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PHYTOCHEMICAL STUDY OF THE THREE MAIN ONION VARIETIES (ALLIUM CEPA L.) GROWN IN ALGERIA

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Abstract

Three species of Allium cepa (red, purple, yellow) were evaluated for their secondary metabolite content, free radical scavenging activity (DPPH), and ferric reducing ability (FRAP). Our findings confirm that the dry matter content in all varieties ranged from 12.3% to 18.1%, rendering them suitable for extended storage. The ethanolic extract of red onion (EERO) displayed the highest yield (3.86%). Similarly, the levels of total phenols (TPC), total flavonoid content (TFC), hydroxycinnamic acid (HTC), and cysteine-containing peptide (CTC) varied between 0.14 and 0.27 mg/g GAE, 0.20 and 0.29 mg/g QE, 0.25 and 0.29 mg/g GAE, and 0.015 and 0.102 mg CE/g DW, respectively. Furthermore, ethanolic extract of yellow onion (EEYO) demonstrated a significant iron reduction capacity (0.75 ± 0.06 mM Fe2+) and an IC50 of 0.159 ± 0.18 mg/ml, indicating enhanced antioxidant potential.

Keywords: Allium Cepa L – variety – secondary metabolites – DPPH - FRAP

1. INTRODUCTION

Originating in Central Asia, the onion (Allium cepa L.) is cultivated as an agricultural crop for its foliage and bulbs (Pitrat & Fourny [1]). It is a fundamental ingredient in recipes worldwide (Mézrog & Baumgartner [2]). The bulb, typically spherical or pear-shaped, with a wide array of features, serves as a nutrient reserve organ supporting the vegetative propagation of the species (Bhattacharjee et al. [3]). The major onion bulb producers in the western region of Africa encompass Niger, Nigeria, Senegal, and Burkina Faso (Schmelzer & Gurib-Fakim [4]). Allium cepa bulbs are a well-vascularized variety and an essential component of many African condiments and sauces. It's one of the world's most prized veggies. Nutritionally, it provides all sorts of vitamins (B vitamins, vitamin C, provitamin A), inorganic salts (potassium, sodium, zinc, iron, phosphorus, selenium, magnesium, manganese, calcium), fats, proteins, carbohydrates, essential oils, organic acids, fibers (Ciqual [5]). Therapeutically, multiple studies have revealed that systematic intake of fresh onions influences blood coagulation, protects against several metabolic health problems (hypcholesterolemia, hypolipidemia, antithrombic effects), colorectal, stomach and prostate cancers (Bianchini & Vainio; You & Li; Graf et al. [6-8]). The preventive effect relies on organosulfur compounds (thiosulfimates), thath impede carcinogenic effects. (Corzo-Martínez et al. [9]). In this context, our research focuses on assessing the antioxidant properties through Ferric Reducing Antioxidant Power and 2,2-diphenyl-1-picrylhydrazyle tests, alongside quantifying secondary metabolites in three various cultivars (red, purple and yellow) widely cultivated in Algeria -Sidi Bel Abbès-.

2. MATERIALS AND METHODS

Biological material
Three Allium cepa varieties - red (Rd), yellow (Ylw), and violet (Prl) - were selected. Freshly harvested bulbs, uniform and flawless, were stored at 4°C before analysis. In the lab, bulbs were cleaned, peeled, diced, and oven-dried (Ecocell) at 40°C for 4 days. Subsequently, they were ground using an electric grinder (IKA), and the powder was collected after sieving.

Non-biological materials
The Folin-Ciocalteu reagent (99.8% purity), quercetin (99%), ascorbic acid (99%) and DPPH (92%) are sourced by Sigma Chemical Co (St. Louis, MO, USA), while the high-purity chemicals (>98%) are by Merck (Darmstadt, Germany). Physical and chemical analyses
Determination of dry matter
5 gr of onion slices were weighed in baskets before and after oven drying at 105°C overnight (16-18 h). The overall solid content was calculated by subtracting the water content.

Ash content
For ash determination, 5 g of onion slices were calcined in a muffle furnace at 550°C for 30 minutes.

Acidity
Thirty milliliters of 95% ethanol were mixed with five grams of ground onion, and the combination was agitated at 25°C for an hour. Subsequently, it was centrifuged for five minutes at 3500 rpm. For qualitative
phytochemical screening, 20 milliliters of the resulting supernatant were titrated with 0.1N NaOH, employing a color indicator (Trease and Evans, 1987 [10]).

**Phytochemical screening (qualitative)**

To extract, 5 grams of each sample powder were boiled in 100 ml of water. After a 15-minute, the suspension was filtered to obtain 100 cc of filtrate (Trease & Evans [10]).

**Hydrolysable tannins test**

5 ml of 5% infusion from each sample was placed in a glass container, followed by the addition of 1 ml of 1% FeCl₃. The emergence of a greenish or blue-black color indicates reactivity.

**Condensed tannins test**

To assess condensed tannins, 1 ml concentrated hydrochloric acid was mixed with 5 ml infusion of each sample and boiled for 10 minutes. The presence of a red precipitate, soluble in amyl alcohol, is a sign of responsiveness.

**Anthocyanins test**

Ammonium hydroxide (NH₄OH) and sulfuric acid (5 ml each) were added to 5 ml of infusion of each sample. The coloration is enhanced by acidity when anthocyanins are present, and in a basic medium, the coloration changes to purplish blue.

**Free quinones test**

Add 0.1 milliliter of 1% NaOH to 1 milliliter of extract from all of the samples. There are free quinones exist when the color turns yellow, red, or purple.

**Anthraquinone**

To 1ml of extract of each sample, 1ml of ammonia (NH₄OH 10%) is added. After shaking, the purple coloration indicates their presence.

**Saponosides**

Samples (1%) were decocted by boiling 1 g of powder in 100 ml water for 15 minutes. The filtered suspension (1-10 ml) was added incrementally to ten test tubes filled with water. The tubes were vigorously shaken horizontally for 15 seconds, and foam height was measured after 15 minutes. For each tube, the foam height was evaluated.

**Quantification of different compounds**

**Producing the alcohol-based extract**

10 g onion powder of each species was soaked in 100 ml of water. After a 15-minute, the suspension was filtered to obtain 100 cc of filtrate (Trease & Evans [10]).

**Total polyphenols content (TPC)**

0.2 ml of each extract was added to a solution consisting of 1 ml diluted Folin-Ciocalteu reagent and 0.8 ml 7.5% Na₂CO₃, according to Singleton et al. [12] process. The reading was taken at 765 nm with Gallic Acid as a reference. Values were reported in milligrams of gallic acid equivalents (GAE) per gram of dry extract, calculated from the equation of the calibration curve

\[
y = 46.41 x + 0.063; R^2 = 0.988.
\]

**Total flavonoids content (TFC)**

The AlCl₃ colorimetric method involves diluting 0.5 ml of each extract in 2 ml of distilled water, then successively adding 0.15 ml of 5% NaNO₂, 0.15 ml of 10% AlCl₃ and 2 ml of 4% NaOH. The volume was adjusted to 5 ml with distilled water. Absorbance at 510 nm was read after 15 minutes of incubation (Kanazawa et al. [13]). Quercitrin served as the reference for the calibration curve. Results, calculated using the curve's equation (y = 46.41 x + 0.063; R² = 0.988), were expressed as mg quercitrin equivalent per gram of dry extract (mg QE/g DE).

**Hydrolysable tannins content (HTC)**

For Hydrolysable Tannin Content (HTC) involved mixing tannin extract with FeCl₃, creating a red-purple complex via Fe³⁺ ion interaction. A solution containing FeCl₃ (0.01M) and HCl (0.001M) (v/v) was combined with each extract. Absorbance was read at 660 nm after 15 seconds (Mole & Waterman [14]). HTC calculation

\[
HT\% = OD_{\text{emolates.SW}} \times \frac{M \times V}{\text{Emolates}}
\]

**Condensed tannins content (CTC)**

Three milliliters of 4% vanillin were mixed with volumes of each sample ranging from 0.1 to 0.5 milliliters. 1.5 cc of HCl was swiftly introduced and thoroughly shaken after the initial vigorous shaking. After 20 minutes of incubation, absorbance was taken at 500 nm (Julkunen-Titto [15]). The outcomes were presented to be mg of catechin equivalent per gram of dry extract (mg CE/g DE) utilising a calibration curve with catechin as a reference under comparable conditions.

**Antioxidant activity via DPPH free radical scavenging**

DPPH radical scavenging was assessed following Benhamou et al.'s method [16]. Five distinct concentrations were made available and 50 µl of samples was introduced into 1.95 ml of freshly made methanolic DPPH solution (0.0025 g/l). A negative control was created similarly using 50 µl of methanol with DPPH. After incubation in darkness for 30 minutes at ambient conditions, readings were taken at 515 nm.

**Ferric reducing antioxidant power (FRAP)**

FRAP test, as outlined by Oyaizu et al. [17], utilized 0.25 ml of individual extract concentrations. These were mixed with 0.625 ml of 0.2 M phosphate buffer (pH: 6.6) and 0.625 ml of 1% K₃Fe(CN)₆ solution. After 20 minutes at 50°C, 0.625 ml of 10% TCA halted the reaction. Subsequently, the supernatant (0.625 ml) was blended with 0.625 ml distilled water and 0.125 ml of a 0.1% FeCl₃ solution. Measured at 700 nm using ascorbic acid as the reference.

**Statistical analysis** was conducted via Microsoft Excel 2010. Feedings were reported as mean ± standard
deviation, with significance set at \( p < 0.05 \). All measurements were performed in triplicate.

3. RESULTS AND DISCUSSIONS

Physico-chemical characteristics

The outcomes concerning the physical and chemical characteristics of the three onion types are detailed in Table 1.

Table 1. Physicochemical parameters of the different varieties of *Allium cepa*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RO</th>
<th>YO</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (g/100g)</td>
<td>12.3±0.0</td>
<td>17.6±0.0</td>
<td>18.1±0.0</td>
</tr>
<tr>
<td>Ashes (g/100g)</td>
<td>1.9±0.005</td>
<td>2.0±0.006</td>
<td>1.1±0.006</td>
</tr>
<tr>
<td>Acidity (%)</td>
<td>3.3±0.15</td>
<td>3.2±0.15</td>
<td>3.1±0.2</td>
</tr>
</tbody>
</table>

DM: Dry matter; RO: Red onion; YO: Yellow onion; PO: Purple onion

Based on our findings, the three varieties have DM contents that vary between (12.3 to 18.1%), these values remain comparable to those reported by (Stadlmayr [18]) (12 to 13%) and (Konate et al. [19]), (9.63±0.38 to 17.97±0.45%). Several studies indicate that the levels of dry matter (DM) typically fluctuate between 7% and 18% of fresh weight based on the onion variety (Abhayawick et al. [20]). Interestingly, a positive relationship has been established between an onion's capacity for long-term storage and the dry matter content within the bulbs (Stadlmayr [18]).

Onions containing higher levels of dry matter tend to exhibit greater firmness, thus offering increased resistance during transportation and handling, reducing susceptibility to damage (Silué et al. [22]). Such damages, if incurred, could serve as entry points for parasites or microorganisms, ultimately leading to bulb rot. Consequently, the varieties under investigation demonstrate characteristics suggesting they possess significant potential for extended storage.

Regarding ash content, the onion varieties exhibited variations ranging from 1.1% to 2%. These findings differ from those reported by (Nwinuka et al. [23]) and (Konate et al. [19]), where ash content was reported to range from 4.8±0.15% to 2.50% to 6.64%, respectively. The acidity levels among the various varieties appear comparable, ranging from 3.1 to 3.3%.

Nevertheless, these readings are notably higher compared to those documented by (Konate et al. [19]), which ranged from 0.27 to 0.40%. This discrepancy might arise from the abundance of acid (propanethial-S-oxide) in our varieties. When this compound is exposed to ocular moisture, it transforms into sulfuric acid.

Phytochemical screening (qualitative)

The findings from the phytochemical examination of the three onion varieties are depicted in Table 2.

Table 2. Phytochemical screening of different varieties of *Allium cepa* (Mean ± SD; \( n=3 \)).

<table>
<thead>
<tr>
<th>Phytochemical substances</th>
<th>RO</th>
<th>YO</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tannins</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids/Anthocyanins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Quinones</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Saponosides</td>
<td>Foam height (cm)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Foam index</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

RO: Red onion; YO: Yellow onion; PO: Purple onion; Very positive: +++ ; moderately positive: ++; positive: +; negative: -

Our findings indicate the existence of distinctive secondary constituents including tannins, anthracene derivatives and saponins, which are distinctive of onions. These outcomes align with research conducted by (Ngadi et al. [24]) and (Arora et al. [25]). Likewise, (Oyewusi et al. [26]) upheld the presence of flavonoids, alkaloids, tannins and saponins in the methanolic extract of onions *Allium cepa* L. However, anthocyanins, known for their antioxidant properties beneficial for UV protection and plant health, were undetected in our extracts.

Phytochemical screening (quantitative)

Table 3 shows the quantification of the various secondary metabolites of the three onion varieties.

Table 3. Secondary metabolites of various *Allium cepa* L. extracts (Mean ± SD; \( n=3 \)).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EERO</th>
<th>EEYO</th>
<th>EEPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC mg EAG/g DM</td>
<td>0.23±0.005</td>
<td>0.14±0.03</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>TPC mg QE/g DM</td>
<td>0.25±0.02</td>
<td>0.20±0.01</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td>HTC mg EAG/g de DM</td>
<td>0.29±0.002</td>
<td>0.25±0.021</td>
<td>0.26±0.031</td>
</tr>
<tr>
<td>CTC mg CE/g DM</td>
<td>0.015±0.008</td>
<td>0.078±0.007</td>
<td>0.102±0.012</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>3.86</td>
<td>6.1</td>
<td>3.79</td>
</tr>
</tbody>
</table>

EERO: ethanolic extract of red onion; EEYO: ethanolic extract of yellow onion; EEPO: ethanolic extract of purple onion; CE: Catechin equivalent; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TPC: Total phenolic content; HTC: Total flavonoid content; CTC: Condensed tannins content

The Total Phenolic Content (TPC) among the three extracts exhibited a range from 0.14 to 0.27 mg, with a maximum of 0.27 ± 0.02 mg EAG/g DM, consistent with findings by (Benkeblia [27]), who reported levels ranging from 0.18 to 0.20 mg/g fresh weight in red onions. Similarly, the concentration of total phenolic compounds varied notably in Allium extracts, spanning from 0.84 to 2.07 mg/g DM, surpassing our recorded results (Nuutila et al. [28]).
Flavonoids, renowned for their anti-inflammatory and analgesic properties in medicinal plants such as onions (Aliyu et al. [29]), exhibit varying contents in our findings, measuring approximately (0.29 ± 0.02 vs 0.20 ± 0.01 vs 0.25± 0.01) for EERO, EEYO, and EEPO, respectively. Tannins, widely prevalent across numerous plant species, serve as a defense against predation and potentially function as pesticides, contributing to growth regulation. The present study confirms the existence of Hydrolysable Tannins (HT) in all three extracts, reaching a peak of 0.29± 0.02 in EERO, while EEPO displayed the highest concentration of Total Tannins (TC) at 0.102± 0.012 mg EC/g DM. The variations observed in bioactive compounds within the three extracts likely stem from variances in genetic makeup, notably the cultivar (Bajaj et al. [30]).

Regarding antioxidant activity, the DPPH test serves as a rapid screening method for identifying antioxidant molecules within plant extracts. Similarly, the assessment of antioxidant potency through iron reduction presents as a facile and reproducible method, widely employed to discern the most active extracts (Rodriguez-Bonilla et al. [31]). The results derived from the DPPH and FRAP tests conducted on the three alcholic extracts of Allium cepa L. are delineated in Table 4.

### Table 4. Free radical-scavenging activities and ferric reducing ability of ascorbic acid and ethanolic extracts of different Allium cepa L (Mean ± 2D; n=3).

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH IC50′(mg/ml)</th>
<th>FRAP** (mM Fe2+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EERO</td>
<td>0.204 ±0.017</td>
<td>0.48 ± 0.09</td>
</tr>
<tr>
<td>EEYO</td>
<td>0.159 ±0.18</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>EEPO</td>
<td>0.265 ±0.014</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>Vit C</td>
<td>0.132 ±0.002</td>
<td>1.37 ± 0.07</td>
</tr>
</tbody>
</table>

Values were the means of three replicates ± standard deviation. *IC50 value (mg/ml of extract), inhibitory concentration 50%; DPPH radicals were scavenged by 50%; **Determined at 1 mg/ml : EERO : ethanolic extract of red onion ; EEYO : ethanolic extract of yellow onion ; EEPO : ethanolic extract of purple onion.

These two trials validate EEYO’s superior antioxidant potential, showcasing an IC50 of (0.159 ±0.18 mg/l) and Ferric Reducing Capacity (0.75 ± 0.06 mM Fe2+), trailed by EERO (0.204 ±0.017 mg/l vs 0.48 ± 0.09 mM Fe2+) and EEPO (0.265 ±0.014 mg/l vs 0.66 ± 0.05 mM Fe2+). This underscores the impact of onion variety on Alliums’ antioxidant prowess. However, the antioxidant performance of Alliums is contingent upon phenolic and sulfur compounds (Miller et al. [32]), cultivation techniques, and extraction procedures (Prakash et al. [33]). Moreover, the varying sensitivity of reagents utilized in the antioxidant assessment methods (DPPH, FRAP) may elucidate the disparities observed among the studied extracts.

### CONCLUSIONS

This investigation underscores the secondary metabolite richness within Algeria's primary onion varieties. The evaluation of antioxidant potential through diverse methodologies demonstrates a convergence in outcomes.

### REFERENCES