JPPIPA 11(5) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Identification of Phenolic Compounds Content in *Tinospora crispa* Stem Decoction by FTIR and UV-Visible Spectrophotometry

Elisabet Sa Wulo[1*], Yunike Kurnia Unda[1], Suparno[1]

[1]Physics Education Study Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, Yogyakarta, Indonesia.

Received: December 16, 2024
Revised: March 06, 2025
Accepted: May 25, 2025
Published: May 31, 2025

Corresponding Author:
Elisabet Sa Wulo
elisabetsa.2024@student.uny.ac.id

DOI: 10.29303/jppipa.v11i5.10069

Abstract: Phenolics are compounds that have a hydroxyl (OH) group attached to an aromatic ring, and are known for various health benefits such as antioxidant, antimicrobial, and anti-inflammatory properties. This study aims to measure the total phenolic content and characterize the functional groups in *Tinospora crispa* stem extract using a decoction method, as well as evaluate its potential as a source of bioactive compounds. Extraction was performed by boiling dried *Tinospora crispa* stems in water. Total phenolic content was quantified using UV-Vis spectrophotometry (λ = 765 nm) via the Folin-Ciocalteu reaction, with a gallic acid calibration curve (R^2 = 0.998). Functional group analysis was conducted using FTIR (4000–400 cm^{-1}). The extract showed high phenolic content (384.909 mg GAE/g), supported by the identification of characteristic functional groups such as O-H (3255.55 cm^{-1}), C=C (2121.59 cm^{-1}), and C-O (1261.45 cm^{-1}) in the FTIR spectrum. These groups are associated with the presence of bioactive compounds such as phenolics. Therefore, *Tinospora crispa* has potential as a source of phenolic compounds, one of which can be obtained using the decoction method.

Keywords: Decoction; FTIR; Phenolic; *Tinospora crispa*; UV-Vis

Introduction

Tinospora crispa is a medicinal plant native to Africa and Southeast Asia that has long been used in various traditional medicine systems, including Ayurveda, Traditional Chinese Medicine (TCM), and Thai herbal formulas such as Tri-Yannarose (Zhang et al., 2018; Llamasares-Castillo et al., 2024; Sanpinit et al., 2023). In Indonesia, particularly in Bengkulu, this plant is one of the most highly valued herbs based on usage frequency and community loyalty (Susanti et al., 2024). The therapeutic potential of *Tinospora crispa* is supported by its diverse phytochemical content; at least 167 compounds from 12 chemical categories—including alkaloids, flavonoids, terpenoids, and phenolic compounds—have been successfully isolated from various parts of this plant (Haque et al., 2022; AP et al., 2023).

Among these bioactive compounds, phenolic compounds are of particular interest due to their role as antioxidants and anticancer agents (Chroho et al., 2022; Suparno et al., 2024). The antioxidant activity of phenolic compounds is closely related to their chemical structure (Pérez et al., 2023). In the context of *T. crispa,* this information can help identify specific phenolic compounds that contribute to anti-inflammatory activity, such as flavonoids and phenolic acids. Phenolic compounds, which contain hydroxyl groups (-OH) bound to aromatic rings, are key components in the plant's defense mechanism against oxidative stress and pathogens. A study by Ibrahim et al. (2011) showed that *Tinospora crispa* methanol extract contains high phenolic content (255.33 ± 10.79 mg GAE/g), which correlates

How to Cite:
Wulo, E. S., Unda, Y. K., & Suparno. (2025). Identification of Phenolic Compounds Content in Tinospora crispa Stem Decoction by FTIR and UV-Visible Spectrophotometry. *Jurnal Penelitian Pendidikan IPA*, *11*(5), 533-540. https://doi.org/10.29303/jppipa.v11i5.10069

with cytotoxic activity against cancer cells. Although extraction methods also influence the biological activity of an extract (Rosidah et al., 2015), extraction methods using organic solvents such as methanol have limitations in large-scale applications due to toxicity and cost factors (Warsinah et al., 2020). Therefore, there is a need for the development of a simpler, safer, and more effective extraction method. One method that can be used is the decoction (boiling) method, especially considering that the traditional use of this plant is often in the form of a decoction (Shah et al., 2021).

Decoction has been shown to improve the solubilization of bioactive compounds compared to maceration, resulting in extracts with higher active compound content (Hidayat & Wulandari, 2021). Herbal decoction can reduce the negative effects of a high-calorie diet, including insulin resistance and fat accumulation in the liver (Castellanos-Jiménez et al., 2022). This opens up opportunities to apply a similar approach to *Tinospora crispa,* given its use in traditional medicine for metabolic conditions. However, comprehensive characterization of the phytochemical profile of *Tinospora crispa* decoction extracts, particularly for phenolic compounds, remains limited (Li et al., 2010). To address this gap, this study combines two analytical techniques: UV-Vis spectroscopy with the Folin-Ciocalteu reagent and FTIR (Fourier Transform Infrared Spectroscopy).

UV-Vis analysis was used in this study for two main purposes: to identify the presence of phenolic compounds through specific absorption patterns, and to quantify the total phenolic content using the Folin-Ciocalteu reagent (Ainsworth & Gillespie, 2007; Bastola et al., 2017; Song et al., 2010). Previous studies have shown that *Tinospora crispa* extract has absorption peaks at 326 nm and 408 nm, which correspond to the characteristics of phenolic compounds such as hydroxycinnamic acid and flavonoid derivatives (Karpagasundari & Kulothungan, 2014). However, the limitations of UV-Vis in identifying specific compounds (due to overlapping absorption peaks) are overcome by combining FTIR for functional group analysis, as well as validation using phenolic standards such as gallic acid through calibration curves.

FTIR (Fourier Transform Infrared Spectroscopy) analysis is an analytical technique used to identify organic materials, polymers, and in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe their chemical properties (RTI laboratories, 2016; Joshi et al., 2021). The test results will provide specific data on biomolecules from two other plant species, as well as how certain peaks in the FTIR spectrum can indicate the presence of various functional groups that are important in the context of phytochemistry (Apriandanu & Yulizar, 2017; Meena & Johri, 2023). FTIR analysis in this study is focused on identifying characteristic functional groups of phenolic compounds in *Tinospora crispa* stem extracts. FTIR technology can detect molecular vibrations from functional groups such as phenolic -OH (3200-3600 cm^{-1}), carboxylic acid C=O (1700-1725 cm^{-1}), and aromatic rings (1450–1600 cm^{-1}), which are important markers of phenolic compounds (Silverstein et al., 2014; Rahman et al., 2020).

The combination of the two tests not only validates the presence of phenolic compounds but also provides more in-depth structural information than previous studies that relied solely on quantitative tests. This opens up opportunities for exploring other bioactive compounds through FTIR analysis. Therefore, the results of this study are expected to strengthen the scientific basis for the use of *Tinospora crispa* in traditional medicine. Additionally, it can provide a more practical extraction method for the development of herbal products.

Method

Sample Preparation

The sample used in this study is a sample of *Tinospora crispa* extract, extracted by the decoction method. 100 grams of *Tinospora crispa* stems are cut into smaller parts approximately 2-3 cm with a diameter of about 1-2 cm. 100 grams of *Tinospora crispa* are boiled in water with a volume of 1000 ml. The ingredients are boiled until the water is reduced to half the initial volume of 500 ml. The resulting extract will be cloudy brown. Figure 1 shows *Tinospora crispa* collected from Bantul Regency, Special Region of Yogyakarta, Indonesia. The resulting extract will be separated into 2 samples, 30 ml for the UV-Vis spectrophotometer test and the other 30 ml will be used for the FTIR test. The picture below shows stem of *Tinaspora crispra* that used in this study

Figure 1. *Tinospora crispa*

Determination of Phenolic Concentrations

The concentration of *Tinospora crispa* was determined using the Folin–Ciocalteu method. Initially, the sample was centrifuged for 10 minutes. Then, 5 mL of the supernatant was taken and placed in a drying device to determine the total solid content of the sample. Once dried and converted to solid form, the sample was weighed to determine its mass. The remaining supernatant was used to prepare various concentrations through dilution. The dilution process followed the formula, $V_1 = (C_2V_2)/C_1$, where V_1 is the required volume of S_2 Stock, C_1 is the concentration of S_2 stock, C_2 is the target concentration, V_2 is the target volume. For the Folin–Ciocalteu reaction, 0.2 mL of Folin–Ciocalteu reagent and 3.16 mL of distilled water were mixed with 0.04 mL of each standard sample. This mixture was left to stand for 8 minutes, after which 0.6 mL of 10% sodium carbonate (Na_2CO_3) solution was added. In this study, the concentrations prepared were 200 ppm, 400 ppm, 600 ppm, and 800 ppm. A calibration curve, as shown in the Table 1, was generated based on these concentrations.

Table 1. Variation of concentration

Concentration (ppm)	Absorbance
0	0.033
200	0.292
400	0.486
600	0.741
800	0.881

Result and Discussion

Total Phenolic Content

UV-Vis analysis was performed using the Folin-Ciocalteu technique. Phenolic concentration was determined by first creating a calibration curve (Delgado, 2022). Absorbance readings were measured at a wavelength of 765 nm using a UV-Vis spectrophotometer (Zugazua-Ganado et al., 2024).

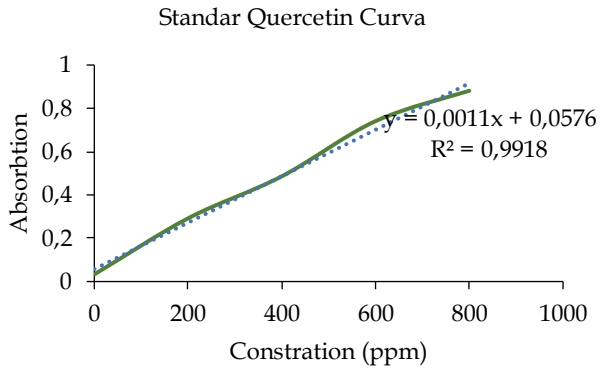

Figure 2. Standard quercetin curve

Figure 2 shows the linearity (R^2) of the standard curve for gallic acid, namely Y = 0.0011 X + 0.0576. X represents the concentration of diluted phenolic compounds, calculated using the equation X = CVDF/m, where C denotes the phenolic concentration in the diluted sample, m is the sample mass, and DF is the dilution factor. Absorbance measurements were performed at a wavelength of 765 nm using a UV-Vis spectrophotometer.

Table 2. Total of phenolic

Sample	Absorbance	V (L)	mg GAE	mg GAE/g
Tinospora c.	0.481	0.005	1.925	384.909

Column 2 in Table 1 shows the absorbance values of the diluted phenolic extract, while column 3 shows the volume used for the 0.005 liter sample. Column 4 shows the total phenolic content in 0.005 L of extract. Column 5 shows that the total concentration of phenolic compounds in milligrams per gram of sample is 384.909 mg GAE/g. With this high phenolic concentration, *Tinospora crispa* has the potential as an alternative for producing high activity for phenolic content (Nguyen et al., 2020).

FTIR Analysis

Figure 3 is the result of the Fourier Transform Infrared Spectroscopy (FTIR) test on *Tinospora crispa* extract which provides important information about the chemical components contained in the sample. The spectrum was measured from 4000 cm^{-1} to 400 cm^{-1} with a total of 3601 data points. The peaks detected in the FTIR spectra indicate the presence of various functional groups along with transmission values (%T).

Tables 3 and 4 are base 1 and base 2 tables that serve to determine the baseline. Baseline correction is a critical step in FTIR data processing to eliminate the influence of background noise. This is because FTIR signals are often distorted by disturbances such as scattering or solvent absorption. The area under the peak (peak area) is calculated relative to the baseline, not the horizontal axis. The baseline aids quantitative interpretation for spectrum normalization, enabling peak intensity comparisons between samples. The first row (Base 1) of the baseline starts at 4000 cm^{-1} and ends at 3999 cm^{-1}. Base2 (continuation baseline) is connected to Base1. The first row of Base2, the baseline is in the region 2985.42–2987.42 cm^{-1} related to the peak at 3255.55 cm^{-1}.

Figure 4 shows the FTIR spectrum indicating the area and height of each peak. The figure shows differences in the area of several peaks, which may indicate the number of functional groups contributing to absorption at that frequency. This may refer to high and low concentrations of functional groups.

For example, a peak with a large area (such as the peak at 3255.55 cm^{-1} may indicate that the O-H group in the sample has a high concentration (Dai et al., 2023).

Meanwhile, peaks with small areas, such as peak 10 with absorption at 839.15 cm^{-1}, indicate that the functional group is likely not dominant due to low absorption intensity. Another possibility is that the small area originates from minor groups or weak signals.

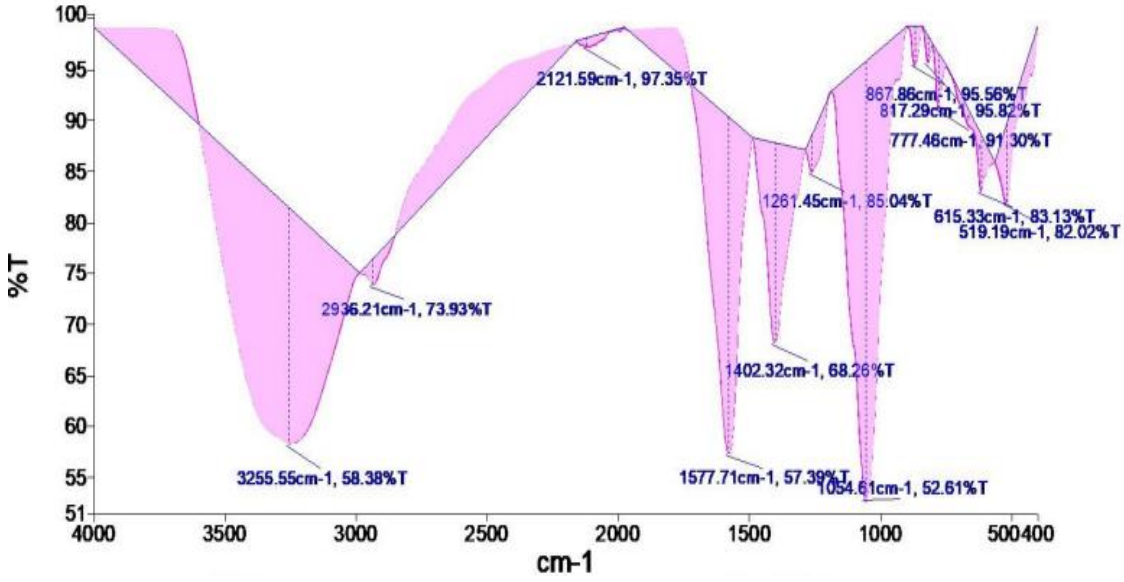

Figure 3. Peak absorption in the infrared spectrum

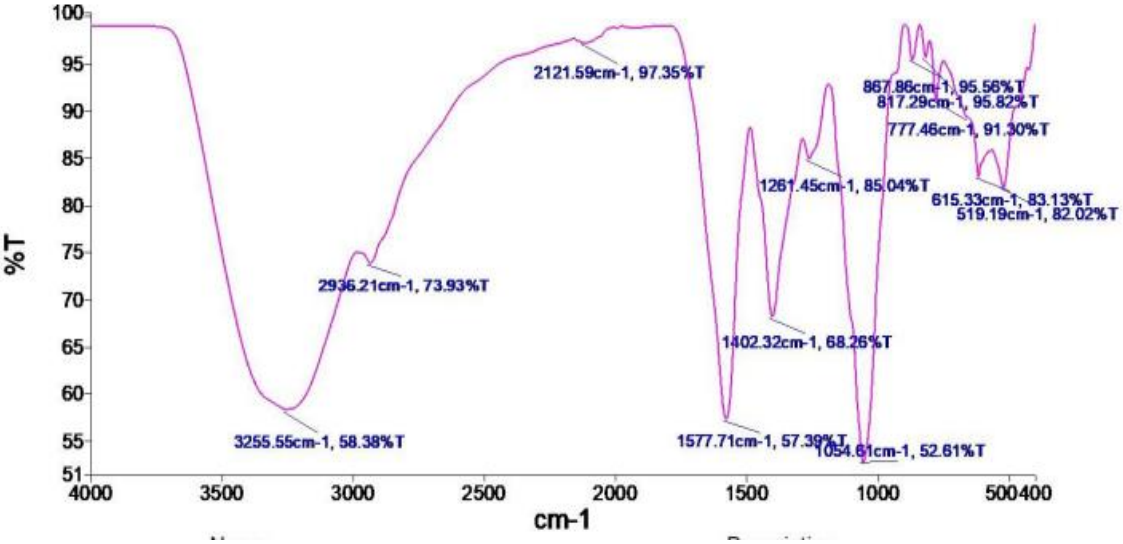

Figure 4. FTIR spectrum on *Tinospora crispa* extract

Phenolic compounds are known to have potential. Table 5 shows a peak around 3255.55 cm^{-1} with a transmittance of 58.38%, indicating the presence of a hydroxyl group (OH), which is commonly found in phenolic compounds and alcohols (Kamacı & Kaya, 2014). Further activity testing is required to confirm its biological effects. At the peak of 2936.2 cm^{-1} with a transmittance of 73.93%, it indicates the possibility of C-H bonds associated with aromatic compounds that have bioactive potential, as shown in previous studies (Paśko et al., 2024). At the peak of 2121.59 cm^{-1} with a transmittance of 97.35%, it indicates C=C stretching, commonly found in unsaturated compounds such as terpenoids or alkenes. Compounds with C=C bonds have been reported to have anti-inflammatory activity in other studies (Aryasa & Sugianta, 2023; Paśko et al., 2024). Furthermore, at the peak of 1577.71 cm^{-1} with a transmittance of 57.39%, it indicates the presence of an aromatic structure, namely bioactive compounds such as flavonoids (Rahayu et al., 2023). At the peak of 1402.32

cm^{-1} with a transmittance of 68.26%, there are no related studies that explain this peak in detail, but it may indicate the presence of symmetric COO^- stretching in samples containing carboxylic acid salts or as CH_3 deformation if there are methyl groups in the molecular structure (Sulastri et al., 2018). Confirmation of its biological activity requires further in vitro/in vivo testing. At the peak of 1261.45 cm^{-1} with a transmittance of 85.04%, it indicates the presence of C-O groups commonly found in glycoside and alkaloid compounds (Costa et al., 2016). This compound has potential pharmacological effects. At the peak of 1054.61 cm^{-1} with a transmittance of 52.61%, it indicates the presence of C-O-C compounds attributed to C–O–C stretching vibrations, which are part of the saponin structure (Rai et al., 2023; Ismail et al., 2022). At the peak of 867.86 cm^{-1} with a transmittance of 95.82%, the peak of 777.46 cm^{-1} with a transmittance of 91.30%, and 615.33 cm^{-1} with a transmittance of 83.13%, the peak positions in this range can provide information about the substitution pattern in the aromatic ring (McMurry, 2023; Tully, 2024). The final peak at 519.19 cm^{-1} with a transmittance of 82.02% indicates the presence of minerals or other elements that require further analysis using techniques such as electron microscopy.

Table 3. Initial baseline

Peak Number	Start	End	Base1	Base1 Left	Base1 Right
1	4000	2986.42	4000	4000	3999
2	2986.42	2160.48	2986.42	2987.42	2985.42
3	2160.48	1977.28	2160.48	2161.48	2159.48
4	1977.28	1487.06	1977.28	1978.28	1976.28
5	1487.06	1286.44	1487.06	1488.06	1488.06
6	1286.44	1187.6	1286.44	1287.44	1285.44
7	1187.6	898.46	1187.6	1188.6	1186.6
8	898.46	839.15	898.46	899.46	897.46
9	839.15	801.55	839.15	840.15	838.15
10	801.55	748.96	801.55	802.55	800.55
11	748.96	564.35	748.96	749.96	747.96
12	564.35	400	564.35	565.35	563.35

Table 4. Advanced baseline

Peak Number	Start	End	Base2	Base2 Left	Base2 Right
1	4000	2986.42	2986.42	2987.42	2985.42
2	2986.42	2160.48	2160.48	2161.48	2159.48
3	2160.48	1977.28	1977.28	1978.28	1976.28
4	1977.28	1487.06	1487.06	1488.06	1486.06
5	1487.06	1286.44	1286.44	1287.44	1285.44
6	1286.44	1187.6	1187.6	1188.6	1186.6
7	1187.6	898.46	898.46	899.46	897.46
8	898.46	839.15	839.15	840.15	838.15
9	839.15	801.55	801.55	802.55	800.55
10	801.55	748.96	748.96	749.96	747.96
11	748.96	564.35	564.35	565.35	563.35
12	564.35	400	400	401	400

Table 5. Peak table result

Peak Number	X (cm^{-1})	Y (%T)
1	3255.55	58.38
2	2936.21	73.93
3	2121.59	97.35
4	1577.71	57.39
5	1402.32	68.26
6	1261.45	85.04
7	1054.61	52.61
8	867.86	95.56
9	817.29	95.82
10	777.46	91.30
11	615.33	83.13
12	519.19	82.02

Conclusion

This study shows that *Tinospora crispa* extract contains significant amounts of phenolic compounds, as quantified by UV-Vis analysis at 384.909 mg GAE/g, indicating its potential as a source of bioactive compounds. The FTIR spectrum shows characteristic functional groups such as O-H at 3255.55 cm^{-1}, C=C at 2121.59 cm^{-1}, and C-O at 1261.45 cm^{-1}, consistent with the phytochemical profile of phenolic-rich plants. However, further bioactivity tests, such as the DPPH assay for antioxidants or cytotoxicity assays for anticancer potential, are needed to support the presence of therapeutic activity. These findings support further research on *Tinospora crispa* for pharmaceutical or nutraceutical applications, particularly in optimizing extraction and isolation methods for active compounds.

Acknowledgments

I would like to thank all parties involved in this research, in particular I would like to thank Suparno M.App.Sc., Ph.D. as my supervisor in the Physics Education study program at Yogyakarta State University who has provided opportunities and encouragement to his students to have a big heart in conducting research.

Author Contributions

Conceptualization, formal analysis, E.S.W. and S.; initial draft writing, results and discussion, Y.K.U.; supervision, conclusion and review, S.

Funding

This research did not receive funding support from any external party and was fully funded through personal financial resources by the researcher. All costs associated with this research, including data collection, analysis, and report preparation, were borne independently without the involvement of sponsors or outside agencies.

Conflicts of Interest

The author declares no conflict interest to publish the results.

References

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin–Ciocalteu Reagent. *Nature Protocols*, *2*(4), 875-877. https://doi.org/10.1038/nprot.2007.102

AP, P., Murugan, K., V, V., & AP, L. (2023). Comparison of UV-Visible Spectrophotometric and FTIR Analysis of Tinospora Crispa (L.) Hook. F. and Thomson Leaves and Stem. *Acta Scientific Veterinary Sciences*, *5*(4), 12-16. https://doi.org/10.31080/asvs.2023.05.0644

Apriandanu, D. O., & Yulizar, Y. (2017). The Role of Aqueous Leaf Extract of Tinospora crispa as Reducing and Capping Agents for Synthesis of Gold Nanoparticles. *IOP Conference Series: Materials Science and Engineering*, *188*, 012013. https://doi.org/10.1088/1757-899x/188/1/012013

Aryasa, I. W., & Sugianta, I. K. (2023). In Silico Study of Derivative Compounds of Galangal Plants as Anti-Inflammatory. *Jurnal Penelitian Pendidikan IPA*, *9*(8), 6531-6539. https://doi.org/10.29303/jppipa.v9i8.3042

Bastola, K. P., Guragain, Y. N., Bhadriraju, V., & Vadlani, P. V. (2017). Evaluation of Standards and Interfering Compounds in the Determination of Phenolics by Folin-Ciocalteu Assay Method for Effective Bioprocessing of Biomass. *American Journal of Analytical Chemistry*, *08*(06), 416-431. https://doi.org/10.4236/ajac.2017.86032

Castellanos-Jiménez, A. K., Reynoso-Camacho, R., Rocha-Guzmán, N. E., Corella-Madueño, M. A., Ríos, E. A. D. L., & Salgado, L. M. (2022). Effect of Herbal Decoctions Used in Mexican Traditional Medicine Attenuate the Adverse Effects of a Hypercaloric Diet. *Phytomedicine Plus*, *2*(1), 100213. https://doi.org/10.1016/j.phyplu.2021.100213

Chroho, M., Bouymajane, A., Majdoub, Y. O. E., Cacciola, F., Mondello, L., Aazza, M., Zair, T., & Bouissane, L. (2022). Phenolic Composition, Antioxidant and Antibacterial Activities of Extract from Flowers of Rosa damascena from Morocco. *Separations*, *9*(9), 247. https://doi.org/10.3390/separations9090247

Costa, R. A., Pinheiro, M. L., Oliveira, K. M., Barison, A., Salomé, K. S., Iank, J. R., Silva, N. G. D., Cabral, T. S., & Costa, E. V. (2016). Structural, Vibrational, and Electronic Properties of the Glucoalkaloid Strictosidine: A Combined Experimental and Theoretical Study. *Journal of Chemistry*, *2016*, 1-16. https://doi.org/10.1155/2016/1752429

Dai, F., Zhuang, Q., Huang, G., Deng, H., & Zhang, X. (2023). Infrared Spectrum Characteristics and Quantification of OH Groups in Coal. *ACS Omega*, *8*(19), 17064-17076. https://doi.org/10.1021/acsomega.3c01336

Delgado, R. (2022). Misuse of Beer–Lambert Law and Other Calibration Curves. *Royal Society Open Science*, *9*(2). https://doi.org/10.1098/rsos.211103

Haque, E., Bari, M. S., Khandokar, L., Anjum, J., Jantan, I., Seidel, V., & Haque, M. A. (2022). An Updated and Comprehensive Review on the Ethnomedicinal Uses, Phytochemistry, Pharmacological Activity and Toxicological Profile of Tinospora crispa (L.) Hook. F. & Thomson. *Phytochemistry Reviews*, *22*(1), 211-273. https://doi.org/10.1007/s11101-022-09843-y

Hidayat, R., & Wulandari, P. (2021). Methods of Extraction: Maceration, Percolation and Decoction. *Eureka Herba Indonesia*, *2*(1), 73-79. https://doi.org/10.37275/ehi.v2i1.15

Ibrahim, M. J., Wan-Nor Izzah, W. M., & Narimah, A. H. (2011). Anti-Proliperative and Antioxidant Effects of Tinospora crispa (Batawali). *Biomedical Research*, *22*(1), 57-62. Retrieved from https://www.researchgate.net/publication/261879595

Ismail, I, I., Irawan, C., Sukiman, M., Putri, I. D., Utami, A., Zalni, M. I., & Putri, R. K. (2022). Optimization of Ultrasound-Assisted Extraction of Andrographis paniculata Nees Leaves, Phytochemical Screening, Total Phenolic Content and Anti-Gout Potential Activity. *Pharmacognosy Journal*, *14*(2), 432-438. https://doi.org/10.5530/pj.2022.14.55

Joshi, R., Sathasivam, R., Park, S. U., Lee, H., Kim, M. S., Baek, I., & Cho, B. (2021). Application of Fourier Transform Infrared Spectroscopy and Multivariate Analysis Methods for the Non-Destructive Evaluation of Phenolics Compounds in Moringa Powder. *Agriculture*, *12*(1), 10. https://doi.org/10.3390/agriculture12010010

Kamacı, M., & Kaya, İ. (2014). Synthesis, Thermal and Morphological Properties of Polyurethanes Containing Azomethine Linkage. *Journal of Inorganic and Organometallic Polymers and Materials*, *24*(5), 803-818. https://doi.org/10.1007/s10904-014-0046-8

Karpagasundari, C., & Kulothungan, S. (2014). Analysis of Bioactive Compounds in Physalis Minima Leaves Using GC MS, HPLC, UV-VIS and FTIR Techniques. *Journal of Pharmacognosy and Phytochemistry*, *3*(4), 196-201. Retrieved from https://www.phytojournal.com/archives/2014.v3.i4.435/analysis-of-bioactive-compounds-in-physalis-minima-leaves-using-gc-ms-hplc-uv-vis-and-ftir-techniques

Li, S., Lai, S., Song, J., Qiao, C., Liu, X., Zhou, Y., Cai, H., Cai, B., & Xu, H. (2010). Decocting-Induced Chemical Transformations and Global Quality of

Du–Shen–Tang, the Decoction of Ginseng Evaluated by UPLC–Q-TOF-MS/MS Based Chemical Profiling Approach. *Journal of Pharmaceutical and Biomedical Analysis*, *53*(4), 946-957. https://doi.org/10.1016/j.jpba.2010.07.001

Llamasares-Castillo, A., Uclusin-Bolibol, R., Rojsitthisak, P., & Alcantara, K. P. (2024). In Vitro and In Vivo Studies of the Therapeutic Potential of Tinospora crispa Extracts in Osteoarthritis: Targeting Oxidation, Inflammation, and Chondroprotection. *Journal of Ethnopharmacology*, *333*, 118446. https://doi.org/10.1016/j.jep.2024.118446

McMurry, J. (2023). *Organic Chemistry*. Houston, Texas: OpenStax. Retrieved from https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter

Meena, R., & Johri, A. (2023). Identification and Comparison of Biomolecules in Medicinal Plant Oxystelma esculentum R. BR. by Using FTIR. *International Journal of Green and Herbal Chemistry*, *12*(1). https://doi.org/10.24214/ijghc/gc/12/1/06570

Nguyen, T. P., Bang, L. H., Nguyen, T. T., & Nguyen, T. P. (2020). Bioactive Compounds Analysis and Antioxidant Activities of Tinospora crispa MIERS Stem Extract. *The Scientific Journal of Tra Vinh University*, *1*(40), 58-69. https://doi.org/10.35382/18594816.1.40.2020.617

Paśko, P., Galanty, A., Dymerski, T., Kim, Y., Park, Y., Cabrales-Arellano, P., & Gorinstein, S. (2024). Physicochemical and Volatile Compounds Analysis of Fruit Wines Fermented with Saccharomyces cerevisiae: FTIR and Microscopy Study with Focus on Anti-Inflammatory Potential. *International Journal of Molecular Sciences*, *25*(11), 5627. https://doi.org/10.3390/ijms25115627

Pérez, M., Dominguez-López, I., & Lamuela-Raventós, R. M. (2023). The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of (Poly)Phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. *Journal of Agricultural and Food Chemistry*, *71*(46), 17543-17553. https://doi.org/10.1021/acs.jafc.3c04022

Rahayu, P. P., Widyastuti, E. S., Nurwahyuni, E., Yunita, C. N., & Hakim, L. (2023). Characterization of Andrographis Paniculata Extract Obtained by Microwave-Assisted Extraction (MAE) Method with Radiation Time. *Jurnal Penelitian Pendidikan IPA*, *9*(12), 11289-11295. https://doi.org/10.29303/jppipa.v9i12.5624

Rahman, M., Rahman, M. H., & Chowdhury, T. A. (2020). Phytochemical and Biological Activity Studies of Tinospora crispa Stem. *Dhaka University Journal of Science*, *68*(2), 167-170. https://doi.org/10.3329/dujs.v68i2.54616

Rai, S., Kafle, A., Devkota, H. P., & Bhattarai, A. (2023). Characterization of Saponins from the Leaves and Stem Bark of Jatropha curcas L. for Surface-Active Properties. *Heliyon*, *9*(5), e15807. https://doi.org/10.1016/j.heliyon.2023.e15807

Rosidah, I., Bahua, H., Mufidah, R., & Pongtuluran, O. B. (2015). Pengaruh Kondisi Proses Ekstraksi Batang Brotowali (Tinospora crispa (L) Hook.f & Thomson) Terhadap Aktivitas Hambatan Enzim Alfa Glukosidase. *Media Penelitian dan Pengembangan Kesehatan*, *25*(4). https://doi.org/10.22435/mpk.v25i4.4586.203-210

RTI laboratories. (2016). *Environmental, Chemical & Materials Testing*. Retrieved from https://rtilab.com/techniques/ftir-analysis/

Sanpinit, S., Wetchakul, P., Chonsut, P., Prommee, N., Punsawad, C., Han, J., & Net-anong, S. (2023). Evaluation of Chemical Compositions and the Antioxidant and Cytotoxic Properties of the Aqueous Extract of Tri-Yannarose Recipe (Areca catechu, Azadirachta indica, and Tinospora crispa). *Antioxidants*, *12*(7), 1428. https://doi.org/10.3390/antiox12071428

Shah, Z. M., Hasan, M. K., Kadir, K. K., Arshad, M. S., & Amom, Z. (2021). The Effects of Extraction Conditions on Extraction Yield and Syringin Content in Producing Standardized Tinospora crispa Aqueous Extract with High Antioxidant Activity. *Asian Food Science Journal*, 106-120. https://doi.org/10.9734/afsj/2021/v20i430291

Silverstein, R. M., Webster, F. X., Kiemle, D. J., & Bryce, D. L. (2014). *Spectrometric Identification of Organic Compounds*. John Wiley & Sons.

Song, F., Gan, R., Zhang, Y., Xiao, Q., Kuang, L., & Li, H. (2010). Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants. *International Journal of Molecular Sciences*, *11*(6), 2362-2372. https://doi.org/10.3390/ijms11062362

Sulastri, L., Syamsudin, S., & Simanjuntak, P. (2018). Karakterisasi Senyawa Penghambat Polimerisasi Hemedari Batang Brotowali (Tinospora crispa(L.). *Biopropal Industri*, *9*(2), 79-86. https://doi.org/10.36974/jbi.v9i2.3778

Suparno, S., Lestari, E. S. A., & Grace, D. (2024). Antibacterial Activity of Bajakah Kalalawit Phenolic Against Staphylococcus aureus and Possible Use of Phenolic Nanoparticles. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-70799-4

Susanti, D., Putra, A. D., Safrina, D., Wijaya, N. R., Adi, M. B., Mujahid, R., Rukmana, R. M., Subositi, D., Haryanti, S., Siswanto, U., & Widiyastuti, Y. (2024). Antimalarial Medicinal Plants Used by Traditional Healers in Bengkulu Province of Indonesia. *BIOTROPIA*, *31*(3), 402-421. https://doi.org/10.11598/btb.2024.31.3.2318

Tully, D. (2024). 15.7 Spectroscopy of Aromatic Compounds. In *Organic Chemistry: A Tenth Edition – OpenStax Adaptation 1*. Retrieved from https://ncstate.pressbooks.pub/organicchem/chapter/spectroscopy-of-aromatic-compounds/

Warsinah, W., Baroroh, H. N., & Harwoko, H. (2020). Phytochemical Analysis and Antioxidant Activity of Brotowali (Tinospora crispa L. Mier) Stem. *Molekul*, *15*(2), 73. https://doi.org/10.20884/1.jm.2020.15.2.533

Zhang, Q., Lin, L., & Ye, W. (2018). Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. *Chinese Medicine*, *13*(1). https://doi.org/10.1186/s13020-018-0177-x

Zugazua-Ganado, M., Bordagaray, A., Ezenarro, J., Garcia-Arrona, R., Ostra, M., & Vidal, M. (2024). Adaptation of the Folin-Ciocalteu and Fast Blue BB Spectrophotometric Methods to Digital Image Analysis for the Determination of Total Phenolic Content: Reduction of Reaction Time, Interferences and Sample Analysis. *LWT*, *193*, 115756. https://doi.org/10.1016/j.lwt.2024.115756