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ORIGINAL RESEARCH PAPER

A COMPARATIVE STUDY ON ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF FIVE ALGERIAN EGGPLANT CULTIVARS

Chérifa Boubekri, Touhami Lanez*, Assia Djouadi

Université d'El Oued, VTRS Laboratory, PO Box 789, 39000, El Oued, Algeria

*Corresponding author: touhami-lanez@univ-eloued.dz

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Abstract: Total phenolic content and antioxidant activity were measured in the whole fruit of dark-purple eggplant cultivars from five different regions of east Algeria using, respectively, the Folin-Ciocalteu method, spectrophotometrical and electrochemical assays. Total phenolic contents were highest in sample from Jijel, followed by Skikda cultivars, finally sample from Guelma with the lowest phenolic contents. Total phenolic content was positively associated with total antioxidant activity in eggplant extracts. These results demonstrate that phenolic compounds have a significant contribution to the total antioxidant activity, which varies considerably depending on the region of the fruit cultivar analyzed. Antioxidant activity was highly correlated with total phenolic contents (R^2 = 0.714).

Keywords: *cyclic voltammetry, DPPH radicals, Folin-Ciocalteu test, phenolic contents, Solanum melongena L.*

INTRODUCTION

Eggplants/aubergines *(Solanum melongena L.)* are a tropical fruit originated from Asia. They vary in colours from dark purplish black to pale purple, white, orange and green, and can be solid colours, striped or mottled. Moreover, they have diverse shapes from egg-shaped to sausage-shaped and pear-shaped [1]. Eggplant consumption has been associated with reduced risk of degenerative diseases, such as cancer and cardiovascular diseases [2, 3]. This association is often attributed to the phenolics antioxidants contained in eggplant, which can protect the human body against oxidative stress by scavenging oxygen free radicals [4]. Eggplant consumption is also believed to lower blood cholesterol [5], prompting research into its possible effects on cardiovascular disease.

The chemical composition and antioxidant capacities of eggplant from many countries have been widely studied by several groups [6 - 9], but only a few reports can be found in literature on Algerian eggplants. This motivated us to explore the antioxidant activity and the total phenolic contents of Algerian eggplants.

The aim of this work is to evaluate the phenolic contents and the antioxidant activity of the ethanolic extract of 5 dark-purple eggplant cultivars from 5 states in east of Algeria using, respectively, spectrophotometrical and electrochemical techniques [10 - 15].

MATERIALS AND METHODS

Chemical

Ethanol (99 %) was purchased from Sigma-Aldrich Co. Ascorbic acid (99.7 %) and sodium carbonate (99 %) were both purchased from Merck & Co. Folin Ciocalteu reagent was purchased from Biochem Chemopharma Co (Canada). All other reagents used were of analytical grade.

Plant material

Plant materials used in this study consisted of five varieties of dark purple eggplant fruits (Table 1). They were collected fresh from five regions in east of Algeria during the harvesting period, from October to November 2011. Immediately after receiving, all samples were peeled using a kitchen knife. Each fresh eggplant was cleaned, air-dried under shade, ground into fine powder using a food mixture and kept at room temperature for future analysis.

Table 1. *Varieties of common eggplant used in a study of total phenolic contents and antioxidant activity*

Varity	Origin	Fruit type*
ELO	El-Oued	Semilong
JIJ	Jijel	Round
GUL	Guelma	Round
SKI	Skikda	Semilong
BAT	Batna	Long

*The round, semilong, and long types considered here are distinguished by the length-to-breadth ratio of the fruit (≈1 to 1.5 for the round, > 1.5 and < 2 for the semilong, and > 2 for the long)

Extraction of eggplant constituents

The powdered samples of each variety (5 g) were extracted with 100 mL of ethanol for 2 hours using a Soxhlet extractor. The extracts were then filtered under suction using Whatman filter No. 4 paper; the filtrate was recovered and evaporated at reduced temperature and pressure. All dry fractions were sealed and stored at -5 °C for further use. The yield was 2.27 – 12. 84 %, Table 2.

Table 2. *Extraction yield from different cultivars of eggplant*

Variety	Mass [g]	Yield [%]*
ELO	0.3463	6.93
JIJ	0.1133	2.27
GUL	0.6421	12.84
SKI	0.2604	5.21
BAT	0.3022	6.04

* (weight of extracts/ weight of dried eggplant) × 100

Total phenolic content

The total phenolic contents were measured using a colorimetric Folin-Ciocalteu method [16]. Briefly, 1 mL of extracts of each part of eggplant was diluted with bidistilled water to a volume of 10 mL in a volumetric flask, and 1 mL of Folin-Ciocalteu reagent was then added. After 3 minutes, 2 mL of 20 % sodium carbonate solution was added to the mixture. The solution was incubated in darkness at room temperature for 30 minutes. The Absorbance of the reaction mixture was read at 760 nm using Shimadzu UV-Vis-1800 spectrophotometer. The measurement was compared to a standard curve of prepared gallic acid solutions (10 points from 3 to 300 $mg \cdot L^{-1}$) and expressed as milligrams of gallic acid (GA) equivalents per 100 g of dry extract (DE). The equation obtained from the linear calibration graph in the studied concentration range for gallic acid is as follows: $y = 3.54\ x + 0.039$ (where y represents the value of the anodic current density and x the value of standards concentration, expressed as mg GA/100g DE), with a correlation coefficient of $r^2 = 0.996$.

Evaluation of antioxidant activity

The measurement of the antioxidant activity of the studied eggplant samples was performed using a spectrophotometrical and electrochemical assays based on cyclic voltammetry techniques. Cyclic voltammetry measurements were conducted using PGP301 potentiostat (radiometer analytical SAS) connected to an electrochemical cell with a volumetric capacity of 50 mL containing a glassy carbon working electrode having area 0.013 cm^2, a Pt wire counter electrode and an Hg/Hg_2Cl_2 reference electrode. The potential was swept in direct scanning mode starting from -200 to +800 mV with a scanning rate of 100 $mV \cdot s^{-1}$. The antioxidant activity was obtained using the current density of the anodic curve of the voltammogram. The calibration graph is obtained by plotting the current density of the anodic curve of the voltammogram of each sample of the standard versus its concentration. Ascorbic acid was used as a standard in the calculation of antioxidant activity, considering its wide spreading in

nature and the fact that its anodic current density displays excellent linearity toward ascorbic acid concentrations [17, 18]. The equation obtained from the linear calibration graph in the studied concentration range for ascorbic acid is as follows: $y = 132.1\ x + 1.189$ (where y represents the value of the anodic current density and x the value of standards concentration expressed as $g \cdot L^{-1}$), with a correlation coefficient of $r^2 = 0.997$.

RESULTS AND DISCUSSION

Total phenolic content

Large differences in phenolic contents were found among the different varieties of the studied materials, with a range from 91.42 ± 15.48 mg GA/100g DE in JIJ variety to 50.98 ± 5.93 mg GA/100g DE in GUL variety which corresponds to a difference of nearly twofold Table 3.
This study showed that the total phenolic contents of the different varieties were significantly different from each other. This might take into account the influence of climatic conditions of the place of cultivation of *Solanum melongena L.* plant.

Table 3. *Total phenolic content (mg gallic acid equivalents/100 g dry extract) of different eggplant cultivars*

Cultivar	Total phenolic content
ELO	52.51 ± 7.88
JIJ	91.42 ± 15.48
GUL	50.98 ± 5.93
SKI	61.02 ± 3.91
BAT	59.53 ± 18.54

Evaluation of antioxidant activity using spectrophotometrical assays

The free radical scavenging capacity of ethanolic eggplant extract was measured in terms of hydrogen donating or free radical scavenging ability by using the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) (Molyneux, 2004), eggplant extract and standard gallic acid solution (0.1 mL) of different concentrations viz. 0.1, 0.2, 0.4, 0.6, 0.8 and 1 $mg \cdot L^{-1}$ was added to 1 mL of a 0.004 % methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition capacity was calculated from the following relation (1).

$$\%\ inhibition = \frac{A_0 - A_1}{A_0} \times 100 \qquad (1)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The antioxidant capacity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in $\mu g.mL^{-1}$) of extracts that inhibits the formation of DPPH radicals by 50 %. All the tests were performed in triplicate and the graph was plotted with the average of three observations.
The equation obtained from the linear calibration graph in the studied concentration range for gallic acid is $y = 271.04\ x$ (where y represents the value of absorbance and x,

the value of gallic acid concentration, expressed as g·L^{-1}) with a correlation coefficient of r^2 = 0.9942. Under the same conditions the equation of the calibration graph for different eggplant cultivars is represented in Table 4.

Table 4. *Comparison of DPPH free radical inhibitory of the eggplant extract and gallic acid*

Samples	Equation	r^2 values	IC_{50} values [g.L^{-1}]
Gallic acid	$y = 271.04\ x$	0.9942	0.18447 ± 0.0231
ELO	$y = -68.316\ x + 93.551$	0.6921	0.5151 ± 0.1318
JIJ	$y = -99.035\ x + 95.013$	0.926	0.4334 ± 0.0783
GUL	$y = -76.75\ x + 96.363$	0.9564	0.6644 ± 1.1508
SKI	$y = -44.356\ x + 92.385$	0.9475	1.1492 ± 0.5794
BAT	$y = -32.685\ x + 93.114$	0.6167	0.6708 ± 0.2458

As it can be seen from Table 4, the ethanolic extracts of JIJ samples have the highest antioxidant activity followed by ELO, and then GUL and BAT have the same antioxidant activity and finally SKI with the lowest antioxidant activity.

Evaluation of antioxidant activity of eggplant samples using electrochemical assays

In order to express the antioxidant activity of different parts of the eggplant extracts in equivalent terms of ascorbic acid equivalent antioxidant capacity (AEAC), different concentrations of the standards ascorbic acid (0.018 to 0.190 g.L^{-1}) were plotted versus the anodic current density obtained from different cyclic voltammograms at *p*H 7 in 0.2 M phosphate buffer solution as a supporting electrolyte using a 3 mm-diameter glassy carbon electrode.

The total antioxidant activity was calculated based on the following equation (2),

$$TAA = \frac{C_1}{C_2} \qquad (2)$$

where *TAA* (mg·g^{-1}) is the total antioxidant activity, C_1 is the eggplant sample extract concentration (g·mL^{-1}) and C_2 is the sample concentration in the electrochemical cell (g·mL^{-1}) calculated by replacing the current density obtained from different voltammograms of sample extracts in the equation $y = 132.1\ x + 1.189$, obtained from the linear calibration graph in the studied concentration range for ascorbic acid.

Figure 1 shows different voltammograms of different parts of dark purple from El-Oued. Each voltammogram shows one oxidation peak and one reduction peak. This reversible electrochemical behavior may indicate that under this electrochemical condition, the ethanolic eggplant extract contains a different polyphenolic content of that of the standard ascorbic acid.

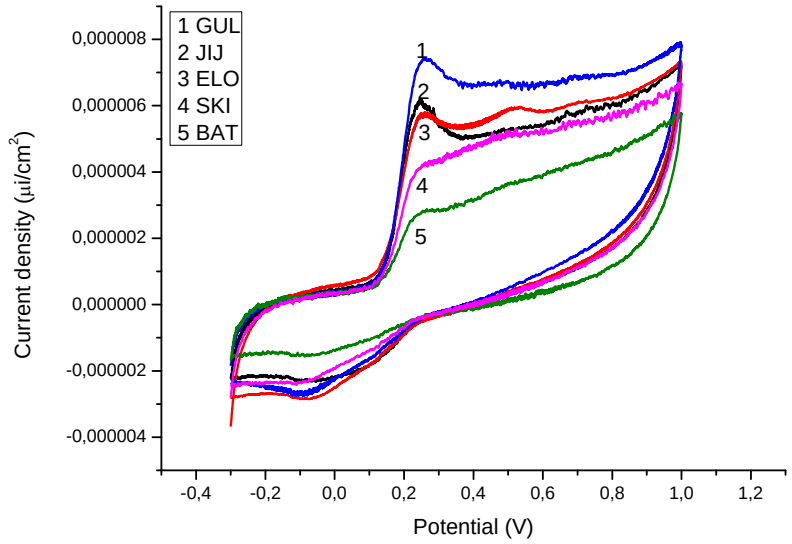

Figure 1. *Cyclic voltammograms of eggplant cultivars in pH 7, 0.2 M phosphate buffer solution at scan rate 100 mV.s^{-1}*

Cyclic voltammetry results indicate that ethanolic extracts from different eggplant cultivars do not reveal similar electrochemical responses to that of gallic acid, suggesting a different electroactive chemical composition and oxidation potential more positive than that of the standard gallic acid. Electrochemical data obtained from voltammograms of all studied eggplant samples are summarized in Table 5.
The total antioxidant activity expressed as mg of ascorbic acid per 100 g of dry extract (mg AA/100g DE) of different parts of eggplant was calculated from the equation $y = 132.1\ x + 1.189$, obtained from the calibration graphs for ascorbic acid (Table 5).

Table 5. *Electrochemical data and antioxidant capacities (mg ascorbic acid/100 g of dry extract) of different eggplant extracts*

Cultivar	ip_a [µA]	$E_{1/2}$ [mV]*	Antioxidant activities
ELO	2.505	125.57	18.64 ± 2.97
JIJ	3.123	134.24	8.84 ± 1.54
GUL	7.006	90.48	18.63 ± 1.40
SKI	7.529	100.68	10.90 ± 3.28
BAT	3.380	112.49	17.90 ± 3.03

*$E_{1/2} = (Ep_a + Ep_c)/2$

Correlation between total phenolic contents and total antioxidant activity

A direct correlation between total phenolic contents and total antioxidant activity was demonstrated by linear regression analysis (Figure 2). The correlation coefficient between the concentration of phenolic contents determined at 760 nm was very high (r = 0.976) in pulp and in the whole fruit (R^2 = 0.714), while a relatively low correlation coefficient was observed between total phenolic contents and antioxidant activities in peels (r = 0.533). Therefore, the total phenolic content could serve as a useful indicator

for the antioxidant activities of different parts of eggplants. These results demonstrate that phenolic contents and antioxidant activities of eggplant varied by parts and region.

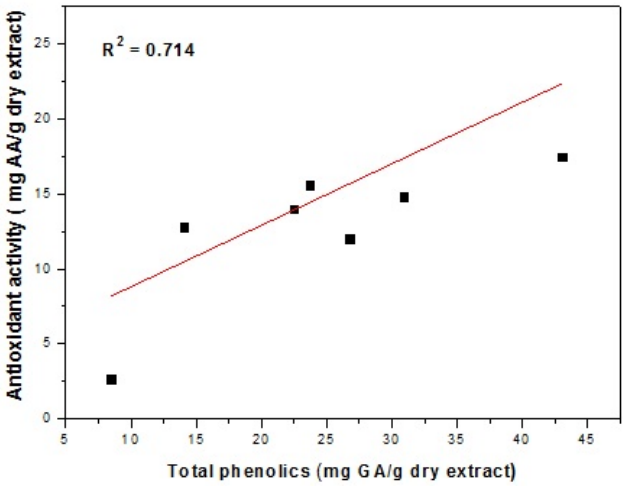

Figure 2. *Correlation of total phenolic content and antioxidant activity*

CONCLUSION

The present study demonstrated that dark purple eggplant cultivars from different regions of eastern Algeria have very strong antioxidant activity and high amounts of phenolic contents. The total phenolic content and antioxidant activity vary considerably depending on the eggplant cultivar analysed. The Folin-Ciocalteu and cyclic voltammetry assays showed that Jijl eggplant extract has higher phenolic contents and higher antioxidant values as compared to the other studied varieties. The correlation between total phenolic content and antioxidant activities was strong (R^2 = 0.714).

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