ASEAN Journal of Chemical Engineering 2022, Vol. 22, No. 2, 274 – 283

Comparison of Maseration and Sonication Method on Flavonoid Extraction from Mango Leaves: Effect of Solvent Ratio

***Alifiana Permata Sari*[1]**
***Nur Layli Amanah*[2]**
***Awalia Wardatullathifa*[1]**
***Agung Nugroho**[,1]**
[1]*Department of Chemical Engineering, Universitas Pertamina*
Jalan Teuku Nyak Arief, Simprug, Kebayoran Lama, Jakarta 12220, Indonesia
[2]*Department of Materials Science and Engineering, National Taiwan University of Science and Technology, No.43, Sec. 4, Keelung Road, Taipei 10607, Taiwan*
**e-mail:* agung.n@universitaspertamina.ac.id

Submitted 19 April 2022 *Revised* 19 October 2022 *Accepted* 3 November 2022

Abstract. Mango leaf extract has proven to contain flavonoids that serve as antioxidants. In this study, a comparison between traditional maceration and sonication on flavonoid extraction from mango leaf was investigated. The various ratios of ethanol and acetone were utilized as solvents (1:5, 1:10, and 1:15). The sonication process, which uses an ultrasonic cleaning bath set at 40 ^{o}C, takes 30 minutes as contrasted to the maceration procedure of 36 hours treatment at room temperature. The flavonoid test using aluminum (III) chloride ($AlCl_3$) colorimetric technique shows that acetone provides greater solvent power than ethanol. According to this study, the optimal ratios for the maceration and sonication procedures are 1:10 and 1:15, respectively. The maceration process resulted in the optimum extract of 0.186 mgQE/g dry leaves. Meanwhile, using a 1:15 acetone solvent ratio and the sonication method, the highest concentration of flavonoid components was discovered, reaching 0.143 mgQE/g dry material with 54 times shorter time.

Keywords: Extraction, Flavonoid, Mango Leaves, Optimum Ratio, Solvent

INTRODUCTION

Free radicals harm the human body, causing damage to DNA, RNA, proteins, carbohydrates, lipids, and blood vessels, which can lead to degenerative disorders (Pisoschi et al., 2021). Thus, antioxidant chemicals are needed and must be balanced in the human body to neutralize free radicals due to their stable nature, which may donate electrons, thereby lowering the capacity of free radicals to harm cells (Pisoschi et al., 2021).

Mangiferin, phenolics, flavonoids, and benzophenones, which come from various plant and fruit kinds, are reported to have antioxidant and free radical scavenging properties. Antioxidant sources can be derived from leaves, flowers, roots, bark, seeds, fruit peels, and wood (Dorta et al., 2012, Zhang et al., 2011).

Currently, the processing of bioactive,

DOI: 10.22146/ajche.74204

primarily phenolic compounds, partially flavonoids, is gaining ground in the pharmaceutical and cosmetic sectors. Flavonoids inhibit several enzymes while stimulating those with antioxidant activity, including cyclo-oxygenase, lipooxygenase, NADPH-oxidase, xanthine-oxidase, and phospholipase (Al-Khayri et al., 2022). In addition to affecting capillary permeability and acting as exogenous antioxidants, flavonoids have antiviral, anti-inflammatory, and anticancer properties. They may also bind to and deactivate (such as catalase and superoxide dismutase). Flavonoids thus aggressively inhibit the generation and propagation of free radicals (Calado et al., 2015, Tohidi et al., 2017).

Antioxidants can be employed as non-consumption substances in addition to being present in food. Essential oils are now valued secondary metabolites in the pharmaceutical industry due to their ability to reduce oxidative damage caused by highly reactive chemicals like reactive oxygen species (ROS). These substances are essential in preventing the chronic disease from occurring by measures taken externally to the body (Gharibi et al., 2015).

Flavonoids have been found in mango fruit trees in previous studies. In 2012, Dorta.m et al. isolated flavonoids with an estimated flavonoid yield of 17–50% from mango peel and seed. Overall, every component of the mango fruit has the potential to contain flavonoids. Therefore, this study aimed to determine the number of flavonoids in mango leaves (Dorta et al., 2012).

Flavonoids were extracted using various techniques, including sonication, reflux, maceration, and soxhletation (Zhang et al., 2018). The kind of solvent used is another factor that impacts the extraction process (Sasadara and Wirawan, 2021). The type of solvent employed depends on the amount of plant material extracted and its flavonoid concentration. In previous research, Minh Phuoc Nguyen, in early 2020, completed his work on extracting flavonoids and phenolics compounds, which stated his success in the influence of material retention of antioxidant stability using ethanol solvent (Nguyen, 2020). Based on these reasons, the authors are also interested in comparing the ethanol solvent used with the acetone solvent for higher results. This research aims to compare the results of mango leaf extraction using two distinct methods: maceration and sonication. In addition, different ratios of samples and solvents were used to analyze their effect on flavonoid levels in mango leaves.

EXPERIMENTAL METHOD

The details of the materials in the research series can be seen as follows: ethanol solvent (C_2H_5OH) gradient grade (CAS: 64175) Sigma Aldrich, acetone 99.5% (C_3H_6O) (CAS: 179124) Sigma Aldrich, quercetin ($C_{15}H_{10}O_7$), (≥95% HPLC, solid) Sigma Aldrich (CAS: 117395), magnesium pure 99% (CAS: 7439954) Sigma Aldrich, aluminum chloride $AlCl_3$ reagent plus 99% (CAS: 7446700) Sigma Aldrich, and hydrochloric acid 37% (CAS: 7647010) Sigma Aldrich.

The sample preparation starts with mango leaves as the main ingredient was washed until clean and dried before the experiment. Furthermore, the size of the mango leaves was reduced and put in an oven at 100 °C for 24 hours to reduce the water content. So that the mango leaves were dry and ready to be ground and the size was equalized to 40 mesh with a sieve. The whole experimental technique is depicted in Fig. 1.

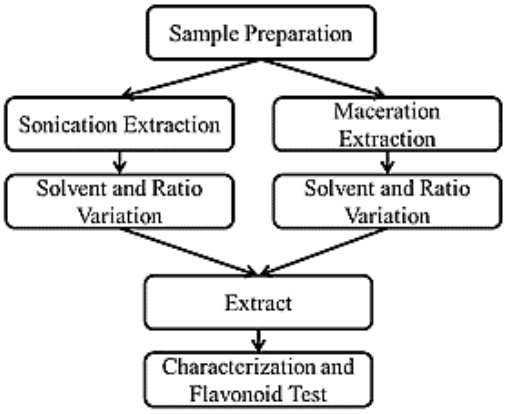

Fig. 1: Experimental method flow chart

Extraction Process

Sonication and maceration were the two procedures used in the extraction process. Variations in the solvent and the ratio of components and solvents were used to carry out the extraction procedure. Ethanol and acetone were utilized as solvents, with changes in the ratios of components and solvents shown in Table 1.

Table 1. Variations in the ratio of the number of sample and solvent ratios

Variation Number	The ratio of sample (g): solvent (mL)
1	1:5
2	1:10
3	1:15

Sonication Extraction

Extraction was performed using a stainless-steel SUS 304/316 ultrasonic cleaning bath Ovan, 40kHz and 28kHz, thermostated ultrasound bath, and temperature controlled by the microprocessor, as the scheme shown in Figure 2. A ten-gram sample was placed in a beaker, then ethanol and acetone were added in equal amounts. The extraction procedure lasted 30 minutes at a stable temperature of 40 °C. The sample was filtered, the pulp was separated, and the extract was kept in a dark area.

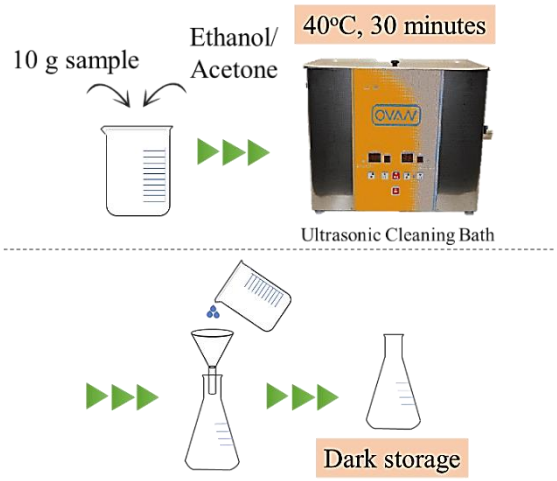

Fig. 2: Steps of sonication extraction diagram

Maceration Extraction

Ten grams of the sample were placed in a glass beaker, and ethanol and acetone were added to the ratio. The glass beaker was carefully sealed, covered in aluminum foil, and kept in the refrigerator for 36 hours at ambient temperature (Safdar et al., 2017). At the end of fermentation, maceration occurs in the presence of a high ethanol concentration, enhancing flavonoid solubility (Morata et al., 2019). After completing the filtration, the sample was stirred every 8 hours. Filtrates that had been reconstituted had been adequately diluted with the solvent to the necessary concentrations (Jovanović et al., 2017). Maintain the filtrate in a dim region.

Characterization and Flavonoid Test

Flavonoid characterization was carried out using a UV-VIS spectrophotometer (Masturi et al., 2019). The standard curve was made using the standard solution's absorbance data, and the flavonoid content was obtained from linear regression of the quercetin standard curve (Masturi et al.,

2020). Quercetin is categorized as a flavonol group with a different phenolic group (OH) and glycan sugar (Martín and Ramos, 2021).

A qualitative test of flavonoids was carried out on 2 ml of extract and 1 ml of 96% ethanol in one tube. Add 0.1 grams of magnesium and ten drops of HCl. The color observed changed from red to purple, and the extract contained flavonoid compounds (Shen et al., 2018). Also, using $AlCl_3$ (Aluminum Chloride), the color will change to become yellow (Shraim et al., 2021).

The quantitative test could be determined through the quercetin curve standard). The value of y is the absorbance, and x is the concentration of the quercetin solution. The concentration of flavonoids in the extract can be determined by measuring the absorbance of the extract and compared with a standard solution of quercetin (Phuyal et al., 2020). The absorbance value of the extract solution obtained was substituted for the value to obtain the x value or flavonoid concentration in the extract (ppm) (Cornard and Merlin, 2002, Kalita et al., 2013). The flavonoid concentration was then converted into mg QE (quercetin equivalent) per g extract using Eq. (1).

$$Percent\ Flavonoid\ (\%) = \frac{C \times V \times f}{m} \quad (1)$$

By multiplying the concentration (C) of the yield extract by the volume of solvent employed (V) and the dilution factor (f), which is divided by the mass of the dry sample under test, one may calculate the flavonoid content (%) (Maungchanburee et al., 2020).

RESULTS AND DISCUSSION

Extraction Flavonoid

Extraction was carried out by the two methods mentioned above. The ratio of ingredients and solvents is the main variety used to determine the optimal amount of flavonoid extract. Due to the difference in concentration between the cell wall and the interior of the cell, the solvent will enter the cell wall during the maceration process and dissolve the active ingredient (Chemat et al., 2017). The greater solvent concentration within the cell will progressively be replaced by a lower solvent concentration inside the cell wall, a process known as diffusion until it reaches a saturation point or a balanced concentration (Tanaka et al., 2017). This process was carried out without light for 36 hours and stirred every 8 hours to avoid the degradation process of flavonoid compounds (Chaves et al., 2020). The degradation of flavonoid compounds is not only sensitive to temperature but also sensitive to sunlight. The mechanism of degradation of flavonoid compounds of the flavonol group can be seen in Figure 3.

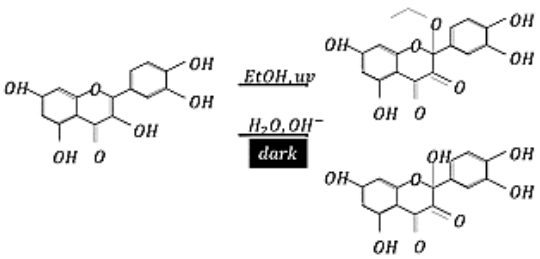

Fig. 3: Degradation of flavonoid compounds (Quercetin) (Dall'Acqua et al., 2012)

Figure 3 shows the degradation reaction of the quercetin compound in ethanol solvent, which will be formed entirely to obtain flavonol derivatives of the OH group and glycan sugar as a hydrolysis process. When it is stored in a dark place, on the other hand, if it is contaminated with UV rays, the quercetin derivatives will fail to be obtained.

Meanwhile, sonication extraction is carried out by utilizing ultrasonic waves emitted by an ultrasonic cleaning bath to produce a cavitation process around the

extracted sample which results in particle movement resulting in a heating process and the release of flavonoid compounds from the sample (Singla and Sit, 2021). The movement of particles due to ultrasonic waves breaks down cell walls, accelerating the mass transfer process to maximize the diffusion of the desired compound from the sample (Syahir et al., 2020).

Characterization Flavonoid Test

The levels of flavonoids contained in the extract were determined by adding $AlCl_3$ Colorimetric (Shraim et al., 2021). A color change reaction could identify the formation of complex compounds to yellow. In principle, a complex compound will be formed between $AlCl_3$ with a ketone group on the C-4 atom and a hydroxy group on the C-3 or C-5 atom, neighboring the flavone and flavonoid groups (Syahir et al., 2020). The presence of a complex compound causes a shift in wavelength to the visible region. In addition, potassium acetate is also added to maintain the wavelength in the visible region (Hamidu et al., 2018).

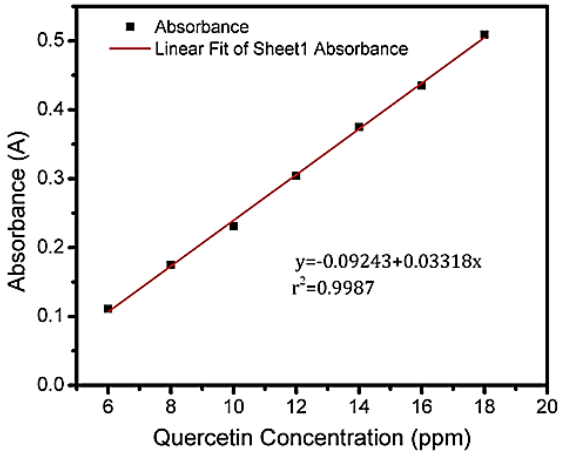

Fig. 4: Linear regression equation graph of flavonoid extraction

From the curve, the linear regression equation y=-0.09243+003318x is obtained (Figure 4). The concentration number may be calculated from this equation by the existence of the absorbance number using the previously indicated quantitative test. The data are shown in Table 2 as % flavonoids and extract concentration in milligram Quercetin Extract per gram of dry sample (mgQE/g).

Table 2. The result percent number of flavonoids

Batch	Ratio	Maceration				Sonication			
		Ethanol		Acetone		Ethanol		Acetone	
		(%)	mgQE/g	(%)	mgQE/g	(%)	mgQE/g	(%)	mgQE/g
I	1:05	0.372	0.037	0.223	0.022	0.275	0.028	0.423	0.042
	1:10	0.278	0.028	0.664	0.066	0.278	0.028	0.692	0.069
	1:15	0.225	0.023	0.440	0.044	0.388	0.039	0.715	0.072
II	1:05	0.744	0.074	1.328	0.133	0.549	0.055	0.846	0.085
	1:10	0.451	0.045	1.861	0.186	0.556	0.056	1.385	0.139
	1:15	0.446	0.045	0.880	0.088	0.776	0.078	1.431	0.143

The results of the flavonoid test showed that the extract was positive for flavonoids, which was marked by a change in color to red (Chen et al., 2021). Flavonoids are polar compounds with more than one hydroxyl group, so flavonoids are soluble in polar solvents such as ethanol and acetone (Hapsari et al., 2022). Polar solvents function to free flavonoids from their salt form (Figure 5). Concentrated hydrochloric acid protonates flavonoids to form flavonoid salts (Chaves et al., 2020). The addition of magnesium makes the color of the solution red or orange (Cruz et al., 2022). The color change is due to the formation of flavylium salts due to the benzopyran core in the flavonoid structure being reduced by magnesium and concentrated hydrochloric acid (Cruz et al., 2022).

The extraction using two methods shows that acetone solvent can produce higher total flavonoid content than ethanol with the same concentration. The naked eye can also observe that the extract with acetone solvent has a more concentrated color than ethanol. Acetone and ethanol have different levels of polarity, which are described by different dielectric constant values (Sasadara and Wirawan, 2021). The dielectric constant is the repulsive force between two electrically charged particles in a molecule. The dielectric constants of acetone and ethanol are 21 and 24, respectively (Sasadara and Wirawan, 2021).

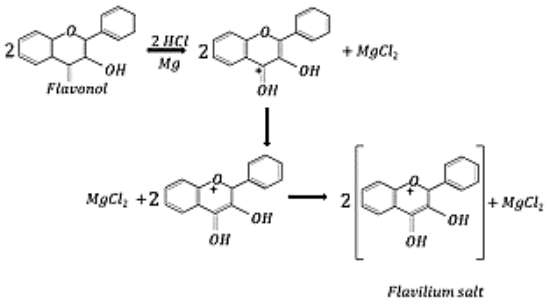

Fig. 5: Reaction of flavonoids with magnesium and hydrochloric acid, flavonoid qualitative test

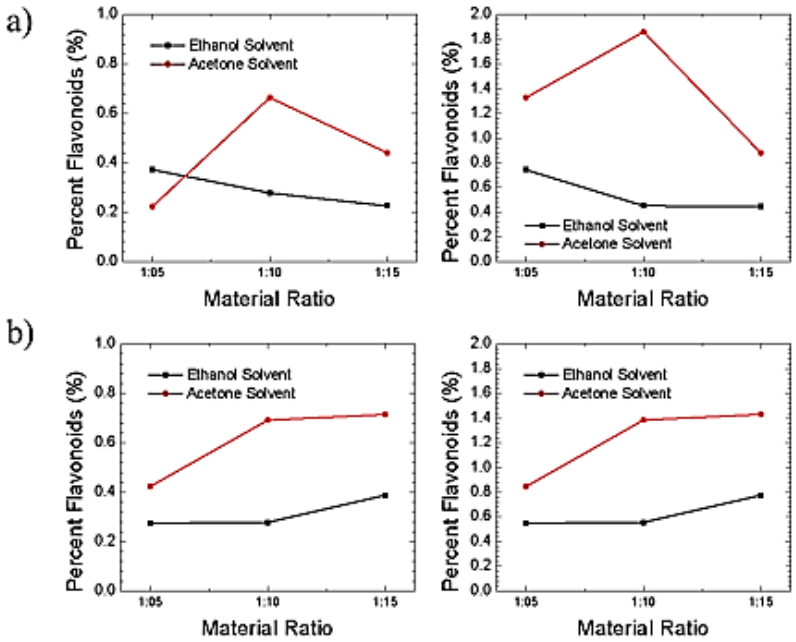

Fig. 6: Result of total flavonoid levels in mango leaf extract with sonication (a) and maceration methods (b)

Figure 6 shows the ratio of ingredients and solvents to total flavonoid levels in the mango leaf extract with maceration and sonication methods. Acetone solvent was able to produce higher total flavonoid content. The maximum flavonoid concentration was produced utilizing the maceration process at a solvent material ratio of 1:10, resulting in 0.451% or 0.0451 mgQE/ dry leaves for ethanol solvent and 1.861 % or 0.1861 mgQE/g dry leaves for acetone solvent. Meanwhile, in the sonication process, the highest concentration was obtained at a ratio of 1:15 for both solvents, with levels reaching 0.776% or 0.0776 mgQE/g dry leaves for acetone and 1.431% or 0.1431 mgQE/g dry leaves for ethanol.

The total flavonoid content generated between the material ratio in the sonication extraction with acetone solvent was detected: solvents 1:5 and 1:10 have a significant difference. This is probably because the solvent has almost reached the saturation point or the maximum limit of the solvent's ability to extract the active substance in the sample. In addition, another cause is that the amount of substance present in the sample has been exhausted or has been completely extracted.

CONCLUSIONS

Based on the research, it can be stated that the sonication extraction method is the best. It may generate a similar total flavonoid content as the maceration approach while taking 54 times less time. In addition, the polarity value of the solvent close to the flavonoid compound will produce a higher total flavonoid content, but after reaching the saturation point, the extract of the flavonoid compound produced tends to be constant. Using the sonication process and acetone as the solvent, the researchers achieved optimum flavonoid levels of 0.1431mgQE/g (1.431%) dry leaf with a 1:15 ratio of material and solvent.

ACKNOWLEDGEMENT

The authors thank all the Chemical Engineering Laboratory staff of Universitas Pertamina for their help in conducting research at the Chemical Engineering Laboratory.

REFERENCES

Al-Khayri, J.M., Sahana, G.R., Nagella, P., Joseph, B. V, Alessa, F.M., and Al-Mssallem, M.Q., 2022. "Flavonoids as potential anti-inflammatory molecules: A review." *Molecules*, 27 (9), 2901 https://doi.org/10.3390/molecules27092901

Calado, J.C.P., Albertão, P.A., de Oliveira, E.A., Letra, M.H.S., Sawaya, A.C.H.F., and Marcucci, M.C., 2015. "Flavonoid contents and antioxidant activity in fruit, vegetables and other types of food." *Agric. Sci., 6*, 426.

Chaves, J.O., de Souza, M.C., da Silva, L.C., Lachos-Perez, D., Torres-Mayanga, P.C., Machado, A.P. da F., Forster-Carneiro, T., Vázquez-Espinosa, M., González-de-Peredo, A.V., Barbero, G.F., and Rostagno, M.A., 2020. "Extraction of flavonoids from natural sources using modern techniques." *Front. Chem., 8*. 507887. https://doi.org/10.3389/fchem.2020.507887

Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., and Abert-Vian, M., 2017. "Ultrasound assisted extraction of food and natural products. Mechanisms, techniques,

combinations, protocols and applications. A review." *Ultrason. Sonochem., 34*, 540–560. https://doi.org/https://doi.org/10.1016/j.ultsonch.2016.06.035

Chen, Q., Wang, X., Yuan, X., Shi, J., Zhang, C., Yan, N., and Jing, C., 2021. "Comparison of phenolic and flavonoid compound profiles and antioxidant and α-glucosidase inhibition properties of cultivated soybean (glycine max) and wild soybean (glycine soja)." *Plants, 10(4), 813*. https://doi.org/10.3390/plants10040813

Cornard, J.P., and Merlin, J.C., 2002. "Spectroscopic and structural study of complexes of quercetin with Al(III)." *J. Inorg. Biochem., 92*, 19–27. https://doi.org/https://doi.org/10.1016/S0162-0134(02)00469-5

Cruz, L., Basílio, N., Mateus, N., de Freitas, V., and Pina, F., 2022. "Natural and synthetic flavylium-based dyes: the chemistry behind the color." *Chem. Rev., 122*, 1416–1481.https://doi.org/10.1021/acs.chemrev.1c00399

Dall'Acqua, S., Miolo, G., Innocenti, G., and Caffieri, S., 2012. "The photodegradation of quercetin: Relation to oxidation." *Molecules*, 17(8), 8898-8907. https://doi.org/10.3390/molecules17088898

Dorta, E., Lobo, M.G., and Gonzalez, M., 2012. "Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties." *J. Food Sci. 77*, C80-8. https://doi.org/10.1111/j.1750-3841.2011.02477.x

Gharibi, S., Tabatabaei, B.E.S., and Saeidi, G., 2015. "Comparison of essential oil composition, flavonoid content and antioxidant activity in eight achillea species." *J. Essent. Oil Bear. Plants, 18*, 1382–1394. https://doi.org/10.1080/0972060X.2014.981600

Hamidu, L., Ahmad, A.R., and Najib, A., 2018. "Qualitative and quantitative test of total flavonoid buni fruit (*Antidesma bunius (L.) Spreng*) with UV-Vis spectrophotometry method." *Pharmacogn. J. 10* (1), 60-63

Hapsari, S., Yohed, I., Kristianita, R.A., Jadid, N., Aparamarta, H.W., and Gunawan, S., 2022. "Phenolic and flavonoid compounds extraction from Calophyllum inophyllum leaves." *Arab. J. Chem. 15 (3)*, 103666. https://doi.org/https://doi.org/10.1016/j.arabjc.2021.103666

Jovanović, A.A., Đorđević, V.B., Zdunić, G.M., Pljevljakušić, D.S., Šavikin, K.P., Gođevac, D.M., and Bugarski, B.M., 2017. "Optimization of the extraction process of polyphenols from *Thymus serpyllum L.* herb using maceration, heat- and ultrasound-assisted techniques." *Sep. Purif. Technol., 179*, 369–380. https://doi.org/https://doi.org/10.1016/j.seppur.2017.01.055

Kalita, P., Tapan, B., Pal, T., and Kalita, R., 2013. "Estimation of total flavonoids content (TFC) and anti oxidant activities of methanolic whole plant extract of *Biophytum sensitivum Linn*." *J. Drug Deliv. Ther., 3(4), 33-37*. https://doi.org/10.22270/jddt.v3i4.546

Martín, M.Á., and Ramos, S., 2021. "Dietary flavonoids and insulin signaling in diabetes and obesity." *Cells, 10 (6), 1474*. https://doi.org/10.3390/cells10061474

Masturi, Alighiri, D., Edie, S.S., Drastisianti, A., Khasanah, U., Tanti, K.A., Susilawati, Maghfiroh, R.Z., Kirana, K.G.C., and Choirunnisa, F., 2020. "Identification of

flavonoid compounds and total flavonoid content from biowaste of local durian shell (*Durio zibethinus*)." *J. Phys. Conf. Ser., 1567*, 42084. https://doi.org/10.1088/1742-6596/1567/4/042084

Masturi, Alighiri, D., Nuzulina, K., Rodhiyah, M., and Drastisianti, A., 2019. "Optimization of condition extraction in quantification of total flavonoid content in the seeds of the Arummanis (Mangifera indica L.) mango from Indonesia." *J. Phys. Conf. Ser., 1321*, 22041. https://doi.org/10.1088/1742-6596/1321/2/022041

Maungchanburee, S., Phongseeput, S., Thongsri, O., Maijuy, M., and Chaithada, P., 2020. "Study of antioxidant activities, total phenolic content and total flavonoid content of the extracts of *Monochoria vaginalis* and *Cissus repens Lamk*. using different solvents." *J. Pharm. Sci. Res,. 12*, 356–359.

Morata, A., González, C., Tesfaye, W., Loira, I., and Suárez-Lepe, J.A., 2019. "Chapter 3 - Maceration and Fermentation: New Technologies to Increase Extraction," in: Morata, A.B.T.-R.W.T. (Ed.), . Academic Press, pp. 35–49. https://doi.org/https://doi.org/10.1016/B978-0-12-814399-5.00003-7

Nguyen, M.P., 2020. "Efficacy of some variables of extraction to the total phenolic and flavonoid content in young mango (*Mangifera indica L.*) leaf." *Ann. PHYTOMEDICINE-AN Int. J., 9*, 113–115. https://doi.org/10.21276/ap.2020.9.1.13

Phuyal, N., Jha, P.K., Raturi, P.P., and Rajbhandary, S., 2020. "Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC." *Sci. World J., 2020*, 8780704. https://doi.org/10.1155/2020/8780704

Pisoschi, A.M., Pop, A., Iordache, F., Stanca, L., Predoi, G., and Serban, A.I., 2021. "Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status." *Eur. J. Med. Chem., 209*, 112891. https://doi.org/https://doi.org/10.1016/j.ejmech.2020.112891

Safdar, M.N., Kausar, T., and Nadeem, M., 2017. "Comparison of ultrasound and maceration techniques for the extraction of polyphenols from the mango peel." *J. Food Process. Preserv., 41*, e13028. https://doi.org/https://doi.org/10.1111/jfpp.13028

Sasadara, M.M. V, and Wirawan, I.G.P., 2021. "Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of Bulung Sangu (*Gracilaria sp.*) Seaweed." *IOP Conf. Ser. Earth Environ. Sci., 712*, 12005. https://doi.org/10.1088/1755-1315/712/1/012005

Shen, J., Zou, Z., Zhang, X., Zhou, L., Wang, Y., Fang, W., and Zhu, X., 2018. "Metabolic analyses reveal different mechanisms of leaf color change in two purple-leaf tea plant (*Camellia sinensis L.*) cultivars." *Hortic. Res., 5*, 7. https://doi.org/10.1038/s41438-017-0010-1

Shraim, A.M., Ahmed, T.A., Rahman, M.M., and Hijji, Y.M., 2021. "Determination of total flavonoid content by aluminum chloride assay: A critical evaluation." *LWT, 150*, 111932. https://doi.org/10.1016/j.lwt.2021.111932

Singla, M., and Sit, N., 2021. "Application of ultrasound in combination with other technologies in food processing: A review." *Ultrason. Sonochem., 73*, 105506. https://doi.org/https://doi.org/10.1016/j.ultsonch.2021.105506

Syahir, A., Sulaiman, S., Mel, M., Othman, M., and Zubaidah Sulaiman, S., 2020. "An overview: Analysis of ultrasonic-assisted extraction's parameters and its process." *IOP Conf. Ser. Mater. Sci. Eng.*, *778*, 12165. https://doi.org/10.1088/1757-899x/778/1/012165

Tanaka, S., Seki, M., Miki, T., Umemura, K., and Kanayama, K., 2017. "Solute diffusion into cell walls in solution-impregnated wood under conditioning process III: effect of relative humidity schedule on solute diffusion into shrinking cell walls." *J. Wood Sci.*, *63*, 263–270. https://doi.org/10.1007/s10086-017-1613-8

Tohidi, B., Rahimmalek, M., and Arzani, A., 2017. "Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran." *Food Chem.*, *220*, 153–161. https://doi.org/https://doi.org/10.1016/j.foodchem.2016.09.203

Zhang, L., Ravipati, A.S., Koyyalamudi, S.R., Jeong, S.C., Reddy, N., Smith, P.T., Bartlett, J., Shanmugam, K., Münch, G., and Wu, M.J., 2011. "Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds." *J. Agric. Food Chem.*, *59*, 12361–12367. https://doi.org/10.1021/jf203146e

Zhang, Q.-W., Lin, L.-G., and Ye, W.-C., 2018. "Techniques for extraction and isolation of natural products: a comprehensive review." *Chin. Med.*, *13*, 20. https://doi.org/10.1186/s13020-018-0177-x