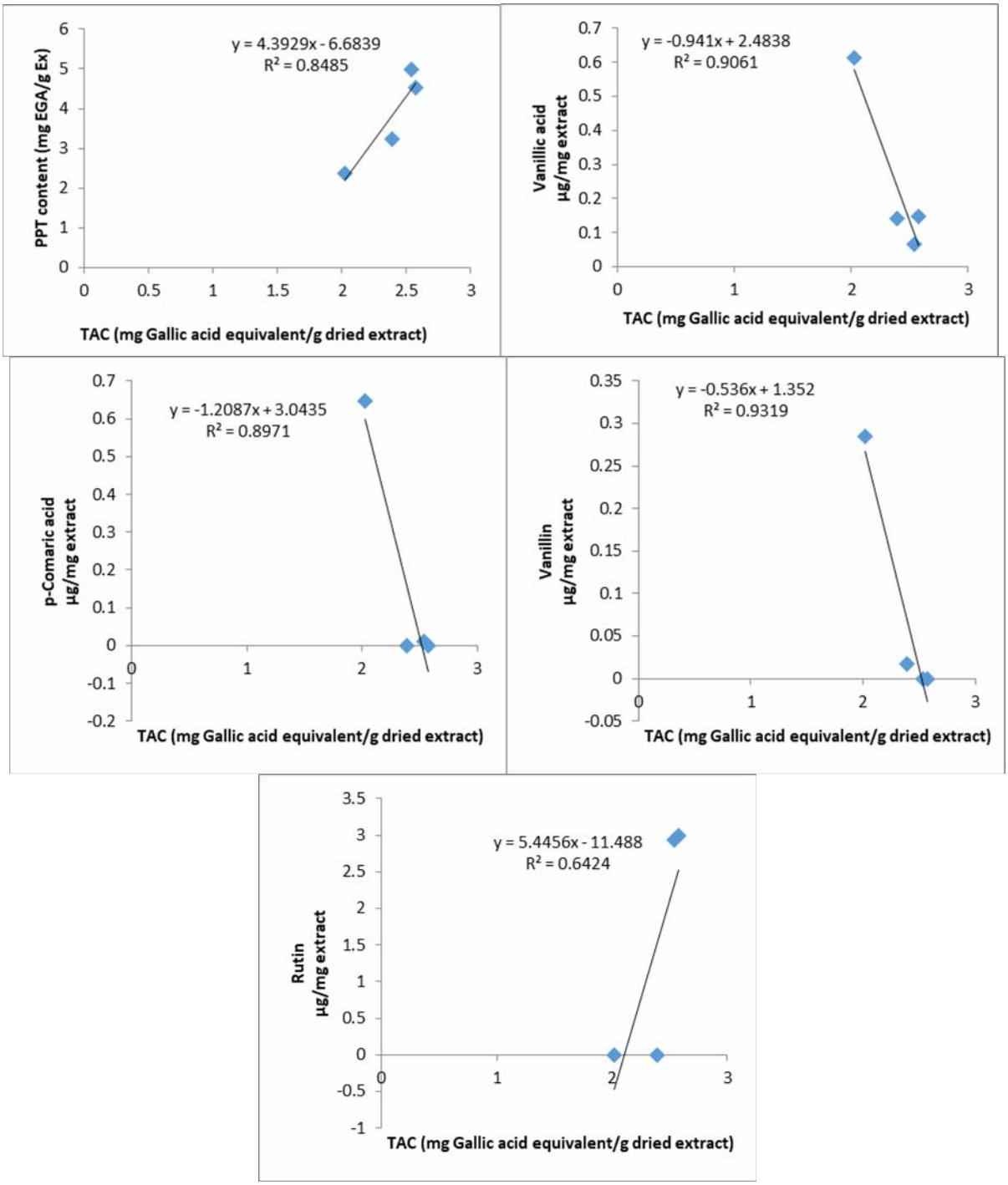

Figure 7. The correlation between Total Antioxidant Capacity (TAC) and (Vanillin, Vanillic acid, p-Comaric acid, PPT content and Rutin respectively) of the extracts of *C. comosum*.

to abiotic stress characterized by the frequency of drought and soil salinization (Pooja & Rajesh 2015), induced the presence of free radicals that damage macromolecules such as proteins (Sbartai 2008). According to many authors, water and salt stress can reduce photosynthesis as well as photochemical activity by producing different deamination in protein residues (Zhong et al. 2017).

In general, catalase activity is stimulated with low doses and inhibited with high doses, this change in enzyme activity is dependent on the severity of stress, varieties and stage of development (Ashraf et al. 2012). Studies have shown that treatment of plants with saline solutions has reduced the activity of this enzyme in lawn grasses and tomatoes (Doudech et al. 2008, Burcu et al., 2010). A decrease in catalase activity under intense stress conditions has been attributed to the inactivation of certain enzymes caused by free radicals. The emission of free radicals is then greater than the detoxification capacity of the enzyme and may contribute to reduce its activity, decrease enzyme synthesis or disruption at the time of assembly of enzymatic protein subunits (Lü et al. 2010).

Through the results obtained by high-performance liquid chromatography (HPLC), which showed the appearance and

absence of many phenolic compounds, the hypothesis that: geographical location, environmental and climatic changes in plant growth areas (local climate, soils, companion plants etc.) have an active role in the quantitative and qualitative diversity of phenolic compounds.

The quantitative and qualitative changes of these compounds in plant extracts can be attributed to environmental conditions; exposure of the plant to biotic and abiotic stress; allelopatic phenomenon (Heidi 1993).

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