# Rapid Detection and Identification of Dioscorine Compounds in *Dioscorea hispida* Tuber Plants by LC-ESI-MS

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Studies have revealed that *Dioscorea hispida* tubers contain a poisonous substance called alkaloid dioscorine. The method for removing alkaloid dioscorine in *Dioscorea hispida* is explored in this research through a soaking process. The tubers were peeled, washed, sliced, and soaked for 5 days in either 1.0 M sodium chloride (NaCl) or distilled water. The aim of this study was to firstly identify the amount of toxic dioscorine remaining after soaking for 5 days, and then determine the best solution for removing dioscorine compounds in the tubers that were obtained from a tropical area in Peninsular Malaysia. The liquid chromatography electrospray ionization mass spectrometric (LC-ESI-MS) systems were used to identify the presence of alkaloid dioscorine compounds within *Dioscorea hispida* tubers. The analysis showed that no dioscorine compounds were present in day 5 for samples soaked in the NaCl solution. However, the relative abundance in the distilled water solution at day 5 was 281000, indicating a 95% decrease of the relative abundance value of the dioscorine compounds. This work aimed to determine the minimum days needed to remove the poisonous element before *Dioscorea hispida* tubers could be used for food consumption or for any other applications.

*Keywords: Dioscorea hispida; Dioscorine compounds; Alkaloid; Foods; LC-ESI-MS*

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## INTRODUCTION

*Dioscorea hispida*, locally known as ubi gadong in Malaysia, is a seasonal plant found in tropical regions such as Malaysia, Indonesia, Vietnam, Thailand, and India. The plant can grow up to 20 m in height and has thorny stems. It is a wild plant with diverse shapes, such as round or oval, and with yellowish-brown skin (Sami and Fata 2019). Recent studies have shown that the *Dioscorea* species can be eaten after undergoing cooking methods such as baking, frying, and boiling (Kumoro *et al*. 2019). This plant belongs to the Dioscoreaceae family and is not frequently consumed by the public because it is a toxic species. It contains the dioscorine alkaloid compound, which can be harmful to health (Napisah and Rosma 2020).

The main challenge faced by researchers is the problem of detoxification. Mlingi *et al*. (1995) state that the safest amount of tubers that can be consumed should not exceed 10 mg of hydrogen cyanide (HCN) per kg of body weight. Dioscorine can be extremely

harmful to humans and may result in poisoning with symptoms ranging from vomiting, nausea, stomach pains, and health complications (Gunawan *et al*. 2019). Dioscorine is a water-soluble alkaloid compound within *Dioscorea hispida* and has the molecular formula of $C_{13}H_{19}O_2N$, which is exceptionally toxic. It is well established regarding the tuber's toxicity in the environment and is generally consumed after undergoing a toxic removal process of the dioscorine compound (Irmayadani *et al*. 2019).

In recent times, there has been growing attention in the agricultural sector for developing biodegradable plastics using *Dioscorea hispida* tubers, sugar palm (Ilyas *et al.* 2018, 2019a, 2019b), sago (Halimatul *et al.* 2019a, 2019b), bengkuang (Syafri *et al.* 2019), potato (Jumaidin *et al.* 2019a), cassava (Jumaidin *et al.* 2019b, 2020), and corn starch (Edhirej *et al*. 2017). The main form of carbohydrates in natural tubers is starch, which has a high percentage of energy contribution to the human diet (Sanyang *et al*. 2018; Ilyas and Sapuan 2020; Nazrin *et al.* 2020). However, plastics developed from *Dioscorea hispida* starch have definite drawbacks in their structural stability compared to conventional plastics, which are known for their stiffness (Navia and Villada 2005). The *Dioscorea hispida* tubers are natural fibers with an abundant source of starch and contain lignin, cellulose, hemicellulose, and fibers (Hamid *et al*. 2019). There are currently only a few published studies focusing on the toxicity removal in *Dioscorea hispida* tubers and their applications. However, there is no information on the specific duration for removing dioscorine from *Dioscorea hispida* tubers through a soaking method with sodium chloride or distilled water. It has been reported that sodium chloride solution for soaking process caused differences in osmosis pressure outside and inside the material. The osmosis diffusion occurred from the inner parts of soaked *Dioscorea hispida* tubers due to the different osmosis pressure after the material being soaked in the sodium chloride solution (Kresnadipayana and Waty 2019).

Liquid chromatography-mass spectrometry is commonly used in many research fields such as in food and safety, environmental, pharmaceuticals, and industrial material purposes (Lee *et al*. 2019). Studies explain liquid chromatography as the separation of compound elements of a sample according to their retention strength for immobile or mobile phases by identifying the separated elements using electrical conductivity followed by LC-MS specifications (Su *et al*. 2019). Recent research has been largely exploratory in nature for the detection of primary substances as references, using time retention and peak intensity (Barbieri and Heard 2019). An early example of chromatography research includes determining the best resolution of qualifying and quantifying substances. However, the chromatography process can be tough for simultaneous multiple analyses (Vinale *et al*. 2020). Twenty cohort study analyses examined the relationship between mass spectrometry (MS), which uses strongly sensitive identification techniques, and the ionization of sample components using multiple methods (Jalal *et al*. 2018). It was found that the separation of resulting ions in a vacuum was according to the mass charge ratios as well as the intensity of each ion (Brown and Carmical 2019).

The role of the mass spectra provided by MS is to specify the concentration position of ions and has shown that mass is the precise information for certain molecules, together with the ability of MS to obtain direct information for identifying a single component. However, it is different for multiple components to analyze the spectra (Naz *et al*. 2019). Although some previous research has been carried out on the method to identify dioscorine compounds such as ultraviolet-visible spectrometry (Kresnadipayana and Waty 2019), TLC densitometry, and TLC image analysis (Sasiwatpaisit *et al*. 2014), no studies have been found which used LC-MS technique. Hence, the combination of LC-MS systems can

be used to identify the alkaloid dioscorine compounds in *Dioscorea hispida* tubers (Hajšlová *et al*. 2018). This is important for determining the amount of dioscorine compounds in *Dioscorea hispida* tubers that can be used for food consumption or any other applications (Leete and Michelson 1988). To date, little reliable evidence is present on the types of solutions that can be used for removing dioscorine in *Dioscorea hispida*. This study aimed to determine the dioscorine toxic compound present in *Dioscorea hispida* tubers after 5 days of soaking through an LC-MS analysis. The *Dioscorea hispida* tubers were obtained from tropical areas in Peninsular Malaysia and their potential use in the development of biocomposites was investigated.

## EXPERIMENTAL

### Materials

*Dioscorea hispida* was obtained from fresh tubers from Kuala Terengganu, Malaysia. For the experiment, these tubers went through a process for removing the poisonous element, alkaloid dioscorine. The *Dioscorea hispida* tubers were extracted following the method employed by Mohd. Hori *et al*. (2016). Two different types of solutions were prepared: 1.0 M sodium chloride (NaCl) and distilled water. The tuber pieces were soaked for 5 days in each solution. The NaCl was supplied by Evergreen Engineering and Resources in Sdn Bhd., Malaysia and was used as the solution for removing the toxic elements in *Dioscorea hispida*.

### Sample Preparation

The *Dioscorea hispida* (Fig. 1a,b) was cleaned (Fig. 1c) and cut into pieces using a slicer blade having 3 mm thickness, as shown in Fig. 1d. The pieces were weighed using a digital weighing scale into 25 g portions, and 5 samples were prepared for the 1.0 M NaCl and distilled water solutions. The samples were then soaked for 5 days in 500 mL of the selected solutions (Fig. 1e). After the soaking process, the samples of *Dioscorea hispida* tubers went through a blending process (Philips NL 9206AD) to produce a solution mixture as preparation for LC-MS (Agilent 6520 Accurate-Mass Q-TOF) alkaloid dioscorine detection.

### Liquid Chromatography

Liquid chromatography-mass spectrometry (LC-MS) was applied to identify the alkaloid dioscorine compound in the solution mixture of *Dioscorea hispida* tubers after soaking completion based on days 1, 2, 3, 4, and 5. An Agilent 1290 Infinity LC system Santa Clara, CA, USA) coupled with an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source was used, and the concentration samples were spun at 15000 rpm for 15 min.

The chromatography process was a compound separation method in which two or more elements were separated and optionally spread between two unmixable stages. Liquid chromatography (LC) was performed on an Agilent Zorbax Eclipse XDB-C18 column (narrow-bore 2.1 x 150 mm, 3.5-micron particle size) at 25 °C. The combination of ultra-purified water ($H_2O$) and acetonitrile ($CH_3CN$) was employed as the mobile phase. The chromatography was conducted using an isocratic mixture and a linear gradient of ACN, which were 5% at 0 to 5 min, 5 to 100% at 5 to 20 min, and 5 to 100% at 20 to 25 min. The column was flushed with acetonitrile at the end of the chromatographic process and re-

equilibrated for 5 min between individual runs. The flow rate was 0.5 mL/min and the injection volume was 1.0 µL.

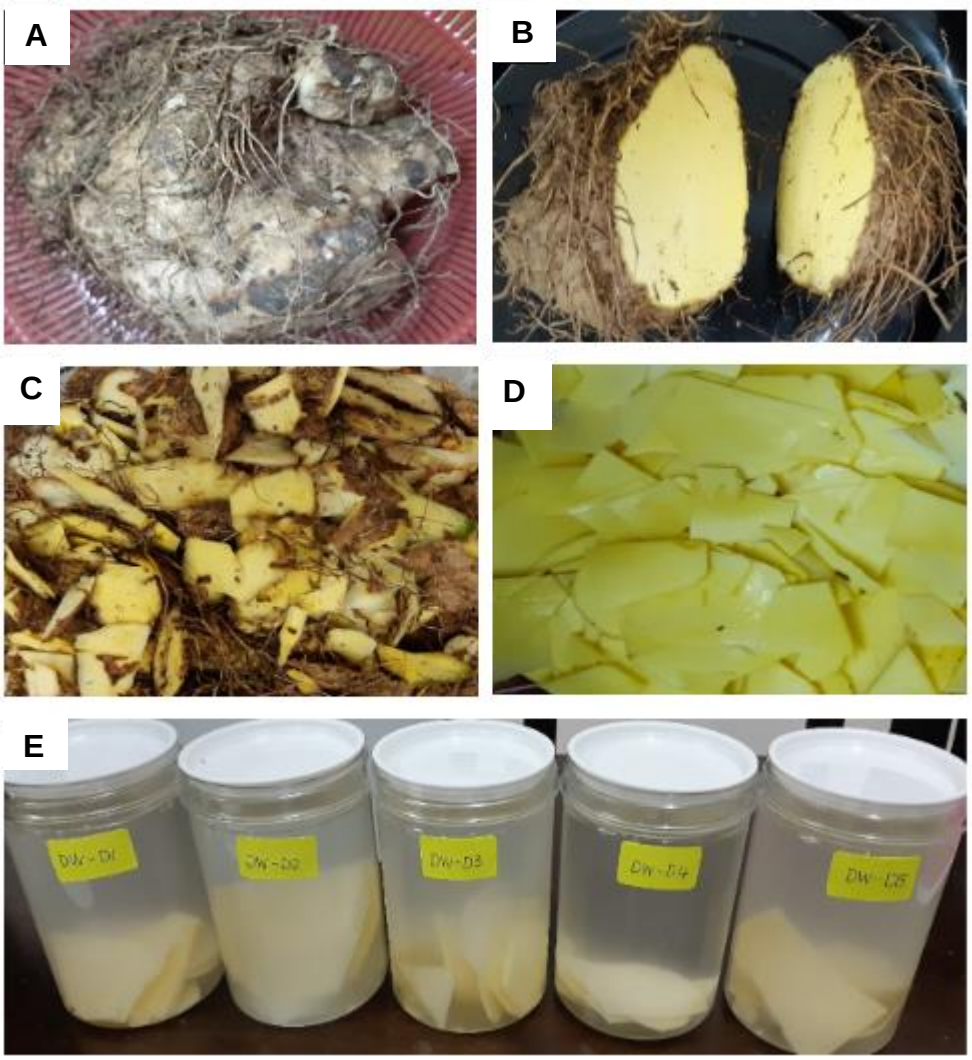


**Fig. 1.** (A) Irregular-shaped tubers, (B) yellowish-brown *Dioscorea hispida,* (C) peels of *Dioscorea hispida* tubers, (D) slices of *Dioscorea hispida* tubers, (E) soaking process of tubers in 1.0 M NaCl and distilled water solutions

## Mass Spectrometry

The positive and negative ion LC-MS spectra were obtained on an ion trap Agilent MassHunter Qualitative Analysis B.07.00 using electrospray ionization (ESI). The analysis identified the polarity after optimizing the instrument settings on the ion charge condition and scanning speed. Nitrogen was applied as a drying gas with a flow rate of 10 L/min, as well as a nebulizer gas at a pressure of 412 kPa. The nebulizer temperature was fixed at 300 °C. The mass spectrometer was recorded in the range between 100 to 3200 m/z, the fragmentor voltage was set at 125 V, and the skimmer voltage was at 65 V.

## Compound Identification

In the compound database structure, the MS data of the original compounds were kept in a library as storage data to be used in the Agilent software. Every sample of the

specimen was analyzed by MS and the process data were recorded. The fragmentation patterns from the LC-MS results of the unknown compound were cross-checked against those in the library for positive compound identification. Figure 2 presents the compound structure of dioscorine in the *Dioscorea hispida* tubers.

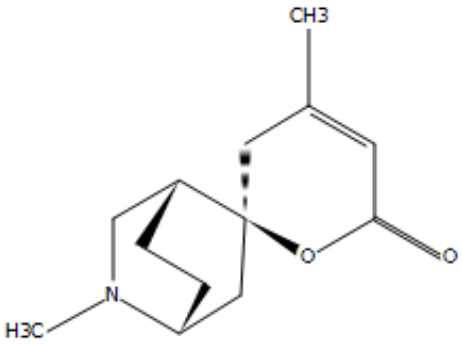


**Fig. 2.** Dioscorine compound structure (Abiodun *et al*. 2009)

## RESULTS AND DISCUSSION

### Dioscorine Identification by LC-ESI-MS Analysis

Table 1 provides the experimental data of the *Dioscorea hispida* soaked in distilled water for 5 days. The results indicate that the dioscorine compound composition clearly showed a decreasing trend of relative abundance values. The dioscorine compound screening used a positive polarity mode from day 1 until day 5. However, there was a 95% significant difference between the amount of relative abundance of dioscorine compound for the sample DW-D1 (5373789) to DW-D5 (280835.53). Strong evidence of retention time ($R_{t)}$ was shown in Table 1, resulting in the positive correlation that the number of days for the soaking process should be increased to remove the toxic elements.

Table 2 provides the experimental data of the soaking process for the *Dioscorea hispida* samples in the NaCl solution. A substantial difference can be seen at SC-D1 (375851.63) and SC-D4 (7141.75). However, the sample SC-D5 detected no dioscorine compound on day 5. The result of soaking *Dioscorea hispida* tubers in the NaCl solution presented more successful findings compared to the distilled water solution. The NaCl solution removed the alkaloid dioscorine more rapidly (approximately 98% amount of relative abundance) compared to distilled water (Kresnadipayana and Waty 2019). The correlation factor is related to different pH of the solutions. Sodium chloride was more neutral with a pH 7, while distilled water was slightly acidic with a pH of 5.8 (Hudzari *et al*. 2012). The abundance amount of dioscorine from these findings specifically supports the hypothesis that the NaCl solution is a better medium for the soaking process and can efficiently remove the poisonous element in *Dioscorea hispida* tubers (Biswal and Bozormer 1991).

As shown in Tables 1 and 2, the retention time ($R_t$) increased for samples DW-D1 (1.648) to DW-D5 (1.715) in distilled water and SC-D1 (1.693) to SC-D4 (1.718) in the NaCl solution. These results are consistent with the data obtained from the time measurements taken when the solute passed through the column during the LC-MS analysis. Figure 3 presents a graph comparison regarding the amount of relative abundance in *Dioscorea hispida* tubers and the different days and solutions used.

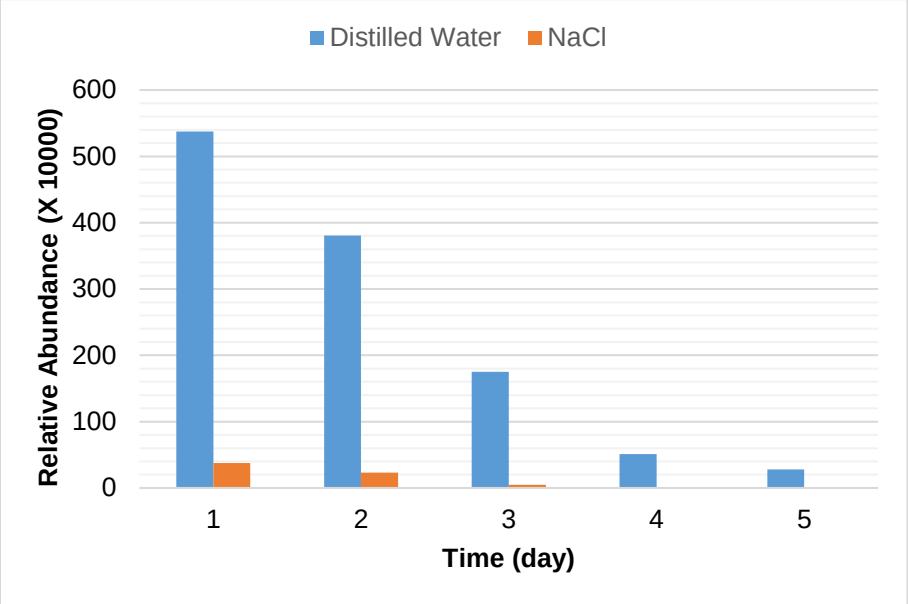


**Fig. 3.** Relative abundance values in D*ioscorea hispida* for different days and solutions

**Table 1.** Distilled Water Samples: Polarity, Relative Abundance, Retention Time at MS Obtained with Dual ESI Sources of Dioscorine Compound

| Samples | Compound | Polarity | Relative Abundance | Retention Time ($R_t$) |
|---|---|---|---|---|
| DW-D1 | Dioscorine | Positive | 5373789 | 1.648 |
| DW-D2 | Dioscorine | Positive | 3806964.25 | 1.661 |
| DW-D3 | Dioscorine | Positive | 1751439.63 | 1.679 |
| DW-D4 | Dioscorine | Positive | 511627.91 | 1.707 |
| DW-D5 | Dioscorine | Positive | 280835.53 | 1.715 |

**Table 2.** Sodium Chloride Samples: Polarity, Relative Abundance, Retention Time at MS Obtained with Dual ESI Sources of Dioscorine Compound

| Samples | Compound | Polarity | Relative Abundance | Retention Time ($R_t$) |
|---|---|---|---|---|
| SC-D1 | Dioscorine | Positive | 375851.63 | 1.693 |
| SC-D2 | Dioscorine | Positive | 231581.36 | 1.705 |
| SC-D3 | Dioscorine | Positive | 46796.39 | 1.713 |
| SC-D4 | Dioscorine | Positive | 7141.75 | 1.718 |
| SC-D5 | Dioscorine | Positive | - | - |

## LC-ESI-MS Spectrum and Fragmentation Pathways for Dioscorine

The LC-MS analysis using ESI is the most popular for identifying the dioscorine compound (Della-Flora *et al*. 2019). Following the present results, these studies have demonstrated that the dioscorine compound had been protonated to the positive mode. It is possible that the mobile phase of 0.1% formic acid enhanced the sensitivity and protonation. The soft ionization techniques produced very little fragmentation, which provides evidence on the molecular mass of the compound (Naz *et al*. 2019).

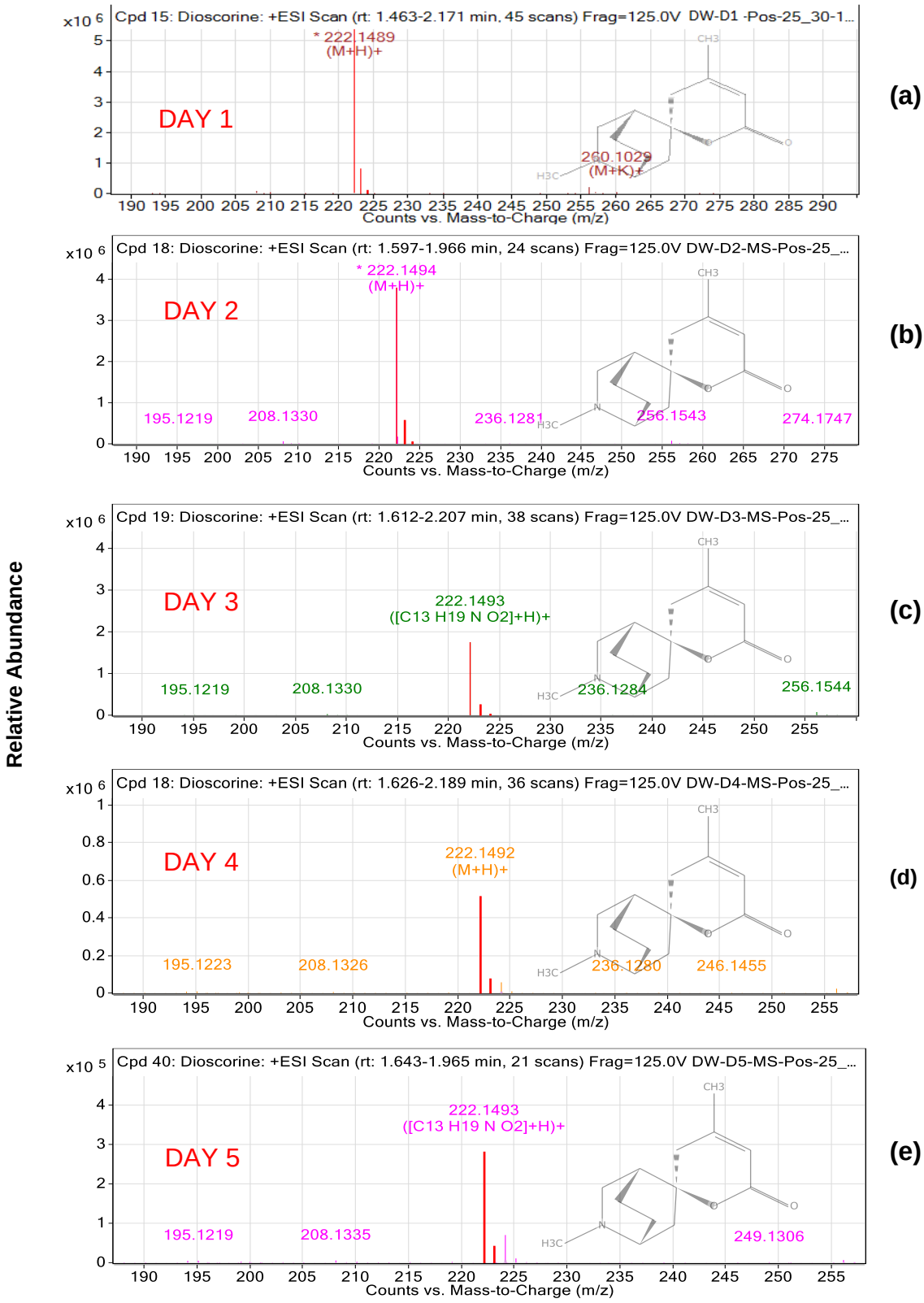


**Fig. 4.** Fragment ion spectra in MS analysis of dioscorine compound for distilled water: (a) DW-D1, (b) DW-D2, (c) DW-D3, (d) DW-D4, and (e) DW-D5

Another possible explanation for the results obtained from the MS analysis (Tables 1 and 2), could be that the liquid-liquid protocols coupled with the LC-ESI-MS based on the selected particle size column for the toxic compounds. The dioscorine compound was identified in the positive ion mode ESI-MS/MS spectra and transferred into the library by using the matches fit score from the dioscorine compound value. The chromatographic separation process in the *Dioscorea hispida* tuber samples was set by the mass spectrometer to scan the mass range with the upper mass limit. It was identified by the highest molecular weight of the subject compound analysis and the smaller limit by the background of ions. The final scanning process of the samples was stored, and the total ion intensities was produced. The total ion current was stored and computed for the result analysis (Badoud *et al*. 2010). These are important findings for the retention times of the longest retained component, which were scanned by the mass spectrometer to obtain the data for processing. This is particularly useful for subsequent spectral analyses, which were based on the total ion (TIC) trace obtained from the *Dioscorea hispida* tuber mixtures. These are due to the appropriate time by retaining the longest retention time and producing the data available for processing. Figure 4 presents the mass spectrometer analysis obtained from the investigation of *Dioscorea hispida* tubers in the distilled water solution. The first set of analyses examined the identification of the alkaloid dioscorine after 5 days of soaking. The relative abundance amount remarkably decreased from DW-D1 to DW-D5.

Interestingly, the *Dioscorea hispida* in the NaCl solution also presented a more rapid decrease of relative abundance from SC-D1 to SC-D4. These results are likely to be related to the osmosis diffusion action when the immersion of *Dioscorea hispida* tubers using sodium chloride (NaCl) solution is carried out. The osmosis reaction is influenced by the concentration gradient. The higher concentration gradient of sodium chloride, the faster the osmosis reaction will occur (Kresnadipayana and Waty 2019). Table 2 shows that the dioscorine alkaloid content was not detected in sample SC-D5, indicating that the *Dioscorea hispida* tubers are safe for consumption or to be used for other purposes. The *m*/*z* ratios coverage of the mass spectrum was produced, whereby the single mass spectrum was provided with complete analytical information for each *Dioscorea hispida* tuber sample (Müller *et al*. 2000). These results are consistent with the research and suggest that the use of the sodium chloride solution is quicker in removing the alkaloid in *Dioscorea hispida* tubers compared to distilled water solution, as shown in Fig. 5 (Oellig and Schmid 2019), which is crucial because the compound may cause food poisoning depending on the toxicity content of the tuber.

The availability of toxic dioscorine was a concern, but the usage of sodium chloride made it easier and faster to remove. This method can effectively minimize the time of preparing *Dioscorea hispida* compared to soaking in a flowing river water for 7 days, which was demonstrated as a technique for removing the dioscorine compound (Roslan *et al.* 2012). Recent studies have been accomplished regarding the application of pesticides, which can benefit from the soaked water technique for *Dioscorea hispida*. The advancements of food products from *Dioscorea hispida* will produce a safer outcome for human consumption (Hudzari *et al*. 2012).

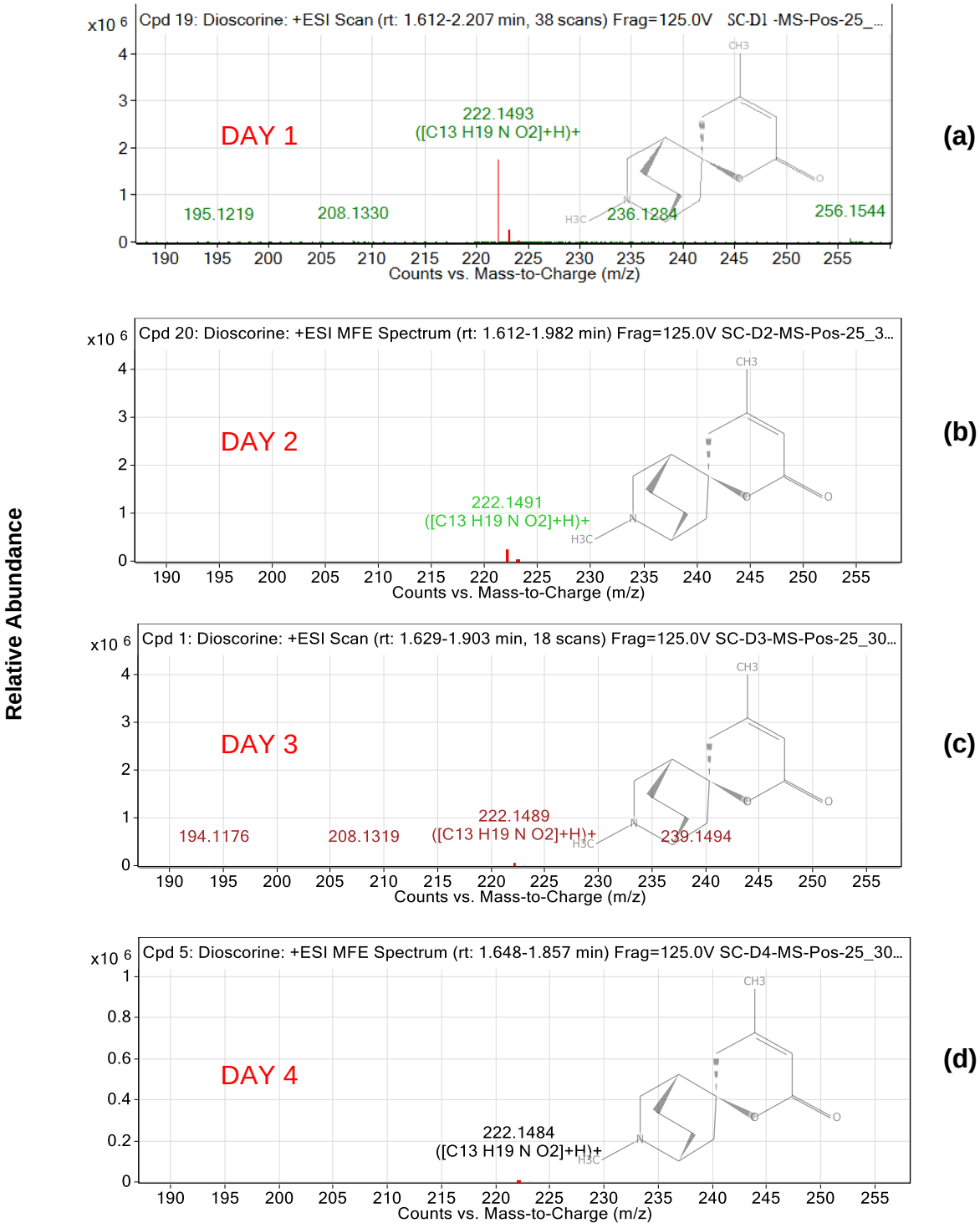


**Fig. 5.** Spectra of fragment ions in MS analysis of dioscorine compound for NaCl: (a) SC-D1, (b) SC-D2, (c) SC-D3, (d) SC-D4

## CONCLUSIONS

1. The present study was designed to determine the minimum days for removing alkaloid dioscorine in *Dioscorea hispida* tubers. It was found that the dioscorine compound was removed faster by using the sodium chloride 1.0 M NaCl solution compared to distilled water.
2. The results from the LC-ESI-MS analysis showed that on day 5, there was no dioscorine detected for soaking in 1.0 M NaCl compared to 281000 of relative abundance amount in the distilled water solution.
3. This study established a qualitative LC-MS framework for detecting the dioscorine compound, and this approach has proven useful in expanding the understanding of toxic removal in *Dioscorea hispida* tubers.

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## REFERENCES CITED

Abiodun, O. A., Adegbite, J. A., and Oladipo, T. S. (2009). "Effect of soaking time on the pasting properties of two cultivars of trifoliate yam (*Dioscorea dumetorum*) flours," *Pakistan J. Nutr*. 8(10), 1537-1539. DOI: 10.3923/pjn.2009.1537.1539

Badoud, F., Grata, E., Perrenoud, L., Saugy, M., Rudaz, S., and Veuthey, J. L. (2010). "Fast analysis of doping agents in urine by ultra-high-pressure liquid chromatography-quadrupole time-of-flight mass spectrometry. II: Confirmatory analysis," *J. Chromatogr. A* 1217(25), 4109-4119. DOI: 10.1016/j.chroma.2009.11.001

Barbieri, M., and Heard, C. M. (2019). "Isolation of punicalagin from *Punica granatum* rind extract using mass-directed semi-preparative ESI-AP single quadrupole LC-MS," *J. Pharmaceut. Biomed.* 166, 90-94. DOI: 10.1016/j.jpba.2018.12.033

Biswal, R. N., and Bozormer, K. (1991). "Equilibrium data for osmotic concentration of potato in nacl water solution," *J. Food Process Eng.* 14(4), 237-245. DOI: 10.1111/j.1745-4530.1991.tb00134.x

Brown, S., and Carmical, J. (2019). "Phospholipid depletion techniques in LC - MS bioanalysis," in: *Sample Preparation in LC-MS Bioanalysis*, W. Li, W. Jan, and Y, Fu (eds.), John Wiley & Sons, Inc., Hokoben, NJ. pp. 52-67. DOI: 10.1002/9781119274315.ch4

Della-Flora, A., Becker, R. W., Benassi, S. F., Toci, A. T., Cordeiro, G. A., Ibáñez, M., Portolés, T., Hernández, F., Boroski, M., and Sirtori, C. (2019). "Comprehensive investigation of pesticides in Brazilian surface water by high resolution mass spectrometry screening and gas chromatography – mass spectrometry quantitative

analysis," *Sci. Total Environ.* 669, 248-257. DOI: 10.1016/j.scitotenv.2019.02.354

Edhirej, A., Sapuan, S. M., Jawaid, M., and Zahari, N. I. (2017). "Preparation and characterization of cassava bagasse reinforced thermoplastic cassava starch," *Fiber Polym.* 8, 162-171. DOI: 10.1007/s12221-017-6251-7

Gunawan, S., Aparamarta, H. W., Anindita, B. P., and Antari, A. T. (2019). "Effect of fermentation time on the quality of modified gadung flour from gadung tuber (*Dioscorea hispida* Dennst.)," in: *Broad Exposure to Science and Technology 2019*, Bali, Indonesia. DOI: 10.1088/1757-899X/673/1/012002

Hajšlová, J., Schulzová, V., Botek, P., and Lojza, J. (2018). "Natural toxins in food crops and their changes during processing," *Czech J. Food Sci.* 22, S29-S34. DOI: 10.17221/10606-cjfs

Halimatul, M. J., Sapuan, S. M., Jawaid, M., Ishak, M. R., and Ilyas, R. A. (2019a). "Effect of sago starch and plasticizer content on the properties of thermoplastic films: Mechanical testing and cyclic soaking-drying," *Polimery*. 64(6), 32–41. DOI: 10.14314/polimery.2019.6.5

Halimatul, M. J., Sapuan, S. M., Jawaid, M., Ishak, M. R., and Ilyas, R. A. (2019b). "Water absorption and water solubility properties of sago starch biopolymer composite films filled with sugar palm particles," *Polimery*. 64(9), 27–35. DOI:10.14314/polimery.2019.9.4

Hamid, Z. A. A., Idris, M. H. M., Arzami, N. A. A. B., and Ramle, S. F. M. (2019). "Investigation on the chemical composition of *Discorea hispida dennst* (Ubi Gadong)," *AIP Conf. Proc. 2068.* DOI: 10.1063/1.5089340

Hudzari, R. M., Ssomad, M. A. H. A., Rizuwan, Y. M., Asimi, M. N. N., and Abdullah, A. B. C. (2012). "Development of automatic alkaloid removal system for *Dioscorea hispida*," *Front. Sci.* 1(1), 16-20. DOI: 10.5923/j.fs.20110101.03

Ilyas, R. A., and Sapuan, S. M. (2020). "The preparation methods and processing of natural fibre bio-polymer composites," *Current Organic Synthesis*. 16(8), 1068-1070. DOI:10.2174/1570179416082001201056l6

Ilyas, R. A., Sapuan, S. M., Atiqah, A., Ibrahim, R., Abral, H., Ishak, M. R., Zainudin, E. S., Nurazzi, N. M., Atikah, M. S. N., Ansari, M. N. M., Asyraf, M. R. M., Supian, A. B. M., & Ya, H. (2019a). "Sugar palm ( *Arenga pinnata* [Wurmb .] Merr ) starch films containing sugar palm nanofibrillated cellulose as reinforcement: Water barrier properties," *Polymer Composites*. 41(2), 459-467. DOI: 10.1002/pc.25379

Ilyas, R. A., Sapuan, S. M., Ibrahim, R., Abral, H., Ishak, M. R., Zainudin, E. S., Atikah, M. S. N., Mohd Nurazzi, N., Atiqah, A., Ansari, M. N. M., Syafri, E., Asrofi, M., Sari, N. H., and Jumaidin, R. (2019b). "Effect of sugar palm nanofibrillated cellulose concentrations on morphological, mechanical and physical properties of biodegradable films based on agro-waste sugar palm (*Arenga pinnata (Wurmb.) Merr*) starch," *Journal of Materials Research and Technology*, 8(5), 4819-4830. DOI: 10.1016/j.jmrt.2019.08.028

Ilyas, R. A., Sapuan, S. M., Ishak, M. R., and Zainudin, E. S. (2018). "Development and characterization of sugar palm nanocrystalline cellulose reinforced sugar palm starch bionanocomposites," *Carbohydrate Polymers*. 202, 186-202. DOI: 10.1016/j.carbpol.2018.09.002

Irmayadani, T. M. I., Yopi, and Febriani. (2019). "Preliminary study of bioethanol production by Saccharomyces cerevisiae BTCC12 utilizing hydrolysis products of *Dioscorea hispida* tubers," in: *The 3rd International Conference on Natural and Enviornmental Sciences,* Banda Aceh, Indonesia. DOI: 10.1088/1755-

1315/364/1/012004

Jalal, R. S., Weber, J. F. F., and Manshoor, N. (2018). "Dereplication of oligostilbenes in dipterocarpaceous plants using LCMS-ESI-Ion trap-database," *J. Liq. Chromatogr. R. T.* 41(4), 161-169. DOI: 10.1080/10826076.2018.1428200

Jumaidin, R., Ilyas, R. A., Saiful, M., Hussin, F., and Mastura, M. T. (2019a). "Water transport and physical properties of sugarcane bagasse fibre reinforced thermoplastic potato starch biocomposite," *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*. 61(2), 273-281.

Jumaidin, R., Khiruddin, M. A. A., Asyul Sutan Saidi, Z., Salit, M. S., and Ilyas, R. A. (2020). "Effect of cogon grass fibre on the thermal, mechanical and biodegradation properties of thermoplastic cassava starch biocomposite," *International Journal of Biological Macromolecules*. 146, 746-755. DOI: 10.1016/j.ijbiomac.2019.11.011

Jumaidin, R., Saidi, Z. A. S., Ilyas, R. A., Ahmad, M. N., Wahid, M. K., Yaakob, M. Y., Maidin, N. A., Rahman, M. H. A., and Osman, M. H. (2019b). "Characteristics of cogon grass fibre reinforced thermoplastic cassava starch biocomposite: Water absorption and physical properties," *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 62(1), 43-52.

Kumoro, A. C., Retnowati, D. S., Ratnawati, R., and Widiyanti, M. (2019). "Effect of temperature and reaction time on the swelling power and solubility of gadung (*Dioscorea hispida* dennst) tuber starch during heat moisture treatment process," *J. Phys. Conf. Ser.* 1295. DOI: 10.1088/1742-6596/1295/1/012062

Kresnadipayana, D., and Waty, H. (2019) "The concentration of NaCl soaking to decreasing cyanide levels in Gadung (*Dioscorea hispida* Dennst)," *Jurnal Teknologi Laboratorium.* 8(1), 36-40 DOI: 10.29238/teknolabjournal.v8i1.156

Lee, B. I., Park, M., Shin, S., Byeon, J., Park, Y., Kim, N., Choi, J., and Shin, Y. G. (2019). "Quantitative analysis of tozadenant using liquid plasma and its human pharmacokinetics prediction pharmacokinetic modeling," *Molecules* 24(7), 1295. DOI: 10.3390/molecules24071295

Leete, E., and Michelson, R. H. (1988). "Biosynthesis of dioscorine from trigonelline," *Phytochemistry* 27(12), 3793-3798. DOI: 10.1016/0031-9422(88)83019-X

Mlingi, N. L. V., Bainbridge, Z. A., Poulter, N. H., and Rosling, H. (1995). "Critical stages in cyanogen removal during cassava processing in southern Tanzania," *Food Chem.* 53(1), 29-33. DOI: 10.1016/0308-8146(95)95782-2

Mohd Hori, N. A. F., Mohd Nasir, N. F., Mohd, N. A., Cheng, E. M., and Sohaimi, S. N. (2016). "The fabrication and characterization of hydroxyapatite-ubi gadong starch based tissue engineering scaffolds," in: *2016 - IEEE-EMBS Conf. Biomed. Eng. Sci.*, Kuala Lumpur, Malaysia, pp. 220-225. DOI: 10.1109/IECBES.2016.7843446

Müller, C., Vogt, S., Goerke, R., Kordon, A., and Weinmann, W. (2000). "Identification of selected psychopharmaceuticals and their metabolites in hair by LC/ESI-CID/MS and LC/MS/MS," *Forensic Sci. Int.* 113(1-3), 415-421. DOI: 10.1016/S0379-0738(00)00202-4

Napisah, H., and Rosma, A. (2020). "Fermentation of *Dioscorea hispida* oligosaccharides as prebiotic potential by lactic acid bacteria and bifidobacterium," in: *The Third Bioprocessing and Biomanufacturing Symposium*, Perak, Malaysia. DOI: 10.1088/1757-899X/716/1/012005

Navia, D. P., and Villada, H. S. (2005). "Thermoplastic cassava flour," in: *Thermoplastics Elastomers*, A. El-Sonbati (ed.), Intech Open, London, UK. DOI: 10.5772/34217

Naz, A., Iqtadar, R., Ahmed, F., and Ul-haq, Z. (2019). "Degradation kinetics of fluvoxamine in buffer solutions : In silico ADMET profiling and identification of degradation products by LC-MS / ESI," *Arab. J. Chem.* 13(2), 4134-4146. DOI: 10.1016/j.arabjc.2019.06.001

Nazrin, A., Sapuan, S. M., Zuhri, M. Y. M., Ilyas, R. A., Syafiq, R., and Sherwani, S. F. K. (2020). "Nanocellulose reinforced thermoplastic starch (TPS), polylactic acid (PLA), and polybutylene succinate (PBS) for food packaging applications," *Frontiers in Chemistry*. *8*(213), 1-12. DOI: 10.3389/fchem.2020.00213

Oellig, C., and Schmid, S. (2019). "Polyethyleneimine as weak anionic exchanger adsorbent for clean-up in pesticide residue analysis of fruits and vegetables," *J. Chromatogr. A* 1597, 9-17. DOI: 10.1016/j.chroma.2019.03.020

Roslan, S., Razali, M. H. H., and Ismail, W. I. W. (2012). "An overview for the application of multispectral device for determination of alkaloid level in *Dioscorea hispida*," *Sci. J. Rev.* 1(3), 53-57.

Sami, M., and Fata, K. (2019). "Application of janeng fruit (Dioscorea hispida dennst) on mortality killing the golden," in: *International Conference on Science and Innovated Engineering (I-COSNE)*, Aceh, Indonesia. DOI: 10.1088/1757-899X/536/1/012153

Sanyang, M. L., Ilyas, R. A., Sapuan, S. M., and Jumaidin, R. (2018). "Sugar palm starch-based composites for packaging applications," in: *Bionanocomposities for Packaging Applications*, M. Jawaid, S. K. Swain (eds.), Springer International Publishing, New York, NY. pp. 125-147.

Sasiwatpaisit, N., Thitikornpong, W., Palanuvej, C., and Ruangrungsi, N. (2014). "Dioscorine content in *Dioscorea hispida* dried tubers in Thailand by TLC-densitometry and TLC image analysis," *J. Chem. Pharm. Res*. 6(4), 803-806.

Su, H., Liu, K. T., Chen, B. H., Lin, Y. P., Jiang, Y. M., Tsai, Y. H., Chang, F. R., Shiea, J., and Lee, C. W. (2019). "Rapid identification of herbal toxins using electrospray laser desorption ionization mass spectrometry for emergency care," *J. Food Drug Anal.* 27(2), 415-427. DOI: 10.1016/j.jfda.2018.11.001

Syafri, E., Sudirman, Mashadi, Yulianti, E., Deswita, Asrofi, M., Abral, H., Sapuan, S. M., Ilyas, R. A., and Fudholi, A. (2019). "Effect of sonication time on the thermal stability, moisture absorption, and biodegradation of water hyacinth (*Eichhornia crassipes*) nanocellulose-filled bengkuang (*Pachyrhizus erosus*) starch biocomposites," *Journal of Materials Research and Technology* 8(6), 6223-6231. DOI: 10.1016/j.jmrt.2019.10.016

Vinale, F., Salvatore, M. M., Nicoletti, R., Staropoli, A., Manganiello, G., Venneri, T., Borrelli, F., Dellagreca, M., Salvatore, F., and Andolfi, A. (2020). "Identification of the main metabolites of a marine-derived strain of *Penicillium brevicompactum* using LC and GC MS techniques," *Metabolites*10(2), 55. DOI: 10.3390/metabo10020055

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