Article

In-Vitro Evaluation of the Antioxidant and Anti-Inflammatory Activity of Volatile Compounds and Minerals in Five Different Onion Varieties

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Abstract: Onions contain high antioxidants compounds that fight inflammation against many diseases. The purpose was to investigate some selected bioactive activities of onion varieties (Yellow, Red, Green, Leek, and Baby). Antioxidant assays and anti-inflammatory activities such as NO production with the addition of some bioactive components were determined and analyzed by using a spectrophotometer. Gas chromatography and mass spectrometry (GC–MS) was used for the volatile compounds, while an Atomic absorption spectrometer was used for mineral determinations. Red variety achieved the highest antioxidant activities. The total flavonoids were between (12.56 and 353.53 mg Quercetin/gin dry weight) (dw) and the total phenol was (8.75–25.73 mg/g dw). Leek, Yellow and Green extracts achieved highly anti-inflammatory values (3.71–4.01 µg/mL) followed by Red and Baby extracts, respectively. The highest contents of sodium, potassium, zinc, and calcium were established for Red onions. Furfuraldehyde, 5-Methyl-2-furfuraldehyde, 2-Methyl-2-pentenal, and 1-Propanethiol were the most predominant, followed by a minor abundance of the other compounds such as Dimethyl sulfide, Methyl allyl disulfide, Methyl-trans-propenyl-disulfide, and Methyl propyl disulfide. The results recommend that these varieties could act as sources of essential antioxidants and anti-inflammatories to decrease inflammation and oxidative stresses, especially red onions that recorded high activities.

Keywords: onions; bioactive activities; antioxidant; anti-inflammatory; volatile compounds; minerals

1. Introduction

Onions are vital industrial marketable vegetable crops grown on a large scale in different parts of the world [1]. It has many medicinal uses such as cardiovascular diseases, nutrients such as carbohydrates, proteins, some vitamins as vitamin C, B₆, and folic acid [2]. It has high effective nutrients as sugars (glucose, fructose, galactose, arabinose) and minerals (Ca, Fe, S) with some flavonoid and polyphenol components [3,4]. It is low in sodium and fats with an energy value of 40 kcal/100 g [5,6]. The antioxidant activities can be estimated using several in-vitro methods [7,8]. Every method is based on different antioxidant activity, as the facility for free radicals scavenging or lipid peroxidation inhibition. However,

the total antioxidant activity is difficult to be estimated by one single method because of the phytochemicals complex nature [9,10]. 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Fluorescence recovery after photobleaching (FRAP, and Free radical scavenging) assay (DPPH) assays are regularly used for measuring the antioxidant activities [11,12]. Inflammation plays a vital function in pathogenesis and cardiovascular diseases [13,14]. The redox stress is responsible for the activation of the immune cells to reactive nitrogen, oxygen, and relief pro-inflammatory cytokines that cause reparation in pathological and physiological pathways [15,16]. Nitric oxide (NO) is a free radical that always results in pathological consequences as chronic inflammation [17]. Besides, the volatile compounds, essential minerals, and phenolic compounds depend on a certain variety to maintain optimal health [5]. Volatile compounds exhibit anticancer, antioxidant, antimicrobial, anti-cholesterol, and anti-inflammatory activities. Sulfur compounds such as thiosulfonates, thiosulfonates, mono-, di-, trisulfides, and thiopropanal S-oxide are responsible for the flavor, odor after cutting, and mastication [18].

The current study aimed to evaluate a comparative analysis of several bioactive compounds such as flavonoid and phenolic contents, antioxidant activities such as ABTS, DPPH, FRAP, anti-inflammatory activity such as NO production, volatile compounds, and mineral contents of onion varieties.

2. Material and Methods

2.1. Chemical and Cells

Quercetin, Trolox, Folin-Ciocalteu reagent, Griess reagent, Ascorbic acid, FRAP, ABTS, DPPH, DMEM, DMSO, MTT, LPS, RAW 264.7 cells, and other reagents were from (Sigma, St. Louis, MO, USA).

2.2. Onion Preparation

The analyzed samples were five different onion varieties: Yellow and Red (*Allium cepa*), Green (*Allium fistulosum*), Leek (*Allium ampeloprasum*), and Baby (*Allium ampeloprasum* var. *sectivum*), analyzed in January 2020. Onions were obtained from a commercial supermarket in Taif from the Kingdom of Saudi Arabia. Onion samples (1 kg/variety) were selected in uniform size, with no sign of microbial infections and mechanical damage. Onions were cleaned, cut into pieces and freeze-dried, milled, packed in N₂, and stored at −80 °C until the end of the experiments.

2.3. Antioxidant Extraction

Onion powder (2 g) was extracted in 10 mL of 80% methanol, sonicated for 30 min and centrifuged at $\sim 4000 \times g$ for 30 min, and the supernatant was kept at 4 °C for antioxidant evaluations.

2.4. Flavonoid Content

A total of 30 μ L aliquots of onion extracts were diluted with a mixture of (90 μ L methanol, 6 μ L of 10% aluminum chloride, and 6 μ L of 1 mol/L potassium acetate) [13,19]. The absorbance was calculated at 415 nm by using Spectra (Max M2, California, CA, USA) with Quercetin as a standard (mg Qe/g dw). The flavonoid content was evaluated in triplicate.

2.5. Phenolic Content

The total phenolic content was evaluated in 10 mL of 80% methanol. In total, 0.1 mL of 2% sodium carbonate, 5 μ L of Folin-Ciocalteu reagent, and 50 μ L of the onion extract were mixed, incubated in the dark for 2 h, measured at 750 nm, and calculated as gallic acid equivalents (mg/g dw) [9,20].

2.6. Total Antioxidant Capacity

An aliquot of 20 μ L of onion extracts was blended with a mixture of 0.2 mL of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid, and 4 mM ammonium molybdate) [19,21]. The absorbance was evaluated after cooling at 695 nm and calculated as Ascorbic Acid Equivalent per mg (μ gAAE/mg).

2.7. Antioxidant Activity Determinations

2.7.1. FRAP Assay

Of the onion extracts, 100 μ L were vortexed and incubated at 37 °C for 30 min in a water bath then added to 1.9 mL FRAP reagent. FRAP reagent included a mixture of (300 mM acetate buffer, 10 mM tripyridyl-s-triazine, 40 mM hydrogen chloride and 20 mM ferric chloride in the ratio 10:1:1) [15,22]. Results were measured after incubation for 10 min at 593 nm, and calculated as (μ mol TE/g dw).

2.7.2. DPPH Assay

An amount of 0.2 mL aliquots of DPPH solution between 50 and 2000 μ M, 20 mL methanol were mixed with 0.04 mL of onion extracts and left in the dark for 30 min at the ambient temperature [9,12]. The results were measured at 517 nm, and calculated as (μ mol TE/g dw).

2.7.3. ABTS Assay

A (7 mM) ABTS solution was mixed with 0.04 mL of onion extracts under regular shaking at 300 rpm until reaching the green radical. The supernatants were collected and the results were measured at 734 nm and calculated as (μ mol TE/g dw) [13,23].

2.8. Anti-Inflammatory Activity

2.8.1. Extraction for Cell Culture

In total, 4 g aliquots of onion samples were mixed and homogenized in 20 mL methanol 80% by using Digital Homogeniser (IkaWerke, Staufen, Germany), combined, and evaporated until dry. Onion extracts were dissolved for a final concentration of 20 mg/mL in DMSO solution.

2.8.2. Cell Viability (MTT)

Mitochondrial respiration of cells has been detected by incubation (1×10^5 cells/mL) for 4 h with 5 mg/mL of MTT in 96-well plates after solubilizing in DMSO as 150 μ L per well and evaluated at 490 nm [19,24].

2.8.3. NO Production

Approximately 0.2 mL aqueous extract of onion varieties with Griess reagent, incubated for 24 h and NO production was evaluated at 540 nm [13,25].

2.9. Determination of Mineral Compositions

An aliquant of (2 g) onions powder of each variety was ashed at 500–550 °C. The white-ash residue was dissolved in 15 mL of 2% HNO₃, filtered through Whatman 40 filter paper, and adjusted to 10 mL with distilled water for mineral determinations. Atomic absorption spectrometer (PerkinElmer, AAnalyst 800) was used for (Na, K, Mg, Zn, Fe, and Ca) evaluations [11]. Phosphorus content was evaluated by a spectrophotometer (Pharmacia biotech Ultraspec 4000, Shanghai, China) at 440 nm.

2.10. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis for Volatile Compounds

The volatile compounds were detected by GC-MS system by GC 7890A and MSD 5975C (Agilent Technologies, Palo Alto, CA, USA). An aliquant of (1 g) onions powder of each variety was kept in a warm water, during the equilibration and the extraction periods 15 and 40 min, respectively. HP-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) was

used for the evaluations, while helium gas was the carrier gas at a flow rate of 1 mL/minute. The temperature was performed at 70 °C for 2 min and raised to 200 °C at a rate of 3 min for 15–18 min [26]. Onion extracts were injected in splitless mode. The individual peaks of volatile compounds were identified by the injection of commercial standards with the help of spectra comparisons such as Wiley 9 MS and NIST 14 (Sigma-Aldrich, Milano, Italy) by comparison of the retention indices against the reference data from the literature and the authentic samples. Data were gained electronically in triplicate from the GC peak areas with no need for any correction factors.

2.11. Statistical Analysis

All the experiments were applied to (ANOVA) and analyzed by using SPSS, version 20 (SPSS, California, CA, USA). Significant differences were evaluated as ($p < 0.05$).

3. Results and Discussion

3.1. Total Flavonoid, Phenol, and Antioxidant Contents

Flavonoid values ranged from (12.56 to 353.53 mgQe/g dw), Leek achieved the highest total flavonoid contents followed by Red and Yellow varieties (Table 1). Green and Baby varieties had lower flavonoid contents of 12.56 and 81.13 mg Qe/g dw, respectively. Gonzales-Barron and Butler [27] reported similar results for onion total flavonoid content. Total phenol contents ranged from 8.75 mg/g dw in Green to 25.73 mg/g dw in Red. Leek and Yellow varieties achieved similar results as 20.35 and 21.61 mM (TEAC), respectively. In a comparison with onion varieties, results were similar to the onion values used in the other study [28]. Leek extract achieved the highest total antioxidant activity of 12.80 µg AAE/mg dw due to the polyphenolic and flavonoids compounds [29].

Table 1. Total flavonoid, phenol, and antioxidant.

	Total Flavonoid mg Qe/g dw	Total Phenol mg/g dw	Total Antioxidant µg AAE/mg
Yellow	163.20 ± 11.83 ^c	21.61 ± 1.37 ^b	11.16 ± 0.35 ^c
Red	253.55 ± 23.15 ^b	25.73 ± 0.74 ^a	12.27 ± 0.22 ^b
Green	12.56 ± 0.36 ^e	8.75 ± 0.62 ^d	5.47 ± 0.08 ^e
Leek	353.53 ± 5.64 ^a	20.35 ± 0.78 ^b	12.80 ± 0.08 ^a
Baby	81.13 ± 3.55 ^d	17.54 ± 2.16 ^c	9.63 ± 0.46 ^d

Different superscript letters a, b, c, d, e show significant differences by statically analysis ($p \leq 0.05$).

3.2. Antioxidant Activity (FRAP-DPPH-ABTS)

Figure 1 shows that FRAP assay of onion varieties were (9.61–41.26 µmol TE/g dw) for Green and Red onions, respectively. The values were in good arrangement with the onion report [30]. DPPH assay results were (6.83–67.10 µmol TE/g dw) for Yellow and Red onions, respectively. The study finding was in agreement for DPPH assay [31]. ABTS⁺ assay results were (1.16–7.10 µmol TE/g dw) for Green and Red onions, respectively. Santaset al. [30] had reported two different white onions (*Calot de Valls* and *var. Fuentes del Ebro*) purchased in a local market which achieved 2.42 and 6.13 µmol TE/g dw as (TEAC), respectively. However, antioxidant values depend on different factors as heredities, production processes, environmental surroundings, and genetics [32].

Figure 1. Antioxidant activities of onion varieties.

3.3. Anti-Inflammatory Activity

3.3.1. MTT Reduction

The cytotoxicities of onion extracts were evaluated in a range of (0–200 $\mu\text{g/mL}$) (Figure 2). Results show that the concentration range of the cell used did not cause any cytotoxic effect.

Figure 2. Cell viability of onion varieties.

3.3.2. NO Production

NO production formed a slight quantity of the nitrite after placing the cells for 24 h in the medium. The medium concentration increased obviously after incubation 24 h with onion extracts. More NO macrophage production represents a possible toxic effect that causes disease progression [13]. A significant concentration for No production was detected for the five onion extracts (Figure 3). Anti-inflammatory values of onion extracts were in a range of (0–100 μg). Leek, Yellow, and Green onions achieved highly anti-inflammatory activity followed by Red and Baby extracts, respectively. These results showed that onion has an obvious influence on free radicals scavenging [33]. NO production grew in the same way with the dose of all onion extracts except in Green onion, while the Green variety showed a different reaction at 50 $\mu\text{g/mL}$ [25].

Figure 3. NO production of onion varieties.

3.4. Mineral Compositions

The mineral compositions of the five onion varieties are shown in Figure 4. The quantity of the minerals: sodium, potassium, magnesium, zinc, iron, phosphorus, and calcium have values ranging between (10.7–49.54), (196–266.23), (32.03–60.36), (2.3–15.56), (3.8–8.32), (234.66–343.43), and (399.32–762.65) (mg/100 g DW), respectively. The highest contents of sodium, potassium, zinc, and calcium were established for Red onions. The calcium composition of onion bulbs was high which meant that it can enhance the osmotic regulation, catalytic functions, and energy provisions [5]. Baby onions had the highest content of phosphorus (343.43 mg/100 g DW). Increases in phosphorous and sodium intake have an important role in lowering blood pressure [34,35]. Magnesium and iron compositions in Yellow onions were higher by (60.36 and 8.32 mg/100 g DW) when compared with that in other onion varieties. Magnesium and potassium are very essential for reducing stroke and coronary heart diseases. Iron is an important mineral due to its effective function as catalytic cofactors for protein [25]. Akinwande and Olatunde [36] reported similar values for some mineral compositions in some varieties of onion bulbs.

Figure 4. Mineral compositions.

3.5. Volatile Compounds

Onion varieties contain cysteine sulfoxides as volatile compounds stored in the intact bulb cytoplasm. After an injury, the alliinase enzyme cleaves cysteine sulfoxides

into the sulfinic acids then to thiosulfates that are chemically unstable and responsible for the strong onion odor [37]. Table 2 presents a total of 26 volatile compounds identified by (GC-MS) of five onion varieties. Differences in volatile compounds were noted among onion varieties; Furfuraldehyde, 5-Methyl-2-furfuraldehyde, 2-Methyl-2-pentenal, and 1-Propanethiol were the most predominant, followed by a minor abundance of the other compounds such as Dimethyl sulfide, Methyl allyl disulfide, Methyl-trans-propenyl-disulfide, and Methyl propyl disulfide. Abundant compounds in Green onions were Sulfur dioxide (0.44 mg/kg DW) and Methanethiol (0.23 mg/kg DW) accompanied with a minor abundance of 2-Methylpentanal (0.14 mg/kg DW) and Dimethyl disulfide (0.13 mg/kg DW). In comparison with Yellow onions, the abundance of several essential compounds was higher, such as Dimethyl sulfide (1.18 mg/kg DW), Methyl allyl disulfide (2.53 mg/kg DW), and Methyl-trans-propenyl-disulfide (1.32 mg/kg DW). Red onions have the highest in Carbon disulfide, 1-Propanethiol, and S-Propyl ethanethioate. 2-Methyl-2-pentenal component was the highest in the Leek variety (65.87 mg/kg DW). Moreover, some compounds of Baby onions were present with the highest values, such as Furfuraldehyde (166.32 mg/kg DW), and 5-Methyl-2-furfuraldehyde (105.55 mg/kg DW). On the other hand, some compounds were in lower values or not detected such as 3,3-Thiobis-1-propene, and Dimethyl disulfide. Liu et al. [38] reported that 1-propanethiol was the most abundant volatile compound in onion (14.1%) which was in agreement with the current study. The amount composition of volatile compounds is responsible for the biological activity and flavor [18,24].

Table 2. Volatile compounds.

	Yellow	Red	Green	Leek	Baby
Ethyl sulfide	n.d.	0.01 ± 0.00 ^c	0.07 ± 0.00 ^b	0.06 ± 0.01 ^b	0.20 ± 0.29 ^a
Dimethyl sulfide	1.18 ± 0.26 ^a	0.19 ± 0.55 ^c	0.06 ± 0.00 ^d	0.17 ± 0.06 ^c	1.12 ± 0.27 ^b
3,3-Thiobis-1-propene	0.07 ± 0.08 ^a	n.d.	n.d.	0.04 ± 0.04 ^b	0.01 ± 0.07 ^c
n-Propyl cis-1-propenyl sulfide	0.06 ± 0.34 ^a	0.02 ± 0.54 ^b	n.d.	0.03 ± 0.05 ^b	0.01 ± 0.34 ^b
Methyl allyl disulfide	2.53 ± 0.54 ^a	1.89 ± 0.88 ^b	1.45 ± 0.88 ^c	0.06 ± 0.09 ^e	0.99 ± 0.53 ^d
Diethyl disulfide	n.d.	0.02 ± 0.29 ^a	0.01 ± 0.55 ^b	0.02 ± 0.09 ^a	0.01 ± 0.01 ^b
Methyl-trans-propenyl-disulfide	1.32 ± 0.21 ^a	1.19 ± 0.29 ^b	0.98 ± 0.55 ^c	0.07 ± 0.00 ^d	1.30 ± 0.09 ^a
Methyl propyl disulfide	1.12 ± 0.41 ^c	2.22 ± 0.07 ^b	0.04 ± 0.44 ^d	2.17 ± 0.05 ^b	5.21 ± 0.00 ^a
Propionaldehyde	16.87 ± 0.59 ^b	6.44 ± 0.34 ^d	5.98 ± 0.54 ^e	12.66 ± 0.44 ^c	48.16 ± 1.05 ^a
2-Methyl-2-pentenal	n.d.	3.87 ± 0.53 ^d	46.88 ± 0.88 ^b	65.87 ± 1.54 ^a	39.00 ± 1.03 ^c
Furfuraldehyde	39.65 ± 0.17 ^e	82.65 ± 0.53 ^d	113.65 ± 0.88 ^b	98.65 ± 0.88 ^c	166.32 ± 1.05 ^a
5-Methyl-2-furfuraldehyde	88.87 ± 0.29 ^b	68.86 ± 0.29 ^c	13.65 ± 0.55 ^e	35.77 ± 0.55 ^d	105.55 ± 0.19 ^a
1-Propanethiol	8.09 ± 0.07 ^e	14.11 ± 0.07 ^a	10.08 ± 0.00 ^c	13.10 ± 0.55 ^b	9.19 ± 0.55 ^d
Dimethyl disulfide	0.12 ± 0.34 ^b	0.10 ± 0.03 ^c	0.13 ± 0.00 ^a	n.d.	n.d.
2-Methylpentanal	0.10 ± 0.53 ^c	0.11 ± 0.05 ^b	0.14 ± 0.00 ^a	0.10 ± 0.50 ^c	n.d.
(E)-Hex-2-enal	0.11 ± 0.20 ^e	0.32 ± 0.02 ^a	0.28 ± 0.01 ^b	0.21 ± 0.55 ^d	0.24 ± 0.88 ^c
(Z)-Hex-3-en-1-ol	0.20 ± 0.56 ^d	0.24 ± 0.00 ^a	0.21 ± 0.00 ^c	0.20 ± 0.00 ^d	0.22 ± 0.29 ^b
(E)-Hex-3-en-1-ol	0.75 ± 0.52 ^b	0.90 ± 0.01 ^a	0.71 ± 0.01 ^c	0.54 ± 0.05 ^d	0.32 ± 0.29 ^e
Propyl hydrosulfide	0.32 ± 0.44 ^c	0.33 ± 0.04 ^b	0.31 ± 0.06 ^d	0.40 ± 0.44 ^a	0.22 ± 0.07 ^e
S-Propyl ethanethioate	0.18 ± 0.54 ^e	1.50 ± 0.05 ^a	0.99 ± 0.04 ^d	1.33 ± 0.54 ^b	1.30 ± 0.34 ^c
2,5-Dimethylthiophene	0.20 ± 0.51 ^b	0.10 ± 0.01 ^d	0.21 ± 0.06 ^c	0.20 ± 0.55 ^b	0.23 ± 0.87 ^a

Different superscript letters a, b, c, d, e show significant differences by statically analysis ($p \leq 0.05$).

4. Conclusions

The results in-vitro studies show the onion potentials to reduce inflammation and oxidative stresses. This study discovers that onion varieties have a highly bioactive effect due to the antioxidant behavior that can be beneficial against inflammation. Volatile components were dominated by Furfuraldehyde, 5-Methyl-2-furfuraldehyde, 2-Methyl-2-pentenal, and 1-Propanethiol, while calcium was the most abundant mineral.

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