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Statistical Optimization of Nilavembu Kudineer using RSM and Its Antibacterial Activity

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The study focused on statistical optimization of Nilavembukudineer using RSM and its antibacterial activity. The combined interactive effect of different variables on Nilavembukudineer production was studied by Response Surface Methodology (RSM). The efficiency of the antibacterial compound was tested against clinical isolate of *Salmonella typhi*. The variables are *Andrographis paniculata, Piper nigrum, Plectranthusvetti veroides, Zingiber officinale, Santalum album, Cyperus rotandus, Hedyotis corymboa, Trichosanthes cucumerina* and *Vetiveria zizanoides*.This study suggest that RSM mediated optimization can be a good method for the enhancement of antimicrobial activity of Nilavembukudineer. In future, this method can be very good alternative method of siddha traditional based optimization.

Key words: Nilavembukudineer, *Salmonella typhi*, antibacterial activity, RSM.

Siddha is the ancient medicinal system having potential therapeutic agent. The Indian subcontinent which is enriched by a variety of flora both medicinal and aromatic plants. Plants have been an integral part of the human society, since the start of civilization. India having rich heritage of traditional medicine constituting with its different components like *Ayurveda, Siddha, Unani*. Numerous siddha formulation are indicated for the suram (fever) in siddha text. Fever is classified into 64 type according to the causative factors in siddha literatures [Dr. Kuppusamy Mudaliyar, 2005]. Many awful viral fevers have been reported recently in India and other ancient countries. Mortality rate of some of dreadful fever dengue, chickungunya and swine flu other than fever. Nilavembu is also given for in the treatment of diabetes as a decoction. Fever arises as an unknown origin as a symptom with the enormous increase in the body temperature. Most frequently children, old age peoples affected for fever. Fever is also caused by some microbial infection of unknown origin. Nilavembukudineer is used as a first line therapy and general remedy [Kuppusamy mudaliyar, 2009]. These chooranam are usually prescribed in the form of kudineer(2 – 4gms hotwater decoction twice a day) in siddha medicine [Deva aasirvatham saamuvel, 2000]. The inappropriate dose and dosing schedule, makes the necessitates for modern scientific approach. The paper present involves the amendment of a new dosage form for improving the therapy of Nilavembukudineer. Therfore a modified technique was adopted for Nilavembukudineer which can improve the patient compliance, increases the bioavailability and therapeutic efficacy of the drug.

In siddha treatment, all the medicine were

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prepared by manual method. Still, traditionally optimized values was using in drug preparation. But, this is a time consuming process and doesn't explain the combined interactive effect of drugs involved in the Nilavembukudineer. To overcome these confines, different initiatives are undertaken. Among these initiatives, statistical optimization which is commonly known as Response Surface Methodology (RSM) was applied. RSM is a collection of statistical and mathematical techniques useful for optimizing stochastic functions. It is an industrially efficient tool for the production of commercially important drugs (Khurana S, 2007). RSM will give 3D plots for response and it will help for visualization of the parameter interaction in a better manner (Zambare 2011).

There are no reports on RSM mediated optimization of Nilavembukudineer. Hence, in the present study we describe response surface methodology mediated optimization of the variables for an economical production of Nilavembukudineer

MATERIALS AND METHODS

Media and drugs

The media was purchased from Hi media Pvt. Ltd, Mumbai. The dried raw drugs were obtained from Raw drug store in Salem, Tamilnadu, India. The identity of the drug were confirmed by Botanist of Medicinal botany lab in Sivaraj Siddha Medical College,Salem.

Drugs involved

1. Nilavembu dry
2. Milagu
3. Vilamichanver
4. Chukku
5. Santhanam
6. Korai kizhangu
7. Parpatakam
8. Pei pudal
9. Vetti ver. [Vaithiyarathinam Murugesa mudaliar, 1997]

Purification of the drug

The other sticks and other foreign matters are removed and dust particles are cleaned manualy.

Preparation of the polyherbal decoction

Herbs were coarsely chopped using grinding machine and the drugs were taken in different ratios for all 65 sets of drugs, then they are soaked in water which is 16 times more when compared with weight of combination of drug as mentioned in the siddha literatures[Deva aasirvatham saamuvel,2000] and boiled and mixed well. The submerged herbs were boiled for about 15-20 minutes with frequent mixing and then waited until the quantity get reduced to 1 part then allowed to set at room temperature for sometimes and filtered through filter, were considered as 100% polyherbal decoction.

Optimization by Response Surface Methodology (RSM)

In the present study, statistically designed experiments were executed to optimize the nilavembukudineer. The nine independent variables chosen for optimization were Nilavembu dry (A), Milagu (B), Vilamichanver (C), Chukku (D), Santhanam (E), Koraikizhangu (F), Parpatakam (G), Pei pudal (H), Vettiver (J) The total number of experiments suggested by the model was 65. The range of Nilavembukudineer ingredients were shown in Table 1. Each variable was set at two levels (-1 and +1). The experimental design was developed using Design Expert, version 7.0.7.1.

Screening for antibacterial activity by Agar well diffusion method

The standard NilavembuKudineer and RSM suggested values (65 sets) were screened for antibacterial activity against *S.typhi* by agar well diffusion method (Gaurav Kumar *et al*, 2010)

RESULTS AND DISCUSSION

Optimization of media

Nilavembukudineer showed very good antibacterial activity. This is the first report on RSM

Table 1. Range of media components for RSM

Ingredients	Low (-1)	High (+1)
Andrographispaniculata (A)	2.5	7.5
Vetiveriazizaniodes(B)	2.5	7.5
Plectranthusvettiveroides(C)	2.5	7.5
Santalum album (D)	2.5	7.5
Trichosanthescucumerina(E)	2.5	7.5
Cyperusrotundus(F)	2.5	7.5
Zingeberofficinale(G)	2.5	7.5
Piper nigrum (H)	2.5	7.5
Hedyotiscorymbosa (J)	2.5	7.5

Optimization of Nilavembukudineer. ANOVA Analysis for response surface quadratic model gives the following equation. *Andrographis paniculata*, *Vetiveria zizaniodes*, *Plectranthus vettiveroides*, *Santalum album*, *Trichosanthes cucumerina*, *Cyperus rotundus*, *Zingiber officinale*, *Piper nigrum* and *Hedyotis corymbosa* are indicated as A, B, C, D, E, F, G, H, J

R1 =+3.48+0.15* A-0.68* B-0.47* C+0.80* D-0.67* E-0.65* F-0.39* G+0.049* H-1.17* J+0.17* A * B+0.19* A* C+1.51* A* D-0.031* A * E-0.55* A* F-0.36* A * G-0.56* A * H
-0.20* A * J-0.28* B * C-0.013* B * D-0.31* B * E+0.79* B * F+0.32* B * G+0.048* B * H
+0.74* B * J-0.30* C * D-0.090* C * E+0.69* C * F+0.47* C * G-0.39* C * H-0.46* C * J-0.32* D * E+0.35* D * F+0.78* D * G-0.094* D * H+0.77* D * J+0.10* E * F-0.15* E * G+ 0.47* E * H+ 0.29 * E * J-0.92 * F * G+0.036* F * H-2.477E-003* F * J-1.05* G * H+ 0.28 * G * J-0.42* H * J-2.26* A^2+2.42* B^2+1.97* C^2-2.76* D^2+1.55* E^2+3.65* F^2+ 4.32* G^2+1.31* H^2-1.95* J^2

The "Model F-value" of 1.84 implies the model is not significant relative to the noise. There is a 14.84 % chance that a "Model F-value" this

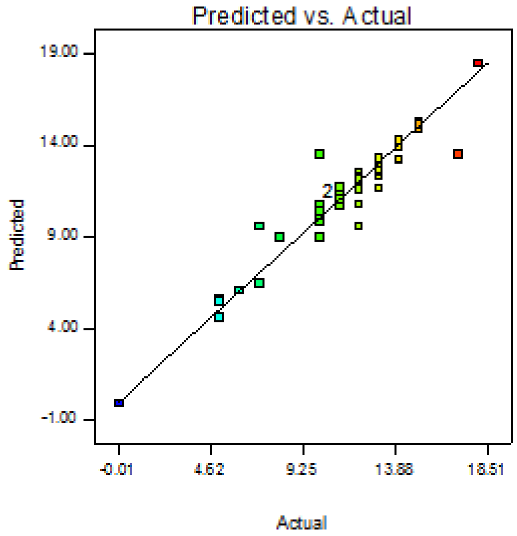

Fig. 1. Predicted response versus actual value

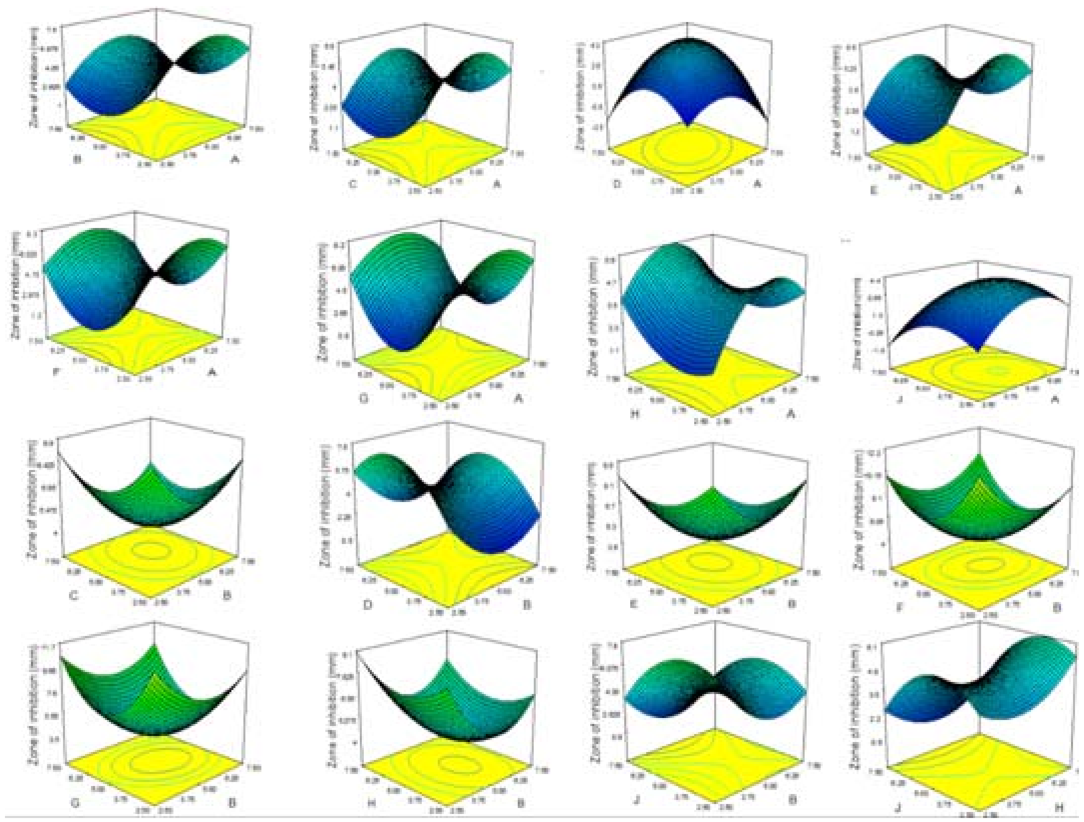

Fig. 2. Effects of Different composition of NilavembuKudineer and their interactive effect on the antibacterial activity

large could occur due to noise.Values of "Prob> F" less than 0.0500 indicate model terms are significant.

In this case J, AD, G2 are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 0.20 implies the Lack of Fit is not significant relative to the pure error. There is a 94.74% chance that a "Lack of Fit F-value" this large could occur due to noise.

The coefficient of determination (R2) for antibacterial activity was calculated as 0.9086. The actual response values agree well with the predicted response values was represented in Figure 1. This indicated that the statistical model explained 90.86% of correlation between the predicted and observed values. Since the R^2 value has shown to be closer to 1, there is a good agreement between the experimental and predicted values of antibacterial activity. The interaction effects of independent variables on antibacterial activity were studied by plotting 3D surface curves. The 3D curves of the calculated antibacterial activity for the interactions between the variables are shown in Figures 2. The optimized values of the variables for antibacterial production were as follows:

Andrographis paniculta	-	7.5g;
Vetiveria zizanoides	-	7.5g;
Plectranthus vettiveroides	-	2.5g;
Santalum album	-	7.5g;
Trichosanthes cucumerina	-	7.5g
Cyperus rotundus	-	7.5g
Zingiberofficinale	-	7.5g
Piper nigrum	-	7.5g
Hedyotis corymbosa	-	5g

The RSM designs have broad application in biotechnology field. Many scientists have reported using RSM technique, satisfactory optimization of antibacterial compound production from microbial sources [Gan & Latiff, 2010: Kiassos *et al*,2009]. In the present study, RSM was shown to be for the optimization of the increase the efficient of antibacterial activity of Nilavembukudineer. In the vision of siddha medicine utility of the NilavembuKudineer, compare to traditional method of optimization, RSM showed significant improvement of antibacterial activity. The zone of inhibition of Nilavembukudineer (traditional optimized) was observed as 20 mm. However, after optimization of the drug, the zone of inhibition increased to 26 mm. Therefore it can be seen that optimization of the drug has also increased the productivity of the antimicrobial agent.

Employment of RSM techniques in the field of siddha is the first time to document the investigation on Nilavembukudineer. Previously many researches have been conducted for Nilavembukudineer for its antibacterial activity. In 2012, Uma *et al* (2012) reported in Vitro Antimicrobial Activity Of The Siddha Drugs Seenthil Sarkarai and NilavembuKudineer Against Leptospiraand Balakrishnan *et al* (2013) also reported Pharmacognosy and Chromatographic analysis of NilavembuChooranam And Nilavembu Tablet.

Hence it can be concluded that RSM can be used for siddha drug preparation to enhance the efficacy of drugs.

CONCLUSION

Comparison of predicted and experimental values revealed good correspondence between them, implying that empirical models derived from RSM can be used to adequately describe the relationship between the factors and response in Nilavembu kudineer optimization. The author demonstrated that optimal condition of Nilavembukudineer is successfully predicted by RSM.

The nilavem bukudineer was optimized by experimental method for the better flow of mixture and found to have agreeable parameters. The results of uniformity and disintegration test indicates its aptness. This scale up process also signifies positive attribute for industrial production.

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