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Classification of Makasar Fruit Extract (*Brucea javanica* L. Merr.) Based on The Level of Ripeness Using a Combination of FTIR and UV-Vis Spectroscopy with Chemometrics

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Abstract

Makasar fruit (*Brucea javanica* L. Merr) is a medicinal plant frequently utilized in Indonesia for its anti-malarial, anti-diabetic, antioxidant, and antibacterial properties. This research aims to classify extracts from *B. javanica* fruits based on their ripeness levels using a combination of FTIR and UV-Vis spectroscopy with chemometrics. Makasar fruits were extracted using methanol assisted by ultrasonic technology, resulting in a higher yield of extract from ripe fruit (black in color) than unripe fruit (green in color). The FTIR spectrum was measured at wavenumbers ranging from 4000 to 600 cm^{-1}, while the UV-Vis spectrum was measured at wavelengths ranging from 200 to 800 nm. Notably, the UV-Vis spectrum revealed absorption peaks at 206 nm, attributed to the quassinoid group, and 265 nm, associated with the flavonoid group. Simultaneously, the FTIR spectrum showed various functional groups, including O-H, C-H, δ-lactone, C=C aromatic stretching vibrations, and C-O-C bending vibrations. Before conducting chemometric analysis, the FTIR and UV-Vis spectra were pre-processed as baseline and SNV. The research findings demonstrate that by employing UV-Vis and FTIR spectra in combination with chemometrics, it has been possible to distinguish Makasar fruit according to its ripeness level. Ripe and unripe fruit extracts were grouped with PC values of 88% for UV-Vis and 90% for FTIR, indicating distinct profiles between ripe and unripe fruit extracts of *Brucea javanica*.

1. Introduction

Plants can function as traditional medicines because they contain bioactive secondary metabolite compounds [1]. The abundance of plant species in Indonesia provides great potential for medicinal plants. Among these, the Makasar plant (*Brucea javanica* L. Merr.) is a notable candidate for medicinal use. This plant is native to Asia and thrives in Indonesia, Thailand, and Malaysia, belonging to the Brucea genus within the Simaroubaceae family, often reaching a height of up to 3 meters [2]. Various parts of the *B. javanica* plant, including its leaves, fruit, and seeds, have long been employed for medicinal purposes. Traditionally, in Bengkulu, the fruit of *B. javanica* is used as an anti-malarial remedy, while in other regions, it is utilized as an anti-diabetic agent. Prior studies have revealed its potential as an anti-malarial [3, 4] and antibacterial, antioxidant, and anti-diabetic properties [2]. The active compounds that have been successfully isolated from plants are quassinoids, bruceine A, B, C, D, E, F, G, H, and I, brusatol A, dihydrobrusatol B, bruceoside A, B, C, D, E, F, G, I, J, K, L, P, and S, brusatol amarissima E2-glucosidase, brusatol ketoacid, and bruceen. These compounds are used for

their anticancer, antitumor, anti-inflammatory, and antiviral properties [5]. The content of bioactive compounds and the quality and quantity of medicinal plants can vary depending on several factors, including environmental factors (geographical location, climate, temperature, and humidity), internal plant factors (e.g., roots, stems, leaves, fruit, and flowers), fruit ripeness, harvesting timing, and other relevant variables. Therefore, it is necessary to conduct a method for analyzing the distinct maturity levels of Makasar fruit (*B. javanica*) that adheres to simple and precise criteria.

A common approach for identifying and classifying natural materials involves assessing the levels of one or more specific compounds [6]. Various methods can be used for this purpose, including high-performance liquid chromatography (HPLC) [7], gas chromatography (GC) [8], thin-layer chromatography (TLC) [9], and spectroscopic techniques such as UV-Vis, FTIR, and NMR [10, 11, 12]. These analytical techniques can be complemented by chemometric analysis.

Among those methodologies, spectroscopy, particularly FTIR and UV-Vis, may be an excellent choice due to their numerous advantages, including ease of operation, cost-effectiveness, and rapid measurement procedures [6]. The data derived from FTIR spectroscopy is intricate, providing information that characterizes the identity of a natural material. Hence, the FTIR spectrum can be utilized to identify various characteristics of natural products, such as variations in fruit ripeness levels, even in cases where the precise chemical composition is unknown [10]. Previous research reported that FTIR spectra combined with chemometrics can classify carobs origin [13], FTIR and UV-Vis spectral data supported by chemometric tools to control the quality of mint species [14]. This approach holds promise for establishing a fingerprinting method to distinguish Makasar fruit based on ripeness levels. Variations in fruit ripeness are believed to yield different characteristics and variations in bioactive compounds, which can serve as references for quality control in medicinal plant assessments.

Nevertheless, there are notable challenges associated with using this instrument, mainly the abundance of data and the fairly high complexity of the data. To address this issue, a combined approach involving multivariate analysis, specifically chemometric analysis, can be employed to extract the information present in the FTIR and UV-Vis spectra, enabling the derivation of conclusions [15]. Consequently, this study categorized Makasar fruit extracts according to the ripeness level of the fruit. This classification was accomplished by integrating FTIR spectroscopy, UV-Vis, and chemometric techniques. This research can provide an overview of fingerprint analysis of *B. javanica* fruit based on the level of fruit ripeness and provide alternative information for society and industry on the use of the maturity levels of *B. javanica* fruit.

2. Experimental

2.1. Equipment and Materials

The equipment in this research was an ATR Alpha Fourier Transform Infra-Red (FTIR) Spectrophotometer (Bruker, Germany), a UV-Vis Spectrophotometer (Agilent Technologies Carry 60), a rotary evaporator (Buchi, Flawil, Switzerland), an oven, freeze dryer Power Dry LL1500 (Thermo Scientific), 50 mesh sieve, and standard laboratory glassware. Meanwhile, the materials were Makasar fruit (*Brucea javanica* L. Merr.), methanol (Merck), acetone (Merck), and distilled water.

2.2. Collection and Preparation of Sample

The samples were collected from the same area in Bengkulu City, Bengkulu Province (no difference in altitude and coordinates). These fruits were selected based on their ripeness and categorized into two distinct groups: unripe (green in color) and ripe fruits (black in color) (Figure 1). Subsequently, the harvested fruits underwent a drying process. Following the drying phase, the samples were homogenized and sieved through a 50-mesh sieve.

2.3. Sample Extraction

A total of 7 grams of unripped *B. javanica* fruit simplicia was extracted using 75 mL of methanol, employing the sonication method in an ultrasonic bath operating at 35 kHz and a temperature of 30°C. The same simplicia underwent a repeat sonication to yield a cumulative volume of 150 mL of methanol extract. This identical procedure was repeated for the ripe fruit. The extraction process was repeated ten times for each type of *B. javanica* fruit, resulting in 20 extracts. The absorbance of the freshly obtained extracts was measured using a UV- Vis spectrophotometer at a wavelength range of 200 to 800 nm. Subsequently, the extracts were concentrated using a rotary evaporator and dried using a freeze-dryer in preparation for FTIR analysis.

2.4. UV-Vis Spectra Measurement

The UV-Vis spectra were recorded from freshly prepared extracts after the extraction process. A 15 µL of liquid extract was placed in a cuvette, and 3 mL of methanol was added. Readings were then taken within a 200-800 nm wavelength range, with 1 nm intervals. The obtained spectra were converted into a format suitable for subsequent analysis using MS Excel.

Figure 1. (a) Makasar plant, (b) unripe fruit, (c) ripe fruit

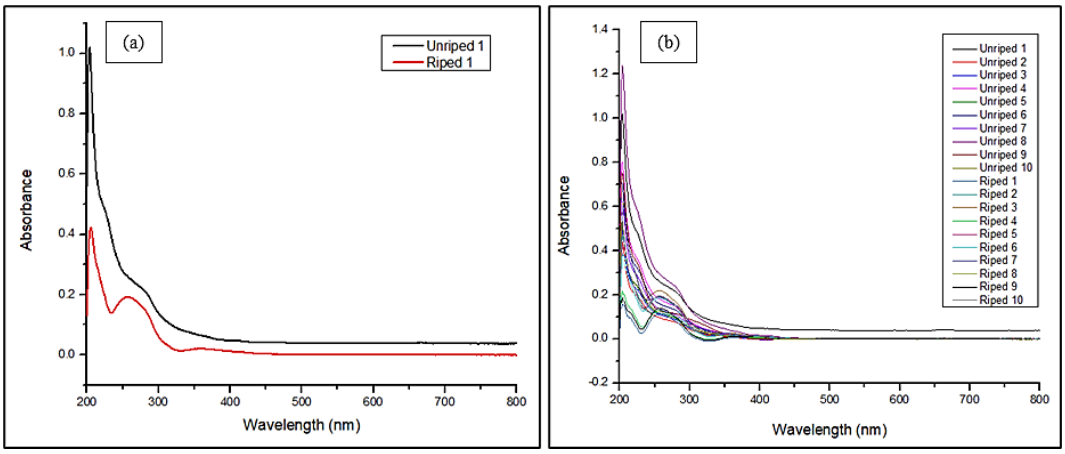

Figure 2. UV-Vis spectra of methanol extract of ripe and unripe fruits of *B. javanica* (a) representative, (b) all samples

2.5. FTIR Spectra Measurement

The FTIR spectra were obtained using an FTIR spectrophotometer controlled by OPUS v7.5 software. For analysis, a 100 mg sample was placed on the ATR crystal and forced down with the swivel press to provide optimal contact between the sample and the crystal, and subsequently scanned at the wavenumber range of 4000-600 cm^{-1}, with a scanning speed of 32 scans/spectrum and a resolution of 4 cm^{-1}. FTIR measurements for the Makasar fruit samples, both unripe and ripe, were replicated ten times.

2.6. Chemometrics Analysis

Chemometric analysis was conducted through Principal Component Analysis (PCA), utilizing Orange software version 3.29.3 to classify unripe and ripe fruit in methanol extracts. Before classification, the spectral data underwent preprocessing in the form of baseline correction and Standard Normal Variate (SNV) transformation.

3. Results and Discussion

3.1. Extraction of *B. javanica* Fruit

When people utilize it as a medicinal plant, it is common to combine unripe and ripe fruits (Figure 1). Differences in the fruit's ripeness levels can potentially influence the extract's yield, quality, and medicinal effectiveness. Data presented in Table 1 highlights variations in methanol extract yield from Makasar fruit based on the fruit's ripeness. Notably, ripe fruit exhibited a significantly higher yield, at 30.61% ± 0.80, compared to unripe fruit, which yielded 27.35% ± 0.32.

3.2. UV-Vis Spectra of *B. javanica* Fruit Extract

Figure 2 displays the UV-Vis spectra of the methanol extract from *B. javanica* fruit. Notably, there are differences between ripe and unripe fruit extracts, indicating variations in metabolite profiles. This aligns with prior research, which has established that plant age contributes to differing metabolite profiles [16]. Multiple absorption peaks observed in the extracts of unripe and ripe fruits can be attributed to the absorption of chromophore groups associated with quassinoids and flavonoids (as detailed in Table 2).

Table 1. Yield of *B. javanica* fruit extract

B. javanica fruit type	Yield (%) ± SD
Ripe fruit	30.61 ± 0.80
Unripe Fruit	27.35 ± 0.32

Note: The values presented have been derived from 10 repetitions.

Table 2. UV-Vis spectra of methanol extract of ripe and unripe fruits of *B. javanica*

B. javanica fruit type	Absorption peak (nm)	Approximation of chromophore groups
Ripe fruit	206	Quassinoid [17]
	265	Flavonoid [18]
Unripe fruit	206	Quassinoid [17]

3.3. FTIR Spectra of *B. javanica* Fruit Extract

The FTIR spectra illustrate the vibrational bonds of compounds within the methanol extracts of both unripe and ripe *B. javanica* fruits. These bond vibrations can interpret the existence of bonds between atoms at different energy levels. As seen in Figure 3, the FTIR spectra of the methanol extracts from ripe and unripe *B. javanica* fruits exhibit similarities, with characteristic absorption occurring within the wavenumber range of approximately 1000 to 1750 cm^{-1}. Nevertheless, variations exist in terms of intensity and wavenumber position. This indicates differences in the composition and levels of compounds in the extract with differences in the level of fruit ripeness [16].

Based on the FTIR spectra, the same peaks can be seen from 3369 to 3012 cm^{-1}, which is the stretching vibration of O-H, and at 2857 cm^{-1} and 2922 cm^{-1}, indicating the stretching vibration of C-H. Meanwhile, C- O-C bending vibrations are seen at a wavenumber of 1026 cm^{-1}. The stretching vibration of aromatic C=C bonds at 1600 cm^{-1} is observed in unripe and ripe fruits. Furthermore, the stretching vibration of δ-lactone bonds at 1732 cm^{-1} is present in both unripe and ripe fruits (as detailed in Table 3).

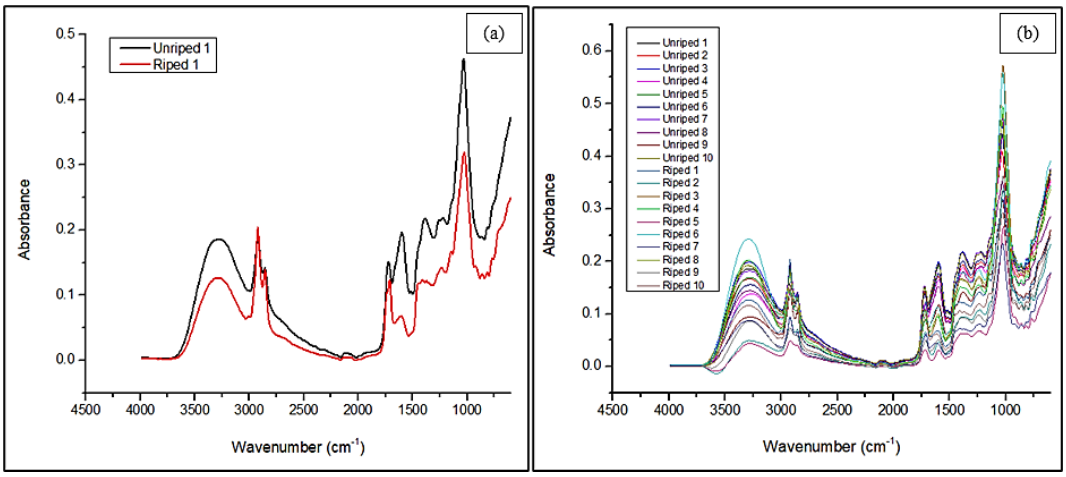

Figure 3. FTIR spectra of methanol extract of ripe and unripe fruits of *B. javanica* (a) representative, (b) all samples

3.4. Classification of *B. javanica* Fruit Extracts

This study aimed to classify Makasar fruit extracts based on their ripeness level, using a combination of FTIR and UV-Vis spectra combined with chemometrics. This classification aims to establish a fingerprint analysis model for Makasar fruit extracts, differentiating between those from unripe and ripe fruits. In the FTIR spectra, distinguishing differences between the extracts from unripe and ripe fruits were somewhat limited. However, more discernible differences became apparent through chemometric analysis, particularly in the PCA score plots of FTIR and UV-Vis spectra (Figure 4). These plots revealed a PCA score of 88% (PC1 and PC2) for FTIR spectra and 90% (PC1 and PC2) for UV-Vis spectra. Higher total PCA values indicate better explanatory power for the components, with a total PCA value exceeding 70% considered good [16].

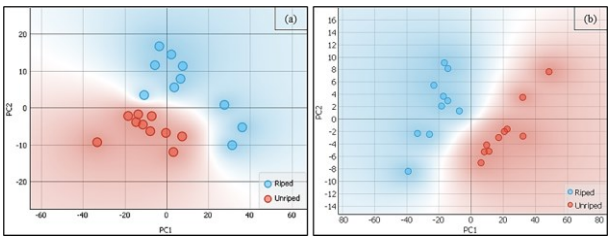

Figure 4. PCA score plot (a. FTIR spectra, b. UV-Vis spectra) of *B. javanica* fruit extract based on differences in fruit ripeness levels

The PCA score plot of FTIR spectra illustrates the positioning of methanol extracts, with the ripe fruit extract appearing in the positive quadrant of PC1 and PC2, while the unripe fruit extract is situated in the negative quadrant of PC1 and PC2. Similarly, a clear separation is observed in the PCA score plot of UV-Vis spectra, with the methanol extract of ripe fruit positioned in the positive quadrant of PC1 and the unripe fruit extract in the negative quadrant of PC1.

Further validation through a confusion matrix (Figure 5) provides additional insights. It demonstrates that PCA using UV-Vis spectra offers a more accurate description, correctly predicting all unripe and ripe fruit extracts. However, in the case of PCA using FTIR spectra, there was a single instance where an unripe fruit extract was incorrectly predicted as a ripe fruit extract (refer to Figure 5a). ROC analysis was conducted to reinforce these findings, yielding an AUC value of 0.95 for PCA with FTIR spectra and 0.99 for PCA with UV-Vis spectra. These values indicate an excellent classification model with an AUC of 0.9 to 1.0 [19].

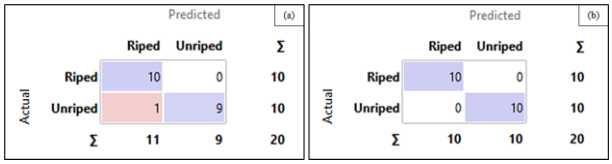

Figure 5. Confusion matrix PCA (a. FTIR spectra, b. UV- Vis spectra)

Table 3. FTIR spectra data of unripe and ripe fruit extracts of *B. javanica*

Wavenumber (cm^{-1})	Vibration type
3369-3012	Stretching vibration of O-H
2922	Stretching vibration of C-H
2857	Stretching vibration of C-H
1732	Stretching vibration of δ-lactone
1600	Stretching vibration of aromatic C=C
1026	Bending vibration of C-O-C

4. Conclusion

The FTIR and UV-Vis spectra revealed similarities in the profiles of methanol extracts obtained from ripe and unripe *B. javanica* fruits. Notably, the extract yield from ripe fruit surpassed that of unripe fruit. However, when both FTIR and UV-Vis spectra were combined with chemometrics, a clear distinction emerged between the methanol extracts of ripe and unripe *B. javanica* fruits, with a PC assessment of 88% (UV-Vis spectra) and 90% (FTIR spectra). In further analysis employing a confusion matrix, all samples were accurately classified in the PCA UV-Vis spectra. In the case of PCA FTIR spectra, there was a single instance where an unripe fruit sample was incorrectly categorized as ripe fruit.

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References

[1] Wulandari Lestyo, Nia Kristiningrum, Fracilia Arinda Ratnasari, Rapid Determination of Total Phenol in Leaf Extracts of a Medicinal Plant using Infrared Spectroscopy and Chemometric Methods, *Journal of Analytical Chemistry*, 75, 4, (2020), 479-486 https://doi.org/10.1134/S1061934820040176

[2] Simamora Adelina, Timotius Kris Herawan, Santoso Adit Widodo, Antidiabetic, Antibacterial and Antioxidant Activities of Different Extracts from *Brucea javanica* (L.) Merr Seeds, *Pharmacognosy Journal*, 11, 3, (2019), 479-485 http://dx.doi.org/10.5530/pj.2019.11.76

[3] Helmi Henny, Susanti Idha, Phytochemical Tested and In Vitro Screening Anti-Malaria Activity of Belilik (*Brucea Javanica* (L)). Merr Against *Plasmodium Falciparum*, *Berkala Penelitian Hayati*, 19, 1, (2014), 1-4

[4] Melanie J. O'Neill, Dorothy H. Bray, Peter Boardman, Kit L. Chan, J. David Phillipson, David C. Warhurst, Wallace Peters, Plants as Sources of Antimalarial Drugs, Part 4: Activity of *Brucea javanica* Fruits Against Chloroquine-Resistant *Plasmodium falciparum* in vitro and Against *Plasmodium berghei* in vivo, *Journal of Natural Products*, 50, 1, (1987), 41-48 https://doi.org/10.1021/np50049a007

[5] Meiwan Chen, Ruie Chen, Shengpeng Wang, Wen Tan, Yangyang Hu, Xinsheng Peng, Yitao Wang, Chemical components, pharmacological properties, and nanoparticulate delivery systems of *Brucea javanica*, *International Journal of Nanomedicine*, 8, 1, (2013), 85-92 https://doi.org/10.2147/IJN.S31636

[6] Edy Djauhari Purwakusumah, Mohamad Rafi, Utami Dyah Safitri, Waras Nurcholis, Muhammad Agung Zaim Adzkiya, Identifikasi dan Autentikasi Jahe Merah Menggunakan Kombinasi Spektroskopi FTIR dan Kemometrik, *agriTECH*, 34, 1, (2014), 82-87

[7] Aryani Sabir, Mohamad Rafi, Latifah K. Darusman, Discrimination of red and white rice bran from Indonesia using HPLC fingerprint analysis combined with chemometrics, *Food Chemistry*, 221, (2017), 1717-1722 https://doi.org/10.1016/j.foodchem.2016.10.114

[8] Silvia López-Vidal, Vicente Rodríguez-Estévez, Santiago Lago, Lourdes Arce, Miguel Valcárcel, The Application of GC–MS and Chemometrics to Categorize the Feeding Regime of Iberian Pigs in Spain, *Chromatographia*, 68, (2008), 593-601 https://doi.org/10.1365/s10337-008-0752-x

[9] Mohamad Rafi, Eti Rohaeti, Ali Miftahudin, Latifah K. Darusman, Differentiation of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar* by Thin Layer Chromatography Fingerprint Analysis, *Indonesian Journal of Chemistry*, 11, 1, (2011), 71-74

[10] Mohamad Rafi, Edy Djauhari Purwakusumah, Taopik Ridwan, Baba Barus, Atang Sutandi, Latifah Kosim Darusman, Geographical classification of Java Tea (*Orthosiphon stamineus*) from Java Island by FTIR Spectroscopy Combined with Canonical Variate Analysis, *Jurnal Sains dan Matematika*, 23, 1, (2015), 25-31

[11] Rachmawati, E. Rohaeti, M. Rafi, Combination of near infrared spectroscopy and chemometrics for authentication of taro flour from wheat and sago flour, *Journal of Physics: Conference Series*, 835, (2017), 012011 https://doi.org/10.1088/1742-6596/835/1/012011

[12] Eti Rohaeti, Khoirotul Muzayanah, Dewi Anggraeni Septaningsih, Mohamad Rafi, Fast Analytical Method for Authentication of Chili Powder from Synthetic Dyes Using UV-Vis Spectroscopy in Combination with Chemometrics, *Indonesian Journal of Chemistry*, 19, 3, (2019), 668-674 https://doi.org/10.22146/ijc.36297

[13] Chrysanthi Christou, Agapios Agapiou, Rebecca Kokkinofta, Use of FTIR spectroscopy and chemometrics for the classification of carobs origin, *Journal of Advanced Research*, 10, (2018), 1-8 https://doi.org/10.1016/j.jare.2017.12.001

[14] Kamila Kucharska-Ambrożej, Agnieszka Martyna, Joanna Karpińska, Anna Kiełtyka-Dadasiewicz, Aleksandra Kubat-Sikorska, Quality control of mint species based on UV-VIS and FTIR spectral data supported by chemometric tools, *Food Control*, 129, (2021), 108228 https://doi.org/10.1016/j.foodcont.2021.108228

[15] Heshmatollah Ebrahimi-Najafabadi, Riccardo Leardi, Paolo Oliveri, Maria Chiara Casolino, Mehdi Jalali-Heravi, Silvia Lanteri, Detection of addition of barley to coffee using near infrared spectroscopy and chemometric techniques, *Talanta*, 99, (2012), 175-179 https://doi.org/10.1016/j.talanta.2012.05.036

[16] Antonio Kautsar, Wulan Tri Wahyuni, Utami Dyah Syafitri, Syifa Muflihah, Nursifa Mawadah, Eti Rohaeti, Zulhan Arif, Bambang Prajogo, Muhammad Bachri Amran, Abdul Rohman, Mohamad Rafi, Data Fusion of UV-Vis and FTIR Spectra Combined with Principal Component Analysis for Distinguishing of *Andrographis paniculata* Extracts Based on Cultivation Ages and Solvent Extraction, *Indonesian Journal of Chemistry*, 21, 3, (2021), 753-760 https://doi.org/10.22146/ijc.60321

[17] Zhiwei Su, Jing Hao, Zhifang Xu, Riming Huang, Nenling Zhang, Shengxiang Qiu, A new quassinoid from fruits of *Brucea javanica*, *Natural Product*

Research, 27, 21, (2013), 2016-2021 https://doi.org/10.1080/14786419.2013.821119

[18] Tom J. Mabry, K. R. Markham, M. B. Thomas, The Ultraviolet Spectra of Flavones and Flavonols, in: T.J. Mabry, K.R. Markham, M.B. Thomas (Eds.) *The Systematic Identification of Flavonoids*, Springer Berlin Heidelberg, Berlin, Heidelberg, 1970, https://doi.org/10.1007/978-3-642-88458-0_5

[19] Nugroho Adi, Gumelar Agustinus Bimo, Sooai Adri Gabriel, Sarvasti Dyana, L. Tahalele Paul, Perbandingan Performansi Algoritma Pengklasifikasian Terpandu Untuk Kasus Penyakit Kardiovaskular, *Jurnal RESTI (Rekayasa Sistem dan Teknologi Informasi)*, 4, 5, (2020), 998-1006 https://doi.org/10.29207/resti.v4i5.2316