A novel HPLC method for simultaneous quantification of eleven phenolic antioxidants in commercial personal care and food products which contain extracts of green apple, pomegranate and argan oil

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ABSTRACT

In this study, a simple, fully validated and rapid reversed-phase HPLC with photodiode array detector method was developed for the simultaneous determination of 11 selected phenolic antioxidants over 33 min in personal care and food samples containing extracts of green apple, pomegranate (Ponca granatum) and argan oil (Argania spinosa). The method was performed using NUCLEODUR C18 column 5 μm particle size and 12.5 cm length. The HPLC mobile phase was prepared as follows, solution A: 1% aqueous acetic acid and solution B: Acetonitrile. The method was gradient at flow rate 1.0 mL/min with a simple mobile phase, minimal sample preparation, and diminished organic solvent usage (3% acetonitrile for almost 90% of the run time). The detection was carried out at 278 nm. The method presented good precision and accuracy with RSD% values ranged between 0.33 and 1.94% and wide linear range. The developed method was successfully applied on 67 personal care and food products present in Egyptian market and can be used for routine screening in laboratory for the regular quality control of the antioxidant content for products containing the mentioned extracts.

KEYWORDS

antioxidants, argan oil, green apple, HPLC, pomegranate

INTRODUCTION

Antioxidants are widely introduced into meals, drugs and cosmetics due to their basic role in improvement of body health. Antioxidants researches have become one of the primary interests for scientists in these days and their biological impacts introduce great concern to many specialists [1]. The scientific specialists community explains such attention for certain reasons, including the broad information about free radicals and reactive oxygen species.
which cause the formation of anatomical–functional damage to our bodies detected with ageing. Ingredients that contain a phenolic group play a significant role in cosmetics, pharmaceutical products and food, to delay and inhibit cell ageing.

Various techniques of analysis have been made for quantitative determination of antioxidants. Most current researches demonstrated that natural origin phenolic antioxidants show more appreciated profile compared with synthetic antioxidants, as synthetic antioxidants are known to be cancer causing and the broad utilization of such materials may lead to wellness risks [2, 3]. Today, exceptional interest has been routed to green apple, pomegranate, and argan oil extracts as a great source for natural antioxidants which were extensively used in different nourishment drinks, pharmaceuticals, and make up products [4].

Green apple is known to contain ten phenolic antioxidants, including catechin, epi-catechin, quercetin, gallic acid, ferulic acid, caffeic acid, vanillic acid, syringic acid, kaempferol, luteolin [5–9]. Pomegranate (Punica granatum) extract is known to contain eleven natural phenolic antioxidants namely: resorcinol, vanillic acid, gallic acid, catechin, syringic acid, epi-catechin, ferulic acid, caffeic acid, luteolin, quercetin, kaempferol [10, 11]. While ten natural phenolic antioxidants are commonly found in argan oil which are resorcinol, vanillic acid, gallic acid, catechin, syringic acid, epicatechin, ferulic acid, caffeic acid, luteolin, quercetin [12, 13]. The particular literature reveals many approaches for the determination of these mentioned antioxidants in green apple, pomegranate and argan oil extracts. HPLC is considered to be the most widely applicable method for the analysis of these active compounds in green apple, pomegranate and argan oil. For green apple, some of these antioxidants were analyzed concurrently by utilizing RP-HPLC [14–18], HPLC-PDA [19], LC-MS [20–25], and GC-MS [26]. For pomegranate, some of these compounds had been determined simultaneously using HPLC [27–32], HPLC-PDA [11, 33], LC-MS [34]. For argan oil some of these compounds were simultaneously analyzed using LC-MS [35–37] and using GC-MS [38, 39]. Great interest in determining the levels of these bioactive compounds in various matrices, created a requirement for simple analytical methods.

According to the past referred literature, clearly, some of the selected antioxidants were determined with each other or with other antioxidants, but there is no reported method for concurrent analysis of all of the selected antioxidants in green apple, pomegranate and argan oil extracts. For green apple, pomegranate and argan oil extracts, the main destination of this work were to create and validate a practical, simple, and rapid reversed-phase HPLC-PDA gradient method for the concurrent determination of these selected eleven antioxidants. The identity of the antioxidants and levels of these compounds in personal care and food products were confirmed after simple extraction procedures implemented as routine screening in laboratory for the regular quality control of the antioxidant content of these products.

Through this work, the developed method was accurately applied to quantify the selected antioxidants in 67 commercial personal care and food products found in the Egyptian market, which includes one – step extraction or simply dilution of the sample and analysis by reversed phase HPLC-PDA technique.

### EXPERIMENTAL

#### Materials and reagents

Original standards of the selected antioxidants were brought from the Sigma – Aldrich (Steinheim, Germany) and labeled to contain 99.0, 98.0, 97.0, 90.0, 97.0, 95.0 98.0, 99.0, 97.0, 99.6 and 95.0% of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA. Methanol and Acetonitrile were of HPLC grade from Riedel-de Haën™ Honeywell (Germany), and acetic acid was analytical grade brought from BDH Laboratory Supplies (Poole, England).

#### HPLC conditions

HPLC was performed utilizing a waters 2,690 Alliance HPLC system provided with Waters 996 photodiode array detector (200–600 nm). This system included an auto sampler with a sample loop of 100 μL and a capacity of 120 vials, quaternary pump with low pressure mixing system, vacuum degasser, empower software for data acquisition and analysis. The chromatographic column was (EC 125/3 NUCLEODUR C18 Gravity, 5 μm). The HPLC mobile phase was consisting of eluent A (1% aqueous acetic acid) and eluent B (acetonitrile). Mobile phase was run using gradient elution: at the time 0–30 min 3% B; 30–35 min gradient up to 35% B. After 10 min the gradient was back to the initial condition and the analytical column was reconditioned for 10 min. The analysis was done at room temperature with injection volume 20 μL, mobile phase flow rate 1.0 mL/min., and the column temperature ambient (28 °C). The selected compounds were detected at 278 nm.

#### Standard solutions and calibration graphs

Stock standard solutions of RS, GA, VA, CA, CF, SY, EC, FA and QU were made by dissolving 25 mg of each compound into 25 mL methanol. Stock standard solutions of KA and LU were made by dissolving independently 25 mg of each compound into 25 mL ethanol. All stock standard solutions were kept in a 4 °C refrigerator. The working solutions used were made by diluting of stock solutions with methanol to achieve the concentration range 0.05–15 μg mL⁻¹ to each
compound. Preceding the analysis, caution was taken to guarantee the stability of CA, EC and LU, because of their sensitivity to light. For that after preparation immediately, all stock standard solutions were kept into 25 mL amber colored volumetric flasks, while all working solutions prepared in 10 mL transparent volumetric flasks were wrapped by aluminum foil.

**Preparation of the samples**

To apply the developed method, 67 commercial products were used. For green apple extract, personal care products selected were: 1 shower gel, 1 foaming bath and 1 hair shampoo, 1 lip balm, 1 body butter, 1 body scrub, 1 body gel lotion and 1 hand cream and food products chosen were 4 apple juices, 5 apple vinegars, and 3 tea bags. For pomegranate extracts, 1 hair shampoo, 1 hair conditioner, 1 shower gel, 1 body wash, 1 bubble bath, 2 face creams and 1 face toner. Beside analysis of some food products and beverages including, 7 juices, 1 pomegranate vinegar, 7 molasses and one tea bag. Finally for argan oil extracts, personal care products analyzed were: 7 shampoos, 1 conditioner, 4 hair oils, 5 hair serums 1 hair cream, 1 hand cream, 1 body oil, 1 body spray, 1 body lotion and 1 body cream. All of these samples collected as commercial products from local Egyptian market. Every sample was typically analyzed for three times.

**Cosmetic preparations.** An accurate amount of samples 1.0 mL for liquid form, 1.0 g for solid and semisolid forms were simply diluted to 10 mL by methanol and put for 30 min in ultrasonic bath till the mixture was dispersed, then filtering through hard filter paper (15.0 cm) and transfer to freezer for 3–5 min to separate the fat layer, then filtering the liquid supernatant through a 0.22 μm syringe filter, 2 mL of the filtrate were placed in an amber HPLC vial for injection into HPLC apparatus for analysis.

**Juice and vinegar samples.** Juice suspensions and vinegar samples were filtered by using whatman hard filter paper. The resulting filtrate was filtered using 0.22 μm syringe filters, 2 mL of this filtrate were kept in an amber HPLC vial for injection into HPLC apparatus.

**Molasses samples.** Molasses sample (1.0 g) was diluted to 10 mL by distilled water, then filtered using 0.22 μm syringe filters, 2 mL of this filtrate were kept in an amber HPLC vial for injection into HPLC apparatus.

**Tea samples.** Tea samples were extracted by weighing 1 g from each sample and macerated in distilled water (25 mL) at 95 to 100 °C for around 5 min, then filtered by using whatman hard filter paper. The resulting filtrate was finally filtered by 0.22 μm disposable syringe filter; 2 mL of this filtrate were placed in an amber HPLC vial for injection into HPLC apparatus [40].

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**Table 1.** The system suitability test results of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA

| Compound | Retention time (min)b | Capacity factor (K) | Selectivity (a)b | Resolution (R)s | %RSDc of retention time | Tailing factor |
|----------|-----------------------|---------------------|-----------------|----------------|-------------------------|---------------|
| RS       | 3.50                  | 3.12                | 2.29 (a1)       | 5.01 (b1)      | 1.53                    | 1.10          |
| GA       | 6.91                  | 7.13                | 1.84 (a2)       | 8.55 (b2)      | 1.66                    | 0.98          |
| VA       | 12.00                 | 13.12               | 1.08 (a3)       | 1.51 (b3)      | 1.50                    | 1.01          |
| CA       | 12.90                 | 14.18               | 1.13 (a4)       | 2.72 (b4)      | 0.62                    | 1.10          |
| CF       | 14.52                 | 16.08               | 1.08 (a5)       | 1.73 (b5)      | 0.75                    | 0.99          |
| SY       | 15.55                 | 17.29               | 1.15 (a6)       | 3.78 (b6)      | 0.52                    | 0.98          |
| EC       | 17.80                 | 19.94               | 1.16 (a7)       | 4.54 (b7)      | 0.40                    | 1.01          |
| FA       | 20.50                 | 23.12               | 1.44 (a8)       | 12.65 (b8)     | 0.55                    | 1.10          |
| LU       | 29.10                 | 33.24               | 1.10 (a9)       | 4.71 (b9)      | 0.95                    | 1.05          |
| QU       | 31.90                 | 36.53               | 1.02 (a10)      | 1.76 (b10)     | 0.80                    | 1.04          |
| KA       | 32.50                 | 37.24               |                 |                | 0.90                    | 1.03          |

b, Relative standard deviation.

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*Fig. 1. Typical HPLC chromatograms of 20 μL injection obtained from analysis of synthetic mixture containing 1 μg mL⁻¹ of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA using Nucleodur column C18, 12.5 cm length, and gradient elution system of acetonitrile- 1% aqueous acetic acid based mobile phase*
Validation of the HPLC method

**Linearity and range.** The linearity of the method to determine RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA was assessed by analyzing different concentrations of each compound. The working solutions used were made by diluting of stock solutions with methanol to achieve the concentration range 0.05–15 μg mL⁻¹ to each compound. Seven concentrations were chosen, ranging between 0.05 and 15 μg mL⁻¹ for the selected antioxidants. Each of these concentrations was repeated three times, to give information on the variation in peak area values between samples of same concentration.

**Limits of detection (LOD) and quantification (LOQ).** Following the International Conference on Harmonization (ICH) recommendations [41], it was based on the standard deviation (S.D.) of the response and the slope was utilized to determine the detection and quantitation limits.

**Accuracy and precision.** The intra-day precision and also accuracy were determined by simply by analyzing three concentration levels of working solutions on the same day (each concentration was repeated five times). Inter-day precision and also accuracy were determined by analyzing three concentration levels of working solutions on five successive days. The concept of acceptability of the data included accuracy stated as relative error (RE%) and precision stated as relative standard deviation (RSD%). Accuracy was also assessed in terms of recovery by adding known concentration of the studied compounds to a known concentration of the commercial products (standard addition method). The resulting mixtures were analyzed, and the mean percentage recoveries for three replicates were calculated.

**RESULTS AND DISCUSSION**

**HPLC-PDA method optimization**

Many trials were performed for efficient separation of the selected antioxidants. Different columns and several mobile phases were tried. Some of these trials will be discussed. One of these trials to develop a separation was made using a C₁₈ column thermoscientific 25 cm length and gradient elution system of organic mobile phase methanol and 1% aqueous acetic acid over 50 min run time. The analytes were detected at 254 nm. Eight compounds has been separated with bad resolution while the other three have not appeared, long time of separation over 37 min is needed. A luna column C₁₈ with 15 cm length was tried and reaching the results.

**Table 2. Analytical parameters for the analysis of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU, and KA by the proposed HPLC analytical method**

| Parameter | RS | GA | VA | CA | CF | SY | EC | FA | LU | QU | KA |
|-----------|----|----|----|----|----|----|----|----|----|----|----|
| Linearity range (μg mL⁻¹) | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 | 0.05–15.0 |
| Correlation coefficient (r) | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 |
| LOD (μg mL⁻¹) | 0.0239 | 0.0222 | 0.0250 | 0.0202 | 0.0203 | 0.0268 | 0.0282 | 0.0266 | 0.0271 | 0.0210 | 0.0285 |
| LOQ (μg mL⁻¹) | 0.0796 | 0.0739 | 0.0834 | 0.0674 | 0.0677 | 0.0894 | 0.0940 | 0.0880 | 0.0904 | 0.0702 | 0.0951 |
| Regression equation (Y):Slope (b) | 1.54 × 10⁻³ | 7.13 × 10⁻³ | 2.39 × 10⁻³ | 5.37 × 10⁻³ | 5.35 × 10⁻³ | 4.72 × 10⁻³ | 5.33 × 10⁻³ | 5.33 × 10⁻³ | 5.33 × 10⁻³ | 5.33 × 10⁻³ | 5.33 × 10⁻³ |
| Standard deviation of the slope (Sb) | 1.57 × 10⁻² | 2.55 × 10⁻² | 2.62 × 10⁻² | 4.69 × 10⁻² | 4.69 × 10⁻² | 4.72 × 10⁻² | 4.72 × 10⁻² | 4.72 × 10⁻² | 4.72 × 10⁻² | 4.72 × 10⁻² | 4.72 × 10⁻² |
| Intercept (a) | 505.48 | 1,047.39 | 1,313.90 | 2,872.22 | 2,872.22 | 1,636.60 | 1,636.60 | 1,636.60 | 1,636.60 | 1,636.60 | 1,636.60 |
| Relative standard deviation (% interpolating) | 1.02 | 1.07 | 1.10 | 1.07 | 1.13 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 |
| Standard deviation of the intercept (Sa) | 1.20 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 | 1.14 |
| **Notes:** Y = a + bC, where C is the concentration in μg mL⁻¹ and Y is the peak area.
a gradient elution system of acetonitrile and 1% aqueous acetic acid mobile phase over 45 min run time, detection at 278 nm. Long separation time over 40 min was observed. Finally, best separation was reached on 33 min using Nucleodur column C18, 5 μm particle size with 12.5 cm length. The HPLC mobile phase was composed of solvent A: 1% Acetic acid in water, solvent B: Acetonitrile, the mobile phase was run using gradient elution: at the time 0–30 min 3% B; 30–35 min gradient up to 35% B. The flow rate was 1.0 mL/min and the injection volume was 20 μL, and the separation was done at room temperature. Quantitation was achieved with detection at 278 nm based on peak area.

The specificity of the HPLC method is expressed in Fig. 1 where entire separation of the studied antioxidants is observed. The average retention time ± S.D. for RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA were found to be 3.50 ± 0.05, 6.91 ± 0.11, 12.00 ± 0.18, 12.90 ± 0.08, 14.52 ± 0.11, 15.55 ± 0.08, 17.80 ± 0.07, 20.50 ± 0.11, 29.10 ± 0.28, 31.90 ± 0.26 and 32.50 ± 0.29 min., respectively and these results were for seven replicates. The peaks obtained were clearly sharp and the results of system suitability parameters were given in Table 1.

HPLC-PDA method validation

Linearity and range. The calibration graph was constructed by plotting peak area measured against corresponding concentrations RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA. The calibration plots for RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA were linear over the calibration range 0.05–15 μg mL⁻¹ for each using square linear regression analysis. The linearity of the calibration graphs was evaluated by the high value of the correlation coefficient. The regression equations and their own parameters of the HPLC method were provided in Table 2.

Limits of detection and quantification (LOD and LOQ). - With respect to (ICH) recommendations [41], LOQ and...
Table 4. Determination of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA in a) personnel care products and b) food products using the proposed HPLC method

| Extract          | a) Product name                        | Average concentration (mg/final volume of each product) |
|------------------|----------------------------------------|--------------------------------------------------------|
|                  |                                        | RS    | GA   | VA   | CA   | EC   | CF   | SY   | FA   | LU   | QU   | KA   |
| Apple            | Body shop® shower gel (250 mL)         | –     | –    | 36.83| –    | 0.15 | 15.77| 0.18 | –    | 7.26 | –    | –    |
|                  | Body shop® foaming bath (300 mL)       | –     | –    | 35.88| –    | –    | –    | 0.41 | –    | 42.57 |–    | –    |
|                  | Palmolive® green apple shampoo (380 mL)| –     | 0.86 | 0.58 | –    | 0.23 | 0.40 | 0.52 | –    | 0.22 | 0.23 | –    |
|                  | Body shop® lip balm (20 mL)            | –     | –    | 2.77 | –    | 0.01 | 2.86 | 0.08 | –    | 0.92 | –    | 0.02 |
|                  | Body shop® body butter (200 mL)        | –     | 0.13 | 28.01| –    | 0.08 | 28.95| –    | 1.45 | 0.15 | –    | –    |
|                  | Body shop® body scrub (250 mL)         | –     | 0.13 | 30.97| 0.15 | 0.13 | –    | 39.12| 20.84 |–    | 0.37 | –    |
|                  | Body shop® body gel lotion (250 mL)    | –     | 0.15 | 21.49| –    | 0.19 | 35.00| –    | 21.36 |–    | 0.15 | –    |
|                  | Fresh fruit® hand cream (150 mL)       | –     | –    | 0.12 | 16.72| 0.08 | 0.12 | 5.79 | –    | 0.10 | –    | 3.92 |
| Pomegranate      | Herbal essences® shampoo (400 mL)      | 0.22  | –    | 61.30| –    | 0.28 | 17.84| –    | 1.77 | 0.31 | 0.35 | 0.25 |
|                  | Herbal essences® conditioner (400 mL)  | –     | –    | 58.02| –    | –    | –    | 0.30 | 1.25 | 0.20 | 0.23 | –    |
|                  | Bonjourn® shower gel (1,000 mL)        | –     | –    | –    | –    | 0.86 | 92.51| 2.07 | 44.88 |1.49 | 4.15 | –    |
|                  | Dove® body wash (190 mL)               | 1.71  | 0.78 | 0.17 | 14.52| 0.10 | 0.10 | 0.23 | –    | 0.10 | 0.12 | 0.29 |
|                  | Avon® bubble bath (1,000 mL)           | 0.61  | –    | 1.68 | –    | 0.57 | –    | –    | 23.28 |0.59 | 0.83 | 0.53 |
|                  | Avon® day face cream (50 mL)           | 0.58  | –    | 0.55 | 0.03 | 0.08 | 0.03 | –    | 0.03 | 0.05 | –    | –    |
|                  | Avon® night face cream (50 mL)         | 0.05  | –    | 0.05 | 0.04 | 0.04 | 0.09 | –    | 0.06 | 0.61 | 0.08 | –    |
|                  | Oriflame® pure skin face toner (150 mL)| 0.21  | –    | –    | –    | 0.10 | –    | –    | 0.11 | –    | –    | –    |
| Argan oil        | Herbal essences® my shine shampoo (400 mL) | –     | –    | 1.64 | 0.33 | 0.37 | 0.91 | 0.71 | –    | 0.35 | 0.46 | –    |
|                  | Avon® advance techniques hair shampoo (250 mL) | 3.81 | –    | 0.45 | 0.27 | 0.21 | 0.20 | –    | 0.13 | 0.22 | –    | –    |
|                  | Professional® hair shampoo (300 mL)    | –     | –    | 0.45 | 0.19 | 0.24 | 2.90 | 0.80 | 0.68 | 0.17 | 0.37 | –    |
|                  | Skin doctor® shampoo (400 mL)          | –     | –    | 57.29| –    | 0.25 | 0.48 | 1.65 | 1.32 | 0.31 | 1.13 | –    |
|                  | Sun silk® shampoo (190 mL)             | 0.75  | –    | 0.57 | 0.72 | 0.13 | 0.68 | 1.05 | –    | 0.16 | 0.85 | –    |
|                  | Palmolive® mediterranean moments argan hair shampoo (500 mL) | 0.48  | 0.60 | 66.32| –    | –    | 0.37 | 73.57| 0.31 | 0.27 | –    |
|                  | Herba vita® shampoo (400 mL)           | 0.39  | 0.28 | 0.74 | –    | 0.26 | 0.60 | 1.08 | –    | 1.42 | 1.08 | –    |
|                  | Avon® advance techniques hair conditioner (250 mL) | –     | 0.84 | 0.45 | –    | 0.17 | 0.89 | 0.38 | 0.07 | 0.24 | –    | –    |
|                  | Argan® hair oil (50 mL)                | 0.35  | 0.08 | 0.21 | 0.08 | 0.10 | –    | 0.21 | –    | 0.12 | 0.09 | –    |
|                  | Skin doctor® argan oil hair oil (50 mL)| 0.03  | –    | –    | 0.03 | 0.03 | 0.04 | –    | 0.03 | 0.03 | 0.04 | –    |
|                  | Oriflame® argan oil hair oil (50 mL)   | –     | –    | 0.04 | 0.03 | 0.03 | –    | –    | 0.07 | 0.03 | –    | –    |
|                  | Herba vita® balsam argan oil (400 mL)  | –     | –    | 0.92 | 0.31 | 0.41 | 0.42 | 0.74 | –    | 0.32 | 0.82 | –    |
|                  | Avon® advance techniques hair serum (30 mL) | 0.05  | –    | 0.09 | 0.05 | 0.03 | 0.05 | 0.09 | 0.03 | 0.03 | 0.06 | –    |
|                  | Oriflame® hair serum (30 mL)           | –     | –    | 0.07 | 1.52 | 0.02 | 0.03 | 0.07 | –    | 0.03 | 0.03 | –    |
|                  | Every strand® polisher hair serum (175 mL) | 0.11  | 0.14 | –    | –    | 26.28| –    | 0.09 | 0.11 | 0.13 | –    | –    |
|                  | No frizz® argan oil hair serum (50 mL) | –     | –    | 0.17 | –    | 0.07 | 0.13 | 0.18 | 0.03 | 0.06 | 0.09 | –    |
|                  | Vital care® luxury argan hair serum (100 mL) | –     | 0.32 | 0.08 | 0.06 | 2.66 | –    | 0.08 | 0.11 | 0.16 | –    | –    |

(continued)
Table 4. Continued

| Extract | a) Product name | RS | GA | VA | CA | EC | CF | SY | FA | LU | QU | KA |
|---------|----------------|----|----|----|----|----|----|----|----|----|----|----|
| Lorys® hair cream (1,000 mL) | 0.55 | 0.58 | 0.96 | - | 0.20 | 3.14 | - | 0.58 | 0.53 | - | - |
| Avon® hand cream (30 mL) | 0.30 | 0.16 | 0.05 | 1.80 | 0.01 | 0.02 | - | 0.02 | 0.02 | 0.03 | - |
| Avon® skin soft 1 body spray (150 mL) | - | 0.08 | 27.60 | - | 0.20 | 0.74 | 0.28 | 0.08 | 0.20 | 0.26 | - |
| Oriflame® body lotion (250 mL) | 0.74 | 0.14 | 0.15 | 4.25 | 0.01 | 0.02 | - | 0.83 | - | 0.20 | - |
| Avon® body cream (200 mL) | 0.27 | 0.11 | 0.52 | - | 0.17 | 0.20 | 0.47 | - | - | 20.73 | 0.19 | - |

| Extract | b) Product name | Average concentration (mg/final volume of each product) |
|---------|----------------|------------------------------------------------------|
| Apple Vitrac® apple juice concentrate (650 mL) | - | 77.42 | 51.48 | 35.89 | 2,983.88 | 222.63 | 2,651.89 | 36.32 | 114.91 | 57.70 | - |
| Lamar® apple juice (230 mL) | - | 52.72 | 35.80 | 15.83 | 224.87 | 68.85 | 100.74 | 19.39 | 13.16 | 20.31 | - |
| Easy mouzuo® apple juice (200 mL) | - | 14.74 | 33.17 | 233.71 | - | 17.34 | 194.03 | 16.32 | 18.68 | - | 13.48 |
| Rani® spark apple juice (330 mL) | 1961.16 | 15.13 | 46.92 | - | 20.09 | 18.90 | 64.03 | 17.12 | 24.54 | - | 253.45 |
| Teebmars® apple vinegar (250 mL) | - | 20.84 | 13.83 | 100.23 | 22.42 | 88.94 | - | 13.36 | - | - |
| Healthy® apple vinegar (250 mL) | - | 12.94 | 13.01 | 39.10 | - | 17.35 | 13.75 | - | - | - |
| Negmet el zeitoun® apple vinegar 6% (500 mL) | - | 7,014.42 | 60.81 | 4,203.88 | 2,400.45 | 2,475.57 | 5,492.71 | 1,491.25 | 400.28 | 93.71 | - |

| Extract | Average concentration (µg/final weight of each product) |
|---------|------------------------------------------------------|
| Pomegranate Juicy® pomegranate juice (300 mL) | 5,338.36 | 10,144.07 | 31,022.31 | - | 1,040.61 | - | 195.54 | - | - |
| Juhayna pure® pomegranate juice (235 mL) | 1,046.12 | 112.52 | 29.69 | 401.31 | - | 1,434.79 | 88.74 | 12.75 | - | - |
| Domty® pomegranate juice (235 mL) | - | 509.83 | 139.42 | 5,103.99 | 56.03 | 80.38 | 516.80 | 28.55 | 15.25 | - |
| Sheepps® pomegranate juice (350 mL) | 23.21 | 17.77 | 28.47 | 23.94 | 17.89 | 42.99 | - | 72.60 | 66.21 | - |
| Barbican® pomegranate juice (250 mL) | 954.35 | 15.75 | 21.45 | 33.07 | 27.24 | 33.02 | 241.83 | 15.92 | 35.66 | - |
| Lamar® pomegranate juice (230 mL) | 3,432.59 | 2,837.35 | 1,542.35 | 2,711.05 | 347.24 | 624.11 | 246.95 | 11.22 | - | 57.51 |
| Juhayna® classic pomegranate juice (235 mL) | 1,657.90 | 32.12 | 23.40 | 1,522.88 | 3,336.48 | 173.96 | 12.51 | - | - |
| Al hasad® vinegar pomegranate (250 mL) | 185.23 | 905.34 | 1,396.51 | 371.19 | 239.13 | 225.70 | 545.72 | 20.09 | 14.33 | 12.51 | 30.66 |

| Molasses | Average concentration (µg/final weight of each product) |
|---------|------------------------------------------------------|
| Pomegranate Durra® pomegranate intensive molasses (235 g) | - | 1,113.39 | 457.75 | - | 123.47 | 249.77 | - | 149.76 | 284.87 | - |
| El arab® pomegranate molasses (325 g) | 2,957.86 | - | 590.14 | 177.07 | 173.10 | 417.16 | 244.16 | - | 172.98 | 181.42 | - |
| Cortas® pomegranate molasses (420 g) | - | 3,665.19 | 30,030.34 | 5,460.00 | 777.83 | 9,180.17 | 867.60 | - | 123.80 | 220.03 | - |
| Healthy® pomegranate molasses (325 gm) | 333.93 | 3,721.21 | 11,353.83 | 18,297.18 | 557.09 | 234.46 | 261.19 | 189.39 | 321.07 | - |
| Nareksilisos® pomegranate molasses (330 g) | - | 721.12 | 371.77 | 5,717.37 | 174.85 | 187.82 | 642.77 | - | 180.67 | 218.25 | 167.17 |

(continued)
LOD were calculated. The results were summarized in Table 2, which reveals the good sensitivity which the developed HPLC method offers.

**Selectivity.** Selectivity of the proposed method was assessed by setting up different laboratory prepared mixtures of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA at concentrations particularly inside the linearity range and analyzing these mixtures. The results were satisfactory and the percentage recovery of the compounds ranged from 98.51 to 100.91% revealing the acceptable selectivity of the method.

**Accuracy and precision.** The intra-day and inter-day of precision values (RSD%) and the accuracies (RE%) of the analytes were demonstrated in Table 3, in which RSD % values were lower than 2% and RE% values were lower than ±9% revealing acceptable accuracy and precision. The mean percentage recoveries for three replicates calculated by standard addition method ranged between 98.43 and 100.86%.

**Stability of analytical solutions.** The stability of stock standard solutions, working solutions and real samples extracted during analysis was evaluated by leaving the solutions in tightly capped volumetric flasks protected from light on a laboratory bench or in a refrigerator 4°C or in a freezer −20°C. The stock solutions and working solutions were stable and showed no chromatographic changes, for about 1 month when kept in a freezer at −20°C, for 12 h when kept at room temperature (25°C) and for 2 days when stored at 4°C while the real herbal samples extracted were stable for 3 h when kept at room temperature (25°C) and 6 h when stored at 4°C. All of the tested commercial products were examined by our method before the expiry dates mentioned and stored according to the instructions mentioned by their producers.

**Analysis of herbal samples**

The proposed HPLC-PDA method was typically applied to the concurrent determination of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA in 67 commercial products without interference of the other active components/exipients present in herbal samples. The concentrations of the antioxidants found in these products were shown in Table 4 and Fig. 2. Then, some of the juices and vinegars samples containing GA, CA, CF, EC, FA and QU were analyzed using the reported HPLC method [32], in this reported method GA, CA, CF, EC, FA and QU were analyzed in pomegranate juices. The results calculated for GA, CA, CF, EC, FA and QU using our proposed method were statistically compared with those calculated from the reported HPLC method using the student t-test. The calculated t and F values, shown in Table 5, were less than the theoretical ones at 95% confidence limit indicating there was no significant difference between the proposed method and the reported method with regard to accuracy and precision.

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**Table 4. Continued**

| Extract | Average concentration (mg/ml) |
|---------|------------------------------|
| RS      | VA  | CA  | GA  | EC  | CF  | SY  | FA  | LU  | QU  | KA  |
| Pomegranate molasses (410 g) | 40.180 | 2.779 | 59.655 | 2.562 | 1.189 | 35.93 | 6.823 | 19.633 | –   | 675.37 |
| Pomegranate molasses (550 g) | 37.690 | 6.19  | 39.16  | 5.028 | 7.19  | 37.69  | 6.19  | 5.028 | –   | 96.69  |

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CONCLUSION

The proposed HPLC method provides precise and reproducible quantitative analysis for determination of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA. It allows accurate quantitation of RS, GA, VA, CA, CF, SY, EC, FA, LU, QU and KA in their pure form and in commercial formulations without interference from other constituents/excipients in

![Typical HPLC chromatograms of 20 µL injection of (a) Sun silk® argan oil shampoo, (b) Lamar® pomegranate juice (c) Healthy® pomegranate molasses and (d) Lipton® apple tea](image)

Table 5. Comparison of the proposed HPLC method with the reported method [32] for some juices and vinegars samples

| Product name                  | Mean ±S.D.* | GA   | CA   | EC   | CF   | FA   | QU   |
|-------------------------------|-------------|------|------|------|------|------|------|
| Domty pomegranate juice (235 mL) |             |      |      |      |      |      |      |
| Proposed method               | 510.92 ± 0.83 | 138.22 ± 0.92 | 5,103.88 ± 1.20 | 56.33 ± 0.92 | 516.88 ± 0.72 | 15.11 ± 0.12 |
| Reported method               | 511.92 ± 0.96 | 139.22 ± 0.83 | 5,102.28 ± 1.12 | 57.14 ± 0.94 | 517.04 ± 0.84 | 15.24 ± 0.14 |
| t (2.18)b                     | 2.08        | 2.16 | 1.60 | 1.63 | 0.62 | 1.87 |
| F (4.28)b                     | 1.00        | 0.99 | 1.00 | 0.97 | 1.00 | 0.98 |
| Vitrac apple juice concentrate (650 mL) |             |      |      |      |      |      |      |
| Proposed method               | 77.62 ± 0.62 | 36.24 ± 0.54 | 2,983.22 ± 0.67 | 222.55 ± 0.57 | 37.00 ± 0.44 | 57.94 ± 0.67 |
| Reported method               | 78.24 ± 0.64 | 35.72 ± 0.57 | 2,984.11 ± 0.94 | 223.18 ± 0.54 | 36.58 ± 0.47 | 58.66 ± 0.64 |
| t (2.18)b                     | 1.84        | 1.75 | 2.04 | 2.12 | 1.73 | 2.06 |
| F (4.28)b                     | 0.98        | 0.97 | 1.00 | 0.99 | 1.00 | 0.98 |
| Hala apple vinegar (250 mL)   |             |      |      |      |      |      |      |
| Proposed method               | –           | 22.58 ± 0.48 | 28.58 ± 0.37 | –     | 15.38 ± 0.48 | 17.89 ± 0.28 |
| Reported method               | –           | 23.11 ± 0.44 | 29.01 ± 0.39 | –     | 14.99 ± 0.49 | 18.11 ± 0.29 |
| t (2.18)b                     | 2.15        | 2.12 | 1.50 | 1.44 |     |     |
| F (4.28)b                     | 0.95        | 0.97 | 1.05 | 0.98 |     |     |
| Healthy apple vinegar (250 mL) |             |      |      |      |      |      |      |
| Proposed method               | 12.88 ± 0.57 | –    | 13.38 ± 0.38 | 39.55 ± 0.58 | –     | 13.32 ± 0.46 |
| Reported method               | 13.22 ± 0.54 | –    | 12.99 ± 0.43 | 40.11 ± 0.69 | –     | 12.89 ± 0.33 |
| t (2.18)b                     | 1.15        | 1.80 | 1.64 | 2.01 |     |     |
| F (4.28)b                     | 0.95        | 1.06 | 0.97 | 1.07 |     |     |

*Mean concentration given in µg per final volume of each product ± S.D. for seven determinations.

b Theoretical values for t and F at P = 0.05.
about 33 min. The method is gradient with a simple mobile phase, minimal sample preparation. The method presented is rather safe to the environment due to the diminished organic solvent usage. This is an HPLC procedure based on a reverse phase C$_{18}$ column with a gradient elution composed of acidic water and acetonitrile. This procedure is likely the first choice for any work dealing with phenolic antioxidants.

Therefore, this method is ideally suited for analysis of the selected antioxidants in formulations containing green apple, pomegranate and argan oil extracts with proper run time. The present work is important from screening and quality control point of view as it could help the researchers to have information about antioxidants found in herbal extracts of argan oil, pomegranate and green apple and finally help consumers to choose the better products by knowing the antioxidants' amounts present from the labels.

Conflict of interest statement: The authors have declared no conflict of interest.

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