**Identification of bioactive compounds in Carica papaya peel**

**Table 1: Table: Retention time (tR), UV-visible absorption maxima and mass spectral characteristics of bioactive compounds identified in extracts from Carica papaya peel.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Peak number* | *tR (min)* | *λmax (nm)* | *[M-H]-* | *MS/MS fragments* | *Molecular formular* | *Proposed compound* |
| Organic acids |  |  |  |  |  |  |
| 1 | 2.96 | 280 | 191 | 111(91) | C6H8O7 | Citric acid |
| 2 | 1.84 | 245 | 133 | 115(70), 89(45) | C4H6O5 | Malic acid |
| 3 | 2.35 | 243 | 175 | 115(48), 87 (15) | C6H8O6 | Ascorbic acid |
| 4 | 1.54 | 249 | 195 | 179 (5) | C6H12O7 | Gluconic acid |
| Hydroxycinnamic acid derivatives |  |  |  |  |  |  |
| 5 | 16.79 | 265, 317 | 163 | 119(29), 59(23) | C9H8O6 | ρ-coumaric acid |
| 6 | 18.22 | 323 | 193 | 149(29), 134(62) | C10H1004 | Ferulic acid |
| 7 | 15.33 | 282, 312, 338 | 341 | 179(2), 85(29) | C15H18O9 | Caffeic acid glucoside |
| Hydroxybenzoic acid derivatives |  |  |  |  |  |  |
| 8 | 8.70 | 280 | 167 | 108(11), 123(12), 152(29) | C8H8O4 | Vanillic acid |
| 9 | 18.22 | 323 | 223 | 164(29), 208(6) | C11H12O5 | Sinapic acid |
| 10 | 8.16 | 280, 312 | 315 | 153 (13), 109(5) | C13H16O9 | Protocatechuic acid-4-glucoside |
| 11 | 14.42 | 327 | 295 | 133 (100), 179 (33) | C13H11O8 | Acetyl salicylate derivative |
| 12 | 16.84 | 265, 288 | 311 | 59(56) | C15H20O7 | 2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate |
| 13 | 11.05 | 274, 282, 289 | 299 | 254 (100), 137(95) | C13H16O8 | Salicylic acid β-D-glucoside |
| 14 | 13.58 | 326 | 401 | 269(99), 161(35), 101(21) | C18H26O10 | Benzyl alcohol hexoside-pentoside |
| Flavonoid derivatives |  |  |  |  |  |  |
| 15 | 17.41 | 352 | 609 | 301(8),300(8) | C27H30O16 | Rutin |
| 16 | 15.70 | 254, 352 | 755 | 301(1), 609(1) | C33H40O20 | Quercetin-3-O-rhamnosyl rutinoside |
| 17 | 17.51 | 287, 289, 302, 351 | 769 | 314 (2), 315 (2) 605(2), 623 (1) | C34H42O20 | Isorhamnetin-3-O-dirhamnosyl glucoside |
| Glucosinolate |  |  |  |  |  |  |
| 18 | 9.17 | 302 | 408 | 97(36), 96(30) | C14H18NO9S2 | Benzyl glucosinolate |

**Organic acids identified**

The compound labelled at peak 1 with a retention time of 2.96 min, molecular ion at m/z 191 and UV-vis absorption wavelength of 280 nm (Table 1) was identified as citric acid. Fragmentation of the acid showed a main product ion with m/z 111 which corresponded to the loss of both a CO2 unit (-44 amu) and 2H2O- groups (-36 amu) (Al Kadhi et al., 2017). The fragmentation pattern is illustrated in Figure 1 and its mass spectrum is shown in Appendix A: A2.

The compound labelled as peak 2 with a retention time of 1.84 min, UV-vis absorption wavelength of 245nm and an [M-H]- ion at m/z 133 was tentatively identified as malic acid (Table 1). The compound produced two main ionic fragments at m/z 115 [due to loss of a water molecule (H2O; -18amu)]and m/z 89 [ due to loss of a carbondioxide (CO2; -44 amu)] (Al Kadhi et al., 2017). The fragmentation patterns are illustrated in Figure 1 and its mass spectrum shown in Appendix A: A3.

The compound labelled as peak 3 with retention time 2.35 min, a UV-vis absorption wavelength of 243nm and molecular ion at m/z 175 was identified as ascorbic acid (Table 1). The main fragment ion produced by the compound was shown at m/z 115, corresponding to the loss of an H2OC=CH2O unit (-60 amu) (Boonpangrak et al., 2016) as illustrated in Figure 1. Its mass spectrum is shown in Appendix A: A4.

The compound labelled as peak 4 (tR = 1.54, λmax = 249) with molecular ion at m/z 195 (Table 1) was tentatively identified as gluconic acid. Fragmentation produced an ion at m/z 179 due to loss of an oxygen atom (-16 amu) as shown in Figure 1. Its mass spectrum is shown in Appendix A: A5.

 

**89**

**115**

**115**

**Malic acid (m/z 133) Ascorbic acid (m/z 175)**

 

**45 amu**

**179**

**111 m/z (HCOO-, 2H2O)**

**Gluconic acid (m/z 195) Citric acid (m/z 191)**

**Figure 1: Proposed fragmentation patterns for organic acids and their derivatives identified in Carica papaya peel.**

**Phenolic acids and their derivatives identified**

The compound labelled as peak 5 with retention time 16.79 min, molecular ion at m/z 163 and maximum UV-vis absorption wavelengths at 265 and 317 nm (Table 1) was identified as ρ-coumaric acid. It produced a main fragment at m/z 119 [ due to loss of a carbondioxide (CO2; -44 amu)] (Ibrahim et al., 2015). The fragmentation pattern is illustrated in Figure 2 and its mass spectrum is shown in Appendix A: A6.

The compound labelled as peak 6 with retention time 18.22 min, molecular ion at m/z 193 and maximum UV-vis absorption wavelength 323 (Table 1) was tentatively identified as ferulic acid. Fragmentation produced ions at m/z 149 corresponding to the loss of a carbondioxide molecule (-44 amu) (Sinosaki et al., 2020), m/z 134 which was due to the loss of a CH3COO ̵ (-59 amu) (Xiang et al., 2019). The fragmentation pattern is illustrated in figure 2 and its mass spectrum shown in Appendix A: A7.

The compound labelled as peak 7 with retention time 15.33 min, molecular ion at m/z 341 and maximum UV-vis absorption wavelengths of 282, 312 and 338 (Table 1) was identified as a caffeic acid glucoside. The compound produced fragment ions at m/z 179 which corresponded to loss of a glucose (C6H11O6 ̵) unit (Li et al., 2016) and m/z 85 which was due to loss of a CH3CH=CHCOO ̵ unit as illustrated in Figure 2. The mass spectrum is shown in Appendix A: A8

The compound labelled as peak 8 (tR = 8.70, λmax = 280) with molecular ion at m/z 167 (Table 1) was tentatively identified as vanillic acid. Fragmentation produced ions at m/z 152 [due to loss of a CH3 unit (-15 amu)], m/z 123 [ due to the loss of a carbondioxide unit (-44 amu)] (Li et al., 2016) and m/z 108 due to loss of a CH3COO ̵ unit (-59 amu) (Singh et al., 2019) as shown in Figure 2. Its mass spectrum is shown in Appendix A: A9.

The compound labelled as peak 9 (tR = 18.22, λmax = 323) with molecular ion at m/z 223 (Table 1) was tentatively identified as sinapic acid. Fragmentation produced ions at m/z 164 [due to loss of a CH3COO ̵ unit (-59 amu)] (Lee et al., 2018) and m/z 208 due to loss of a CH3 unit (-15 amu) (Oszmiański, Kolniak-Ostek & Wojdyło., 2013) as shown in Figure 2. Its mass spectrum is shown in Appendix A: A10

The compound labelled as peak 10 (tR = 8.16, λmax = 280 and 312) with molecular ion at m/z 315 were tentatively identified as protocatechuic acid 4-glucoside. Fragmentation produced ions at m/z 153 which corresponded to a protocatechuic acid (due to loss of the glucose unit) and m/z 109 (due to loss of a glucose and carbondioxide) (Li et al., 2016) as shown in figure 2. The mass spectrum of the compound is shown in Appendix A: A11

The compound labelled as peak 11 (tR = 14.42, λmax = 327) with molecular ion at m/z 295 (Table 1) was tentatively identified as a derivative of acetyl salicylate. Fragmentation produced a main ion at m/z 133 (C4H5O5 ̵ unit) and at m/z 179 (acetyl salicylate; C9H7O4̵) as shown in Figure 2. Its mass spectrum is shown in Appendix A: A12

The compound labelled as peak 12 (tR = 16.84, λmax = 265 and 288) with molecular ion at m/z 311 (Table 1) was tentatively identified as 2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate. Fragmentation produced a main ion at m/z 59 (CH3COO ̵ unit) as shown in Figure 2. Its mass spectrum is shown in Appendix A: A13

The compound labelled as peak 13 with retention time 11.05 min, molecular ion at m/z 299 and maximum UV-vis absorption wavelengths of 274, 282 and 289 (Table 1) was identified as salicylic acid β-D-glucoside. The compound produced fragment ions at m/z 254 which corresponded to loss of a carbondioxide (CO2; -44 amu) unit and m/z 137 which corresponded to a salicylic acid unit (Abu-Reidah et al., 2013) as shown in Figure 2. Its mass spectrum is shown in Appendix A: A14

The compound labelled as peak 14 with retention time 13.58 min, molecular ion at m/z 401 and maximum UV-vis absorption wavelength of 326nm (Table 1) was identified as benzyl alcohol hexoside-pentoside. The compound produced fragment ions at m/z 269 which corresponded to a benzyl hexoside unit (C13H17O6 ̵) and m/z 161 which corresponded to a C6H9O3 ̵unit(Russo et al., 2013) as shown in Figure 2. Its mass spectrum is shown in Appendix A: A15.

**149**

 

**134**

**119**

**ρ-coumaric acid (m/z 163) Ferulic acid (m/z 193)**



**179**

**85**

**caffeic acid glucoside (m/z 341)**

 

**208**

**164**

**152**

**108**

**123**

**Vanillic acid (m/z 167) Sinapic acid (m/z 223)**



**59**

**2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate (m/z 311)**

 

**179**

**133**

**153**

**109**

**Protocatechuic acid-4- glucoside (m/z 315) Acetyl salicylate derivative (m/z 295)**

 

**161**

**269**

**137**

**254**

**Salicylic acid β-D-glucoside (m/z 299)** **Benzyl alcohol hexoside-pentoside**

**Figure 2: Proposed fragmentation patterns for phenolic acids and their derivatives identified in Carica papaya peel.**

**Flavonoids and their derivatives identified**

The compound identified as peak 15 with retention time 17.41 min, a UV-vis absorption wavelength of 352 nm and molecular ion at m/z 609 was tentatively identified as rutin (Table 1). Fragmentation produced ions at m/z 301 (corresponding to a quercetin unit) and at m/z 300 (corresponding to two glucoside units) (Kumar, Singh & Kumar., 2017) as shown in Figure 3. The mass spectrum is shown in Appendix A: A16.

The compound identified as peak 16 with retention time 15.70 min, UV-vis absorption wavelengths of 254 and 352 nm and molecular ion at m/z 755 was tentatively identified as quercetin-3-O-rhamnosyl rutinoside (Table 1). Fragmentation produced ions at m/z 301 (corresponding to a quercetin unit) and at m/z 609 which corresponded to a rutin unit as shown in Figure 3 (Won et al., 2018). The mass spectrum is shown in Appendix A: A17.

Compound at peak 17 with retention time 17.51 min, UV-vis absorption wavelengths 287, 289, 302 and 351 nm exhibited a [M-H] ̵ ion at m/z 769, which, after the MS/MS experiments produced ion fragments at m/z 623 ((Negri et al., 2018), due loss of the rhamnose moiety. Fragmentation also produced another ion at m/z 605 which was due to the loss of rhamnose unit (Figure 3). Other fragment ions at m/z 315 corresponded to deprotonated isorhamnetin (due to loss of the glucosyl unit) and m/z 314 which was due to loss of the glycosyl groups (Ding et al., 2018). The mass spectrum is shown in Appendix A: A18



**301**

**Rutin (m/z 609)**



**609**

**301**

**Quercetin-3-O-rhamnosyl rutinoside (m/z 755)**



**605**

**315**

**Isoharmnetin-3-O-dirhamnosylglucoside (m/z 769)**

**Figure 3: Proposed fragmentation patterns for flavonoids and their derivatives identified in Carica papaya peel.**

**Glucosinolate identified**

The compound identified as peak 18 with retention time 9.17 min, UV-vis absorption wavelength of 302 nm and molecular ion at m/z 408 was tentatively identified as benzyl glucosinolate (Table 1). Fragmentation produced two main ions at m/z 97 (corresponding to a hydrogen sulphate unit; HSO4 ̵ ) and at m/z 96 which corresponded to a sulphate (SO4 ̵ ) unit as shown in Figure 4 (Castro‐Vargas, Baumann & Parada‐Alfonso., 2016). The mass spectrum is shown in Appendix A: A119.



**97**

**96**

**Benzyl glucoside (m/z 408)**

**Figure 4: Proposed fragmentation patterns for benzyl glucosinolate and its derivatives identified in Carica papaya peel.**

**TPC AND TFC RESULTS**

TPC- 6864.50 mg GAE/g sample (dry basis)

TFC- 2629.91 mg QE/g sample (dry basis)

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**APPENDIX A**

16

2

**Chart, histogram

Description automatically generated**

10

8 18

12 15 6 9

6 9

17

5 12 15

11

7

14

13

1

3

4

retention time (min)

**Figure A 1: UPLC-MS chromatogram of extracts from Carica papaya peel. 1= Citric acid, 2= Malic acid, 3= Ascorbic acid, 4= Gluconic acid, 5= p-coumaric acid, 6= Ferulic acid, 7= Caffeic acid glucoside, 8= Vanillic acid, 9= Sinapic acid, 10= Protocatechuic acid-4-glucoside, 11= Acetyl salicylate derivative, 12= 2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate, 13= Salicylic acid-β-D-glucoside, 14= Benzyl alcohol hexoside-pentoside, 15= Rutin, 16= Quercetin-3-O-rhamnosyl rutinoside, 17= Isoharmnetin-3-O-dirhamnosylglucoside, 18= Benzyl glucosinolate.**

Chart, scatter chart

Description automatically generated

**Figure A 2: Mass spectrum of citric acid (191 m/z) peak 1**

Chart, scatter chart

Description automatically generated

**Figure A 3: Mass spectrum of malic acid (133 m/z) peak 2**

Chart, scatter chart

Description automatically generated

**Figure A 4: Mass spectrum of ascorbic acid (175 m/z) peak 3**

Timeline

Description automatically generated

**Figure A 5: Mass spectrum of gluconic acid (195 m/z) peak 4**

Application

Description automatically generated with low confidence

**Figure A 6: Mass spectrum of p-coumaric acid (163 m/z) peak 5**

Chart

Description automatically generated

**Figure A 7: Mass spectrum of ferulic acid (193 m/z) peak 6**

A picture containing application

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**Figure A 8: Mass spectrum of caffeic acid glucoside (341 m/z) peak 7**

Chart, scatter chart

Description automatically generated

**Figure A 9: Mass spectrum of vanillic acid (167 m/z) peak 8**

Chart, scatter chart

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**Figure A 10: Mass spectrum of sinapic acid (223 m/z) peak 9**

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**Figure A 11: Mass spectrum of protocatechuic acid-4-glucoside (m/z 315) peak 10**

Graphical user interface

Description automatically generated with medium confidence

**Figure A 12: Mass spectrum of acetyl salicylate derivative (295 m/z) peak 11**

A picture containing chart

Description automatically generated

**Figure A 13: Mass spectrum of 2-acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate (311 m/z) peak 12**

A picture containing application

Description automatically generated

**Figure A 14:** **Mass spectrum of salicylic acid β-D-glucoside (299 m/z) peak 13**

Graphical user interface, Word

Description automatically generated with medium confidence

**Figure A 15: Mass spectrum of benzyl alcohol hexoside pentoside (401 m/z) peak 14**

A picture containing text

Description automatically generated

**Figure A 16: Mass spectrum of rutin (609 m/z) peak 15**

A picture containing chart

Description automatically generated

**Figure A 17: Mass spectrum of quercetin-3-O-rhamnosyl rutinoside (755 m/z) peak 16**

A picture containing graphical user interface

Description automatically generated

**Figure A 18: Mass spectrum of Isorhamnetin-3-O-dirhamnosyl glucoside (769 m/z) peak 17**

Graphical user interface, application

Description automatically generated

**Figure A 19: Mass spectrum of benzyl glucosinolate (408 m/z) peak 1**