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# Investigation and identification of chemical changes in ginger tea using Ion Trap Mass Spectrometry

Abide Mo[1,2], A.F.M Motiur Rahman[2]

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**Abstract**

Green tea and ginger have been consumed both separately and together for thousands of years and are considered to have numerous health benefits. Consuming these two together has long been acknowledged to have many improved health benefits, but it is unclear whether this is due to the synergistic effects of the components in each separate substance or the formation of novel compounds (or both). In this study, to investigate if there were any new compounds formed, we compared the chemical compositions of green tea, ginger, and a mixture of green tea with ginger using ion trap liquid chromatography-mass spectrometry (LC-MS). Using this technique, we identified one novel compound of molecular weight 301. While the characteristics, potential positive effects on health, and mechanism of action of this compound require further research, our findings suggest that adding ginger to tea may lead to the formation of novel compounds with potential health benefits. Future studies will aim to isolate and/or synthesize this compound and test for its biological activity.

**Keywords**

Green tea, Ginger, Ginger tea, Mass spectrometry, Peonidin, Ion trap liquid chromatography, Anthocyanins, Glycosides, Ion chromatogram

[1]Abide Mo, Bangladesh International School English Section, Riyadh 12221, Saudi Arabia.
[2]Corresponding author: A. F. M. Motiur Rahman, Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia, afmrahman@ksu.edu.sa

## Introduction

Tea is a widely consumed plant products that have been shown to have a wide range of health benefits (1-4). Tea is rich in polyphenols (5-6), which have antioxidant and anti-inflammatory properties (7-8) and have been associated with a reduced risk of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (9-10). Ginger, a plant that has been used for over 2000 years as a spice, tea, and medicine, is a popular addition to commercial foods, beverages, and supplements due to its high concentration of pungent constituents, particularly gingerol-related compounds (11-12). On the other hand, ginger contains bioactive compounds such as gingerols and shogaols, which have anti-inflammatory, antiplatelet activator, antioxidative and anti-cancer properties (13-20). Various forms of ginger such as dried ginger and ginger charcoal, has unique properties that can help alleviate respiratory and digestive symptoms by affecting different meridians, and are often prescribed by clinicians for related conditions (21). While each has unique health benefits, it's not clear whether the enhanced health effects from consuming them together is attributed to the synergistic effects of the exclusive chemical components from each substance or the formation of new compounds in the combination (or both). There are a large number of studies focused on identifying the chemical components of natural products including green tea and ginger using modern techniques such as liquid chromatography-mass spectrometry (LC-MS) and many others (22-34). In order to investigate the hypothesis of the formation of novel components, we herein analysed green tea, ginger and ginger tea using LC-MS.

## Materials and Methods

All the chemicals and the solvent were reagent grade and were used without further purification. Green tea packets and ginger were obtained from a local market in Riyadh, Saudi Arabia. Milli-Q connected to Elix Millipore water purification system (Millipore, USA) was used to obtain HPLC grade water. Acetonitrile (ACN) was HPLC grade. Electrospray ionization (ESI) mass spectrometry (MS) experiments were conducted using an Agilent 1200 series HPLC connected with an Agilent 6320 ion trap mass spectrometer equipped with an electrospray ionization (ESI) ion source (Agilent Technologies, Palo Alto, CA, USA) with direct injection as well as using column. Eclipse plus C18 (4.6 mm X 150 mm, 3.5 micron) column was used (Agilent Technologies, Palo Alto, CA, USA)). The mobile phase was a gradient prepared from 5 mM ammonium formate and 0.1% formic acid in water (A) and acetonitrile (B). The gradient program for the HPLC was as follows: 0−5 min, 5−20% B; and 5−60 min, 20−95% B, the flow rate was 0.3 mL $min^{-1}$. The injection volume was 10 µL, and the column temperature was maintained at 25°C. Run time was set to 60 min. Mass spectra in the m/z range 50−1000 were obtained by an electrospray ionization with a

positive/negative-ion mode. The mass spectrometric conditions were optimized as follows: the voltage was maintained at 4.5kV, the capillary temperature at 350°C, and the drying gas flow rate at 10.0 L $min^{-1}$; the nebulizer gas pressure was set to 60 psi. Mass fragmentation was accomplished by a triple quadrupole detector (TQD) mass spectrometer (Waters Corporation, Milford, MA USA).

*Sample preparation procedure*

The sample preparation procedure followed the following simple method. Three green tea bags (Ahmad Tea London; each bag = 2g) were added to boiling water (500 mL) and kept boiling for 5 minutes to allow the tea to dissolve in the water. The same procedure was then repeated for ginger (fresh raw ginger, 20 g) and ginger tea (fresh raw ginger, 20g + 3 tea bags). After cooling to room temperature followed by filtration, the filtrate was extracted with ethyl acetate (3 times, 50 mL each), dried over anhydrous $Na_2SO_4$, filtered to remove the drying agent, and evaporated with a reduced pressure rotatory evaporator at 100 rpm and 60℃ till dryness. The resulting extracts were reconstituted in an acetonitrile and water mixture (1:1) and analysed using LC-MS.

**Results and Discussion**

To investigate whether new chemical species formed in the ginger tea, we separately boiled green tea bags and ginger slices, as well as the mixture of the two. We then extracted the three mixtures using ethyl acetate as an organic solvent, and the extracted samples were subjected to analysis by ion trap liquid chromatography-Mass spectrometry (LC-MS). All three samples were scanned in LC-MS using C18 column in both positive and negative modes. The spectra are shown in figures (Figure 1 and 2) and the data are summarized in Tables 1-3.

In brief, analysis of the ethyl acetate extract of the tea sample revealed 11 distinguishable peaks in positive mode (Figure 1A, 4-52 min) and 24 peaks in negative mode (Figure 2A, 3.5-58 min). From the spectra, 21 previously reported components were detected. 12 of them originated from the positive mode: caffeine and/or pectin (*m/z* = 195 $[M+H]^+$, 4.7 min), (-)-gallocatechin and/or (-)-epigallocatechin and/or gallocatechol (*m/z* = 307 $[M+H]^+$, 5.1 min), vitamin E (*m/z* = 432 $[M+H]^+$, 5.3 min), (+)-catechin and/or (-)- epicatechin (*m/z* = 291 $[M+H]^+$, 5.7 min), (-)-gallocatechin gallate and/or (-)-epigallocatechin gallate (*m/z* = 459 $[M+H]^+$, 6.2 min), (-)-epicatechin gallate and/or (-)-catechin gallate (*m/z* = 443 $[M+H]^+$, 7.2 min), cellulose (162 per glucose unit, *m/z* = 163 $[M+H]^+$, 25.3 min) , and quinine acid (*m/z* = 204 $[M+H]^+$, 48.1 min); the other 9 originated from the negative mode: (-)-gallocatechin and/or (-)-epigallocatechin and/or gallocatechol (*m/z* = 305 $[M-H]^-$, 4.8 min), (-)-gallocatechin gallate and/or epigallocatechin gallate (*m/z* = 457 $[M-H]^-$, 5.4 min), delphinidin (*m/z* = 302 $[M-H]^-$, 7.7 min), ellagic acid and/or quercetin (*m/z* =

301 [M-H]$^-$, 29.6 min), gallic acid (*m/z* = 213 [M-H]$^-$, 36.9 min), kaempferol and/or vitamin A (*m/z* = 285 [M-H]$^-$, 37.3 min), and 10-shogaol (*m/z* = 331 [M-H]$^-$, 37.7 min) (Table 1).

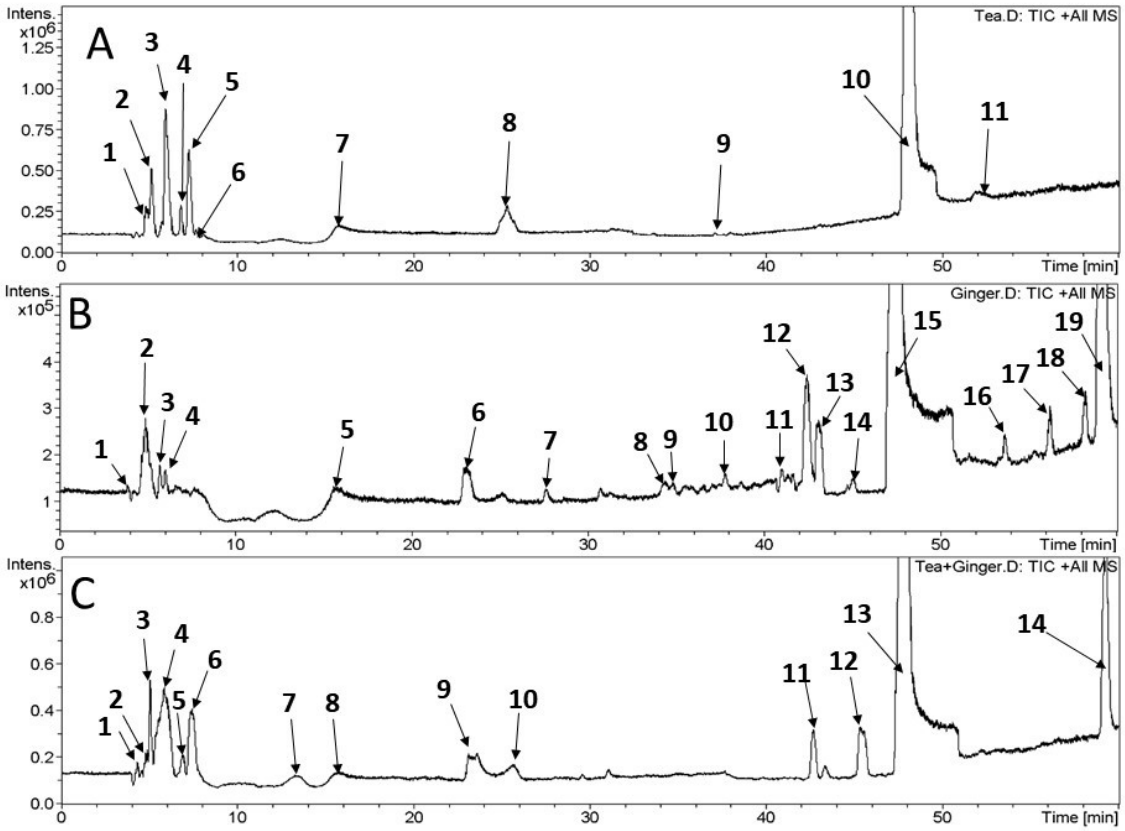



Figure 1. Total Ion Chromatogram (TIC) in positive mode: Green tea (A); Ginger (B); Ginger tea (C).

Table 1. Analysis of green tea samples in positive and negative mode

| Green tea sample | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Positive Ion mode | | | | | Negative Ion mode | | | | |
| Peak no. | Compounds Name (Chemical Formula) | RT (min) | MW | *m/z* $[M+H]^+$ | Peak no. | Compounds Name (Chemical Formula) | RT (min) | MW | *m/z* $[M-H]^-$ |
| 1 | Caffeine ($C_8H_{10}N_4O_2$) | 4.7 | 194 | 195 | 1 | Gallocatechol ($C_{15}H_{14}O_7$) | 4.8 | 306 | 305 |
| | | 4.8 | | 239 | 2 | Epigallocatechin gallate ($C_{22}H_{18}O_{11}$) | 5.4 | 458 | 457 |
| | | 4.9 | | 283 | | | | | |
| | | 4.9 | | 300 | | | | | 383 |
| | | 5.0 | | 344 | 3 | | 5.7 | | 442 |
| 2 | (-)-Gallocatechin (-)-epigallocatechin ($C_{15}H_{14}O_7$) | 5.1 | 306 | 307 | | | | | 458 |
| | | | | | | | | | 471 |
| | | 5.3 | | 432 | 4 | | 5.8 | | 441 |
| | | 5.6 | | 187 | 5 | | 6.3 | | 427 |
| 3 | (+)-Catechin (-)- epicatechin ($C_{15}H_{14}O_6$) | 5.7 | 290 | 291 | | | | | 539 |
| | | | | | | | | | 555 |
| | | | | | 6 | | 6.7 | | 425 |
| | (-)-gallocatechin gallate (-)-epigallocatechin gallate ($C_{22}H_{18}O_{11}$) | 5.9 | | 289 | | | | | 455 |
| | | 6.2 | | 433 | 7 | | 7.1 | | 451 |
| | | 6.2 | 458 | 459 | 8 | | 7.4 | | 481 |
| | | | | | 9 | Delphinidin ($C_{15}H_{11}O_7^+$) | 7.7 | 303 | 302 |
| 4 | | 6.8 | | 289 | | | | | 409 |
| | | 6.8 | | 473 | | | | | 516 |
| 5 | (-)-epicatechin gallate (-)-catechin gallate ($C_{22}H_{18}O_{10}$) | 7.2 | | 123 | 10 | | 8.3 | | 471 |
| | | | | 273 | 11 | | 24.9 | | 585 |
| | | | 442 | 443 | | | 25.3 | | 435 |
| | | | | | 12 | Ellagic acid | 29.6 | 302 | 301 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 6 | | 7.6 | | 331 | | ($C_{14}H_6O_8$) / Quercetin ($C_{15}H_{10}O_7$) | | | 415 |
| | | | | 191 | | | | | |
| 7 | | 15.6 | | 187 | | | | | |
| 8 | Cellulose (C12H20O10)n 162 g/mol per glucose unit | 25.3 | 162 | 163 | 13 | | 33.2 | | 449 |
| | | | | 197 | | | | | 583 |
| 9 | | 37.1 | | 359 | 14 | | 34 | | 483 |
| 10 | Quininic acid ($C_{11}H_9NO_3$) | 48.1 | 203 | 204 | | | 6.9 | | 113 |
| | | | | | 15 | Gallic acid ($C_7H_6O_5$) 10-Gingerol ($C_{21}H_{24}O_4$) | | 170 | 169 |
| 11 | | 51.9 | | 340 | | | | | 329 |
| | | | | 438 | | | | 350 | 349 |
| | | | | | 16 | Kaempferol ($C_{15}H_{10}O_6$) | 37.3 | 286 | 285 |
| | | | | | | | | | 464 |
| | | | | | 17 | | 37.7 | | 331 |
| | | | | | 18 | | 38.1 | | 467 |
| | | | | | 19 | 10-Gingerol ($C_{21}H_{34}O_4$) | 42.6 | | 249 |
| | | | | | | | | 350 | 349 |
| | | | | | 20 | | 44 | | 517 |
| | | | | | 21 | | 49.5 | | 358 |
| | | | | | 22 | | 52.2 | | 255 |
| | | | | | 23 | | 55.5 | | 256 |
| | | | | | 24 | | 56.4 | | 499 |

Analysis of the ginger extract sample yielded 19 peaks in positive mode (Figure 1B, 3.8-60 min) and 25 peaks in negative mode (Figure 2B, 3.8-60 min). From the spectra, 17 previously reported components were detected. 9 of them originated from the positive mode: zingerone (*m/z* = 195 $[M+H]^+$, 4.7 min), gingerenone-A (*m/z* = 357 $[M+H]^+$, 42.4 min), zingiberene, β-bisabolene, α-farnesene, and/or β-sesquiphellandrene (*m/z* = 205 $[M+H]^+$, 47.5 min), chlorogenic acid (*m/z* = 339 $[M+H]^+$, 56.2 min), and 6-shogaol (*m/z* = 277 $[M+H]^+$, 59.5 min); the other 8 originated from the negative mode: 10-gingerol (*m/z* = 349 $[M-H]^-$, 4.0 min), 6-gingerol (*m/z* = 293 $[M-H]^-$, 47.1 min), 4-gingerol (*m/z* = 265 $[M-H]^-$, 50.1 min), and 8-gingerol (*m/z* = 321 $[M-H]^-$,

58.5 min), 1-dehydro-6-gingerdione (*m/z* = 317 $[M-H]^-$, 4.0 min), 1-dehydro-12-gingerdione (*m/z* = 373 $[M-H]^-$, 32.4 min), acetoxy-[10]-gingerol (*m/z* = 391 $[M-H]^-$, 7.2 min), 3- or 5-Acetoxy-[6]-gingerdiol (*m/z* = 338 $[M-H]^-$, 45.0 min) and methyl diacetoxy-[10]-gingerdiol (*m/z* = 449 $[M-H]^-$, 7.5 min) (Table- 2).

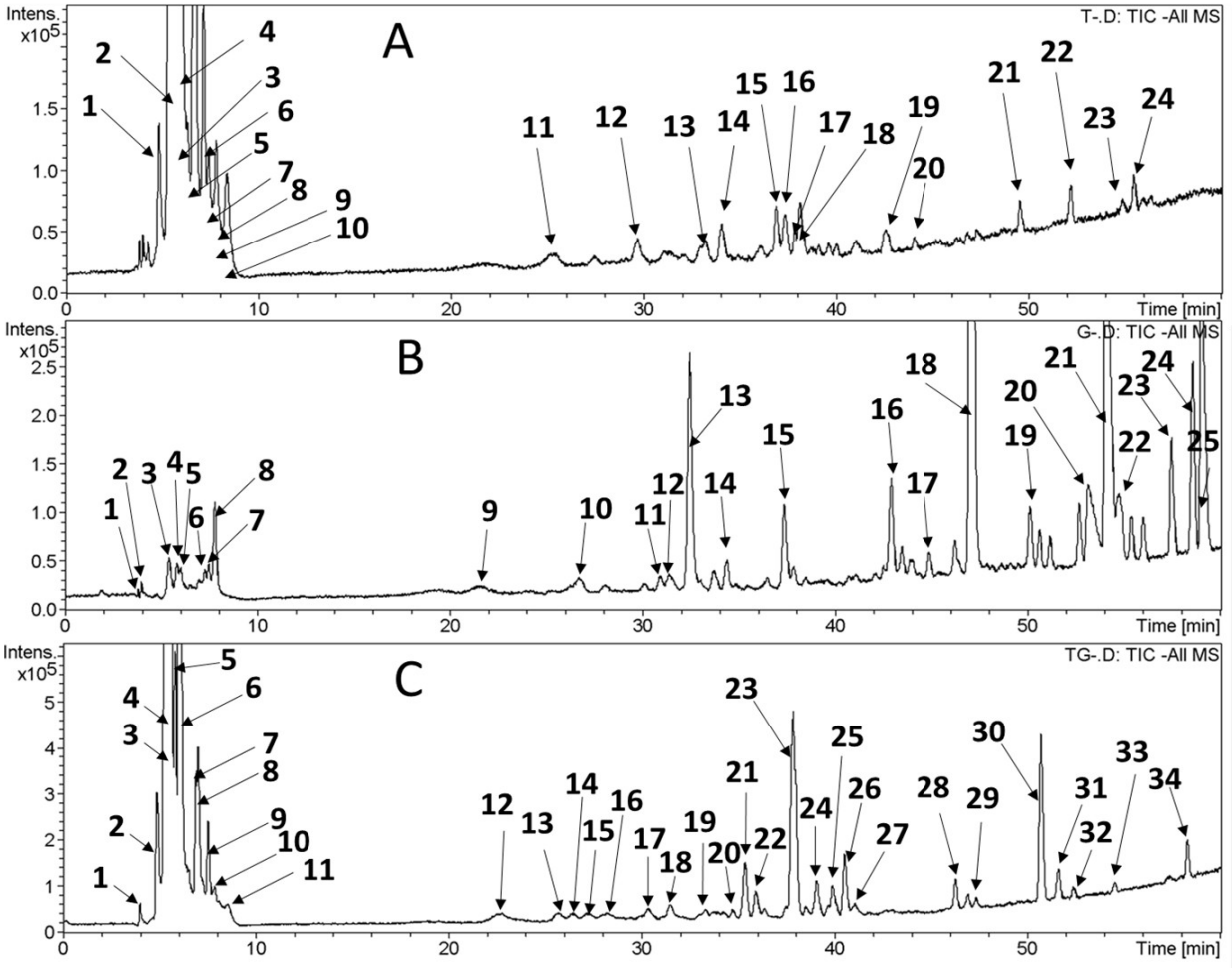



Figure 2. Total Ion Chromatogram (TIC) in negative mode: Green tea (A); Ginger (B); Ginger tea (C).

Table 2. Analysis of ginger samples in positive and negative mode

| Ginger sample | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Positive Ion mode | | | | | Negative Ion mode | | | | |
| Peak no. | Compounds Name (Chemical Formula) | RT (min) | MW | *m/z* $[M+H]^+$ | Peak no. | Compounds Name (Chemical Formula) | RT (min) | MW | *m/z* $[M-H]^-$ |
| 1 | | 3.9 | | 187 | 1 | 1-Dehydro-6-gingerdione ($C_{17}H_{22}O_4$) | 3.8 | | 175 |
| 2 | Zingerone ($C_{11}H_{14}O_3$) | 4.6 | | 151 | | | | | 213 |
| | | 4.7 | | 187 | | | | 290 | 289 |
| | | | 194 | 195 | | | | | 305 |
| | | 4.8 | | 239 | 2 | 1-Dehydro-8-gingerdione ($C_{19}H_{26}O_4$) 10-Gingerol ($C_{21}H_{34}O_4$) | 4.0 | | 249 |
| | | | | 256 | | | | 318 | 317 |
| | | 4.9 | | 283 | | | | 350 | 349 |
| | | | | 300 | 3 | | 5.4 | | 457 |
| | | 5.0 | | 327 | 4 | | 5.8 | | 441 |
| | | | | 344 | | | | | 555 |
| | | | | 388 | 5 | | 6.0 | | 405 |
| | | 5.2 | | 432 | 6 | Acetoxy-[10]-gingerol ($C_{24}H_{38}O_4$) | 7.2 | 390 | 389 |
| 3 | | 5.7 | | 230 | 7 | Methyl diacetoxy-[10]-gingerdiol ($C_{26}H_{42}O_6$) | 7.5 | | 309 |
| 4 | | 6.0 | | 195 | | | | | 361 |
| 5 | | 15.5 | | 187 | | | | 450 | 449 |
| 6 | Cellulose $(C_{12}H_{20}O_{10})_n$ 162 g/mol per glucose unit | 23.2 | 162 | 163 | | | | | 475 |
| 7 | | 27.6 | | 274 | 8 | | 7.8 | | 389 |
| 8 | | 34.2 | | 373 | 9 | | 21.5 | | 213 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 9 | | 34.8 | | 343 | | | | | 359 |
| 10 | | 37.8 | | 341 | | | | | 473 |
| 11 | | 40.9 | | 387 | 10 | | 26.7 | | 375 |
| | | | | 422 | 11 | | 30.9 | | 403 |
| 12 | Gingerenone-A ($C_{21}H_{24}O_5$) | 42.4 | 356 | 357 | 12 | | 31.4 | | 343 |
| | | | | | | | | | 491 |
| 13 | | 43.0 | | 249 | 13 | 1-Dehydro-12-gingerdione ($C_{23}H_{34}O_4$) | 32.4 | 374 | 373 |
| 14 | 3- or 5-Acetoxy-[6]-gingerdiol ($C_{19}H_{30}O_5$) | 45.0 | 338 | 339 | | | | | |
| | | | | 341 | 14 | | 34.4 | | 433 |
| 15 | Zingiberene<br>$\beta$-Bisabolene<br>$\alpha$-Farnesene $\beta$-Sesquiphellandrene ($C_{15}H_{24}$) | 47.5 | 204 | 205 | 15 | | 37.3 | | 431 |
| | | | | | | | | | 545 |
| | | | | | | | 37.8 | | 401 |
| | | | | | 16 | | 42.9 | | 342 |
| | | | | | | | | | 475 |
| | | | | | | | 43.8 | | 445 |
| | | | | | 17 | | 44.8 | | 296 |
| 16 | | 53.6 | | 262 | | | | | 341 |
| | | | | 300 | 18 | 6-Gingerol ($C_{17}H_{26}O_4$) | 47.1 | 294 | 293 |
| 17 | Chlorogenic acid ($C_{16}H_{18}O_9$) | 56.2 | | 339 | 19 | 4-Gingerol ($C_{15}H_{22}O_4$) | 50.1 | 266 | 265 |
| | | | | 341 | 20 | | 53.1 | | 456 |
| | | | 354 | 355 | | | | | 556 |
| | | | | 454 | 21 | 4-Gingerol ($C_{15}H_{22}O_4$) | 54.2 | 266 | 265 |
| | | | | 494 | | | | | 531 |
| 18 | | 58.2 | | 278 | 22 | | 54.7 | | 279 |
| | | | | 296 | 23 | | 57.5 | | 279 |
| 19 | | 59.5 | | 177 | 24 | 8-Gingerol ($C_{19}H_{30}O_4$) | 58.5 | 322 | 321 |
| | 6-Shogaol | | 276 | 277 | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ($C_{17}H_{24}O_3$) | | | | 25 | | 59.0 | | 279 |

In addition, analysis of the targeted ginger tea extract observed 14 detectable peaks in positive mode (Figure 1C) and 34 peaks in negative mode (Figure 2C). All the detected components which were seen in ginger tea were observed in the green tea and ginger tea except for a new compound at RT=45.5 and 37.8 min. in positive and negative mode, respectively (Table 3).

Table 3. Analysis of ginger tea samples in positive and negative mode

| Ginger tea sample | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Positive Ion Mode | | | | | Negative Ion Mode | | | | |
| Peak no. | Compounds Name (Chemical Formula) | RT (min) | MW | *m/z* $[M+H]^+$ | Peak no. | Compounds Name (Chemical Formula) | RT (min) | MW | *m/z* $[M-H]^-$ |
| 1 | | 4.3 | | 187 | 1 | 1-Dehydro-8-Gingerdione ($C_{19}H_{26}O_4$) 10-Gingerol ($C_{21}H_{34}O_4$) | 4.0 | | 249 |
| | | 4.5 | | 123 | | | | 318 | 317 |
| | | | | 151 | | | | | |
| 2 | | 4.8 | | 239 | | | | 350 | 349 |
| | | 4.9 | | 283 | 2 | Gallocatechol ($C_{15}H_{14}O_7$) | 4.8 | 306 | 305 |
| | | | | 300 | | | | | 383 |
| 3 | (-)-Epigallocatechin (-)-Gallocatechin Gallocatechol ($C_{15}H_{14}O_7$) | 5.0 | | 289 | 3 | (-)-Epigallocatechin (-)-Gallocatechin Gallocatechol ($C_{15}H_{14}O_7$) | 5.2 | 306 | 305 |
| | | | 306 | 307 | 4 | Epigallocatechin gallate ($C_{22}H_{18}O_{11}$) | 5.5 | 458 | 457 |
| 4 | (+)-Catechin (-)- epicatechin ($C_{15}H_{14}O_6$) | 5.7 | 290 | 291 | 5 | | 5.8 | | 471 |
| | | | | | 6 | | 6.0 | | 441 |
| | | | | | 7 | | 6.9 | | 455 |
| | | 5.9 | | 289 | 8 | | 7.0 | | 425 |
| | (-)-gallocatechin gallate (-)-epigallocatechin | 6.2 | 458 | 459 | 9 | | 7.5 | | 451 |
| | | | | | 10 | | 7.8 | | 481 |
| | | | | | 11 | Quercetin ($C_{14}H_6O_8$ | 8.5 | 302 | 301 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | gallate ($C_{22}H_{18}O_{11}$) | | | | | / $C_{15}H_{10}O_7$) | | | 409 |
| 5 | | 6.9 | | 289 | | | | | 113 |
| | | | | 473 | | | | | 213 |
| 6 | | 7.4 | | 273 | 12 | Gallic acid ($C_7H_6O_5$) | 22.7 | 170 | 169 |
| | (-)-Epicatechin gallate / (-)-Catechin gallate ($C_{22}H_{18}O_{10}$) | | 442 | 443 | | | | | 471 |
| | | | | | | 10-Gingerol ($C_{21}H_{34}O_4$) | | 350 | 349 |
| 7 | Caffeine ($C_8H_{10}N_4O_2$) / Zingerone ($C_{11}H_{14}O_3$) | 13.4 | 194 | 195 | 13 | | 25.3 | | 389 |
| | | | | | 14 | | 26.4 | | 359 |
| | | | | | | | | | 473 |
| | | | | | 15 | | 27.2 | | 585 |
| 8 | | 15.5 | | 187 | 16 | | 28.2 | | 435 |
| 9 | Cellulose ($C_{12}H_{20}O_{10})_n$ 162 g/mol per glucose unit | 23.1 | 162 | 163 | 17 | | 30.3 | | 375 |
| | | | | 187 | | | | | 389 |
| | | | | 197 | 18 | Ellagic acid ($C_{14}H_6O_8$) / Quercetin ($C_{15}H_{10}O_7$) | 31.5 | 302 | 301 |
| 10 | Cellulose ($C_{12}H_{20}O_{10})_n$ 162 g/mol per glucose unit | 25.6 | 162 | 163 | | | | | |
| | | | | 187 | 19 | | 33.3 | | 483 |
| | | | | | 20 | Diacetoxy-[10]-gingerdiol ($C_{25}H_{40}O_6$) | 34.7 | 436 | 435 |
| 11 | Gingerenone-A ($C_{21}H_{24}O_5$) | 42.6 | 356 | 357 | | | | | 491 |
| | | | | | 21 | 1-Dehydro-12-gingerdione ($C_{23}H_{34}O_4$) | 35.3 | 374 | 373 |
| 12 | New | 45.5 | 301 | 302 | 22 | | 35.9 | | 483 |
| 13 | Zingiberene *β*-Bisabolene *α*-Farnesene *β*-Sesquiphellandrene | 47.4 | 204 | 205 | 23 | New | 37.8 | | 256 |
| | | | | | | | | 301 | 300 |
| | | | | | 24 | Kaempferol ($C_{15}H_{10}O_6$) | 39.0 | 286 | 285 |
| | | | | | 25 | | 39.9 | | 467 |
| | | | | | 26 | | 40.5 | | 431 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ($C_{15}H_{24}$) | | | | | | | | |
| 14 | 6-Shogaol ($C_{17}H_{24}O_3$) | 59.5 | 276 | 177 | 27 | | 41.0 | | 401 |
| | | | | 277 | 28 | | 46.3 | | 475 |
| | | | | | 29 | | 47.3 | | 445 |
| | | | | | 30 | 6-Gingerol ($C_{17}H_{26}O_4$) | 50.7 | 294 | 293 |
| | | | | | 31 | | 51.6 | | 358 |
| | | | | | 32 | | 52.4 | | 329 |
| | | | | | 33 | | 54.5 | | 501 |
| | | | | | 34 | 4-Gingerol ($C_{15}H_{22}O_4$) | 58.3 | 266 | 265 |

As expected, by stacking all three TIC scans of the corresponding extracts in both positive and negative modes, a new peak appeared at 45.5 min with *m/z* of 302 Da in positive mode (Figure 3A) and at 37.8 min with *m/z* of 300 Da in negative mode (Figure 3B), for the ginger tea extract (red spectra), indicating the possible existence of a newly formed compound.

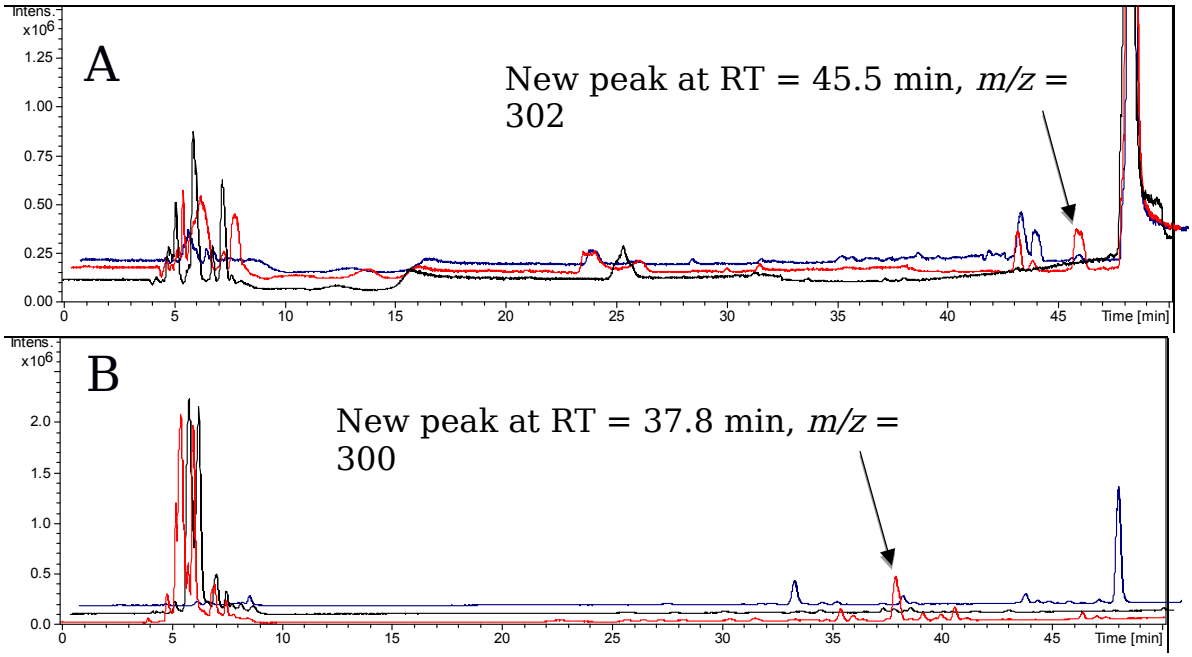



Figure 3. Stacked spectra: In positive mode: ginger (in blue), green tea (in black) and ginger tea (in red) (A); in negative mode: ginger (in blue), green tea (in black) and ginger tea (in red) (B)

The extracted ion chromatogram (EIC) spectrum of the ginger tea in positive mode at 302 (Figure 4A) was compared with EIC spectra of green tea (Figure 4B) and ginger (Figure 4C) samples at the same ion of interest. It was found that the obtained peak extracted at 302 Da in the ginger tea was not observed in either the green tea (Figure 4B) or in the ginger sample (Figure 4C).

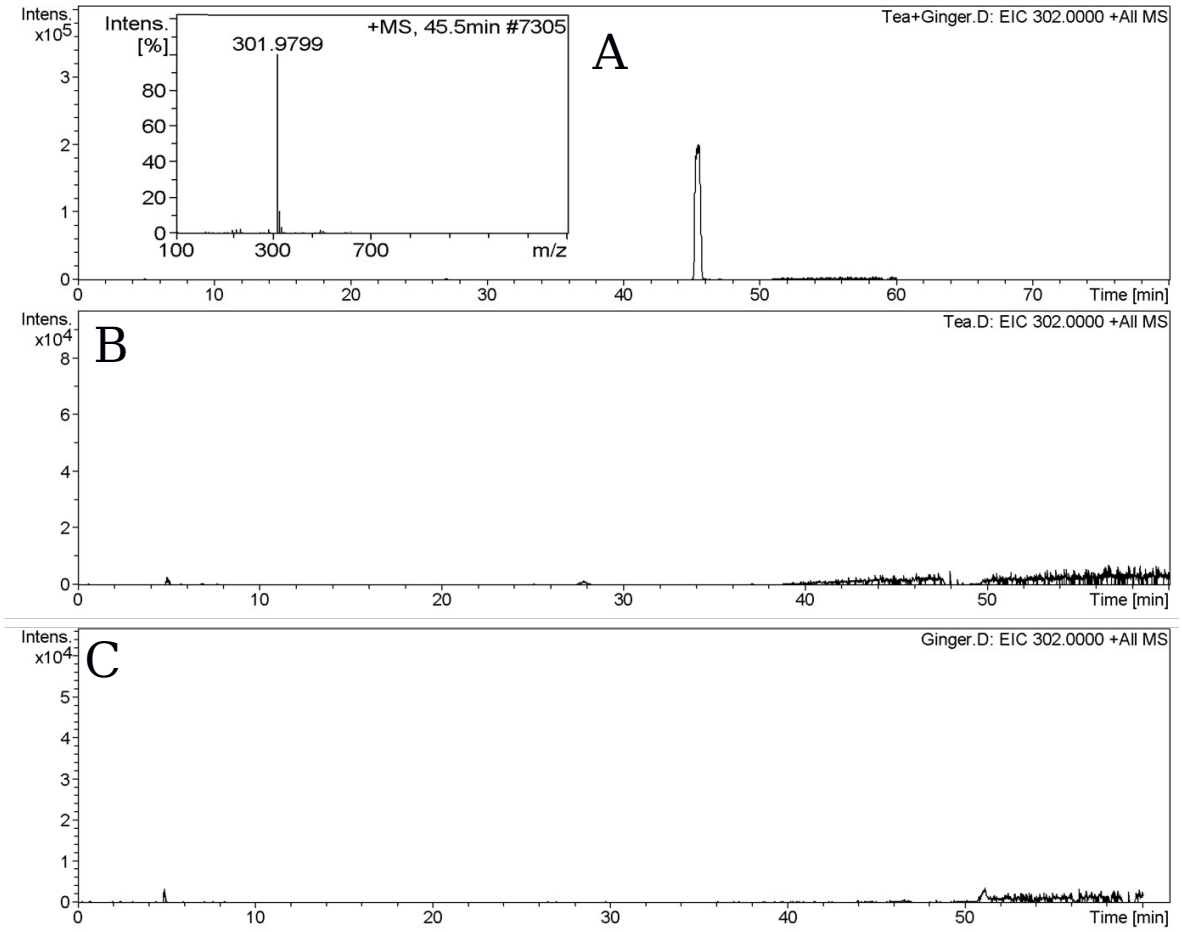



Figure 4. Extracted Ion Chromatogram (EIC) in positive mode: Ginger tea (A); Green tea (B); Ginger (C).

For further confirmation, the EIC spectrum of ginger tea in negative mode was also studied. Extraction at 300 (Figure 5A) generated a prominent peak as well. This peak was compared with the corresponding EIC spectra of green tea (Figure 5B) and ginger (Figure 5C) samples. As with the positive mode, the obtained peak was, once

again, not observed in either the green tea or the ginger sample.

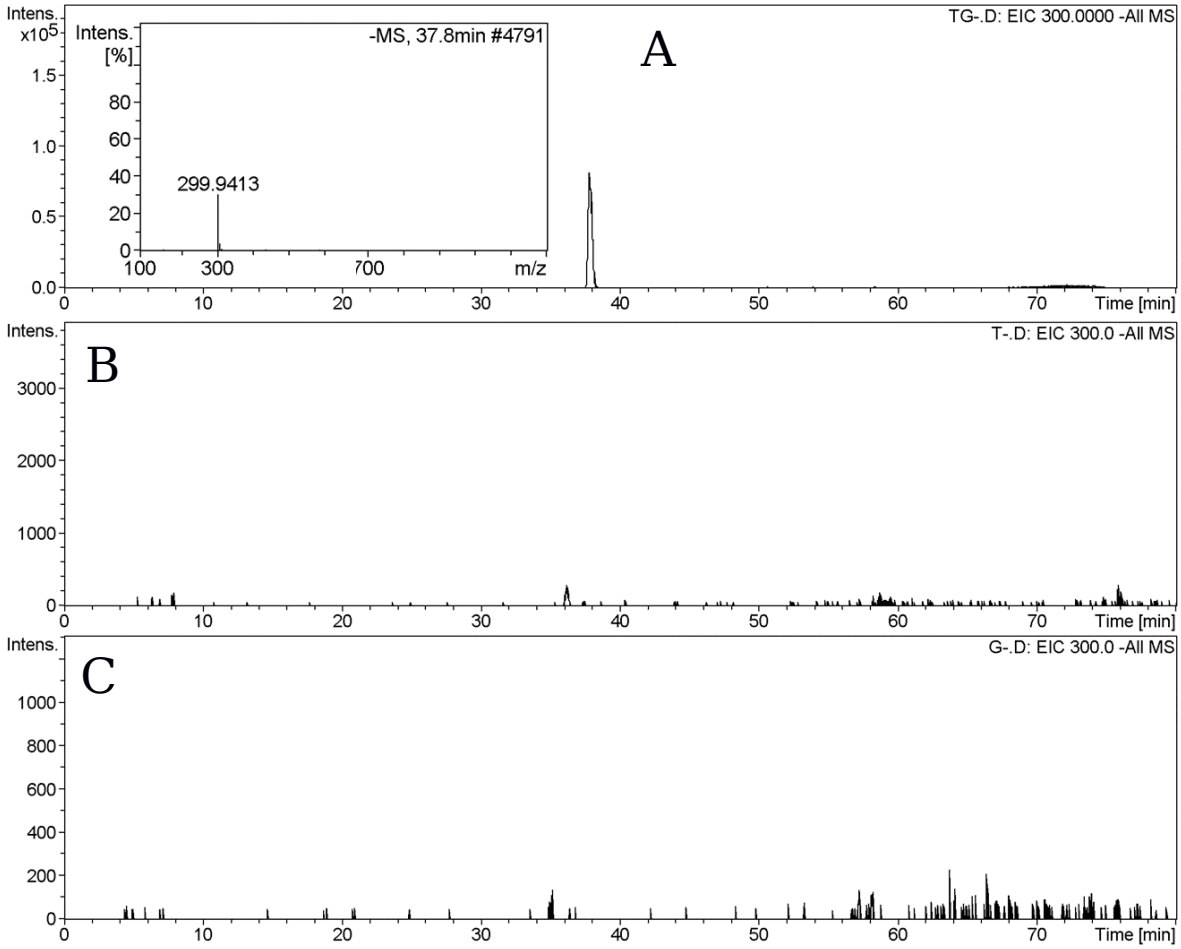



Figure 5. Extracted Ion Chromatogram (EIC) in negative mode: Ginger tea (A); Green tea (B); and Ginger (C).

From the above analysis, it appears possible that a compound with molecular mass of 301 was formed *in-situ* in the ginger tea. In order to further confirm our assumption, a mass spectral fragmentation experiment was performed. Direct injection of the ginger tea sample was scanned and a peak at 302.09 was observed (Figure 6A), fragmentation of that peak yielded several daughter ions at *m/z* = 185, 167, 159, 144, 140, 139, 113, and 112 Da (Figure 6B), respectively.

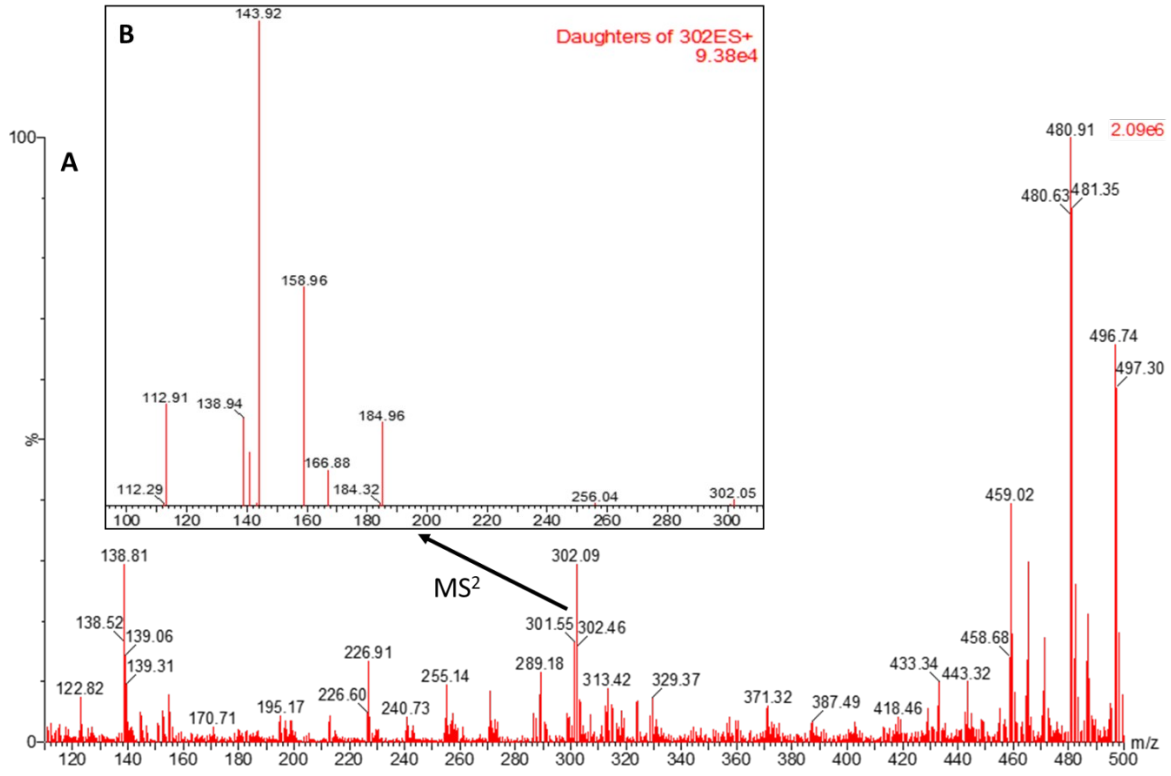
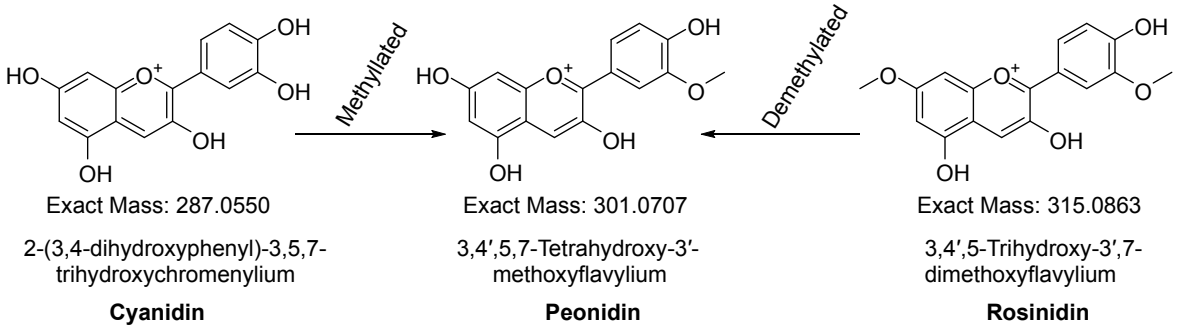


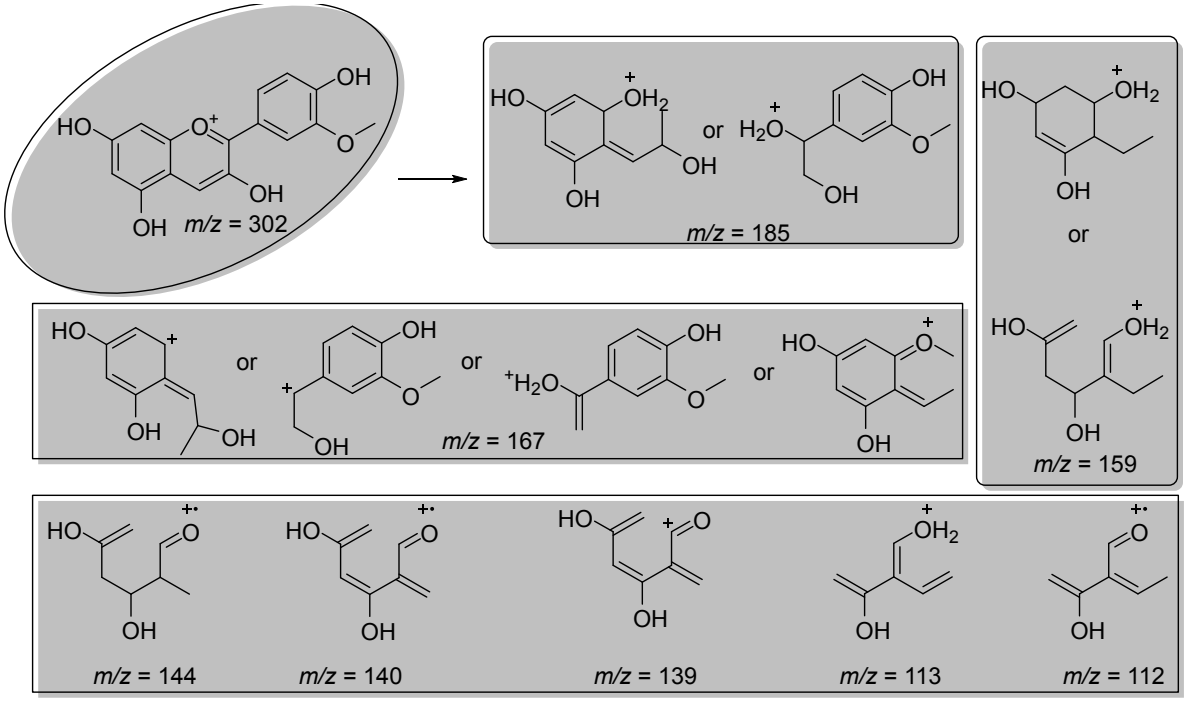

Figure 6. Mass spectra of ginger tea: MS scan (A); $MS^2$ spectra (B)

Based on the fragmentation pattern, we concluded that the identified compound could be the natural product peonidin (Scheme 1). The new compound may have formed during the heating of green tea with ginger, or it could potentially be a metabolite of cyanidin (MW = 286) when methylated, or resinidin (MW = 315) when demethylated. It is also known that methyl transferases are present in plants (35). Additionally, the pH changes (ginger tea pH ~ 7-7.5) that occur when green tea (~ 7-10) is heated with raw ginger (pH ~ 3-5) could have contributed to the formation of this compound *via* the methylation of cyanidin. While a peonidin standard would unequivocally identify the unknown peak being attributable to peonidin, time and resource constraints do not permit us to perform this experiment in the near future. Hence, we cannot definitively assign the peak to peonidin at the present time. However, further research is needed to investigate the mechanism behind the formation of new compounds, which will be explored in due course. It should be noted that, no significant trace of similar mass was present in the ginger or green tea alone.




Scheme 1. Possible structure of identified natural component.

We attempted to analyse the obtained fragmentation patterns based on Figure 6B. All the fragments matched our predicted fragmentation pattern (Scheme 2).




Scheme 2. Possible fragmentation pattern of identified natural component peonidin.

In addition to identifying a new compound, we also observed the disappearance of several peaks from the green tea and ginger extracts, which we have summarized in Tables 1-3. This was confirmed by using extracted ion chromatograms for all

individual peaks and comparing them with the peaks identified in green tea and ginger, as well as those in ginger tea. Further research is needed to investigate the possible chemical changes or modifications that occur during the preparation of ginger tea.

## Conclusion

In conclusion, our findings suggest that consuming a tea and ginger mixture may lead to the formation of novel compounds with potential health benefits that warrant further investigation. The individual health benefits of tea and ginger, as well as the potential synergistic effects of consuming them together, make a tea and ginger mixture a promising area of research. Based on our analysis, the potential formation of the novel compound, with a molecular weight of 301 Da, was tentatively identified as peonidin. Future research is required to determine the characteristics, health benefits, and mechanism of action in the body of this novel compound, and it is hoped that it may be isolated and/or synthesized for use in therapeutic purposes. In addition, further studies using peonidin standard are needed to confirm the proposed structure.

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