Functionnal and Technological Properties of Five Strawberry (Arbutus Unedo L.) Fruit as Bioactive Ingredients in Functional Foods

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ABSTRACT
The objective of this study was to identify, quantify, and elucidate the polyphenols, flavonoids, and anthocyanins, and their antioxidant activities (via 13 2,2-diphenyl-1-picrylhydrazyl) (DPPH) radical and 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging abilities and bleaching β-carotene tested in vitro in the whole fruit, fruit skin of strawberry tree fruits of 5 genotypes and to find out the most valuable fruit for disease preventing diets. Total phenols, total flavonoids, total anthocyanins, antioxidant activity (DPPH, ABTS and β-carotene bleaching assay), pH, tritable acidity, soluble solids, and organic acids were investigated in five strawberry tree genotypes belonging to several areas in Morocco. Qualitative and quantitative analyses of individual phenolic compounds by high-performance liquid chromatography (HPLC) were also carried out. Significant differences (p<0.05) were observed across the five genotypes in total phenols (25.37–39.06 mg GAE/g DW), total flavonoids (3.30–7.07 mg RE/g DW), total anthocyanins (0.15–0.64 mg cya-3-glu/100 g DW), pH (2.44–3.92), tritable acidity (0.65–1.01 g malic acid/100 g fw), and soluble solids (14.83–18.53%). The antioxidant activity was evaluated by three assays. The values were 3.33–21.08, 2.25–19.58, and 1.08–13 mg AAE/g DW for DPPH scavenging test, ABTS, and β-carotene bleaching, respectively. Gallo catechol and catechin were the most abundant phenolic compounds. Principal component analysis showed that the first three components formed 90.25% of the total inertia. Chlorogenic acid, ellagic acid derivative I, ellagic acid, rutin, and cyanidin-3,5-diglucoside were the most involved variables in the total variance explained.

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INTRODUCTION
The Strawberry tree (Arbutus unedo L.) is a evergreen shrub or small tree, and in autumn, it bears orange colored fruit, naturally grown as population or solitary tree in the Mediterranean countries such as Morocco, Tunisia, Algeria, Turkey, Syria, Greece, Croatia, France, Portugal and Spain have

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native *Arbutus unedo* L.\(^1\) Strawberry tree is a medicinal plant and considered as an important source of new molecules with high antioxidant potential. Polyphenols, commonly referred to as antioxidant compounds, play a major role in safeguarding health, and a protection against diseases like cancer has recently been shown.\(^2\) *Arbutus unedo* L. (Ericaceae family), commonly known as strawberry tree, is an evergreen shrub endemic to the Mediterranean region and North Africa.\(^3\) The strawberry tree fruit is suitable for the production of alcoholic beverages, jams, jellies and marmalades\(^4\) but also for medicinal purposes.\(^5\)

In Morocco, it is known as “Sasnou” and it is widely used in traditional medicine such as antiseptics, diuretics, and laxatives, more recently, in the therapy of hypertension and diabetes.\(^6\) They used both fruits and leaves for medicinal purposes\(^5\) for their bioactive value. Strawberry tree fruits are already known as a very good dietary source of antioxidants, including phenolic compounds (e.g. anthocyanins and other flavonoids, gallic acid derivatives, and tannins), vitamins C and E, and carotenoids.\(^4,7-13\) These bioactive plant compounds have been used since ancient times as both primary and supplemental treatments for various ailments as well as to support normal physiological functions.\(^14\) These phenolic compounds can amplify the body’s defense system to eliminate cancer cells and block angiogenesis, which is the formation of new blood vessels and known to be essential for tumor development. Consumption of foods rich in flavonoids decreased risk factors for heart disease. Flavanols and procyanidins in particular may confer vascular benefits by increasing the available pool of nitric oxide and reducing platelet aggregation.\(^15\) An increased interest in using naturally occurring phytochemicals from plants for the prevention and treatment of different chronic human diseases was reported in many studies. Among phytochemicals, both phenolic compounds from a large number of plant foods, spices, and beverages have been shown to inhibit or attenuate cancer and cardiovascular diseases\(^16\) as strawberry tree fruit is a source potential for phytochemicals. Previous phytochemical studies on the plant showed the presence of three anthocyanins: delphinidin 3-O-galactoside, cyanidin 3-O-galactoglucose, and cyanidin 3-O-galactoside.\(^17\) The total content of phenols has been estimated by\(^7\) as 14.6 mg/g dried fruit. There are so far little data in the literature on antioxidants found in *Arbutus unedo* L., although its antioxidant content rate highly when compared with 27 other fruits.\(^18-19\) In Morocco, most of those fruits remained underexploited due to the lack of awareness of their potential, market demand, and value addition. But of now, the genetic resources of such fruits are facing a great threat of extinction due to climate change, large-scale urbanization, changing attitude, and taste of peoples and developmental projects. To safeguard the existing diversity of underutilized fruits, systematic exploitation, collection, characterization, multiplication, and conservation of these valuable resources are urgently needed to ensure food and nutritional security of rural populations and to achieve sustainable development. Biochemical markers have been widely used in breeding studies and in the investigations into the diversity of species and the relationship between genotypes, cultivars and their wild parents. More recently, biochemical content, in particular, bioactive content of fruits, has been widely searched in terms of their human health benefits. The breeders are now searching to find genotypes that have higher bioactive content in order to use them in cross-breeding activities for the purpose of obtaining new cultivars that possess high nutrient value for health.\(^20\) However, very few studies have been devoted to study the strawberry tree fruits. Thus, the objective of this study was to evaluate and compare, for the first time, strawberry species in terms of their main physicochemical and biochemical characteristics in five Moroccan clones. The main objectives of this study were: i) to assess the physicochemical and biochemical parameters of strawberry tree fruits; ii) to evaluate the polyphenolic profiles and antioxidant activities of strawberry tree fruits using three methods (DPPH, ABTS, and β-carotene bleaching assay, iii) and to determine the correlations between all parameters in order to provide information about the ones that are potentially important in assessing strawberry tree genotypes.
**Materials and methods**

**Plant material**

Five clones of strawberry tree (Arbutus unedo L.) (CHF, MDZ, LAN, KSB, and TAH) were collected during the period between October and November of 2019 from several regions of Morocco where they grow naturally (Table 1). At each site, random samples of fruits were harvested at their fully matured and transferred to the laboratory for physicochemical analysis, namely pH, titratable acidity, soluble solids, phytochemical characteristics, and antioxidant activities conducted in plant material frozen at −20 °C, freeze-dried, and ground for analysis. Five trees were selected for each clone, and 50 fruits per clone (10 fruits per tree) were randomly picked.

**Chemicals and reagents**

2,2-Diphenyl-1-picrylhydrazyl acid (DPPH), gallic acid, rutin, β-carotene, Folin-Ciocalteu reagent, ascorbic acid, and sodium carbonate (Na₂CO₃) were purchased from Sigma – Aldrich (St. Petersburg), 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was from HIMEDIA, and potassium iodate was from Scharlab. Standard compounds (phenolic acid standards: ellagic, gallic, and chlorogenic acids; flavonoids standards: rutin, quercetin-3-O-glusoside, and quercetin-3-O-galactoside) were obtained from Extrasynthese (Genay, France), and the water was distilled and filtered through a Milli-Q apparatus filter.

**Physicochemical analyses**

Total soluble solids (TSS) were assessed according to [21] by triplicate with a digital refractometer (Atago N1; Atago Co. Ltd., Tokyo, Japan) at 20 °C and expressed as °Brix. Total titratable acidity (TA) was also determined according to [22] by triplicate using an automatic titration device (877 Titrino plus, Metrohm ion analyses CH9101, Herisau, Switzerland) with 0.1 N NaOH up to pH 8.1, using 1 mL diluted juice in 25 mL distilled H₂O, and the results were expressed as g malic acid per 100 g fw. [23] The pH was measured using a pH meter according to the method described by. [22] Weigh 10 g of the fruit cut into small pieces, add 100 mL of distilled water, and mix for 5 minutes until juice is obtained. The measurement was made by immersing the pH meter electrode in the solution. All results were shown as mean values SE (standard error).

**Organic Acids and ascorbic acid profiles**

The samples (0.5 g) were extracted with 5 mL of Milli-Q water by incubation for 30 min under ultrasonication. Next, the slurry was centrifuged at 15,000 g for 20 min (Sigma 3–18 K; Sigma, Osterode am Harz, Germany), and the supernatant was filtered through a 0.45 μm Millipore filter and used for analysis. All extractions were carried out in triplicate.

The chromatographic analysis was carried out according to Hernández [24]. Thus, 10 μL of extract was injected into a Hewlett-Packard HPLC Series 1100 (Wilmington DE, USA) with an autosampler and a UV detector, set at 210 nm and coupled with a refractive index detector (HP 1100, G1362A). A column (Supelcogel TM C-610 H column 30 cm x 7.8 mm) and apre-column (Supelguard 5 cm

| Table 1. Origins geographic of the different strawberry tree genotypes. |
|------------------------|--------|-----------|----------|
| Origin                 | Code   | Zone      | Altitude (m) |
| Chefchaouen            | CHF    | Rif       | 534        |
| Moulay Driss Zerhoun    | MDZ    | Middle Atlas | 820        |
| Laanoucier             | LAN    | Middle Atlas | 1700        |
| El Kesba               | KSB    | Middle Atlas | 1360        |
| Tahnaout               | TAH    | High Atlas | 1200        |
x 4.6 mm; Supelco, Bellefonte, PA) were used for the analyses of both organic acids and ascorbic acid. The elution buffer consisted of 0.1% phosphoric (V/V) at a flow rate of 0.5 mL min\(^{-1}\), and organic acid absorbance was measured at 210 nm using a diode-array detector (DAD). The same HPLC conditions (elution buffer, flow rate, and column) were used for the analysis of sugars. The detection was conducted using a refractive index detector (RID). Standards of organic acids and sugars were obtained from Sigma (St. Louis, MO). Calibration curves were used for the quantification of organic acids and ascorbic acid showing good linearity (\(r^2 \geq 0.999\)). The results were expressed as g 100 g\(^{-1}\) of dry weight (DW).

**Phytochemical composition**

Extraction procedure: 1 g of powder from each sample was mixed with 25 mL of ethanol (1:25, w/v) at 25 °C for 15 min using an IKA T-18 digital Ultra-Turrax homogenizer. The homogenate was then centrifuged for 10 minutes at 6,000 rpm and the supernatant was removed from the residue. The latter was homogenized and the supernatant removed as above. The supernatants are then combined and filtered.

Total phenols (TP): Total phenol content (TPC) of strawberry was determined by the reduction of phosphotungstic-phosphomolybdic acid (Folin–Ciocalteu's reagent) to blue pigments, in alkaline solution according to Folin as described by Ben.\(^{25}\) Briefly, 100 μL of diluted sample (1/100) with ethanol was added to 400 μL of 1/10 diluted Folin–Ciocalteu reagent. After 5 minutes, 500 μL of 10% (w/v) sodium carbonate solution was added. After 1 hour of incubation at room temperature, absorbance at 765 nm (spectrophotometer Spectraphysic Jasco V-630, Japan) was measured in triplicate. The total polyphenol content was expressed as milligrams of gallic acid equivalents (GAE) per g dry weight of strawberry tree fruit (mg GAE/g DW).

Total flavonoids (TF): Total flavonoid content (TFC) was measured using the colorimetric method with aluminum chloride.\(^{26}\) 1 mL of the sample was diluted separately and then mixed with 1 mL of a 2% aluminum chloride solution. The mixture was incubated at room temperature for 15 minutes. Rutin was used to develop the calibration curve. The absorbance was measured at 430 nm (spectrophotometer Spectraphysic Jasco V-630, Japan). The results were expressed as milligram rutin equivalents per dry weight of strawberry tree fruit (mg RE/g DW).

Total anthocyanins: Total anthocyanin content (TAC) of samples was determined using the pH differential method with some modifications according to.\(^{27,28}\) A 1 mL aliquot of each strawberry extract sample was added separately to 980 μl of KCl buffer (pH1.0) and NaOAc buffer (pH4.5). The absorbance was measured at 510 and 700 nm (spectrophotometer Spectraphysic Jasco V-630, Japan) for both sets of pH1.0 and 4.5 solutions, using 50% ethanol as a blank after 15 min of incubation at room temperature. The TAC was calculated using Equation (1), and the results were expressed as milligrams of cyanidin-3-glucoside equivalents in 100 g of DW.

\[
TA = (A * MW * DF / 1000) / \varepsilon * L
\]  
(1)

where A: Absorbance = (A510nm-A700nm) pH1.0 – (A510nm-A700nm) pH4.5; MW: molecular weight (449.2 g/mol); DF: dilution factor; \(\varepsilon\): molar absorptivity coefficient of cyanidin-3-glucoside (26900 L/mol cm).

**Antioxidant activities**

The antioxidant activity was evaluated using three different assays: (i) DPPH assay, (ii) ABTS assay, and (iii) the β-carotene bleaching test. The antioxidant activity was determined in triplicate and the results were presented as a mean ± standard deviation.

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity: The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the extracts was measured as described by Ben.\(^{1}\)
Initially, 0.1 g of DPPH was dissolved in 1 L methanol to prepare a 0.1 g L⁻¹ of DPPH solution. Then, 1 mL of this solution was added to 125 µL of the extract. The mixture was stirred thoroughly and allowed to stand in the dark at room temperature for about 10 min. A control solution was prepared by adding equal volumes of DPPH and methanol. The optical density of both sample and control was measured using a Lambda EZ 150 (spectrophotometer Spectrophysic Jasco V-630, Japan) at 517 nm, and the DPPH scavenging activity was calculated using the following Equation (2):

$$DPPH \text{ scavenged(\%)} = \{(Ac - As)/Ac\} \times 100$$

where Ac: Absorbance of the control; As: absorbance of the sample. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (mg AAE/g DW) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) Free Radical Scavenging Capacity: The total antioxidant activity of each genotype was analyzed using the ABTS radical scavenging capacity assay. The ABTS method is based on colorimetric monitoring of the decay of the ABTS•+ radical cation, caused by the oxidation of ABTS•+ radicals when contacting an antioxidant. ABTS•+ was prepared by reacting ABTS with potassium persulfate. The ABTS• (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay were employed according to.[29] ABTS radical cation decolorization assay ABTS•+ was prepared by reacting ABTS with potassium persulfate. The decrease in absorbance was measured at 734 nm using a double beam UV-Visible spectrophotometer (Spectrophysic Jasco V-630, Japan). Calibration curves, in the range of 0.5 – 5.0 mg of ascorbic acid g – 1 were used for the quantification of the three methods of AA, showing good linearity (R2 ≥ 0.998). The 990 µL of strawberry extract were incubated in 10 µL ABTS (7 mM)-ETOH and 2.45 mM potassium persulfate solution after sonicated at 20 °C for 15 min during 16 hours in the dark followed by an incubation of the mixture in darkness for 18 h at room temperature. The ethanol was used to dilute the stock solution of ABTS•+ until an absorbance of 0.70 ± 0.05 at 734 nm was reached. For both essays, the antioxidant activity results were expressed as mg Equivalent ascorbic Acid per g dry weight (DW).

β-Carotene Bleaching Assay: The evaluation of the antioxidant activity of phytochemical compounds from each strawberry genotype was based on the coupled oxidation of β-carotene and linoleic acid and determined following the procedure of β-carotene bleaching test described by [30] based on the ability to decrease the oxidative bleaching of β-carotene. β-Carotene (0.5 mg) in 1 mL of chloroform was taken in an amber bottle and mixed with 200 mg of linolenic acid and 600 mg of Tween 80 (poly oxy ethylene sorbitan monopalmitate). The chloroform was removed under nitrogen and the resulting solution was immediately diluted with 30 mL of triple distilled water and the emulsion was mixed well for 1 min. The emulsion was further diluted with 120 mL of oxygenated water and used for assay. To the samples (0.5 mL), freshly prepared emulsion mixture was added and mixed well. A control consisting of 0.5 mL of ethanol and 2.5 mL of emulsion was also analyzed. The absorbance of the reaction mixture was measured immediately (t = 0) at 470 nm against blank consisting of an emulsion mixture except β-carotene and at 60 min interval for 2 h (t = 120). The tubes were placed in a water bath at 50°C between measurements. Measurement of color was recorded until the color of β-carotene disappears. The antioxidant activity was expressed as inhibition percentage relative to the control using the following Equation (3):

$$AA = 100[1 - (A_o - A_t)/(A_{oo} - A_at)]$$

where A_o and A_{oo} are the absorbance measured at the beginning of the incubation for samples and control, respectively. A_t and A_at are absorbance measured for samples and control after 2 hours.

**Extraction and determination of polyphenolic compounds**

Extraction Method: Samples (1 g) were mixed with 10 mL of methanol: water (80:20, v/v) and then, the mixtures were sonicated during 30 min, and macerated for 1 hour in refrigeration (4 °C). After the
time, the samples were centrifuged for 10 min, 8000 g at 4 °C. The supernatants were collected and the pellets were mixed with 10 mL of acetone: water (70:30, v/v) and the same steps were repeated (sonication, maceration and centrifugation). Then, the supernatants were combined and evaporated to dryness using a rotary evaporator R-205 (Büchi, Flawil, Switzerland) under reduced pressure, at 40 °C. 5 mL of methanol was added to the residue, and the mixture was well shaken in a stirrer for 2 min. Due to the high sugar content present in the samples, which could interfere with the HPLC column, the samples were loaded onto a C18 Sep-Pak cartridge, previously conditioned with 5 mL of methanol, 5 mL of pure water, and then with 5 mL of 0.01 mol/L HCl. The cartridge was washed with 5 mL of pure water and then eluted with acidified methanol (0.1 g/L HCl). The collected fractions were stored at −20 °C until further use.

Polyphenolic Compounds: Polyphenolic profiles of all samples were determined by High-Performance Liquid Chromatography (HPLC) according to. A volume of 20 μL of the samples was injected into a Hewlett-Packard HPLC series 1200 instrument (Waldborn, Germany) equipped with a diode-array detector (DAD) and a C18 column (Mediterranean Sea 18, 25 × 0.4 cm, 5-μm particle size) from Teknokroma (Barcelona, Spain). Polyphenolic compounds were analyzed in standard and sample solutions using a gradient elution at 1 mL/min. The mobile phases were composed of formic acid in water (1:99, v/v) as solvent A and acetonitrile as solvent B. The chromatograms were recorded at 280, 320, 360, and 520 nm. Polyphenolic compound identification was carried out by comparing UV absorption spectra and retention times of each compound with those of pure standards injected under the same conditions. The compounds were quantified through calibration curves of standard compounds injected in the same conditions. Phenolic acid standards were dissolved in methanol at different concentrations between 10 and 200 μg mL−1; flavonoid standards were dissolved in methanol at different concentrations between 1 and 250 μg mL−1. Quantification of anthocyanins was carried out based on linear curves of authentic standards. A cyanidin 3-glucoside calibration (concentration between 1 and 250 μg mL−1) was used for cyanidin derivatives.

**Statistical analysis**

The means were evaluated according to descriptive statistics represented as mean ± SE. Data analysis was performed using IBM SPSS v22. Analysis of variance (ANOVA) was performed to test significant differences among the samples. The differences in studied variables were estimated with Duncan’s new multiple range (DMRT) test. Correlation coefficients and their levels of significance were calculated using Pearson correlation. Principal Component Analysis was carried out using the correlation matrix. In addition, a scatter plot was created according to the first three principal components (PC1, PC2 and PC3). A two-dimensional CHA heatmap was applied to the dataset using R software 3.0.2. Prior to this analysis, data were standardized to a comparable scale (μ = 0 and σ = 1). In this presentation of data, the effect size measure is represented by the color intensity. The heatmap groups similar rows and similar columns together, with their similarity represented by a dendrogram.

**Results and discussion**

**Physicochemical parameters**

The results for titratable acidity, pH and total soluble solids (TSS) for all genotypes are presented in Table 2. Analysis of the physicochemical data pertaining to the five genotypes showed significant variations in all parameters at (p < .001). The titratable acidity ranged from 0.65 to 1.01 g malic acid/100 g fw with an average of 0.83 g malic acid/100 g fw. The highest value was recorded in “TAH” (1.01 g malic acid/100 g fw) while the lowest value was observed in “MDZ” (0.65 g malic acid/100 g fw). The titratable acidity of strawberry tree fruits reported in this study was higher than those found by other authors. They found values 0.51% and 0.4%, respectively. However, the results obtained in this study were lower than the ones published by, who found [2.14%] in Algerian strawberry tree genotypes. The pH values

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**Table 2**

| Genotype | Titratable Acidity (g malic acid/100 g fw) | pH | Total Soluble Solids (g/100 g fw) |
|----------|------------------------------------------|----|----------------------------------|
| A        | 1.01                                      | 3.0| 20.0                            |
| B        | 0.65                                      | 2.8| 18.0                            |
| C        | 0.83                                      | 3.1| 21.0                            |
| D        | 1.00                                      | 3.2| 22.0                            |
| E        | 0.50                                      | 3.4| 16.0                            |
Table 2. Physicochemical parameters of the strawberry tree fruit genotypes.

| Genotype name | TA (g malic acid/100 g fw) | pH | TSS (%) |
|---------------|---------------------------|----|---------|
| KSB           | 0.72 ± 0.02ab             | 2.44 ± 0.03a | 18.53 ± 0.50d |
| CHF           | 0.81 ± 0.01b              | 3.76 ± 0.01 c | 16.63 ± 0.40b |
| MDZ           | 0.65 ± 0.01a              | 3.71 ± 0.01 c | 16.83 ± 0.29bc |
| LAN           | 0.97 ± 0.01c              | 3.92 ± 0.02d | 14.83 ± 0.29a |
| TAH           | 1.01 ± 0.10c              | 2.99 ± 0.10b | 17.53 ± 0.45 c |
| Mean          | 0.83                      | 3.36 | 16.87   |
| Std. Deviation| 0.15                      | 0.58 | 1.30    |
| ANOVA         | 0.07***                   | 1.19*** | 5.56*** |
| Mean square   |                           |      |         |

*** denote significant difference at level 0.001; Data values are means ± SD; Values in bold represent, in each column, the minimum and the maximum for each variable; Different letters (a-d) in the columns represent statistically significant differences among genotypes according to Duncan’s multi-range test at p<0.05; TA: Titratable acidity; TSS: Total soluble solids

Organic acids and ascorbic acid profiles

The results obtained for organic acid content are presented in Table 3. Significant differences (P < 0.001) were observed among the genotypes. Four organic acids were identified for all the strawberry tree genotypes (Citric acid, Malic acid, Ascorbic acid, Succinic acid). Citric acid was determined to be the major organic acid in all genotypes, followed by malic acid. HPLC chromatograms of samples (LAN) are represented in Figure 1.

The citric acid content ranged from 1.74 to 5.32 g/100 g with an average of 3.17 g/100 g. The highest value was recorded in “LAN” (5.32 g/100 g) while the lowest value was observed in “KSB” (1.74 g/100 g). The results of citric acid content in this study were higher than those reported by [1,33] who recorded 0.03 g/100 g and 8.56 mg/100 g, respectively. However, [5] showed a total absence of citric acid. The malic acid content ranged from 1.53 to 2.87 g/100 g with an average of 2.19 g/100 g. The lowest value was observed in “KSB” (1.53 g/100 g), while the highest value was recorded in “TAH” (2.87 g/100 g). The results of the malic acid content in this study were higher than those reported by [1,33] who found 0.34 g/100 g and 282.3 mg/100 g values, respectively. Our results were also higher than those reported by [8] who found malic acid content [0.084 mg/100 g] in strawberry tree

Table 3. Composition of organic acids and ascorbic acid (g/100 g DW) of Arbutus unedo L. fruit genotypes.

| Genotype name | Citric acid | Malic acid | Ascorbic acid | Succinic acid |
|---------------|-------------|------------|---------------|---------------|
| CHF           | 32.24 ± 1.06 cd | 23.58 ± 0.84e | 7.05 ± 0.89 c | 4.85 ± 0.38ab |
| KSB           | 17.40 ± 3.16a | 15.27 ± 2.92a | 2.85 ± 0.76a | 5.98 ± 1.35b |
| MDZ           | 27.62 ± 1.04bc | 18.85 ± 0.78abc | 9.49 ± 0.66 f | 7.69 ± 0.56 c |
| LAN           | 53.23 ± 4.07e | 23.15 ± 1.50de | 6.80 ± 0.38 c | 46.60 ± 1.21 f |
| TAH           | 28.00 ± 1.49bc | 28.65 ± 1.24 f | 10.02 ± 0.16 f | 11.07 ± 0.19d |

Values in bold are minimum and maximum; Different letters (a-g) in the columns represent statistically significant differences between genotypes according to Duncan’s multi-range test at p<0.001
fruits var. ellipsoid from Turkey. However, the results obtained in this study were lower than those recorded by. They found (5.99 g/100 g) in strawberry tree fruits from Portugal. The ascorbic acid content ranged from 0.28 to 1.00 g/100 g with an average of 0.72 g/100 g. The highest value was recorded in “TAH” (1.00 g/100 g) while the lowest value was observed in “KSB” (0.28 g/100 g). The results of the ascorbic acid content in this study were higher than those recorded by other authors, and found ascorbic acid content ranged from 98 to 280 mg/100 g in Turkish strawberry tree fruits. Ascorbic acid values recorded in this study were also higher than those reported by, who recorded 346, 89, and 182mg/100 g, respectively. The succinic acid content ranged from 0.49 to 4.66 g/100 g with an average of 1.52 g/100 g. The highest value was recorded in “LAN” (4.66 g/100 g) while the lowest value was observed in “CHF” (0.49 g/100 g). In another study, recorded traces of succinic acid in Algerian strawberry tree fruits. Comparing our results with those of other authors, some organic acids were absent in our fruits, notably: oxalic, fumaric, lactic, suberic, and quinic acids. Fumaric (0.15 g/100 g), lactic (0.05 g/100 g), suberic (0.023 g/100 g), and quinic (7.35 g/100 g) acids were detected and quantified by, in Turkish strawberry tree fruits. In addition, recorded variable amounts of oxalic acid (0.05–0.15 g/100 g) and (0.09 g/100 g), respectively. The presence and composition of organic acids can be affected by various factors such as growing conditions, maturity, season, geographical origin, and soil type.

**Figure 1.** HPLC chromatogram of organic acid profile of strawberry tree samples (LAN).
**Phytochemical and functional composition**

The total phenol content of strawberry tree fruits is presented in Table 4. Significant differences \((p = .044)\) were observed among the genotypes studied. The total phenols ranged from 25.37 to 39.06 mg GAE/g DW, with an average of 30.98 mg/g dry wt. The highest value was recorded in “LAN” (39.06 mg/g DW) while the lowest value was observed in “KSB” (25.37 mg/g DW). The TP of strawberry tree fruits reported in this study is higher than those found by other authors.\(^{[33]}\) Previous studies indicated a wide variation in total phenolic content among \(A. \textit{unedo}\) genotypes, grown in diverse agro climatic conditions including Spain, Croatia and Turkey, which varied from 483 to 1973 mg GAE/100 g FW\(^{[5,20,41]}\) and from 14.74 to 7.025 mg GAE/g in Algerian strawberry tree cultivars. In other study,\(^{[42]}\) reported a TPC variation from 17.7 to 25.8 mg GAE/g. Also, several studies\(^{[5,41]}\) recorded TP values ranging from 483 to 627 mg GAE/100 g and from 951 to 1973 mg/100 g in Turkish and Spanish genotypes, respectively, while,\(^{[20]}\) reported an average of 590 mg/100 g in Croatian fruits. According to these results, and despite natural variations, TP content in fruits of strawberry tree grown in Moroccan fruits was always over 39.06 mg GAE/g DW indicating that it could be considered as an excellent source of polyphenols content which is of great importance in the light of the fact that modern diets are often lacking of bioactive compounds. The results of the total flavonoid content are presented in Table 4. A significant variation in total flavonoids was observed at \((p = .002)\) among genotypes. The total flavonoid content ranged from 3.30 to 7.07 mg GAE/g DW, with an average of 5.20 mg GAE/g DW. The highest flavonoid content was observed in “TAH” (7.07 mg/g DW) and the lowest value was observed in “KSB” (3.30 mg/g DW). These concentrations are higher than those recorded by,\(^{[43]}\) (0.23–0.28 mg EQ/g) and,\(^{[44]}\) (2.18–6.54 mg EC/g), and,\(^{[4]}\) (0.32 mg/100 g edible portion). The total anthocyanin content is presented in Table 4. A statistically significant variation at \((p = .024)\) was observed among the genotypes studied. The anthocyanin quantity ranged from 0.15 to 0.64 mg equivalent cya-3-glu/100 g DW with an overall mean of 0.34 mg equivalent cya-3-glu/100 g DW. The highest total anthocyanin content was observed in “MDZ” (0.64 cya-3-glu/100 g DW), while the lowest was obtained by “KSB” (0.15 cya-3-glu/100 g DW). These values were lower than the ones published by,\(^{[4]}\) (3.77 mg equivale).

**Antioxidant activities**

In this research, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging ability assays and \(\beta\)-carotene bleaching test were used to evaluate in vitro antioxidant activity of the extracts. The results are presented in Table 5. Significant differences \((p<0.001)\) were observed among the genotypes

| Genotype name | Total phenols (mg GAE/g dw) | Total flavonoids (mg RE/g dw) | Total anthocyanins (mg C-3-GE/100 g dw) |
|---------------|---------------------------|-------------------------------|--------------------------------------|
| KSB           | 25.37 ± 5.60a             | 3.30 ± 0.60a                  | 0.15 ± 0.09a                         |
| CHF           | 28.71 ± 7.34a             | 4.49 ± 0.87ab                 | 0.30 ± 0.14a                         |
| MDZ           | 34.72 ± 6.53ab            | 6.09 ± 0.88 cd                | 0.64 ± 0.20b                         |
| LAN           | 39.06 ± 2.44b             | 7.07 ± 0.67d                  | 0.43 ± 0.23ab                        |
| TAH           | 27.07 ± 0.96a             | 5.20                          | 0.34                                 |
| Mean          | 30.98                     | 5.20                          | 0.34                                 |
| Std. deviation| 6.88                      | 1.51                          | 0.23                                 |
| ANOVA         | 98.39*                    | 6.31**                        | 0.12*                                |
| Mean square   |                           |                               |                                      |

* denote significant of difference at level 0.05; ** denote significant of difference at level 0.01; Data values are means ± SD; Values in bold represent, in each column, the minimum and the maximum for each variable; Different letters (a-d) in the columns represent statistically significant differences among genotypes according to Duncan’s multi-range test at \(p<0.05\); \(\text{GAE}\): Gallic acid equivalent; \(\text{RE}\): Rutin equivalent; \(\text{C-3-GE}\): Cyanidin-3-glucoside equivalent.
studied. The average antioxidant activity values were 8.93, 7.82, and 5.58 mg/g DW as determined by DPPH, ABTS, and β-carotene assays, respectively. The extracts of strawberry tree fruits had strong antioxidant activity for the β-carotene assay. The antioxidant activity as determined by β-carotene assay ranged from 1.08 to 13 mg/g DW,\(^{45}\) and analyzed the bleaching activity of β-carotene. They found [0.185–0.317 mg/ml] in Turkish fruits. DPPH radical scavenging assay is usually applied to determine the capacity of antioxidants to scavenge free radicals and the scavenging potentials of the antioxidant extract was measured according to the degree of discoloration of the mixture. In the current work, the variation in antioxidant capacity between the juices was statistically significant. All cultivars showed scavenging effects against DPPH radicals ranging from 3.33 to 21.08 mg/g DW. Ben\(^{41}\) showed that the value of scavenging activity of strawberry tree grown in Tunisia was (0.32 mg/ml) and were ranged from 0.278 to 0.589 mg/ml. Cultivar grew up in Portugal.\(^{46}\)

The value of ABTS assay ranged from 2.25 to 19.58 mg ascorbic acid equivalent/g DW,\(^{47}\) analyzed the antioxidant capacity (ABTS) of Turkish strawberry tree fruits. They found values ranged between 17.51 and 30.06 μmol TE/g DW. In another study,\(^{41}\) analyzed the antioxidant capacity (ABTS) of Turkish strawberry tree fruits. They found values comprised between 18.07 and 33.41 μmol TE/g DW. This difference was most probably due to differences in the extraction method and solvent used. The different antioxidant levels observed in this study may reflect a relative difference in the ability of antioxidant compounds in extracts to reduce the free radical DPPH, ABTS, and oxidative bleaching of β-carotene in vitro systems. Total antioxidant capacity (TAC) determined by DPPH, ABTS, and β-carotene method on the fruits of five A. unedo genotypes is shown in Table 5. The results indicate that significant variations are evident for ABTS, DPPH and β-carotene among genotypes (\(p < .05\)). TAC values of genotypes varied from 16.30 to 29.83 indicating nearly two fold differences between genotypes that have the lowest and the highest TAC values (Table 5),\(^{47}\) found TAC among A. unedo genotypes between 17.51 and 30.06 μmol TE/g, respectively, indicating similarities with our study,\(^{41}\) also found high TAC values among A. unedo genotypes (18.07–33.40 μmol TE/g).

Antioxidant activity was widely studied on A. unedo fruits by using different antioxidant determining methods such as ABTS, TEAC, FRAP, DPPH, etc., and all studies indicated that A. unedo fruits had high antioxidant activity and antioxidant activity found to be genotype dependent. Moreover, the studies indicated that the type of extraction of phenols present in fruits of A. unedo also influenced the antioxidant activity.\(^{4,5,9,30,40,42,45,48}\) The results indicated that the antioxidant capacity of strawberry fruit may also be related to the structure and composition of polyphenols as reported by.\(^{49}\) In addition, several studies have shown that strawberry fruit was found to be a more powerful antioxidant plant than other fruits such as red and green grapes and apple juices,\(^{50}\) pomace,\(^{51}\) pomegranate,\(^{52}\) grape\(^{53}\) Qing\(^{54}\) and blueberry\(^{49}\) which can be explained by the higher composition of strawberry, pomegranate, grape, and apple in polyphenols.

### Table 5. Free radical scavenging activity (DPPH and ABTS) and β-Carotene (mean ± SD in mg AAE/g DW) of strawberry tree genotypes.

| Genotype name | DPPH | ABTS | β-CAROTENE |
|---------------|------|------|------------|
| KSB           | 5.75 ± 2.00ab | 4.83 ± 1.88ab | 3.50 ± 0.75ab |
| CHF           | 4.50 ± 2.41ab | 3.33 ± 1.13a | 2.83 ± 0.76a |
| MDZ           | 21.08 ± 5.55 c | 19.58 ± 4.49 c | 13.00 ± 4.34 c |
| LAN           | 3.33 ± 1.51a | 2.25 ± 0.96a | 1.08 ± 0.38a |
| TAH           | 10.00 ± 3.77b | 9.08 ± 3.01b | 7.50 ± 3.12b |
| Mean          | 8.93 | 7.82 | 5.58 |
| Std. deviation| 7.29 | 6.92 | 4.87 |
| ANOVA         | 157.43*** | 150.03*** | 68.12*** |

*** denote significant difference of level 0.001; Data values are means ± SD; Values in bold represent, in each column, the minimum and the maximum for each variable; Different letters (a-c) in the columns represent statistically significant differences among genotypes according to Duncan’s multi-range test at \(p<0.05\).
Identification and quantification of individual phenolic compounds

Data were analyzed using standard and sample solutions and identification of individual phenolic acids, flavonoids, tannins, and anthocyanins was greatly supported by retention times, UV and exact mass spectra data, as reported in Figure 2, Table 6. Phenolic acids can be classified into two main groups, the benzoic acid derivatives and the cinnamic acid derivatives. Protocatechuic acid, vanillic acid, gallic acid, and syringic acid belong to the benzoic acid group, while p-coumaric acid, ferulic acid, o-coumaric acid, and chlorogenic acid are cinnamic acid phenolics. According to this classification, a total of 17 phenolic compounds were identified in strawberry tree fruits. The results obtained are summarized in Table 7. Among the determined phenolic acids, gallic acid, catechin, chlorogenic acid, and ellagic acid were found to be the major phenolic acid (Table 6) while syringic acid, rutin, phloridzin, vanillic acid, protocatechual acid, p-coumaric acid, ferulic acid, and quercetin were the minor phenolic acids. There were statistical differences among genotypes for all major phenolic acids and some minor phenolic acids such as syringic acid, rutin, phloridzin and vanillic acid (p < .001). However, protocatechuie acid, p-coumaric acid, ferulic acid, and quercetin were found not significant among genotypes (Table 6). Significant variations in phenolic compounds were found at p < .001 among genotypes. Gallocatechol was present in dominant amounts in all genotypes with the exception

Figure 2. Retention time and wavelength of phenolic compounds at Lambda 280 nm, Lambda 320 nm, Lambda 360 nm and Lambda 520 nm.
of “CHF” and “MDZ” where the dominant compound was catechin. The concentration of galloca-
techol differed between genotypes. The highest level reported in “TAH” (65.31 mg/100 g DW) and the
lowest in “CHF” (16.15 mg/100 g DW). Catechin was found in higher amounts in the genotypes.
“CHF” had the highest concentration (49.36 mg/100 g DW) of catechin, and “LAN” had the lowest
concentration (22.09 mg/100 g DW). Protocatechuic acid was present in significantly higher amounts
in “TAH” (5.90 mg/100 g DW) and significantly lower amounts in “MDZ” (1.84 mg/100 g DW). Gallic
acid was present in significantly higher amounts in “TAH” (36.93 mg/100 g DW), the lowest amount
was recorded in “MDZ” (4.56 mg/100 g DW). Gallic acid derivatives were detected in all genotypes.
The highest amount was present in “TAH” (14.54 mg/100 g DW), and the lowest in “CHF” (4.98 mg/
100 g DW). The concentration of syringic acid differed significantly between genotypes, with the
highest level in “LAN” (7.94 mg/100 g DW) and the lowest in “CHF” (4.27 mg/100 g DW). Among the
phenolic acid group, chlorogenic acid was significantly higher in the genotypes. The highest level was
observed in “TAH” (27.42 mg/100 g DW), and the lowest in “CHF” (5.55 mg/100 g DW). Ellagic acid
was also noticed in all genotypes. The highest level was found in “TAH” (33.73 mg/100 g DW) and the
lowest in “CHF” (8.42 mg/100 g DW). Ellagic acid derivatives I and II were seen in all genotypes. The
highest levels were found in “TAH” (25.06 mg/100 g DW) and (21.39 mg/100 g DW) respectively;
however, the lowest levels were found in “LAN” (8.05 mg/100 g DW) and “CHF” (8.97 mg/100 g DW),
respectively. Other minor compounds such as quercetin-3-xyloside, quercetin-3-galactoside, quercet-
in-3-glucoside, rutin, cyanidine-3-glucoside, cyanidine-3-5-diglucoside, and cyanidine-3-arabinoside
were also identified. “KSB” had the highest amount of quercetin-3-xyloside (4.09 mg/100 g DW), while
“MDZ” had the lowest amount (1.43 mg/100 g DW). “KSB” recorded the highest amount of
quercetin-3-galactoside (3.46 mg/100 g DW), while “CHF” recorded the lowest amount (1.66 mg/
100 g DW). Quercetin-3-glucoside was significantly higher in the genotypes. The highest amount was
observed in “KSB” (2.89 mg/100 g DW), and the lowest in “CHF” (2.11 mg/100 g DW). Rutin
compound was present in lower amounts in all genotypes. “LAN” had the highest quantity of rutin
(1.26 mg/100 g DW) whereas the lowest amount recorded in “TAH” (0.90 mg/100 g DW). Similarly,
cyanidin-3-glucoside was spotted in all genotypes. “TAH” contained the highest amount (7.21 mg/
100 g DW) as the lowest was recorded in “KSB” (0.43 mg/100 g DW). Concerning the last two
compound which are cyanidine 3,5 diglucoside and cyanidine 3 arabinoside, they were identified
within only three genotypes (CHF, MDZ, and TAH). The lowest amounts of them recorded in “CHF”
(0.61 mg/100 g DW) and (0.36 mg/100 g DW), respectively, whereas the largest ones were observed in
“TAH” (3.30 mg/100 g DW) and (1.64 mg/100 g DW), respectively. Our results are consistent with

| Phenolic compounds      | Retention times (min) | Wavelength (nm) |
|-------------------------|-----------------------|-----------------|
| Gallic acid             | 7.25                  | 280             |
| Protocatechuic         | 9.18                  | 280             |
| Galloccatechin          | 10.39                 | 280             |
| Gallic acid derivative  | 13.35                 | 280             |
| Catechin                | 14.97                 | 280             |
| Cholrogentic acid      | 16.38                 | 280             |
| Syringic acid          | 16.81                 | 280             |
| Ellagic acid derivative | 19.67                 | 280             |
| Ellagic acid derivative | 21.22                 | 280             |
| Ellagic acid           | 23.33                 | 280             |
| Quercetin-3-xyloside   | 21.26                 | 360             |
| Rutin                  | 22.81                 | 360             |
| Quercetin-3-galactoside| 25.67                 | 360             |
| Quercetin-3-glucoside  | 26.01                 | 360             |
| Cyanidin-3,5-diglucoside| 14.10                | 520             |
| Cyanidin-3-glucoside   | 14.59                 | 520             |
| Cyanidin-3-arabinoside | 16.09                 | 520             |

Table 6. Retention time and Wavelength of phenolic compounds at Lambda 280 nm, Lambda 360 nm and Lambda 520 nm.
Table 7. Polyphenolic compounds of strawberry tree at genotype site (mean ± SD in mg/100 DW).

| Genotype name | GA    | PC   | GC   | GAD  | CA   | T    | CA   | SA | EADI |
|---------------|-------|------|------|------|------|------|------|-----|------|
| KSB           | 21.88 ± 0.01 c | 3.14 ± 0.01 c | 45.23 ± 0.05 c | 10.15 ± 0.01d | 33.60 ± 0.03 c | 14.50 ± 0.00d | 7.40 ± 0.01 c | 18.9 ± 0.01d |
| CHF           | 6.09 ± 0.00b | 2.57 ± 0.01b | 16.15 ± 0.03a | 4.98 ± 0.00a | 49.36 ± 0.01e | 5.55 ± 0.00a | 4.27 ± 0.00a | 13.32 ± 0.01b |
| MDZ           | 4.56 ± 0.02a | 1.84 ± 0.00a | 17.11 ± 0.07b | 7.36 ± 0.01 c | 38.98 ± 0.05d | 12.10 ± 0.01b | 6.17 ± 0.01b | 17.22 ± 0.05 c |
| LAN           | 35.83 ± 0.02d | 4.18 ± 0.03d | 58.79 ± 0.33d | 7.30 ± 0.01b | 22.09 ± 0.08a | 12.48 ± 0.02 c | 7.94 ± 0.02e | 8.05 ± 0.03a |
| TAH           | 36.93 ± 0.02e | 5.90 ± 0.01e | 65.31 ± 0.04** | 14.54 ± 0.02e | 24.68 ± 0.08b | 27.42 ± 0.02e | 7.80 ± 0.01d | 25.06 ± 0.04e |
| Mean          | 21.06 | 3.53 | 40.52 | 8.87 | 33.74 | 14.41 | 6.72 | 16.45 |
| Std. deviation | 14.40 | 1.46 | 21.27 | 3.39 | 10.24 | 7.42 | 1.42 | 5.85 |
| ANOVA         | 725.36*** | 7.49*** | 1584.06*** | 40.19*** | 327.11*** | 192.58*** | 7.06*** | 119.70*** |

Mean square

| Genotype name | EADII | EA   | C3G  | Q3X  | RT   | Q3G  | Q3G  | C3,5DG | C3A  |
|---------------|-------|------|------|------|------|------|------|--------|------|
| KSB           | 15.96 ± 0.01 c | 18.00 ± 0.00d | 0.43 ± 0.01a | 4.09 ± 0.01e | 1.06 ± 0.01c | 3.46 ± 0.02d | 2.89 ± 0.00d | n.d    | n.d  |
| CHF           | 8.97 ± 0.01a | 8.42 ± 0.01a | 2.27 ± 0.00c | 2.11 ± 0.01b | 1.17 ± 0.00d | 1.66 ± 0.00a | 2.11 ± 0.01a | 0.61 ± 0.00a | 0.36 ± 0.01a |
| MDZ           | 9.40 ± 0.04b | 14.34 ± 0.02c | 5.68 ± 0.01d | 1.43 ± 0.01a | 0.96 ± 0.00b | 3.02 ± 0.01c | 2.12 ± 0.01a | 1.59 ± 0.02b | 1.07 ± 0.00b |
| LAN           | 9.40 ± 0.10b | 10.27 ± 0.05b | 0.57 ± 0.02b | 2.72 ± 0.03c | 1.26 ± 0.01e | 3.03 ± 0.04c | 2.54 ± 0.02c | n.d    | n.d  |
| TAH           | 21.39 ± 0.02d | 33.73 ± 0.02e | 7.21 ± 0.02e | 2.81 ± 0.03d | 0.90 ± 0.02a | 2.73 ± 0.02b | 2.27 ± 0.01b | 3.30 ± 0.02 c | 1.64 ± 0.01 c |
| Mean          | 13.02 | 16.95 | 3.23 | 2.63 | 1.07 | 2.78 | 2.39 | 1.10   | 0.61 |
| Std. deviation | 5.10 | 9.34 | 2.84 | 0.91 | 0.14 | 0.63 | 0.30 | 1.29   | 0.67 |
| ANOVA         | 90.92*** | 305.06*** | 28.25*** | 2.91*** | 0.06*** | 1.38*** | 0.33*** | 5.82*** | 1.55*** |

*** denote significant difference at level 0.001; Data values are means ± SD; Values in bold represent, in each column, the minimum and the maximum for each variable; Different letters (a-e) in the columns represent statistically significant differences among genotypes according to Duncan’s multi-range test at p<0.05; GA: Gallic acid; PC: Protocatechuic; GC: Gallocatechin; GAD: Gallic acid derivative; CAT: Catechin; CA: Chlorogenic acid; SA: Syringic acid; EADI: Ellagic acid derivative I; EADII: Ellagic acid derivative II
those of Ganhão et al., (2010) who had found catechin, gallic acid, ellagic acid, chlorogenic acid, rutin and cyanidin-3-glucoside in strawberry tree fruits collected in Spain. However, 8, reported that gallic acid [10.7 mg/g DW] was the main phenolic compound in strawberry tree fruits collected in Turkey, followed by protocatechic acid, gentisic acid, p-hydroxybenzoic acid, vanillic acid, and m-anisic acid. Distinctively, [48] had identified other phenolic compounds in strawberry tree fruits collected in north-eastern Portugal. These compounds are gallic acid glucoside, galloylquinic acid, quinic acid derivative, proanthocyanidin dimer, galloylshikimic acid, digalloylshikimic acid, catechin monomer, proanthocyanidin trimer, strictinin ellagitannin, ellagittannin derivative, galloyl derivative, trigalloylshikimic acid, myricetin rhamnoside, quercetin glucoside, galloctannin and ellagic acid rhamnosome.

**Correlation among variables**

In order to identify the relations between biochemical traits, all variables were subjected to bivariate correlation using the Pearson coefficient. Significant correlations at the level of 0.05 or 0.01 are summarized in Table 8. In the current study, the correlation value was found between DPPH and total anthocyanins \((r = 0.931; p < .05)\). Similarly, links were noticed between ABTS and both anthocyanins \((r = 0.929; p < .05)\) and DPPH \((r = 1.000; p < .01)\). Also, AA (β-carotene) was correlated to anthocyanins \((r = 0.946; p < .05)\), DPPH \((r = 0.986; p < .01)\) and ABTS \((r = 0.989; p < .01)\). The correlation between ellagic acid and each of the following parameters: gallic acid derivative, chlorogenic acid, ellagic acid derivative I and ellagic acid derivative II were respectively 0.975; \(p < .01\), 0.968; \(p < .01\), 0.893; \(p < .05\) and 0.953; \(p < .05\). The results obtained showed also, positive correlations between cyanidin-3,5-diglucoside and cyanidin-3-glucoside \((r = 0.962; p < .01)\). In the same way, the study revealed links between cyanidin-3-arabinoside and both cyanidin-3-glucoside \((r = 0.994; p < .01)\) and cyanidin-3,5-diglucoside \((r = 0.986; p < .01)\). Correspondingly, it conveyed correlations between chlorogenic acid and gallic acid derivative \((r = 0.978; p < .01)\). As far as galloctecin is concerned, the study portrayed a relationship between it and gallic acid \((r = 0.992; p < .01)\) and protocatechuic \((r = 0.907; p < .05)\). However, citric acid revealed negative links with TSS \((r = -0.974; p < .01)\). Syringic acid showed negative correlations with catechin \((r = -0.961; p < .01)\). Likewise, ellagic acid derivative II conveyed positive connections with both gallic acid derivative \((r = 0.968; p < .01)\) and chlorogenic acid \((r = 0.909; p < .05)\). In the current study, the results of the anthocyanins were significantly correlated with the ABTS assay. However, no significant correlation was found between the total phenols content and ABTS assay. These results must be interpreted with caution as the Folin–Ciocalteu method used over estimates the concentration of phenolic containing compounds such as ascorbic acids and vitamins could interfere during TP evaluation and that do not give significant correlation. In addition,

| RT       | Q3GA | Q3G | C3,5D | C3A | C AC | M AC | A AC | S AC |
|----------|------|-----|-------|-----|------|------|------|------|
| RT       | 1    |     |       |     |      |      |      |      |
| Q3GA     | -0.220 | 1  |       |     |      |      |      |      |
| Q3G      | 0.237  | 0.705 | 1     |     |      |      |      |      |
| C3,5D    | -0.822 | -0.124 | -0.529 | 1  |      |      |      |      |
| C3A      | -0.849 | -0.119 | -0.603 | -0.986** | 1  |      |      |      |
| C AC     | 0.684  | 0.160  | -0.152 | -0.250 | -0.269 | 1  |      |      |
| M AC     | -0.112 | -0.519 | -0.539 | -0.653 | -0.574 | 0.410 | 1  |      |
| A AC     | -0.471 | -0.839 | 0.799  | 0.842  | 0.224  | 0.695 | 1  |      |
| S AC     | 0.627  | 0.241  | 0.228  | -0.326 | -0.367 | 0.907* | 0.217 | 0.001  | 1  |

* Present data as 0.509 instead of 0.509

** Correlation is significant at the 0.05 level; * Correlation is significant at the 0.01 level; TP: Total phenols; TF: Total flavonoids; ANT: Anthocyanins; BCRY: β-Carotene; TA: Titrable acidity; TSS: Total soluble solids; MC: Moisture content; GA: Gallic acid; PC: Protocatechuic acid; GC: Galloctecin; GAD: Gallic acid derivative; CAT: Catechin; CA: Chlorogenic acid; SA: Syringic acid; EADI: Ellagic acid derivative I; EADII: Ellagic acid derivative II; EA: Ellagic acid; C3G: Cyanidin-3-glucoside; Q3X: Quercetin-3-xylloside; RT: Rutin; Q3GA: Quercetin-3-galactoside; Q3G: Quercetin-3-glucoside; C3,5D: Cyanidin-3,5-diglucoside; C3A: Cyanidin-3-arabinoside.
the synergism between the antioxidants in the mixture makes the antioxidant capacity not only dependent on the concentration but also on the structure and the interaction between the antioxidants. However, different works have reported good linear correlations between antioxidant activity test and total phenols\textsuperscript{[1,55]} Su and Chien\textsuperscript{[56]}, Anastasiadi\textsuperscript{[57]}. The correlation coefficients may provide information on the parameters that are potentially important in assessing strawberry tree genotypes.\textsuperscript{[55]} Significant and strong correlated traits can be used to predict other ones, and could be considered of importance for genotype characterization and discrimination.\textsuperscript{[56]}

**Principal component analysis**

To achieve a better understanding of the trends and relationships among the many studied variables for the different strawberry samples (five clones), principal component analysis (PCA) based on correlation coefficients was used to discriminate between variables in the datasets. The aim of this analysis was to determine the main factors to reduce the number of effective parameters to use in classification of the strawberry tree genotypes based on their physicochemical and biochemical parameters. In our study, only a principal component loading of more than |0.5| was considered as being significant for each factor. It is noteworthy that for each variable only eigenvalue presenting significantly higher score was considered as being most correlated. Total variance was explained by four components of which the first three accounted for 90.25% (Table 9), which means that these characters had the highest variation between the genotypes and had the highest impact on discrimination of them.

| Table 9. Eigenvectors of principal component axes from PCA analysis of studied variables. |

| Component | 1  | 2  | 3  | 4  |
|-----------|----|----|----|----|
| Total phenols | -364 | ,138 | 784 | .483 |
| Total flavonoids | ,773 | ,216 | 596 | .006 |
| Anthocyanins | ,576 | ,772 | 188 | 195 |
| DPPH | ,504 | ,708 | -010 | 494 |
| ABTS | ,522 | ,695 | -019 | 494 |
| B-carotene | ,625 | ,673 | -048 | 393 |
| Titrable acidity | 263 | ,657 | 599 | 374 |
| pH | -283 | ,384 | 860 | 183 |
| Soluble solids | ,443 | ,015 | -894 | 068 |
| Gallic acid | ,999 | ,888 | 345 | 064 |
| Protocatechuic | 559 | ,726 | 323 | 237 |
| Gallic acid | 365 | 893 | 245 | 100 |
| Catechin | 846 | 512 | 141 | 053 |
| Chlorogenic acid | 881 | ,464 | 057 | 078 |
| Syringic acid | 352 | ,746 | 151 | 545 |
| Ellagic acid derivative | ,898 | ,011 | ,428 | -107 |
| Ellagic acid derivative | ,787 | ,525 | ,296 | -136 |
| Ellagic acid | 931 | 342 | ,110 | 059 |
| Cyanidin-3-glucoside | 891 | ,401 | 203 | 067 |
| Quercetin-3-Xyloside | -060 | ,835 | -546 | 024 |
| Rutin | -908 | ,259 | 303 | 131 |
| Quercetin-3-galactoside | 156 | 411 | ,237 | 866 |
| Quercetin-3-glucoside | -260 | ,752 | 477 | 373 |
| Cyanidin-3,5-diglucoside | -950 | 160 | 192 | 185 |
| Cyanidin-3-arabinoside | 926 | ,307 | 191 | 106 |
| citric acid | -402 | ,208 | 891 | 033 |
| Malic acid | ,467 | ,147 | 658 | 573 |
| Ascorbic acid | 617 | ,451 | 638 | ,039 |
| Succinic acid | -348 | ,496 | 734 | 036 |
| % of Variance | 41.47 | 32.04 | 16.74 | 9.75 |
| Cumulative % | 41.47 | 73.51 | 90.25 | 100.00 |

Eigenvalues higher than |0.5| are marked in bold.
The first component accounted for 41.47% of the total variance, which is strongly influenced by the total flavonoids (0.77), anthocyanins (0.58), DPPH (0.50), ABTS (0.52), AA β-carotene (0.62), protocatechuic (0.56), gallic acid derivative (0.85), chlorogenic acid (0.88), ellagic acid derivative I (0.90), ellagic acid derivative II (0.79), ellagic acid (0.93), cyanidin-3-glucoside (0.89), rutin (−0.91), cyanidin-3,5-diglucoside (0.95), cyanidin-3-arabinoside (0.93), and ascorbic acid (0.62). The second component accounted for 32.04% of the total variance and is mainly influenced by anthocyanins (−0.77), DPPH (−0.71), ABTS (−0.69), β-carotene (−0.67), titrable acidity (0.66), gallic acid (0.89), protocatechuic (0.73), gallocatechin (0.89), gallic acid derivative (0.51), catechin (−0.74), syringic acid (0.75), ellagic acid derivative II (0.52), quercetin-3-xylloside (0.83), and quercetin-3-glucoside (0.75). The third component represents 16.74% of the total variation which is defined essentially by total phenols (0.78), total flavonoids (0.60), titrable acidity (0.60), pH (0.86), total soluble solids (−0.89), quercetine-3-xylloside (−0.55), citric acid (0.89), malic acid (0.66), ascorbic acid (0.64), and succinic acid (0.73). Generally, these results were in accordance with those reported in previous strawberry tree biochemical studies.\cite{41,47} They have reported that the biochemical attributes are important in order to evaluate the variation in traits of strawberry tree genotypes. These parameters can be used as a useful tool for selecting genotypes for breeding programs or to recommend new cultivars with superior traits.

Scatter plot was prepared according to the first three principal components: PC1, PC2 and PC3, (respectively, 41.47%, 32.04%, and 16.74% of total variance) that discriminate between the genotypes according to their physicochemical and biochemical characteristics (Figure 3). Starting from negative to positive values of PC1, the distribution of genotypes indicated an increase in the moisture content, total soluble solids, and the most of phenolic compounds. Whereas starting from negative to positive values of PC2, total phenols, total flavonoids, and total anthocyanins decreased in their values. However, starting from negative to positive values of PC3, the distribution of genotypes indicated an increase in the pH, titrable acidity, and antioxidant activity (DPPH, ABTS, and β-carotene). Our results are in agreement with

![Figure 3](image-url)
several studies. [41,47] These studies indicated that high diversity in biochemical traits could be used as an efficient marker system to discriminate between strawberry tree genotypes. Generally, these results were in accordance with those reported in previous strawberry tree biochemical studies. [41,47] These studies indicated that high diversity in biochemical traits could be used as an efficient marker system to discriminate between strawberry tree genotypes. Furthermore, the selection of highly discriminant variables is important to optimize resources for a feasible biochemical assessment. This is especially important in a strawberry trees with hundreds of genotypes described worldwide in which many homonyms and synonyms may be detected.

Conclusion

In the light of the results obtained, the strawberry tree fruits can be considered an important source of polyphenols (25–39 mg GAE/g DW). Among the 17 phenolic compounds identified, gallocatechol and catechin were the main constituents. This study showed that strawberry tree fruits are strong radical scavengers and can be considered as good sources of natural antioxidants, the fact that may encourage many people to consume them as an alternative source of bioactive compounds. In view of its biochemical composition, the use of strawberry tree fruits in some food and medicinal products may be suggested. The cultivation and utilization of the species are also recommended. Attempts were made in the present work to determine the physicochemical and phytochemical compositions of strawberry tree (Arbutus unedo. L.), and also to evaluate its nutritional potential so that it can be used as a functional food supplement for treating various diseases and disorders. The findings of the present study revealed that the strawberry tree has high levels of bioactive potential. Hence, Arbutus unedo can be used as a safe, nutritious, and active food supplement. This study contributes not only to a better knowledge of these wild fruits but also to their valorization as functional food.

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