

# **Systematical Ingredient Investigations of *Ficus tikoua* Bur. Fruit and Immunoregulatory and Antioxidant Effects of Different Fractions**

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## CONTENTS

|   |    |
|---|----|
| Abbreviations.....  | 3  |
| <b>Figure S1.</b> The picture of <i>Ficus tikoua</i> fruit .....  | 5  |
| <b>Figure S2.</b> TIC LC-MS spectrum of the methanol extract of <i>F. tikoua</i> fruit.....   | 6  |
| <b>Figure S3.</b> HPLC spectrum of the methanol extract of <i>F. tikoua</i> fruit.....  | 6  |
| <b>Table S1.</b> Compounds identified by LC-MS from the methanol extract of <i>F. tikoua</i> fruit.....   | 7  |
| <b>Figure S4.</b> TIC GC-MS spectrum of the hexane extract of <i>F. tikoua</i> fruit.....   | 9  |
| <b>Table S2.</b> Compounds identified by GC-MS from the hexane extract of <i>F. tikoua</i> fruit.....   | 10 |
| <b>Table S3.</b> Crude protein, amino acids, total polysaccharides, polyphenols, flavonoids, and vitamins contents of <i>F. tikoua</i> fruit..... | 12 |
| <b>Figure S5.</b> The HPLC analysis result of the vitamin composition of <i>F. tikoua</i> fruit .....   | 14 |
| <b>Figure S6.</b> The result of the monosaccharide composition of <i>F. tikoua</i> fruit .....  | 15 |

## **Abbreviations**

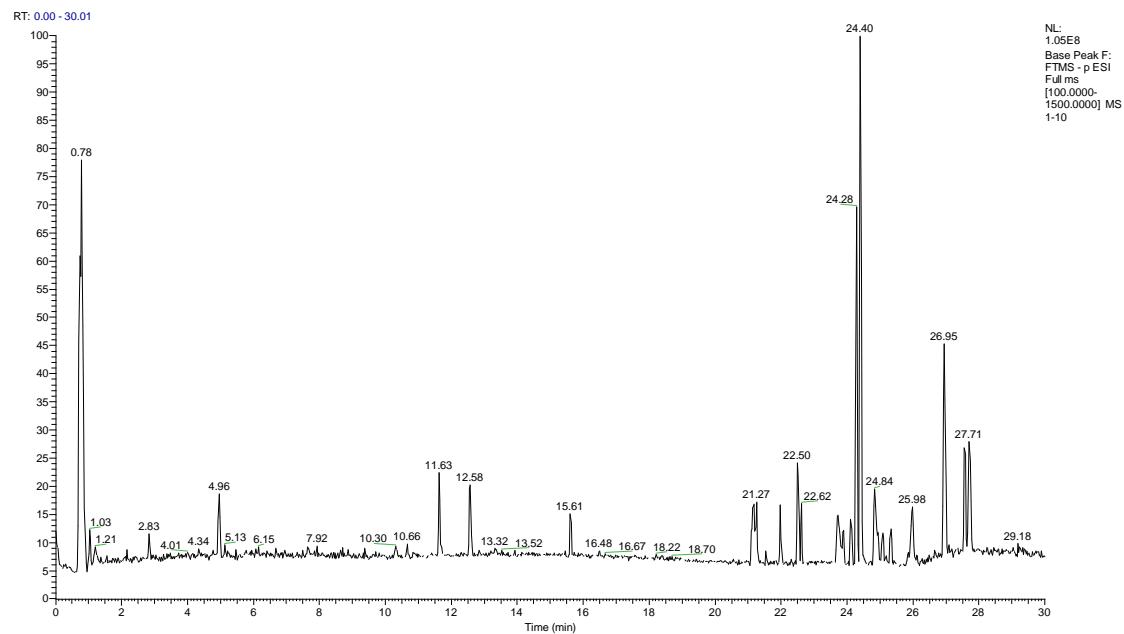
|                  |  |
|------------------|--|
| ABTS             | 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)     |
| Ara              | arabinose  |
| BCA              | bicinchoninic acid   |
| BSA              | bovine serum albumin                                       |
| DPPH             | 2,2-diphenyl-1-picrylhydrazyl radical                      |
| ECL              | enhanced chemiluminescence                                 |
| ELISA            | enzyme linked immunosorbent assay                          |
| FBS              | fetal bovine serum   |
| FRAP             | ferric reducing antioxidant power                          |
| Gal              | galactose  |
| GalA             | galacturonic acid  |
| GC-MS            | gas chromatography-mass spectrometry                       |
| Glc              | glucose  |
| GlcA             | glucuronic acid  |
| HPLC             | high performance liquid chromatography                     |
| HRP              | horseradish peroxidase                                     |
| IC <sub>50</sub> | half maximal inhibitory concentration                      |
| LC-MS            | liquid chromatography-mass spectrometry                    |
| LPS              | lipopolysaccharide   |
| Man              | mannose  |
| MTT              | 3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide |

|                  |                                |
|------------------|--------------------------------|
| NMR              | nuclear magnetic resonance     |
| NO               | nitric oxide                   |
| PGE <sub>2</sub> | prostaglandin E2               |
| PMP              | 1-phenyl-3-methyl-5-pyrazolone |
| PVDF             | poly vinylidene fluoride       |
| Rha              | rhamnose                       |
| SDS              | sodium dodecyl sulfate         |
| TFA              | trifluoroacetic acid           |
| TFC              | total flavonoids content       |
| TPC              | total polyphenols content      |
| TPTZ             | tri-2-pyridyl-s-triazine       |
| UV               | ultraviolet                    |
| Xyl              | xylose                         |

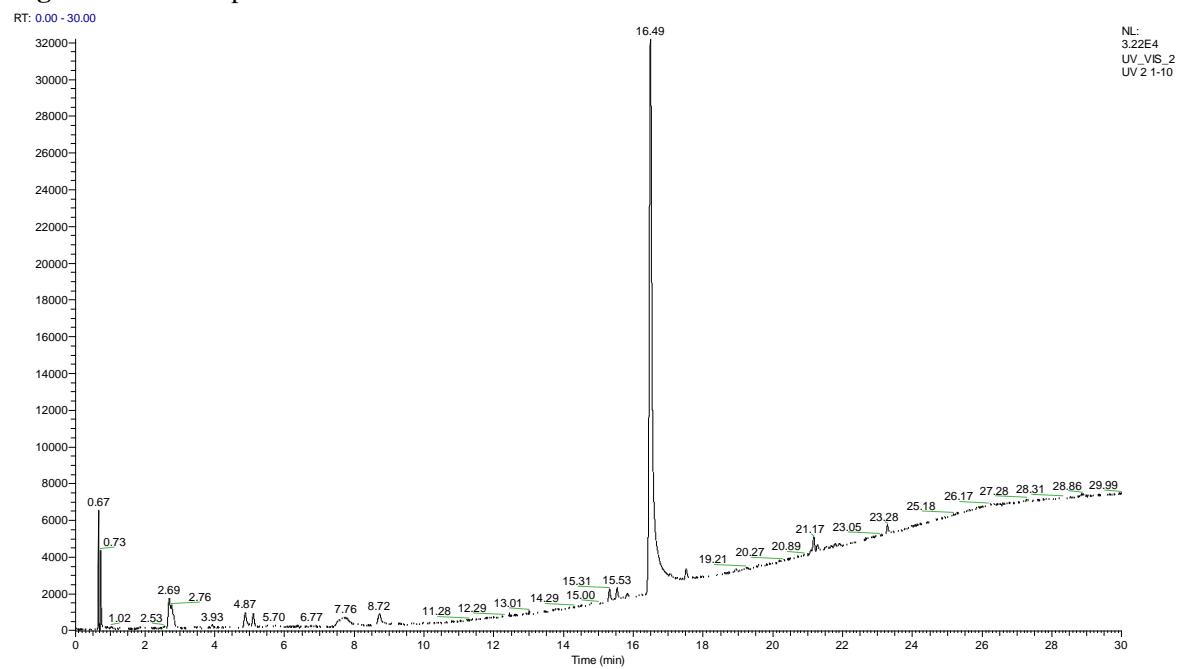
**Figure S1.** The picture of *Ficus tikoua* fruit.



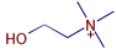
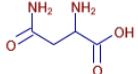
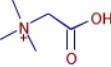
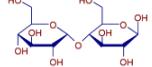
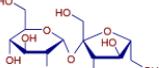
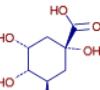
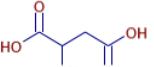
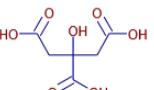
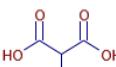
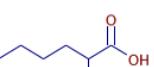
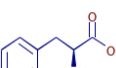
**Figure S2.** TIC LC-MS spectrum of the methanol extract of *F. tikoua* fruit.

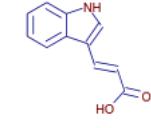
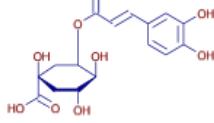
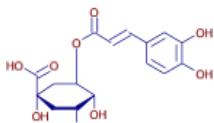
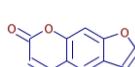
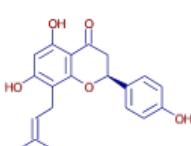
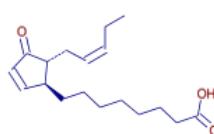
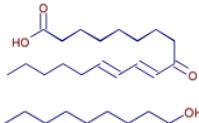
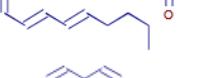
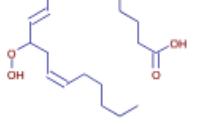
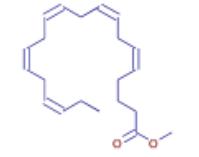
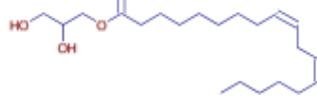
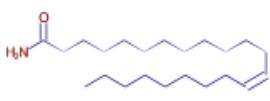


**Figure S3.** HPLC spectrum of the methanol extract of *F. tikoua* fruit.

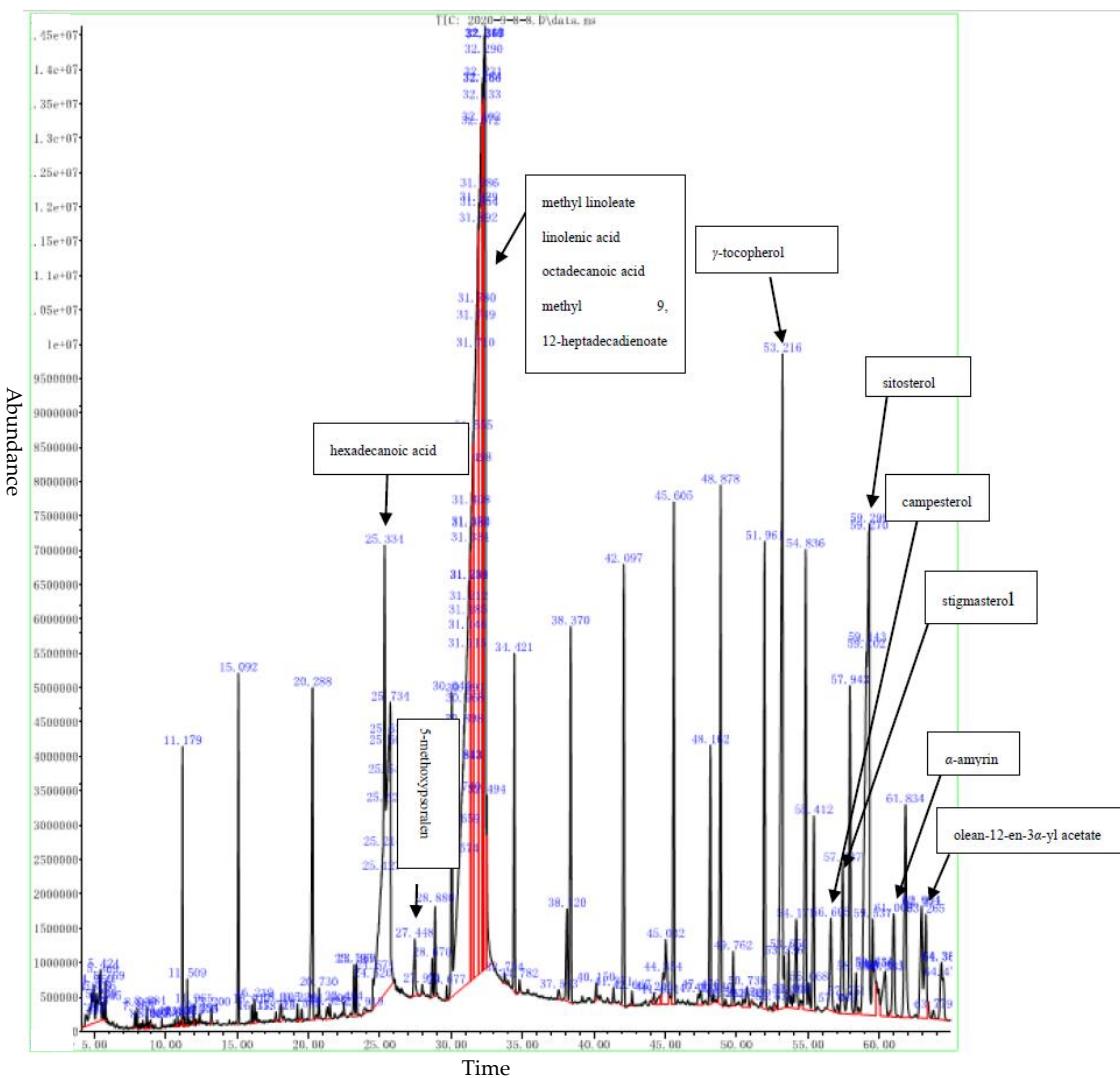


**Table S1.** Compounds identified by LC-MS from the methanol extract of *F. tikoua* fruit.

| No. | <i>t</i> <sub>R</sub><br>(min) | compound name             | molecular weight | molecular formula   | structure   |
|-----|--------------------------------|---------------------------|------------------|---|---|
| 1   | 0.7                            | choline                   | 103.1            | C <sub>5</sub> H <sub>13</sub> NO                           |    |
| 2   | 0.7                            | asparagine                | 132.1            | C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub> |    |
| 3   | 0.73                           | betaine                   | 117.1            | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>              |    |
| 4   | 0.74                           | <i>D</i> -(+)-proline     | 133.1            | C <sub>5</sub> H <sub>9</sub> NO <sub>2</sub>               |    |
| 5   | 0.74                           | <i>D</i> -(+)-maltose     | 364.1            | C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>             |    |
| 6   | 0.74                           | sucrose                   | 342.1            | C <sub>11</sub> H <sub>22</sub> O <sub>11</sub>             |    |
| 7   | 0.74                           | <i>D</i> -(-)-quinic acid | 192.1            | C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>               |   |
| 8   | 0.77                           | pipecolic acid            | 129.1            | C <sub>6</sub> H <sub>11</sub> NO <sub>2</sub>              |  |
| 9   | 0.79                           | <i>DL</i> -malic acid     | 134.0            | C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>                |  |
| 10  | 1.03                           | citric acid               | 192.0            | C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>                |  |
| 11  | 1.19                           | methylmalonic acid        | 118.0            | C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>                |  |
| 12  | 1.23                           | <i>DL</i> -norleucine     | 131.1            | C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>              |  |
| 13  | 2.06                           | <i>L</i> -phenylalanine   | 165.1            | C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>              |  |
| 14  | 2.84                           | 8-hydroxyquinoline        | 145.1            | C <sub>9</sub> H <sub>7</sub> NO                            |  |
| 15  | 2.84                           | 4-indolecarbaldehyde      | 145.1            | C <sub>9</sub> H <sub>7</sub> NO                            |  |

|    |       |  |       |  |   |
|----|-------|--|-------|--|---|
| 16 | 3.46  | indole-3-acrylic acid                                    | 187.1 | C <sub>11</sub> H <sub>9</sub> NO <sub>2</sub> |    |
| 17 | 5.57  | neochlorogenic acid                                      | 354.1 | C <sub>16</sub> H <sub>18</sub> O <sub>9</sub> |    |
| 18 | 6.44  | chlorogenic acid   | 354.1 | C <sub>16</sub> H <sub>18</sub> O <sub>9</sub> |    |
| 19 | 11.44 | psoralen   | 186.0 | C <sub>11</sub> H <sub>6</sub> O <sub>3</sub>  |    |
| 20 | 19.44 | 8-prenylnaringenin                                       | 340.1 | C <sub>20</sub> H <sub>20</sub> O <sub>5</sub> |    |
| 21 | 21.22 | 9S,13R-12-oxophytodienoic acid                           | 292.2 | C <sub>18</sub> H <sub>28</sub> O <sub>3</sub> |   |
| 22 | 21.82 | 9-oxo-10( <i>E</i> ),12( <i>E</i> )-octadecadienoic acid | 294.2 | C <sub>18</sub> H <sub>30</sub> O <sub>3</sub> |  |
| 23 | 21.99 | $\alpha$ -eleostearic acid                               | 278.2 | C <sub>18</sub> H <sub>30</sub> O <sub>2</sub> |  |
| 24 | 22.00 | (+/-)12-HpETE  | 318.2 | C <sub>20</sub> H <sub>32</sub> O <sub>4</sub> |  |
| 25 | 23.43 | eicosapentaenoic acid methyl ester                       | 322.2 | C <sub>21</sub> H <sub>32</sub> O <sub>2</sub> |  |
| 26 | 24.46 | 1-linoleoyl glycerol                                     | 354.3 | C <sub>21</sub> H <sub>38</sub> O <sub>4</sub> |  |
| 27 | 26.96 | erucamide  | 337.3 | C <sub>22</sub> H <sub>43</sub> NO             |  |
| 28 | 28.44 | docosanamide   | 339.3 | C <sub>22</sub> H <sub>45</sub> NO             |   |

**Figure S4.** TIC GC-MS spectrum of the hexane extract of *F. tikoua* fruit.



**Table S2.** Compounds identified by GC-MS from the hexane extract of *F. tikoua* fruit.

| <i>t<sub>R</sub></i> (min) | compound name  | CAS No.    | relative area % |
|----------------------------|--|------------|-----------------|
| 10.827                     | 2,6-di-tert-butylbenzoquinon                             | 719-22-2   | 0.02            |
| 11.507                     | 2,4-di-tert-butylphenol                                  | 96-76-4    | 0.1             |
| 13.198                     | <i>n</i> -hexadecane                                     | 544-76-3   | 0.03            |
| 16.017                     | <i>n</i> -heptadecane                                    | 629-78-7   | 0.05            |
| 18.106                     | tetradecanoic acid                                       | 544-63-8   | 0.09            |
| 19.227                     | octadecane   | 593-45-3   | 0.05            |
| 19.540                     | 6,10,14-trimethyl-2-pentadecanone                        | 502-69-2   | 0.1             |
| 21.323                     | pentadecanoic acid                                       | 1002-84-2  | 0.07            |
| 22.487                     | nonadecane   | 629-92-5   | 0.03            |
| 23.192                     | 7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione | 82304-66-3 | 0.18            |
| 23.370                     | hexadecanoic acid, methyl ester                          | 112-39-0   | 0.16            |
| 23.921                     | 16-methyl-oxacyclohexadecan-2-one                        | 4459-57-8  | 0.04            |
| 25.128                     | hexadecanoic acid  | 57-10-3    | 1.52            |
| 27.450                     | 5-methoxypsalen  | 484-20-8   | 0.3             |
| 27.971                     | 1-nonadecene   | 18435-45-5 | 0.03            |
| 28.670                     | methyl linoleate   | 112-63-0   | 0.13            |
| 28.878                     | linolenic acid, methyl ester                             | 301-00-8   | 0.33            |
| 29.675                     | methyl stearate  | 112-61-8   | 0.05            |
| 30.661                     | linoelaidic acid   | 506-21-8   | 0.57            |
| 30.845                     | linolenic acid   | 463-40-1   | 3.09            |
| 30.900                     | linolenyl alcohol  | 506-44-5   | 0.61            |
| 30.967                     | linoleic acid  | 60-33-3    | 0.84            |
| 31.378                     | methyl 9, 12-heptadecadienoate                           | 29305-60-0 | 0.38            |
| 32.493                     | octadecanoic acid  | 57-11-4    | 0.74            |
| 33.737                     | eicosane   | 112-95-8   | 0.04            |
| 34.785                     | phytane  | 638-36-8   | 0.04            |
| 37.542                     | tetracosane  | 646-31-1   | 0.06            |
| 40.152                     | pentacosane  | 629-99-2   | 0.06            |
| 44.852                     | 2-linoleoyl glycerol                                     | 3443-82-1  | 0.21            |
| 48.161                     | squalene   | 111-02-4   | 1.01            |
| 50.734                     | $\delta$ -tocopherol                                     | 119-13-1   | 0.14            |
| 53.216                     | $\gamma$ -tocopherol                                     | 7616-22-0  | 4.52            |
| 53.651                     | 1-docosene   | 1599-67-3  | 0.22            |
| 53.804                     | nonacos-1-ene  | 18835-35-3 | 0.04            |
| 56.604                     | campesterol  | 474-62-4   | 0.65            |
| 57.437                     | stigmasterol   | 83-48-7    | 0.95            |
| 59.104                     | sitosterol   | 83-46-5    | 5.36            |
| 59.539                     | 28-isofucosterol   | 481-14-1   | 0.48            |
| 61.010                     | $\alpha$ -amyrin   | 638-95-9   | 0.74            |

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|        |   |            |      |
|--------|---|------------|------|
| 62.952 | olean-12-en-3 $\alpha$ -yl acetate          | 33055-28-6 | 0.58 |
| 64.337 | urs-12-en-24-oic acid, 3-oxo-, methyl ester | 20475-86-9 | 0.26 |

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**Table S3.** Crude protein, amino acids, total polysaccharides, polyphenols, flavonoids, and vitamins contents of *F. tikoua* fruit.

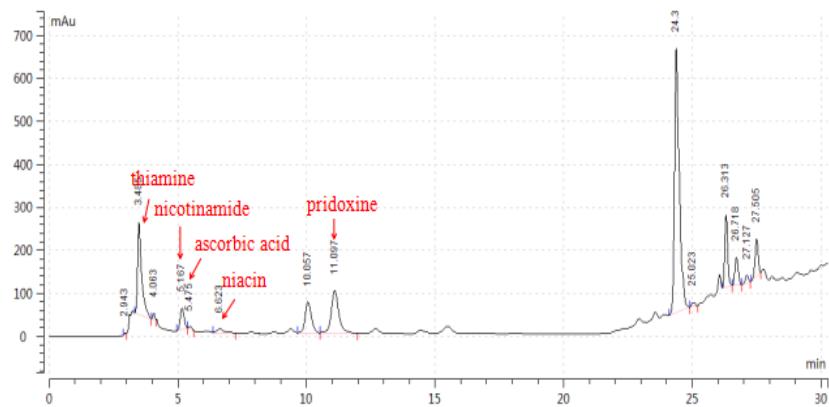
| phytochemical  | standard curve line | result                 |
|----------------|---------------------|------------------------|
| crude Protein  | -                   | 9.41 ± 0.03 (g/100g)   |
| ash            | -                   | 10.29 ± 0.08 (g/100g)  |
| vitamin        | -                   | -                      |
| pridoxine      | -                   | 66.67 ± 2.3 (mg/100g)  |
| thiamine       | -                   | 205.42 ± 5.4 (mg/100g) |
| riboflavin     | -                   | 0                      |
| ascorbic acid  | -                   | 10.00 ± 0 (mg/100g)    |
| cyanocobalamin | -                   | 0                      |
| niacin         | -                   | 3.20 ± 0.4 (mg/100g)   |
| folic acid     | -                   | 0                      |
| nicotinamide   | -                   | 15.40 ± 1.3 (mg/100g)  |
| amino acids    | -                   | 9.28%                  |
| threonine      | -                   | 0.35%                  |
| proline        | -                   | 0.51%                  |
| isoleucine     | -                   | 0.40%                  |
| leucine        | -                   | 0.69%                  |
| phenylalanine  | -                   | 0.48%                  |
| lysine         | -                   | 0.51%                  |
| aspartic acid  | -                   | 1.48%                  |
| serine         | -                   | 0.50%                  |

|                         |                               |                           |
|-------------------------|-------------------------------|---------------------------|
| glutamate               | -                             | 1.40%                     |
| alanine                 | -                             | 0.49%                     |
| glycine                 | -                             | 0.44%                     |
| cystine                 | -                             | 0.21%                     |
| methionine              | -                             | 0.12%                     |
| tyrosine                | -                             | 0.24%                     |
| histidine               | -                             | 0.35%                     |
| arginine                | -                             | 0.62%                     |
| proline                 | -                             | 0.47%                     |
| total polysaccharide    | $Y = 17.902x + 0.05, R^2 =$   | $1.25 \pm 0.04$ (g/100g)  |
| total polyphenols (TPC) | 0.9970                        | $0.86 \pm 0.01$ (g/100g)  |
| total flavonoids (TFC)  | $Y = 28.611x - 0.424, R^2 =$  | $8.00 \pm 0.11$ (mg/100g) |
|                         | 0.9951                        |                           |
|                         | $Y = 3.4967x - 0.0467, R^2 =$ |                           |
|                         | 0.9969                        |                           |

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Each value was expressed as mean  $\pm$  SE ( $n = 3$ ).

**Figure S5.** The HPLC analysis result of the vitamin composition of *F. tikoua* fruit.



**Figure S6.** The result of the monosaccharide composition of *F. tikoua* fruit. (A) The result of the HPLC spectrum of the PMP-labeled monosaccharide standard. (B) The result of the monosaccharide composition of *F. tikoua* fruit.

