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Contribution to the quantification and antioxidant activity of polyphenols from cultivated and spontaneous garlic that grow in the western of Algeria

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ABSTRACT

Background: Garlic plant is a traditional medicinal plant that have noumerous biological activity due to their secondary metabolism.**. Objective:** write t The present work is a comparative study between two varieties of *allium sativum* (white and red) and other spontaneous garlic (*allium vineal*) for this we have quantify total phenolic using folin sicalteu method and total flavonoids using trichlorure aluminum method and condensed tannins using vanillin method then we have estimate the antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl method. **Results**: Our result highlight that the spountanous garlic is the most concentrated in phenolic compound and that the red garlic is more concentrated then the white. We have demonstrated that the antioxidant activity is important in the red garlic**. Conclusion:** this work can validate the difference between the importance traditional uses of this plant.

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INTRODUCTION

Potential health benefits of vegetables belonging to the *allium* sp species, particularly garlic have been used from ancient times to treat various disorders, but still extensive research is required to establish the claimed benefits (Galeone *et al*., 2006).

Garlic is a common plant in algeria includes the species *Allium sativum which* is cultivated such as weed however we can find others species spontaneously such as *allium vineal* (baba issa,2011)

In the present work we compared between two varieties (red and white) of Alium sativum and spontaneous plant of *allium vineal* witch grows in the western of Algeria, for this we have used spectroscopic method to quantify total phenols, total flavonoids ,condensed tannins and antioxyant activity

MATERIAL AND METHODS

Sampling Method:

Cultivars garlic (white and red) were collected from the insitue technics in june,2013 situated in Sidi Bel Abbes city (Algeria NW), the spountanous variety is collected in the same time from Ain nahla region which is situated in Tlemcen. Bubbles were dried in the shield and ambient temperature for 2 weeks.

Extraction Method:

Each 2 g of dried bubbles were grinding by mortar in 10 minutes, then was placed in an Erlenmeyer with 20 ml of methanol (96 %) during 24 hours. After filtration (using paper watt man 1ml), the methanolic solutions were evaporated under a reduced pressure in a rotary evaporator standard Buchi r-200 at 40°C. The weighed dry residues were taken again by 3 ml of methanol (matkowski & piotrowska, 2006).

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Total phenols assay:

A volume of 200 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagent diluted 10 times with water and 0.8 ml of a 7.5 % sodium carbonate solution in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm by using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. (Boizot & Charpentier, 2006).

Total Flavonoids assay:

At zero time, 0.3 ml 5 % sodium nitrite was added to the flask. After 5 min, 0.3 ml of 10 % aluminium chloride was added. At 6 min, 2 ml of 1 m sodium hydroxide was added to the mix. At one time, the mixture was diluted to volume with the addition of 2.4 ml distilled water and thoroughly mixed. The absorbance of the mixture, pink in color, was determined at 510 nm versus a blank containing all reagents except samples of extracts or fractions. Catechin was used as a standard for the calibration curve. (Zhishen *et al*., 1999)

Condensed tannin assay:

Condensed tannins were determined by vanillin under acidic conditions (price *et al*., 1978). This method is based on the ability of reacting with the vanillin units of condensed tannins in the presence of acid to produce a colored complex measured at 500 nm. The reactivity of vanillin with tannins involved only the first unit of polymer. The amounts of tannins were estimated by: counted 0,1ml sample condensation packed into a wrapped reaction tube, then enhanced by 3 ml vanilin 4% (b/v) then merged using a vortex mixer, immediately enhanced by 1,5 condensed HCL meal and mixed. Absorbance of the sample was read at λ 500 nm after incubation during 20 minutes at room temperature. Obstetrical of condensed tannin was expressed as catechin equivalent in mg/g of dry or fresh matter and catechin equivalent in mg/g of methanolic extract matter.

DPPH scavenging assay:

The hydrogen atom's donation ability of chemical compounds was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical.Fifty microliter of various concentrations of the extracts in methanol were added to 1,950 ml of a 0.025 g/l methanol solution DPPH.

The incubation was doing in 30 minute at room temperature after that the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was estimated utilizing the following recipe:

DPPH scavenging activity (%) = (A blank- A sample/A blank) X 100:

Where a blank is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC50) was estimated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid methanol solution was applied as positive control. (benhammou *et al*, 2006)

RESULTS AND DISCUSSION

in medicinal plants research , different organs may have a different concentration in phenolic compound (Macheix *et al*., 2005). However, it is difficult to compare the results with those of the literature for the use of different methods of extraction and quantification because that reduced reliability of a comparison of the studies (Lee *et al*. 2003).

Total phenols:

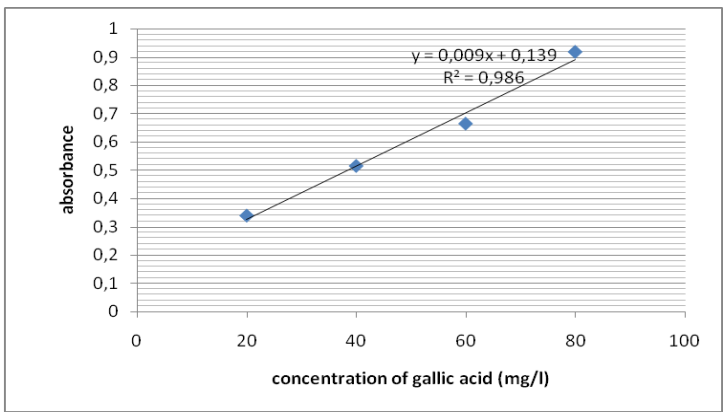

Fig. 1: calibration curve of gallic acid

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Total phenol compounds are reported as Gallic acid equivalents by reference to a standard curve (y = y = 0,0001 x + 0,135, R^2 = 0,991.). the total phenolic content was expressed as milligrams of gallic acid equivalents per gram of bubls and milligrams of gallic acid equivalents per g of methanolic extract

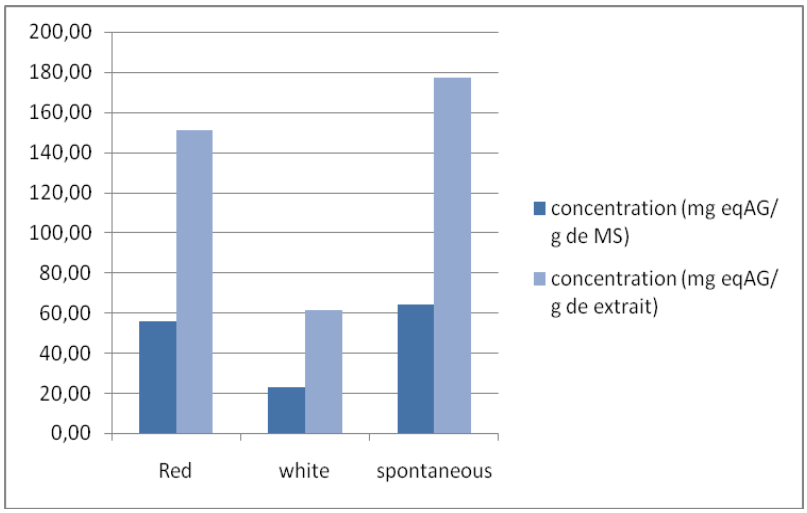

Fig. 2: total phenols concentrations of different garlic

Quantification of total phenols highlights that the spontaneous has the best concentration with (63,94±2,64 mg /g of dry buble ,177,13±7,31 mg/g of extract) and the red garlic is more concentrate then white garlic with (55,73±0,88 mg/g of dry buble, 150,70±2,39 mg/g of extract ; 22,63±1,33 mg/g of dry bulbe, 61,08±3,58mg/g of extract) respectively.

Total flavonoids:

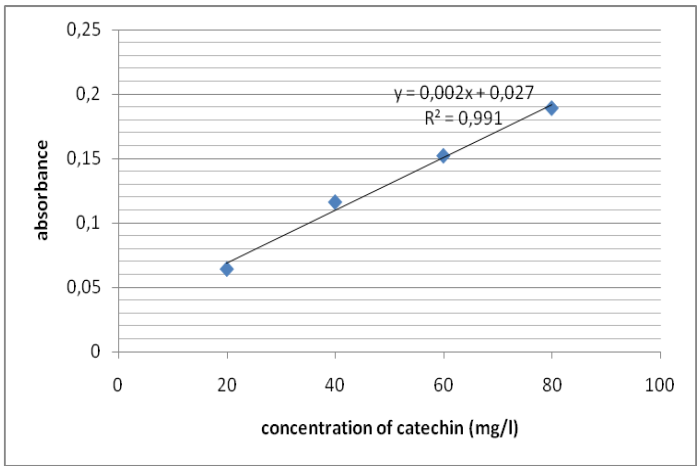

Fig. 3 calibration curve of catechin

The total flavonoid content of the extracts and fractions were expressed by reference to a standard curve (y = 0,0021x + 0,0275, R^2 = 0,9917) as catechine mg equivalents per gram of bubles and per gram of methanolic extract.

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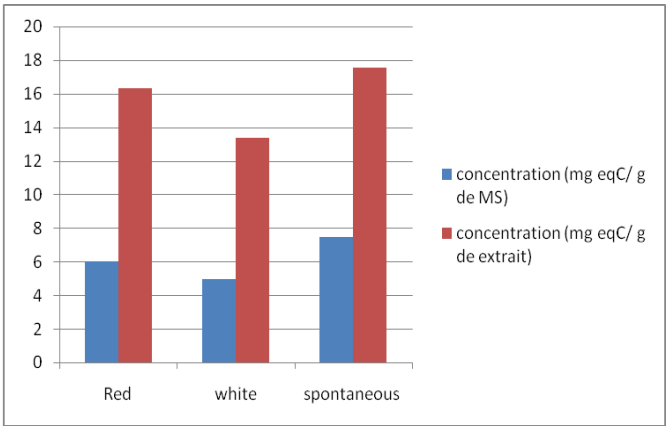

Fig. 4: Total flavonoids concentrations of different garlic

Quantification of total flavonoid highlights that the spontaneous have the best concentration with (7,44±0,71mg/g of dry bulbe,17,53±1,68mg/g of extract) and the red garlic is more concentrate then white garlic with (6,03±0,02mg/g of dry bulbe, 16,30±0,06 mg/g of extract; 4,96±0,82 mg/g of dry bulbe, 13,38±2,21mg/g of extract) respectively

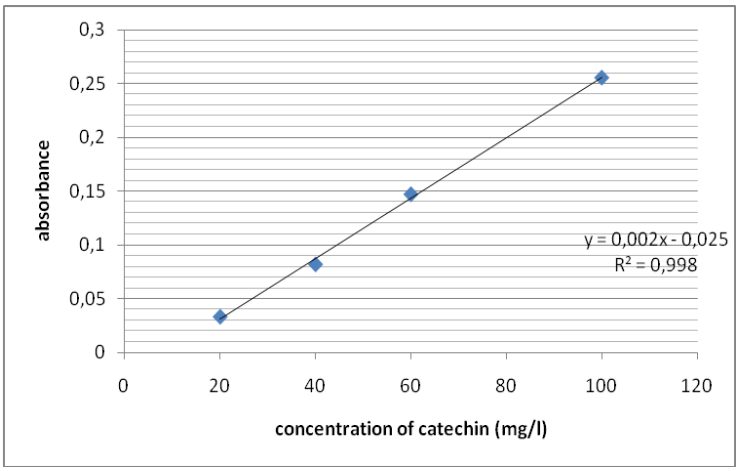

Fig. 5: calibration curve of catechin

Curve calibrates were drawn up by catechin equivalents by references to a standard curve (y = 0,0028x - 0,0253, R^2 = 0,9983) as standard

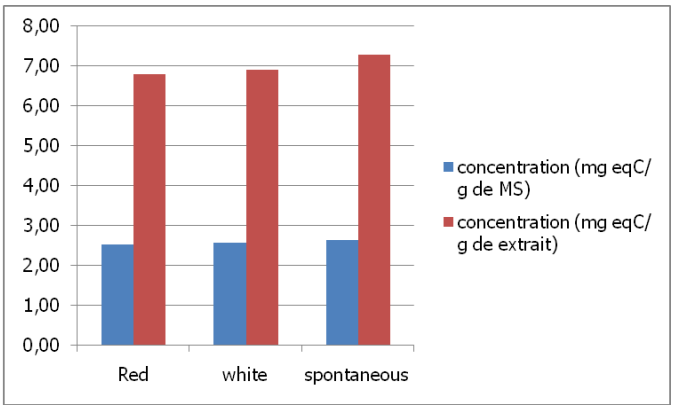

Fig. 6: condensed tannins concentrations of different garlic

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Quantification of condensed tannins highlights that the spontaneous have the best concentration with (2,62±0,36 mg/g of dry bulbe, 7,29± 0,97 mg/g of extract) and the red garlic is more concentrate then white garlic with (2,56± 0,05mg/g of dry bulbe, 6,91 ±0,13 mg/g of extract; 2,51±0,01 mg/g of dry bulbe, 6,79±0,02 mg/g of extract) respectively

Antioxidant activity

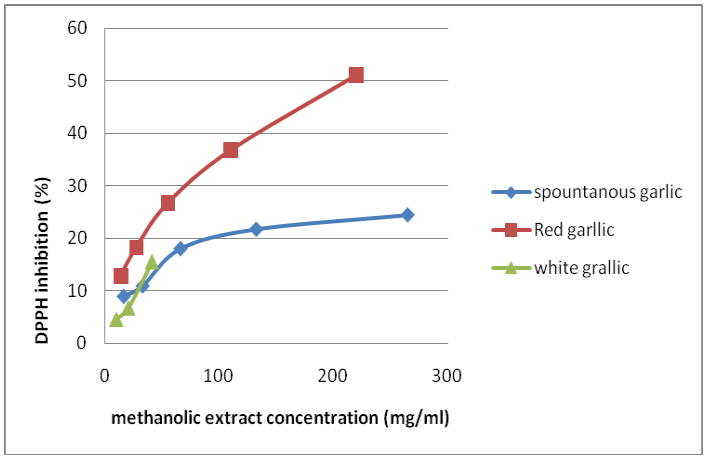

Fig. 7: DPPH inhibition of different methanolic concentrations of garlic

Our results highlight that the red garlic have more antioxidant activity then the white, and the spontaneous garlic have the best. Various factors, such as genotype of cultivar, growing season, postharvest treatment, and cultivation places are responsible for the variation in the antioxidant acivity in garlic (Beato *et al*., 2011; Bozin *et al*., 2008; Sangwan *et al*., 2010; Bhandari, 2014).

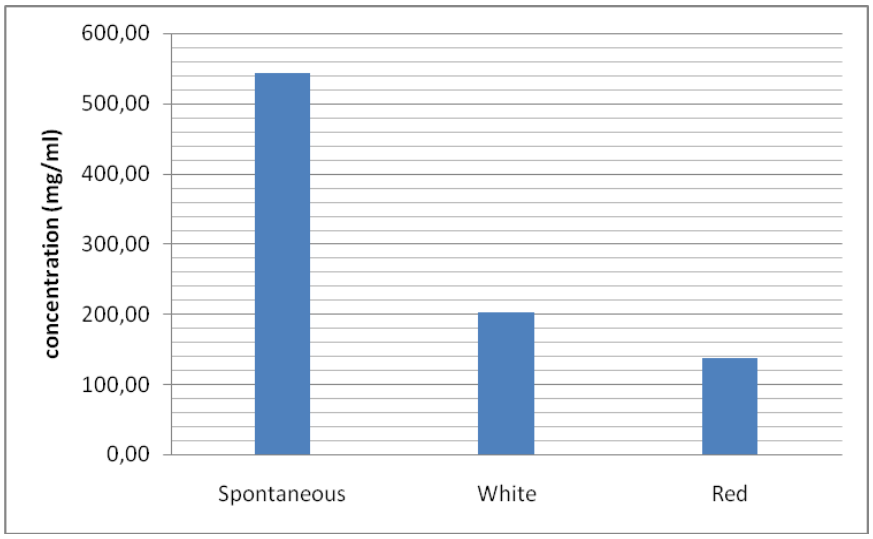

Fig. 7: the antioxidant of different garlic evaluated as IC 50

Conclusion:

In this study we have demonstrated the difference between cultivars and spontaneous varieties of common garlics that are situated in the western of Algeria

Phenolic compound quantification and antioxidant activity measured can validate the traditional use of this medicinal plant

In the future we look to work deeply in this research and to identify these molecules and prove there others biological activities.

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