Appendix
APPENDIX

The following steps were taken for calculation of LC_{50} of the oils.

I. The log values of concentrations of dose of oil used were determined with the help of log table. This is denoted as X. the actual concentration used is presented in column 1 and log values (x) in column 2 of the table. The dose of table 25 and 26 were multiplied by 20 to making log value positive and LC_{50} were divided by 20.

II. Number of the mice (n) used for bioassay at a particular concentration is presented in column 3 of the table with the help of data on mortality of test mice, the percentage of mortality was calculated. The percentage was corrected (column 5) by applying the Abott's formula.

\[
\text{Corrected percentage mortality} = \frac{T - C}{100 - C} \times 100
\]

Where, \(T\) = %mortality in the treatment
\(C\) = % mortality in the control

III. With the help data of corrected mortality, empirical probit values were determined from the standard probit values given in standard book following Finney (1977). These are presented in column 6.

IV. The empirical probit values were plotted on a graph keeping the data on log concentration of essential oils on the horizontal line i.e., on x axis, while corresponding data vertical line i.e. y axis (Fig- 25).
V. The position of the values on the graph was assessed with core and an eye fit line was drawn. After drawing the eye fit line the next step was to find out the expected probit by shifting the actual points plotted on the graph on the eye fit line itself. The expected probit value was denoted by capital Y and it was written in column 7.

VI. Using the value of expected probit corresponding with natural percentage of mortality, weighting coefficient was observed (Finney, 1977). The weighting coefficient is denoted by ‘w’ (column 8).

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**Fig- 25**: Expected value for *Mentha piperita* oil by eye fit line

**Fig- 26**: Expected value for *Citrus medica* oil by eye fit line
VII. With the help of data on expected probit and corrected percentage of mortality the value of working probit was determined with the help of table IV of Finney’s book. The value of working probit are denoted by \( y \) and written in column 9.

VIII. In column 10 of the table the values of \( nw \) has been written. They are evidently obtained by multiplying values of \( n \) (from column 3) with the values of \( w \) from (column 8).

IX. In column 11, 12, 13, 14 and 15 the following corresponding values have been written after calculating them with the help of the data already obtained.

<table>
<thead>
<tr>
<th>Column</th>
<th>Data of be calculated</th>
<th>Mode of calculation. Value in x value in x value in</th>
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<td>Column 3 \times column 8 \times column 9</td>
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<td>Column 3 \times column 8 \times (column 2)^2</td>
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<td>nwy^2</td>
<td>Column 3 \times column 8 \times (column 9)^2</td>
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<td>15</td>
<td>Nwxy</td>
<td>Column 3 \times column 8 \times column 2 \times column 9</td>
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X. For the value of \( \bar{x} \) and \( \bar{y} \) following formula were used

\[
\bar{x} = \frac{\text{Snwx}}{\text{Snwy}} \quad \text{i.e. total number of column 11 \over \text{total number of column 10}}
\]

\[
\bar{y} = \frac{\text{Snwy}}{\text{Snw}} \quad \text{i.e. total number of column 12 \over \text{total number of column 10}}
\]

XI. The value of regression coefficient was calculated with the help of following formula

\[
b = \frac{\text{Sxy}}{\text{Sxx}}
\]

where, \( \text{Sxy} = \text{Snwxy} - \frac{(\text{Snwx})(\text{Snwy})}{\text{Snw}} \)
i.e. Total no. of column 15 - Total no. of column 11 - Total no. of column 12

\[ S_{xx} = S_{xx}^2 - \frac{(S_{nx})(S_{ny})}{S_{nwy}} \]

XII. Applying the following regression equation the value of x was recorded

\[ Y = y + b(x - \bar{x}) \]

Where, \( Y \) = probit of 50% mortality (from table no. 1 of Finney's book)
\( \bar{y} \) = mean (from x)
\( b \) = regression coefficient (from xi)
\( x \) = log concentration (unknown)
\( \bar{x} \) = mean (from x)

XIII. The antilog of the value of x was noted which concentration was of does capable of killing 50 percent population of test animal, LC50 of the oil.

**Calculation of LC50 Mentha piperita (Table-25)**

For the value of x and y following formula were used

\[ \bar{x} = \frac{S_{nx}}{S_{nw}} \quad \text{i.e.} \quad \frac{\text{Total no. of column 11}}{\text{Total no. of column 10}} = \frac{18.6122}{31.1932} = 0.5966 \]

\[ \bar{y} = \frac{S_{nx}}{S_{nw}} \quad \text{i.e.} \quad \frac{\text{Total no. of column 12}}{\text{Total no. of column 10}} = \frac{154.0068}{31.1932} = 4.9371 \]

The value of regression coefficient was calculated with the help of following formula:

\[ b = \frac{S_{xy}}{S_{xx}} \]

where, \( S_{xy} = S_{nxy} - \frac{(S_{nx})(S_{ny})}{S_{nwy}} \)

i.e. Total no. of column 15 - Total no. of column 12

\[ 95.335 - 18.6122 \times \frac{154.0068}{31.1932} = 3.4430 \]

\[ S_{xx} = S_{nx}^2 - \frac{(S_{nx})}{S_{nw}} \]
i.e. Total number of column 13 - \( \frac{(\text{Total no. of column 11})^2}{(\text{Total no. of column 10})} \)

\[
11.791 - \frac{3464139}{311932} = 0.6855
\]

\[b = \frac{S_{xy}}{S_{xx}} = \frac{3.4430}{0.6855} = 5.0226\]

Applying the following regression equation the value of \( x \) was recorded

\[Y = \bar{y} + b(x - \bar{x})\]

where, \( Y \) = probit of 50% mortality (from table no. 1 Finney's book)

\( \bar{y} \) = means (from \( x \))

\( b \) = regression coefficient (from \( x_i \))

\( x \) = log concentration (unknown)

\( \bar{x} \) = mean (from \( x \))

\[Y = \bar{y} + b(x - \bar{x})\]

\[
5 = 4.9371 + 5.0226(x-0.5960) = 0.61095756
\]

Antilog of 0.61095756 = 4.08279

\[
408279 \quad \frac{ml}{l} = 0.204139
\]

\[LC_{50} = 0.204139 \times \frac{1000}{40} = 5.103475 \text{ ml/kg body weight}\]

**Calculation of \( LC_{50} \)** *Citrus medica* (Table-26)

For the value of \( x \) and \( y \) following formula were used

\[
\bar{x} = \frac{\text{Snwx}}{\text{Snw}} \quad \text{i.e.} \quad \frac{\text{Total no. of column 11}}{\text{Total no. of column 10}} = \frac{17961}{28534} = 0.62946
\]

\[
\bar{y} = \frac{\text{Snwy}}{\text{Snw}} \quad \text{i.e.} \quad \frac{\text{Total no. of column 12}}{\text{Total no. of column 10}} = \frac{138989}{28534} = 4.87099
\]

The value of regression coefficient was calculated with the help of following formula:

\[b = \frac{S_{xy}}{S_{xx}}\]

where, \( S_{xy} = \text{Snwxy} - \frac{(\text{Snwy})(\text{Snw})}{\text{Snw}} \)

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\[
S_{xx} = \frac{\sum_{i} x_i^2 - \left(\sum_{i} x_i\right)^2 / n}{\sum_{i} w_i}
\]

i.e. Total no. of column 13 - \frac{(\text{Total no. of column 11})^2}{(\text{Total no. of column 10})}

\[
11.791 - \frac{322.5975}{28.534} = 0.4844
\]

\[
b = S_{xy} \times \frac{1}{S_{xx}} = \frac{2.1190}{0.4844} = 4.3745
\]

Applying the following regression equation the value of x was recorded

\[
Y = y + b(x - \bar{x})
\]

where, \( Y \) = probit of 50% mortality (from table no. 1 Finney's book)

\( \bar{y} \) = mean (from x)

\( b \) = regression coefficient (from x_i)

\( x \) = log concentration (unknown)

\( \bar{x} \) = mean (from x)

\[
Y = y + b(x - \bar{x})
\]

\[
5 = 4.87099 + 4.3745(x - 0.62946) = 0.65895
\]

Antilog of 0.65895 = 4.55985

\[
\frac{4.55985}{20} \text{ ml/l} = 0.22799
\]

\[
LC_{50} = 0.22799 \times \frac{1000}{40}
\]

= 5.6998 ml/kg body weight.
Table-25: Determination of LC50 Mentha piperita

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<th>Dose or conc. of oil x20</th>
<th>Dose or conc. of oil</th>
<th>Log of Conc.</th>
<th>Total no. of animal</th>
<th>Number of dead animal</th>
<th>%age mortality (r)</th>
<th>Corrected % mortality</th>
<th>Empirical probit</th>
<th>Expected Probit (Y)</th>
<th>Weighting coefficient (w)</th>
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<th>Corrected % mortality</th>
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Table-26: Determination of LC$_{50}$ *Citrus medica*

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<th>Dose or conc. of oil</th>
<th>Dose or conc. of oil $\times 20$</th>
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<th>Total no. of animal (n)</th>
<th>Number of dead animal</th>
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Appendix 129