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MICROWAVE-ASSISTED EXTRACTION IMPACTS ON PHARMACOLOGICAL ACTIVITY OF *Vernonia amygdalina* Delile LEAF EXTRACTS AND FT-IR APPLICATION FOR PHYTOCHEMICALS ANALYSIS

P. A. Z. Hasibuan[1,✉], J. M. Keliat[2] and M. F. Lubis[3]

[1]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20222, Indonesia

[2]Department of Pharmaceutical and Food Analysis, Faculty of Vocational, Universitas Sumatera Utara, Medan, 20222, Indonesia

[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20222, Indonesia

✉Corresponding Author: poppyanjelisa@usu.ac.id

ABSTRACT

Vernonia amygdalina (VA) is a native plant from Africa that is widely distributed to Asia, especially in Indonesia. One of the factors that can affect the pharmacological activity of natural ingredients is the extraction process. This study aims to show the effect of extraction methods in the VA leaves extraction process on phytochemical content and their correlation with pharmacological activities. VA leaves extract in this study was obtained using different extraction methods. The extracts were investigated for total phenolic content (TPC) and total flavonoid content (TFC) using a calorimetry assay. Principal Component Analysis (PCA) was used to group the extracts based on FTIR data. Antioxidant activity of the extracts was done using DPPH and ABTS assay. While the cytotoxic activity of the extracts was carried out using an MTT assay on T47D, WiDr, and HeLa cells. The cytotoxic activity of extracts was described using the IC50 value. There were significantly different total phenolic content and total flavonoid content of extracts ($p<0.05$). The microwave-assisted extraction (MAE) extract was identified to have the highest TFC, DPPH, ABTS, and cytotoxic values of 11.06±0.77 mg QE/g dry powder, 63.05±3.21 µg/mL, 32.19±1.23 µg/mL, and 133.922±3.22 µg/mL (T47D), 194.547±3.43 µg/mL (WiDr), and 1275.436±11.42 µg/mL (HeLa), respectively ($p<0.05$). This is supported by PCA analysis which shows that there are differences in extracts based on FTIR data. This study reports that each extract of VA leaves gives a distinct phytochemical profile (TPC, TFC, and FTIR spectrum) that contributes to the antioxidant activity and cytotoxic activity.

Keywords: *Vernonia amygdalina* Delile, Extraction, Phytochemicals, Antioxidant, Cytotoxic.

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INTRODUCTION

Plants are a valuable source of bioactive chemicals that can be used in medication development. Isolated bioactive molecules are used as both starting materials for drug synthesis in the lab and as a model for producing physiologically active chemicals.[1] Phytochemical processing of raw plant materials is necessary for optimizing the concentration of recognized ingredients while also preserving their activity. Extraction is a critical step in the phytochemical processing pathway for identifying bioactive compounds in plant materials.[2] The choice of an appropriate extraction process is particularly vital for the standardization of herbal products since it is used to remove desirable soluble elements while leaving out those that aren't needed using solvents.[3] Furthermore, for upscaling reasons, such as from bench scale to pilot plant level, selecting a proper extraction method and optimizing different parameters is crucial. Traditional extraction processes such as maceration, percolation, infusion, decoction, and hot continuous extraction are among the most often utilized.[4] During the last three decades, alternative approaches such as ultrasound-aided extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extractions (SFE) have gotten a lot of attention.[5] MAE is a novel extraction technology that extracts secondary metabolites in less time while using less solvent and energy.[6] The molecular interactions between the electric component of the microwave field and the dipolar molecules and ionic species present in the extraction mix account for

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MAE's significant capacity to improve extraction yield (solvent-sample).[7] The use of green extraction techniques such as MAE for the phytochemical processing of medicinal plants has been rapidly and steadily rising across the world since these procedures are faster than traditional approaches. In terms of solvent and energy use, these approaches are also ecologically benign.[8] The yield is equivalent to that of traditional extraction, and in certain circumstances, it is considerably higher. However, various investigations have found that the extract yield as well as the bioactivities of extracts generated using different extraction procedures vary.[9,10] *Vernonia amygdalina* (VA) is a woody shrub that grows up to 5 meters tall and belongs to the Asteraceae family.[11] VA is widespread across Asia but is most prevalent in Indonesia, Singapore, and Malaysia. The leaves have a distinct odor and bitter taste, which explains the popular English term 'bitter leaf.' Due to a lack of documentation, multiple local names for this plant have been used in different countries, including Etidod or Ewuro in Nigeria, and South African leaf in Malaysia, Kenya, and Indonesia.[12,13] The plant's medical effects, on the other hand, are well known. In Nigeria, it is one of the plants that make up a large part of the naturalist's pharmacopeia.[14] VA has been proven to have anti-malarial, anti-microbial (anti-bacterial, anti-fungal, anti-plasmodial, and other anti-microbial) anti-diabetic, and anti-cancer properties.[15-17] The current study was conducted with the goal of discovering a link between extraction techniques, yield, total phenolics, and cytotoxic activity.[18] Since alcohol is used as a solvent in most herbal preparations, extraction was done with ethanol as a solvent using MAE.[19] For the comparison of extract yield and phytochemical characteristics, a traditional extraction method utilizing refluxing and maceration was used.

EXPERIMENTAL

Plant Materials and Extracts Preparation

VA leaves were obtained in the Indonesian Deli Serdang, in the province of Sumatera Utara. In the shade, it was air-dried. An electric grinder was used to finely powder the leaves. In a round bottom flask, 5 g of powdered plant material was combined with 50 mL of ethanol and refluxed for roughly 5 hours at 100°C for traditional extraction (refluxing). The maceration procedure was completed by immersing 50 g of powder in a glass container for 3 days in a 5 L ethanol solution.[20] In Pyrex beakers, 5 g of powdered plant material was combined individually with 50 ml of ethanol for MAE. Microwave radiation (Samsung Electronics, Model ME731K) was used to extract the beakers, which were placed in the center of the oven over a rotating dish and subjected to it for 7 minutes at 450 W. Vacuum filtration was used to separate the liquid extracts from the solid residue, and a rotary evaporator was used to concentrate them. At 4 °C, dried extract samples were stored in an airtight container.[21]

Determination of Total Phenolics in Extracts

The phenolic content is calculated using the curve absorbance of a standard gallic acid solution. The procedure was, however, somewhat altered in this study. In brief, 1 mL of each concentration series from the sample was obtained, followed by 0.4 mL of Folin-Ciocalteu reagent. The mixture was then agitated for 8 minutes before adding 4 mL of Na_2CO_3 7%. After homogeneity was achieved, 10 mL of methanol was added. After 30 minutes, the mixture was discarded. The absorbance was then measured at a wavelength of 647 nm using a UV-Vis spectrophotometer. Each sample concentration series was measured three times to ensure reproducibility. Gallic Acid Equivalents are used to measure total phenolic content (GAE).[22]

Determination of Total Flavonoids in Extracts

The colorimetric technique was used to determine the total flavonoid content. The procedure was somewhat changed in this study, with 10 mg of material being dissolved in methanol p.a. to 10 mL. Following that, 1 mL and 3 mL of pure methanol were added, followed by 0.2 mL of 10% aluminum chloride and 0.2 mL of 1 M potassium acetate. To create 10 mL, distilled water was added. For 30 minutes, the mixture was incubated. The absorbance was then measured at a wavelength of 439 nm using a UV-Vis spectrophotometer. The test was repeated three times. Quercetin Equivalents are used to measure total flavonoid content (QE).[23]

DPPH Technique for Radical Scavenging Activity

Using the DPPH test, the radical scavenging activity of VA extracts produced by maceration, refluxing, and MAE was assessed. In test tubes, extracts were obtained at different concentrations (corresponding to

200, 400, 600, 800, and 1000 ppm). Methanol was used to bring the total amount down to 8.5 ml. In these tubes, 5.0 ml of a 0.1 mM methanolic DPPH solution was added and well-mixed using a vortex mixer. For 20 minutes, the tubes were held at room temperature. The blank was made in the same way as the extract, but without the extract, and the baseline correction was done with methanol. The absorbance of the extract samples was measured using a UV–visible spectrophotometer at 517 nm. The inhibition percentage of radical scavenging activity (RSA) was estimated using the following formula.[24]

%Radical Scavenging Activity = [(Absorbance of Control- Absorbance of Sample)]/[Absorbance of Control] X 100

The Capacity to Scavenge Free Radicals by Using a Stable ABTS Radical Cation

The ABTS radical cation decolorization test was used to assess free radical scavenging activity. Before use, ABTS was dissolved in water to a concentration of 7 µM, and radical cation (ABTS+) was created by reacting the ABTS solution with 2.45 µM potassium persulphate at ambient temperature in the dark (12–16 h). The ABTS+ solution was diluted with water to achieve an absorbance value of 0.70 ± 0.02 at 734 nm for the test. After 6 minutes, absorbance was measured after adding 3.0 ml of diluted ABTS+ solution to 100 µl of extracts solutions.[25]

Calculation of Inhibitory Concentration 50 (IC_{50})

From the curve of RSA percentage against extract concentration, the extract concentration corresponding to 50% inhibition (IC^{50}) was derived. As a control, quercetin was utilized. For each concentration, each sample was analyzed three times.

Determination of Chemical Compounds Using FT IR

Around 2 mg of sample, extract was combined with 200 mg KBr and manually pushed into the disc for 10 minutes. In the Tensor 37 FTIR spectrophotometer (Ettlingen, Germany), the disc was put. The detector utilized was deuterated triglycine sulfate (DTGS). FTIR measurements were taken in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and a sampling rate of 32 s/min using OPUS 4.2 software. A data point database was used to store FTIR spectra.[26]

Cytotoxic Activity

The test was carried out at the Universitas Gadjah Mada's Integrated Laboratory of Parasytology. In this test, 3 different types of cancer cells were used, namely T47D, WiDr, and HeLa cells. The method used in the test for cytotoxic activity is the micro tetrazolium (3- [4,5 – dimethylthiazol - 2yl] 2,5-diphenyl tetrazolium bromide) (MTT) assay. Cell suspension in full medium (density 10,000 cells/well) was injected into plate 96 and cultured for 24 hours in a CO_2 5% incubator. The medium in each well is withdrawn at the conclusion of incubation, and the new medium and 100 µL. the sample is put in each well, resulting in a final grade of the sample with a fluctuation of specific levels (samples: 500, 250, 125, 62.5, and 31.25 µg/mL). The plate is then incubated at 37 °C for 24 hours in a 5% CO_2 incubator. After the incubation period, the medium in each well was removed, and cleaned with 100 µL PBS, and 100 µL MTT 5 mg/mL in PBS was added. At 37° C, the plate was incubated for another 4 hours. MTT interacts with living cells to create purple formazan. The synthesis of formazan was inhibited by a 10% solution of SDS (Sodium Dodecyl Sulphate) in 0.01N HCl, then incubated at room temperature. The absorbance was measured with an ELISA reader at 550 nm at the conclusion of the incubation time.[27] The fraction of live cells was determined using the following formula:

= [(Absorbance of Control- Absorbance of Sample)]/[Absorbance of Control] X 100

Data Analysis

The experimental data was gathered in triplicate measurements and given as mean standard deviation. A one-way analysis of variance (ANOVA) was used to do statistical comparisons, followed by the Tukey HSD test, with a significant difference being recognized at the 95 percent confidence level ($p<0.05$). FTIR spectra were preprocessed using standard normal variate before being utilized to cluster the sample extracts. The absorbance values utilized to categorize the extracts were in the ranges of 3200-2800 cm^{-1} and 1800-

400 cm^{-1}. PCA (Principal Component Analysis) was used to cluster the extracts according to the extracting process. Minitab version 19 was used to determine this data.

Tabel-1: Extraction Yields, Total Phenolic Contents, Total Flavonoid Contents, and Antioxidant Activities of *Vernonia amygdalina* Leaves Extracts ($p<0.05$)

Extraction Methods	Extraction Yield (%)	Total Phenolic Content (mg GAE/g dried powder)	Total Flavonoid Content (mg QE/g dry powder	IC_{50} (µg/ml) ± SD	
				DPPH	ABTS
Maceration	20.33±1.30	62.81±1.62	6.83±0.53	71.21±2.34	73.59±3.21
Refluks	15.32±1.15	48.91±1.54	10.12±0.61	103.15±4.55	134.53±4.32
MAE	9,21±0.41	38.78±1.21	11.06±0.77	63.05±3.21	32.19±1.23

The reported values are mean ± SD of the triplicate assay for each sample

RESULTS AND DISCUSSION

Yield of Extracts

The yields from three different extracting methods (maceration, reflux, and MAE) (Table-1). The maceration method gave the highest yield (%) was 20.33±1.30, followed by the reflux method and MAE method of 15.32±1.15, 9,21±0.41 respectively. The obtained result indicated that phytochemicals present in VA extract are different according to extraction methods. We found that from the turkey HSD test, the yield of the maceration method and reflux method were not significantly different ($p<0.05$), while both methods were significantly different ($p>0.05$) with the MAE method. This result suggests that the extraction type of the extracted compounds depends on the extraction method.

Total Phenolics Content

Phenolic acids, flavonoids, anthocyanins, lignin, and tannins are examples of phenolic chemicals found in plants. In terrestrial plants, phenolic compounds are the most common secondary metabolites.[28] The antioxidant capabilities of the phenolic molecule are known to be due to reduction, free radical scavengers, metal chelators, and electron donor processes. However, not all phenolic compounds have the potential to be antioxidants.[29] The Folin-Ciocalteu technique was used to quantify total phenolic content. The Folin-Ciocalteu reagent interacts with phenolic substances alone. As the aromatic rings of the phenolic compounds convert phosphomolybdate-phosphotungstate in the Folin-Ciocalteu reagent to blue molybdenum, the reagent, which was initially yellow, turns blue.[30] The capacity of phenolic compounds to halt oxidation processes is used to determine total phenol levels, and it is thought that there is a close link between total phenolic content and antioxidant activity.[31] The extract's total phenolic contents range from 38.78 to 62.81 GAE/g dry extract (Table-1), with the highest in the extract using the maceration method to the lowest in were reflux method > MAE extracts. The extract's total phenolic contents showed a significant difference between extraction methods at a 5% confidence level. Based on these findings, the maceration method for extracting phenolic chemicals from VA leaves is the most efficient.

Total Flavonoids Content

The total flavonoid content of VA leaves extracts with different extraction methods are exhibited in Table 1 and there were expressed in terms of quercetin equivalent per mg of dried extracts. From the result obtained, the MAE method is the highest total flavonoid content compared to maceration and reflux methods was 11.06±0.77 mg QE/g sample, 6.83±0.53 mg QE/g sample, and 10.12±0.61 mg QE/g sample, respectively. Our results show that the extraction method affects the total flavonoid content of the extract. The extract's total flavonoid contents showed a significant difference between extraction methods at a 5% confidence level. The results obtained are in accordance with other studies that compare the total flavonoid value of the extract obtained by maceration and MAE methods of Casia alata. MAE method gives a result of 135.18±2.90 mg QE/g sample, while the maceration method was 70.13±4.43 mg QE/g sample ($p<0.05$)[32]. This is due to an increase in both internal and external mass transfer due to microwave irradiation. Since polyphenol extraction is a mass transfer process, it is very important that the extraction yield increases as the mass velocity increases.[33]

Antioxidant Activity of *V. amygdalina* Leaf Extracts

The DPPH approach has several benefits for assessing the antioxidant activities of extracts, including quick but simple analysis and information on sample reactivity toward stable DPPH radicals.[34] The capacity of antioxidants to scavenge DPPH radicals is demonstrated by the color shift of purple DPPH to yellow as a result of the electron transfer process. The amount of antioxidant activity in this investigation was expressed as an IC_{50}, with a low IC_{50} indicating strong antioxidant activity.[35] The extracting method gave a significant difference in the antioxidant activity level (Table-1). We found the highest antioxidant activity was from the MAE method with an IC_{50} of 63.05±3.21 µg/mL, followed by maceration and reflux methods was 71.21±2.34 µg/mL and 103.15±4.55 µg/mL, respectively. Furthermore, at a 5% confidence level, all extract antioxidant activity was substantially varied among methods, suggesting that all extracts may contain varying concentrations of antioxidant components. As a result, various extraction procedures may provide varied degrees of antioxidant activity. To ensure the antioxidant activity of the extracts due to differences in extraction methods, another test was carried out, namely the ABTS test.[36] The ABTS free radical-scavenging activity of a sample suggested that its mode of action was as a hydrogen donor, which stopped the oxidation process by converting free radicals to more stable compounds. Like the DPPH method, the IC_{50} value will be a reference for the strength of the extract as an antioxidant.[37] The test results obtained showed that the activity of the extract obtained by the MAE method was better than the extract from the maceration and reflux method with IC_{50} values of 32.19±1.23 µg/mL; 73.59±3.21 µg/mL; and 134.53±4.32 µg/mL ($p<0.05$). The results of the ABTS test are similar to those of the sample antioxidant test using DPPH, where the extract from the MAE process has better activity than other methods. The antioxidant activity of the extract is highly dependent on the number of active compounds extracted from the VA leaves. Microwaves interact with the polar molecules in the extraction media to generate heat quickly, causing the internal pressure in the plant cell to increase, forcing it to rupture, and thereby enabling the release of the bioactive component.[38]

Extracts Characterization Using FT-IR and PCA Analysis

Classification of VA leaves extracts with different methods of extraction was done using FTIR spectra combined with chemometrics (Fig.-1). The results of the FTIR spectra of each extract were compared and evaluated. The extracts have OH alcohol absorption area which is 3704-3333 cm^{-1}, The aliphatic C-H uptake area of 2962-2853 cm-1, and C=O ketone absorption at 1725-1675 cm^{-1} were identified in extracts too.[39] The rest of the differences in absorption from each extract can be seen in the fingerprint area, namely 666-1667 cm^{-1}. To facilitate the identification process of the extract's FTIR spectrum, the absorption data was processed by the PCA method using the Minitab 19 software, the results of which can be seen in Fig.-2.

Tabel-2: The IC_{50} of Extract Against Cancer Cells ($p<0.05$)

Extraction Methods	IC_{50} (µg/ml) ± SD		
	T47D	WiDr	HeLa
Maceration	540.743±8.31	870.791±11.53	NI
Reflux	811.374±10.17	706.051±6.62	NI
MAE	133.922±3.22	194.547±3.43	1275.436±11.42

Note: NI = Not identified

The first two main components (Fig.-2) of the principal component analysis (PCA) of the FTIR spectrum data were shown in a two-dimensional plot, accounting for PC1 (81.4%) and PC2 (18.6%) of the 99% total variance. PCA demonstrated a pretty strong differentiating capability between VA leaves extracts using three different methods for the extraction procedure in a dependent manner, according to the PCA score plot.[40] It can be seen that the presence of MAE extract, maceration extract, and reflux extract has different places which describe the different components of each extraction process.

Cytotoxic Activity of *V. amygdalina* Leaf Extracts

This test was conducted to determine the cytotoxic potential of VA leaf extract obtained from various extraction methods on 3 cancer cells, namely T47D, WiDr, and HeLa cells. The method used in this cytotoxic test is the MTT method. Measurement basis MTT method is crystal measurement formazan formed. formazan crystal is a purple crystal that is insoluble in water but in SDS 10% soluble[41]. Formation

formazan crystals are the result of the reaction between MTT salt and reductase system tetrazolium succinate is present in the mitochondria of living cells. So living cancer cells will be capable of forming formazan crystals which are more.[42]

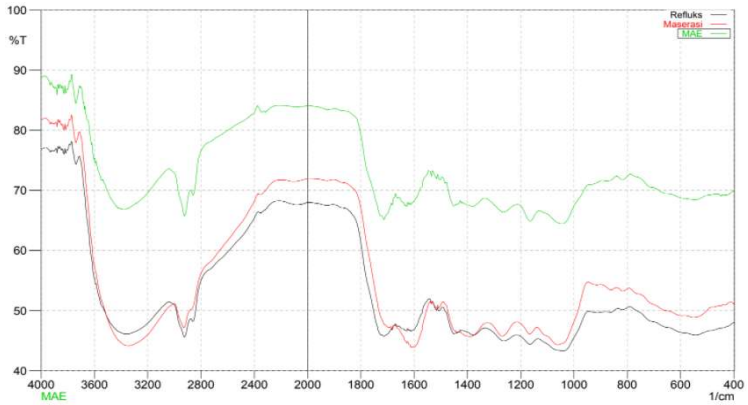

Fig.-1: The Spectrum IR of VA Leaves Extracts. MAE, Maceration, Reflux

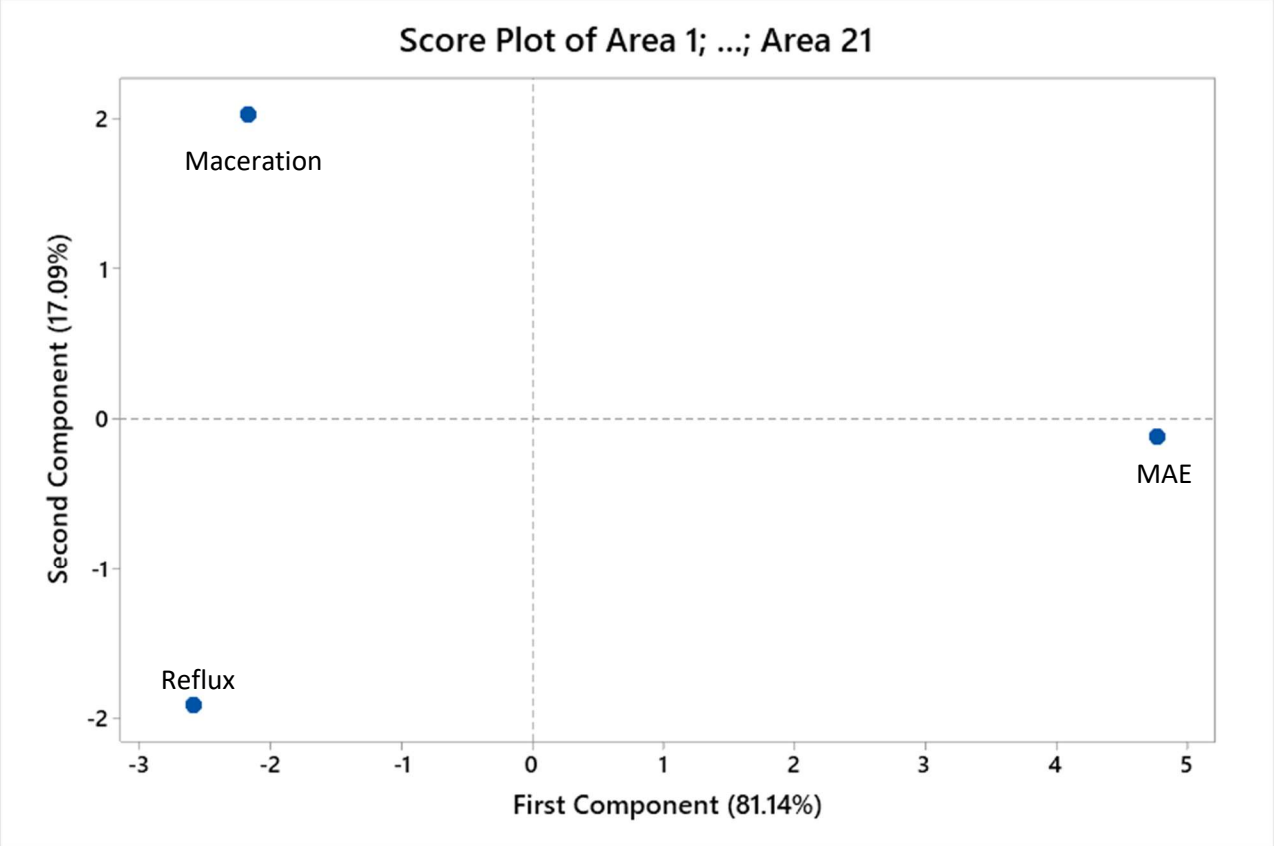

Fig.-2: The Score Plot of PCA Derived from Extract of VA Leaves Using Variable Mid-IR Area (4000-400 cm-^{1})

Observation of cytotoxic potential in cancer cells is by counting the percentage of living cells. Sample concentration made a log to obtain the equation more linear, then an equation is made linear regression between log concentration vs percentage of living cells.[43] The IC_{50} value is the concentration that can kill half of the cancer cells. The magnitude of the cytotoxic potential is described by the small IC_{50} value. The IC_{50} values of each extract against T47D, WiDr, and HeLa cells can be seen in Table-2. The test results showed that the IC_{50} value of each extract had different values in cancer cells. In T47D cells the extract activity obtained from the MAE method was better than the maceration and reflux method with values of 133.922±3.22 µg/ml, 540.743±8.31 µg/ml, and 811.374±10.17 µg/ml, respectively ($p<0.05$). MAE extract also had the best activity when tested against WiDr cells compared to maceration and reflux methods. The

IC_{50} values of each extract were 194.547±3.43 µg/ml, 870.791±11.53 µg/ml, and 706.051±6.62 µg/ml ($p<0.05$). Meanwhile, only the MAE method whose cytotoxic activity can be identified in HeLa cells with an IC_{50} value of 1275.436±11.42 µg/ml. The cytotoxic activity of the VA leaves extract extracted using the MAE method was very dominant. In T47D and WiDr cells, this extract was identified as having quite strong activity because the IC_{50} value was at 100-500 µg/ml.[44] While in HeLa cells it was identified as weak because the IC_{50} value was >500 µg/ml. This result is also in line with the data obtained regarding the total number of flavonoids from the extract and the antioxidant activity of the extract where the extract obtained by the MAE method had the highest total flavonoid content and the best antioxidant activity compared to other extracts.

CONCLUSION

The type of extracting method affected the FTIR spectra profile, secondary metabolite profile, and pharmacological activities. The MAE extract gave the highest total flavonoid content that affected to antioxidant activity and cytotoxic activity of the extract. Using the PCA, all extracts were clustered according to their respective groups with a total PC1 and PC2 of 98%. Based on these results, the MAE method is the most effective method to obtain the best pharmacological activity from VA leaves.

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CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID is given below:

P.A.Z. Hasibuan https://orcid.org/0000-0001-9122-711X
J. M. Keliat https://orcid.org/0000-0002-8366-3795
M.F. Lubis https://orcid.org/0000-0001-9651-904X

REFERENCES

1. A.G. Atanasov, S.B. Zotchev, V.M. Dirsch, C.T. Supuran, *Natural Reviews Drug Discovery*, **20**, 200(2021), https://doi.org/10.1038/s41573-020-00114-z
2. M.A. Acquavia, R. Pascale, L. Foti, G. Carlucci, L. Scrano, G. Martelli, M. Brienza, D. Coviello, G. Bianco, F. Lelario, *Separations*, **8(91)**, 1(2021), https://doi.org/10.3390/separations8070091
3. A.R. Abubakar, M. Haque, *Journal of Pharmacy & BioAllied Sciences*, **12(1)**,1(2020), https://doi.org/10.4103/jpbs.JPBS_175_19
4. M. Maric, A.N. Grassino, Z. Zhu, F.J. Barba, M. Brncic, S.R. Brncic, *Trends in Food Science & Technology*, **76**, 28(2018), https://doi.org/10.1016/j.tifs.2018.03.022
5. F.J. Barba, Z. Zhu, M. Koubaa, A.S. Sant'Ana, V. Orlien, *Trends in Food Science & Technology*, **49**, 96(2016), https://doi.org/10.1016/j.tifs.2016.01.006
6. J.P. Maran, K.A. Prakash, *International Journal of Biological Macromolecules*, **73**, 202(2015), https://doi.org/10.1016/j.ijbiomac.2014.11.008
7. F.C. Ekezie, D. Sun, J. Cheng, *Trends in Food Science & Technology*, **67**, 160(2017), https://doi.org/10.1016/j.tifs.2017.06.006
8. Q. Zhang, L. Lin, W. Ye, *Chinese Medicine*, **13(20)**, 1(2018), https://doi.org/10.1186/s13020-018-0177-x

9. E. Aspe, K. Fernandez, *Industrial Crops and Products*, **32(1)**, 838(2011), https://doi.org/10.1016/j.indcrop.2011.02.002
10. J. Wang, J. Zhang, X. Wang, B. Zhao, Y. Wu, J. Yao, *International Journal of Biological Macromolecules*, **45(5)**, 483(2009), https://doi.org/10.1016/j.ijbiomac.2009.09.004
11. I.I. Ijeh, C.E.C.C. Ejike, *Journal of Medicinal Plants Research*, **5(7)**, 1051(2011), https://doi.org/10.5897/JMPR.9000004
12. S. Danladi, M.A. Hassan, I.A. Masa'ud, I.U. Idris, *Research Journal of Pharmacy and Technology*, **11(9)**, 4187(2018), https://doi.org/10.5958/0974-360X.2018.00768.0
13. E.O. Farombi, O. Owoeye, *International Journal of Environmental Research and Public Health*, **8(6)**, 2533(2011), https://doi.org/10.3390/ijerph8062533
14. O. Kadiri, B. Olawoye, *Turkish Journal of Agriculture – Food Science and Technology*, **4(9)**, 763(2016), https://doi.org/10.24925/turjaf.v4i9.763-768.570
15. D. Asante, E. Effah-Yeboah, P. Barnes, H.A. Abban, E.O. Ameyaw, J.N. Boampong, E.G. Ofori, J.B. Dadzie, *Journal of Diabetes Research*, **8252741**, 13(2016), https://doi.org/10.1155/2016/8252741
16. C.I. Uzoigwe, O.K. Agwa, *African Journal of Microbiology Research*, **5(12)**, 1467(2011), https://doi.org/10.5897/AJMR10.866
17. F.C. Wong, C.C. Woo, A. Hsu, B.K.H. Tan, *Plos One*, **8(10)**, e78021(2013), https://doi.org/10.1371/journal.pone.0078021
18. E.E. Maaiden, S. Bouzroud, B. Nasser, K. Moustaid, A.E. Mouttaqi, M. Ibourki, H. Boukcim, A. Hirich, L. Kouisni, Y.E. Kharrassi, *Molecules*, **27(7)**, 2074(2022), https://doi.org/10.3390/molecules27072074
19. A. Galan, L. Calinescu, A. Trifan, C. Winkworth-Smith, M. Calvo-Carrascal, C. Dodds, E. Binner, *Chemical Engineering and Processing: Process Intensification*, **116**, 29(2017), https://doi.org/10.1016/j.cep.2017.03.006
20. P.A.Z. Hasibuan, D. Munir, D. Pertiwi, D. Satria, M.F. Lubis, *Rasayan Journal of Chemistry*, **13(4)**, 2577(2020), http://dx.doi.org/10.31788/RJC.2020.1345625
21. O.R. Alara, N.H. Abdurahman, O.A. Olalere, *Food and Bioproducts Processing*, **107**, 36(2018), https://doi.org/10.1016/j.fbp.2017.10.007
22. S.M. Sinaga, S. Sudarmi, I. Iksen, K. Kevin, M.P. Sari, *Rasayan Journal of Chemistry*, **11(4)**, 1604(2017), http://dx.doi.org/10.31788/RJC.2018.1144067
23. P.A.Z. Hasibuan, P. Sitorus, D. Satria, R.D. Sibue, *Indonesian Journal of Cancer Chemoprevention*, **10(1)**, 37(2019).
24. A. Dalimunthe, P.A.Z. Hasibuan, J. Silalahi, D. Satria, *Asian Journal of Pharmaceutical and Clinical Research*, **11(1)**, 31(2018), http://dx.doi.org/10.22159/ajpcr.2018.v11s1.26558
25. A. Dalimunthe, D. Pertiwi, M. Muhammad, D. Satria, *E3S Web of Conferences*, **332**, 08005(2021), https://doi.org/10.1051/e3sconf/202133208005
26. M. Rafi, W. Rismayani, R.M Sugiarti, U.D. Syafitri, W.T. Wahyuni, E. Rohaeti, *Indonesian Journal of Pharmacy*, **32(2)**, 132(2021), https://doi.org/10.22146/ijp.755
27. D.M. Syari, R. Rosidah, P.A.Z. Hasibuan, G. Haro, D. Satria, *Open Access Macedonian Journal Medical Sciences*, **7(22)**, 3745(2019), https://doi.org/10.3889/oamjms.2019.495
28. D. Satria, J. Silalahi, G. Haro, S. Ilyas, P.A.Z. Hasibuan, *Asian Pacific Journal of Cancer Prevention*, **18(2)**, 399(2017), https://doi.org/10.22034/APJCP.2017.18.2.399
29. M.F. Lubis, V.E. Kaban, J.O. Aritonang, D. Satria, A.A. Mulina, H. Febriani, H, *Rasayan Journal of Chemistry*, **15(1)**, 256(2022), https://dx.doi.org/10.31788/RJC.2022.1516401
30. A. Blainski, G.C. Lopes, J.C.P. De Mello, *Molecules*, **18**, 6852(2013), https://doi.org/10.3390/molecules18066852
31. R.M. Rover, R.C. Brown, *Journal of Analytical and Applied Pyrolysis*, **104**, 366(2013), https://doi.org/10.1016/j.jaap.2013.06.011
32. Y.Y. Ling, P.S. Fun, A. Yeop, M.M. Yusoff, J. Gimbun, *Materials today*, **19(4)**, 1273(2019), https://doi.org/10.1016/j.matpr.2019.11.133
33. S.B. Bagade, M. Patil, *Critical Reviews in Analytical Chemistry*, **51(2)**, 138(2021), https://doi.org/10.1080/10408347.2019.1686966

34. A.L. Dawidowicz, D. Wianowska, M. Olszowy, *Food Chemistry*, **131(3)**, 1037(2012), https://doi.org/10.1016/j.foodchem.2011.09.067
35. Z. Chen, R. Bertin, G. Froldi, *Food Chemistry*, **138(1)**, 414(2013), https://doi.org/10.1016/j.foodchem.2012.11.001
36. I.R. Ilyasov, V.L. Beloborodov, I.A. Selivanova, R.P. Terekhov, *International Journal of Molecular Sciences*, **21**, 1131(2020), https://10.3390/ijms21031131
37. P. Shah, H.A. Modi, *International Journal For Research in Applied Science and Engineering Technology*, **3(6)**, 636(2015).
38. V. Lovric, P. Putnik, D.B. Kovacevic, M. Jukic, V. Dragovic-Uzelac, *Food Technology Biotechnology*, **55(2)**, 243(2017), https://doi.org/10.17113/ftb.55.02.17.4687
39. P.A. Sahayaraj, J. Gowri, V. Dharmalingam, *International Journal of Nano Corrosion Science and Engineering*, **2(5)**, 322(2015).
40. E. Rohaeti, F. Karunina, M. Rafi, *Indonesian Journal of Chemistry*, **21(1)**, 128(2021), https://doi.org/10.22146/ijc.54577
41. U. Amna, Halimatussakdiah, P. Wahyuningsih, N. Saidi, R. Nasution, *Journal of Advanced Pharmaceutical Technology & Research*, **10(2)**, 51(2019), https://doi.org/10.4103/japtr.JAPTR_373_18
42. S.N. Ostad, A. Rajabi, R. Khademi, F. Farjadmand, M. Eftekhari, A. Hadjiakhoondi, M. Khanavi, *Acta Medica Iranica*, **54(9)**, 583(2016).
43. P. Graidist, M. Martla, Y. Sukpondma, *Nutrients*, **7(4)**, 2707(2015), https://doi.org/10.3390/nu7042707
44. E. Studzinska-Sroka, H. Piotrowska, M. Kucinska, M. Murias, W. Bylka, *Pharmaceutical Biology*, **54(11)**, 2480(2016), http://dx.doi.org/10.3109/13880209.2016.1160936

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