Phytochemical screening of *Psidium guajava* and *Carica papaya* leaves aqueous extracts cultivated in Greece and their potential as health boosters

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Abstract

**Aim:** The scope of the present study was to investigate the phytochemical profile of *Psidium guajava* and *Carica papaya* leaves aqueous extracts, from plants cultivated on Crete island in Greece.

**Methods:** Total phenolic content (TPC) in the aqueous extracts was determined spectrometrically using the Folin-Ciocalteu (F-C) assay. The identification and quantification of different phenolic compounds in the aqueous extracts were conducted using reversed-phase high-performance liquid chromatography (RP-HPLC) analysis. Different metals were also determined (K, Fe, Zn, Ca, Mg, Pb, and Cd) to investigate the potential health claims or hazards in the water extractable infusion using inductively coupled plasma mass spectrometry (ICP-MS) method.

**Results:** TPC in the aqueous extracts was found to be 28.0 g gallic acid equivalent (GAE)/kg dry leaves for *Psidium guajava* leaves aqueous extract and 15.0 g GAE/kg dry leaves for *Carica papaya* leaves aqueous extract. The dominant phenolic compounds in *Psidium guajava* leaves aqueous extract were myricetin (3,852 mg/kg dry sample) and rutin (670 mg/kg dry sample) while the dominant phenolic compounds in *Carica papaya* leaves aqueous extract were salicylic acid (338 mg/kg dry sample) and rutin (264 mg/kg dry sample). Different metals were also determined (K, Fe, Zn, Ca, Mg, Pb, and Cd) to investigate the potential health claims or hazards in the water extractable infusion, and it was found that no toxic metals were extracted whereas some nutritional benefits were achieved.

**Conclusions:** Results proved that *Psidium guajava* and *Carica papaya* can be provided a strong antioxidant activity and can be used as medicinal plants.
Keywords
Psidium guajava leaves, Carica papaya leaves, phytochemical profile, phenolic compounds, health claims, elemental content

Introduction
The use of plants as herbal medicines is widely known since ancient times [1]. Phytochemical constituents of plants are the basis for developing potential medicine or food supplements [2, 3]. The use of plants for the treatment or improvement of various diseases and the improvement of health has important advantages in general. Various diseases have been cured with the use of different plants in folk medicine, and nowadays pharmaceutical, cosmetics, and food industries have turned their attention to the use of medicinal plants and the utilization of their phytochemical properties [4]. Among plant organs, leaves are the greatest accumulators of bioactive compounds and for that reason are used by food, pharmaceutical, and cosmetics industries, to manufacture their products [1].

The Guava (Psidium guajava) tree is a plant that belongs to the Myrtaceae family, and exhibits various nutritional and medicinal properties. Guava tree is cultivated mainly in India, China, Thailand, Pakistan, Mexico, Indonesia, and Brazil. Different parts of Guava tree, e.g., fruits, leaves, roots, stem, and bark are used for treating diabetes, stomachache, diarrhea, and many other illnesses around the world [1]. Guava leaves are dark green in colour, oblong to oval in shape, and average 3–5 cm wide and 7–15 cm long. Guava leaves have been used to treat gastrointestinal and respiratory disorders as well as to boost platelets in patients suffering from dengue fever [5]. Guava leaves are also used for their anti-inflammatory, antispasmodic, cough sedative, anti-obesity, anti-hypertension, and antioxidant properties, mainly due to their content of phenolic compounds [6]. In addition, Guava leaves show antivirus, antibacterial, hepatoprotective, anticancer, and antitumor properties [7, 8].

The bioactive and therapeutic properties of Guava leaves are mainly due to the presence of phenolic compounds, such as gallic acid, ferulic acid, caffeic acid, quercetin, kaempferol, naringenin, rutin, catechins, and epicatechins [1, 9]. Studies have shown that catechin, epicatechin, and gallic acid have cholesterol-lowering activity, because they inhibit pancreatic cholesterol esterase, bind bile acids and decrease the solubility of cholesterol in micelles, resulting in delayed cholesterol absorption [10]. Kaempferol exhibits moderate cytostatic activity [11], naringenin improves endothelial function [12], and rutin shows anti-allergic and anti-inflammatory activity [13].

Papaya (Carica papaya) trees are herbaceous plants that belong to the Caricaceae family [14], and constitute the third most cultivated tropical crop around the world [15]. Although all parts of papaya tree have been studied, leaves and fruits are the main parts that are widely used for various purposes such as in medicines, foods, cosmetics, and pesticides [16]. The nutritional compounds which have been found in papaya leave extracts, include mainly macromolecules (proteins, lipids, carbohydrates), minerals (potassium, phosphorus, magnesium, iron, calcium), fibers, and vitamins (C, B1, B2, B3, beta-carotene) [17]. The phytochemical substances which have been reported in papaya leave extracts include phenolic compounds (gallic acid, protocatechuic acid, kaempferol, apigenin, catechin, deoxykaempferol, deoxyquercetin) [18], flavonoid glycosides, coumarins, quinones, cyanogenic glycosides, phenols and alkaloids [19–24]. Papaya leaves extract is used to treat the fevers caused by virus infections such as malaria, dengue, and chikungunya [17]. In addition, papaya leaves extract shows anti-diabetic, anticancer, antiviral, anti-inflammatory, antiplasmodial, and antitrichochramal activity [25–27]. Recently, it has been proved that plants with high content of flavonoids can be also used in the case of diabetes and hyperglycemia [28].

The aim of this study is to define the phytochemical profile of Psidium guajava and Carica papaya leaves extracts and their potential to constitute high-value products in terms of their health benefits. The phenolic profile of Psidium guajava and Carica papaya leaves extracts was determined by using high-performance liquid chromatography (HPLC) analysis, the total phenolic content (TPC) by using the Folin-Ciocalteu (F-C) method. The determination of metal content in the infusion was performed using an inductively coupled
plasma mass spectrometry (ICP-MS) instrumentation. To the best of our knowledge, it is for the first time that a study was focused on the nutritional potential of the infusion of *Psidium guajava* and *Carica papaya* based on the special terroir of the cultivated area.

**Materials and methods**

**Reagents, standards, and solvents**

For the HPLC system, water (Fisher Scientific, HPLC grade), methanol (Fisher Scientific, HPLC grade), and acetic acid were used. For the extraction of phenolic compounds, ethyl acetate (concentration 99%, Scharlab, analytical grade) was used. For the standard stock, solutions were used with the following phenolic compounds standards.

- Syringic acid (purity 95%), taxifolin (purity 99%), myricetin (purity 99%), galangin (purity 99%), chrysin (purity 99%), chlorogenic acid (purity 99%), kaempferol (purity 99%), naringenin (purity 99%), homogentisic acid (purity 95%) and rutin (purity 99%) were purchased from Extrasynthèse (Genay, France).
- p-hydroxybenzoic acid (purity 99%), salicylic acid (purity 98%), quercetin (purity 98%), gallic acid (purity 98%), p-coumaric acid (4-hydroxycinnamic acid; purity 98%) and ferulic acid (purity 98%) were purchased from Sigma-Aldrich® (Steinheim, Germany).
- Vanillin (purity 99%), caffeic acid (purity 99%), protocatechuic acid (purity 97%), and apigenin (4,5,7-trihydroxyflavone; purity 97%) were purchased from Alfa Aesar (Karlsruhe, Germany).
- Luteolin (purity 98%) was purchased from Santa Cruz (Dallas, USA).
- Biotechnologies, *trans*-cinnamic acid (purity 99%), 2-cis-4-*trans*-abscisic acid (purity 98%) was purchased from Merck (Hohenbrunn, Germany).
- Hesperidin (purity 86.6%) was purchased from LGC, Germany.

For metal determination, the following reagents were used throughout this work. K, Fe, Zn, Ca, Mg, Pb, and Cd in 1% HNO₃ were purchased from Merck, Germany, and diluted to appropriate concentrations with ultra-pure water (MilliQ® water, Millipore, Bedford, MA, USA) and stock solutions of In, Th, Sc (10 mg/L) were also used as internal standards.

The phenolic content was determined according to the F-C method and the results were expressed as g Gallic acid per 100 g of dry leaves [20].

**Sample collection**

Fresh leaves of *Psidium guajava* and *Carica papaya* leaves were collected from Crete, a Greek island. The cultivation area was close to the sea and soil had a high salinity and conductivity, pH higher than 7.5, and high calcium carbonate content. In general, *Psidium guajava* and *Carica papaya* are not well grown in these kinds of soil properties, and therefore the great success of the producer was that managed to develop the cultivation providing a special terroir.

**Sample preparation**

Leaves of both plants were washed in running water and kept for shade drying for 10 days. The dried leaves were pulverized into powder, and 1 g of the obtained powder was weighed accurately in an analytical balance, and subjected to infusion in 100 mL boiling water for 30 min. After the end of the extraction, the pulverized leaves were filtered and the aqueous extracts obtained were stored in a refrigerator for later use [29, 30].

The phenolic compounds were extracted from the aqueous extracts by ethyl acetate. Specifically, 5 mL of each aqueous extract was introduced into a centrifuge tube, and followed by three successive extractions with 5 mL of ethyl ethane (EtAc) each time. Centrifugation was performed between the successive extractions to achieve better separation. The organic layer from the three extractions was placed in test tubes and was dried with Na₂SO₄. The organic layer from each tube was then placed in 30 mL beakers and was dried under nitrogen gas until all solution was evaporated. Then 800 μL of methanol/water solution (50% methanol/50% water) is added to each beaker, and the beaker was stirred for 1 min. The sample was then taken from the beaker with the help of a syringe, filtered through 45 μm polytetrafluoroethylene (PTFE) filters (Whatman filters Z134260), and finally injected into the HPLC system for analysis.
For the elemental determination in the infusion, the following protocol was used: 0.2500 g of the powdered sample (leaves) was put in 30 mL of boiled water for about 5 min and, after filtering the sample, the final volume was adjusted to 50 mL with 1% [volume/volume (v/v)] nitric acid.

Finally, for TPC the F-C method was followed after the extraction of phenolic content by boiled-ultra-pure water [20].

**HPLC analysis**

Shimadzu LC-2030C Plus HPLC system was used for the determination of 23 phenolic compounds in the samples, equipped with a diode array detector (DAD), and the detection wavelengths were set from 230 nm to 360 nm. The phenolic compounds gallic acid, homogentisic acid, protocatechuic acid, p-hydroxybenzoic acid, chlorogenic acid, cis-caffeic acid, syringic acid, vanillin, p-coumaric acid, taxifolin, ferulic acid, salicylic acid, hesperidin, 2-cis-4-trans-abscisic acid, and naringenin were identified and quantified at 273 nm, while the phenolic compounds rutin, myricetin, quercetin, luteolin, kaempferol, apigenin, chrysin, and galangin were identified and quantified at 360 nm. A reverse phase column, Poroshell 120 EC-C18 (4.6 mm × 100 mm, 2.7 μm) was used at a temperature of 40°C. The flow rate of the gradient elution was set at 0.5 mL/min and remained constant throughout the analysis. The injection volume was 20 μL. The binary mobile phase consisted of a solution of 1.0% (v/v) acetic acid in deionized water, HPLC grade (solvent A), and methanol, HPLC grade (solvent B). Elution from the column was achieved with the following gradient: 0–40 min solvent B increased from 10% to 85%; 40–41 min solvent B decreased from 85% to 10% and then kept constant (isocratic elution with 10% solvent B) till 50 min, in order to achieve column equilibration.

For the identification of each peak, and the construction of calibration curve, 1,000 × 10–6 of stock solution of each individual standard was prepared, by dissolving the phenolic compounds standards in a solution of methanol/water (50% methanol/50% water). The identification of each phenolic compound was attained by injecting 20 μL of each stock solution in the HPLC system. By comparing the retention times each phenolic compound could be identified. Stock standard solutions were diluted into five different concentrations 1 mg/L, 4 mg/L, 8 mg/L, 12 mg/L, and 20 mg/L, and were used for the construction of calibration curve of each phenolic compound. Typical linear correlations of $R^2 \geq 0.993$ were obtained from the calibration curves of all phenolic compounds.

In order to examine the matrix effect, two different types of calibration curves were constructed per phenolic compound, correlating the peak areas of the absorption signals to each phenolic compound concentration in standard solutions and in spiked sample extracts. The matrix effect was found less than 10%. In addition, a $t$-test comparison of the slopes of both calibration curves was performed (confidence level 95%) and no significant difference was detected.

**ICP-MS analysis**

For K, Fe, Zn, Ca, Mg, Pb, and Cd determination, an Agilent 7,500 s ICP-mass spectrometer equipped with a Fassel torch, a peristaltic pump, and an autosampler was used. The ICP-MS operating conditions were as follows: radio-frequency (rf) power of 1,350 W; nebulizer argon flow, 1.15 L/min; number of replicates, ten. Between samples or standards, the sampling system was rinsed with 1% HNO₃ (v/v). The (a) $^{45}$Sc, (b) $^{115}$In, and (c) $^{232}$Th (50 μg/L) were used as internal standards for the determination of (a) K, Fe, Zn, Ca, Mg, (b) Cd, and (c) Pb, respectively.

For the validation of ICP-MS method, recovery tests were performed. The accuracy was checked by spiking subsamples at three different concentration levels of each analyte and following the whole procedure. The precision was also checked by calculating the (%) relative standard deviation (RSD) of six independent infusion preparations. The calculated recoveries [% recovery ± standard deviation (SD), $n = 6$] were found in the range of (95.3% ± 1.5%; K) to (101.0% ± 4.1%; $^{114}$Cd), and the RSD values ($n = 6$) was ranged from 1.3% (for Ca) to 10% (for Cd). The quantification was performed using internal standard method calibration curves in order to avoid ICP-MS signal drift and typical linear correlations of $R^2 \geq 0.998$ were obtained for all analytes determined (calculated by Microsoft Excel 365).
Results
Phenolic compound analysis
Using the aforementioned analysis procedure, the phenolic compounds present in *Psidium guajava* and *Carica papaya* leaves extracts were separated and quantified. The Figure S1 shows a typical chromatogram of *Psidium guajava* leaves aqueous extract at 273 nm. Quantification was done using external standards calibration method. The amounts of phenolic compounds detected in the samples are shown in Table 1. Results are expressed in mg/kg of dry sample. The most abundant phenolic acids in *Psidium guajava* leaves extract, were gallic acid (256.63 mg/kg), protocatechuic acid (78.41 mg/kg), *p*-coumaric acid (36.66 mg/kg), and *p*-hydroxybenzoic acid (256.63 mg/kg) while the most abundant flavonoids were myricetin (3,852.07 mg/kg), rutin (669.68 mg/kg), and quercetin (54.73 mg/kg). The most abundant phenolic acids in *Carica papaya* leaves extract, were salicylic acid (338.16 mg/kg), *cis*-caffeic acid (86.16 mg/kg), *p*-coumaric acid (50.30 mg/kg), and *p*-hydroxybenzoic acid (25.30 mg/kg) while the most abundant flavonoids were rutin (263.81 mg/kg), quercetin (51.47 mg/kg), and taxifolin (29.54 mg/kg). TPC in the aqueous extracts was found to be 28.0 g gallic acid equivalent (GAE)/kg for *Psidium guajava* dry leaves aqueous extract and 15.0 g GAE/kg for *Carica papaya* dry leaves aqueous extract.

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th><em>Psidium guajava</em> (mg/kg)</th>
<th><em>Carica papaya</em> (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>670 ± 15</td>
<td>264 ± 15</td>
</tr>
<tr>
<td>Myricetin</td>
<td>3852 ± 39</td>
<td>ND</td>
</tr>
<tr>
<td>Quercetin</td>
<td>54.73 ± 1.22</td>
<td>51.5 ± 1.3</td>
</tr>
<tr>
<td>Luteolin</td>
<td>3.29 ± 0.45</td>
<td>ND</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>3.32 ± 0.13</td>
<td>9.20 ± 0.89</td>
</tr>
<tr>
<td>Apigenin</td>
<td>4.02 ± 0.78</td>
<td>ND</td>
</tr>
<tr>
<td>Chrysin</td>
<td>7.81 ± 0.99</td>
<td>ND</td>
</tr>
<tr>
<td>Galangin</td>
<td>2.82 ± 0.12</td>
<td>ND</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>257 ± 12</td>
<td>18.6 ± 1.5</td>
</tr>
<tr>
<td>Homogentisic acid</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>78.4 ± 1.8</td>
<td>17.6 ± 1.2</td>
</tr>
<tr>
<td><em>p</em>-hydroxybenzoic acid</td>
<td>25.1 ± 1.3</td>
<td>25.3 ± 2.1</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>23.0 ± 1.8</td>
<td>ND</td>
</tr>
<tr>
<td><em>cis</em>-caffeic acid</td>
<td>4.42 ± 0.16</td>
<td>86.2 ± 2.5</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>16.8 ± 1.0</td>
<td>ND</td>
</tr>
<tr>
<td>Vanillin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>p</em>-coumaric acid</td>
<td>36.7 ± 1.8</td>
<td>50.3 ± 2.1</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>ND</td>
<td>29.5 ± 1.3</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>ND</td>
<td>20.4 ± 1.1</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>21.2 ± 1.6</td>
<td>338 ± 10</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>ND</td>
<td>20.3 ± 1.2</td>
</tr>
<tr>
<td>2-<em>cis</em>-4-<em>trans</em>-abscisic acid</td>
<td>ND</td>
<td>8.45 ± 0.12</td>
</tr>
<tr>
<td>Naringenin</td>
<td>ND</td>
<td>6.86 ± 0.21</td>
</tr>
</tbody>
</table>

ND: not detected

Papers about phenolic profile of *Psidium guajava* and *Carica papaya* leaves aqueous extracts are very scarce in the literature. A study focused on *Psidium guajava* leaves aqueous extracts reported that gallic acid content in these samples varies from 240 mg/kg to 260 mg/kg and these findings are in agreement with current study [31]. In addition, this study reported that was not detected quercetin in the examined aqueous extracts, while in the current study, the content of quercetin was 54.73 mg/kg. According to Alvarez et al. [32] study, the content of myricetin in *Psidium guajava* leaves extracts varies from 45 mg/kg to 247 mg/kg, while in the current study, the content of myricetin is 3,852.07 mg/kg. Canini et al. [20] study reported that the content of quercetin in *Carica papaya* leaves is 40 mg/kg and this value is in agreement with current study (51.47 mg/kg).
The findings of different studies vary mainly due to climatic conditions and soil composition in different areas. For this reason, it is important to investigate the phytochemical profile of the same plant species, cultivated in different areas.

The present study reveals that *Psidium guajava* leaves extract contains myricetin and rutin in high concentration. Myricetin shows antidiabetic, anticancer, analgesic, cardiovascular, immunomodulatory, and antihypertensive properties. Also, myricetin exhibits neuroprotective activity, revealing preclinical activities on Parkinson’s, Alzheimer’s, and Huntington’s diseases [33]. Furthermore, myricetin can be used as a food preservative, in foods containing fats and oils due to its ability to protect lipids against oxidation [34].

Rutin, also known as rutoside, shows a number of pharmacological activities. In the central nervous system, rutin exhibits neuroprotective effect on brain ischemia, promotes neural crest cell survival, possesses anticonvulsant activity, shows sedative and antidepressant activity. In addition, rutin shows analgesic and antiarthritic activities, anti-hypercholesterolemic and cardioprotective effects. In gastrointestinal system, rutin presents antiulcer activity, in the respiratory system shows antiasthmatic activity, in eyes antiarterial activity and in reproductive system improves sperm quality. Furthermore, rutin shows retinoprotective activity, protective effect on lung tissue, hepatoprotective activity, nephroprotective activity, and protective effect on blood vasculature [35].

Salicylic acid is a phytochemical that is present in plants, fruits, and vegetables. The most significant effect of salicylic acid is the inhibition of prostaglandin synthesis. Salicylic acid shows anti-inflammatory activity via suppression of transcription of genes for cyclooxygenase and antioxidative activity since it is an inhibitor of oxidative stress [36]. This phenolic acid consists of an aspirin ingredient, is responsible for the anti-inflammatory properties of aspirin, and may be the reason for the reduced risk of colorectal cancer observed in people who take aspirin [37]. In addition, salicylic acid is used to treat various skin disorders such as acne vulgaris, photodamage, melasma, freckles, and lentigines [38].

### Elemental content determination

The results for elemental determination in the infusion are provided in Table 2. Potassium, calcium, and magnesium contents in the infusion were higher in *Psidium guajava* than in *Carica papaya*, whereas zinc and iron were found to be higher in *Carica papaya*. In both samples, no lead or cadmium was detected. According to Regulation [European Commission (EC)] No 1169/2011, a beverage can be a source of a significant amount of minerals when it provides a percentage of 7.5% of the nutrient reference values per 100 mL. The reference values for potassium, iron, zinc, calcium, and magnesium are 2,000 mg, 14 mg, 10 mg, 800 mg, and 375 mg, respectively [39].

<table>
<thead>
<tr>
<th>Element</th>
<th><em>Psidium guajava</em> infusion</th>
<th><em>Carica papaya</em> infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.45 (% w/w) on dry weight or 72.5 mg/L in the infusion</td>
<td>1.5 (% w/w) on dry weight or 75.4 mg/L in the infusion</td>
</tr>
<tr>
<td>Fe</td>
<td>70.0 mg/kg on dry weight or 0.35 mg/L in the infusion</td>
<td>84.0 mg/kg on dry weight or 0.42 mg/L in the infusion</td>
</tr>
<tr>
<td>Zn</td>
<td>30.0 mg/kg on dry weight or 0.15 mg/L in the infusion</td>
<td>50.0 mg/kg on dry weight or 0.25 mg/L in the infusion</td>
</tr>
<tr>
<td>Ca</td>
<td>0.91 (% w/w) on dry weight or 45.3 mg/L in the infusion</td>
<td>0.65 (% w/w) on dry weight or 32.4 mg/L in the infusion</td>
</tr>
<tr>
<td>Mg</td>
<td>0.065 (% w/w) on dry weight or 3.25 mg/L in the infusion</td>
<td>0.043 (% w/w) on dry weight or 2.12 mg/L in the infusion</td>
</tr>
<tr>
<td>Cd</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pb</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

w/w: weight/weight; ND: not detected

According to the Commission Regulation [European Union (EU)] No 432/2012 of 16 May 2012, there is a list of allowed health claims that can be written on food labeling. According to these health claims, potassium contributes to the normal functioning of the nervous system, to normal muscle function, and to the maintenance of normal blood pressure [40]. Iron contributes to normal cognitive function, the normal energy-yielding metabolism, the normal formation of red blood cells and haemoglobin, the normal oxygen transport in the body, the normal function of the immune system, the reduction of tiredness and fatigue, and finally, has a role.
in the process of cell division. Zinc contributes to normal acid-base metabolism and carbohydrate metabolism, contributes to normal cognitive function, normal DNA synthesis, normal fertility, and reproduction, to the normal macronutrient, fatty acid, and vitamin A metabolism, to normal protein synthesis, maintenance of normal hair; nails, and skin, to the maintenance of normal testosterone levels in the blood, the maintenance of normal vision, the normal function of the immune system, the protection of cells from oxidative stress, and has a role in the process of cell division. Calcium contributes to normal blood clotting, normal energy-yielding metabolism, normal muscle function, neurotransmission, and normal function of digestive enzyme, and has a role in the process of cell division and specialization. Finally, calcium is needed for the maintenance of normal bones and teeth. Magnesium contributes to the reduction of tiredness and fatigue, to electrolyte balance, to the normal energy-yielding metabolism, normal functioning of the nervous system, and muscle function, to the normal protein synthesis and psychological function, to the maintenance of normal bones, and teeth, and finally, it has a role in the process of cell division.

According to the results achieved from the current work the consumption of 100 mL of the infusion provides for about 7.5 mg of potassium, 0.04 mg of iron, 0.02 mg of zinc, 4 mg of calcium, and 0.25 mg of magnesium, which corresponds to less than 1% of the recommended daily value. This means that the majority of the elements found in the *Psidium guajava* and in *Carica papaya* can cover only a small percentage of the recommended daily values of an adult daily diet. When compared with total element content as presented in study of Thomas et al. [41], it can be proved that the water-extracted elements are less than the total digested elements. For example, in their study Ca, K, Fe, and Mg were found equal to 1,660 mg, 1,602 mg, 13.50 mg, and 440 mg per 100 g of Guava leaf dry weight, respectively, when in the current study the water-extractable metal content was found equal to 910 mg, 1,450 mg, 7.0 mg, 65 mg per 100 g of Guava leaf dry weight, respectively. This means that, in general, Guava leaves have a significant source of elemental content but are not easily can be extracted in water infusions. Similar results are achieved for *Carica papaya* [41].

**Discussion**

This study managed to provide for the first time a detailed phytochemical profile of *Psidium guajava* and *Carica papaya* leaves aqueous extracts, cultivated in Greece, revealed their content in a large number of phenolic compounds as well as that the leaves aqueous extracts of both plants have important content in various phenolic acids and flavonoids, so they can be further checked for their medicinal properties *in vitro*. Leaves of both *Psidium guajava* and *Carica papaya* provided a high phenolic content and can have many medicinal uses. TPC in *Psidium guajava* leaves was found to be higher than in *Carica papaya* leaves. Various metals and metalloids were water extractable but their content in aqueous extracts was found to be less than 1% of the recommended daily value. The study has a strong future aspect since these plants are of great importance and since it is a new cultivation in Greece. A full project regarding cultivation process must be undertaken to investigate the possible enhancement of the health claims.

**Abbreviations**

F-C: Folin-Ciocalteu  
GAE: gallic acid equivalent  
HPLC: high-performance liquid chromatography  
ICP-MS: inductively coupled plasma mass spectrometry  
TPC: total phenolic content  
v/v: volume/volume

**Supplementary materials**

The supplementary material for this article is available at: [https://www.explorationpub.com/uploads/Article/file/10102_sup_1.pdf](https://www.explorationpub.com/uploads/Article/file/10102_sup_1.pdf).
Declarations

Author contributions
DDN, INP, and KGR: Formal analysis, Investigation, Writing—original draft, Writing—review & editing, Conceptualization, Validation, Methodology. GD: Project administration, Resources. CP: Project administration, Visualization.

Conflicts of interest
The authors declare that they have no conflicts of interest.

Ethical approval
Not applicable.

Consent to participate
Not applicable.

Consent to publication
Not applicable.

Availability of data and materials
Not applicable.

Funding
Not applicable.

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References


