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# Papaya Seed Extract and Recovery of Some Main Constituents

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**Abstract:** *Carica papaya* Linn. is one of the most common plants in almost all tropical countries. Its fruit is widely used as food or an ointment in traditional medicine. It is one of the few tropical fruits to contain glucotropaeolin (benzyl glucosinolate) detected in great quantity, mainly in the seeds of papaya. When cells in plant tissue are damaged, glucotropaeolin is hydrolysed by the enzyme myrosinase to benzyl isothiocyanate. The anticancer activity of this latter compound has been widely evidenced, but the metabolic profile of the papaya seed extracts is not reported in the literature. The objective of this study is to investigate the seed extracts of *C. papaya* L. by UHPLC-PDA ESI/MS, with and without the inactivation of myrosinase, and compare the recovery of some main components using two different inactivation procedures. The extracts (methanol/water, 60:40, *v*/*v*) were studied in negative and positive ionisation modes. Separations were carried out on an Acquity BEH C18 (50 × 2.1 mm i.d.) 1.7 µm analytical column, and 0.02% formic acid in water and acetonitrile was used as the mobile phase at a flow rate of 0.6 mL $min^{-1}$. Beyond the amino acids and glucotropaeolin already detected in papaya seeds, 4-hydroxybenzoic acid, which has never been detected in papaya seeds with this technique before, was identified. Moreover, mono-, di- and tri-glycosides of 4-hydroxybenzoic acid were putatively assigned. Glucotropaeolin, 4-hydroxybenzoic and tryptophan were quantified in seeds extracted after myrosinase deactivation. These three components were more effectively recovered using an oven than microwave myrosinase inactivation.

**Keywords:** *Carica papaya* Linn.; seed extracts; glucotropaeolin; 4-hydroxybenzoic acid; tryptophan



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## 1. Introduction

The fruit of *Carica papaya* Linn., simply known as papaya, is one of the most common fruits in almost all tropical American countries. It was discovered by the Spanish in the region between Southern Mexico and Northern Nicaragua and was widely spread to other countries [1]. Now, it is one of the most cultivated and consumed fruits in tropical and subtropical regions [2].

The interest in papaya constituents is focused not only on the presence of pro-vitamin A, ascorbic acid, potassium, folate and fibre but also flavonoids and phenolic acids in the pulp, which tend to decrease during ripening [3,4]. Nevertheless, not only the pulp at all different stages of maturation is exploited but also the other parts of the plant, such as the seeds, leaves, roots and peel of ripe and unripe fruit, which are used as food as well as ointment in traditional medicine for several diseases [5]. Constituents of *C. papaya* L. have been investigated in the entire plant, and the variation in phytochemicals by cultivar, location, agricultural practice, plant part, maturity and sunlight exposure has also been studied [4,6,7].

*C. papaya* L. is one of the few tropical fruit species to contain glucosinolates (GSLs, *O*-sulphated *S*-glucosyl thiohydroximates), a group of secondary metabolites mainly found

in the Cruciferae family, Brassicaceae [8]. Several other plant families, such as Capparaceae, Resedaceae, Caricaceae, Limnanthaceae and Tropaelaceae, have been found to possess GSLs [9]. GSLs are organic anions, which produce different decomposition products when plant cell tissues are damaged. In fact, GSLs present in vacuoles are hydrolysed by the enzyme myrosinase (thioglucoside glucohydrolase), and the hydrolysis products include substituted isothiocyanates (ITCs), thiocyanates, nitriles, epithionitriles and oxazolidinethiones, which vary depending on plant species, side-chain substitution, cell pH and cell iron concentration [10].

Plants usually possess four or five GSLs, but in *C. papaya* L., only glucotropaeolin (GTL, benzyl GSL) has been identified [9,11]. Benzylisothiocyanate (BITC) has been reported as its unique isothiocyanate degradation product [12]. GTL and BITC have been quantified in the peel, pulp and seeds of papaya during development and ripening [13].

Many GSL degradation products are of interest because of their biological activities [14]. Several of these products have biocidal activity against a wide variety of organisms, such as insects, plants, fungi and bacteria [9,15]. Others have human health benefits [16,17]. The GTL content determination and anticancer activity of BITC in *C. papaya* L. have been published [18–20].

When the papaya fruit is sliced, small black gelatinous seeds fill the centre of the fruit when ripe, which are whitish when unripe (Figure 1). The seeds are edible when dried or fermented, and they are used as a food ingredient. The seeds of papaya are a rich source of proteins, lipids and crude fibre. The seed oil is low in iodine value, free fatty acids and carotene [21,22]. This suggests that the seeds have important nutritional properties, and they can be useful in the treatment of diseases. Many health benefits, such as antibacterial, antifungal and anthelmintic activity, have been reported, while a loss of fertility was observed in animal models treated with papaya seeds [23–26]. They are rich in papain, a protease enzyme used in tenderising meat and for digestive action on nitrogenous substances [27].

**Figure 1.** Papaya fruit cut in two halves; the black seeds are visible.

Despite the numerous beneficial effects of papaya seeds, information about their chemical composition is still quite scarce [5]. Lipids, amino acids, organic acids, carbohydrates and phenolics, both in the leaves and the seeds of papaya, have been detected [7]. Since papaya seeds make up 22% of the waste product of the papaya fruit, a better knowledge of the composition of these seeds could help to evaluate the possible utilisation of the residues and encourage their use as food supplements or for other purposes [28].

As far as we are aware, there is no research on the metabolic profile of the seeds of *C. papaya* L. fruit using HPLC or UHPLC-PDA-ESI/MS. Although GTL was quantified by HPLC, the UHPLC-PDA-ESI/MS profile of seed extracts has never been studied before [19]. The aim of this paper is to describe the metabolic profile of the seeds of *C. papaya* L. extracts with and without the inactivation of myrosinase and gain insight into the recovery of some of the main metabolites by comparing two different inactivation procedures.

## 2. Materials and Methods

### *2.1. Chemicals and Reagents*

Hypergrade acetonitrile for LC-MS was purchased from Merck (Darmstadt, Germany) and HPLC grade methanol from Sigma-Aldrich. Water was prepared with a Direct-Q 3 (Millipore, Vimodrone, Italy) purification system. Formic acid (98%) was purchased from J. T. Baker. Leucine enkephalin, the reference compound, was from Sigma-Aldrich (Milano, Italy).

Tryptophan (Trp), tyrosine, leucine, phenylalanine, proline, valine, 4-hydroxybenzoic acid (4-HBA), 4-cumaric acid, caffeic acid, 5-caffeoylquinic acid, ferulic acid, isoferulic acid, shikimic acid, quinic acid, syringic acid, vanillic acid, quercetin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, quercetin 3-rutinoside, myricetin, myricetin 3-rhamnoside, kaempferol, luteolin 7-glucoside, avicularin, hesperidin, catechin, (-) epicatechin, (-) epigallocatechin, 1,3,6-trigalloylglucose, penta-O-galloyl-β-D-glucose and rhamnetin, were purchased from Sigma-Aldrich (Milano, Italy). Glucotropaeolin potassium salt (GTL) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). All chemicals were of reagent grade and used without further purification.

### *2.2. Sample Preparation*

Papaya fruits from Brazil were purchased from Italian markets. Fruits were sliced into halves, and their seeds were separated from the pulp and carefully wiped with paper to eliminate pulp residual. The fruits were selected at the same stage of ripening (completely mature) after being cut, based on the colour of the pulp (Figure 1). Seeds were stored at 4 °C until use.

To prepare seed extracts without myrosinase inactivation, 1 g of seeds (FW) was pounded in a ceramic pestle, and 5 mL of methanol/water (60:40, *v*/*v*) was added. Samples were vortex-mixed for 3 min and centrifuged for 3 min at 4000 g, and the supernatant was filtered before injection (0.22 µm). Samples were prepared in triplicate.

To inactivate myrosinase, the two procedures described in Sections 2.3 and 2.4 were carried out prior to crushing the seeds. The seeds were then processed like seeds without myrosinase inactivation with the addition of the same volume of methanol/water (60:40, *v*/*v*), vortex-mixed, centrifuged and filtered before injection. The samples were prepared in triplicate.

### *2.3. Microwave Drying*

To inactivate myrosinase, a procedure already described in the literature was applied with some modifications [12]. A total of 1 g of seeds (FW) was microwaved for 3 min at 600 W without water.

### 2.4. Oven Drying

A drying process was carried out based on the deactivation procedure already described [29]. A total of 1 g of seeds (FW) was placed in an oven at 75 °C for 30 min.

### 2.5. Calibration Standard Solutions

Standard stock solutions of Trp and 4-HBA were prepared separately in methanol, while the solution of GTL was prepared in water at the concentration of 1 mg $mL^{-1}$.

Working solutions of Trp and 4-HBA at concentrations of 0.5, 1, 2, 5, 10, 20, 50 and 100 μg $mL^{-1}$ and GTL working solutions at concentrations of 50, 100, 150, 200, 300 and 500 μg $mL^{-1}$ were prepared by diluting the stock solutions with methanol/water (60:40, *v*/*v*).

Peak integration was performed at λ = 254 nm. The calibration samples were prepared in triplicate and analysed in duplicate in two independent runs. The calibration curves were calculated with equal weighted least-squares linear regression analysis of the peak area against the standard nominal concentration. The content of metabolites is the mean of three samples.

### 2.6. UHPLC-PDA-ESI-TOF/MS Instrumentation and Conditions

A Waters Acquity UPLC ™ system (Milford, MA, USA), including a binary pumping system, an autosampler with thermostat control set at 10 °C, a high-temperature column heater set at 30 °C and a photodiode array (PDA) detector, was used for chromatographic analysis. The PDA scan was in the λ = 210–500 nm range, with a frequency of 20 Hz. Separations were carried out on a Waters Acquity BEH C18 (50 × 2.1 mm i.d.) 1.7 μm analytical column, and A = water/0.02% formic acid and B = acetonitrile were used as the mobile phase at a flow rate of 0.6 mL $min^{-1}$. The UPLC-PDA system was coupled with a high-resolution Waters Micromass LCT Premier XE time-of-flight (TOF) mass spectrometer (MS), and experiments were performed in negative and positive ionisation modes (nESI and pESI, respectively). The chromatographic and mass conditions have been previously described [30]. LC-MS analysis of intact GSLs is usually performed in nESI, and GSLs are detected as deprotonated molecules $[M\text{-}H]^-$. In this study, analyses were carried out in both nESI and pESI.

Data acquisition, instrument control and data handling (elemental composition, isotope modelling distribution and molecular calculator) were performed by MassLynx Software 4.1v. The injection volume was 2 μL.

## 3. Results

The UHPLC-PDA metabolic profiles obtained with and without myrosinase deactivation are depicted in Figure 2a,b, while the same profiles in UHPLC-MS (nESI) are depicted in Figure 3a and Figure 3b, respectively.

Metabolites and their corresponding retention time (RT, min), $\lambda_{max}$ (nm) and $m/z$ ratio of deprotonated $[M\text{-}H]^-$ and/or protonated molecules $[M+H]^+$ in nESI and pESI, respectively, are listed in Table 1. RTs, UV and MS spectra were used to assign (with analytical standards) or putatively assign (without analytical standards) the secondary metabolites present in the seed extracts.

### 3.1. Amino Acids

The following amino acids were identified according to the literature [5,7]: proline, RT = 0.23 min at $[M+H]^+$ = 116 $m/z$; valine, RT = 0.27 min at $[M+H]^+$ = 118 $m/z$; tyrosine, RT = 0.43 min at $[M+H]^+$ = 182 $m/z$; leucine, RT= 0.47 min at $[M+H]^+$ = 132 $m/z$; phenylalanine, RT= 1.01 min in both polarities at $[M\text{-}H]^-$ = 164 $m/z$ and $[M+H]^+$ = 166 $m/z$, respectively; and Trp RT = 1.61 min detected in both polarities, with $[M\text{-}H]^-$ = 203 $m/z$ and $[M+H]^+$ = 205 $m/z$ [4].

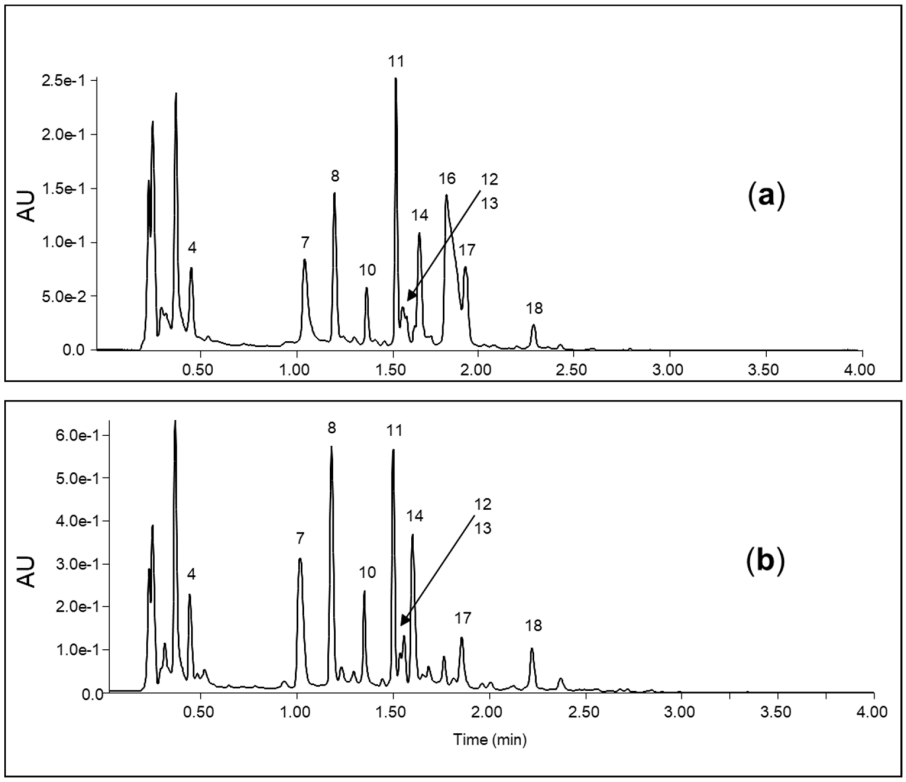


**Figure 2.** UHPLC-PDA base peak chromatograms of seed extracts (methanol/water, 60:40, *v*/*v*): (**a**) with myrosinase deactivation: GTL detectable (**16**); (**b**) without myrosinase deactivation: GTL not detectable; tyrosine (**4**); phenylalanine (**7**); 4-HBA glycosides (**8**) (**10**) (**11**); Trp (**14**); 4-HBA (**17**).

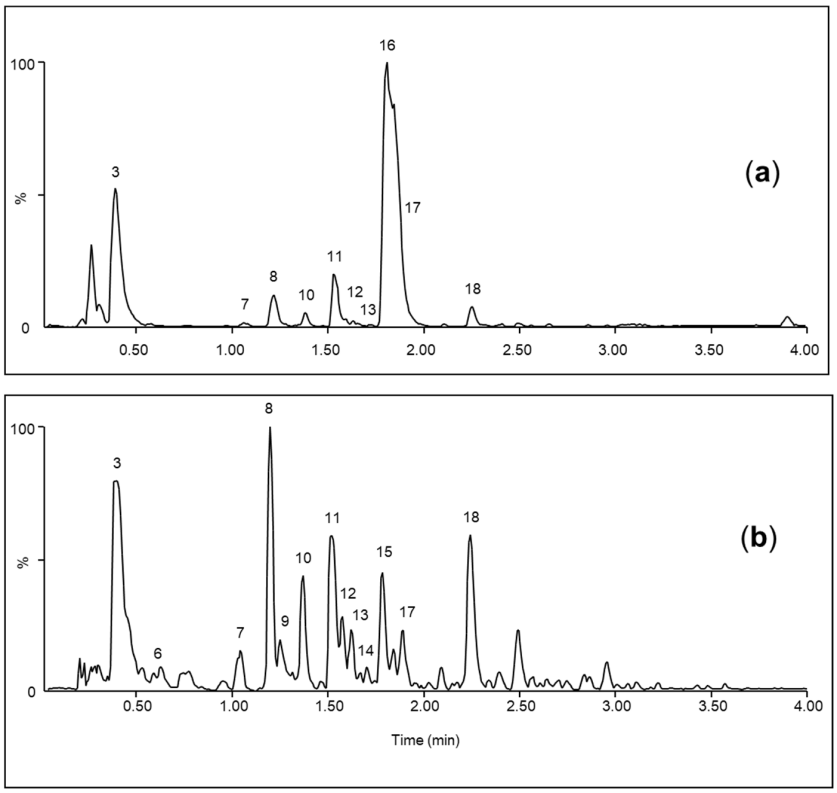


**Figure 3.** UHPLC-MS base peak chromatograms (nESI) of seed extracts (methanol/water, 60:40, *v*/*v*): (**a**) with myrosinase deactivation: GTL detectable (**16**); (**b**) without myrosinase deactivation: GTL not detectable; citric acid (**3**); phenylalanine (7); 4-HBA glycosides (**8**) (**10**) (**11**); Trp (**14**); 4-HBA (**17**).

**Table 1.** Retention time (RT, min), UV absorption wavelength ($\lambda_{max}$, nm), monoisotopic mass of deprotonated $[M-H]^-$ and protonated $[M+H]^+$ molecules ($m/z$) of compounds in nESI and pESI modes and chemical formulae.

| No. | RT (min) | $\lambda_{max}$ (nm) | $[M-H]^-$ *m/z* | $[M+H]^+$ *m/z* | Chemical Formula | Compound |
|---|---|---|---|---|---|---|
| **1** | 0.23 | | | 116.07 | $C_5H_9NO_2$ | Proline * |
| **2** | 0.27 | | | 118.09 | $C_5H_{11}NO_2$ | Valine * |
| **3** | 0.35 | | 191.02 | | $C_6H_8O_7$ | Citric acid * |
| **4** | 0.43 | 223/273 | | 182.08 | $C_9H_{11}NO_3$ | Tyrosine * |
| **5** | 0.47 | | 130.09 | 132.10 | $C_6H_{13}NO_2$ | Leucine * |
| **6** | 0.59 | | 292.14 | 294.15 | | Unknown |
| **7** | 1.01 | 257 | 164.07 | 166.09 | $C_9H_{11}NO_2$ | Phenylalanine * |
| **8** | 1.17 | 248 | 299.08 | 301.09 | $C_{13}H_{16}O_8$ | 4-hydroxybenzoic acid glycoside ** |
| **9** | 1.25 | | 326 | | | Unknown |
| **10** | 1.33 | 248 | 461.13 | $[M+Na]^+$ 485.13 | $C_{19}H_{26}O_{13}$ | 4-hydroxybenzoic acid diglycoside ** |
| **11** | 1.49 | 248 | 623.18 | $[M+Na]^+$ 647.18 | $C_{25}H_{36}O_{18}$ | 4-hydroxybenzoic acid triglycoside ** |
| **12** | 1.54 | 247/295 | 801.21 | | $C_{34}H_{42}O_{22}$ | Rhamnetin triglycoside 1 ** |
| **13** | 1.61 | 244/310 | 801.21 | | $C_{34}H_{42}O_{22}$ | Rhamnetin triglycoside 2 ** |
| **14** | 1.64 | 218/279 | 203.08 | 205.10 | $C_{11}H_{12}N_2O_2$ | Tryptophan * |
| **15** | 1.74 | | 313.09 | | | Unknown |
| **16** | 1.76 | 240 sh | 408.04 | $[M+H-SO_3]^+$ 330.10 | $C_{14}H_{19}NO_9S_2$ | Glucotropaeolin * |
| **17** | 1.85 | 254 | 137.02 | | $C_7H_6O_3$ | 4-hydroxybenzoic acid * |
| **18** | 2.20 | 230 | 236.06 | 238.07 | | Unknown |

* Assigned with chemical standard. ** Putatively assigned.

### 3.2. Organic Acids

Based on the analytical standards, citric acid (**3**) (RT = 0.35 min with $[M-H]^-$ at $m/z$ = 191) was identified (Figure 3), whereas the isobaric quinic acid observed by another author was not identified [7]. The peak **17** at RT= 1.85 min (Figure 2), with UV spectra at λ = 254 nm (Figure 4b) and $[M-H]^-$ at 137 $m/z$, was identified as 4-HBA.

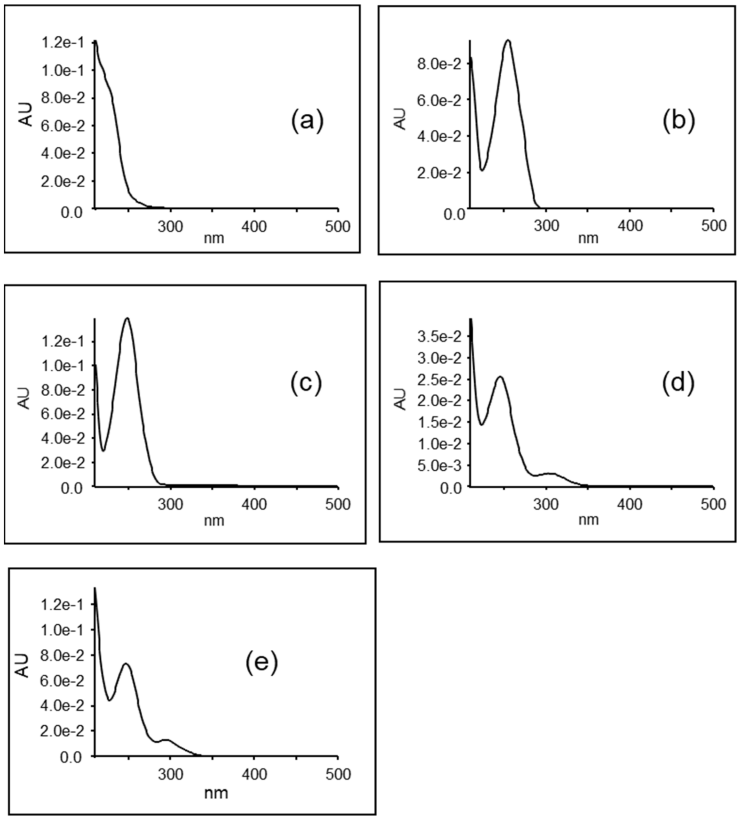


**Figure 4.** UV spectra of the main metabolites. (**a**) GTL (**16**); (**b**) 4-HBA (**17**); (**c**) 4-HBA glycosides (**8**, **10**, **11**); (**d**) putatively assigned isorhamnetin triglycoside (**12**); (**e**) putatively assigned isorhamnetin triglycoside (**13**).

### 3.3. Glucotropaeolin and Other Glycosides

GTL (**16**) was identified at RT = 1.76 min (Figure 2a) with the analytical standard and showed the deprotonated molecule $[M\text{-}H]^-$ = 408 $m/z$ in nESI and $[M+H\text{-}SO_3]^+$ = 330 $m/z$ in pESI. The UV spectrum is displayed in Figure 4a. As expected, the GTL peak is displayed only in the chromatograms of the seeds with myrosinase deactivation (Figures 2a and 3a), and it is absent in the chromatograms of seeds pounded without myrosinase deactivation.

Three main peaks in the PDA chromatogram at RT = 1.17 min (**8**), 1.33 min (**10**) and 1.49 min (**11**), respectively, show the same UV peak, with $\lambda_{max}$ = 248 nm (Figure 4c). The base peaks of **8**, **10** and **11** in the MS spectra are $[M+H]^+$ = 299, 461 and 623 $m/z$, respectively, with a Δm = 162 amu; they were putatively assigned as a glycoside, di-glycoside and tri-glycoside of 4-HBA.

In the MS spectra of **8**, beyond $[M\text{-}H]^-$ = 299 $m/z$, the formic adduct $[M+HCOO]^-$ = 345 $m/z$ and the dimer $[2M\text{-}H]^-$ = 599 $m/z$ peaks are evident, while in pESI, $[2M+H]^+$ = 601 $m/z$ is present. This putative assignment is supported by the MS/MS spectra, where the $m/z$ 137 fragment of 4-HBA is evident.

The peak **10** shows $[M+HCOO]^-$ = 507 $m/z$ and $[2M\text{-}H]^-$ = 923 $m/z$ beyond the base peak at $[M\text{-}H]^-$ = 461 $m/z$.

The peak **11** gives rise to MS peaks at $[M\text{-}H]^-$ = 623 $m/z$.

The elution order of these three glycosides is reversed compared to the elution order of glycosidic flavonoids, such as quercetin and myricetin, whose RT decreases as the number of glycosides in the molecule increases [30].

### 3.4. Flavonoids

To investigate the presence of flavonoids, the UV chromatogram was extracted at λ = 324 ± 4 nm. Two main peaks at RT= 1.53 and 1.55 min (**12** and **13**) are evident. The UV absorption spectrum of **12** displays two maxima at $\lambda_{max}$ = 247 and 295 nm (Figure 4d), while **13** displays two maxima at $\lambda_{max}$ = 244 and 310 nm (Figure 4e), characteristic of band I and band II of flavonoids [31]. The MS peaks at 801, 639, 477 and 315 $m/z$ correspond to both **12** and **13** in nESI, all with Δm = 162 amu; **12** and **13** have been putatively assigned to $[M\text{-}H]^-$ = 801 $m/z$ and the other peaks to $[M\text{-}H\text{-}glu]^-$, $[M\text{-}H\text{-}2glu]^-$ and $[M\text{-}H\text{-}3glu]^-$ as fragments of two isobaric flavonoid triglycosides. No other flavonoids or phenolics were found in the seeds analysed, with the exception of traces of cumaric acid. This difference with other studies may be due either to a different stage of ripeness of the fruit or a different extraction procedure.

### 3.5. Unknown Metabolites

In the PDA and MS chromatograms, three main unknown metabolites were detected. The first one (**6**) at RT = 0.59 min shows a base peak in the MS spectra, with $[M\text{-}H]^-$ = 292 $m/z$ and $[M+H]^+$ = 294 $m/z$; the second one (**15**) at RT = 1.74 min, shows base peaks in the MS spectra, with $[M\text{-}H]^-$ = 313 $m/z$ and $[M+Na]^+$ = 337 $m/z$; and the third one (**18**) was detected at RT = 2.20 min (**18**), with $[M\text{-}H]^-$ = 236 $m/z$ and $[M+Na]^+$ = 238 $m/z$ (Table 1).

### 3.6. UHPLC-PDA Quantification of Trp, GTL and 4-HBA

The metabolite content in the solution was calculated based on the calibration curves calculated with the analytical standard UV peak area (λ = 254 nm) versus the analytical standard nominal concentration (μg $mL^{-1}$). The linear equations were **Trp:** y = (96.2 ± 1.5) x + (153 ± 65) $r^2$ = 0.9994; **GTL:** y= (14.2 ± 0.1)x + (58.7 ± 29.2) $r^2$ = 0.9998; and **4-HBA:** y = (710 ± 8) + (1495 ± 289) $r^2$ = 0999. The metabolite content was then expressed in (μg $g^{-1}$) as the mean of three samples.

### 3.7. Recovery

The recovery of GTL, Trp and 4-HBA as the main metabolites identified was evaluated in methanol/water (60:40, $v/v$). The recovery was first evaluated in water, but despite the

inactivation of myrosinase, GTL was not stable in this solvent as it hydrolyses rapidly and could not be quantified. Conversely, in the methanol/water mixture, GTL was stable for at least two hours. The results of the extractions are shown in Table 2. The comparison of the two methods of myrosinase inactivation shows that the recovery of the three metabolites is lower by myrosinase inactivation in a microwave (about 80% for GTL and Trp and 76% for 4-HBA) than by drying in an oven at 75 °C for 30 min. The process is reproducible, as shown by the standard deviation of the three extractions. The amount of GTL recovered in the extracts with myrosine deactivation at 75 °C for 30 min was 308 ± 31 μg $mL^{-1}$, which is comparable, although lower than the amount of GTL reported in the literature [12,19]. The amount of Trp and 4-HBA was 85 ± 5 μg $mL^{-1}$ and 74 ± 19 μg $mL^{-1}$, respectively.

**Table 2.** Recovery of Trp, GTL and 4- HBA (μg $g^{-1}$) by extraction of papaya seeds with methanol/water (60:40, *v*/*v*) after myrosinase deactivation in oven at 75 °C for 30 min and microwave at 600 W for 3 min.

| Myrosinase Deactivation | Trp | GTL | 4-HBA |
|---|---|---|---|
| Microwave | 69 ± 1 | 245 ± 72 | 56 ± 13 |
| Oven | 85 ± 5 | 308 ± 31 | 74 ± 19 |

## 4. Discussion

Some amino acids identified (Table 1) are not visible in Figure 2a,b due to their lack of absorbance in the UV region. They are also not visible in Figure 3a,b because they are not sensitive to nESI. Phenylalanine (**7**) and Tyr (**14**) are visible in both Figures 2 and 3, while tyrosine (**4**) is only visible in Figure 2 for the aforementioned reason. The seed extract chromatogram in Figure 5a was compared to a solution of analytical standards (**7** and **14)** (Figure 5b), while in Figure 6a,b, the MS spectra of the same metabolites are displayed. Amino acids have been found in C. Papaya before [5,7].

In Figure 5b, 4-HBA (**17**) was identified by comparing the chromatogram of seed extracts to a solution of 4-HBA analytical standard. The RTs (Figure 5b), UV and MS spectra (Figures 4b and 6c) matched [32]. Interestingly, as far as we are aware, this metabolite has never been reported in papaya seeds before with this technique.

Liquid chromatography is the official technique for analysing GSLs after the enzymatic desulphation of samples [33]. More recently, analyses of GSLs have been performed by LC-MS and LC-MS/MS either with or without the usual pre-treatment of samples with sulphatase solution [34–37]. In this study, no sulphatase pre-treatment was performed. At all stages of ripening, papaya seeds contain GTL, which is hydrolysed and transformed in BITC if myrosinase is not deactivated [12,19]. Therefore, to observe GTL in the extracts of the seeds of C. papaya, it was necessary to preventively deactivate myrosinase. GTL (**17**) can be seen only in chromatograms where myrosinase was inactivated (Figures 2a and 3b). The chromatograms of seed extracts and the solution of GTL analytical standard (Figure 5a,c) and UV and MS spectra (Figures 4a and 6b) matched.

The two inactivation procedures already described in the literature were compared. The procedure described in Section 2.3 was previously applied to papaya seeds [12], while the procedure described in Section 2.4 was previously applied to mustard seeds [29]. The comparison revealed that the process of oven deactivation permitted a greater recovery of the three metabolites under investigation.

Figure 2 shows that the relative abundance of **8**, **10** and **11** changes before and after myrosinase deactivation. After deactivation, **8** is more abundant than **11**. This suggests that the heat during deactivation increases the monoglycoside content and decreases the triglycoside content.

Phenolic compounds were observed in the flesh of "Maradol" papaya fruit, while other authors observed phenolics in greater concentration in leaves than in seeds [4,7]. The presence of isorhamnetin has been previously reported in papaya flesh, but in the

extracts analysed, this flavonoid was not detected. Nevertheless, based on the molecular ions and UV spectra (Figure 4e), the two peaks were putatively assigned as two isomers of isorhamnetin triglycoside [4].

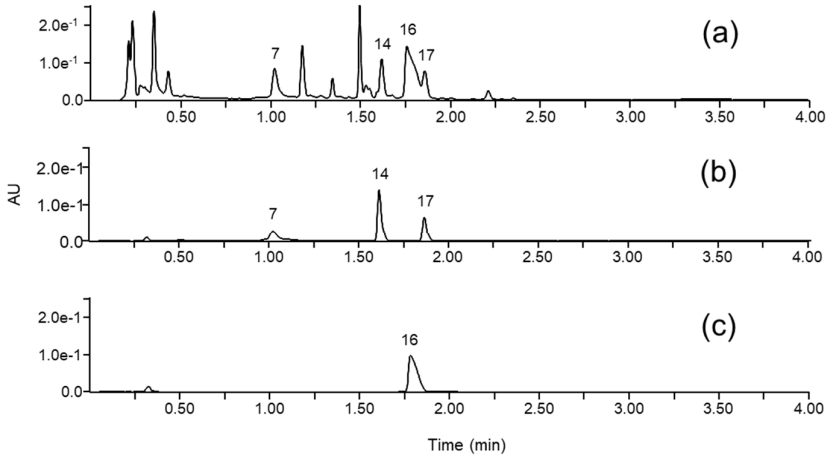


**Figure 5.** UHPLC-PDA base peak chromatograms of (**a**) seed extracts with myrosinase deactivation; (**b**) analytical standard solution of phenylalanine (**7**), Tyr (**14**), (**16**) and 4-HBA (**17**) at 10 μg $mL^{-1}$; (**c**) GTL analytical standard solution (**16**) at 100 μg $mL^{-1}$.

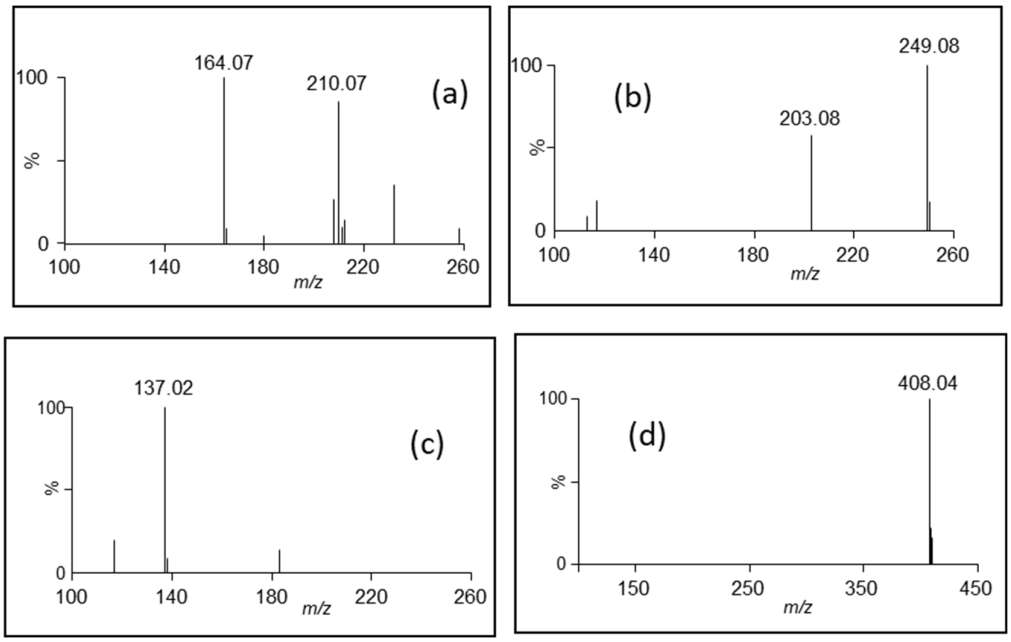


**Figure 6.** MS spectra of metabolites: (**a**): phenylalanine (**7**); (**b**): Trp (**14**); (**c**): 4-HBA (**17**); (**d**) GTL (**16**). In (**a**–**c**), the molecular ion and the formic adduct [M+HCOO]- are visible.

It is estimated that global papaya production is approximately 13 million tons, with India accounting for 40% of this output. The remaining major producers in the Americas are Brazil, the Dominican Republic, Mexico and Colombia. It is noteworthy that approximately 30% of the weight of this fruit is discarded, comprising seeds and peel [38]. The recovery of antioxidants from papaya has already been studied [3–5]. Given the high levels of GTL found in seeds, it may be interesting to recover this compound as a BITC precursor from papaya seeds. This could be a promising opportunity to obtain high-value bioactive compounds from agro-industrial residues, especially given the potential for BITC recovery.

In fact, BITC, in conjunction with sulforaphane and allyl isothiocyanate, represents one of the most extensively researched naturally occurring ITCs. The high activity of

BITC against chemical- and inflammation-related carcinogenesis has been demonstrated in numerous studies [39,40]. In particular, there has been a notable increase in research interest in the anticancer activity of BITC in recent years, as evidenced by a significant number of publications in this field. A recent review demonstrated that the anticancer activity of BITC was investigated in 14 distinct types of cancer, with over a quarter (27%) of the research examining its efficacy against breast cancer, pancreatic cancer (19%) and lung cancer (8%). Colon and urinary cancers (both 7%) were the next most frequently studied, followed by brain, liver, head and neck cancers [41–46]. In most of these preclinical studies, BITC was used at concentrations below 10 μM. In some cases, it was used at higher concentrations (up to 80 μM).

The available evidence from in vitro and in vivo studies (on mice by gavage) indicates that BITC can effectively modulate a number of signalling pathways, including those involved in apoptosis, cell proliferation, cell cycle arrest, metastasis, angiogenesis and autophagy. Furthermore, the use of BITC in combination with commercially available drugs has also been shown to have a beneficial effect.

Seeds are a rich source for future experiments. This is based on the concentration of BITC used for preclinical experiments and the recovery of GTL from papaya seeds.

Despite promising preclinical data, BITC has yet to be tested clinically for its anticancer effect. The evidence of its efficacy suggests that BITC could be a promising anticancer agent, either alone or in combination with other chemotherapeutics and radiotherapy. In the absence of further data to translate the efficacy of BITC, further research is required to determine the optimal therapeutic dose of BITC as a dietary or therapeutic supplement [41].

## 5. Conclusions

In this study, the metabolic profile of methanol/water extracts of the seeds of *C. papaya* Linn. was investigated by UHPLC-PDA or UPHLC-ESI/MS with and without the inactivation of myrosinase.

In addition to some of the amino acids previously observed, other new compounds were also found. 4-HBA and mono-, di- and tri-glycosides of the same acid were observed. GTL was also identified. As this metabolite can only be recovered through the deactivation of myrosinase, two deactivation procedures were employed, and the recovery of the major metabolites (GTL, 4-HBA and Trp) with the two techniques was compared. The recovery was higher in the extracts obtained after myrosinase deactivation in an oven at 75 °C for 30 min than in a microwave at 600 W for 3 min for all metabolites. (The microwave recovery was approximately 80% for GTL and Trp, and 76% for 4-HBA.)

GTL is the precursor of BITC, which has been attributed with several health benefits, including recently, broad-spectrum and stable bacteriostatic activity [20]. However, like all isothiocyanates, BITC can react with nucleophiles, such as alcohols, due to its electrophilicity, and organic solvents, such as hexane, are required for its recovery [47]. On the other hand, GTL can be recovered with a lower percentage of organic solvents. Considering GTL can be hydrolysed in the intestinal tract by microflora with myrosinase-like activity, the recovery of GTL from papaya seeds may be more convenient than the recovery of BITC [48]. Simple treatment with oven or microwave-assisted extraction, followed by solvent extraction, seems a promising technique for the recovery of BITC.

In conclusion, these extracts have the potential for application in the pharmaceutical industry as well as in the nutraceutical and food sectors [49], either with myrosinase deactivation, which makes it possible to recover GTL as a BITC precursor or without myrosinase deactivation, which makes it possible to recover BITC.

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