

Modelling of Soxhlet Extraction of Lemongrass Oil

Samson Onoriode Okpo^{1*} and Ipeghan Jonathan Otaraku²

¹Department of Chemical Engineering Technology, Delta State Polytechnic, Ozoro, Delta State, Nigeria

²Department of Chemical Engineering, University of Port Harcourt, Nigeria

ABSTRACT

Soxhlet extraction of lemongrass oil using ethanol as a solvent was carried out, and regression modeling was done with Microsoft Excel 2010 and SPSS version 23. The results obtained from the extraction process were fitted into different regression models to select an appropriate model for the extraction process using their coefficients of regression (R^2) and significance values (p -value) as the basis for selection. The effects of particle size, contact time, and solvent volume on oil yield were considered in the modeling. The proposed regression model for effect of particle size, contact time and solvent volume on yield are $y = -0.246\ln(x) + 1.4147$, $y = -0.00002510x^2 + 0.01548x - 0.6898$ and $y = -0.0000001714x^2 + 0.000113x + 1.600$ respectively. The optimum yield on applying the proposed models was 1.586 for 0.5cm particle size, 1.696 for 300 minutes contact time, and 1.6179 for 300ml solvent volume. To maximize the lemongrass's oil yield using Soxhlet extraction, a particle size of 0.5cm, contact time of 300 minutes, and solvent volume of 300ml is recommended. Finally, the proposed model equations can be used satisfactorily to predict any value of yield for Soxhlet extraction of lemongrass essential oil within the defined experimental range of values.

Keywords: Essential oil, Lemongrass, Modelling, Operating parameters, Soxhlet extraction

I. INTRODUCTION

Lemongrass is a tropical perennial (all seasons) plant belonging to *Graminae*(Poaceae) family and genus *Cymbopogon*. The plant has long, thin leaves and is largely cultivated as a medicinal plant in parts of tropical and subtropical areas of Asia, Africa, Australia, Europe, and America ([11]; [21]; [25]; [5]). The leaves of lemongrass and its oil have a lemon-like flavor due to its citral content. Dry leaves of lemongrass contain approximately 1%-2% essential oil [3]. The oil has a light yellow color. The essential oil composition of lemongrass does vary with agronomic treatment, climatic conditions, and geographical locations. Many techniques of extracting essential oil of plant origin include steam distillation, solvent extraction, Soxhlet extraction, hydro-distillation, hydro-diffusion, enfleurage,

maceration, expression, destructive distillation ([9]; [11]; [7]).

Soxhlet extraction techniques involve solid/liquid contact for removing one or more chemical compounds from solid materials by dissolution in liquid reflux. In a conventional Soxhlet extractor, the solid material is put into the thimble of the extractor. It is gradually filled up with the extracting liquid phase by condensing the vapors from the distillation flask. When the solvent gets to a particular level, a siphon pulls the thimble contents into the distillation flask, thus carrying the extracts into the bulk liquid [20]. The process is continued for the chosen contact time, and each is replicated.

Moreover, [4] has reported that many industrial applications require mathematical models for design and effective systems control. Models are simplified mathematical representations of systems at a particular point in time and intended to promote understanding of the real system [2]. Therefore, process modeling involves relating together the properties of a system that are influenced by the process. The outcome is a set of mathematical equations, which is the process model [27]. The process model comprises a set of mathematical formulations or equations that permit us to predict a chemical process's dynamics. Sometimes, to optimize or maximize process operating variables, engineers cannot choose the best operating variables that will minimize operating costs or maximize the profit of a chemical process plant. In a situation like this, the process model and appropriate economic information are used to analyze the prevailing situation and determine the most profitable process conditions [2]. It is worthy to note that mathematical models are useful in developing scale-up procedures from laboratory scale up to pilot plant scale and then industrial scale-up allowing alternative strategies to evaluate the selection of the process variable conditions [18]. In modeling with Microsoft Excel, different trendlines, including linear, polynomial (quadratic and cubic), exponential, logarithmic, and power regression models, can be obtained. But, Middleton [14] suggested that the exponential and power model transform data before the fit, resulting in inaccurate best fit and regression (R^2). In addition, both power and exponential curves are used to fit data that increase or decrease at a high rate, and

neither curve can fit harmful data or data equal to zero. Much research work had been conducted on kinetic modeling of the steam distillation technique, but information regarding Soxhlet extraction modeling of lemongrass oil is scarce in the literature. Therefore, this study aims to formulate Soxhlet extraction models to help achieve maximum lemongrass oil yield by fitting the observed experimental data into the different regression models. Their respective coefficient of determination (R^2) and significance value (p-value) will be the basis for selection.

II. MATERIALS AND METHODS

A. Samples collection and preparation

Fresh lemongrass leaves used for this research work were harvested from a private garden in Ozoro located at 5° 32' 18" N, 6° 12' 58" E, Delta State, Nigeria. The solvent used for this study was ethanol. Samples were washed and dried for eight (8) hours in an oven to reduce the moisture content. The dried lemongrass leaves were kept in a sealed bag to avoid direct sunlight. After that, the dried lemongrass leaves were cut with a knife into various sizes of 0.5cm, 1.0cm, 1.5cm, 2.0cm, and 2.5cm to increase the plant matrix's contact area.

B. Experimental method

The experiment was performed according to the method described by [16] using 500 ml Shuniu GG-17 Soxhlet extractor. 100g of 0.5cm particle size lemongrass samples were measured using weighing balance. The weighed sample was put into an extractor thimble, and 300ml of ethanol were added into the flask. The heating mantle was set at a pre-determined temperature of 78°C according to the solvent's boiling point. The experiment was conducted considering the operating variables, as stated below:

- The effect of particle (solid material) size on oil yield was done using 300ml of different solvents and 100g of particle sizes - 0.5, 1.0, 1.5, 2.0, and 2.5cm of lemongrass sample for 60 minutes. The results were recorded accordingly.
- The effect of contact time on yield was done using 300ml of different solvents and 100g of 0.5cm particle size lemongrass sample. Five different contact times of 60 minutes intervals were used to study the effect of contact time on yield. The results were recorded accordingly.
- The effect of the volume of solvent on oil yield was performed using 100g of 0.5cm particle size lemongrass sample in 100ml, 150ml, 200ml, 250ml, and 300ml of solvent

for 1 hour. The results were recorded accordingly.

After that, the experimental set up was dismantled, and the extracted oil-solvent mixture was collected, recovered, and distilled to obtain solvent-free oil. The oil yield obtained was weighed, and the percentage of oil yield was calculated.

C. Extraction Process modeling

The experimental data results were subjected to a linear, polynomial (quadratic and cubic), exponential, logarithmic, and power regression model using Microsoft Excel 2010 version and SPSS 23 version. The accuracy of the model was determined by evaluating the coefficient of regression (R^2) and significance level, which provide a measure of how much interaction occurs between independent and dependent variables.

III. RESULTS AND DISCUSSION

A. Effects of particle size on oil yield

The results of experimental data fitted into different Microsoft Excel regression models are presented in table 1. As reported by [14], exponential and power models transform data before fit; thereby resulting in inaccurate best fit and regression (R^2); therefore, they were not considered in this discussion.

TABLE 1: MODEL EQUATIONS FOR EFFECT OF PARTICLE SIZES ON YIELD

Model type	Model equation	R^2	p-value
Linear	$y = -0.1826x + 1.623$	78.84%	0.044
Quadratic	$y = 0.1363x^2 - 0.5915x + 1.862$	94.21%	0.058
Cubic	$y = -0.1227x^3 + 0.6883x^2 - 1.315x + 2.1196$	97.41%	0.204
Exponential	$y = 1.6328e^{-0.131x}$	81.50%	0.036
Logarithmic	$y = -0.246\ln(x) + 1.4147$	92.76%	0.008
Power	$y = 1.4057x^{-0.175}$	94.15%	0.006

Each model's fitness was determined by the coefficient of determination (R^2) and the significance value. The closer the value of the coefficient of regression (R^2) to 1, the better the empirical model fits the experimental data ([29]; [13]; [12]). It was reported that R^2 value should be 80% and above to have a better fit of a regression model ([17]; [26]; [29]; [10]). Table 1 considers linear, quadratic, cubic, and logarithmic models, the logarithmic model has R^2 of 92.76% and a significance value of 0.008, which is less than 0.05 significance levels. This means the logarithmic model is more appropriate as it adequately approximates the response variable. It can be satisfactorily used to predict any value of the response variable within the experimental data range. The obtained R^2 value of 92.76% is more than 80% recommended. It shows that 92.76% of the response

variable change was explained by the independent variable (particle size), confirming that the regression model fits the data. The predicted R^2 value is in reasonable agreement with an adjusted R^2 of 90.3%. Besides, the significance value (p-value) of 0.008 is less than the significance level of 0.05. This result also confirms that the model is statistically valid and significant. The proposed logarithmic regression model for the effect of particle size on yield is as given in Equation 1:

$$y = -0.246 \ln(x) + 1.4147 \quad (1)$$

From the model equation, 1% increase in particle size (x) will result in a 0.00246% decrease in oil yield (y). This inference is also in agreement with [22] report, which affirmed that as particle size decreases, oil yield increases. This finding was also observed in figure 1 as particle size increased, oil yield decreased, or vis-versa. The experimental oil yield predicted oil yield and residual output of particle size are presented in Table 2. The predicted results and the residual output were obtained on fitting the experimental data into the proposed model.

TABLE 2: EFFECT OF PARTICLE SIZES ON OIL YIELD

Particle Size (cm)	Experimental yield (%)	Predicted oil yield (%)	Residual yield (%)
0.5	1.625	1.586	0.039
1.0	1.345	1.415	-0.070
1.5	1.319	1.315	0.004
2.0	1.236	1.244	-0.008
2.5	1.223	1.189	0.034

From Table 2, the highest experimental oil yield was 1.625 for 0.5cm particle size, and this is in agreement with 1.586 predicted yields. The results obtained from the experimental results agreed with the predicted results in all cases of particle sizes selected. This has also proved the reliability of the proposed model if employed in large scale operations. The plot of the experimental oil yield against particle size is presented in Figure 1.

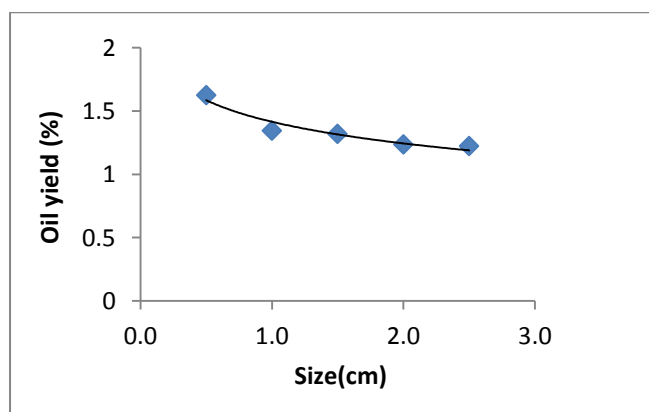


Figure 1: Oil yield against particle size line fit plot

B. Effect of contact time on oil yield

The experimental data results fitted into different Microsoft Excel regression models on the effects of contact time on yield are presented in Table 3. As reported in the preceding discussion, exponential and power models were not considered. From Table 3, among linear, quadratic, cubic, and logarithmic models, the quadratic model was found to be better in terms of R^2 and significance value. This polynomial (quadratic) model equation was found to be adequate for prediction within the range of experimental data as its coefficient of determination (R^2) was found to be 95.62%.

TABLE 3: MODEL EQUATIONS FOR EFFECT OF CONTACT TIME ON YIELD

Model type	Model equation	R^2	p-value
Linear	$y = 0.006448x - 0.0573$	88.84%	0.016
Quadratic	$y = -0.00002510x^2 + 0.01548x - 0.6898$	95.62%	0.044
Cubic	$y = -0.0000025x^3 + 0.000110x^2 - 0.00576x + 0.2174$	98.11%	0.174
Exponential	$y = 0.2016e^{0.0082x}$	83.24%	0.031
Logarithmic	$y = 0.9856 \ln(x) - 3.8756$	93.13%	0.008
Power	$y = 0.0012x^{1.3059}$	95.08%	0.005

The predicted R^2 value for the quadratic model was 95.62%, and its p-value is 0.044, which is less than 0.05 significance levels. This value indicates that contact time explain 95.62% of the changes in oil yield. The obtained R^2 is higher than 80% recommended for a good fit model ([17]; [10]). This means that 95.62% of the response variable change was explained by the independent variable (contact time), confirming that it fit the regression model. The predicted R^2 value is in reasonable agreement with an adjusted R^2 of 91.2%. Besides, the significance value (p-value) of 0.044 is less than the significance level of 0.05. According to [28], a small p-value implies a more significant effect on the corresponding response variable. This result also confirms that the model is statistically valid and significant. The proposed second-order quadratic regression model for the effects of extraction time on yield is given in Equation 2;

$$y = -0.00002510x^2 + 0.01548x - 0.6898 \quad (2)$$

It has been reported that coefficients of polynomial regression equations such as quadratic model equations cannot be easily or readily interpreted partly because they are not comparable ([8]; [24]). Notwithstanding, from the model equation, 0.6898 is the y-intercept, and since the coefficient of the quadratic term is negative, its curve turned downward (concave shape), as shown in Figure 2. To test the significance of the coefficients, the p-value was used to check each coefficient's significance. It was found that the linear coefficients (x) have a p-value of

0.016, less than 0.05, which indicates its significance, while the quadratic term coefficient (x^2) has a p-value of 0.220, which was greater than 0.05. Therefore, quadratic term coefficients were not significant since it is greater than 0.05 significance levels. The quadratic model equation is simply interpreted as follows; for every 60 minutes, the average yield will increase by 0.0154 in linear term(x) while the yield will decrease by 0.00002510 in the quadratic term (x^2). The experimental oil yield predicted oil yield, and residual output of the extraction time effect are presented in Table 4. The predicted results and the residual output were obtained on fitting the experimental data into the proposed model.

TABLE 4: EFFECTS OF EXTRACTION TIME ON OIL YIELD

Time (minute)	Experimental yield (%)	Predicted oil yield (%)	Residual yield (%)
60	0.235	0.149	0.086
120	0.592	0.807	-0.215
180	1.412	1.284	0.128
240	1.625	1.581	0.044
300	1.653	1.696	-0.043

Table 4 shows that the highest experimental oil yield observed was 1.653 for an extraction time of 300 minutes, which is in agreement with 1.696 yields predicted using the quadratic regression model. It was observed that the oil recovery increases with time for experimental and predicted yield; this finding is in agreement with [15]. A plot of experimental yield against time is presented in Figure 2.

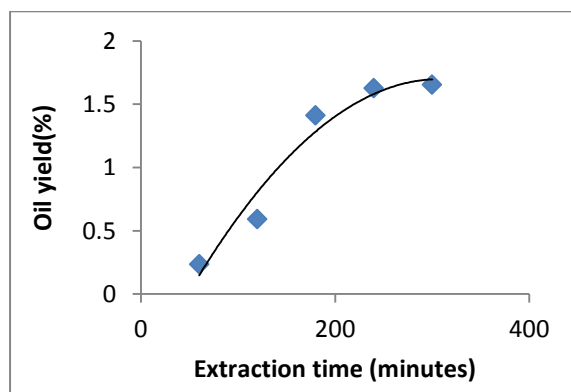


Figure 2: Oil yield versus extraction timeline fit plot

C. Effect of solvent volume on oil yield

The experimental data results fitted into different Microsoft Excel regression models on the effect of solvent volume on yield are presented in Table 5. As reported in the proceeding variable, exponential and power models were not also considered in this discussion. From Table 5, among linear, quadratic, cubic, and logarithmic models, the quadratic model

was found to be better in terms of R^2 and significance value. This polynomial (quadratic) model equation was adequate for prediction within the range of experimental data as its coefficient of regression (R^2) was found to 99.55%.

TABLE 5: MODEL EQUATIONS FOR EFFECT OF SOLVENT VOLUME ON YIELD

Model type	Model equation	R^2	p-value
Linear	$y = 0.000044x + 1.606$	94.53%	0.006
Quadratic	$y = -0.0000001714x^2 + 0.000113x + 1.600$	99.55%	0.004
Cubic	$y = 0.0000000007x^3 - 0.0000006x^2 + 0.0002x + 1.5954$	99.75%	0.064
Exponential	$y = 1.6056e^{3-05x}$	94.50%	0.006
Logarithmic	$y = 0.0082\ln(x) + 1.5714$	99.30%	0.000
Power	$y = 1.572x^{0.0051}$	99.28%	0.000

The predicted R^2 value for the quadratic model was 99.55%, and its p-value is 0.004, which is less than 0.05 significance levels. This value indicates that the solvent volume causes 99.55% of the changes in oil yield. In other words, 0.45 % of the changes could not be explained by the model due to other factors. The obtained R^2 is higher than 80% recommended for a good fit model ([17]; [10]). The predicted R^2 value is in reasonable agreement with an adjusted R^2 of 99.1%. Besides, the significance value (p-value) of 0.004 is less than the significance level of 0.05, which implies a more significant effect on the corresponding response variable [28]. This result also confirms that the model is statistically valid and significant. The proposed second-order quadratic regression model for the effect of solvent volume on yield is given in Equation 3:

$$y = -0.0000001714x^2 + 0.000113x + 1.600 \quad (3)$$

According to ([8]; [24]), the coefficients of polynomial regression equations such as quadratic model equations are challenging to interpret partly because they are not comparable. Notwithstanding this claim, from the model equation (3), 1.600 is the y-intercept, and since the coefficient of the quadratic term is negative, its curve is turned downward (concave shape), as shown in Figure 3. To test the significance of the coefficients, the p-value was used to check the significance of each coefficient. It was found that the linear coefficients (x) have a p-value of 0.006 ($p < 0.05$), which indicates it is significant, and the quadratic term coefficient (x^2) has a p-value of 0.042 ($p < 0.05$). Since the linear and quadratic terms have a p-value of less than 0.05 significance levels, they are significant. This result has indicated the model's efficacy as a good representation of the interaction between the variables considered within the experimental data. The quadratic model equation is simply interpreted as follows: for every 100ml, the average yield will increase by 0.000113 in linear term(x) while the yield will decrease by

0.0000001714 in the quadratic term (x^2). The experimental oil yield, predicted oil yield, and residual output of solvent volume is presented in Table 6. The predicted results and the residual output were obtained on fitting the experimental data into the proposed model.

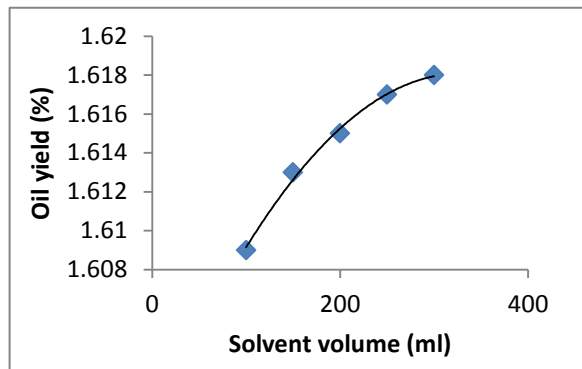


Figure 3: Oil yield against solvent volume line fit plot

TABLE 6: EFFECTS OF SOLVENT VOLUME ON OIL YIELD

The volume of solvent (ml)	Experimental yield (%)	Predicted oil yield (%)	Residual yield (%)
100	1.609	1.6091	-0.0001
150	1.613	1.6126	0.0004
200	1.615	1.6153	-0.0003
250	1.617	1.6170	0.0000
300	1.618	1.6179	0.0001

From Table 6, the values of solvent volume, when applied in the proposed model equation, the predicted extraction yield ranges between 1.6091% - 1.6179%. Thus, predicted values from fitted equations and observed values were in agreement. These results also show the reliability of the proposed model when applied in large scale production.

IV. CONCLUSION

The predicted oil yield results on applying the various model equations were 1.586 for 0.5cm particle size, 1.696 for 300 minutes contact time, and 1.6179 for 300ml solvent volume. Finally, the studied operating parameters have a healthy relationship concerning oil yield. The fitted models adequately approximate the response variable (yield). Therefore the model equations can be used satisfactorily to predict any value of yield for Soxhlet extraction of lemongrass essential oil within the defined experimental range of values.

REFERENCES

[1] Amenaghawon, N. A., Okhueleigbe, K. E., Ogbeide, S. E. and Okieimen, C. O. (2014). "Modeling the Kinetics of Steam Distillation of Essential Oils from Lemon Grass (*Cymbopogon Spp.*)". International Journal of Applied Science and Engineering, 12(2), p. 107-115

- [2] Amiya, K.J. (2011). "Chemical Process Modelling and Computer Simulation", Second Edition. PHI Learning Private Limited, 111 Patparganj Industrial Estates. New Delhi, India.
- [3] Carlson, L. H. C., Machado, R. A. F., Spricigo, C. B., Pereira, L. K. and Bolzan, A., (2001). "Extraction of lemongrass essential oil with dense carbon dioxide." Journal of Supercritical Fluids, 21, p. 33-39.
- [4] Ceylan, H. (2007). "Control and Simulation Studies for a Multicomponent Batch Packed Distillation Column", Turkey: Graduate School of Natural and Applied Sciences of Middle East Technical University.
- [5] Chantal, S., Prachakoli, S., and Ruangviriyachai, C. (2012). "Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass and lemongrass grown in Thailand". Journal of AOAC International, 95: 763-772.
- [6] Desai, M.A., Parikh, J., and De, A. K. (2014). "Modeling and optimization studies on extraction of lemongrass oil from *Cymbopogon flexuosus* (Steud.) Wats". Chemical Engineering Research and Design, 92, p.793-803. <http://dx.doi.org/10.1016/j.cherd.2013.08.011>
- [7] Djouahri, A., Boudarene, L., Meklati, B.Y. (2013). "Effect of extraction method on chemical composition, antioxidant and anti-inflammatory activities of essential oil from the leaves of Algerian *Tetraclinis articulata* (Vahl) Masters". International Journal of Industrial crops and products, 44, p.32-36, <https://doi.org/10.1016/j.indcrop.2012.10.021>
- [8] Edwards, J.R., and Parry, M.E. (1993). "On the use of polynomial regression equations as an alternative to difference scores in organizational research". Academy of management journal, 36(6): p.1577-1613.
- [9] Hesham, H.A., Abdurahman, H.N. and Rosli, M.Y. (2016). "Techniques for extraction of essential oils from plants: A review". Aust. J. Basic & Appl. Sci., 10(16), p. 117-127.
- [10] Joglekar, M. and May, A. T. (1987). "Product excellence through design of experiments", "Cereal Foods World, 32, p. 857- 868.
- [11] Joy, P.P., Mathew, S., Skaria, B.P., Mathew, G., Joseph, A. and Sreevidya, P.P. (2006). "Lemongrass Aromatic and Medicinal Plants Research Station", Odakkali, Asamannoor, Kerala, India, p.1-32.
- [12] Lee, C. L., and Wang, W. L. (1997). "Biological Statistics (Beijing, People's Republic of China, Ed.)". Science Press.
- [13] Lee, W.C., Yusof, S., Hamid, N.S.A., and Baharin, B.S. (2006). "Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM)". Journal of Food Engineering, 73, p.55-63.
- [14] Middleton, M.R. (2010). Better Exponential Curve Fitting using Excel. <http://www.DecisionToolworks.com/ExpCurveFit2010.pdf>
- [15] Nguyen, D.B.T., Ta, H. D. and Nguyen, T.D. (2017). "Kinetics and modeling of oil extraction from vietnam lemongrass by steam distillation". Vietnam Journal of Science and Technology, 55(5A), p. 58-65.
- [16] Okpo, S.O and Otaraku, I.J. (2020). "GC-FID and FT-IR Characterization of Lemongrass Oil Extracted with Soxhlet Extraction Apparatus Using Ethanol as Solvent." IOSR Journal of Engineering (IOSRJEN), 10(5), pp. 33-38.
- [17] Oyekunle, D. T., and Oyekunle, D. O. (2018). "Biodiesel production from yellow oleander seed oil via heterogeneous catalyst: performance evaluation of minitab response surface methodology and artificial neural network." Journal of Materials and Environmental Sciences, 9(8), p.2468-2477
- [18] Reverchon, E. and Marco, I.D. (2006). "Supercritical fluid extraction and fractionation of natural matter". Journal of supercritical fluids, vol. 38, p. 146-166.
- [19] Sayyar, S., Zainal, A.Z., Yunus, R. And Muhammad, A. (2009). "Extraction of oil from *Jatropha* Seeds optimization and kinetics". American Journal of Applied Sciences, 6(7), p.1390-1395.
- [20] Schantz, M. M. and Hawthorne, S.B. (1998). "Comparison of Supercritical Fluid Extraction and Soxhlet Extraction for

- the determination of polychlorinated biphenyls in Environmental Matrix Standard Reference Materials". *Journal of Chromatography A*, 816: p.213-220.
- [21] Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., and Mann, A., (2011). "Scientific basis for the therapeutic use of *Cymbopogon citratus*, Stapf (Lemon grass)", *J. Adv. Pharm. Technol. Res.*, 2 (1), p.3-8.
- [22] Shah, M., Shrivastava, P. and Singh, N. (2014). "Microwave-assisted extraction of lemongrass essential oil: Study of the influence of extraction method and process parameters on extraction process". *Journal of Chemical and Pharmaceutical Research*, 6(11), p.385-389. Available online www.jocpr.com
- [23] Sirisompong, W., Jirapakkul, W. and Klinkesorn, U. (2011). "Response surface optimization and characteristics of rambutan (*Nephelium lappaceum* L.) kernel fat by hexane extraction". *LWT Food Science and Technology* 44 (9), p.1946-1951.
- [24] Stimson, J.A., Carmines, E.G. and Zeller, R.A.(1978). "Interpretation of Polynomial Regression", *Sociological methods and research*, volume 6, p. 515-534.
- [25] Suryawanshi, M.A., Mane, V.B. and Kumbhar, G.B. (2016). "Methodology to Extract Essential Oils from Lemongrass leaves: solvent extraction approach". *International Research Journal of Engineering and Technology (IRJET)*, 3 (8):1775-1780.
- [26] Temitayo, O.D. (2017). "Optimization of Oil Extraction from *Thevetia Peruviana* (Yellow Oleander) Seeds: A Case Study of Two Statistical Models". *International Journal of Engineering and Modern Technology*, 3(4), 2504-8856.
- [27] Upreti, S. R. (2017). "Process Modelling and Simulation for Chemical Engineers: Theory and Practice", Chichester, UK; Hoboken, NJ: John Wiley & Sons.
- [28] Yolmeh, M., Habibi, N.M. and Farhoosh, R. (2014). "Optimization of Ultrasound-assisted Extraction of Natural Pigment from Annatto Seeds by Response Surface Methodology (RSM)". *Food Chem* 155: 319-324
- [29] Zaibunnisa, A.H., Norashikin, S., Mamot, S., and Osman, H. (2009). "An experimental design approach for the extraction of volatile compounds from turmeric leaves (*Curcuma domestica*) using pressurised liquid extraction (PLE)". *LWT, Food Science and Technology*, 42: 233-238.
- [30] Jack, I. R. and Nwachoko, N, "Constituents of the ethanolic extract of *Bryophyllum Pinnatum* leaves" *SSRG International Journal of Applied Chemistry* 3.1 (2016): 1-4.