Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/12858
DOI URL: http://dx.doi.org/10.21474/IJAR01/12858

RESEARCH ARTICLE

OPTIMIZATION SEQUENTIAL MICROWAVE ULTRASONIC ASSISTED EXTRACTION TO EXTRACT POLYPHENOL FROM MORINGA OLEIFERA LEAVES USING MULTIRESPONSE SURFACE METHODOLOGY

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Manuscript Info

Manuscript History
Received: 15 March 2021
Final Accepted: 19 April 2021
Published: May 2021

Key words:-
Moringa Oliefera, Microwave, Ultrasound, Phenolic, RSM

Abstract

Moringa leaves contain phenolic compounds and flavonoids which are useful as antioxidants. The disadvantages of traditional extraction methods, such as maceration, include a lengthy extraction process and the use of excessive solvent. Therefore, in this study, moringa leaf extraction was carried out using sequential microwave ultrasonic-assisted extraction (MUAE) to speed up the extraction time, and get more yields. This study aimed to improve the extraction of Moringa leaves with MUAE by adjusting the extraction time, temperature, and ethanol percentage. The conditions varied at extraction time (0, 10, 15, 20, 30 minutes), temperature (30, 40, 50, 60, 80 ^{o}C), and ethanol concentration (0, 30, 50, 70, 90%). The findings revealed that the best conditions for extraction Moringa leaves with sequential microwave-ultrasonic assisted extraction were at a time of 15 minutes, a temperature of 55^{o}C, and an ethanol percentage of 54%. In this condition, the extract yield was 12.95%, total phenolic 330.38 GAE mg / 100 g, flavonoids in total 298.15 QE mg / 100 g, the $IC_{50}$for antioxidant activity is 78.37 ppm.

Introduction:-

The medicinal application plant diets and the study is being scaled up in the current scenario due to higher concentrations of a substance of phytochemicals in plant that are therapeutic and may aid in the progression of innovative treatments [Tungmunnithum et al, 2018]. The majority of phytochemicals derived from plants, such as carotenoids, alkaloids, glucosinolates, phenolic acids, flavonoids, tannins, and saponins, have been shown to improve global health [Chhikara et al, 2020]. Flavonoids, which are secondary plant metabolites, are among the dietary phytonutrients found in moringa that have anti-inflammatory and antioxidant properties [Mengfei et al. 2018]. Flavonoids, for example, are responsible for one of moringa's antioxidant effects [Akhavan et al, 2015]. Flavonoids have anti-inflammatory, antibacterial, antiviral, and anti-cancer properties. [Kumar et al, 2016].

There are various conventional solvent extraction methods commonly used to extract bioactive compounds, namely maceration, percolation, reflux, soxhletation, infusion, decoc, distillation and backflow [Hanani, 2014]. Green technology-based extraction alternatives include ultrasound-assisted extraction (UAE), extraction of supercritical fluid (SCFE), microwave-assisted extraction (MAE), and extraction of pressurized liquid (PLE) [Ameer et al, 2017]. Combining two or more extraction techniques, such as sequential microwave-ultrasonic extraction (MUAE), saves

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time and energy [Wang et al, 2017]. Extraction with the help of micro-ultrasonic waves,The mass transfer mechanism is gradually improved, and the extraction time is reduced. The high activation energy or impact energy required for the extraction process can be obtained using this technique. [Chan et al, 2011]. Due to the acoustic cavitation effect of ultrasonic, bioactive compounds are extracted, and this is followed by internal heating in plant cells caused by microwave irradiation [Pongmalai et al, 2015].

Bucić-Kojić et al, [2011] stated that the temperature and time of extraction are crucial in phenolic compound extraction. Some of the factors that influence ultrasonic wave assisted extraction are temperature, time, and the concentration of the solvent used. To determine the effect of each factor, the interaction between factors, and optimization, the response surface methodology (RSM) can be used. RSM is a modeling in an empirical approach to determine the relationship and problems of several variables by finding the optimum value of a response (the dependent variable). RSM uses mathematical and statistical procedures to model and analyze experimental results to determine significant parameters that affect the optimization of the resulting response [Ahmad et al, 2020]. The goal of this research was to extract Moringa leaves by sequential microwave-assisted ultrasonic extraction at variations in temperatures, extraction times, and ethanol percentage. Extraction optimization was completed utilizing with the response surface methodology. This research is very useful to obtain information about an easy extraction process in order to produce optimal extraction quality.

Experimental Section

Reagents and plant material

The leaves of M. oleifera were collected in Semarang, and determined by the Ecology and Biosystematics Laboratory, Department of Biology, (UNDIP, Semarang). In a stone mortar, the leaves were crushed, dry and cool conditions were maintained for the powder in the dark until treatment. The chemical such as Ethanol (96% CAS 64-17-5), $AlCl_3$ (CAS 7446-70-0), Potassium Acetate (CAS 127-08-2), and Standard Kursetin (CAS 117-39-5) were purchased from Merck (Germany) for this study. DIPO Pure Undip prepared the aquadest.

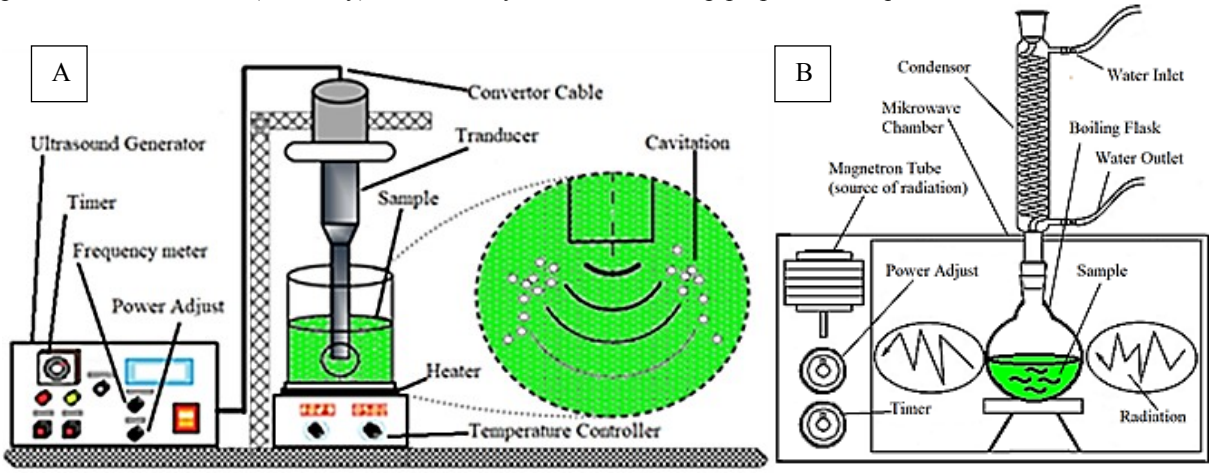

Fig. 1:- (a) Ultrasonic extraction, and (b) Microwave extraction.

Sequential Microwave Ultrasonic-assisted extraction (MUAE)

MUAE extraction was carried out by microwave and ultrasonic extractor consecutively. The ultrasonic extractor (model JP-010S) had a 2L tank, a 0-30 minute timer, an ultrasonic with an output of 80 watts and a frequency of 40 kHz. The customized microwave extractor with a 20 L internal capacity and a digital timer to control the extraction time. The customized microwave extraction (Beko-MGC20100S) operating at a frequency of 2455 MHz and a maximum power of 200 W In 20 mL extraction cells, 1000 ppm M. oleifera leaves was placed in the test tube. The yield of the extraction is well known to be highly dependent on the solvent used. A mixture of two different green solvents was used in this experiment (water, and ethanol) was used with 30-90% ethanol variations.The extract was decanted following the extraction process, followed by filtering with filter paper (Whatman No. 42). Crude extracts were collected in a round glass bottle and concentrated under vacuum pressure in a rotary evaporator (Rotavapor® Buchi R-114, Switzerland) at 70 °C until semi-liquid extracts were obtained. The powder was measured using an analytical balance (PX 224E, Ohaus). The extracts were collected and preserved at cooling temperatures in amber glass bottles.Figure 1 depicts a ultrasonic and microwave device schematic.

Determination of % yield
The percentage of extract yield is calculated according to the formula to get the extract yield adopted from Kunarto et al, [2019] with a formula that is the dry extract weight divided by the dry sample weight then multiplied by 100.

Total Phenolic Content Determination (TPC)
Hapsari et al, [2018] used the Folin–Ciocalteu assay to determine the TPC of extracts. First, 100 ppm of ethanolic reference solutions of gallic acid, 0,2 mL of each sample, 2,6 mL of aquadest, 0,2 mL of 50 percent Folin–Ciocalteu reagent was incorporated into the mix. After 10 min, 1 mL Na_2CO_3 7,5% were poured into each of the wells. A UV-Vis spectrophotometer was used to perform the analysis (Shimadzu ENF-24 / F). The level of absorbance was determined at 765 nm, after 30 minutes of incubation in darkness. The TPC were expressed in milligrams (mg) a gallic acid equivalent (GAE) per 100 gram of dry leaves.

Total FlavonoidDetermination (TF)
The TF content was evaluated,The colorimetric modifying assay for aluminum chloride has been modified fromStankovic et al, [2011]. First, 100 ppm of quercetin ethanolic reference solutions,2 mL of each sample, 1mL of $AlCl_3$2%, and 1 mL potassium asetat 120 mM were added to each well.A UV-Vis spectrophotometer was used to perform the analysis (Shimadzu ENF-24 / F). The level of absorbance was determined at 430 nm, after 30 minutes of incubation in darkness. The TF content were expressed in mg of quercetin equivalent (QE) per 100 g of dry leaves.

Assays of antioxidant activity
The DPPH scavenging method was used to determine antioxidant activity modified from Tristantini et al, [2016]. Initially a 1 mM DPPH solution was prepared by dissolving 3.8 mg in 100 ml of ethanol. Next, 100 ppm of the ethanol reference solution of quercetin and 2 mL of each sample were added to 2mL of 1 mM DPPH. The absorbance level was determined at 517 nm, after 30 minutes of incubation in darkness. Analysis using a UV-Vis spectrophotometer (Shimadzu ENF-24 / F). The remaining DPPH concentration in each well is determined using a calibration curve, and the proportion of remaining DPPH is then in comparison to the concentration of extract to ascertain the number of samples needed to derive the initial DPPH reduction of 50%, or IC_{50}. Triplicate evaluations were taken. The value of a good IC_{50} is below 200 mg / L.

Experiment designs by RSM
RSM was used in order to improve extraction conditions and to figure out how the independent variables (extraction time, temperature, and ethanol percentage) are related to the responses chosen (extract yield, TPC, TF, and antioxidant activity).In the case of MUAE, Three central points were used in a Box–Behnken design. These studies' range and central point values for three, Theindependent variable ranges and levels are listed in Table 1.

Independent variables such as extraction time (0, 10, 15, 20, 30 minutes), temperature (30, 40, 50, 60, 80 ^oC), and the solvent concentration of ethanol (0, 30, 50, 70, 90%) were studied. Regressions with multiple linear variables were used to determine the best MUAE extraction conditions using Statistica 10.0 software (Hamburg, Germany). The optimal surface response obtained is in the form of a stationary point and the optimum response value is shown based on the contour plot and surface plot.

Table 1:- Independent Variable Ranges and Levels.

Independent variable	Range and Levels		
	Low Level (-1)	Level (0)	High Level (+1)
Time (t)	0	15	30
Temperature (T)	30	55	80
Ethanol(%)	0	45	90

Results And Discussion:-

Multiresponse Surfaces Analysis
Three independent variables, such as the ethanol percentage (water:ethanol ratio), extraction temperature, and extraction time, were used.RSM was used to calculate the best best extract yield, TPC, TF, and antioxidant activity. The findings for the correspondence of the MUAE method are summarized in Table 2. One of the most thoroughly researched factors is the type of extraction solvent used. In the food and nutraceutical industries, The most suitable

solvents are ethanol and water because they're easy to remove from the finished item and increase phenolic, flavonoid, glycosides, catechols, and tannin extraction efficiency from raw plant materials. However, using solvents in a mixture with a restricted range of compositions can improve the soluble nature of these substances [Rodriguez et al, 2016]. The statistical model was adjusted and optimized using an ANOVA for each response.

Table 2:-Response Values based on the Box-Behnken design.

Spl	Run	Time (min)	Temp (^oC)	Ethanol (%)	Nilai Respon			
					Yield	TPC	TF	IC_{50}
3	1	30	55	0	8.95	217.99	185.81	127.42
15	2	30	55	90	9.33	227.25	193.95	121.13
8	3	15	55	45	12.96	332.00	300.00	79.07
14	4	15	80	90	10.33	232.49	242.60	104.39
4	5	0	55	0	8.11	188.07	168.17	140.41
5	6	15	30	0	10.01	231.19	234.72	108.61
9	7	30	30	45	7.88	167.55	164.29	139.45
2	8	30	80	45	7.80	161.71	162.61	140.98
12	9	15	55	45	12.93	330.00	298.00	79.05
13	10	0	30	45	7.15	144.55	148.69	153.66
1	11	15	80	0	9.91	223.11	232.32	109.81
11	12	15	55	45	12.90	327.00	295.00	79.02
7	13	0	80	45	7.08	139.51	147.17	155.35
10	14	0	55	90	8.46	196.05	175.55	133.48
6	15	15	30	90	10.44	240.91	245.10	103.25

Table 2. Shows that the yield of moringa oleifera leaf extract ranged from 7.08 - 12.96%. Moringa extract contains phenolic compounds ranging from 139.51 - 332.00GAE mg / 100g, total flavonoids ranging from 147.17 - 300.00 QE mg / 100g, DPPH antioxidant activity with IC_{50} of 155.35 - 79.07 ppm. The leaves of moringa oleifera extracted using ethanol solvent by Rodriguez et al, [2016] contain phenolics of 59 ± 6 GAE mg / g, flavonoids of 6.5 ± 0.2 QE mg / g, and inhibition of DPPH radicals (IC_{50}) of 21 ± 3 ppm. The different results of this study are due to several factors, including the variety, age, location and environmental conditions of moringa growth and maintenance. The relationship between as extraction time, temperature, and ethanol concentration on extract yield response, total phenolic, total flavonoids, IC_{50} is shown in Table 3. All responses showed R^2 value greater than 0.75, and lack of fit greater than 0.05. shows that the mathematical model on the response is quite good because it shows the suitability between the response data and the model [Li et al, 2016].

Table 3:-Quadratic equation models.

Response	Quadratic equation	R^2	P-Value	Lack of fit
Yield extract	$-1.45 + 0{,}49A + 0.35B + 0.04C - 0.015A^2 - 0.003B^2 - 0.0004C^2$	99.69	<0.001	0.208
Total phenolic	$-170.51 + 14.28A + 13.23B + 1.07C - 0.446A^2 - 0.121B^2 - 0.01C^2$	98.93	<0.001	0.363
Total flavonoids	$-33.43 + 13.87A + 7.368B + 0.848C - 0.44A^2 - 0.067B^2 - 0.0083C^2$	99.67	<0.001	0.794
Antioxidant (IC_{50})	$245.87 - 6.61A - 3.86B - 0.307C + 0.205A^2 + 0.035B^2 + 0.0026C^2$	99.64	<0.001	0.052
Note: A for extraction time, B for temperature, C for ethanol concentration				

Yield extract, TPC, and TF resulted from Moringa oleifera extract

The surface plot response graph is shown in Figure 2 (other parameter response graphs, have been analyzed but are not shown because they have elements of the same graphic form).

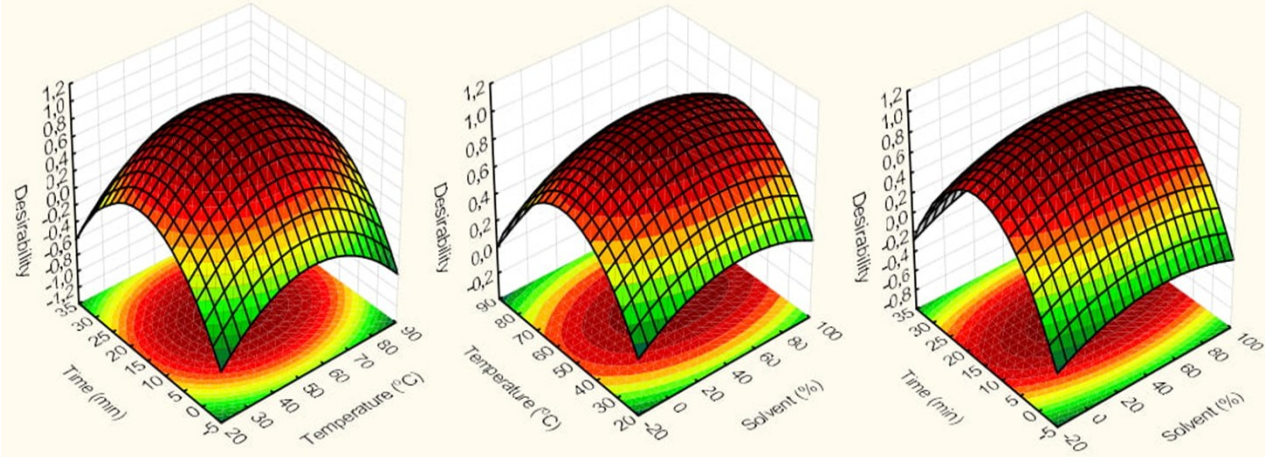

Fig.2:-Maximum surface response for time-temperature interaction; interaction of temperature-ethanol percentage; and the interaction of time-ethanol percentage.

In the graph of the response to the interaction between temperature and time, and the concentration of solvent in the time span of 5-25 minutes, temperature 30-70 ^{o}C, and ethanol concentration 20-80, the highest extract yield was obtained, namely 12.95%; the highest obtained TPC was 330.38 GAE mg / 100 g; The highest obtained on TF was 298.15 QE mg / 100 g.The contour graph for maximum response point is shown in Figure 3.

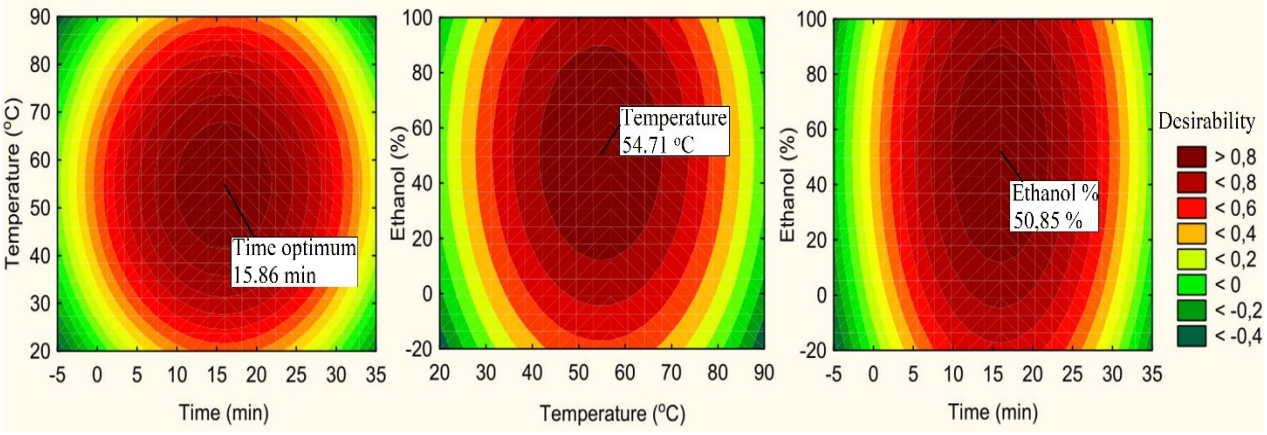

Fig.3:- Countour plot (max response point) for time-temperature interaction; interaction of temperature-ethanol percentage; and the interaction of time-ethanol percentage.

The extraction time can increase and decrease the phenolic and flavonoid compound in Moringa olievera extract. The increase in phenolic and flavonoid compound is due to the longer the process, the solvent can damage the cell walls to remove substances from the plant tissue, so that the extracted phenolic and flavonoid compounds will increase in number. The longer the extraction takes, the extraction content will gradually increase and reach the highest value. About 15-20 minutes is usually a longer time for the extraction process with the help of microwaves [Akhtar et al, 2019]. With a longer microwave-ultrasonic extraction period, there is a risk that these compounds will be degraded, resulting in a decrease in phenolic and flavonoid content.

DPPH radical inhibition (IC_{50}) from Moringa oleifera extract

Determination of antioxidant activity in this study using the DPPH method to get IC_{50} value of the plant extract. The IC_{50} value is the sample concentration required to inhibit 50% of free radicals. The surface plot response graph for the IC_{50} extract is shown in Figure 4.

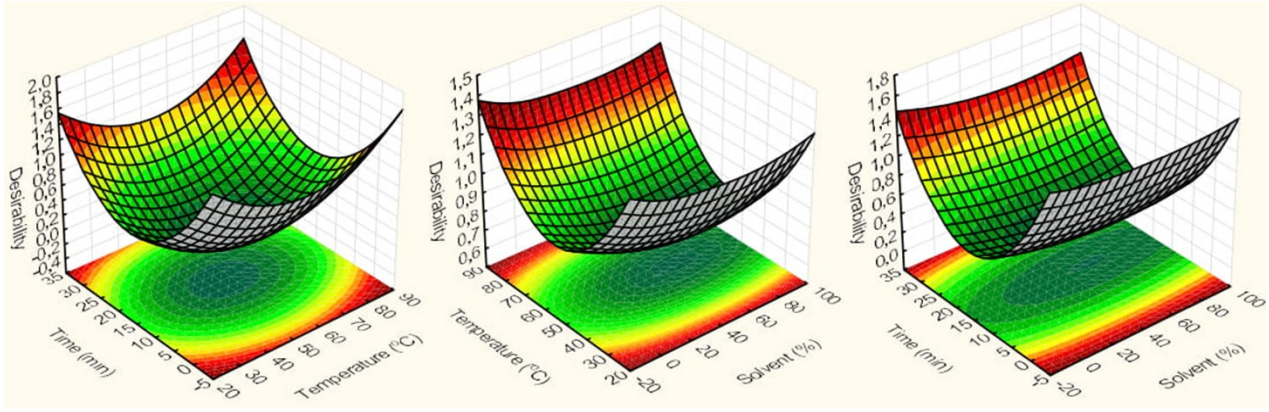

Fig.4:- Minimum surface response for time-temperature interaction; interaction of temperature-ethanol percentage; and the interaction of time-ethanol percentage.

In the graph of the response to the interaction between temperature with time, and the concentration of solvent in a time span of 10-20 minutes, a temperature of 40-60 ^{o}C, and a concentration of 40-70 ethanol, the highest IC_{50} is 78.37 ppm. A compound's antioxidant activity is described as "extremely strong" if its IC_{50} value is less than 50 ppm, strong at 50-100 ppm, moderate at 100-150 ppm, and weak at 150-200 ppm [Haerani et al, 2019]. So that the DPPH test results in this study note that moringa oleifera extracted from sequential microwave ultrasonic has strong antioxidant power.The contour graph for minimum response point in DPPH radical inhibition is shown in Figure 5.

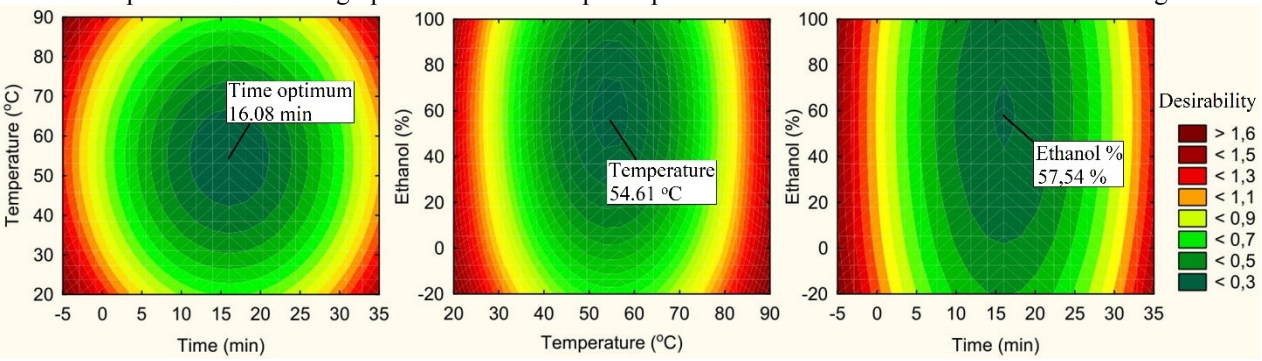

Fig.5:- Countour plot (min response point) for time-temperature interaction; interaction of temperature-ethanol percentage; and the interaction of time-ethanol percentage.

Green extraction methods such as microwave (MAE) provides the advantage of obtaining extracts with a greater amount of bioactive substances, high antioxidant activity, reducing extraction time, and reducing energy expenditure [Hanula et al, 2020]. Lestari et al. [2020] used the UAE method to extract flavonoids from tempuyung leaves and found that 30 minutes of extraction produced the highest antioxidant activity of 58.60 percent with an IC_{50} of 262.82 ppm. Riskayanti et al. [2017] discovered that macerating Moringa leaves with ethanol extract has antioxidant potential, with an IC_{50} value of 22.18 ppm, which is higher than the IC_{50} value of 57.54 ppm for Moringa leaves water extract. The standard IC_{50} value for vitamin C is 8.8 ppm. Vitamin C has a higher IC_{50} value than Moringa leaf extract, indicating that it is a more powerful antioxidant.

Table 4:-Optimization value criteria.

Variable	Target	Lower limit	Upper limit
Time (A)	Range	0	30
Temperature (B)	Range	30	80
Concentration (C)	Range	0	90
Yield extract	Maximum	7.08	12.96
Total phenolic	Maximum	139.51	332.0
Total flavonoids	Maximum	147.17	300.0
Antioxidant (IC_{50})	Minimum	155.35	79.07

Extraction optimization

The criteria used to obtain the optimization value are shown in Table 4. The factors used are only within a predetermined range. The extract yield, TPC, and TF parameters were determined by the maximum response point, while the antioxidant activity IC_{50} was determined by the minimum response point. All factors have the same level of importance so that no factors or parameters are calculated in advance. The optimization results show that the optimal conditions for sequential microwave-ultrasonic extraction of Moringa oleifera are at 15.97 ± 0.076 minutes, a temperature of 54.66 ± 0.0522 ^{o}C, and 54.19 ± 1.29 %ethanol: water.

Table 5 demonstrates the expected response values and the actual response values on the basis of the best extraction conditions. The resulting desirability is 0.996, which means that using time, temperature and ethanol concentration can produce a parameter / response value of 99.6%. Based on the calculation, the standard error (SE) residual value is 0.02-1.8%, which demonstrates that there is no discernible difference between the expected and the actual response value. According to Sulaiman et al, [2017] the standard error residual of less than 5% shows the results are not significantly different.

Table 5:-Predictive response values, true values, and standard error (SE).

Response	Predicted value	True value	Residual SE (%)
Yield extract	13,00421	12,93000	0,028868
Total phenolic	334,300363	329,666667	1,80258651
Total flavonoids	300,594225	297,666667	1,13886981
Antioxidant (IC_{50})	80,0208836	79,0466667	0,378986954
Optimized desirability 0,99644; extraction condition: 16 min, 55 ^{o}C, 54% ethanol			

Conclusion:-

Optimization of sequential microwave ultrasonic extraction of Moringa oleifera using the response surface method has been successfully carried out and can produce optimal values based on extraction time at 15.97 ± 0.076 minutes, temperature at 54.66± 0.0522^{o}C, and 54.19 ± 1.29% ethanol concentration.

Conflict Of Interest

The authors have no conflict of interest.

Author Contributions

G. Restu Prinanda conducted the experiment, Wahyudi conducted the RSM calculations, DwiPurwati wrote manuscript, and AjiPrasetyaningrum revised the manuscript. All authors agreed to the final version of this manuscript.

References:-

[1] Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A. (2018). Flavonoids, and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview,Medicines.5 (93), 1-16.doi:10.3390/medicines5030093.

[2] Chhikara, N., Kaur, A., Mann, S., Garg, M.K., Sofi, S.A., Panghal, A. (2020). Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L. an update review, Nutrition and Food Science. p. 1-24. doi:10.1108/NFS-03-2020-0087.

[3] Lin, M., Zhang, J.,Chen, X. (2018). Bioactive flavonoids in Moringa oleifera and their health-promoting properties, Journal of Functional Foods. 47 (2018), 469–479. doi:10.1016/j.jff.2018.06.011.

[4] Akhavan, H., Barzegar, M., Weidlich, H., Zimmermann, B.F. (2015). Phenolic Compounds and Antioxidant Activity of Juices from Ten Iranian Pomegranate Cultivars Depend on Extraction, Journal of Chemistry. ID 907101, p. 1-7. doi:10.1155/2015/907101

[5] Kumar, P., Rao, N.B.,Roja, R.A. (2016).Antimicrobial, Antioxidant Activity and Phytochemical Screening of Acalypha indica Crude Leaf Extract,International Journal of Pharmaceutical and Clinical Research. 8(6), 583-588.ISSN- 0975 1556.

[6] Hanani, E.(2014).Analisis Fitokimia, PenerbitBukuKedokteran EGC, Jakarta. p. 1-272.

[7] Ameer, K., Shahbaz, H.M., Joong-Ho. (2017). Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review, Food Science and Food. 16 (2017), 295-315. doi:10.1111/1541-4337.12253.

[8] Wang, T., Li, W., Li, T. (2017). Microwave-Ultrasonic Synergistic Extraction of Crude Se-Polysaccharides from Se-Enriched Tea, Key Engineering Materials. 737, 360-366. doi:10.4028/www.scientific.net/KEM.737.360.

[9] Chan, C.H., Yusoff, R., Ngoh, G.C., Kung, F.W.L. (2011). Microwave-Assisted Extractions of Active Ingredients from Plants, J. Chromatogr. A. 1218 (37), 6213–6225. doi:10.1016/j.chroma.2011.07.040.

[10] Pongmalai, P., Devahastin, S., Chiewchan, N., Soponronnarit, S. (2015). Enhancement of Microwave-Assisted Extraction of Bioactive Compounds from Cabbage Outer Leaves via The Application of Ultrasonic Pretreatment, Sep. Purif. Technol. 144, 37–45. doi:10.1016/j.seppur.2015.02.010.

[11] Bucić-Kojić, A., Planinić, M., Tomas, S., Jokić, S., Mujić, I., Bilić, M., Velić, D. (2011).Effect of Extraction Conditions on the Extractability of Phenolic Compounds from Lyophilised Fig Fruits (Ficus Carica L.),Pol. J. Food Nutr. Sci. 61 (3), 195-199. doi:10.2478/v10222-011-0021-9.

[12] Ahmad, I., Prabowo, W.C., Nur, Y., Ardana, M., Rahayu, B.P., Herman. (2020). OptimasiMetodeEkstraksiBerbantuMikrowavedenganPelarut Hijau (AsamSitrat-Glukosa) Terhadap Kadar Polifenol Total dariDaunKadamba (Mitragyna Speciosa Korth. Havil) menggunakan Response Surface Methodology, MajalahFarmasi dan Farmakologi(MFF). 24(1), 11-16. doi:10.20956/mff.v24i1.9456.

[13] Kunarto, B., Sutardi., Supriyanto., Anwar, C. (2019). Optimization of Ultrasonic-assisted Extraction of "MelinjoKerikil" (Gnetumgnemon L. 'Kerikil') Seeds using Response Surface Methodology, JurnalAplikasiTeknologiPangan. 8 (3), 1-8.doi.org/10.17728/jatp.5122.

[14] Hapsaria, A.M., Masfria., Dalimunthe, A. (2018).PengujianKandungan Total FenolEkstrakEtanolTempuyung (Shoncus arvensis L.),Tripical Medicine (TM): Conference Series, 01 (2018), 284–290.doi:10.32734/tm.v1i1.75.

[15] Stankovic, M.S., Niciforovic, N., Topuzovic, M., Solujic, S. (2011).Total Phenolic Content, Flavonoid Concentrations and Antioxidant Activity, of The Whole Plant and Plant Parts Extracts from Teucrium Montanum L. Var. Montanum, F. Supinum (L.) Reichenb,Biotechnology and Biotechnological Equipment. 25 (1), 2222-2227.doi:10.5504/BBEQ.2011.0020.

[16] Tristantini, D., Ismawati, A., Pradana, B.T., Jonathan, J.G. (2016). PengujianAktivitasAntioksidanMenggunakanMetode DPPH pada DaunTanjung (Mimusopselengi L), Prosiding Seminar Nasional Teknik Kimia, Yogyakarta, Indonesia, p. 1-7. ISSN 1693-4393

[17] Rodríguez-Pérez, C., Gilbert-Lpoez, B., Mendiola, J.A., Quirantes-Piné, R., Segura-Carretero, A., and Ibanez, E. (2016). Optimization of Microwave-Assisted Extraction and Pressuried Liquid Extraction of Phenolic Compounds from Moringa oleifera leaves by Multiresponse Surface Methodology,Electrophoresis. 00, 1–9 doi:10.1002/elps.201600071.

[18] Li, H.Z., Zhang, Z.J., Xue, J., Cui, L.X., LI, X.J., Chen, T. (2016). Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants and rosmarinic acid from perilla leaves using response surface methodology, Food Science and technology. 36 (4), 686-693. doi:10.1590/1678- 457x.13516.

[19] Akhtar, I., Javad, S., Yousaf, Z., Iqbal, S., Jabeen, K. (2019). Microwave-assisted extraction of phytochemicals an efficient and modern approach for botanicals and pharmaceuticals - A Review, Pak. J. Pharm. Sci. 32 (1), 223-230. PMID: 30772814.

[20] Haerani, A., Chaerunisa, A.Y.,Subarnas, A. (2019). Antioxidant Activities of Muntingiacalabura, Syzygiumcumini, Ocimumbasilicum, and Eleutherine bulbosa using DPPH Method,Indo J Pharm.1 (2), 57-61.doi:10.24198/idjp.v1i2.21531.

[21] Hanula, M., Wyrwisz, J., Moczkowska, M., Horba´nczuk, O.K., Pogorzelska-Nowicka, E., Wierzbicka, A. (2020). Optimization of Microwave and Ultrasound Extraction Methods of Açai Berries in Terms of Highest Content of Phenolic Compounds and Antioxidant Activity,Appl. Sci. 10 (8325), 1-12. doi:10.3390/app10238325.

[22] Lestari, N.M.M., Yusa, N.M., Nocianitri, K.A. (2020). Effect of Ultrasonic Extraction Time on Antioxidant Activity of Tempuyung leaf (Sonchus arvensis L.) Ethanol Extract, Jurnal ITEPA. 9 (3), 321-326. ISSN : 2527-8010 (e).

[23] Rizkayanti., Diah, A.W.M., Jura, M.R. (2017).Antioxidant Activity Tests of Water and Ethanol Extracts of Moringa (Moringa oleifera LAM) Leaves, JurnalAkademika Kimia. 6(2), 125-131. ISSN 2302-6030 (p), 2477-5185 (e).

[24] Sulaiman, I.S.C., Basri, M., Masoumi, H.R.F., Chee, W.J., Ashari, S.E.,Ismail, M. (2017). Effects of Temperature, Time, and Solvent Ratio on The Extraction of Phenolic Compounds and The Antiradical Activity of Clinacanthus nutans Lindau leaves by Response Surface Methodology,Chemistry Central Journal. 11(54), 1-11. doi:10.1186/s13065-017-0285-1.