IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

ISOLATION OF ESSENTIAL OIL FROM BUDS OF SYZYGIUM AROMATICUM USING HYDRODISTILLATION: MULTI-RESPONSE OPTIMIZATION AND PREDICTIVE MODELLING

Sagar Kapadiya[1] Meghal A. Desai[2]

[1]*Chemical Engineering Department, Shroff S. R. Rotary Institute of Chemical Technology, Bharuch, Gujarat, (India)*

[2] *Department of Chemical Engineering, Sardar Vallabhbhai National Institute of Technology, Surat, Gujarat, (India)*

ABSTRACT

Clove oil is a high value spice utilized in food industries as preservatives, flavor and antioxidants, and in pharmaceutical industries. In this research article, multi-response optimization of process parameters of hydrodistillation for extraction of essential oil from clove buds using the Taguchi method and grey relational analysis was implemented. Yield of clove oil, yield of eugenol and zone of inhibition were selected as the responses. Optimum condition was achieved as 20 g of solid loading, 300 mL volume of water, 565 micron size of clove buds and 45 minute of extraction time. Under these optimized conditions, extraction yield of clove oil (11.35 %, w/w), yield of eugenol (10.30 %, w/w) and zone of inhibition (21 mm) were achieved. Artificial neural network was applied for predictive modelling, which provided better accuracy in case of 4-8-3 topology and tangent sigmoidal transfer function for hidden layer (mean squared error = 0.236).

Keywords: Artificial neural network, Grey relational analysis, Multi-response optimization, Taguchi method

I. INTRODUCTION

Many parts of plant like flower, fruit, leaves comprise “essential oils” which are typically rich in organic compounds with high boiling points. These oils pertain an important place in medicines, perfumes, flavoring agents, etc. [1]. Oil extracted from clove buds falls under the category of essential oil. *Syzygium aromaticum*, commonly named as clove, is widely cultivated in Indonesia, India, Malaysia, Srilanka, Madagascar and Tanzania. Indonesia is the largest producer of clove with an average of 66 % of the total world produces (63,700 tons) annually [2]. According to data from Spices board, Government of India, India has produced 1160 tons of Clove in year 2010-11 (Vision 2050, Indian Council of Agricultural Research). A Gross market value was US$ 30–70 million per annum through its function as flavor and fragrance ingredient and antibacterial agent [3]. Clove is one of the key natural sources of phenolic compounds. Eugenol (4-allyl-2-methoxy phenol) is the most active component of clove oil which is a phenylpropene, an allyl chain-substituted guaiacol [4-5]. Other

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

phenolic acids like caffeic, ferulic, elagic and salicylic acids are found in clove. Quercetin, kaempferol and its derivatives as flavonoids are presents in lower proportion in clove oil [6].

Clove oil containing a higher amount of eugenol has shown strong antimicrobial activity against multi-resistant *Staphylococcus epidermidis* and pathogenic bacteria including *Herpes simplex* and hepatitis C viruses [7]. Clove oil could inhibit the growth of the larvae, *Aedes aegypti* (Diptera: Culicidae) which is responsible for dengue [6]. Clove bud oil is used as a local anesthetic in dentistry, ingredient in dental cement for temporary filling [8] and mucosal inflammations. Bioactivity studies have shown that various compound in clove bud oil possess antifungal, antiseptic, anesthetic, antispasmodic, carminative, anti-inflammatory, cytotoxic, and insect repellant activities [7, 9-11].

Driven by the potential use of the essential oil in various sectors, clove oil had been extracted by solvent extraction using dichloromethane and hexane [12], steam distillation [13], hydrodistillation [11, 14-17]. However, consumption of organic solvents, extended time for extraction, labour intensiveness, higher energy consumption and greater environmental impact are the downsides of these techniques. For greener production, supercritical carbon dioxide extraction [2, 4, 18-19] was employed giving improved yield and purity. This technique has a major limitation of high operating pressure which creates safety concern as well as cost ineffectiveness.

It is expected that short term and medium term demand of clove oil should be satisfied with proper quality. Extraction processes suffering from few drawbacks need to be improved. Also, optimization of process variables is required to improve profitability. Till now all the experiments on hydrodistillation for clove oil were performed to study the yield and components at a fixed condition. There is no multi-response optimization study performed which is very important to find industrial scope. The primary objective of this study was to perform multi-objective optimization incorporating yield of essential oil, yield of eugenol and zone of inhibition as responses.

By aiming this, the first step was to execute a parametric study which could explain the effect of different parameters viz. solid loading, water volume, particle size and time on the responses. Multi-response optimization was performed using the Taguchi method and Grey relational analysis. To study the effect of each parameter on response, analysis of variance (ANOVA) study was performed, in qualitative as well as quantitative manner. Moreover, analytical study was performed to assess the quality of extracted clove oil. The results obtained by experiments were used to train the artificial neural network (ANN) model that would give prediction for each response at a given condition. The results show that the application of ANN offers acceptable prediction for any combination of parameters for clove oil extraction using hydrodistillation. The results obtained from the present work would be beneficial for higher scale studies.

II. MATERIALS AND METHODS

2.1 Materials

Dried clove buds (*Syzygium aromaticum*) were bought from a local market of Surat, Gujarat, India and stored at a room temperature in the moisture free environment. The samples were ground by domestic grinding machine to get the different particle size distribution, measured by mechanical sieves. Eugenol (98% pure) was procured from Spectrochem Pvt. Ltd., Mumbai, Maharastra, India and Methanol (HPLC grade) was purchased from

Lombart Fine Chem. Ltd., Surat, Gujarat, India for the analysis purpose. The Mueller Hinton agar media was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

2.2 Methods

2.2.1 Hydrodistillation

For the hydrodistillation, circulatory Dean and Stark type apparatus (1 L capacity) was used. The rehydrated raw material was kept in a round bottom flask along with water. During hydrodistillation, vaporization of essential oil would occur which was carried along with water vapour towards the condenser. The condensed water and clove bud oil were then decanted and separated. The whole extraction process was operated at atmospheric pressure and temperature attained inside the system was boiling point of water. Experiments were performed in duplication to check accuracy and reproducibility of the process. After removing moisture from the oil, it was stored at 2 °C. Detailed investigation was performed to study the effect of major parameters (solid loading, solid to water ratio, size of the clove buds and time of extraction) on the extraction yield (Percentage yield, Y = mass of oil collected, g × 100/ mass of clove bud, g), eugenol content, and antimicrobial activity in terms of the zone of inhibition.

2.2.2 Parametric study

Based on literature survey, four effective independent variables were selected for their impact on responses, viz., solid loading (10-50 g), volume of water (12-20 mL per g of material), particle size of clove buds (whole buds to 565 micron) used and extraction time. Clove buds were soaked in water for 15 minute for rehydration in order to fasten the hydrodistillation. The power was kept constant at 360 W.

2.2.3 Design of experiment

Design of Experiment is an effective tool to optimize the process parameters. Using the Taguchi method, well-defined sets of experiments were planned and performed for four factors, i.e., solid loading, water volume, particle size and extraction time with four levels. Details of various factors and their levels are mentioned in the TABLE 1. Minitab 17.0 (Minitab Inc., PA, U.S.A.) was used for planning of experiments and calculation of S/N ratio. Grey relational analysis (GRA) was used to combine all the responses and convert them into a single response. Based on GRA a set of optimum conditions were found and ANOVA was performed [20-21].

TABLE 1 Factors and their levels

Factors	Levels			
	1	2	3	4
A: Solid loading (g)	40	30	20	10
B: Extraction time (minute)	15	25	35	45
C: Particle size* (micron)	1	2	3	4
D: Water volume (mL)	300	400	500	600

*1: 565 micron, 2:926 micron, 3: 1500 micron, 4: Whole clove buds

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

2.2.4 Artificial neural network (ANN)

ANN is a useful tool to establish and analyze a nonlinear relationship in the system. These models are very important to correlate and predict the diverse performance [22]. The concept of artificial neural network for the prediction was reported in the literature in different fields [23-30]. However, there is no research article reporting prediction of multi-response objective in extraction of essential oil.

Results from the preliminary investigation and L_{16} array were utilized to evaluate the behaviour of response function. The data were randomly divided into training, testing and validation set. The commercial neural network toolbox of MATLAB R2014a (The Mathworks Inc., Ver., 8.3, Natick, Massachusetts, USA) was utilized for ANN studies. A multilayer perceptron neural network (MLPNN) was designed which could relate multiple responses to control variables. Architecture of a neural network consists of an input layer, hidden layer and output layer. Neurons are arranged in two different layers (hidden layer and output layer). Hidden layer with any sigmoid transfer function and output layer with linear activation function will have capability to approximate any function [26].

In present study, a number of neurons in hidden layers were varied from 4 to 10 and based on trial and error, optimum neurons were retrieved to obtain minimum mean squared error (MSE) and the highest regression coefficient (R^2) [24]. Four independent variables, i.e., solid loading, water volume, size and time were considered in input layer. Yield of clove oil, yield of eugenol and zone of inhibition were selected as output for modelling of ANN. Based on input and output layer, 4-x-3 topology was selected with 80 data sets where x is a number of neurons in the hidden layer.

2.2.5 Gas chromatography (GC)

The essential oil was analyzed by GC (TRACE 1110, Thermo Fisher Scientific India Pvt Ltd, Navi Mumbai, Maharashtra, India) using AB-5 column (30 m × 0.25 mm × 0.25 μm). The conditions maintained for obtaining GC spectra were: carrier gas N_2 with the flow rate of 1.2 mL/minute, splitless injection mode with flame ionization detector (FID) and sample injection volume 1 μL. Injector and detector temperature were set at 225 °C and 270 °C, respectively. Oven temperature was kept at 110 °C with hold time of 8 minute and then increased to 220 °C at a rate of 15 °C per minute and then hold at 220 °C for 2 minute [31].

2.2.6 Antimicrobial activity

The antimicrobial activity of clove oil was determined by modified Kirby-Bauer well diffusion method. Briefly, pure isolate of *Bacillus subtilis* (ATCC 6633, MTCC 441) was first subcultured in nutrient broth at 37 °C for 24 hours. One hundred microliters (100 μL) of the standardized inoculum (0.5 Mac-Farland) of *Bacillus subtilis* was spread with the help of sterile spreader on to a sterile Mueller-Hinton agar (4 mm depth or 60 to 70 mL) so as to achieve a confluent growth. The plates were allowed to dry and four wells of 5 mm diameter each were made in the agar petriplates. Subsequently, a 50 μL volume of the known concentrated oil (5 %) was introduced in wells into Mueller-Hinton agar plate. The plates were allowed to stand for at least 1 hour for diffusion to take place and then incubated at 37 °C for 16 hour. If the plates were satisfactorily streaked and inoculum was correct, the resulting zone of inhibition (ZOI) will be uniformly circular. The diameter of the zone of complete inhibition was measured, known as the zone of inhibition.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

III. RESULTS AND DISCUSSION

3.1 Parametric Study

3.1.1 Effect of solid loading:

Solid loading plays an important role in maximizing the yield of essential oil and eugenol with improved antimicrobial activity during HD. The effect of different solid loadings was studied to observe its impact on various responses while keeping other parameters constant (size of plant material: 1003 micron; solid to water ratio: 1:14; power: 360 W; extraction time: 45 minute) as shown in Fig. 1. As the amount of solid loading was increased from 10 g to 50 g, a decrease in yield of clove oil was observed after 20 g of solid loading. As solid loading increases, energy distribution per particle will get reduced. Further, heating power was kept constant resulting in less heating phenomena which reduced the recovery of oil. Similar observation was made by Parikh and Desai [32]. Yield of eugenol followed the similar extraction pattern as of yield of clove oil. However, a decrease in yield was observed after 30 g of solid loading.

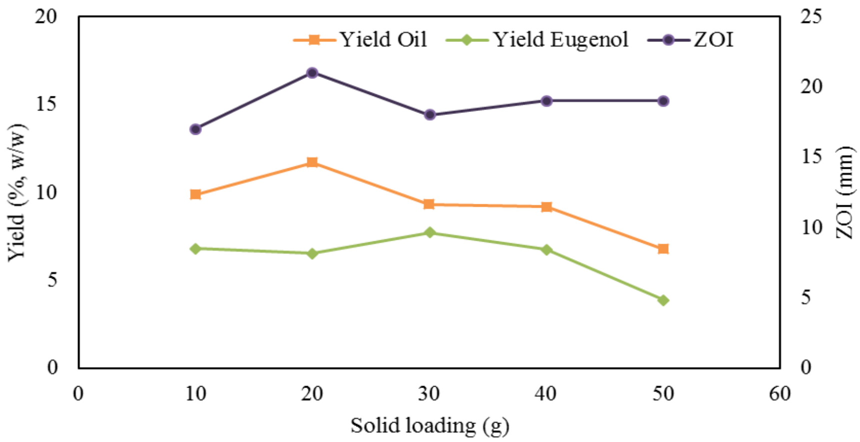

Fig. 1. Effect of solid loading on extraction efficiency in HD (Constant parameters: solid to water ratio 1:14; particle size 1003 micron; time 45 minute; power 360 W)

A zone of inhibition for *Bacillus subtilis* microbe was maximum at 20 g of solid loading and remained almost constant for other cases. ZOI depends not only eugenol but all other phenolic compounds, which were present in varying quantity. Apart from eugenol, eugenyl acetate, caryophyllene and caryophyllene oxide are main components found in clove oil which inhibit growth of *Bacillus subtilis*. The highest yield of oil (11.68 %, w/w) and ZOI (21 mm) were observed at 20 g of clove. Hence, further experiments were performed using 20 g of material.

3.1.2 Effect of water volume

The volume of water to be added should be selected in such a way that proper hydration of plant material should take place as well as water should remain in adequate amount so that it can be a barrier to avoid overheating of plant material, thus avoid charring. The amount of water was increased from 240 mL to 400 mL at 20 g solid loading, 360 W power, 1003 micron size material and 45 minute of extraction time.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

In case of 240 mL water volume, a lower yield of clove oil was observed (Fig. 2). Further charring would affect the quality of essential oil. A higher amount of eugenol (7.758 %, w/w) was obtained at 360 mL volume of water. At this condition, water was not enough to complete the extraction and charring was observed leading to a lower yield of oil and eugenol. However, an increase in water volume increases the amount of heat required to reach boiling point of water, leading to incomplete extraction in a given time period. Also, a hydrolytic effect might have contributed to the lower yield [33].

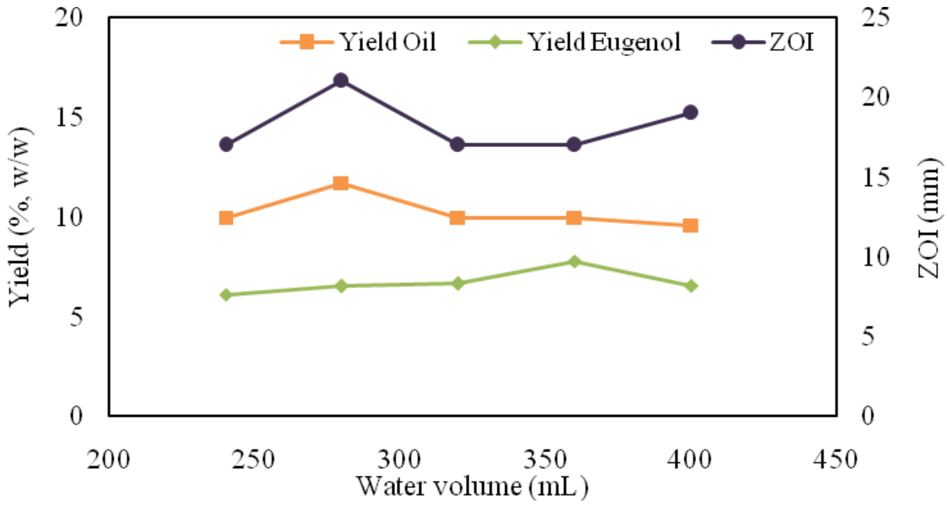

Fig. 2 Effect of water volume on extraction efficiency in HD (Constant parameters: solid loading 20 g; particle size 1003 micron; time 45 minute; power 360 W)

Antimicrobial activity of oil was found to be the maximum for the highest clove oil yield, which showed that a mixture of all phenolic compounds affects more on inhibitory effect of oil. Based on two responses, viz., clove oil yield (11.68 %, w/w) and zone of inhibition (21 mm), 280 mL of water volume was selected for further studying other parameters.

3.1.3 Effect of particle size

Size of clove buds is one of the most important parameter. Extraction was started using whole clove buds and 4 size variations were made with the lowest size of 565 micron (Fig. 3). As the particle size of buds decreases, an increase in yield of essential oil and eugenol was observed. This could be explained based on the fact that an increase in the available surface area with decrease in particle size, leads to a better heat and mass transfer. For large size particles, heat would not distribute into whole material. Some part of clove bud might remain unused, which could result in decreased extraction yield. The highest zone of inhibition (21 mm), extraction yield of clove oil (12.074 %, w/w) and eugenol (8.094 %, w/w) were achieved for the 565 micron size of particles, which was selected for further experiments.

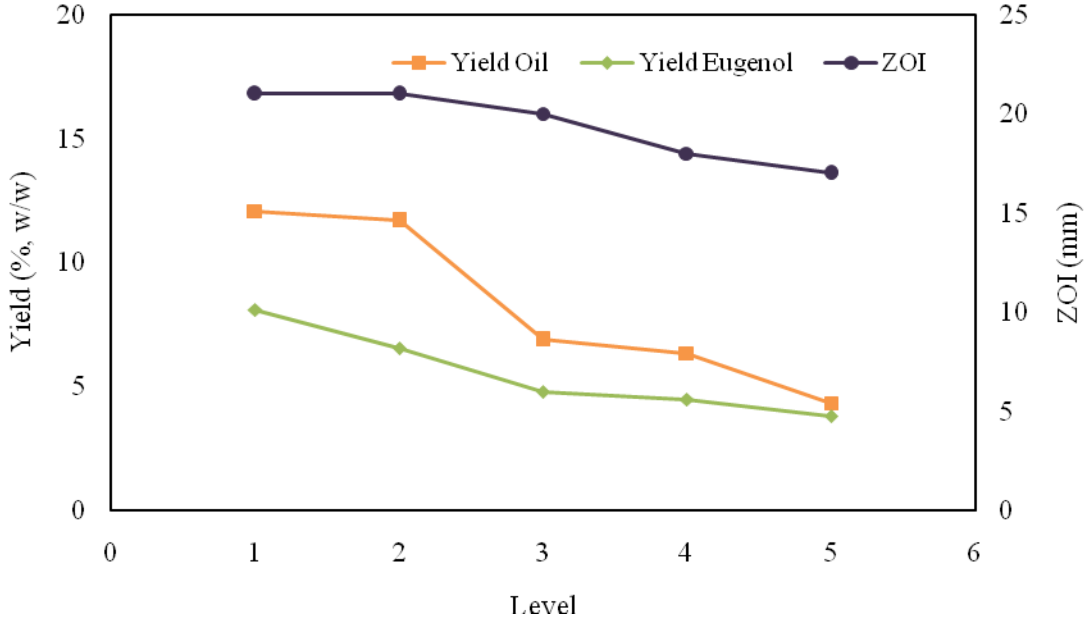

Fig. 3 Effect of particle size on extraction efficiency in HD (Level 1- 4. 565 – 1003 – 1500 – 2400 micron; Level 5. whole clove buds; Constant parameters: 20 g solid loading; 280 mL water volume; time 45 minute; power 360 W)

3.1.4 Effect of time

Time is the very important criteria, makes more impact on the efficacy of extraction. The rate of extraction was high at the initial stage of process and then reduced after 25 minute of time (Fig. 4). To measure the total time required for complete extraction, experiment was performed till 50 minute but charring was observed after 45 minute of extraction time. A decrease in yield of eugenol was observed after 35 minute of extraction, while ZOI was constant after 40 minute of extraction. The highest zone of inhibition (21 mm), extraction yield of clove oil (12.074 %, w/w) and eugenol content (8.094 %, w/w) were achieved after 45 minute. Prolonging the extraction time period did not make improvement in yield of essential oil, but it has increased power consumption and wastage of biomass due to charring.

3.2 Multi-Response Optimization

3.2.1 Optimization of extraction conditions

Using Taguchi techniques, experimental planning was done using L_{16} array (TABLE 2) and accordingly experiments were performed in duplication. Yield of clove oil, yield of eugenol and zone of inhibition were selected as responses. All the responses (S/N ratios) were converted to a single response (Grey relational grade-GRG) for optimization of process parameters (TABLE 3) and ANOVA studies. The procedure is outlined in the supplementary material. Based on GRG, level total of GRG was found for each factor at each level as shown in the TABLE 4. The level corresponding to the maximum value of total GRG among four levels would be selected for the optimal set of parameters. From the TABLE 4, it can be seen that the optimum parameters are 20 g of solid loading, 300 mL volume of water, 565 micron size of clove buds and extraction time of 45 minute.

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 01 , January 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

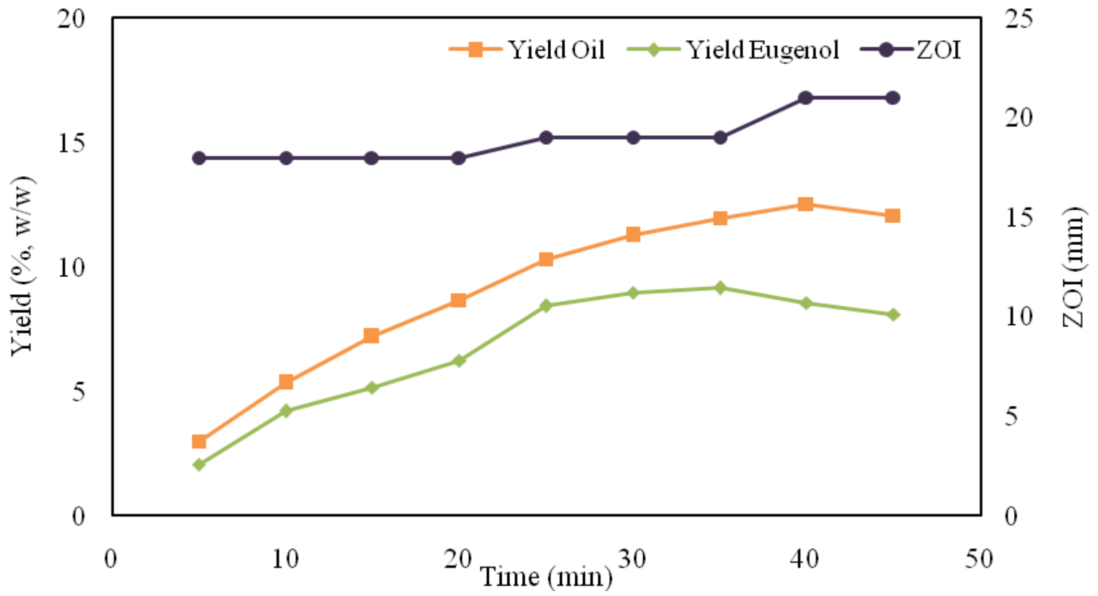

Fig. 4 Effect of extraction time on extraction efficiency in HD (Constant parameters: solid loading 20 g; water volume 280 mL; particle size 565 micron; power 360 W)

3.2.2 Analysis of variance (ANOVA)

ANOVA was performed to identify the significant effect of each parameter on three responses, viz., extraction yield of clove oil and eugenol, and zone of inhibition. *F*-test was performed using variance of factor and variance of error. Higher value for the calculated *F*, the more significant was the corresponding coefficient. As seen from the TABLE 5, the calculated *F*-value for B (extraction time) was higher than *F*-values for A, B and D (water volume) while factor D was found to be statistically insignificant. Percentage contribution of factor B (extraction time) was 41.0061 while for factor D (water volume) it was 5.1979. The order of factors based on their magnitude of influence is as follows: Extraction time > Solid loading > Particle size > Water volume. Hence, a change in extraction time can have the highest impact on the responses followed by solid loading and particle size.

TABLE 2 Experimental layout using L16 or thogonal array and the responses

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 01 , January 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

3.2.3 Confirmation experiment

Multi-response optimization is more advantageous than a single response, in which single optimized condition

Exp. No.	Factors				Yield of essential oil		Yield of eugenol		ZOI (mm)
	A	B	C	D	Y_1 (%, w/w)	Y_2 (%, w/w)	y_1 (%, w/w)	y_2 (%, w/w)	
1	40	15	1	300	4.6115	5.0325	2.7711	2.6002	19
2	40	25	2	400	5.8585	5.7610	4.1565	4.1761	18
3	40	35	3	500	5.9767	6.5173	4.4129	4.8504	19
4	40	45	4	600	3.7430	3.8730	3.2864	3.2160	18
5	30	15	2	500	4.1480	3.9590	2.8689	2.8997	18
6	30	25	1	600	7.3210	7.4240	5.3138	5.4024	20
7	30	35	4	300	3.7193	3.7536	3.8031	3.9004	19
8	30	45	3	400	7.2347	7.7140	6.0153	6.0181	19
9	20	15	3	600	3.9270	4.4995	3.2606	3.1667	18
10	20	25	4	500	3.6855	3.5305	2.7914	2.8233	21
11	20	35	1	400	9.4840	9.7560	6.1361	6.0733	18
12	20	45	2	300	8.4320	8.9995	5.8879	6.1097	20
13	10	15	4	400	1.1570	1.0790	0.7454	0.6782	17
14	10	25	3	300	6.3710	6.3040	3.9558	3.7866	19
15	10	35	2	600	4.2400	4.4990	2.8138	2.7340	17
16	10	45	1	500	7.8750	7.9270	4.2820	4.3686	17

can be achieved for the best experimental results for all three responses. In order to validate the optimized condition obtained, verification experiment was performed at conditions: 20 g solid loading, 300 mL volume of water, 565 micron size of particles and 45 minute extraction time. The results of confirmation experiments represent the highest yield of clove oil (11.35 %, w/w) as well as eugenol (10.30 %, w/w) with an acceptable zone of inhibition (21 mm).

3.2.4 Prediction using artificial neural network

Neural network tool of MATLAB was used to design ANN. Total 80 data sets were divided in 70 %, 15 % and 15 % for training, testing and validation respectively. Pure linear was selected as a transfer function for the output layer. Variations were made by selecting different transfer functions (pure linear, log sigmoidal, tangent sigmoidal) for neurons of the hidden layer. Among these, tangent sigmoidal was selected as the transfer function which produced the best results as shown in the TABLE 6. Levenberg-Marquardt back propagation was selected as a training algorithm because it is robust, fast and accurate to reach the target value. The gradient descent momentum was adapted as a learning algorithm to change weight values of the neural network. Performance of the neural network was measured using the mean squared error.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

TABLE 3 S/N Ratios and Grey Relational Grade

Exp. No.	$(S/N\ ratio)_Y$	$(S/N\ ratio)_y$	$(S/N\ ratio)_{ZOI}$	GRG
1	13.6397	8.5677	25.5750	0.7195
2	15.2822	12.3949	25.1054	0.7459
3	15.8890	13.2856	25.5750	0.7986
4	11.6101	10.2393	25.1054	0.6836
5	12.1495	9.2003	25.1054	0.6777
6	17.3516	14.5793	26.0206	0.8817
7	11.4489	11.7110	25.5750	0.7324
8	17.4580	15.5871	25.5750	0.8555
9	12.4321	10.1372	25.1054	0.6899
10	11.1393	8.9655	26.4443	0.8072
11	19.6609	15.7129	25.1054	0.8594
12	18.7923	15.5569	26.0206	0.9199
13	0.9530	-2.9820	24.6089	0.5
14	16.0380	11.7505	25.5750	0.7804
15	12.7971	8.8592	24.6089	0.6544
16	17.9535	12.7189	24.6089	0.7594

TABLE 4 Response table for grey relational grade

Factors	GRG			
	Level 1	Level 2	Level 3	Level 4
A: Solid loading	2.9477	3.1475	3.2765	2.6944
B: Extraction time	2.5872	3.2155	3.0449	3.2185
C: Particle size	3.2202	2.9981	3.1246	2.7233
D: Water volume	3.1524	2.9609	3.0431	2.9098

TABLE 5 ANOVA results

Factors	Sum of squares	DOF	*F*	Contribution (%)
A: Solid loading	0.0483	3	12.4243	29.8527
B: Extraction time	0.0664	3	17.0661	41.0061
C: Particle size	0.0347	3	8.9650	21.5406
D: Water volume	0.0084	3	2.1633	5.1979
Error	0.0039	3	1	2.4045

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 01 , January 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

TABLE 6 Comparison between different transfer functions

Transfer function	R^2			MSE
	Training	Testing	Validation	
Tangent sigmoidal	0.9956	0.9909	0.9871	0.236
Logistic sigmoidal	0.9953	0.9907	0.9944	0.341
Pure linear	0.9779	0.9786	0.9757	1.61

The best number of neurons (x) in the hidden layer was found by trial and error for 4-x-3 topology. The minimum values of error for training, validation and testing data sets with high R^2 value were obtained with 8 neurons in the hidden layer. Coefficients of regression (R^2) for training, validation and testing were 0.9956, 0.9871 and 0.9909, respectively, suggesting the best fit for experimental and predicted data as shown in supplementary material. A comparison between experimental and predicted results after training of MLP is shown graphically in Fig. 5.

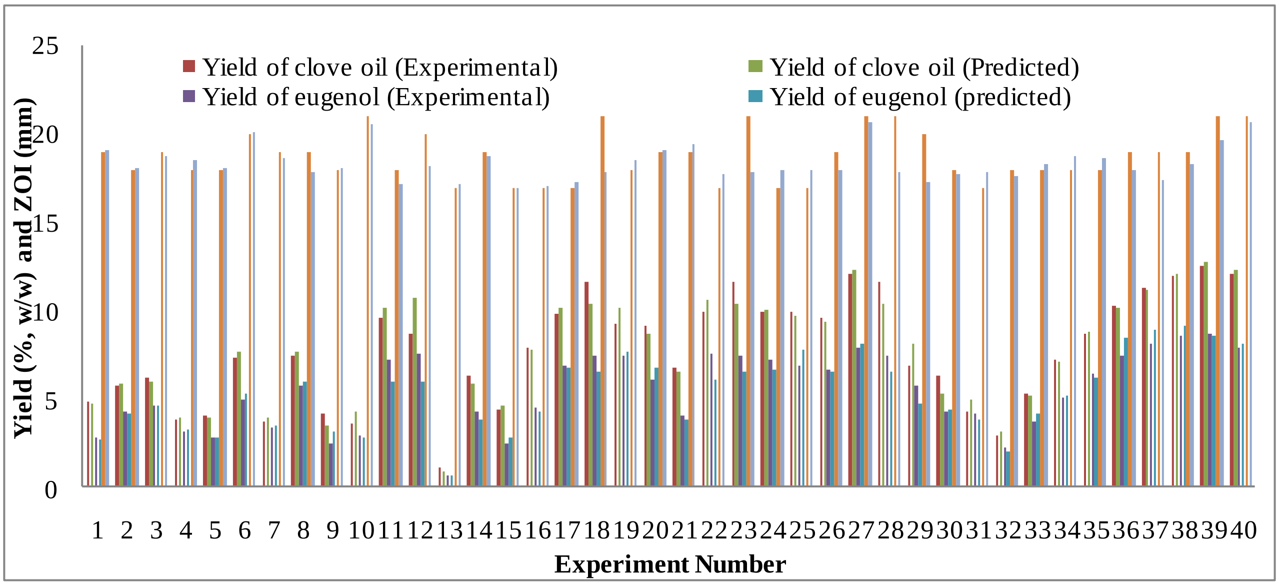

Fig. 5 Comparison between experimental and predicted data

IV. CONCLUSION

In the present study, hydrodistillation was employed for the extraction of clove oil and eugenol as a primary component. The extract exhibited different yield of clove oil and eugenol at different levels with respective zone of inhibition for *Bacillus subtilis* microbes. The hydrodistillation method for extraction of clove oil was found to be the effective method with some improvements to achieve higher yield and antimicrobial activity. Optimum condition was achieved as 20 g of solid loading, 300 mL of water volume, 565 micron size of clove buds and 45 minute of total extraction time with constant 360 W heating power. Under these conditions, extraction yield of

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

clove oil (11.35 %, w/w), yield of eugenol (10.30 %, w/w) and zone of inhibition (21 mm) were achieved. Prediction using Taguchi and ANN methodology imparts applicability to the higher scale study.

REFERENCES

[1] Lubbe and R. Verpoorte, Cultivation of medicinal and aromatic pants for specialty industrial materials, Industrial Crops and Products, 34, 2011, 785-801.

[2] G. Wenqiang, L. Shufen, Y. Ruixiang, T. Shaokun and Q. Can, Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods, Food Chemistry, 101, 2007, 1558–1564.

[3] H. J. Bohnert, H. R. Nguyen and N. G. Lewis, Bioengineering and molecular biology of plant pathways (Vol. 1), Elsevier, California, U.S.A, 2011.

[4] D. Chatterjee and P. Bhattacharjee, Supercritical carbon dioxide extraction of eugenol from clove buds, Food and Bioprocess Technology, 6, 2012, 2587–2599.

[5] G. P. Kamatou, I. Vermaak and A. M. Viljoen, Eugenol-from the remote Maluku islands to the international market place: A review of a remarkable and versatile molecule, Molecules, 17, 2012, 6953–6981.

[6] D. F. Cortes-Rojas, C. R. F. De Souza and W. P. Oliveira, Clove (Syzygium aromaticum): a precious spice, Asian Pacific Journal of Tropical Biomedicine, 4, 2014, 90–96.

[7] K. Chaieb, T. Zmantar, R. Ksouri, H. Hajlaoui, K. Mahdouani, C. Abdelly and A. Bakhrouf, Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. Mycoses, 50, 2007, 403–406.

[8] K. Markowitz, M. Moynihan, M. Liu and S. Kim, Biologic properties of eugenol and zinc oxide-eugenol: A clinically oriented review, Oral Surgery, Oral Medicine and Oral Pathology, 73, 1992, 729– 737.

[9] L. Dhara, and A. Tripathi, Antimicrobial activity of eugenol and cinnamaldehyde against extended spectrum beta lactamase producing enterobacteriaceae by in vitro and molecular docking analysis, European Journal of Integrative Medicine, 5, 2013, 527–536.

[10] H. J. D. Dorman, S. G. Deans, L. Merr and P. Myrtaceae, Antimicrobial agents from plants : antibacterial activity of plant volatile oils, Journal of Applied Microbiology, 88, 2000, 308-316.

[11] M. A. Hossain, S. R. Al Harbi, A. M. Weli, Q. Al-Riyami and J. N. Al-Sabahi, Comparison of chemical constituents and antimicrobial activities of three essential oils from three different brands' clove samples collected from Gulf region, Asian Pacific Journal of Tropical Biomedicine, 4, 2014, 262–268.

[12] M. Sudarma, E. Yuanita and I. W. Suana, Markovnikov addition of chlorosulfuric acid to eugenol isolated from clove oil, Indonesian Journal of Chemistry, 13, 2013, 181–184.

[13] M. H. Alma, M. Ertas, S. Nitz and H. Kollmannsberger, Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum (L.)), Bioresources, 2, 2007, 265-269.

[14] L. Santos, G. O.Chierice, K. S. Alexander, A. Riga and E. Matthews, Characterization of the raw essential oil eugenol extracted from Syzygium aromaticum (L.), Journal of Thermal Analysis and Calorimetry, 96, 2009, 821–825.

[15] N. I. Bhuiyan, J. Begum, N. C. Nandi and F. Akter, Constituents of the essential oil from leaves and buds of clove (Syzigium caryophyllatum (L.) Alston), African Journal of Plant Science, 4, 2010, 451–454.

[16] J. A. Pino, R. Marbot, J. Aguero and V. Fuentes, Essential oil from buds and leaves of clove (Syzygium aromaticum (L.)) grown in Cuba, Journal of Essential Oil Research, 13, 2001, 278–279.

[17] K. Srivastava, S. K. Srivastava and K. V. Syamsundar, Bud and leaf essential oil composition of Syzygium aromaticum from India and Madagascar, Flavour and Fragrance Journal, 20, 2005, 51–53.

[18] E. Reverchon and C. Marrone, Supercritical extraction of clove bud essential oil: Isolation and mathematical modelling, Chemical Engineering Science, 52, 1997, 3421–3428.

[19] G. L. Zabot, M. N. Moraes, A. J. Petenate and M. A. A. Meireles, Influence of the bed geometry on the kinetics of the extraction of clove bud oil with supercritical CO_2, Journal of Supercritical Fluids, 93, 2014, 56–66.

[20] N. Haq, P. Marimuthu and R. Jeyapaul, Multi response optimization of machining parameters of drilling Al/SiC metal matrix composite using grey relational analysis in the Taguchi method, The International Journal of Advanced Manufacturing Technology, 37, 2008, 250-255.

[21] K. Krishnaiah and P. Shahabudeen, Applied design of experiments and Taguchi methods, PHI learning Pvt Ltd, New Delhi, India, 2012.

[22] H. E. Reynel-Avila, A. Bonilla-Petriciolet and G. De la Rosa, Analysis and modelling of multicomponent sorption of heavy metals on chicken feathers using Taguchi’s experimental designs and artificial neural networks, Desalination and Water Treatment, 2014, 1–15.

[23] M. Akintunde, S. O. Ajala and E. Betiku, Optimization of Bauhinia monandra seed oil extraction via artificial neural network and response surface methodology: A potential biofuel candidate, Industrial Crops and Products, 67, 2015, 387–394.

[24] Das, A. K. Golder and C. Das, Enhanced extraction of Rebaudioside-A: Experimental, response surface optimization and prediction using artificial neural network, Industrial Crops and Products, 65, 2015, 415-421.

[25] M. Khajeh, M. G. Moghaddam, and M. Shakeri, Application of artificial neural network in predicting the extraction yield of essential oils of Diplotaenia cachrydifolia by supercritical fluid extraction, Journal of Supercritical Fluids, 69, 2012, 91–96.

[26] R. Noorossana, A. Zadbood, F. Zandi and K. Noghondarian, An interactive artificial neural networks approach to multi response optimization, The International Journal of Advanced Manufacturing Technology, 76, 2014, 765–777.

[27] R. Noorossana, T. S. Davanloo and A. Saghaei, An artificial neural network approach to multiple-response optimization, The International Journal of Advanced Manufacturing Technology 40, 2009, 1227–1238.

[28] J. L. Pilkington, C. Preston and R. L.Gomes, Comparison of response surface methodology (RSM) and artificial neural networks (ANN) towards efficient extraction of artemisinin from Artemisia annua, Industrial Crops and Products, 58, 2014, 15–24.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

[29] K. Rajkovic, M. Pekmezovic and A. Barac, Nikodinovic-Runic, J., Arsić Arsenijević, V., Inhibitory effect of thyme and cinnamon essential oils on Aspergillus flavus: Optimization and activity prediction model development, Industrial Crops and Products, 65, 2015, 7–13.

[30] K. Sinha, P. D. Saha, and S. Datta, Response surface optimization and artificial neural network modelling of microwave assisted natural dye extraction from pomegranate rind, Industrial Crops and Products, 37, 2012, 408–414.

[31] Y. K. Shruthi, B. M. Gurupadayya, K. S. Venkata and T. N. Kumar, Development and validation of GC method for the estimation of eugenol in clove extract, International Journal of Pharmaceutical Science, 6, 2014, 3–6.

[32] J. K. Parikh and M. A. Desai, Hydrodistillation of essential oil from Cymbopogon flexuosus, International Journal of Food Engineering, 7, 2011, Article 11.

[33] M. A. Desai, J. K. Parikh and A. K. De, Modelling and optimization studies on extraction of lemongrass oil from Cymbopogon flexuosus (Steud.) Wats, Chemical Engineering Research & Design, 92, 2014, 793-803.