Jurnal Rekayasa Kimia dan Lingkungan
(Journal of Chemical Engineering and Environment)
Volume 17, Number 2, Page 113 – 120, July - December, 2022
ISSN 1412-5064, e-ISSN 2356-1661
https://doi.org/10.23955/rkl.v17i2.26915

Effect of Solid to Solvent Ratio and Extraction Time on Yield, Phytochemical Content and Physicochemical of Amla (*Phyllanthus emblica*) Seed Oil Extracted using Hexane

Nadia Putri Mauliza[1], Nanda Suriaini[2], Cut Meurah Rosnelly[2], Satriana[3], Muhammad Dani Supardan[2*]

[1] Graduate School of Chemical Engineering, Universitas Syiah Kuala, Jl. Tengku Syech Abdurrauf No. 7, Darussalam-Banda Aceh, Indonesia 2311.
[2] Chemical Engineering Department, Faculty of Engineering, Universitas Syiah Kuala, Jl. Tengku Syech Abdur Rauf No. 7, Darussalam-Banda Aceh, Indonesia 23111
[3] Department of Agricultural Product Technology, Universitas Syiah Kuala, Jalan Tgk. Hasan Krueng Kalee No.3, Darussalam, Banda Aceh 23111, Indonesia
*E-mail: m.dani.supardan@unsyiah.ac.id

Article History
Received: 14 July 2022; Received in Revision: 8 August 2022; Accepted: 9 August 2022

Abstract

Amla (*Phyllanthus emblica*) seeds have only been disposed of as waste and have not been utilized optimally. Utilizing the seeds to produce oil has been regarded as a promising approach for reducing solid wastes and producing value-added products. This study evaluated the effect of solid to solvent ratio and extraction time on yield, phytochemical content and physicochemical of oil extracted from amla seed using hexane as a solvent. The amla seed oil was extracted through the solid-liquid process at different solid to solvent ratios (1:20, 1:25 and 1:30 g/mL) and extraction times (30, 60, 90 and 120 minutes). The highest yield found was 10.97% at a solid to solvent ratio of 1:30 (g/mL) and an extraction time of 120 minutes. The highest concentrations of phenolic compounds and flavonoids were 66.12 mg GAE/g and 95.70 mg QE/g respectively, obtained at a solid to solvent ratio of 1:25 (g/mL) and extraction time of 120 minutes. Physicochemical analysis (pH, density, acid number, free fatty acid, and peroxide number) showed that the quality of extracted oil was better than what the previous study reported. In the amla seed oil, the content of unsaturated fatty acids was higher than saturated fatty acids with linoleate as the main component. These results clarified that solid-liquid extraction method can be used for extracting amla seed oil and also indicated that amla seed oil can be used as an ingredient in health-related industry products.

Keywords: Amla seed oil, fatty acid, functional groups, phytochemical compounds, solvent extraction

1. Introduction

Amla fruit or Indian gooseberry (*Phyllanthus emblica*) is a fruit that comes from India. Amla fruit is widespread in tropical and subtropical countries, which are commonly found in Asia (Sriwatcharakul, 2020). Amla plant height is about 8-18 m, light green and the leaves of this tree are pinnate. The fruit is round (15–20 mm x 18–25 mm) with six starfruit ribs. Amla fruit consists of a smooth outer skin that has a yellow-green color, where the inside of the fruit contains a seed coat and dark brown amla seeds consist of 4-6 seeds in it (Gantait et al., 2021). Amla fruit is not suitable for direct consumption because of its sour taste. Therefore, amla fruit is consumed mainly in processed forms such as sweets, candies, syrups, beverages, pickles, jam, sauces and powder (Alkandari et al., 2019).

Many people only use the flesh of the amla fruit, while the seeds are discarded as organic waste. Amla seeds can be used by taking the oil. It has high nutritional value as antioxidants with free radical scavenging properties (damaged cells) (Dinesh et al., 2016). Amla seeds have numerous nutritional contents of ascorbic acid, magnesium, calcium, potassium, and amino acids which have high nutritional value for health. Amla seeds have antibacterial and antifungal benefits, there is no toxicity or allergy associated with using the seeds as food or medicine, so they have the potential to be used as a source of vegetable oil (Kumar et al., 2015).

Dinesh et al. (2016) previously reported the presence of phytochemical compounds in the form of alkaloids, tannins, phytosterols, and phenols in amla seed extract produced by solid-liquid extraction. They used methanol as a solvent. Furthermore, Keithellakpam and Patel (2018) studied the physicochemical properties of amla seed oil and the effect of storage conditions on the shelf life of the oil. However, research reports published on amla seed oil extraction are still limited. Therefore, this study was conducted to provide additional information about reducing solid wastes and producing value-added products by utilizing the amla seeds to produce oil.

Amla seed oil is used to develop high-impact products such as traditional medicines and cosmetics. Amla seed oil is rich in health benefits and treatment of several diseases such as an anti-anemic agent, enhances fertility, remedy for menstrual disorders, effective in vagina irritations, diabetes disease, remedy for eye disorders, to stop nausea, vomiting and bleeding of the nose and diarrhea disorders (Pria and Islam, 2019). The potential and availability of vegetable oil source (such as amla seed) in our country are extensive, but it has not been able to be balanced with the ability to process it well. This is due to the low mastery of technology in the amla seed oil industry, which hampers its potential to become a source of regional income, especially in Aceh. Knowing these conditions, efforts are needed to develop a good production technology for production a quality amla seed oil more efficiently.

Extraction is separating the desired natural substance from a material/plant. Extraction includes several methods, one of which is solvent extraction or solid-liquid extraction method. This method is the most commonly used method for materials with low oil content (Zhang et al., 2018). Amla seed oil can be obtained by solid-liquid extraction method. The objective of this present study is to evaluate the effect of solid to solvent ratio and extraction time on yield, phytochemical content and physicochemical of oil extracted from amla seed using hexane as a solvent.

2. Methodology

2.1. Materials

Amla seeds were obtained as part of amla fruit obtained from Blang Bintang, Aceh Besar District. Hexane 96% (analytical grade) from KGaA, Darmstadt, Germany was used as a solvent. The solvent was used without further purification.

2.2. Amla Seed Oil Production Process

The amla seed oil production process is presented in Figure 1. The amla seeds was separated from the flesh and shell and then dried using an oven to reduce the water content in the seeds. The drying process was carried out at 105°C for three days until the amla seeds obtained a constant moisture content. Then, the size of amla seeds was reduced using a blender to produce a fine powder. The seeds were sieved with a size of 60 mesh. The seeds with size larger than 60 mesh was used for further oil extraction process.

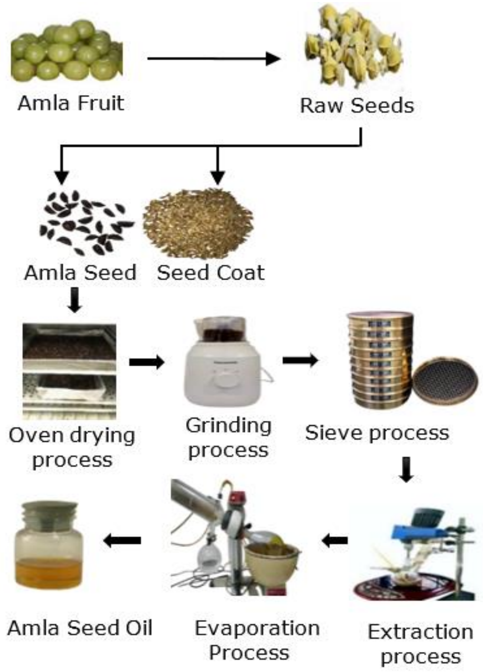


Figure 1. Amla seed oil production process.

The amla seed powder is weighted according to the ratio of solid to solvent (g:mL) of 1:20; 1:25 and 1:30. The mixture of amla seed powder and solvent then put into a 500 mL three-neck flask placed on an infrared electric stove for the extraction process. The extractor is equipped with a stirrer and speed of 400 rpm to maximize the extraction process and a condenser to prevent solvent evaporation. Furthermore, the extraction process was carried out for 30, 60, 90 and 120 minutes at 60°C. After the extraction process is complete, the mixture was filtered using filter paper (Whatman) to separate the amla seed powder that has been removed from the fat and miscella (a solution of amla seed oil dissolved in a solvent). The solvent was separated from the amla seed oil by evaporation using a rotary vacuum evaporator to obtain amla seed oil.

2.2. Amla Seed Oil Analysis

The analysis carried out includes a study in the form of yield. The physicochemical properties was analyzed in the form of peroxide number, acid number, free fatty acid (FFA) according to the SNI 01-3555-1998 procedure and density according to the ISO 6883: 2017. The functional group of the sample was analyzed using a Shimadzu Prestige 6400 Fourier Transform Infrared (FTIR) spectrometer. Phytochemical analysis to determine the phenol content was analyzed using a Folin-Ciocalteu reagent with a wavelength of 725 nm. Flavonoid compounds were calibrated against standard quercetin and expressed in mg QE/g (Quercetin) with a wavelength of 435 nm using a UV-Visible Spectrophotometer (Shimadzu 1700). The surface morphology of the sample was analyzed using Scanning Electron Microscope (SEM) TM 3000 Hitachi, which was presented with a magnification of 5000x. The fatty acid components in amla seed oil were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) Shimadzu QP2010 plus.

3. Results and Discussion

3.1. Effect of Solid to Solvent Ratio and Extraction Time on Oil Yield

Figure 2 shows that the extraction results obtained using 1:30 of solid to solvent ratio have a higher yield than 1:20 and 1:25 solid to solvent ratios. The more solvent used, the more oil can be extracted. This is because of the greater ratio of solvent to amla seeds, the difference in concentration between the solvent and constituents contained in the seeds is also higher so that the yield of the extraction will increase. Additionally, the increase in yield value is also caused by the increase in the contact area between amla seeds and the solvent. The more solvent, the greater the distribution of solvent to solids evenly so that the extracted amla seed oil will be enhanced(Ntalikwa, 2021).

Figure 2 also shows that the longer the extraction time, the higher the amla seed oil yield. It is because the contact time between the solvent and the material is longer so that more oil content is absorbed by the solvent which can obtain a large yield. However, after a certain time of the extraction process, the amount of oil extracted did not increase because the content of oil in the amla seed and solvent used was limited (Zamanhuri et al., 2021).

The highest yield of 10.97% was obtained at the solid to solvent ratio of 1:30 and extraction time of 120 minutes, while the lowest yield was 2.66% at the solid to solvent ratio of 1:20 and extraction time of 30 minutes.

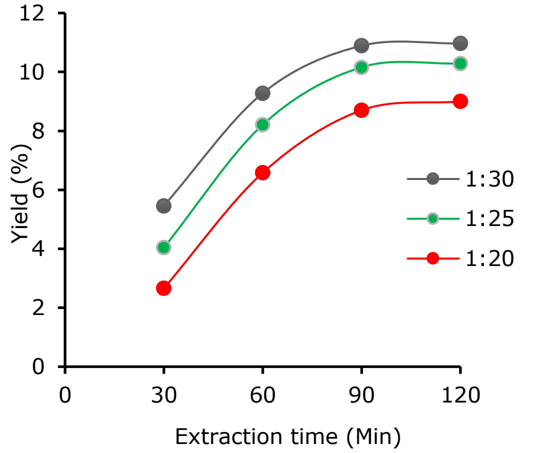
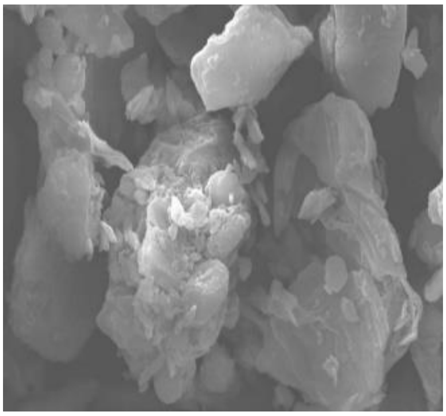
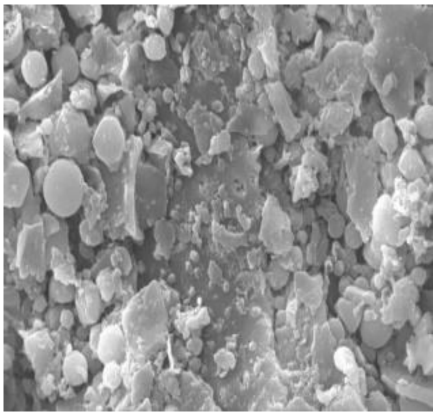


Figure 2. Effect of solid to solvent ratio and extraction time on amla seed oil yield.



(a) 30 minutes



(b) 120 minutes

Figure 3. Morphology of amla seed powder over time extraction.

Morphological structure analysis using SEM can also be used to see how extraction time affects yield. The results of this SEM analysis were carried out on the best samples based on yield analysis. The morphological structure analysis of amla seed powder at extraction times of 30 and 120 minutes can be seen in Figure 3. The figure shows that the 30 minutes extraction treatment still shows many intact surface structures (Figure 3a). Meanwhile, in the extraction time of 120 minutes, the cell wall was degraded and broke to form smaller particles (Figure 3b). Thus, these results confirmed that the extraction time can cause the cell walls to break into tiny particles, significantly facilitating oil extraction, resulting in higher extraction yields.

3.2. Effect of Solid to Solvent Ratio and Extraction Time on Phytochemical Content of Oil

Figure 4 and 5 show the increase in the ratio of solid to solvent from 1:20 to 1:25 has increased the phytochemical content of amla seed oil. The greater the amount of solvent used, the greater the amount of phenol and flavonoids extracted. It is because the more solvents, the more degradation of cell walls and membranes due to the pressure difference between the inside and outside of the cell working optimally. Then it caused more flavonoids and phenols in the cytoplasm are dissolved in the solvent (Predescu et al., 2016).

In addition, the greater the amount of solvent used, the greater the chance of contact between the bioactive components with the extraction solvent. It will cause more compounds come out from the extracted cell wall to make a greater amount of phytochemical extracted. However, the increase in the ratio of solids to solvents will not continue to increase phytochemical content extracted when equilibrium is reached (Elboughdiri, 2018). At a ratio solid to solvents of 1:30, the amount of phenol and flavonoid extracted decreased because it had reached the equilibrium point where the amount of phytochemical in the seed was limited (Sani et al., 2022).

The amount of flavonoid and phenol extracted increased at the extraction time from 30 minutes to 90 minutes (Figure 4 and 5). The longer the extraction time, the more the amount of flavonoids and phenols extracted. It is because a longer contact time between the material and the solvent will enhance the ability of the solvent to take up flavonoid and phenol in the material (Sani et al., 2022).
However, the amount of flavonoid and phenol extracted at 90 minutes is almost close to 120 minutes of extraction time. It is because the extraction process almost reached the saturation point where the rate of diffusion of extracted compounds from the solid surface to the solvent is equal to the rate of flavonoid diffusion from the solvent to the solid surface. The increase extraction time more than 120 minutes to be no longer effective in increasing the amount of phytochemical extracted (Azahar et al., 2017).

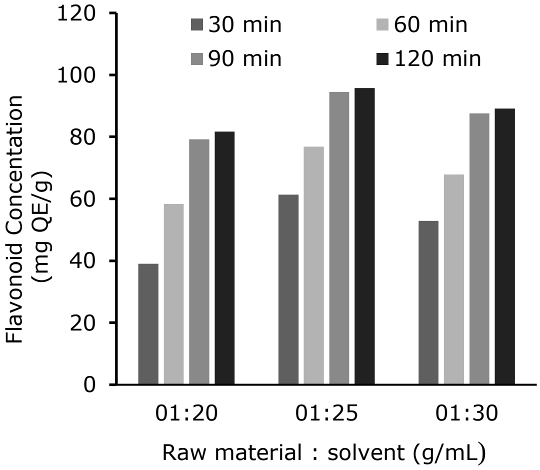


Figure 4. Effect of solid to solvent ratio and extraction time on total flavonoid compounds.

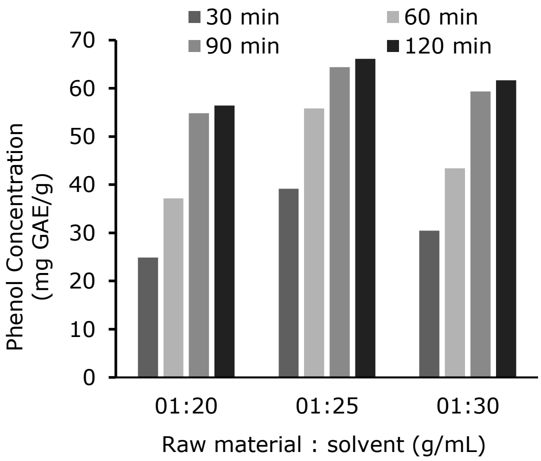


Figure 5. Effect of solid to solvent ratio and extraction time on total phenolic compounds.

The highest amount of flavonoid and phenolic compounds extracted of 95.70 mg QE/g and 66.12 mg GAE/g, respectively, were obtained from the ratio of solid to solvent of 1:25 and extraction time of 120 minutes. Meanwhile, the lowest amount of flavonoids and phenol extracted of 39 mg QE/g and 24.87 mg GAE/g, respectively, were achieved in the treatment of the solid to solvent ratio of 1:20 and the extraction time of 30 minutes.

3.3. Physicochemical Properties of Oil

The physicochemical properties of an oil are crucial for the analysis to determine the quality of a product. This physicochemical analysis were carried out on the oils extracted at the operating condition with the highest yield. Table 1 shows the physicochemical analysis of amla seed oil.

Table 1 shows that the pH tends to be acidic in the extracted oil, which is 5, while in the research of Keithellakpam and Patel (2018) is 6. According to Kumar et al., (2020), most bacteria live at a pH of 6.5-7.5 and are in the optimum growth phase.

Table 1. Physicochemical analysis of amla seed oil.

Parameter	Extracted Oil	Keithellakpam and Patel (2018)
pH	5	6
Density (g/ml)	0.828	0.832
Acid Number (mg KOH/g)	14.94	24.71
Free Fatty Acids (%)	7.60	24
Peroxide Number (meq/kg)	25.74	38.29

The density value of the extracted oil obtained is 0.8228 g/mL, which is not much different from the research of Keithellakpam and Patel (2018), which is 0.832 g/mL. Then the acid number of the extracted oil is 14.94 mg KOH/g, and the results obtained are lower than the research of Keithellakpam and Patel (2018) of 24.71 mg KOH/g. The lower the acid number of oil, the better the quality of oil, and the higher the acid number contained in the oil, the higher the level of damage to the oil (Zhou and Yang, 2021). The acid number indicates the FFA content contained in the oil, where the extracted amla seed oil has an FFA content of 7.60%. This value is still lower than the research reported by Keithellakpam and Patel (2018).

Table 2. Fatty acid components of amla seed oil.

Types of Fatty Acids	Molecular Formula	Rate (%)
Myristate	$C_{14}H_{28}O_2$	6.73
Palmitate	$C_{16}H_{12}O_2$	2.15
Stearate	$C_{18}H_{36}O_2$	5.44
Oleate	$C_{18}H_{33}O_2$	29.86
Linoleate	$C1_8H_{34}O_2$	36.32
Linolenate	$C_{18}H_{32}O_2$	19.51
Total Saturated Fatty Acid		14.31
Total Unsaturated Fatty Acid		85.69

The amount of FFA contained in the oil is a parameter of oil quality, where the higher the FFA content, the lower the quality (Mariana et al., 2020). Meanwhile, the peroxide analysis obtained in the extracted oil was much lower at 25.74 meq/kg compared to the results reported by Keithellakpam and Patel (2018), which was 38.29 meq/kg. A high peroxide value indicates that the oil has been oxidized, characterized by a rancid taste and odour, which means that the oil cannot be reused because it is toxic and can endanger health (Padehban et al., 2018).

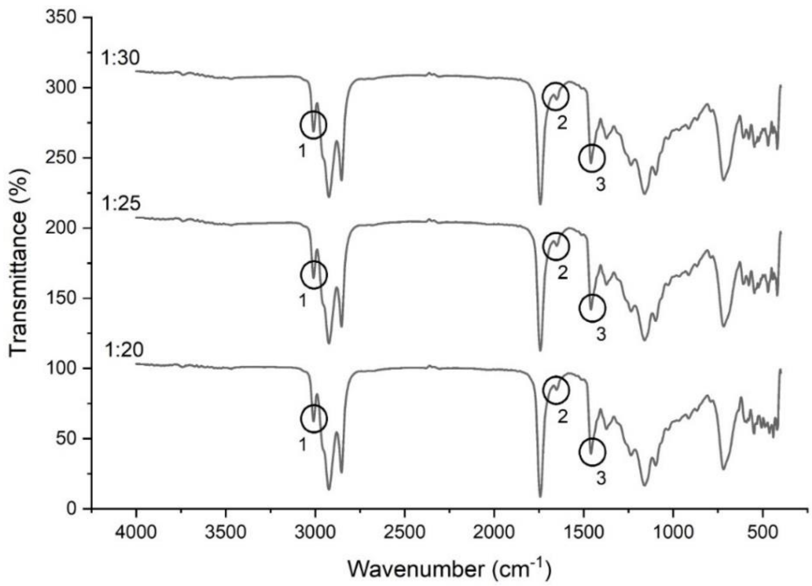


Figure 6. IR spectra of Amla seed oil in regard of solid to solvent ratio.

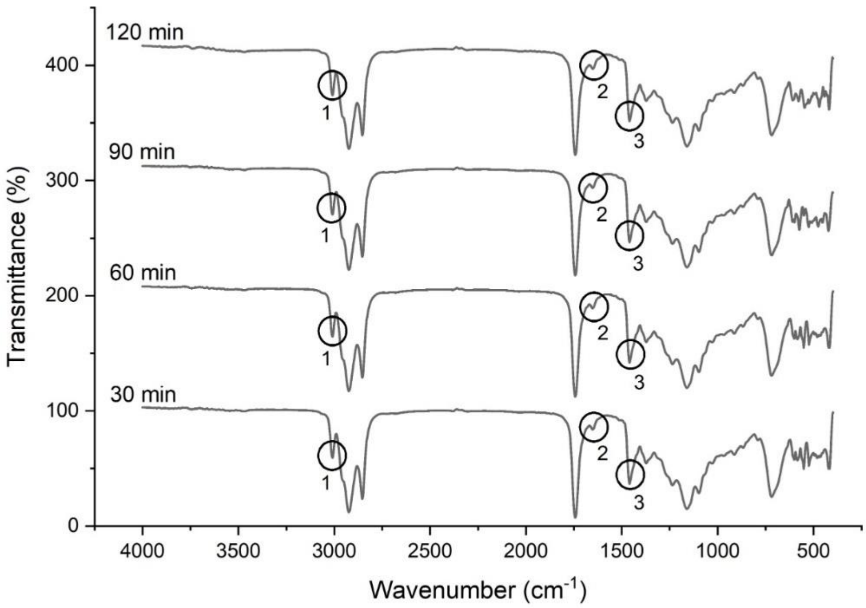


Figure 7. IR spectra of Amla seed oil in regard of extraction times.

The physicochemical properties of extracted oil in this study were different from the previous study due to differences in treatment, such as solvent type, extraction time and the ratio of solid to solvent.

Figure 6 and Figure shows the FTIR results of amla seed oil. The variations in solid to solvent ratio and extraction time showed the same functional group content. Functional groups of amla seed oil showed the presence of: (1) hydroxyl group (O–H) at wave number 2700–3200 cm^{-1}; (2) keton group (C=O) at wavenumber 1600–1690 cm^{-1}; and (3) eter group (C–O) at wave number 1400–1500 cm^{-1}. The existence of O–H, C=O and C–O groups indicated that the presence of polyphenol, ascorbic acid and flavonoid are contained in amla seed oil (Dinesh et al., 2016).

The fatty acid components of amla seed oil can be identified by GC-MS analysis. The analysis of the fatty acid components was conducted on the best samples based on the highest yield. The samples analyzed were extracted oil at a ratio of solid to solvent of 1:30 and extraction time of 120 minutes. Table 1 shows that the component of unsaturated fatty acids is more than saturated fatty acids, where the main component is linoleate with an amount of 36.32%, whereas unsaturated fatty acids can provide many health benefits (Aslam et al., 2022). Other fatty acids component with the highest composition after linoleate are oleate, linolenate, myristate, stearate, and palmitate.

4. Conclussion

The solid to solvent ratio and extraction time affects the yield, phytochemical content and physicochemical of oil extracted from amla seed using hexane as a solvent. The highest yield of 10.97% obtained in the treatment of the solid to solvent ratio of 1:30 and the extraction time of 120 minutes. Surface morphology analysis showed that with increasing extraction time, the morphology of amla seeds increased the number of cell walls that broke into tiny particles, causing the material to be extracted to come out more efficiently so that the extraction yield was greater. The highest concentrations of phenolic compounds and flavonoids, namely 66.12 mg GAE/g and 95.70 mg QE/g, were obtained from the treatment of the ratio of solids to solvents of 1:25 and extraction time of 120 minutes. FTIR analysis with variations in solid to solvent ratios and extraction times showed the presence of the same functional groups in the form of O–H, C=O, and C–O, which indicated the presence of polyphenolic, ascorbic acid and flavonoids compounds in amla seed oil. The results of GC-MS showed that unsaturated fatty acids are more dominant than saturated fatty acids. The physicochemical properties of extracted amla seed oil in the form of pH, density, acid number, FFA, and peroxide number obtained better values than in previous studies.

References

Alkandari, D., Sarfraz, H., Sidhu, J.S., 2019. Development of a functional food (pan bread) using amla fruit powder. J. Food Sci. Technol. 56, 2287–2295. https://doi.org/10.1007/s13197-019-03718-5

Aslam, M., Mughal, H.M., Rauf, S., Jamshaid, N., Pervaiz, S., Nadeem, R., Amjad, H.N., Farooq, M., Khalid, A., Malik, H.S., Jabeen, S., 2022. Impact of essential fatty acids consumption in combating neurological disorders. Pakistan Biomed. J. 5, 2–6. https://doi.org/10.54393/pbmj.v5i2.116

Azahar, N.F., Gani, S.S.A., Mohd Mokhtar, N.F., 2017. Optimization of phenolics and flavonoids extraction conditions of Curcuma Zedoaria leaves using response surface methodology. Chem. Cent. J. 11, 1–10. https://doi.org/10.1186/s13065-017-0324-y

Dinesh, M., Roopan, S.M., Selvaraj, C.I., 2016. Photocatalytic degradation of nitrophenol using biologically active Phyllanthus emblica seed extract. J. Photochem. Photobiol. B Biol. 161, 273–278. https://doi.org/10.1016/j.jphotobiol.2016.05.033

Elboughdiri, N., 2018. Effect of time, solvent-solid ratio, ethanol concentration and temperature on extraction yield of phenolic compounds from olive leaves. Eng. Technol. Appl. Sci. Res. 8, 2805–2808. https://doi.org/10.48084/etasr.1983

Gantait, S., Mahanta, M., Bera, S., Verma, S.K., 2021. Advances in biotechnology of *Emblica officinalis* Gaertn. syn. *Phyllanthus emblica* L.: a nutraceuticals-rich fruit tree with multifaceted ethnomedicinal uses. 3 Biotech 11, 1–25. https://doi.org/10.1007/s13205-020-02615-5

Keithellakpam, L., Patel, A., 2018. Study on extraction and effect of storage condition on shelf life of amla (*Emblica Officinalis*) seed oil. Dissertation. India: Universitas Higgingbottom.

Kumar, N., Shail, Dwivedi, M., 2015. Nutrional importance and traditional uses of amalaki (*Phyllanthus emblica*). Int. J. Pharm. Biol. Arch. 6, 1-6.

Kumar, A., Singh V.P., Singh, P., Mishra, V.K., 2020. *Microbe mediated remediation of environmental contaminants 1st Edition*. Elsevier. Netherlands.

Mariana, R.R., Susanti, E., Hidayati, L., Wahab, R.A., 2020. Analysis of peroxide value, free fatty acid, and water content changes in used cooking oil from street vendors in Malang. AIP Conf. Proc. 2231, 1-7.https://doi.org/10.1063/5.0002656

Ntalikwa, J.W., 2021. Solvent extraction of jatropha oil for biodiesel production: Effects of solvent-to-solid ratio, particle size, type of solvent, extraction time, and temperature on oil yield. J. Renew. Energy 2021, 1–8. https://doi.org/10.1155/2021/9221168

Padehban, L., Ansari, S., Koshani, R., 2018. Effect of packaging method, temperature and storage period on physicochemical and sensory properties of wild almond kernel. J. Food Sci. Technol. 55, 3408–3416. https://doi.org/10.1007/s13197-018-3239-2

Predescu, N.C., Papuc, C., Nicorescu, V., Gajaila, I., Goran, G.V., Petcu, C.D., Stefan, G., 2016. The influence of solid-to-solvent ratio and extraction method on total phenolic content, flavonoid content and antioxidant properties of some ethanolic plant extracts. Rev. Chim. 67, 1922–1927

Pria, F. F., Islam, M. S.,, 2019. *Phyllanthus emblica* Linn. (Amla) - A natural gift to humans: An overview. J. Dis. Med. Plants 5, 1-9. http://dx.doi.org/10.11648/j.jdmp.20190501.11

Sani, M.S.A., Bakar, J., Azid, A., Iqbal, M.J., 2022. Chemometrics-based evaluation on the effect of sonication, contact time and solid-to-solvent ratio on total phenolics and flavonoids, free fatty acids and antibacterial potency of Carica papaya seed against S. enteritidis, B. cereus, V. vulnificus and P. mirabilis. Food Chem. Adv. 1, 1-11. https://doi.org/10.1016/j.focha.2022.100033

Sriwatcharakul, S., 2020. Evaluation of bioactivities of *Phyllanthus emblica* seed. Energy Reports 6, 442–447.

https://doi.org/10.1016/j.egyr.2019.08.088

Zamanhuri, N.A., Rahman, N.A., Bakar, N.F.A., 2021. Effect of microwave power and extraction time on crude palm oil quality using microwave-assisted extraction process. Int. J. Renew. Energy Dev. 10, 495–505. https://doi.org/10.14710/ijred.2021.35402

Zhang, Q.W., Lin, L.G., Ye, W.C., 2018. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Med. 13, 1–26. https://doi.org/10.1186/s13020-018-0177-x

Zhou, F., Yang, K., 2021. The Influence of water content on the acid number of diesel engine lubricant in electrochemical measurement. IOP Conf. Ser. Mater. Sci. Eng. 1043. https://doi.org/10.1088/1757-899X/1043/5/052054