Abstract: Neem is one of the best gift of nature due to large number of applications such as in medicinal, agricultural, cosmetics industry. The oil obtained from neem seeds plays important role in many industries such as insecticide and fertilizer industry. There are many methods for production of neem seed oil, in this study our emphasis was on to optimize the production of neem seed oil by solvent extraction process, to find the optimal values of independent variables (extraction time, volume of solvent, temperature) on our desired response variable (oil yield). For this purpose we used Box-Behnken method which comes under response surface methodology. The software package that was used was Design Expert 10.0.0 trial version. Based on this 17 experimental runs where taken. Mass of sample was kept 50 grams for all runs and solvent used for each run was hexane.

Keywords: Response Surface Methodology; Soxhlet Extraction; Design Expert 10.0.0. Trial Version

I. INTRODUCTION

Biological name of neem is Azadirachta indica. Neem is considered to be one of the best gift of nature we are blessed with. It is mainly found in india and countries that are in vicinity of india such as Pakistan, Bangladesh, sri lanka. Neem is often referred as plant of promise, the oil derived from neem is also very useful and is considered to be key ingredient in many cosmetics. Neem oil has number of applications such as it is used in insecticide, cosmetics, fertilizer industry etc. neem oil has garlic like odor and its color varies from yellowish golden to red to dark brown. [3]

There are many methods for production of neem oil such as mechanical cold pressing, solvent extraction, supercritical fluid extraction, enzymatic extraction etc. However supercritical fluid extraction is very expensive, mechanical cold pressing is very cost effective however the oil obtained from this process is very turbid and is low grade oil because of metal contents. The most widely used method is solvent extraction, this is because it is cost effective as well as less time is required for oil production.[4]

In this study our main focus was to optimize the process variables to obtain best yield. For this process we used mathematical and statistical software design expert 10.0. The tool that we used for optimization is Box-behnken method which falls under Response surface methodology. Three variables (extraction time, volume of solvent fed, temperature) where taken and our response variable was oil yield in ml). In this study we tried to find the optimal process condition at which maximum yield was obtained [4].

Response surface methodology (RSM) is tool for optimization which possess factorial design and regression analysis. Mathematical model obtained from this helps in predicting the value of desired variable in response to our input variable. Three dimensional plots obtained in this methodology is very useful for understanding the interaction of response variable with our input independent variables.

II. MATERIAL AND METHODS

A. Preparation of seeds

Neem seeds where purchased from local market in the nashik city, Maharashatra. This material was treated for further process. Treatment of the seeds include cover removal, water washing, sun drying and crushing. Outer cover of neem seeds was removed, the seeds where washed in water and then they were dried, further this seeds were crushed into particles.[4]

B. Oil Extraction Procedure

The experimental work was carried in labs. The equipment that was used for extraction was soxhlet extractor, 50 grams of crushed neem seeds where placed in thimble. The soxhlet extractor was placed on the round bottom flask at one end and the condenser was attached at the other end. The flask was heated using heating mantle. Initially at the start of the process pure solvent vaporized and passed to condenser where it was condensed and was refluxed to thimble, where it came in contact with seeds and as a result of which solid liquid extraction took place in thimble and the resulting mixture moved in round bottom flask. This cycle repeats over the period of extraction. For extraction the solvent we used for each run was hexane.Based on this 17 runs where performed.

C. Fractionation

The extraction mixture in the round bottom flask was fractionated to separate oil and solvent. The fractionation setup consisted of two round bottom flasks, spiral condenser with cooling water arrangements, heating mantle. The extraction mixture was heated in
one of the round bottom flask which was placed over heating mantle, temperature of flask was kept 60 °C using mantle, and the solvent vaporized and was collected in receiving flask. Oil obtained was stored in culture bottles.

D. Experimental Design

Three-level-three-factor Box-Behnken was used for optimization. Based on this method 17 experimental runs where taken. This included 6 factorial points, 6 axial points and three central points, this helped us in studying curvature effect. The independent variables that we have selected are extraction period, volume of solvent and temperature. The coded and uncoded factors (X1, X2, X3) and the levels used are shown below in the table.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction time (h)</td>
<td>X1</td>
<td>2 3 4</td>
</tr>
<tr>
<td>Volume of solvent (ml)</td>
<td>X2</td>
<td>200 250 300</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>X3</td>
<td>25 45 65</td>
</tr>
</tbody>
</table>

E. Statistical Tools

The quality of model was evaluated using ANOVA (which stands for analysis of variance). Multiple regression was used to fit the polynomial model of response in order to correlate response variable to independent variable.

\[ Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j + e \]

Here y= response variable, i and j denotes linear and quadratic coefficients respectively. \( \beta_0 \) is the intercept, \( \beta_i \) is the first order model coefficient \( k \) is the number of factors and e is the error generated. [4]

III. RESULTS

The final equation in terms of coded factors is as follows

\[ Y = 23.06 + 1.97X_1 + 1.63X_2 + 1.52X_3 + 1.00X_1X_2 - 0.72X_1X_3 - 0.25X_2X_3 - 0.65X_1^2 - 2.83X_2^2 - 3.05X_3^2 \]

This equation is generated by design expert 10.0 after regression analysis. The experimental design by Box-Behnken for three-level-three-factors response surface study is shown below.

Design expert 10.0 was used for collection of data as well as regression analysis

Regression analysis is the method to fit the empirical model via collected response variable data. The result of ANOVA analysis for response surface model shows that Model F-value of 11.6 implies that the model is significant. There is only 0.19 % chance that this large F-value could occur because of large noise. Value of “Prob>F” less than 0.05 indicate the model terms are significant in this case X1, X2, X3, X12,X22,X32 are significant model terms. X1X2, X1X3, X2X3are insignificant model terms."The "’Lack of Fit F-value'" of 0.71 implies the Lack of Fit is not significant relative to the pure error. There is a 59.58% chance that a "’Lack of Fit F-value’" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.[4]

<table>
<thead>
<tr>
<th>std</th>
<th>Run</th>
<th>Factor 1 Extraction (hours)</th>
<th>Factor 2 Volume of solvent (ml)</th>
<th>Factor 3 Temperature (°C)</th>
<th>Response Oil yield (ml)</th>
<th>Actual Value</th>
<th>Predicted Value</th>
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<tr>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>22</td>
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<tr>
<td>2</td>
<td>2</td>
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<td>0</td>
<td>0</td>
<td>25</td>
<td>23.06</td>
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</tr>
<tr>
<td>12</td>
<td>3</td>
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<td>12</td>
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<tr>
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<td>1</td>
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</tr>
</tbody>
</table>

The effect of extraction time, volume of solvent on yield at constant temperature is shown in the fig below.

And the effect of extraction period, temperature on oil yield at constant volume of solvent is shown in the fig below.
Effect of temperature, volume of solvent on oil yield
keeping extraction time constant is as follows

The lowest oil yield of 12 ml and the highest of 25 ml
were recorded. The lowest yield was recorded at
2 hours, 250 ml of solvent and 25 °C temperatures. And
the highest yield was recorded at 4 hours, 250 ml of
solvent and 45°C

CONCLUSION

This study showed the optimization of the solvent
extraction process using response surface methodology.
At constant mass of sample (50 grams), constant coolant
flow rate in condenser, and variable extraction period,
volume of solvent and temperature neem seed oil
extraction process was successfully optimized. Highest
oil yield of 25 ml at the conditions of (4h of extraction
period, 250 ml solvent, and 45 temperatures) was
recorded. Hence we draw the inference that optimized
yield can be obtained at (4h, 250 ml, 45 °C) at constant
mass of neem seed (50 grams) and constant coolant flow
rate. Mathematical model can be used to predict the oil
yield, and graphs can be used to understand the
relationship between variables (extraction period,
volume of solvent, temperature) and response of our
interest (oil yield).

Acknowledgement

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