Identification of Secondary Metabolite Compounds in Two Varieties of Young Winged Beans (*Psophocarpus tetragonolobus* L.) at Two Harvest Ages

Destia Susanti S\(^A\), Maya Melati*\(^B\), Ani Kurniawati\(^B\)

\(^A\) Study Program of Agronomy and Horticulture, Graduate School, IPB University, Jl. Meranti, Campus of IPB Dramaga Bogor 16680, West Java, Indonesia
\(^B\) Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, Campus of IPB Dramaga Bogor 16680, West Java, Indonesia

* Corresponding author; email: maya_melati@apps.ipb.ac.id

Abstract

The winged bean (*Psophocarpus tetragonolobus* L.) is a member of the *Fabaceae* family (beans). Winged bean is developed as a vegetable, and young winged bean pods can be consumed fresh, steamed, fried, or pickled. In Southeast Asia, winged bean pods are generally cooked or consumed as side dishes or salad. Winged bean contains high protein content equivalent to soybean seed. Besides being rich in protein, vitamins, and minerals, the winged bean also has secondary metabolites, including phenolics and flavonoids. This study aimed to identify secondary metabolites in young pods of two varieties, “Fairuz” and “Sandi”, at two different harvest ages of 8 and 10 days after anthesis (DAA) and provide the biological activity on each identified compound. The experiment was conducted at the IPB experimental field at Leuwikopo, Bogor, from September 2020 to February 2021. The identification of secondary metabolites of the young green pods “Fairuz”, and the young purple pods “Sandi”, was performed using the GCMS method at the Regional Health Laboratory (KESDA) DKI Jakarta. The results of the GCMS analysis showed that 1,2-Benzedicarboxylic acid, mono (2-Ethylhexyl) ester was the most abundant compound identified from pods harvested at 8 DAA in both varieties, namely 42.26% in “Fairuz” and 26.66% in “Sandi”. Other compounds, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z) (Linoleic acid ester), were found in “Fairuz”, whereas (9E,12E)-9,12-Octadecadienoic acid (Linoleic acid) was found in “Sandi”; these compounds are hydroxyl group and phenolic glucoside compounds and are found in pods harvested at 10 DAA.

Keywords: antioxidant, biological activity, days after anthesis, green pods, purple pods

Introduction

Winged bean (*Psophocarpus tetragonolobus* L.) is a member of the *Fabaceae* family (beans). Winged beans thrive in hot and humid tropical regions (Mohanty et al., 2013). India, Thailand, Papua New Guinea, and Indonesia (Khan, 1976; Krisnawati, 2016). Winged bean seed contains high protein content equivalent to soybean plants and can be substituted for soybean needs (Amoo et al., 2006; Handayani, 2013; Alalade et al., 2016). Winged bean has much potential as a protein source because, in addition to stems and roots, almost all parts of the plant, including young leaves, flowers, young pods, dried seeds, and tubers, can be consumed (Tanzi et al., 2019; Mohanty et al., 2020). Young winged pods can be consumed fresh, steamed, fried, or pickled, and in Southeast Asia, winged bean pods are generally cooked or consumed as a side dish or salad (Sriwichai et al., 2021). Therefore, winged bean has great potential to be developed as a useful legume crop (Handayani, 2013).

Young winged bean pods contain 3.99% carbohydrates, 0.16% fat, and 93.50% water content (Pangestu, 2020). Young pods contain around 6.1% albumin, globulin, and glutelin, and have a high lysine content, so young pods contain good quality protein compared to other pods (Tadera et al., 1984). Besides being rich in protein, vitamins, and minerals, winged beans also contain secondary metabolites, including phenolics and flavonoids (Singh et al., 2019), and have high tannin content (Mohanty et al., 2013). Immature winged bean pods contain amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, tryptophan, and valine. The pods also contain 5.0% sucrose, 1.0% raffinose, 2.5% stachyose (NRC, 1981). Traditionally, winged beans can be used to prevent cancer, diabetes,
asthma, muscle weakness, overcome migraines, eye diseases, and strengthen the immune system (Singh et al., 2019). The total antioxidant capacity ranges from 1,278 to 1,806 mg AsA/g so winged beans can serve as a good source of antioxidants for humans and animals (Olaiya et al., 2018).

From the many chemical components ingredients in winged bean, studies related to the complete profile of secondary metabolites is not widely known, particularly of the newly developed varieties. Therefore, this study was carried out to investigate the secondary metabolites' profile in young pods of the winged beans. Gas Chromatography-Mass Spectrophotometry (GCMS) was used to analyze the range of secondary metabolites in winged beans. GCMS is one of the best techniques for identifying the constituents of volatiles, long-chain, hydrocarbons, branched-chain, alcohols, acids, and esters (Sermakkani dan Thangapandian, 2012). GCMS has successfully identified the bioactive compounds present in many plant species (Karthikeyan et al., 2019).

There are two new varieties of winged beans, namely “Fairuz” (green pods) and “Sandi” (purple pods). The color difference can indicate the difference in the phytochemical compounds contained in the pods. The phytochemical compounds can also be different from pods harvested at various ages. This study aims to identify phytochemical compounds in young pods of winged bean and compare the results between the two harvest ages 8 and 10 days after anthesis (DAA).

Material and Methods

Experimental Site and Materials

The experiment was conducted at the IPB experimental field Leuwikopo, Bogor, West Java, Indonesia, from September 2020 to February 2021. The analysis of secondary metabolites in winged bean pods was conducted at the Regional Health Laboratory (KESDA), DKI Jakarta.

Two winged bean varieties were used to identify the secondary metabolites in young pods, namely “Fairuz” (green pod) and “Sandi” (purple pod). The young pods were harvested 8 or 10 days after anthesis (DAA).

Sample Preparation

Sample preparation was carried out before injecting test substances into each tool. Samples were dried in an oven at 60°C for approximately 3 hours or completely dry.

Sample Extraction

The dried samples were mashed using a blender and then macerated with methanol for 5x 24 hours. Each macerated sample was then put into a 10 ml tube using a pipette and dried for 1 hour at 60°C. After drying, it was dissolved with the remaining 200 µl of the macerated extract, followed by chemical analysis using a Gas Chromatography-Mass Spectrometer (GCMS).

Analysis of Secondary Metabolites using GCMS

The analysis procedure conducted by the Regional Health Laboratory followed the Research Institute for Spices and Medicinal Plants method (Utami et al., 2017). The GCMS was used to analyze the content of secondary metabolites in the Agilent 7890 gas chromatography technology with automatic sampling and 5975 mass selective detectors and data systems. A total of 5 µl samples of young winged pods was injected into the GCMS, which has a capillary column size of HP Ultra 2 with a length of 30 m x 0.20 mm x 0.11 m film thickness. The oven temperature was set at an initial temperature of 80°C, then rose at 3°C/min to 150°C, held for 1 minute, and finally increased 20°C/min to 280°C and held for 26 minutes. The injection temperature was 250°C, the ion source temperature was 230°C, the interface temperature was 280°C, and the quadrupole temperature was 140°C. Helium gas was used as the carrier gas at a constant flow rate of 1.2 mL/min (8:1 split).

Result and Discussion

GCMS had successfully identified the secondary metabolites or phytochemicals of the winged bean pods (Figure 1, 2). Thirteen (13) 13 different compounds were identified in “Fairuz” harvested at 8 and 10 DAA, and 17 and 15 compounds identified in “Sandi” harvested at 8 and 10 DAA, respectively.

Identification of the secondary metabolites was confirmed based on the peak area, retention time (RT), and molecular formula. The retention time depends on the molecular weight of the compounds; our samples had the retention times of 20-30 minutes. In “Fairuz” harvested at 8 DAA, the first compound with a lower retention time was Hexadecanoic Acid, Ethyl Ester (27.831 minutes). In contrast, the last phytochemical compound identified that required the longest retention time was gamma-Sitosterol (37.084 minutes). Pods harvested at 10 DAA had the lowest retention time was tetracosenoic acid (27.162
Figure 1. Chromatography of GCMS on young green pods ("Fairuz") harvested at 8 days (A) and 10 days (B) after anthesis
Figure 2. Chromatography of GCMS on young purple pods (“Sandi”) harvested at 8 days (A) and 10 days (B) after anthesis.

The phytochemical compounds identified in “Fairuz” and “Sandi” from two harvest dates have biological activity. Therefore, “Fairuz” and “Sandi” are rich sources of secondary metabolites with biological activities (Konovalova et al., 2013).

Two phytochemical compounds were identified in “Fairuz” harvested at 8 DAA with the highest content of >10% (Table 3). The first was 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester (42.26%), a phthalate group chemical. This compound has antifungal, anticancer, immunomodulatory, and antimicrobial activity (Ezhilan dan Neelamegam, 2012; Save et al., 2015; Prawanayoni dan Sudirga, 2020). The
Table 1. Secondary metabolites identified in “Fairuz” harvested at 8 and 10 days after anthesis (DAA) with an abundance >1%

| No | Compound                                                                 | Molecular formula  | Molecular weight (g.mol$^{-1}$) | 8 DAA* Retention time | Peak area % | 10 DAA* Retention time | Peak area % |
|----|--------------------------------------------------------------------------|--------------------|---------------------------------|-----------------------|-------------|------------------------|-------------|
| 1  | TETRADECANOIC ACID                                                      | C$_{14}$H$_{28}$O$_{2}$ | 228.37                          | -                     | -           | 27.162                 | 1.33        |
| 2  | HEXADECANOIC ACID, ETHYL ESTER                                          | C$_{16}$H$_{36}$O$_{2}$ | 284.5                           | 27.831                | 3.97        | 27.748                 | 5.13        |
| 3  | HEXADECANOIC ACID                                                       | C$_{16}$H$_{32}$O$_{2}$ | 256.42                          | -                     | -           | 28.327                 | 6.05        |
| 4  | n-Hexadecanoic acid                                                     | C$_{16}$H$_{32}$O$_{2}$ | 256.42                          | 28.396                | 7.46        | -                      | -           |
| 5  | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)                     | C$_{20}$H$_{34}$O$_{2}$ | 306.5                           | -                     | -           | 28.968                 | 27.53       |
| 6  | ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE                            | C$_{20}$H$_{34}$O$_{2}$ | 306.5                           | 29.010                | 13.69       | -                      | -           |
| 7  | (9E,12E)-9,12-OCTADECADIENOIC ACID                                       | C$_{18}$H$_{32}$O$_{2}$ | 280.4                           | 29.430                | 9.78        | 29.396                 | 14.30       |
| 8  | 9,12-Octadecadienoic acid (Z,Z)-                                       | C$_{18}$H$_{32}$O$_{2}$ | 280.4                           | -                     | -           | 29.865                 | 5.38        |
| 9  | 14-METHYL-8-HEXADECY-1-OL                                               | C$_{17}$H$_{32}$O     | 252.4                           | 29.975                | 2.87        | -                      | -           |
| 10 | (R)-(Z)-14-Methyl-8-hexadecen-1-ol                                       | C$_{17}$H$_{34}$O     | 254.5                           | 30.244                | 1.58        | -                      | -           |
| 11 | 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester                  | C$_{16}$H$_{32}$O$_{4}$ | 278.35                          | 30.872                | 42.26       | -                      | -           |
| 12 | [DIMETHYLSILYL]METHYL(DIMETHYL) SILANE                                   | C$_{4}$H$_{14}$Si$_{2}$ | 130.33                          | -                     | -           | 30.927                 | 9.27        |
| 13 | 2-CHLOROETHYL (9Z,12Z)-9,12-OCTADECADIENOATE                             | C$_{20}$H$_{30}$ClO$_{2}$ | 342.9                           | -                     | -           | 31.375                 | 2.49        |
| 14 | Geranylgeraniol                                                         | C$_{20}$H$_{34}$O     | 290.5                           | -                     | -           | 31.761                 | 12.98       |
| 15 | i-Propyl 9,12-octadecadienoate                                           | C$_{21}$H$_{38}$O$_{2}$ | 322.5                           | 31.768                | 5.39        | -                      | -           |
| 16 | Z,Z-10,12-Hexadecadien-1-ol acetate                                     | C$_{18}$H$_{32}$O$_{2}$ | 2804                            | 32.058                | 1.52        | -                      | -           |
| 17 | PENTACOSANE                                                              | C$_{30}$H$_{62}$      | 352.7                           | 32.258                | 1.63        | -                      | -           |
| 18 | .gamma.-Tocopherol                                                      | C$_{28}$H$_{48}$O$_{2}$ | 416.7                           | 33.775                | 1.83        | -                      | -           |
| 19 | Stigmasterol                                                            | C$_{29}$H$_{50}$O     | 412.7                           | 36.243                | 3.99        | 35.967                 | 3.99        |
| 20 | .gamma.-Sitosterol                                                      | C$_{29}$H$_{50}$O     | 414.7                           | 37.084                | 2.36        | 36.746                 | 3.18        |
| 21 | Pyrene, hexadecahydro-                                                 | C$_{16}$H$_{36}$      | 218.38                          | -                     | -           | 37.174                 | 1.10        |

Note: *DAA= days after anthesis
Table 2. Secondary metabolites identified in winged bean “Sandi” harvested at 8 and 10 days after anthesis (DAA) with an abundance >1%

| No | Compound                                                                 | Molecular formula | Molecular weight (g.mol⁻¹) | 8 DAA* Retention time | Peak area % | 10 DAA* Retention time | Peak area % |
|----|--------------------------------------------------------------------------|-------------------|-----------------------------|------------------------|-------------|-------------------------|-------------|
| 1  | 2,5-FURANDIONE                                                          | C₆H₈O₄            | 144.12                      | 3.794                  | 9.60        |                         |             |
| 2  | 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE                         | C₅H₈NO            | 126.12                      | -                      | 22.459      |                         | 6.50        |
| 3  | 1H-Imidazole-4-carboxamide, 5-amino- $$ Imidazole-4-carboxamide, 5-amino- | C₁₇H₁₄O₃          | 194.18                      | -                      | 27.452      |                         | 13.77       |
| 4  | 1H-Imidazole-4-carboxamide, 5-amino- $$ Imidazole-4-carboxamide, 5-amino- | C₁₇H₁₄O₃          | 284.5                        | 27.845                | 27.721      |                         | 2.22        |
| 5  | ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE                             | C₆H₁₀O₃           | 306.5                        | -                      | 28.893      |                         | 3.92        |
| 6  | Linoleic acid ethyl ester                                                | C₁₈H₃₂O₂          | 280.4                        | 29.038                | 29.293      |                         | 20.02       |
| 7  | ETHYL (9E,12E)-9,12-OCTADECADIENOIC ACID                                  | C₁₈H₃₂O₂          | 280.4                        | 29.437                | 29.293      |                         | 20.02       |
| 8  | 1,2-ETHYLENEDIAMINE, N,N-DIMETHYL-N’-2-PYRIDINYL-N’-(2-PHENYLMETHYL)-      | C₁₄H₂₀CIN₃S       | 297.8                        | 29.748                | -           |                         | -           |
| 9  | 1,2-ETHYLENEDIAMINE, N,N-DIMETHYL-N’-2-PYRIDINYL-N’-(2-PHENYLMETHYL)-      | C₁₄H₂₀CIN₃S       | 280.4                        | 29.982                | -           |                         | -           |
| 10 | (9E,12E)-9,12-OCTADECADIENOIC ACID                                       | C₁₈H₃₂O₂          | 280.4                        | 30.244                | 30.313      |                         | 2.57        |
| 11 | 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester                   | C₆H₁₂O₂            | 182.30                       | 30.596                | 31.623      |                         | 1.80        |
| 12 | 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester                   | C₆H₁₂O₂            | 278.35                       | 30.865                | -           |                         | -           |
| 13 | 4-(4-ETHYL-CYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE                             | C₁₉H₃₄             | 262.5                        | 30.927                | -           |                         | 7.32        |
| 14 | 4-(4-ETHYL-CYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE                             | C₁₉H₃₄             | 262.5                        | 31.534                | -           |                         | 1.51        |
| 15 | 14-HYDROXY-20-OXOPREGNAN-3-YL ACETATE                                    | C₁₈H₃₂O₂          | 280.4                        | 31.444                | -           |                         | -           |
| 16 | 14-HYDROXY-20-OXOPREGNAN-3-YL ACETATE                                    | C₁₈H₃₂O₂          | 396.7                        | 31.782                | -           |                         | 1.55        |
| 17 | 14-HYDROXY-20-OXOPREGNAN-3-YL ACETATE                                    | C₁₈H₃₂O₂          | 410.7                        | 31.906                | -           |                         | 1.10        |
| 18 | 1-CINNAMYL-3-METHYLINDOLE-2-CARBALDEHYDE                                  | C₁₀H₉NO            | 159.18                       | 32.044                | -           |                         | 1.19        |
| 19 | ICOSANE                                                                  | C₂₀H₄₂            | 282.5                        | 32.237                | -           |                         | 1.19        |
| 20 | Stigmasteryl tosylate                                                    | C₃₀H₅₀            | 566.9                        | 33.402                | -           |                         | 3.05        |
| 21 | Ethyl tetracosanoate                                                     | 416.7             | 33.747                       | 2.12                  | -           |                         | -           |
| 22 | Squalene                                                                 | C₁₈H₃₂O₂          | 414.7                        | 36.326                | 35.809      |                         | 2.43        |
| 23 | 1-CINNAMYL-3-METHYLINDOLE-2-CARBALDEHYDE                                  | C₁₀H₉NO            | 159.18                       | 32.044                | -           |                         | 1.19        |
| 24 | Icosane                                                                  | C₂₀H₄₂            | 282.5                        | 32.237                | -           |                         | 1.19        |
| 25 | Stigmasteryl tosylate                                                    | C₃₀H₅₀            | 566.9                        | 33.402                | -           |                         | 3.05        |
| 26 | .gamma.-Tocopherol                                                       | C₁₉H₄₈O₂          | 414.7                        | 36.326                | 35.809      |                         | 2.43        |
| 27 | STIGMAST-5-EN-3-OL                                                       | C₂₉H₄₈O₂          | 414.7                        | 37.250                | 4.56        |                         | -           |
| 28 | Stigmasterol                                                             | C₂₉H₄₈O₂          | 414.7                        | 37.250                | 4.56        |                         | -           |
| 29 | .gamma.-Sitosterol                                                       | C₂₉H₄₈O₂          | 414.7                        | 37.250                | 4.56        |                         | -           |

Note: *DAA= days after anthesis
second compound is Ethyl (9Z, 12Z, 15Z)-9,12,15-Octadecatrienoic acid (13.69%), which belongs to the linolenic acid group. This compound has biological activity as anti-inflammatory, hypocholesterolemic, cancer preventive, antiasthmatic, antiacne, antiarthritic, and anticoronary products (Kumar et al., 2010; Starlin et al., 2019).

There were three significant compounds in “Fairuz” pods harvested at 10 DAA with abundance > 10% (Table 4). The predominant one was 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z) (27.53%). These compounds belong to the Linoleic acid ester compound group and have hypocholesterolemic, antiinflammatory, antiandrogenic, anticoronaory, and anticancer activity. They can also be used as a nematicide and insect repellent (Mamani dan Alhaji, 2019). The compound (9E,12E)-9,12-Octadecadienoic acid (14.30%), belongs to the Linoleic acid group, has the biological activity as anti-inflammatory, hypocholesterolemic, antiasthmatic, antiarthritis, and antiaacne product (Sermakkani dan Thangapandian, 2012). The third compound is Geranygeraniol (12.98%), which belongs to the chioroform group of compounds, has antiacne and antimycobactarial activity (Vik et al., 2007; Sianipar dan Purnamaningsih, 2018).

Similar phytochemical compounds to “Fairuz” variety harvested at 8 DAA are also found in “Sandi” variety harvested at 8 DAA above 10% (Table 5). The phytochemical compound with the highest content was 1,2-Benzenedicarboxylic acid mono(2-Ethylhexyl) ester (26.66%). The second most important product was Linoleic acid ethyl ester (15.30%), which has antiinflammatory, hypocholesterolemic, antiasthmatic, antiarthritis, antiangicemic activities, antiandrogeneric, and anticoronaory compounds (Sermakkani dan Thangapandian, 2012; Sudha et al. 2013). Furthermore (9E,12E)-9,12-Octadecadienoic acid (11.57), which belongs to the linoleic acid group, his activity as an anti-inflammatory, hypocholesterolemic, antiasthmatic, antiarthritis, and antiaacne products (Sermakkani dan Thangapandian, 2012).

Secondary metabolite compounds were abundant in the “Sandi” harvested at 10 DAA, i.e. > 10% (Table 6). The phytochemical compound with the highest content was (9E,12E)-9,12-Octadecadienoic acid (20.02), as found in “Sandi” variety with pods harvested at8 DAA. The next important phytochemical compound was 2,3-dihydroxy-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One (13.97%), which belongs to the hydroxyl group, with activity as an antioxidant (Li et al., 2019). Furthermore, alpha-D-Glucopyranoside, methyl (13.77%), which belongs to the phenolic glucoside group, has apoptotic activity, anticancer, antituberculosis, antioxidant, alpha-amylase inhibitory activity, and as an anticonvulsant (Lyantagaye, 2013; Uchegbu et al., 2017).

The abundance distribution of identified secondary metabolites from these two varieties of winged bean at two harvest ages is shown in the heatmap. The heatmap was based on the distribution of phytochemical with an abundance > 1% (Figure 3). The highest content of secondary metabolite compound from pods harvested at 8 DAA from these two varieties was 1,2-Benzenedicarboxylic acid mono (2-Ethylhexyl) ester. In contrast, at 10 DAA harvest age, the highest abundance compound was 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) in the “Fairuz” and (9E,12E)-9,12-Octadecadienoic acid in the “Sandi”.

Discussion

From the results of the GCMS analysis, it has been shown that the identified compounds in the two winged bean varieties and at harvest ages are very diverse. The compounds with the highest content found in both varieties at each harvest were derived from the linoleic acid compound group of fatty acids. These compounds have biological activity as anti-inflammatory, hypocholesterolemic, antiasthmatic, antiarthritic, antiaacne products, as well as nematicidal and insecticidal properties (Sermakkani dan Thangapandian, 2012).

It has been shown that the dominant secondary metabolites, with abundance > 10%, was 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester, Ethyl (9Z,12Z,15Z)-9,12,15-Octadecatrienoate, Linoleic acid ethyl ester, (9E,12E)-9,12-Octadecadienoic acid, Geranygeraniol, 9,12,15-Octadecatrienoic acid, ethyl ester (Z, Z, Z), 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One, alpha-D-Glucopyranoside, methyl. Therefore, these compounds can be used as specific compounds in winged beans, especially in young pods.

There are compounds in the “Sandi” harvested at 10 DAA that were not found in those harvested at 8 DAA, or in the “Fairuz” harvested at 8 and 10 DAA. These compounds are from the hydroxyl group and phenolic glucoside, with one of their activities being as antioxidants (Uchegbu et al., 2017; Li et al., 2019). “Sandi” has a purple pod, and the color is darker when harvested at 10 DAA than it is at 8 DAA. The darker color can indicate higher content of metabolite compounds. Previous studies reported that purple, red or blue fruits, and vegetables generally contain anthocyanin pigments (Juansah et al., 2013; Reswari
| No | Retention time | Peak area % | Compound | Compound group | Activity | Reference |
|----|----------------|-------------|----------|----------------|----------|-----------|
| 1  | 30.872         | 42.26       | 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester | Phthalate | Antifungal, Anticancer, Immunomodulatory activities, Antimicrobial | (Ezhilan dan Neelamegam 2012; Save et al., 2015; Prawanayoni dan Sudirga 2020) |
| 2  | 29.010         | 13.69       | ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE | Linolenic acid | Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Nematicide, Insectfuge, Antithistaminic, Antiacne, Antiarthritic, Anticoronary | (Kumar et al., 2010; Starlin et al., 2019) |
| 3  | 29.430         | 9.78        | 9,12-Octadecadienoic acid (Z,Z)- | Linoleic acid | Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectfuge, Antithistaminic, Antiarthritic, Antiacne | (Sermakkani and Thangapandian, 2012) |
| 4  | 28.396         | 7.46        | n-Hexadecanoic acid | Palmitic acid | Antioxidant, Nematicide, Pesticide, Antiarboigenic, Flavor, Anti-inflammatory | (Aparna et al., 2012; Sermakkani and Thangapandian, 2012; Korbecki and Bajdak-Rusinek, 2019) |
| 5  | 31.768         | 5.39        | i-Propyl 9,12-octadecenadienoate | - | - | - |
| 6  | 36.243         | 3.99        | Stigmasterol | Steroid | Antimicrobial, Anticancer, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant | (Sudha et al., 2013; Mary and Giri, 2016) |
| 7  | 27.831         | 3.97        | HEXADECANOIC ACID, ETHYL ESTER | Ester | Antioxidant, Flavor, Nematicide, Pesticide, Antiarboigenic | (Sermakkani and Thangapandian, 2012) |
| 8  | 29.975         | 2.87        | 14-METHYL-8-HEXADECYN-1-OL | - | Sclerosant, Spasmolytic, Catechol-O-Methyl-Transferase-Inhibitor, 5-Alpha-Reductase-Inhibitor, Free-Radical Scavenging | (Karthikeyan et al., 2019; Payum, 2020) |
| 9  | 37.084         | 2.36        | .gamma.-Sitosterol | Steroid | Antidiabetic, Anti-aging, Anticancer, Antimicrobial, Anti-inflammatory, Antiviral | (Tripathi et al., 2013a; Tripathi et al., 2013b; Nisha and Rao, 2018) |
| 10 | 33.775         | 1.83        | .gamma.-Tocopherol | - | Antioxidant | (Falk and Munné-Bosch, 2010) |
| 11 | 32.258         | 1.63        | PENTACOSANE | - | - | - |
| 12 | 30.244         | 1.58        | (R)-(·)-(Z)-14-Methyl-8-hexadecen-1-ol acetate | - | Hypoglycemic, Hycholesterolemic, Antioxidant, Anticancerous | (Jain and Rijhwani, 2018) |
### Table 4. Secondary metabolites and biological activity of winged bean “Fairuz” harvested at 10 days after anthesis (DAA)

| No | Retention time | Peak area % | Compound | Compound group | Activity | Reference |
|----|----------------|-------------|----------|----------------|----------|-----------|
| 1  | 28.968         | 27.53       | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) | Linoleic acid ester | Hypcholesterolemic, Nematicide Antiarthritic, Antiandrogenic, Antiinflammatory, Anticancer | (Mamani dan Alhaji, 2019) |
| 2  | 29.396         | 14.30       | (9E,12E)-9,12-Octadecadienoic acid | Linoleic acid | Anti-inflammatory, Hypcholesterolemic, Nematicide, Insectifuge, Antiasthmatic, Antiarthritic, Antiacne | (Sermakkani dan Thangapandian, 2012) |
| 3  | 31.761         | 12.98       | Geranylgeraniol | Chloroform | Anticancer, Antimycobacterial | (Vik et al., 2007; Sianipar and Purnamaningsih, 2018) |
| 4  | 30.927         | 9.27        | [(DIMETHYLSILYL) METHYL](DIMETHYL) SILANE | - | - | - |
| 5  | 28.327         | 6.05        | HEXADECANOIC ACID | Palmitic acid | Antioxidant, Nematicide, Pesticide, Antiandrogenic, Flavor, Anti-inflammatory | (Sermakkani and Thangapandian, 2012; Korbecki and Bajdak-Rusinek, 2019) |
| 6  | 29.865         | 5.38        | 9,12-Octadecadienoic acid (Z,Z)- | Linoleic acid | Anti-inflammatory, Hypcholesterolemic, Nematicide, Insectifuge, Antiasthmatic, Antiarthritic, Antiacne | (Sermakkani and Thangapandian, 2012) |
| 7  | 27.748         | 5.13        | HEXADECANOIC ACID, ETHYL ESTER | Ester Compound | Antioxidant, Flavor, Nematicide, Pesticide, Antiandrogenic | (Sermakkani and Thangapandian, 2012) |
| 8  | 35.967         | 3.99        | Stigmasterol | Steroid | Antimicrobial, Anticancer, Antiarthritic, Antiasthmatic, Diuretic, Anti-inflammatory, Antioxidant | (Sudha et al., 2013; Mary and Giri, 2016) |
| 9  | 30.348         | 3.35        | (9E,12E)-9,12 OCTADECADIENOYL CHLORIDE | Chlorinated aliphatic ketone | Anti-inflammatory, Antiarthritic, Antidiabetic, hypolipidemic, Cytotoxic activities | (Sreeja et al., 2018) |
| 10 | 36.746         | 3.18        | .gamma.-Sitosterol | Steroid | Antidiabetic, Anti-aging, Anticancer, Antimicrobial, Anti-inflammatory, Antiviral | (Tripathi et al., 2013a; Tripathi et al., 2013b; Nisha dan Rao, 2018) |
| 11 | 31.375         | 2.49        | 2-CHLOROETHYL (9Z,12Z)-9,12-OCTADECADIENOATE | Fatty acid | Anticancer, Antioxidant | (Berwal et al., 2019) |
| 12 | 27.162         | 1.33        | TETRADECANOIC ACID | Myristic acid | Antioxidant, Cancer preventive, Nematicide, Hypcholesterolemic | (Rajeswari et al., 2013) |
| 13 | 37.174         | 1.10        | Pyrene, hexadecahydro- | - | Dyes and plasticisers precursor, Biodegradation of organic compounds | (Wei et al., 2015; Salim 2018) |
| No | Retention time | Peak area % | Compound | Compound group | Activity | Reference |
|----|----------------|-------------|----------|----------------|----------|-----------|
| 1  | 30.865         | 26.66       | 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester | Phthalate | Anti-fungal, Anticancer, Immunomodulatory activities, Antimicrobial | (Ezhilan and Neelamegam, 2012; Savé et al., 2015; Prawanayoni and Sudinga, 2020) |
| 2  | 29.038         | 15.30       | Linoleic acid ethyl ester | Linoleic acid ethyl ester | Anti-Inflammatory, Hypcholesterolemic, Nematicide, Insecticidgel, Antihistaminic, Antiarthritic, Anti-inflammatory, Antimicrobial | (Sermakkani and Thangapandian, Thangapandian, 2012) |
| 3  | 29.437         | 11.57       | (9E,12E)-9,12-Octadecadienoic acid | Linoleic acid | Nematicide, Insecticide, Antihistaminic, Anti-inflammatory, Anti-anaphylactic activity | (Sermakkani and Thangapandian, 2012) |
| 4  | 28.451         | 7.91        | HEXADECANOIC ACID | Palmitic acid | Antioxidant, Nematicide, Pesticide, Anti-drogogenic, Flavor, Anti-inflammatory | (Aparna et al., 2012; Sermakkani and Thangapandian, 2012; Korbecki and Bajdak-Rusinek, 2019) |
| 5  | 27.845         | 7.82        | HEXADECANOIC ACID, ETHYL ESTER | Ester | Antioxidant, Flavor, Nematicide, Pesticide, Anti-drogogenic | (Sermakkani and Thangapandian, 2012) |
| 6  | 36.326         | 4.72        | Stigmasterol | Steroid | Antiasthma, Diuretic, Anti-inflammatory, Antioxidant | (Sudha et al. 2013; Mary and Giri, 2016) |
| 7  | 37.250         | 4.56        | .gamma.-Sitosterol | Steroid | Antidiabetic, Anti-aging, Anticancer, Anti-inflammatory, Antiviral | (Tripathi et al., 2013a; Tripathi et al., 2013b; Nisha dan Rao, 2018) |
| 8  | 29.982         | 2.94        | (9E,12E)-9,12-Octadecadienoic acid | Linoleic acid | Nematicide, Insecticide, Antihistaminic, Anti-inflammatory | (Sermakkani and Thangapandian, 2012) |
| 9  | 30.596         | 2.81        | 10-Undecen-1-ol, 2-methyl | - | Antimicrobial and Anti-inflammatory | (Hameed et al., 2016) |
| 10 | 33.747         | 2.12        | .gamma.-Tocopherol | - | Anti-Inflammatory | (Falk and Munné-Bosch, 2010) |
| 11 | 31.444         | 2.02        | 9,12-Octadecadienoic acid (Z,Z) | Linoleic acid | Nematicide, Insecticide, Antihistaminic, Anti-inflammatory | (Sermakkani and Thangapandian, 2012) |
| 12 | 29.748         | 1.83        | 1,2-Ethanediamine, N,N-Dimethyl-N'-2-Pyridinyl-N'-2 Phenylmethy1 | - | - | - |
| 13 | 30.244         | 1.66        | E,E-10,12-Hexadecaadien-1-ol acetate | - | Hypoglycemic, Hycholesterolemic, Antioxidant, Anticancerous, Thyroid inhibiting properties | (Jain and Rijhwani, 2018) |
| 14 | 31.782         | 1.55        | Ethyl tetracosanoate | Ester | Antibacterial | (Igwe and Okwu, 2013) |
| 15 | 32.044         | 1.19        | 1-CINNAMYL-3-METHYLINDOLE-2 CARBADEHYDE | - | - | - |
| 16 | 32.237         | 1.19        | ICOSANE | - | Anti-fungal, Anticancer | (Ahsan et al., 2017; Sianipar and Pumamaningi, 2018) |
| 17 | 31.906         | 1.10        | Squalene | Triterpene | Anticancer, Antimicrobial, Antioxidant, Chemopreventive, Pesticide, Anti-tumor, Antitumoral and Antimicrobial | (Ezhilan and Neelamegam, 2012; Sermakkani and Thangapandian, 2012) |
Table 6. Secondary metabolites and biological activity of winged bean “Sandi” and the harvest age of 10 days after anthesis (DAA)

| No | Retention time | Peak area % | Compound | Compound group | Activity | Reference |
|----|----------------|-------------|----------|----------------|----------|-----------|
| 1  | 29.293         | 20.02       | (9E,12E)-9,12-OCTADECADIENOIC ACID | Linoleic acid | Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insecticuge, Antihistaminic, Antiarthritic, Antiacne | (Sermakkani and Thangapandian, 2012) |
| 2  | 6.642          | 13.97       | 2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE | Hydroxyl group | Antioxidant | (Li et al., 2019) |
| 3  | 27.452         | 13.77       | .alpha.-D-Glucopyranoside, methyl | Phenolic glucoside | Apoptotic activity, Anticancer, Antituberculosis, Antioxidant, Alpha-amylase inhibitory activity, Anticonvulsant | (Lyantagaye 2013; Uchegbu et al., 2017) |
| 4  | 28.279         | 9.92        | n-Hexadecanoic acid | Palmitic acid | Antioxidant, Nematicide, Pesticide, Antiandrogenic, Flavor, Anti-inflammatory | (Aparna et al. 2012; Sermakkani and Thangapandian 2012; Korbecki dan Bajdak-Rusinek, 2019) |
| 5  | 3.794          | 9.60        | 2,5-FURANDIONE | - | - | - |
| 6  | 30.927         | 7.32        | 4-(4-ETHYLCYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE | - | - | - |
| 7  | 22.459         | 6.50        | 5-amino- $$ Imidazole-4-carboxamide, 5-amino- | - | - | - |
| 8  | 28.893         | 3.92        | ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE | Linolenic acid | Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Nematicide, Insecticuge, Antihistaminic, Antiacne, Antiarthritic, Anticoronary | (Kumar et al., 2010; Starlin et al., 2019) |
| 9  | 33.402         | 3.05        | Stigmasterol tosylate | - | - | - |
| 10 | 30.313         | 2.57        | 2-Dodecen-1-yl(-) succinic anhydride | Ethyl acetate | Antineoplastic agents, Antioxidants, Antimicrobial Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant | (Rawal and Sonawani 2016; Saravanan et al., 2018) |
| 11 | 35.809         | 2.43        | Stigmasterol | Steroid | Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant | (Sudha et al., 2013; Mary and Giri, 2016) |
| 12 | 27.721         | 2.22        | HEXADECANOIC ACID, ETHYL ESTER | Ester Compound | Antioxidant, Flavor, Nematicide, Pesticide, Antiandrogenic | (Sermakkani and Thangapandian, 2012) |
| 13 | 31.623         | 1.80        | 14-HYDROXY-20-OXOPREGNAN-3-YL ACETATE | - | - | - |
| 14 | 31.354         | 1.51        | 4-(4-ETHYLCYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE | - | - | - |
| 15 | 33.857         | 1.41        | STIGMAST-5-EN-3-OL | Phytosterols | Anticancer, Reduce blood level of glucose, Hypercholesterolemia | (Sianipar et al., 2016; Yamuna et al., 2017) |
et al., 2019). The anthocyanin pigments are a group of flavonoids whose activity is an antioxidant (Nomer et al., 2019), which in this study was demonstrated in pods harvested at 10 DAA. Many phytochemical compounds with biological activities make young winged pods have good health benefits, and they can be consumed as functional vegetables.

**Conclusion**

The secondary metabolite compounds mostly from the linoleic acid group were identified from the young green pods “Fairuz” harvested at 8 and 10 DAA. Similar compounds were found in the purple pods “Sandi”. The purple pods harvested at 10 DAA have additional compounds with antioxidant activity other than those from the linoleic acid group. 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester was the most abundant compound identified from pods harvested at 8 DAA in both varieties. Therefore, young winged bean pods have benefits when consumed as vegetables.

**References**

Ahsan, T., Chen, J., Zhao, X., Irfan, M., and Wu, Y. (2017). Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express* 7, 1–9. https://doi.org/10.1186/s13568-017-0351-z

Alalade, J.A., Akinlade, J.A., Aderinola, O.A., Fajemisin, A.N., Muraina, T.O., and Amoo, T.A. (2016). Proximate, mineral and anti-nutrient contents in Psophocarpus tetragonolobus (L) DC. (winged bean) leaves. *British Journal of Pharmaceutical Research* 10, 1–7. https://doi.org/10.9734/bjpr/2016/22087

Amoo, I.A., Adebayo, O.T., and Oyeleye, A.O. (2006). Chemical evaluation of winged beans. *African Journal of Food Agriculture Nutrition and Development* 6, 1–12.
Destia Susanti, Maya Melati, Ani Kurniawati

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C., and Haridas, M. (2012). Anti-Inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology and Drug Design 80, 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x

Berwal, R., Vasudeva, N., Sharma, S., and Das, S. (2019). Investigation on biomolecules in ethanol extract of fruits of prosoptis juliflora (sw.) dc. using GC-MS. Journal of Herbs, Spices and Medicinal Plants 25, 172–180. https://doi.org/10.1080/10496475.2019.1579148

Ezhilan, B.P. and Neelamegam, R. (2012). GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. Pharmacognosy Research 4, 11–14. https://doi.org/10.4103/0974-8490.91028

Falk, J. and Munné-Bosch, S. (2010). Tocochromanol functions in plants: antioxidation and beyond. Journal of Experimental Botany 61, 1549–1566. https://doi.org/10.1093/jxb/erq030

Hameed, I.H., Altameeme, H.J., and Idan, S.A. (2016). Artemisia annua: biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. Research Journal of Pharmaceutical, Biological and Chemical Sciences 7, 1843–1868.

Handayani, T. (2013). Kecipir (Psophocarpus tetragonolobus L.) potensi lokal yang terpinggirkan. Jurnal IPTEK Tanaman Sayuran 1, 1–8. https://doi.org/10.1067/mtc.2001.112466

Igwe, O.U. and Okwu, D.E. (2013). GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the stem bark of Brachystegia eurycoma (harms). International Journal of Chemical Sciences 11, 357–371.

Jain, P.K. and Rijhwani, S. (2018). Comparative GC-MS analysis of Cyamopsis tetragonoloba fruit extracts. International Journal of Pharmaceutical Sciences and Research 9, 4236–4242. https://doi.org/10.13040/IJPSR.0975-8232.9(10).4236-42

Juansah, J., Ariyanti, R., and Akhiruddin. (2013). Potensi metode optik untuk pendugaan kandungan antosianin pada buah black mulberry dan stroberi. Jurnal Biofisika 9, 22–30.

Karthikeyan, M., Parthiban, S., and Ramalingam, S. (2019). Phytochemical analysis in economically important Ficus benghalensis L. and Ficus krishnae C.DC. using GC-MS. International Journal of Pharma and Bio Sciences 10, 5–12. https://doi.org/10.22376/ijpbs.2019.10.4.p5-13

Khan, T.N. (1976). Papua a new guinea: a centre of genetic diversity in winged bean (Psophocarpus tetragonolobus (L.) Dc.). Euphytica 25, 693–706.

Konovalova, O., Gergel, E., and Herhel, V. (2013). GC-MS analysis of bioactive components of Shepherdia argentea (pursh.) Nutt. from ukrainian flora. The Pharma Innovation Journal 2, 7-12.

Korbecki, J. and Bajdak-Rusinek, K. (2019). The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms. Inflammation Research 68, 915–932. https://doi.org/10.1007/s00011-019-01273-5

Krisnawati, A. (2010). Keragaman genetik dan potensi pengembangan kecipir (Psophocarpus tetragonolobus L.) di Indonesia [Genetic diversity and development prospects of winged bean (Psophocarpus tetragonolobus L.) in Indonesia]. Jurnal Penelitian dan Pengembangan Pertanian 29, 113–119. https://doi.org/10.21082/jp3.v29n3.2010.p113-119

Kumar, P.P., Kumaravel, S., and Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. African Journal Biochemistry Research 4, 191–195.

Li, H., Wu, C.J., Tang, X.Y., and Yu, S.J. (2019). Insights into the regulation effects of certain phenolic acids on 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one formation in a microaqueous glucose-proline system. Journal of Agricultural and Food Chemistry 67, 1–40. https://doi.org/10.1021/acs.jafc.9b01182

Lyantagaye, S.L. (2013). Methyl-α-D-glucopyranoside from Tulbghia violacea extract induces apoptosis in vitro in cancer cells. Bangladesh Journal of Pharmacology 8, 93–101. https://doi.org/10.3329/bjp.v8i2.13717

Mamani, R. and Alhaji, N.M. (2019). GC-MS analysis of phytocomponents in methanolic extract of Coleus aromaticus. Journal of Pharmacognosy and Phytochemistry 8, 106–109.
Mary, A.P.F. and Giri, R.S. (2016). Phytochemical screening and GC-MS analysis in ethanolic leaf extracts of Ageratum conyzoides (L.). *World Journal of Pharmaceutical Research* **5**, 1019–1029. https://doi.org/10.20959/wjpr20167-6505

Mohanty, C.S., Singh, V., and Chapman, M.A. (2020). Winged bean: an underutilized tropical legume on the path of improvement, to help mitigate food and nutrition security. *Scientia Horticulturae* **260**, 1–8. https://doi.org/10.1016/j.scienta.2019.108789

Mohanty, C.S., Verma, S., Singh, V., Khan, S., Gaur, P., Gupta, P., Nizari, M.A., Dikshit, N., Pattanayak, R., Shukla, A., Niranjan, A., Sahu, N., Behera, S.K., and Rana, T.S. (2013). Characterization of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) based on molecular, chemical and physiological parameters. *American Journal of Molecular Biology* **3**, 187–197. https://doi.org/10.4236/ajmb.2013.34025

Nisha, and Rao, P.B. (2018). Gas chromatography-mass spectrometry analysis for identification of bioactive compounds in selected genotypes of Trigonella foenum-graecum L. *The Pharma-Innovation Journal* **7**, 929–939.

Nomer, N.M.G.R., Duniaji, A.S., and Nocianitri, K.A. (2019). Kandungan senyawa flavonoid dan antosianin ekstrak kayu secang (*Caesalpinia sappan* L.) serta aktivitas antibakteri terhadap vibrio cholerae [Flavonoid and anthocyanin analysis of sappan wood extract (*Caesalpinia sappan* L.) and antibacterial activity against vibrio cholerae]. *Jurnal Ilmu dan Teknologi Pangan* **8**, 216–225. https://doi.org/10.24843/itepa.2019.v08.i02.p12

Payum, T. (2020). Phytoconstituents and proximate composition of Clerodendrum colebrookianum walt.: a widely used anti high blood pressure medicinal food plant in eastern himalayas. *Pharmacognosy Journal* **12**, 1534–1540. https://doi.org/10.5530/pj.2020.12.210

Prawanayoni, S.S. and Sudirga, S.K. (2020). Isolasi dan identifikasi senyawa anti jamur daun jeringau (Acorus calamus Linn.) sebagai pengendali jamur Athelia rolfsii Sacc. penyebab penyakit busuk batang pada tanaman kedelai [Isolation and identification of antifungal compounds of jeringau leaf (Acorus calamus Linn.) as a control of Athelia rolfsii Sacc. fungus that causes stem rot disease of soybean plants]. *Metamorfoса: Journal of Biological Sciences* **7**, 152–158. https://doi.org/10.24843/metamorfosa.2020.v07.i02.p02

Rawal, J.R. and Sonawani, P.R. (2016). Determination of bioactive components of Cynodon dactylon by GC-MS analysis and it’s In vitro antimicrobial activity. *International Journal of Pharmacy and Life Sciences* **7**, 4880–4885.

Rajeswari, G., Murugan, M., and Mohan, V.R. (2013). GC-MS analysis of bioactive components of Hugonia mystax L. (Linaceae). *Journal Of Pharmaceutical And Biomedical Sciences* **29**, 818–824.

Reswari, H.A., Syukur, M., and Suwarno, D.W.B. (2019). Anthocyanin and carotenoid contents, and yield components in various genotypes of purple and green yard long beans. *Indonesian Journal of Agronomy* **47**, 61–67. https://doi.org/10.24831/jai.v47i1.23402

Salim, S.A. (2018). In vitro induction of callus from different explants of Terminalia arjuna (Roxb.) wight and am and detection of its active secondary metabolites using GC-MS analysis. *Plant Archives* **18**, 2519–2527.

Saravanan, M., Senthilkumar, P., Kalimuthu, K., Chinnadurai, V., Vasantharaj, S., and Pugazhendhi, A. (2018). Phytochemical and pharmacological profiling of Turnera subulata Sm., a vital medicinal herb. *Industrial Crops and Products* **124**, 822–833. https://doi.org/10.1016/j.indcrop.2018.08.065

Save, S.A., Lokhande, R.S., and Chowdhary, A.S. (2015). Determination of 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester from the twigs of Thevetia peruviana as
a Colwell biomarker. *Journal Innovations in Pharmaceuticals and Biological Sciences* **2**, 349–362.

Sermakkani, M. and Thangapandian, V. (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal Pharmaceutical and Clinical Research* **5**, 90–94.

Sianipar, N.F. and Purnamaningsih, R. (2018). Enhancement of the contents of anticancer bioactive compounds in mutant clones of rodent tuber (*Typhonium flagelliforme* Lodd.) based on GC-MS analysis. *Pertanika Journal Tropical Agricultural Science* **41**, 305–320.

Sianipar, N.F., Purnamaningsih, R., and Rosaria. (2016). Bioactive compounds of fourth generation gamma-irradiated *Typhonium flagelliforme* Lodd. mutants based on gas chromatography-mass spectrometry. *IOP Conferences Series: Earth and Environmental Science* **41**, 1–10. https://doi.org/10.1088/1755-1315/41/1/012025

Singh, M., Dubey, R.K., Koley, T.K., Maurya, A., Singh, P.M., and Singh, B. (2019). Valorization of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) for food and nutritional security: synthesis of past research and future direction. *Planta* **250**, 911–931. https://doi.org/10.1007/s00425-019-03141-2

Sriwichai, S., Monkham, T., Sanitchon, J., Jogloy, S., and Chankaew, S. (2021). Dual purpose of the winged bean (*Psophocarpus tetragonolobus* (L.) DC.), the neglected tropical legume, based on pod and tuber yields. *Plants* **10**, 1–13. https://doi.org/10.3390/plants10081746

Starlin, T., Prabha, P.S., Kumar, B., Thayakumar, A., and Gopalakrishnan, V.K. (2019). Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. *Bioinformation* **15**, 425–429. https://doi.org/10.6026/97320630015425

Sudha, T., Chidambarampillai, S., and Mohan, V.R. (2013). GC-MS analysis of bioactive components of aerial parts of *Fluggea leucopyrus* willd. (Euphorbiaceae). *Journal of Applied Pharmaceutical Science* **3**, 126–130. https://doi.org/10.7324/JAPS.2013.3524

Tanzi, A.S., Eagleton, G.E., Ho, W.K., Wong, Q.N., Mayes, S., and Massawe, F. (2019). Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) leaves for food and nutritional security: synthesis of past research and future direction. *Planta* **250**, 911–931. https://doi.org/10.1007/s00425-019-03141-2

Tripathi, N., Kumar, S., Singh, R., Singh, C.J., Singh, P., and Varshney, V.K. (2013a). Isolation and identification of γ-sitosterol by GC-MS from the leaves of *Girardinia heterophylla* (Decne). *The Open Bioactive Compounds Journal* **4**, 25–27. https://doi.org/10.2174/187447301004010010025

Uchegbu, R.I., Ahuchaogu, A.A., Amanze, K.O., and Ibe, C.O. (2017). Chemical constituents analysis of the leaves of *Bryophyllum pinnatum* by gc-ms. *American Association for Science and Technology Journal of Chemistry* **3**, 19–22. https://doi.org/10.13005/ojtc/290245

Vik, A., James, A., and Gundersen, L.L. (2007). Screening of terpenes and derivatives for antimycobacterial activity identification of geranylgeraniol and geranylgeranyl acetate as potent inhibitors of *Mycobacterium tuberculosis* in vitro. *Planta Medica* **73**, 1410–1412. https://doi.org/10.1055/s-2007-990238
Wei, C., He, W., Wei, L., Li, C., and Ma, J. (2015). The Analysis of a microbial community in the UV/O3-anaerobic/aerobic integrated process for petrochemical nanofiltration concentrate (NFC) treatment by 454-Pyrosequencing. *PLoS One* **10**, 1–14. https://doi.org/10.1371/journal.pone.0139991

Yamuna, P., Abirami, P., Vijayashalini, P., and Sharmila, M. (2017). GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq. *Journal of Medicinal Plants Studies* **5**, 31–37.