Fingerprinting Analysis of Antioxidant Activity from *Acalypa Indica* using FTIR Metabolomics based on Different Extraction Methods

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Abstract- **The *A. Indica* plant *(Acalypha indica)* is a herbal plant that grows in tropical areas. This plant contains secondary metabolites such as alkaloids, flavonoids, steroids, triterpenoids and tannins. The aim of this research is to determine the FTIR-ATR-based secondary metabolite profile of *A. Indica* based on differences in extraction methods through PCA multivariate analysis and to find out what functional groups contribute actively as antioxidants to *A. Indica* through OPLSDA multivariate analysis. Extraction of secondary metabolites was carried out using ultrasonic, maceration and shaker methods using ethanol absolute solvent, then the method for determining antioxidant activity used was the DPPH method. The results of this study indicate that the highest yield and antioxidant content from different extraction methods were obtained using the ultrasonic method, with a yield of 6.34 grams and an antioxidant level of 140 ppm. PCA analysis showed separation of groups from various extraction methods, suggesting differences in the secondary metabolite profiles produced. Furthermore, the OPLSDA analysis identified four main functional groups acting as antioxidant compounds in the *A. Indica* extract using different extraction methods, namely: 1550, 1735, 1895, 830, dan 3300 cm-1. These wavenumbers correspond to the functional groups C=C *aromatic*, C=O *stretching,* C-H *aromatic*, and O-H *stretching*. These functional groups are components of quercetin, which is one of the compounds with potential antioxidant properties.**

Keyword: Anting -Anting (Acalypha indica), FTIR, Metabolomics, Antioxidants

I. INTRODUCTION

A. Indica is a medicinal plant that grows in tropical regions such as India, Sri Lanka, Thailand, and Pakistan, and has also been found in several regions of Indonesia. It belongs to the Euphorbiaceae family and is often considered a weed with potential medicinal value [1]. Traditionally, the leaves are used to treat diseases such as dysentery, asthma, diarrhea, burns, rheumatic pain, and toothaches [2]. These properties are attributed to its secondary metabolites, including alkaloids, flavonoids, steroids, triterpenoids, tannins, and saponins, which exhibit pharmacological activities such as antioxidant, anti-inflammatory, antiviral, and anticancer effects [3].

The presence of secondary metabolites indicates the pharmacological potential of *A. Indica*, including anticancer, anthelmintic, antimalarial, antibiotic, anti-inflammatory, antiviral, and antioxidant activities [4]. Antioxidant activity can reduce the effects of free radicals and is commonly evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. DPPH is a stable purple radical, and this method is widely used due to its simplicity, speed, and low sample requirement [5]. The primary parameter is IC_{50}, defined as the concentration required to reduce 50% of DPPH radicals. A lower IC_{50} value indicates higher antioxidant activity. Based on Molyneux [6], antioxidant activity is categorized as very strong (IC_{50} < 50 ppm), strong (50–100 ppm), moderate (100–150 ppm), and weak (150–200 ppm).

The extraction method significantly influences the effectiveness of bioactive compound isolation. Methods such as sonication, maceration, and shaker extraction have been widely used to extract secondary metabolites with varying results. Extraction effectiveness is evaluated by yield, duration, and the ability to preserve compound stability. Previous studies report that ultrasonic extraction produces a higher yield compared to maceration, while shaker extraction provides optimal results under specific conditions [7]. To further investigate the metabolite profile and bioactivity of *A. Indica*, a metabolomic approach using FTIR combined with chemometric analysis (PCA and OPLS-DA) was employed. FTIR was chosen due to its simplicity, low cost, rapid analysis, and capability to classify samples based on their biological properties. PCA was used to observe sample grouping based on extraction method, while OPLS-DA identified functional groups associated with antioxidant activity. To date, no studies have specifically applied this approach to classify *A. Indica* extracts, and the findings are expected to provide new insights into the antioxidant potential of its active compounds.

II. MATERIALS AND INSTRUMENT

A. Materials

The materials used in this study included *A. Indica* leaf powder (collected from Sidoarjo, East Java), absolute ethanol (p.a, Merck), distilled water (OneMed), DPPH powder (Sigma Aldrich), and filter paper.

B. Instruments

The instruments used in this research were: a rotary evaporator IKA RV10 (Labfirst Scientific, Shanghai) for solvent evaporation, a UV-Vis spectrophotometer (Genesys 150 Thermo Scientific) for antioxidant activity analysis, and an

FTIR-ATR spectrophotometer (Shimadzu IRSpirit with QATR-S) for functional group identification *A. Indica.*

C. Procedure

1. Sample Preparation

The *A. Indica* leaves were collected from Tuban, Bojonegoro, and Ngawi regions. The leaves were washed, chopped into small pieces, oven-dried, ground into powder using a blender, and sieved with an 80-mesh sieve.

2. Extraction of Active Compounds

A total of 5 grams of *A. Indica* leaf powder was subjected to extraction using three different methods: ultrasonic extraction, maceration, and shaker. For the ultrasonic method, the sample was sonicated at a frequency of 42 kHz for 20 minutes at 25 °C. In the maceration method, the sample was soaked in absolute ethanol for 72 hours at room temperature, while in the shaker method, the sample was agitated using a mechanical shaker for 72 hours at room temperature. All extraction processes used absolute ethanol as the solvent, with a solid-to-solvent ratio of 1:10 (w/v) [8][9]. After extraction, the solutions were filtered using filter paper into vials, and the residues were discarded. Each extraction was performed in four replicates.

3. *FTIR Analysis for Functional Group Identification*

The FTIR instrument was first initialized via the Lab Solutions IR software. The spectrum settings were set to a scan range of 400–4000 cm^{-1}, resolution of 4 cm^{-1}, 10 scans, and % transmittance mode. After background (BG) scanning, a small amount of the extract sample was placed on the cleaned ATR crystal. The sample was then scanned, and the spectrum was saved for further analysis.

4. *Determination of Antioxidant Activity*

A stock solution of 500 ppm was prepared by dissolving 10 mg of concentrated extract in 20 mL of ethanol. The solution was diluted to obtain concentrations of 25, 50, 100, 150, and 200 ppm. For each concentration, 3 mL of extract was mixed with 1 mL of 0.2 mM DPPH solution. The mixture was vortexed and incubated for 10 minutes at 37 °C. Absorbance was measured at 515–517 nm using a visible spectrophotometer. The percentage of radical scavenging activity was calculated using Equation [1]:

$$\%\ inhibition\ = \frac{A\ kontrol - A\ sampel}{A\ kontrol} \times 100\% \qquad [1]$$

The IC_{50} value was determined using a linear regression equation obtained from the relationship between extract concentration and % inhibition in Microsoft Excel, where a lower IC_{50} indicates stronger antioxidant activity [10].

5. *Multivariate Analysis*

The wavenumber data from the FTIR analysis were pre-processed and analyzed using Orange software for data normalization. Further multivariate analysis was conducted using MetaboAnalyst 6.0. Principal Component Analysis (PCA) was used to observe the secondary metabolite profile of *A. indica* based on different extraction methods. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used to identify functional groups that actively contribute to antioxidant activity. Additionally, the Variable Importance in Projection (VIP) score was used to determine characteristic functional groups in the samples.

III. RESULTS AND DISCUSSION

A. Sample Preparation

This study used *Acalypha indica* leaves collected from Sidoarjo, East Java. The leaves were first washed with water to remove impurities, then dried in an oven at a temperature of 40–50 °C. This drying method was chosen as it helps preserve the nutritional content and antioxidant activity optimally. Once dried, the leaves were ground using a blender to increase surface area and facilitate extraction, then sieved to obtain uniform particle size. From 1.66 kg of clean leaves, 0.379 grams of fine green powder was obtained [11].

B. Extraction of A.Indica

The powdered *Acalypha indica* leaves were extracted using ultrasonic extraction, shaker, and maceration methods with ethanol as the solvent. The average yield from each extraction method is presented in Table 1.

TABLE I. RESULTS OF A. INDICA EXTRACT YIELD BASED ON DIFFERENT EXTRACTION METHODS

Differences in Extraction Methods	yield (%)
Maseration	6,11 ± 0.31
Shaker	6,27 ± 0.39
Ultrasonic	6,34 ± 0.52

Based on Table 1, the normality test results indicated that the yield data were normally distributed ($p > 0.05$). The one-way ANOVA test also showed a p-value greater than 0.05, suggesting no significant difference among the extraction methods. Therefore, post hoc analysis was not required. The highest yield was obtained from the ultrasonic method (6.34 ± 0.52%), followed by the shaker method (6.27 ± 0.39%) and maceration (6.11 ± 0.31%). The superiority of the ultrasonic method can be attributed to the cavitation energy that disrupts cell walls and accelerates the release of active compounds. In contrast, the maceration and shaker methods rely solely on passive diffusion without significant cell damage, resulting in comparatively lower yields [12].

C. Antioxidant Activity Test of A. indica Extract Using the DPPH Method

The antioxidant activity test using the DPPH reagent involves a color change from purple to yellow, indicating the donation of a hydrogen atom to the DPPH radical. This color shift is measured using a visible spectrophotometer. A higher percentage of inhibition reflects greater compound capability in neutralizing free radicals. Better antioxidant activity is indicated

by a lower IC50 value. The antioxidant activity results for ethanol extracts of *A. indica* using different extraction methods are presented in Table 2.

TABLE II. RESULTS OF A. INDICA EXTRACT IC 50 BASED ON DIFFERENT EXTRACTION METHODS

Differences in Extraction Methods	IC 50 (ppm)
Maseration	$165 \pm 32{,}9^{b}$
Shaker	$142 \pm 25{,}6^{a}$
Ultrasonic	$140 \pm 8{,}04^{a}$

Based on Table 2, the normality test indicated that the IC_{50} data were normally distributed ($p > 0.05$), while the one-way ANOVA test revealed a significant difference among the extraction methods ($p < 0.05$). Post-hoc analysis showed that the maceration method differed significantly from the others. The IC_{50} values of A. indica extracts indicated moderate to weak antioxidant activity, with the highest activity observed in the ultrasonic method (140 ± 8.04 ppm), followed by shaker (142 ± 25.6 ppm), and the lowest in maceration (165 ± 32.9 ppm).

These differences were influenced by the efficiency of each method. Ultrasonic extraction produced higher activity due to cavitation energy, which accelerates the release of active compounds without degrading them. The shaker method, although similar to maceration, was more effective due to continuous agitation. In contrast, maceration is slower and less efficient. These findings align with previous studies, which reported that ultrasonic extraction results in higher antioxidant activity compared to agitation or maceration. Compared to vitamin C ($IC_{50} = 2.02 \pm 28.73$ ppm), all three extraction methods demonstrated significantly lower antioxidant activity [13][14].

D. Identification of Antioxidant Functional Groups in A. indica Using FTIR Spectrophotometry

In this study, the FTIR-ATR technique was used to identify the functional groups of antioxidant compounds in A. indica extracts obtained from different extraction methods. The FTIR spectrum analysis results are shown in Figure 1:

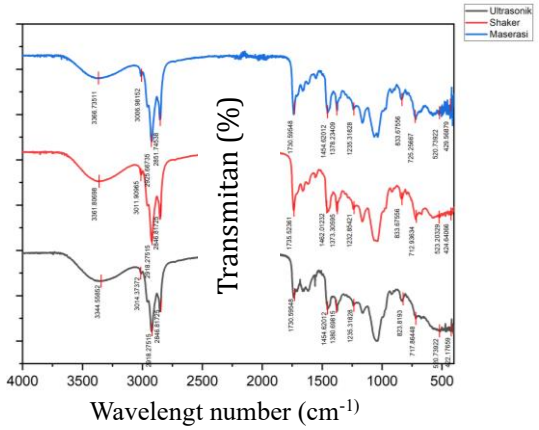

Fig 1. FTIR spectrum of A. indica extract based on different extraction methods

The FTIR spectra in Figure 1 did not show significant differences in the compound profiles. The spectra displayed similar peak patterns in terms of wavenumbers, with only minor variations in % transmittance for each extraction method. Therefore, the FTIR spectra alone were not sufficient to distinguish compound profiles in detail. With the aid of PCA, patterns and differences in secondary metabolite profiles across extraction methods could be more clearly observed and classified into distinct groups.

E. Chemometric Analysis Using Principal Component Analysis (PCA)

PCA is a chemometric method used to simplify high-dimensional data without losing essential information [15]. In this study, the FTIR analysis in Figure 1 alone was not sufficient to differentiate the secondary metabolite profiles of A. indica based on extraction method, as the FTIR spectra showed similar wavenumber patterns and only minor differences in % transmittance. Therefore, PCA was employed to reveal the patterns and distinctions of compound profiles more clearly. The PCA score plot results are shown in Figure 2, displaying groupings based on the extraction methods.

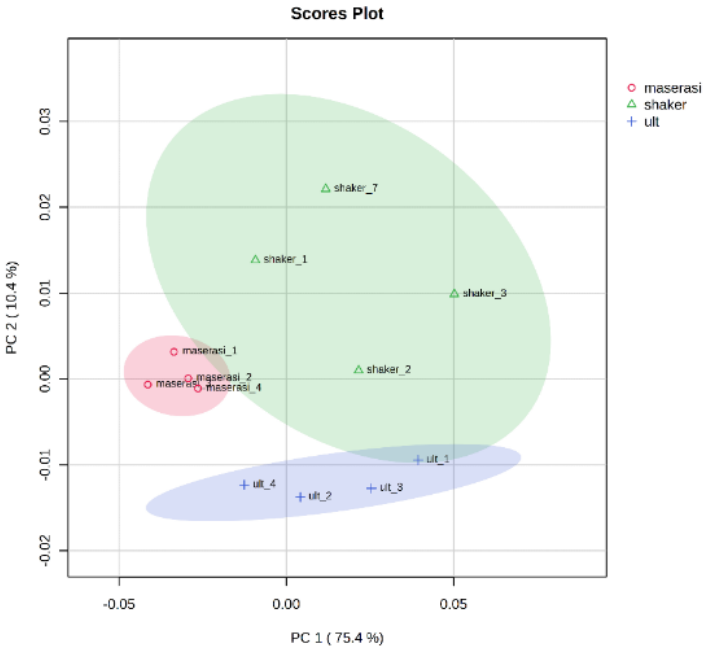

Fig 2. PCA score plot of A. indica extract based on different extraction methods

As shown in Figure 2, the steps to obtain the PCA score plot began with inputting the FTIR data, which includes wavenumbers and % transmittance. These data were then preprocessed using Origin software for normalization, followed by chemometric analysis via MetaboAnalyst 6.0. The resulting score plot visualizes the data clustering. The PCA score plot is used to observe sample differences based on their quadrant positions. Figure 2 demonstrates that A. indica extracts from the

three extraction methods (ultrasonic, maceration, and shaker) were separated into three distinct groups. The two principal components, PC1 and PC2, explained a total variance of 85.8%, with contributions of 75.4% and 10.4%, respectively. PC1 had the highest variance, representing the majority of information in

the dataset. A cumulative variance value exceeding 70% is considered ideal, as it indicates the principal components adequately represent the original data [16]. The grouping observed in Figure 2 indicates that the shaker method is located in quadrant 2, maceration in quadrant 3, and ultrasonic in quadrant 4. This separation suggests differences in secondary metabolite profiles among the extraction methods. Furthermore, differences in compound intensities are indicated by % transmittance values shown in Table 4.3, where each extraction method produced FTIR spectra with distinct characteristics.

F. Chemometric Analysis Using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA)

OPLS-DA is a chemometric technique used to classify data by separating relevant information for distinguishing groups from irrelevant variations [17]. In this study, OPLS-DA was employed to determine the functional groups actively contributing as antioxidants in A. indica extracts across different extraction methods. OPLS-DA identifies these groups based on the highest Variable Importance in Projection (VIP) scores [18]. The OPLS-DA score plot results for A. indica extracts are shown in Figure 3.

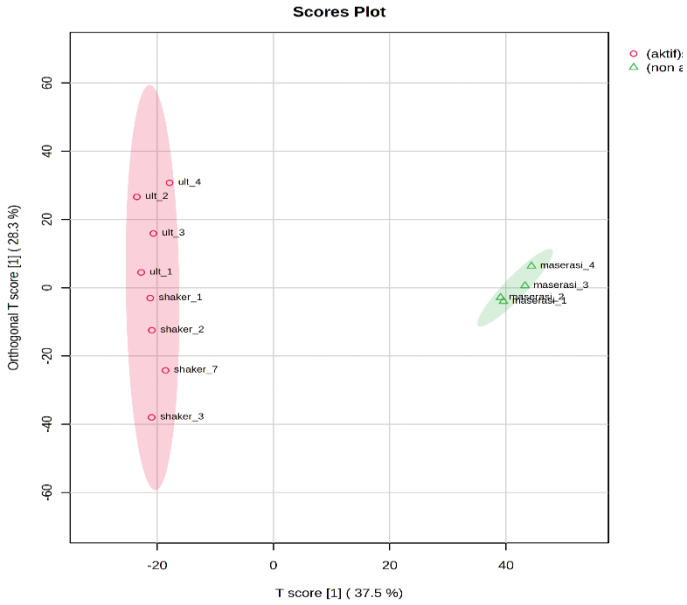

Fig 3. *OPLS-DA* score plot of A. indica extract based on different extraction methods

Figure 3 shows a T-score of 37.5% and an orthogonal T-score of 28.3%, indicating a clear separation between moderate and weak antioxidant activities. The permutation test produced high values for R^2 (0.996) and Q^2 (0.825), demonstrating model stability and strong predictive power [19]. In the score plot, the shaker and ultrasonic extraction methods were located on the left and near the center point (0,0), while maceration was positioned on the right and farther from the origin. According to [20], samples located close to the coordinate center (0,0) on the score plot indicate chemical similarity, whereas greater distances reflect higher chemical differences among samples. The score plot effectively separates each variable's relative contribution to distinguishing antioxidant groups (moderate and weak). In this case, shaker and ultrasonic extraction methods clustered within the moderate category, while maceration was separated, indicating weak antioxidant activity. To further identify the functional groups actively contributing as antioxidant compounds, the loading S-plot in Figure 4 was examined.

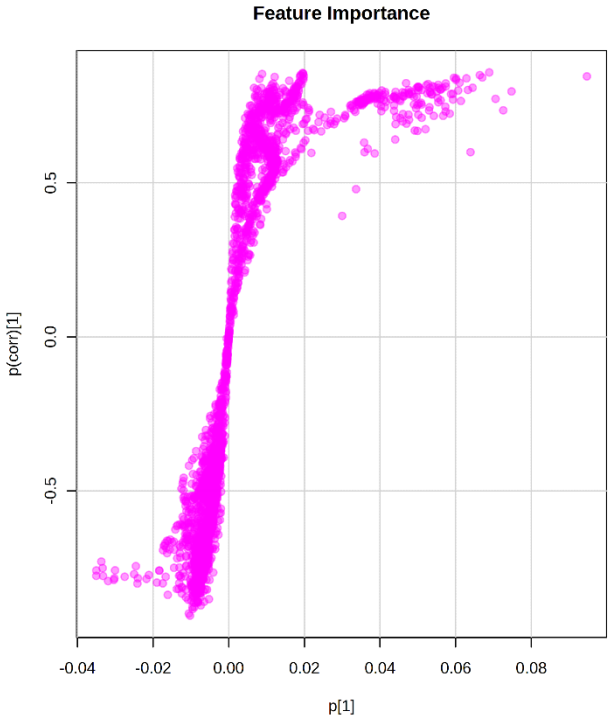

Fig 4. *OPLS-DA* loading S-plot of A. indica extract based on different extraction methods.

Figure 4 reveals the functional groups contributing to the antioxidant activity in A. indica extract by identifying the groups with the highest VIP rankings. Table 4 presents the five major identified functional groups considered to actively contribute to the antioxidant compounds of A. indica across different extraction methods.

Based on Table 4, four main functional groups were identified as actively contributing to the antioxidant activity of *A. indica* extracts obtained from different extraction methods. These functional groups were determined through the Loading S-plot feature in the OPLS-DA analysis by selecting variables located in the lower-left corner of the graph. The selection of variables in this area is justified, as the previous score plot had shown that the most influential functional groups in antioxidant activity were positioned on the left side. Variables with a VIP (Variable Importance in Projection) score above 1 were considered to have

the highest contribution in distinguishing the antioxidant activity of each extract [21].

The highest VIP values in this study were observed at wavenumbers 1550, 1735, 1895, 830, and 3300 cm^{-1}. These wavenumbers correspond to the following functional groups: aromatic C=C, C=O stretching, aromatic C–H, and O–H stretching. According to the study by [22], which investigated antioxidants in eggplant peel extract using FTIR, the functional groups O–H, C=O (stretching), aromatic C=C, aromatic C–H, C–O, and C–O–C were found and identified as components of quercetin. Similarly, in the study by [23] on quercetin quantification using FTIR-ATR, the functional groups O–H, ketonic C=O, aromatic C=C, and C–O were also associated with quercetin. Moreover, a metabolomics study using LC-HRMS by [24] detected the presence of quercetin in *A. indica*. In addition, [25] reported that quercetin has strong potential as an antioxidant compound.

TABLE III. THE MAIN IDENTIFIED FUNCTIONAL GROUPS THAT ARE CONSIDERED TO CONTRIBUTE ACTIVELY TO THE ANTIOXIDANT COMPOUNDS OF A. INDICA EXTRACT ARE DIFFERENT EXTRACTION METHODS

wavelength number (cm^{-1})	*p*-value	VIP value > 1	Fungtional Group	Reference wavelength number (cm^{-1})
1550	-0.0056124	1.0314	C=C *aromatik*	1500-1675
1735 1885	-0.035049 -0.0098189	1.2385 1.1661	C=O *stretching*	1650-1900
830	-0.008138	1.3676	C-H *Aromatik*	670-900
3300	-0.011987	1.1253	O-H *stretching*	3300-3500

IV. CONCLUSION

Based on the conducted research and data analysis, it can be concluded that the PCA analysis results showed that *A. indica* extracts obtained through ultrasonic, maceration, and shaker methods formed distinct groups. This variation is presumably due to differences in the secondary metabolite profiles produced. PCA was able to explain a total cumulative variation of 85.8%, with PC1 accounting for 75.4% and PC2 for 10.4%. Furthermore, the OPLS-DA analysis identified four major functional groups that actively contribute to antioxidant activity: aromatic C=C, C=O stretching, aromatic C–H, and O–H stretching. These functional groups are known components of the quercetin molecule, which is recognized as a potent antioxidant compound.

REFERENCES

[1] Chekuri, S., Lingfa, L., Panjala, S., Bindu, K. S., dan Anupalli, R. R. (2020). *A. Indica* an important medicinal plant: a brief review of its pharmacological properties and restorative potential. *European Journal of Medicinak Plants*. *31*(11):1-10.

[2] Zahidin NS, et al. (2017). A Review of *A. Indica* (*Euphorbiaceae)* as Traditional Medicine Plant and its Therapeutic Potential. *Journal of Ethnopharmacology*. 207:146–173.

[3] Amarnath, K., Dhanabal, J., Agarwal, I., dan Seshadry, S. (2014). Cytotoxicity induction by ethanolic extract of *A. Indica* loaded casein-chitosan microparticles in human prostate cancer cell line in vitro. *Biomed. Prev. Nutr*. 4: 445–450.

[4] Amelia. (2018). *Khasiat A. Indica A*. Majalah Farmasetika. 3(1): 7−11

[5] Molyneux, P. (2004). The Use Of The Stable Free Radical *Diphenylpicrylhydrazyl* (DPPH) For Estimating Antioksidan Activity, Songklanakarin: *Journal Science Technology*. 26(2): 211-219.

[6] Yuniarto, P. F., dan Abd Kadir, M. B. (2020). Optimation Of Carbopol 940 And Glycerin Of Tanaman *A. Indica* Gel An Antioxidant. *Jurnal Inovasi Farmasi Indonesia*. 2(1).

[7] Sayuti, Mohammad. (2017). Pengaruh Perbedaan Metode Ekstraksi, Bagian Dan Jenis Pelarut Terhadap Rendemen Dan Aktifitas Antioksidan Bambu Laut (*Isis Hippuris*). *Technology Science and Engineering Journal.* 1(30): 2549-1601.

[8] Fadhila, S. I., Hayati, E. K., Rafi, M., dan Sabarudin, A. (2023). Effect Of Ethanol-Water Concentration As Extraction Solvent On Antioxidant Activity Of *A. Indica*. *al Kimiya: Jurnal Ilmu Kimia dan Terapan*. *10*(2): 133-142.

[9] Fauziyah, R., Widyasanti, A., dan Rosalinda, S. (2022). Perbedaan Metode Ekstraksi terhadap Kadar Sisa Pelarut dan Rendemen Total Ekstrak Bunga Telang (*Clitoria ternatea L.*). *Jurnal Kimia Padjadjaran*. *1*:18-25.

[10] Aura, C. R., Handayani, R., Kurniaty, R., & Adriani, A. (2024). Uji aktivitas antioksidan teh herbal daun anting-anting (Acalypha indica L.) menggunakan metode DPPH (*1, 1-diphenyl-2-picrylhidrazyl*). *Nutrition and Health Insights*. *1*(2):71-75.

[11] Yasi, R., M., Sih,. R., H., Trisn, T., L., (2022) The Effect of Simplicia Drying Method on the Acquisition of Active Compound Levels of Grinting Grass Simplicia Extract (Cynodon dactylon (L.) Pers.). *Jurnal Universitas Negrei Jember*. 10 (3); 147-154

[12] Hidayati, N., Kurniawan, T., Kusumawardani, N. I., & Sari, R. P. (2018). Comparison of maceration and ultrasonication methods on indigofera tinctoria linn leaf extraction. *Jurnal Bahan Alam Terbarukan*. 7(1): 54-58

[13] Burnaz, N. A. (2021). Evaluation of Ultrasonication and Agitation Extraction Methods at Different Conditions on the Phenolic Composition and Antioxidant Activities of *Mammillaria prolifera. Indian Journal of Pharmaceutical Sciences*. 83(5):1-11

[14] Burnaz, N. A. (2021). Evaluation of Ultrasonication and Agitation Extraction Methods at Different Conditions on the Phenolic Composition and Antioxidant Activities of *Mammillaria prolifera. Indian Journal of Pharmaceutical Sciences*. 83(5):1-11

[15] Arina, Y., Shiyan, S., & Suprayetno, S. (2022). Analisis kemometrik ekstrak akar tunjuk langit (*Helminthostachys zeylanica (L)*) melalui analisis fourier transformed infrared dari berbagai daerah Sumatera Selatan. *Jurnal'Aisyiyah Medika*. *7*(1): 1-8

[16] Ganendra, M. V. E. B., Putro, R. A., Athallah, Z. A., Nasrudin, M., & Trimono, T. (2025). Analisis Jumlah Penduduk Tenaga Kerja Industri Di Jawa Timur Berdasarkan Tahun 2017-2018, Dengan Menggunakan Metode Rm Manova. *Kohesi: Jurnal Sains dan Teknologi*. 8(2): 81-90.

[17] Lestari, O. A., & Dewi, Y. S. K. (2023). Penerapan Analisis Multivariat untuk Memetakan Sifat Fisikokimia dan Mutu Glikemik Madu. *Gorontalo Agriculture Technology Journal*. 6 (1): 25-36.

[18] Umar, A. H., Syahruni, R., Ranteta'dung, I., & Rafi, M. (2023). FTIR-based fingerprinting combined with chemometrics method for rapid discrimination of Jatropha spp.(*Euphorbiaceae*) from different regions in South Sulawesi. *Journal of Applied Pharmaceutical Science*. *13*(1): 139-149

[19] Pan, L., Zhou, C., Jing, J., Zhuang, M., Zhang, J., Wang, K., & Zhang, H. (2022). Metabolomics analysis of cucumber fruit in response to foliar fertilizer and pesticides using UHPLC-Q-Orbitrap-HRMS. *Food Chemistry*. 369:130960.

[20] Khan, M. A., Abbasi, B. H., Shah, N. A., Yücesan, B., & Ali, H. (2015). Analysis of metabolic variations throughout growth and development of adventitious roots in *Silybum marianum L*.(Milk thistle), a medicinal plant. *Plant Cell Tissue and Organ Culture* .123: 501-510.

[21] Mashiane, P., Manhivi, V. E., Shoko, T., Slabbert, R. M., Sultanbawa, Y., & Sivakumar, D. (2021). Cooking African pumpkin leaves (*Momordica balsamina L*.) by stir-frying improved bioactivity and bioaccessibility of metabolites—Metabolomic and chemometric approaches. *Foods*. 10(11),: 2890

[22] Rahman, H., Arini, S. F., dan Utomo, V. (2020). Tannins Extraction of *Tea Leaves* by Ultrasonic Method: [Comparison with The Conventional Method. *Jurnal Teknologi*. *8*(1): 84-95.

[22] Siswarni, M. Z., Putri, Y. I., & Rinda, R. (2017). Ekstraksi kuersetin dari kulit terong Belanda (Solanum betaceum Cav.) menggunakan pelarut etanol dengan metode maserasi dan sokletasi. *Jurnal Teknik Kimia USU*. *6*(1): 36-42.

[23] Winingsih, W., Mursyida, U., O., Suprijana. (2016). Penggunaan FTIR-ATR Znse (Fourier Transform Infra Red) Untuk Penetapan Kadar Kuersetin Dalam Teh Hitam (*Camellia sinensis L*.). *Indonesian Journal of Pharmaceutical Science and Technology*. 5(1).

[24] Hayati, E. K., Sabarudin, A., Rafi, M., & Karomah, A. H. (2024). LCHRMS-based metabolomics on *A. Indica* with different altitude and antioxidant activity. *South African Journal of Botany*.*172*: 710-719.

[25] Noer, S., & Pratiwi, R. D. (2019). Penetapan Kadar Flavonoid Sebagai Kuersetin Dan Aktivitas Antioksidan Ekstrak Metanol Daun Inggu (*Ruta angustifolia L.). Simposium Nasional Ilmiah & Call for Paper Unindra (Simponi)*. *1*(1):1-6

[26] Silverstein, R.M., Webster, F.X. dan Kiemle, D.J.(2005).*Spectrometric Identification of Organic Compounds, Seventh Edition.* New York: John Wiley & Sons, Ltd.

[27] Zin, N. B. M., Azemin, A., Rodi, M. M. M., Rashid, Z. M., & Mohd, K. S. (2019). Application of FTIR fingerprints coupled with chemometric for comparison of stingless bee propolis from different extraction methods. *Malaysian Journal of Fundamental and Applied ScienceS*. *15*. 350-355.