BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 2, October 2026 | Pages: 2369-2373 | DOI: 10.14421/biomedich.2026.152.2369-2373 ISSN 2540-9328 (online)

Caffeine Profiling of *Antidesma bunius* using UHPLC-Q-Orbitrap HRMS Metabolomics

Elsa Putri Rahmawati[1], Dika Putra Wijaya[1,2], Danar Danar[1]*, Nabilla Sevira Endah Prastiwi[1], Marshya Qurrotul Aini Wibowo[1], Cahya Adidharma[1], Agniatun Maulidatun Khasanah[3], Fitri Rosa Amalia[1], Sumari Sumari[1]

[1]Chemistry Department; [3]Biology Department, Faculty of Mathematics and Natural Science, Universitas Negeri Malang
Jl. Semarang No. 5, Malang 65145, Tel. +62-341-330-1130, Fax. +62-341-551921, Indonesia
[2]Quality Control Department, PT Indofood CBP Sukses Makmur FID Pasuruan Tbk.
Jalan Raya Gempol 67155 Gempol East Java, Tel. +62-852-3014-8165, Indonesia.

Corresponding author*
danar.fmipa@um.ac.id

Manuscript received: 12 July 2026. Revision accepted: 25 September 2026, Published: 27 November 2026.

Abstract

Antidesma bunius (L.) Spreng. is an indigenous tropical fruit recognized as a potential source of bioactive compounds with various pharmacological properties. However, comprehensive information regarding its metabolite profile, particularly the occurrence of caffeine, remains limited. This study aimed to identify and characterize caffeine in the ethanol extract of *A. bunius* fruit using an untargeted metabolomics approach based on Ultra-High Performance Liquid Chromatography coupled with Quadrupole-Orbitrap High Resolution Mass Spectrometry (UHPLC-Q-Orbitrap HRMS). Dried fruit powder was extracted by maceration using 96% ethanol, and the resulting extract was analyzed using UHPLC-Q-Orbitrap HRMS operated in Full MS/dd-MS² mode. Compound identification was performed through accurate mass determination, MS/MS fragmentation analysis, and database matching. The analysis successfully identified caffeine as one of the detected secondary metabolites. The compound eluted at a retention time of 2.86 min and exhibited a protonated molecular ion ($[M+H]^+$) at *m/z* 195.08775, corresponding to the molecular formula $C_8H_{10}N_4O_2$. Structural confirmation was supported by a characteristic fragment ion at *m/z* 138.06623 generated through the neutral loss of a methyl isocyanate moiety during high-energy collision dissociation. The excellent agreement between experimental and theoretical mass values demonstrated the high accuracy of the Orbitrap HRMS platform. These findings expand the current phytochemical information available for *A. bunius* and indicate that UHPLC-Q-Orbitrap HRMS is a reliable and efficient analytical platform for metabolite profiling and rapid dereplication of bioactive compounds in indigenous tropical fruits. The results also provide a scientific basis for further investigation of *A. bunius* as a potential functional food and nutraceutical resource.

Keywords: *Antidesma bunius*; caffeine; metabolomics; phytochemical profiling; UHPLC-Q-Orbitrap HRMS.

INTRODUCTION

Antidesma bunius (L.) Spreng., commonly known as buni fruit, is a tropical fruit species belonging to the family Phyllanthaceae that is widely distributed in Southeast Asia, including Indonesia, Thailand, Malaysia, the Philippines, and Vietnam (Islam et al., 2018) (Nguyen-Ngoc et al., 2022). The fruit is characterized by its small size and color changes from red to dark purple-black during ripening, which are associated with the accumulation of pigments and secondary metabolites (Fathimah et al., 2026). Traditionally, buni fruit has been consumed as fresh fruit and processed into various products, such as juices, syrups, jams, fermented beverages, and traditional wines (Kemsawasd & Chaikham, 2021). Due to its nutritional value and diverse phytochemical composition, *A. bunius* has gained attention as a potential source of functional food ingredients and nutraceutical resources (Islam et al., 2018). Phytochemical studies have revealed that *A. bunius* contains various bioactive compounds, including phenolic compounds, flavonoids, anthocyanins, tannins, proanthocyanidins, and phenolic acids (Krongyut & Sutthanut, 2019; Nguyen-Ngoc et al., 2022). These compounds contribute to the characteristic color, taste, and biological properties of the fruit. Several flavonoids, including catechin, procyanidin B1, procyanidin B2, quercetin, and their derivatives, have been identified in *A. bunius* (Nguyen-Ngoc et al., 2022). Furthermore, recent investigations have reported the presence of other specialized metabolites, such as lignans, coumarinolignans, cyclopeptides, and additional phenolic derivatives, demonstrating the chemical complexity of this species (Nguyen-Ngoc et al., 2024). Phenolic compounds and flavonoids are important plant-derived bioactive compounds due to their antioxidant properties (Ardyansyah et al., 2026). These compounds can reduce

oxidative stress through free radical scavenging, hydrogen donation, and inhibition of oxidative reactions. Excessive production of reactive oxygen species has been associated with various chronic diseases, including cardiovascular disorders, diabetes mellitus, cancer, and other degenerative diseases. Therefore, fruits rich in phenolic compounds and flavonoids are considered promising sources for the development of functional foods with health-promoting properties (Udomkasemsab et al., 2018; Zubia et al., 2023).

The antioxidant activity of *A. bunius* has been linked to its high content of phenolic and flavonoid compounds. Extracts from *A. bunius* fruit have demonstrated antioxidant activity and inhibitory effects on digestive enzymes, indicating its potential application in managing oxidative stress-related disorders (Krongyut & Sutthanut, 2019). In addition, processing conditions have been reported to influence the retention of bioactive compounds and antioxidant properties of *A. bunius* juice, supporting its potential as a functional beverage ingredient (Kemsawasd & Chaikham, 2021). Besides antioxidant activity, *A. bunius* also exhibits antibacterial potential. The extract of buni fruit has been reported to inhibit pathogenic bacteria, including *Salmonella typhimurium* and *Bacillus cereus* (Indrawati et al., 2017). The antimicrobial effects of plant phenolic compounds are associated with their ability to disrupt bacterial membranes, interfere with cellular enzymes, and inhibit microbial growth. These properties indicate that *A. bunius* has potential as a natural source of antimicrobial compounds for food and health-related applications. Despite its biological potential, the utilization of *A. bunius* as a high-value bioresource remains limited, particularly in Indonesia. Comprehensive characterization of its chemical composition is required to understand the relationship between metabolites and biological activities. Advanced analytical techniques, including high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS), have been widely applied for plant metabolite identification and metabolomic profiling (Nguyen-Ngoc et al., 2022, 2024). Therefore, this study aimed to evaluate the phytochemical characteristics of *Antidesma bunius* fruit, determine its antioxidant and antibacterial activities, and perform metabolomic profiling using advanced analytical approaches (Azani et al., 2026). This research is expected to provide scientific evidence regarding the potential utilization of buni fruit as a natural bioactive resource and support the development of functional food products based on Indonesia's biodiversity (Wijaya et al., 2026).

MATERIALS AND METHODS

Tool and Material

The tools used in this study were standard laboratory glass, air bubble washer, rhizome chopper, hybrid dryer warehouse, oven (Memmert), food dehydrator, grinding machine (Grinder) with gasoline motor drive (Honda), machine and sieve (Sieve Shaker) no. mesh 12, 18, 30, 60, 70, 120, 170, and 230, and rotary evaporator (IKA RV 10 digital). The materials used in this study were buni fruit, 96% alcohol (Merck), and Whatman filter paper no. 42.

Processing Buni Fruit into Powder

The process began with buni fruit washing using an air bubble washer to remove physical contaminants without damaging the fruit tissue. After wet sorting and size reduction, the fruit's moisture content is significantly reduced through a thermal process using a food dehydrator, oven, or hybrid drying building until it reaches a critical dry point safe from microbial growth. The dried fruit then enters the grinding stage using a grinder machine to reduce its size to fine particles. To produce fruit powder with uniform homogeneity and meet quality standards, the product is sieved using a sieve shaker with the appropriate mesh number (ranging from 12 to 230 mesh). The result was buni fruit powder which is ready for maceration extraction.

Maceration of Buni Fruit Powder

A 250 g sample of buni fruit powder was added to 2500 mL of ethanol (1:10 w/v) and left to stand for 120 hours. The mixture was filtered using Whatman No. 42 paper. Next, use a rotary evaporator at 60°C and 300 rpm. Continue until there is no solvent left in the buni fruit extract to achieve the maximum thickness result.

LC-HRMS Preparation and Testing

Sample preparation was carried out by weighing 10 mg of sample and then dissolving it in 1 mL of HPLC-grade methanol (Fisher Chemical). The mixture was homogenized using a digital vortex (Thermo Scientific) for 2 minutes. Once homogenized, the solution was filtered through a 0.2 µm nylon filter before being introduced into the chromatography system. Metabolomics analysis was performed using a Thermo Scientific™ Vanquish™ Horizon ultra-high-performance liquid chromatography (UHPLC) system equipped with a binary pump. Analyte separation was carried out using a Thermo Scientific™ Accucore™ Phenyl Hexyl column with dimensions of 100 mm x 2.1 mm and a particle size of 2.6 µm. The column temperature was set at 40 °C with a sample injection volume of 5 µL. The mobile phase consisted of (A) MS-grade water with 0.1 % formic acid and (B) MS-grade acetonitrile with 0.1 % formic acid. The flow rate was set at 0.3 mL/min for a total analysis time of 25 minutes. Gradient elution was set as follows: starting at 5 % B, gradually increasing to 90 % B in 16

minutes, maintained at 90 % B for 4 minutes, and returning to the initial 5 % B level until the 25th minute (Kusuma et al., 2025; Rivaldi et al., 2025; Windarsih et al., 2022). Parameters mass detection was performed using a Thermo Scientific™ Orbitrap™ Exploris 240 HRMS. Data acquisition was performed in Full MS/dd-MS2 mode with polarity switching (positive and negative). The spray voltage was set at 3500 V for positive mode and 2500 V for negative mode. The ion source used was Optamax™ NG Heated Electrospray Ionization (H-ESI) with the following gas parameters: sheath gas 35 AU, ux gas 7 AU, and sweep gas 1 AU. The ion transfer tube temperature was set at 300 °C and the vaporizer temperature at 320 °C. Full MS resolution was set at 60,000 FWHM with a scan range of m/z 70-800, while dd-MS2 resolution was set at 22,500 FWHM. Fragmentation was performed using nitrogen gas with variations in Normalized Collision Energy (NCE) of 30, 50, and 70. Data Analysis and Compound Identification Raw data were processed using Thermo Scientific Compound Discoverer 3.3 software. Compound identification was performed through a search of the mzCloud mass spectra library, which includes endogenous metabolites, natural products, toxins, and vitamins. In addition, data matching was carried out with various mass databases such as Arita Lab 6549 Flavonoid, Natural Products Atlas, Lipid Maps, as well as various databases on ChemSpider such as KEGG, ChEBI, and PubChem (Budthapa et al., 2026; Maity & Mitra, 2026).

RESULTS AND DISCUSSION

Based on the characterization using Ultra-High Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry (UHPLC-Q-Orbitrap HRMS) operated in positive electrospray ionization mode (ESI+), a secondary metabolite detected in the ethanol extract of *Antidesma bunius* fruit (F1) was successfully identified as caffeine. The compound eluted at a retention time (RT) of 2.86 minutes, with a sharp chromatographic peak observed at RT 2.852 minutes (Figure 1). The full-scan mass spectrum (MS1) exhibited a dominant protonated molecular ion ($[M+H]^+$) at m/z 195.08775, corresponding to the molecular formula $C_8H_{10}N_4O_2$ with a theoretical molecular weight of 194.08048 Da. Database matching confirmed that the accurate mass and chromatographic behavior were consistent with caffeine, a naturally occurring purine alkaloid. Further structural confirmation was performed using tandem mass spectrometry (MS/MS) with High-energy Collision Dissociation (HCD). The MS/MS spectrum produced a highly abundant base peak at m/z 138.06623, representing the major product ion generated from fragmentation of the precursor ion (Figure 2). The chemical structure of caffeine is presented in Figure 3.

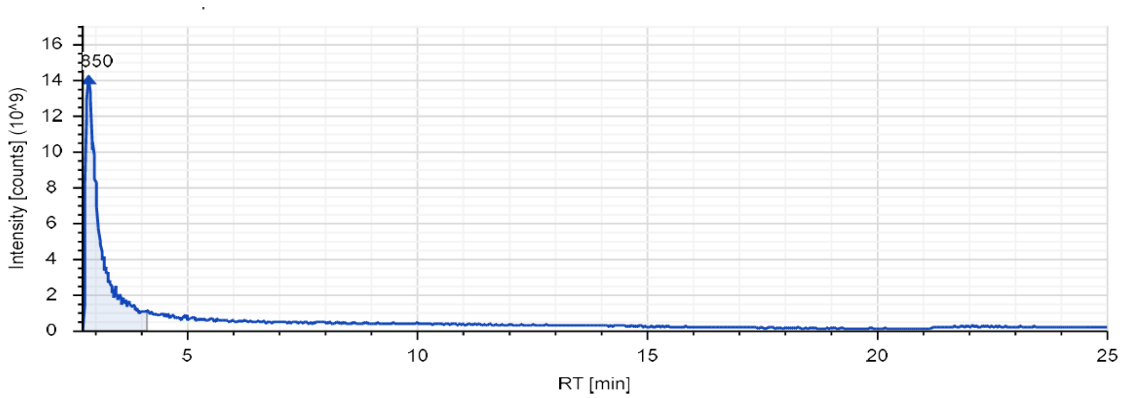

Figure 1. LC-HRMS chromatogram of *Antidesma bunius* fruit extract (F1) showing the caffeine peak at a retention time of 2.86 minutes.

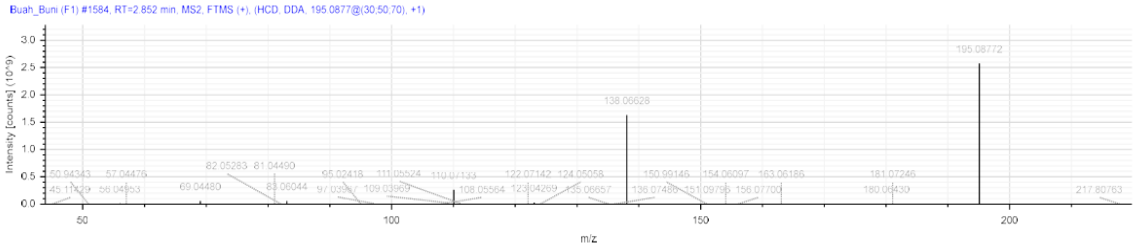

Figure 2. MS/MS fragmentation spectrum of caffeine detected in *Antidesma bunius* fruit extract.

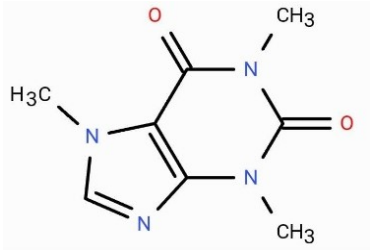

Figure 3. Chemical structure of caffeine ($C_8H_{10}N_4O_2$).

The chromatographic profile demonstrated that caffeine eluted at an early retention time of 2.86 minutes, indicating its relatively high polarity under reversed-phase chromatographic conditions using a C18 column. High-resolution mass spectrometry provided highly accurate mass measurements for molecular formula determination. The detected protonated molecular ion $[M+H]^+$ at m/z 195.08775 showed excellent agreement with the theoretical protonated mass of $C_8H_{11}N_4O_2^+$ (195.08767 Da). The very small mass difference indicates the high mass accuracy of the Orbitrap-based FTMS instrument, allowing reliable confirmation of the elemental composition of the detected compound.

The strongest structural evidence was obtained from the MS/MS fragmentation pattern. The dominant fragment ion at m/z 138.06623 resulted from the neutral loss of 57.02152 Da (195.08775 − 138.06623 = 57.02152 Da). This neutral loss corresponds to the elimination of a methyl isocyanate (C_2H_3NO) moiety, which is widely recognized as a characteristic fragmentation pathway of caffeine. The observed fragmentation pattern is highly consistent with previously reported mass spectra of caffeine and provides strong confirmation of the compound's identity.

The presence of caffeine in *Antidesma bunius* fruit extract contributes additional information regarding the phytochemical composition of this indigenous tropical fruit. Caffeine is a biologically active purine alkaloid that has been extensively reported to possess central nervous system stimulant activity and has also been associated with antioxidant properties and modulation of energy metabolism. Although *A. bunius* is not generally recognized as a major natural source of caffeine, its detection in the present study demonstrates the capability of LC-HRMS metabolomics to identify minor constituents with high confidence. Overall, these findings highlight the effectiveness of LC-HRMS as a sensitive and reliable analytical platform for metabolite profiling and dereplication of bioactive compounds in medicinal and functional plants.

CONCLUSIONS

This study successfully profiled and characterized the major secondary metabolite in the ethanol extract of buni fruit (*Antidesma bunius* (L.) Spreng.) using a non-targeted metabolomics approach based on UHPLC-Q-Orbitrap HRMS. High-resolution chromatographic and mass spectrometric analyses identified caffeine, a biologically active purine alkaloid, with high confidence. The compound eluted at a retention time of 2.86 minutes and was characterized by a protonated molecular ion ($[M+H]^+$) at m/z 195.08775, corresponding to the molecular formula $C_8H_{11}N_4O_2$. Structural confirmation was further supported by the characteristic MS/MS fragment ion at m/z 138.06623, which resulted from the neutral loss of a methyl isocyanate moiety. The identification of caffeine in *A. bunius* expands the current phytochemical profile of this indigenous tropical fruit and provides additional scientific evidence for its potential health benefits, including central nervous system stimulation, antioxidant activity, and metabolic modulation. Overall, the UHPLC-Q-Orbitrap HRMS platform demonstrated excellent sensitivity, selectivity, and mass accuracy, proving to be a reliable and efficient analytical tool for rapid metabolite dereplication and comprehensive phytochemical profiling of local functional foods and medicinal plant resources in Indonesia.

Acknowledgements: We would like to acknowledge and thank the (1) Central Laboratory of State University of Malang, (2) Corpora Science Laboratory, (3) The authors acknowledge and thank the internal funding at Universitas Negeri Malang for the research funding Student Innovation 14.04.102/UN32.14.1/LT/2026.

Authors' Contributions: Elsa Putri Rahmawati, Dika Putra Wijaya, Danar Danar, Nabilla Sevira Endah Prastiwi, Marshya Qurrotul Aini Wibowo, Cahya Adidharma, Agniatun Mulidatun Khasanah, Fitri Rosa Amalia designed the study. Elsa Putri Rahmawati and Cahya Adidharma carried out the laboratory work. Elsa Putri Rahmawati, Nabilla Sevira Endah Prastiwi and Dika Putra Wijaya analyzed the data. Elsa Putri Rahmawati, Dika Putra Wijaya wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: Internal funding at Universitas Negeri Malang for the research funding Student Innovation 14.04.102/UN32.14.1/LT/2026.

REFERENCES

Ardyansyah, A., Wijaya, D. P., Danar, D., Nareswari, T. J., Utami, A. F., Annisaa, A., Nadhifah, D., & Fitrianingrum, L. (2026). Formulation of a Synergistic Herbal Blend of Green Tea (Camelia sinensis) and Traditional Spices for Immune Support and Anxiety Relief. *Biology, Medicine, & Natural Product*

Chemistry, *14*(2), 1561–1568. https://doi.org/10.14421/biomedich.2025.142.1561-1568

Azani, I., Hadiarti, D., & Aulia Fitri, N. (2026). Metabolomics-Based Profiling of Antioxidant Compounds in Kesum (Polygonum minus) via UHPLC-Q-Orbitrap HRMS as Potential Anti-SARS-CoV-2 Candidates: An In Silico Approach. *Biology, Medicine, & Natural Product Chemistry*, *15*(1), 1321–1327. https://doi.org/10.14421/biomedich.2026.151.1321-1327

Budthapa, P., Bista, D., Budhathoki, R., Upadhyaya, H., Sharma, K. R., & Parajuli, N. (2026). Bioactivity-guided metabolite profiling of Pistacia chinensis Bunge gall using LC–HRMS/MS. *Pharmacological Research - Modern Chinese Medicine*, *18*, 100787. https://doi.org/10.1016/j.prmcm.2026.100787

Fathimah, R., Wijaya, D. P., Muniroh, A., Sumari, S., Danar, D., Nafiah, N. I., Az-Zahra, F., Zhafirah, A. G., Monicha, G. D., Sajidah, M. S., Oktaviani, J., Musallamah, F. K., & Febrianti, P. R. (2026). Physicochemical Properties and Fatty Acid Methyl Ester (FAME) Extract of Maggot Black Soldier Fly (Hermetia illucens) as Animal Feed. *Biology, Medicine, & Natural Product Chemistry*, *15*(1), 229–238. https://doi.org/10.14421/biomedich.2026.151.229-238

Indrawati, I., Fitri, A., & Rizki, M. (2017). POTENSI EKSTRAK BUAH BUNI (Antidesma bunius L) SEBAGAI ANTIBAKTERI DENGAN BAKTERI UJI Salmonella thypimurium DAN Bacillus cereus. *Jurnal Biodjati*, *2*(2). http://journal.uinsgd.ac.id/index.php/biodjatiTel/Fax

Kemsawasd, V., & Chaikham, P. (2021). Alteration of Bioactive Compounds and Antioxidative Properties In Thermal, Ultra-High Pressure and Ultrasound Treated Maoberry (Antidesma Bunius L.) Juice during Refrigerated Storage. *Current Research in Nutrition and Food Science*, *9*(3), 904–916. https://doi.org/10.12944/CRNFSJ.9.3.17

Krongyut, O., & Sutthanut, K. (2019). Phenolic profile, antioxidant activity, and anti-obesogenic bioactivity of mao luang fruits (antidesma bunius l.). *Molecules*, *24*(22). https://doi.org/10.3390/molecules24224109

Kusuma, J., Analianasari, Wahyudi, A., Abdullah, M. K., Hasan, A. Z., Asrowardi, I., Fitriani, & Tahir, M. (2025). Diversity of the non-targeted metabolomic data across various varieties of Cloves (Syzygium spp.). *Data in Brief*, *58*, 111237. https://doi.org/10.1016/j.dib.2024.111237

Maity, P., & Mitra, A. K. (2026). Ionic liquid–driven pyran synthesis: Pathways and perspectives. *Journal of Ionic Liquids*, *6*(1), 100200. https://doi.org/10.1016/j.jil.2026.100200

Nguyen-Ngoc, H., Alilou, M., Derbré, S., Blanchard, P., Pham, G. N., Nghiem, D. T., Richomme, P., Stuppner, H., & Ganzera, M. (2022). Chemical constituents of Antidesma bunius aerial parts and the anti-AGEs activity of selected compounds. *Phytochemistry*, *202*. https://doi.org/10.1016/j.phytochem.2022.113300

Nguyen-Ngoc, H., Le-Thi-Phuong, T., Vu-Van, T., Pham-Ha-Thanh, T., & Nguyen-Huu, T. (2024). Phytochemical and Pharmacological Review of the Genus Antidesma. In *Natural Product Communications* (Vol. 19, Number 4). SAGE Publications Inc. https://doi.org/10.1177/1934578X241247990

Rivaldi, M., Frediansyah, A., Aziz, S. A. A., & Nugroho, A. P. (2025). Active biomonitoring of stream ecosystems: untargeted metabolomic and proteomic responses and free radical scavenging activities in mussels. *Ecotoxicology*, *34*(3), 425–443. https://doi.org/10.1007/s10646-024-02846-9

Shariful Islam, M., Sharif Ahammed, M., Islam Sukorno, F., Ferdowsy Koly, S., Morad Biswas, M., & Hossain, S. (2018). A review on phytochemical and pharmacological potentials of Antidesma bunius. *Journal of Analytical & Pharmaceutical Research*, *7*(5). https://doi.org/10.15406/japlr.2018.07.00289

Udomkasemsab, A., Ngamlerst, C., Adisakwattana, P., Aroonnual, A., Tungtrongchitr, R., & Prangthip, P. (2018). Maoberry (Antidesma bunius) ameliorates oxidative stress and inflammation in cardiac tissues of rats fed a high-fat diet. *BMC Complementary and Alternative Medicine*, *18*(1). https://doi.org/10.1186/s12906-018-2400-9

Wijaya, D. P., Rahmawati, K., Bachtiar, A. Y., Ridhwan, A. E. R., Rahmah, A. A., Taufikurohmah, T., & Danar, D. (2026). Formulation and Standardization of Face Wash Using Watermelon Rind Extract (Citrullus lanatus). *Biology, Medicine, & Natural Product Chemistry*, *14*(2), 1569–1578. https://doi.org/10.14421/biomedich.2025.142.1569-1578

Windarsih, A., Suratno, Warmiko, H. D., Indrianingsih, A. W., Rohman, A., & Ulumuddin, Y. I. (2022). Untargeted metabolomics and proteomics approach using liquid chromatography-Orbitrap high resolution mass spectrometry to detect pork adulteration in Pangasius hypopthalmus meat. *Food Chemistry*, *386*, 132856. https://doi.org/10.1016/j.foodchem.2022.132856

Zubia, C. S., Babaran, G. M. O., Duque, S. M. M., Mopera, L. E., Flandez, L. E. L., Castillo-Israel, K. A. T., & Reginio, F. C. (2023). Impact of drying on the bioactive compounds and antioxidant properties of bignay [Antidesma bunius (L.) Spreng.] pomace. *Food Production, Processing and Nutrition*, *5*(1). https://doi.org/10.1186/s43014-022-00122-z

THIS PAGE INTENTIONALLY LEFT BLANK