CHAPTER 4

SUMMARY
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The local name of the oil of Derris Indica is Karanj oil. Medicated karanj oil is used widely by Ayurvedic Practitioners in rural areas of Gujarat for the treatment of rheumatism and curing skin infections. It is used for healing, antiseptic, and haemostatic effect externally by Ayurvedic Practitioners.

Systemic information is not available on the efficacy of karanj oil. The present investigation was aimed to study systematically and scientifically the efficiency of Karanj oil. The formulations of the karanj oil were prepared using pharmaceutical methodology. The activity of the karanj oil, medicated karanj oil, selected optimized formulation of emulsion, microemulsion and cream of karanj oil was studied for wound healing, antibacterial, and haemostatic efficiency.

Formulation optimization:

Emulsion of karanj oil:

To optimize the concentration of emulsifiers for emulsion of karanj oil, a $3^2$ factorial design was adopted using the concentration of Tween 80 ($X_1$) and Span 20 ($X_2$) as independent variables at three levels: low (-1), medium (0) and high (1). The factorial design batches were prepared by dry gum method. The dependent variables were cream height, number of inversions, and globule size. Response surface curvature can be examined when the two variables are investigated at three levels. The design provided the following empirical second-order equation (Full model):
where $Y$ is response, $b_0$ is intercept, $b_1$ and $b_2$ are coefficients of main effects, $b_{12}$ is the coefficient for the interaction term and $b_{11}$ and $b_{22}$ are the coefficients for the second-order quadratic terms. The non-significant estimated coefficients should be dropped from the full model by adopting a significance test for the regression coefficient. Microsoft Excel® was used to identify non-significant terms. A coefficient is significant if $t_i > t_{crit}(v)$, where $v$ is the degrees of freedom of residual variance. The refined model may be used for calculation of residuals or for drawing the contour plot. The full and refined models ($p < 0.05$) of factorial design batches for cream height, number of inversions, and globule size were obtained and contour plots were constructed.

To validate the evolved mathematical models (reduced models for cream height, number of inversions, and globule size), two check points were prepared and evaluated. Good correlation was found between observed and predicted values. Hence, it was concluded that the evolved models may be used for theoretical prediction of responses within the factor space. As per the results of the $3^2$ experimental design, the optimized formulation of karanj oil emulsion contained 30% w/w of karanj oil, 10% w/w of Tween 80 and 5% w/w of Span 20.

Microemulsion of karanj oil:

The microemulsion of karanj oil were prepared using surfactant, co-surfactant, solvent, and co-solvent like Cremophor RH 40, Glyceryl dilaurate, Tween 80, Ethyl oleate, Transutol P, etc. To optimize the
concentration of co-surfactant, surfactant, and solubilizer, a $3^3$ factorial design was adopted using the concentration of glyceryl dilaurate ($X_1$), Cremophor RH 40 ($X_2$), and Transutol P ($X_3$) as independent variables at three levels: low (-1), medium (0) and high (1). The dependent variables were Viscosity (cp), globule size (nm), and Zeta potential (mV).

Response surface curvature can be examined when the variables are investigated at three levels. The design provides the following empirical second-order equation (Full model, FM):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{123}X_1X_2X_3$$

where $Y$ is response, $b_0$ is the intercept, $b_1$, $b_2$ and $b_3$ are regression coefficient of main effects and $b_{12}$, $b_{23}$, $b_{13}$ and $b_{123}$ are regression coefficient for the interaction terms. The coefficients with second-order terms ($b_{11}$, $b_{22}$ and $b_{33}$) indicate the quadratic nature. Microsoft Excel® may be used to identify the non-significant terms. The full and refined models ($p < 0.05$) for Viscosity (cp), globule size (nm), and Zeta potential (mV) were obtained.

Desirability function approach was used for solving the multiple performance characteristics optimization problems, where all the objectives are to attain a definite goal simultaneously. As per the results of the $3^3$ experimental design on the basis of overall optimum characteristics, the optimized formulation of karanj oil microemulsion contained 15%w/w of karanj oil, 12.5% w/w of Glyceryl dilaurate, 22.5% w/w/ of Cremophor RH 40, and 10%w/w of Transcutol P.
Summary

Cream of karanj oil:

The cream containing karanj oil was prepared by hot emulsification method. It contained 30% w/w of karanj oil, 5% w/w of stearic Acid, 10% w/w of ethoxylated cetosteryl alcohol, 5% w/w of stearyl alcohol, and 10% w/w of propylene glycol.

Wound healing activity of Karanj oil Formulations:

Wistar rats of either sex weighing 150-200 gm were used for the study. All the animals were maintained under controlled room temperature (22±2°C) and relative humidity (50±5%) with 12:00 hr light, 12:00 hr dark cycle. The animals were housed in colony cages (three animals per cage) with free access to diet and water ad libitum during the investigation period. All the animals were acclimatized to the laboratory environment for 5 days before the experimentation. Six animals (rats) per group comprising of three males and three females were used in each experiment set. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of C.U. Shah College of Pharmacy and Research, Wadhwan (Gujarat).

Excision wound model was used to evaluate the wound healing efficiency. The rats were inflicted with excision wounds by under light ether anesthesia. A circular wound of about 300 sq. mm and 2 mm depth was made on depilated ethanol sterilized dorsal thoracic region of the rats. The animals were divided into seven groups of six each. The animals of group I was left untreated and considered as control. The rats of Group II were served as reference standard and received 1% w/w framycetin
sulphate cream (FSC), the animal group III and IV were treated with karanj oil and medicated karanj oil, respectively. The animal group V, VI and VII were treated with karanj oil emulsion, karanj oil microemulsion and karanj oil cream, respectively. The karanj oil or its formulation was topically applied once a day, starting from the day of the operation, till complete epithelialisation. The parameters studied were wound closure and epithelialisation time. The wounds were traced on mm$^2$ graph paper on day 0, 4, 8, and 16 and thereafter on alternate days until healing was completed. The period of epithelialisation was the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound

The results of wound healing activity were expressed as mean ± standard deviation of six animals in each group. The data were statistically evaluated by one-way ANOVA followed by Tukey’s pairwise comparison test. The values of p<0.05 were considered as statistically significant. The histogram of wound healing activity of Karanj oil and its formulations were presented. The results of the wound healing activity revealed that the significant promotion of wound healing was observed by karanj oil and its formulations as compared to control group.

**Antimicrobial activity of Karanj oil Formulations:**

Karanj oil and its formulations were evaluated for their potential antibacterial activity by standard agar well diffusion assay. Petri dishes containing agar medium were seeded with microbial suspension of
Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella typhi and media was allowed to solidify. Wells were cut into solidified agar media using a sterilized cup-borer. Each sample was poured in the respective well and the dishes were incubated at 37 °C for 24 hrs. The antibacterial activity was expressed in terms of the mean of diameter of zone of inhibition at the end of incubation period. Sterilized distilled water was served as negative control. 1% w/w framycetin sulphate cream (FSC) ointment diluted and mixed sterilized distilled water was used as standard i.e. positive control.

The karanj oil was found to be most inhibitory against S. aureus, the least inhibition by karanj oil was found against S. subtilis. The medicated karanj oil was found to be most inhibitory against S. aureus, the least inhibitory against S. typhi. The results revealed that karanj oil and its formulations exhibited significant antibacterial activity.

Hemostatic activity of Karanj oil Formulations:

Rats were slightly ether anesthetized, and abdominal hair was carefully removed with electric clipper, they were fasted overnight. The animals were divided into six groups of six rats in each. The animals of group I was left untreated and considered as control. The rats of Group II, III, IV, V, and VI were applied topically karanj oil, medicated karanj oil, emulsion, microemulsion, and cream, respectively, on the abdominal area gently. Rats were anesthetized by intravenous injection of sodium pentobarbital. The caudal caval vein was exposed by a mid-line incision and blood was collected at 0 hr and 24 hrs into a plastic syringe.
containing citrate buffer pH 4.5. The sample was immediately agitated and centrifuged in a plastic tube at 1500 rpm for 10 min. Plasma was transferred to another plastic tube and the coagulation tests for the determination of thromboplastin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were performed within 3 h. The results of present investigation indicated that the karanj oil had shortened the PT, APTT and TT indicating its hemostatic activity.

Conclusions:

The wound healing activity of Karanj oil and its formulations was studied by using excision wound model and significant wound healing activity was observed. The seed oil of Karanj tree inhibited microbial growth of known wound contaminants and accelerated wound healing process. It also exhibited hemostatic activity with accelerated blood coagulation process. The wound healing, antimicrobial and hemostatic activity are interrelated. The results of the present study indicated that karanj oil had good potentials for use in wound care and further provided a rationale for the use of the plant in wound management in traditional medicine practice.