Sterol and Triterpene Profiles of the Callus Culture of Solanum mammosum

Silvy Juliana
Plant Biotechnology Research Group, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia

Suciati
Plant Biotechnology Research Group, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia, suciati@ff.unair.ac.id

Gunawan Indrayanto
Plant Biotechnology Research Group, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia

Follow this and additional works at: https://scholarhub.ui.ac.id/science

Recommended Citation
Juliana, Silvy; Suciati; and Indrayanto, Gunawan (2019) "Sterol and Triterpene Profiles of the Callus Culture of Solanum mammosum," *Makara Journal of Science*: Vol. 23 : Iss. 2 , Article 2.
DOI: 10.7454/mss.v23i2.11044
Available at: https://scholarhub.ui.ac.id/science/vol23/iss2/2

This Article is brought to you for free and open access by the Universitas Indonesia at UI Scholars Hub. It has been accepted for inclusion in Makara Journal of Science by an authorized editor of UI Scholars Hub.
Sterol and Triterpene Profiles of the Callus Culture of Solanum mammosum

Cover Page Footnote
This study was funded by the Faculty of Pharmacy, Airlangga University research grant.

This article is available in Makara Journal of Science: https://scholarhub.ui.ac.id/science/vol23/iss2/2
Sterol and Triterpene Profiles of the Callus Culture of *Solanum mammosum*

Silvy Juliana, Suciati*, and Gunawan Indrayanto

Plant Biotechnology Research Group, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia

*E-mail: suciati@ff.unair.ac.id

Received January 15, 2019 | Accepted April 1, 2019

**Abstract**

This study aimed to compare the sterol and triterpene profiles of two types of *Solanum mammosum* callus cultures, i.e., compact globular structure (CGS) and normal fine (F) calluses. The CGS callus resulted from the differentiation of the F callus culture after many years of subculturing. The growth rate, microscopic characteristics, and morphologies of the two callus types were determined and compared. Sterols and triterpenes were identified through thin-layer chromatography, gas chromatography–flame ionization detection, and gas chromatography–mass spectrometry analyses. The growth rate of the CGS callus was lower than that of the F callus. Microscopic identification revealed that thick, lignin-containing cell walls formed in the CGS callus but not in the F callus. The chromatographic analysis suggested that the CGS and F callus cultures had different sterol and triterpenoid profiles. The sterols and triterpenes produced by the CGC culture were more diverse than those produced by the F callus culture.

**Keywords:** *Solanum mammosum*, callus, tissue culture, sterol, triterpene

**Introduction**

Sterols can be found in all eukaryotic organisms as membrane components that regulate the fluidity and the permeability of phospholipid bilayers. Most plant cells can produce sterols, such as cholesterol, campesterol, and β-sitosterol, via the cycloartenol pathway [1]. Sterols in plants originate from cycloartenol, whereas those in fungi or animals are derived from lanosterol [2]. Sterols are members of the terpenoid family. Specifically, sterols are triterpenoids. Terpenoids are produced by plants and have various basic functions in growth and development but mainly participate in chemical interactions and protection against abiotic and biotic environmental stressors [3]. Several sterols have important biological activities, including anti-inflammatory, antidiabetic, anticancer, and lipid-lowering activities [4]. Triterpenes, such as betullic acid, are natural products that exert activities against a variety of cancer types by directly influencing mitochondrial membrane permeabilization [5].

Some sterols and triterpenes have been identified in several cell cultures of *Solanum* spp. Cholesterol, stigmasterol, β-sitosterol, isofucosterol, lanosterols, lupeol, betulin, betulin aldehyde, and betulnic acid have been identified in callus cultures of *S. laciniatum* [6,7]. Cholesterol, campesterol, stigmasterol, β-sitosterol, 28-isofucosterol, and 24-methylene cycloartenol have also been found in callus cultures of *S. malacoxylon* (current name: *S. glaucophyllum*) [8]. The callus cultures of *S. mammosum* and *S. wrightii* produce cholesterol, campesterol, stigmasterol, and β-sitosterol but not solasodine [7,9]. The application of callus cultures of *S. mammosum* for the production of phytosteroids and the biotransformation of some chemical compounds have been reviewed [10].

Some plant cell cultures can self-develop compact globular structure (CGSs). In general, the secondary metabolite profiles of CGS cultures are different from those of normal callus cultures [11-13]. In addition, CGS cultures exhibit cell wall thickening. Two forms of *S. mammosum* callus cultures, CGS and normal (F), are available in our laboratory (Figure 1). CGS callus cultures were obtained after more than 26 years of subculturing since 1984. To the best of our knowledge, reports on the

![Figure 1. *Solanum Mammosum* Callus Cultures: F Callus (A) and CGS Callus (B)](image)
CGS culture of *S. mammosum* have not yet been published. The present work describes the sterol and triterpene profiles of the CGS and F callus cultures of *S. mammosum*.

**Materials and Methods**

**Preparation of callus cultures.** The CGS and F callus cultures of *S. mammosum* were grown in a 300 mL Erlenmeyer flask containing 50 mL of modified Murashige-Skoog medium supplemented with 7 g/L agar, 30 g/L sucrose, 2 mg/L kinetin, and 0.5 mg/L 1-Naphthaleneacetic acid at 25 °C ± 2 °C under continuous light (ca. 2000 lux). The cultures were subcultured every 4 weeks of incubation as described in our previous work (Indrayanto et al., 1998). Growth rate was determined by measuring the weight of four cultures every week by calculating the ratio of the callus weight at a certain time (n th week) to the initial inoculation weight (n = 0). Data were reported as average rate ± standard deviation (SD).

**Microscopic characterization.** Thin sections of fresh F and CGS callus cultures were observed under light microscopy with 400× magnification. A few drops of phloroglucinol–HCl were added to the CGS callus cultures to identify the presence of lignin.

**Sample preparation.** Extraction was performed in accordance with a previously published method [7]. Oven-dried (40 °C; moisture content 10%) powdered callus (6 g) was extracted with 50 mL of n-hexane by ultrasonification (3 × 15 min). The extract and residue were separated through filtration. The residue was further extracted with acetone (50 mL) using the same procedure. All collected extracts were evaporated to dryness under a nitrogen stream. The dried extracts of n-hexane (200 mg) and acetone (200 mg) were dissolved in 2 mL of chloroform. A total of 5 mg of different standards (cholesterol, campesterol, stigmasterol, β-sitosterol, lupeol, betulonic acid, and betulinic acid) (Sigma) was dissolved in 2 mL of chloroform.

**Thin-layer chromatography.** Standards (β-sitosterol, lupeol, and betulinic acid) and 2 μL aliquots of n-hexane and acetone extracts from the CGS and F callus cultures in chloroform were applied to thin-layer chromatography (TLC) plates (Kieselgel 60 F 254). Two different solvents were used in the TLC analysis: 1) n-hexane : ethyl acetate (4:1) for n-hexane extraction, and 2) chloroform : methanol (6:1) for acetone extraction. Plates were sprayed with anisaldehyde–H2SO4 for visualization.

**Gas chromatography–flame ionization detection.** Samples (2 μL) and standards (2 μL) containing cholesterol, campesterol, stigmasterol, β-sitosterol, lupeol, betulin, or betulinic acid were injected into the gas chromatography (GC) instrument. Acetate derivatization was performed by mixing ca. 1 mg of acetone extract with 2 mL of pyridine and 2 mL of an acetic acid anhydride. The mixture was incubated for 24 h in dark, evaporated into dryness under nitrogen, and then dissolved with 2 mL of ethyl acetate. The solution (2 μL) was then injected into the GC instrument. Gas chromatography–flame ionization detection (GC–FID) was performed with a capillary column 5% phenyl methyl siloxane (30.0 m × 0.25 mm × 0.25 μm) (Agilent Technologies 6890N) at a flow rate 40 mL/min (He) and FID 300 °C. The column temperature was started at 220 °C and then increased to 270 °C at a rate of 10 °C/min.

**Gas chromatography–mass spectrometry analyses.** Gas chromatography–mass spectrometry analyses (GC–MS) analysis was performed using Agilent GC 6890N and Mass Selective Detector (MSD) 5973 equipped with HP-5 column (30 m × 0.250 mm × 0.25 μm i.d.) and completed with Wiley 7n1 database (2004). Inlet temperature was set at 250 °C. Analysis was performed in splitless injection mode at a flow rate of 1 mL/min (He). The oven temperature was programmed as 220 °C–270 °C at 10 °C/min. Electron impact ionization (EI–MS) source temperature was set at 230 °C. The transfer line temperature from oven to the detector was 280 °C. The energy of ionization was set at 70 eV.

**Identification method.** The EI–MS spectra of peaks 1–15 (Figures 5 and 6) were compared with the EI–MS spectra of standards and EI–MS spectra from the Wiley database, the online databases of National Institute of Standard and Technology [14], the Spectral Database for Organic Compounds [15], MassBank [16], and published reports [17–24]. Peaks 1–15 were identified in accordance with the method of Commission Decision 2002/657/EC [25].

**Results and Discussion**

**Microscopic identification and growth rate of CGS and F calluses.** The CGS culture could be easily differentiated macroscopically from the F callus culture. The CGS culture showed a cohesive callus aggregate with diameters of 0.5–2 cm. By contrast, the normal callus culture was highly dispersed. Microscopic examination revealed that the formation of a thick cell wall limited the differentiation of the CGS culture. The thick cell walls of the CGC culture contained lignin as indicated by the development of a red color after the addition of phloroglucinol–HCl reagent. By contrast, F callus cultures lacked cell wall thickening and lignin (Figure 2). The growth rate of CGS cultures was slower than that of the F callus culture (Figure 3).

**Identification of sterol and triterpenes in CGS and F calluses.** Sterols and other triterpenes in the callus cultures were identified through TLC, GC–FID, and GC–MS analyses. The n-hexane and acetone extracts of CGS and F callus cultures were subjected to TLC analysis,
visualized with anisaldehyde sulfuric acid spray reagent, and compared with standards. The results indicate that all n-hexane and acetone extracts contained sterol (β-sitosterol) and triterpene (lupeol). However, betulinic acid was only detected in the acetone extract of the CGS callus culture (Figure 4). Further analysis was performed through GC–FID (Tables 1 and 2). The peaks of the cholesterol, campesterol, stigmasterol, and β-sitosterol peaks at 31.678, 32.692, and 45.096 min were further identified through GC–MS as isofucosterol, cycloartenol, and 24-methylene cycloartenol, respectively. GC–FID analysis indicates that β-sitosterol was the major compound in the n-hexane extract of the F and CGS calluses, whereas β-sitosterol and stigmasterol were the major compounds in acetone extracts of the F and CGS calluses, respectively.

![Figure 2. Microscopy Identification of S. Mammosum Callus Cultures: F Callus (A) and CGS Callus (B) After the Addition of Phloroglucinol–HCl](image)

![Figure 3. Growth Rate of the F and CGS Callus Cultures of S. Mammosum](image)

**Table 1. GC–FID Results for the N-Hexane Extracts of F and CGS Callus Cultures**

| Standard     | Rt std (min) | RRT standarda | Rt callus F (min) | RRT callus F | Rt callus CGS (min) | RRT callus CGS |
|--------------|--------------|---------------|-------------------|--------------|---------------------|---------------|
| Cholesterol  | 23.383       | 1.00          | 23.126            | 1.00         | 23.272              | 1.00          |
| Campesterol  | 27.042       | 1.16          | 27.119            | 1.17         | 27.060              | 1.16          |
| Stigmasterol | 28.256       | 1.21          | 28.395            | 1.23         | 28.294              | 1.22          |
| β-Sitosterol | 30.782       | 1.32          | 31.043            | 1.34         | 30.939              | 1.33          |
| -            | -            | -             | -                 | -            | 31.678b             | 1.36          |
| -            | -            | -             | -                 | -            | 32.692              | 1.40          |
| Lupeol       | 35.145       | 1.50          | 34.608            | 1.50         | 35.138              | 1.51          |
| -            | -            | -             | -                 | -            | 45.096              | 1.94          |

*a* RRT was calculated on the basis of the Rt of the cholesterol standard;  
b later identified by GC–MS as isofucosterol, cycloartenol, and 24-methylene cycloartenol. - not detected.

**Table 2. GC–FID Result for the Acetone Extracts of F and CGS Callus Cultures**

| Standards    | Rt std (min) | RRT standarda | Rt Callus F (min) | RRT Callus F | Rt Callus CGS (min) | RRT Callus CGS |
|--------------|--------------|---------------|-------------------|--------------|---------------------|---------------|
| Cholesterol  | 23.383       | 1.00          | 23.205            | 1.00         | 23.260              | 1.00          |
| Campesterol  | 27.042       | 1.16          | 27.070            | 1.17         | 27.041              | 1.16          |
| Stigmasterol | 28.256       | 1.21          | 28.283            | 1.22         | 28.713              | 1.23          |
| β-Sitosterol | 30.782       | 1.32          | 30.855            | 1.33         | 30.841              | 1.32          |
| Lupeol       | 35.145       | 1.50          | 35.577            | 1.53         | 35.032              | 1.51          |

*a* RRT was calculated on the basis of the Rt of the cholesterol standard.
The acetone extract of the CGS callus was subjected to acetate derivatization to confirm the presence of betulonic acid further. n-Hexane and acetone-derivatized extracts were further subjected to GC–MS analysis. The total ion chromatograms (TICs) of the n-hexane and acetone extracts of the CGS callus exhibited 8 and 7 peaks, respectively (peak 1–15) (Figures 5 and 6). The EI–MS spectra of the extracts were compared with those from the Wiley database available in the GC–MS instrument, as well as those from other online databases, i.e., NIST, SDBS, and MassBank [14-16], to determine the identity of the peaks. The EI–MS spectra of compounds 1, 2, 3, 4, and 7 were identical to those of cholesterol, campesterol, stigmasterol, β-sitosterol, and lupeol, standards, respectively. The EI–MS spectra of their acetate derivatives (9–13) were identical to published EI–MS spectra and EI–MS spectra from three online databases [14-24]. The important EI–MS fragments of compounds 1–15 and their molecular ions [M]+ are presented in Tables 3 and 4. The chemical structures of sterols and triterpenes that were identified in this work are illustrated in Figure 7. Although compounds 5, 6, 8, 14, and 15 lacked standards, their EI–MS spectra were identical to those from the three databases and published data [14-24]. The Commission Decision 2002/657/E states that the identity of a compound can be confirmed if its MS spectrum showed at least four fragments that are identical to those in the MS spectrum of the standard [25]. Compounds 1–15 showed numerous fragments (>4 fragments) that were identical to the fragments of standards, online databases, and published data [14-24]. Thus, the identities of peaks 1–15 were unambiguously confirmed.

Figure 5. Total Ion Chromatogram of the N-Hexane Extract of The CGS Callus

Figure 6. Total Ion Chromatogram of the Acetone Extract of the CGS Callus After Derivatization
Table 3. Molecular Ions and Fragments of the Identified Sterols in CGS Callus

| Molecular ion [M]+ and fragment | Compound (m/z) | R = OH | R = CH₃COO | 1 | 2 | 3 | 4 | 5 | 9 | 10 | 11 | 12 |
|-------------------------------|----------------|--------|-------------|---|---|---|---|---|---|----|----|----|----|
| [M]+                          | 386            | 400    | 412         | 414| 412|  - |  - |  - |  - |  - |  - |  - |
| [M-CH₃]+                      | 371            | 385    | 397         | 399|  - |  - |  - |  - |  - |  - |  - |  - |
| [M-R]+                        | 368            | 382    | 394         | 396|  - |  - |  - |  368 | 382 | 394 | 396 |  - |
| [M-CH₃-H₃]+                   | 353            | 367    | 379         | 381| 379| 379| 379| 355 | 367 | 379 | 381 |  - |
| [M,C₇H₅]+                     | -              | 369    | -           |  - |  - |  - |  - |  - |  - |  - |  - |  - |
| [M,C₇H₅-R]+                   | -              | -      | 351         |  - |  - |  - |  - | 351 |  - |  - |  - |  - |
| [M,C₇H₅-H₃]+                  | 301            | 315    | 327         | 329|  - |  - |  - |  - |  - |  - |  - |  - |
| [M,C₇H₅-I]+                   | -              | -      | 314         |  - |  - |  - |  - |  - |  - |  - |  - |  - |
| [M,C₇H₅-R]+                   | 275            | 289    | 301         | 303|  - |  - |  - |  - |  - |  - |  - |  - |
| [M,C₈H₁₃]+                    | 260            | 274    | 286         | 288|  - |  - |  - | 260 | 274 |  - | 288 |  - |
| [M,C₈H₁₃-R]+                  | 247            | 261    | 273         |  - |  - |  - |  - |  - |  - |  - | 275 |  - |
| [M,C₈H₁₃-2CH₃-R]+             | -              | -      | 281         |  - |  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain]+                | 273            | 273    | 273         | 273|  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain-2H]+             | -              | -      | 271         |  - |  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain-R]+              | 255            | 255    | 255         | 255|  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain-H₂O]+            | -              | -      | 255         |  - |  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain-42]+             | 231            | 231    | 231         | 231|  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain-42-R]+           | 213            | 213    | 213         | 213|  - |  - |  - |  - |  - |  - | 213 | 213 |
| [M-Side chain-44]+             | -              | -      | 229         |  - |  - |  - |  - |  - |  - |  - |  - |  - |
| [M-Side chain-42-H₂O]+         | -              | -      | 213         |  - |  - |  - |  - |  - |  - |  - |  - |  - |

1: Cholesterol; 2: Campesterol; 3: Stigmastanol; 4: β-Sitosterol; 5: Isofucosterol; 9: Cholesterol acetate; 10: Campesterol acetate; 11: Stigmastanol acetate; 12: Sitosterol acetate.

Table 4. Molecular Ions and Fragments of the Identified Triterpenes in CGS Callus

| Molecular ion [M]+ and fragment | Compound (m/z) | R = OH | R = CH₃COO | 6 | 7 | 8 | 13 | 14 | 15 |
|-------------------------------|----------------|--------|-------------|---|---|---|----|----|----|
| [M]+H⁺                        | 427            | 441    |  -           |  - |  - |  - |  - |  - |  - |
| [M]+                          | 426            | 426    | 440         | 468|  - |  526 |  - |  - |  - |
| [M-CH₃]+                      | 411            | 411    | 425         | 453| 483|  - |  - |  - |  - |
| [M-R]+                        | -              | -      | 422         | 408| 438| 466 |  - |  - |  - |
| [M-CH₃-H₃]+                   | 393            | 393    | 407         | 393| 423| 451 |  - |  - |  - |
| [M-3CH₃]+                     | -              | -      |  -           |  - |  - |  - |  423 |  - |  - |
| [M-2R]+                       | -              | -      |  -           |  - |  - |  - |  - |  406 |  - |
| [M-2R-CH₃]+                   | -              | -      |  -           |  - |  - |  - |  - |  - |  391 |
| [M-C₇H₅]+                     | -              | 397    |  -           |  - |  - |  - |  - |  - |  - |
| [M-C₇H₅-R]+                   | -              | 379    |  -           |  - |  - |  - |  - |  - |  - |
| [M-C₇H₅-I]+                   | -              | -      |  453         |  - |  - |  - |  - |  - |  - |
| [M-HCOOH]+                    | -              | -      |  452         |  - |  - |  - |  - |  - |  - |
| [M-HCOOH-CH₃]+                | -              | -      |  395         |  - |  - |  - |  - |  - |  - |
| [M-ring C,D,E]+               | -              | -      |  262         |  - |  - |  - |  - |  - |  - |
| [M-ring C,D,E-2]+             | -              | -      |  249         | 249 |  - |  - |  - |  - |  - |
| [M-ring A,B,C]+               | -              | -      |  248         |  - |  - |  - |  - |  - |  - |
| [M-ring A,B,C-COOH]+          | -              | -      |  203         |  - |  - |  - |  - |  - |  - |
| [M-ring C,D,E-CH₃COO]+         | -              | -      |  189         |  - |  - |  - |  - |  - |  - |
| [M-Side chain-CH₃]            | -              | 370    |  -           |  - |  - |  - |  - |  - |  - |
| [M-Cyclop propane ring cleavage]+ | 286       | 300    |  -           |  - |  - |  - |  - |  - |  - |
| [M-Cyclop propane ring cleavage-CH₃]+ | 271       | 285    |  -           |  - |  - |  - |  - |  - |  - |
| [M-Cyclop propane ring cleavage-C₃H₄]+ | - | 203     |  -           |  - |  - |  - |  - |  - |  - |
| [M-Side chain-ring E-CH₃]+     | -              | 329    |  -           |  - |  - |  - |  - | 371 |  - |
| [M-ring C,D,E]+               | -              | 207    |  -           |  - |  - |  - |  - |  - |  249 |

6: Cycloartenol; 7: Lupeol; 8: 24-methylene cycloartenol; 13: Lupeol acetate; 14: Acetyl betulinic acid; 15: Serratenediol diacetate.
Conclusion

The sterol profile of the CGS culture was more diverse than that of the normal callus culture. The production of several triterpenes, including betullinic acid, by the CGS culture but not by the normal callus culture could be attributed to differentiation by the CGS culture. This is the first report of betullinic acid from the in vitro callus culture of *S. mammosum*.

Acknowledgments

This study was funded by the Faculty of Pharmacy, Airlangga University research grant.

References

[1] Schaller, H. 2003. The role of sterols in plant growth and development. Prog. Lipid Res. 42(3): 163-175. doi: 10.1016/S0163-7827(02)00047-4.
[2] Nes, W.R. 1974. Role of sterols in membranes. Lipids 9(8): 596-612. https://doi.org/10.1007/BF02532509.
[3] Tholl, D. 2015. Biosynthesis and biological functions of terpenoids in plants. In Schrader, J., Bohlmann, J. (eds.), Biotechnology of Isoprenoids Advances. Advances in Biochemical Engineering/Biotechnology, vol. 148. Springer. Cham. pp. 63-106.
Juliana, et al.

[4] Miras-Moreno, B., Sabater-Jara, A.B., Pedreño, M.A., Almagro, L. 2016. Bioactivity of phytosterols and their production in plant in vitro cultures. J. Agric. Food Chem. 64(38): 7049-7058. doi: 10.1021/acs.jafc.6b02345.

[5] Fulda, S. 2009. Betullinic acid: A natural product with anticancer activity. Mol. Nutr. Food Res. 53(1): 140-146. https://doi.org/10.1002/mnfr.200700491.

[6] Hosoda, N., Yatazawa, M. 1979. Sterols, steroidal sapogenin and steroidal alkaloid in callus culture of Solanum laciniatum ait. Agric. Biol. Chem. 43(4): 821-825. https://doi.org/10.1271/bbb1961.43.821.

[7] Indrayanto, G. 1983. Steroide und triterpene in zellkulturen: untersuchungen mit zellkulturen von Solanum laciniatum Ait., Solanum Wrightii Bth. und Costus speciosus (Koen) Sm. Ph.D. Thesis, Universität Tübingen, Germany.

[8] Suardi, M.L., Bernasconi, S., Pelizzoni, F., Racchi, M.L. 1994. In vitro cultures of Solanum malacoxylon Sendt.: nutritional requirements and sterol production. Plant Cell Tissue Organ Cult. 36(1): 9-14. doi: 10.1007/BF00048309.

[9] Sutarjadi, I., Indrayanto, G. 1986. Sterols in callus cultures of Solanum mammosum. Planta Med. 52(5): 413. doi: 10.1055/s-2007-969208.

[10] Indrayanto, G., Sondakh, R., Utami, W., Syahrani, A. 1998. Solanum mammosum: in vitro cultures and the production of secondary metabolites. In Bajaj, Y.P.S. (ed.), Medicinal and aromatic plants. X. Biotechnology in agriculture and forestry, vol. 41. Springer. Berlin. pp. 394-414.

[11] Remotti, P.C. 1995. Primary and secondary embryogenesis from cell suspension cultures of Gladiolus. Plant Sci. 107(2): 205-214. https://doi.org/10.1016/0168-9452(95)04106-5.

[12] Zhao, J., Hu, Q., Guo, Y.Q., Zhu, W.H. 2001. Effect of stress factors, bioregulators, and synthetic precursors on indole alkaloid production in compact callus clusters cultures of Catharanthus roseus. Appl. Microbiol. Biotechno.l 55(6): 693-698. doi:10.1007/s002530000568.

[13] Yusoff, N.F., Alwee, S.S.R.S., Abdullah, M.O., Chai-Ling, H., Namasiyavam, P. 2012. A time course anatomical analysis of callogenesis from young leaf explant of oil palm (Elaeis guineensis Jacq.). J. Oil Palm. Res. 24: 1330-1341.

[14] NIST Chemistry Webbook: NIST Standard Reference Database Number 69. Accessed from: http://webbook.nist.gov/chemistry/, on January 17th, 2017.

[15] Spectral Database for Organic Compounds (SDBS): Accessed from: http://sdb.sdb.db.aist.go.jp/sdb/cgi-bin/crc_index.cgi, on January 17th, 2017.

[16] Massbank: High Quality Mass Spectral Database. Accessed from: http://www.massbank.jp/en/data-base.html, on January 17th, 2017.

[17] Lindgren, B.O., Svahn, C.M. 1966. Separation of trimethylsilyl ethers of triterpenes by thin layer chromatography: Triterpenes in wood from Populus trima ula L. Acta Chem. Scand. 20: 1763-1768.

[18] Knights, B.A. 1967. Identification of plant sterols using combined GLC/mass spectrometry. Ph.D. Thesis. Botany Department Research Laboratory, University of Glasgow, Scotland.

[19] Rogers, I.H., Rozon, L.R. 1970. Neutral terpenes from the bark of Sitka spruce [Picea sitchensis (Bong.) Carr.]. Can. J. Chem. 48(6): 1021-1025. doi: 10.1139/v70-170.

[20] Knapp, F.F., Schroepfer, G.J. 1976. Mass spectrometry of sterols. Electron ionization induced fragmentation of C-4-alkylated cholesterol. Chem. Phys. Lipids. 17(4): 466-500. doi: 10.1016/0009-3084(76)90048-7.

[21] Hembold, H. 1977. Steroide in zellkulturen von Digitalis lanata. Ph.D. Thesis. Eberhard-Karls-Universität zu Tübingen, Germany.

[22] Wahyuono, S. 1985. Phytochemical investigation of Amsonia grandiflora family Apocynaceae. Ph.D. Thesis. The University of Arizona. USA.

[23] Carvalho, T.C.D., Polizeli, A.M., Turatti, I.C., Severiano, M.E., Carvalho, C.E.D., Ambrósio, S.R., Crotti, A.E., Figueiredo, U.S.D., Vieira, P.C., M.A., Almagro, L. 2016. Bioactivity of phytosterols and triterpenoids with potential anticancer activity in bran of black non-glutinous rice. Nutrients 7(3): 1672-1687. 10.3390/nu7031672.

[24] Suttiaiporn, P., Chumposri, W., Mahatheeranont, S., Luangkamin, S., Teepsawang, S., Leardkamolkarn, V. 2015. Structures of phytosterols and triterpenoids with potential anticancer activity in bran of black non-glutinous rice. Nutrients 7(3): 1672-1687. 10.3390/nu07031672.

[25] European Commission. 2002. Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities. L221: 8–36.