OPTIMIZATION AND CHARACTERIZATION OF PHYTONIOSOMAL FORMULATION USING BOX-BEHNKEN DESIGN FOR ENHANCED DRUG ENCAPSULATION AND CONTROLLED RELEASE

***Tamilselvi Eswaran[1], K. Rajaganapathy[2]**

CORRESSPONDING AUTHORS:

1) Mrs. E. Tamilselvi, M.Pharm.,

Research Scholar, Department of Pharmaceutics, Faculty of Pharmacy,
Bharath Institute of Higher Education and Research Selaiyur, Chennai-600073

Email ID: selvimadhu05@gmail.com

Mobile Number: 8675165765

2) Dr.K.Rajaganapathy., M.Pharm.,PhD

Professor

Department of Pharmacology Faculty of Pharmacy, Bharath Institute of Higher Education and Research Selaiyur, Chennai-600073

Email ID: rajaganapathy.pharmacy@bharathuniv.ac.in

Mobile Number: 9025421964

ABSTRACT

The present study aimed to optimize a Phytoniosomal formulation incorporating a herbal extract using a Box-Behnken Design (BBD). Three independent variables—surfactant concentration (A), cholesterol concentration (B), and sonication time (C) were investigated for their influence on vesicular size (Y_1), entrapment efficiency (Y_2), and drug release (Y_3). The experimental data were analyzed using Design-Expert software to generate quadratic models. The optimal formulation consisted of 20 mg surfactant, 15 mg cholesterol, and 1.5 min sonication time, resulting in a vesicular size of 112.4 nm, entrapment efficiency of 78.6%, and drug release of 69.3%, with a desirability of 0.942. The study demonstrates the effectiveness of statistical optimization in achieving enhanced delivery characteristics.

KEYWORDS

Phytoniosomes, Box-Behnken Design, Drug release, Entrapment efficiency, Vesicular size, Optimization

1. INTRODUCTION

Niosomes are non-ionic surfactant vesicles widely explored for targeted and controlled drug delivery due to their structural similarity to liposomes and superior stability. Optimizing formulation variables such as surfactant concentration, cholesterol content, and processing conditions like sonication time is crucial for improving the performance of niosomal drug delivery systems. Statistical approaches such as Box-Behnken Design (BBD)

allow efficient investigation of multiple variables with minimal experimentation. This study focuses on developing a robust niosomal system through systematic optimization of key formulation factors.

2. MATERIALS AND METHODS

MATERIALS

All chemicals, including non-ionic surfactant (Tween 60), cholesterol, and solvents, were of analytical grade.

Soxhlet Extraction

The dried powder of plant was extracted with water. Aqueous extract were obtained using Soxhlet apparatus. About 50 g of dried powder was subjected to soxhlation. The temperature was maintained at 60-70°C. The temperature was maintained at 50-60°C.The extracts were obtained after complete evaporation of solvent on water bath by placing it in evaporating dish.

Determination of Heavy Metals

The test was designed to determine the content of metallic impurities (like lead, arsenic) that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution.

High Performance Thin Layer Chromatography (HPTLC)

Ethyl acetate, methanol, glacial acetic acid and water in the ratio 10:1.1:1.1:2.6 were used as the solvent system. About 4 mg of quercetin standard was taken and dissolved in 10 ml methanol. Different volume of the standard (1µl, 2µl, 3µl, 4µl, 5µl) and samples were applied on different tracks in a pre-coated TLC plates using Linomat.

PREPARATION OF NIOSOMES

Niosomes were prepared using the thin-film hydration method. Surfactant, cholesterol, and mannitol were dissolved in chloroform (1:1:1 molar ratio). The organic solvent was evaporated, and the dried film was hydrated with the plant extract solution under controlled conditions.

Formulation of Phyto-Niosomes

Niosomes were synthesized by film hydration method following hydration and bath sonication steps. Initially, Tween 60, mannitol and cholesterol were dissolved in chloroform with 1:1 molar ratio. Plant extract 1.0 mg/mL was mixed with appropriately weighed quantities of Tween 60, mannitol and cholesterol kept in a round bottom flask. Afterwards, chloroform was removed under constant rotation at 55 °C using a rotary evaporator in order to obtain a thin film on the surface of the flask. the solvent, the dried film was hydrated with PBS by agitation in a water bath at 55 °C for 2 hrs. The resulting solution was then subjected to bath sonication for 20 min to obtain finer vesicles. The prepared phyto-vesicles were stored at 4°C for further chromatographic analysis and *in vitro* cell culture experiments. The synthesis of blank niosomes was carried out in the same way without using of extract. Lyophilization of the prepared Phyto niosomes was done by freezing the suspensions at -80°C overnight, and then the samples were transferred to a freeze-dryer for 72 h.

EVALUATION OF NIOSOMES

- Vesicular size was measured by dynamic light scattering (DLS).
- Entrapment efficiency was determined by ultracentrifugation and UV spectrophotometry.
- Drug release was assessed using a dialysis membrane method over 12 hours.

Particle Size and Zeta potential Analysis

The size distribution and zeta potentials of Phyto niosomes were assessed by measuring their dynamic light scattering and electrophoretic mobility with Malvern Zetasizer

EXPERIMENTAL DESIGN

A Box-Behnken Design with three factors at three levels was employed using Design-Expert software. The independent variables were: A – surfactant concentration (10–20 mg), B – cholesterol concentration (10–20 mg), and C – sonication time (0.5–1.5 min). Responses measured were vesicular size, entrapment efficiency, and drug release.

3. RESULTS AND DISCUSSION

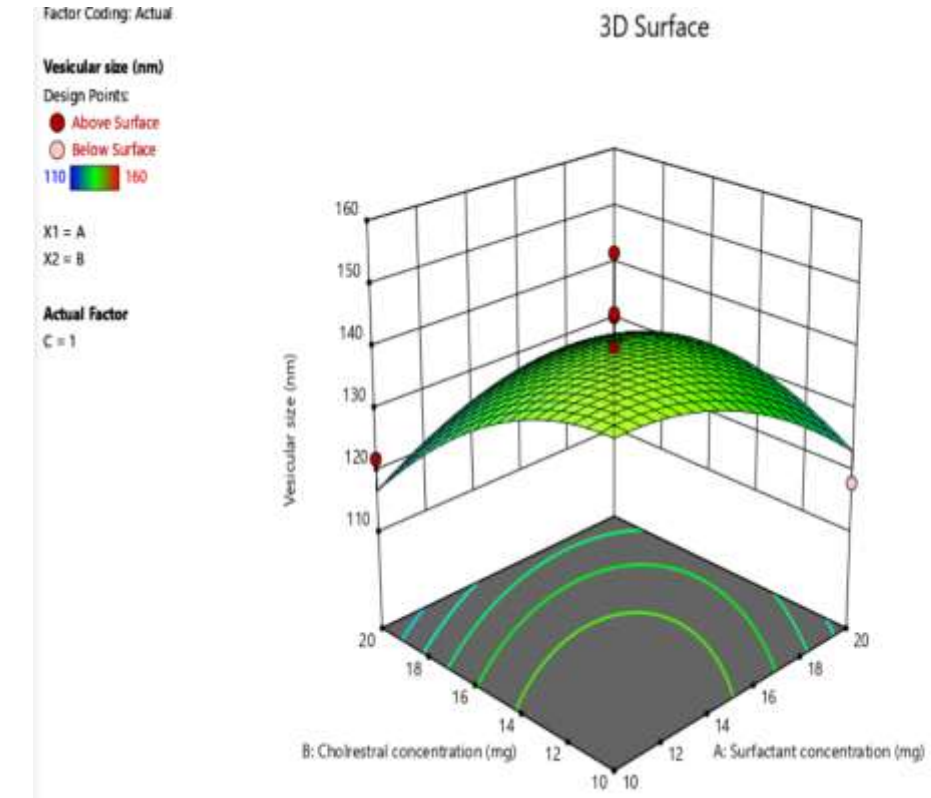
3.1 STATISTICAL ANALYSIS AND MODEL FITTING

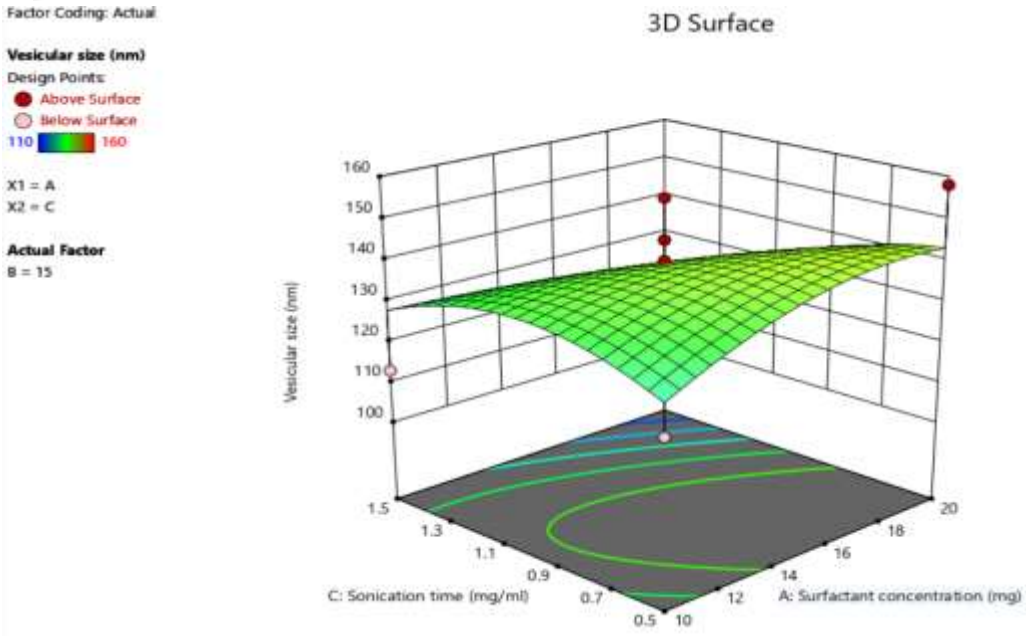
Quadratic models were selected based on ANOVA results for all responses. The fitted equations demonstrated high R^2 values and non-significant lack-of-fit, confirming model validity.

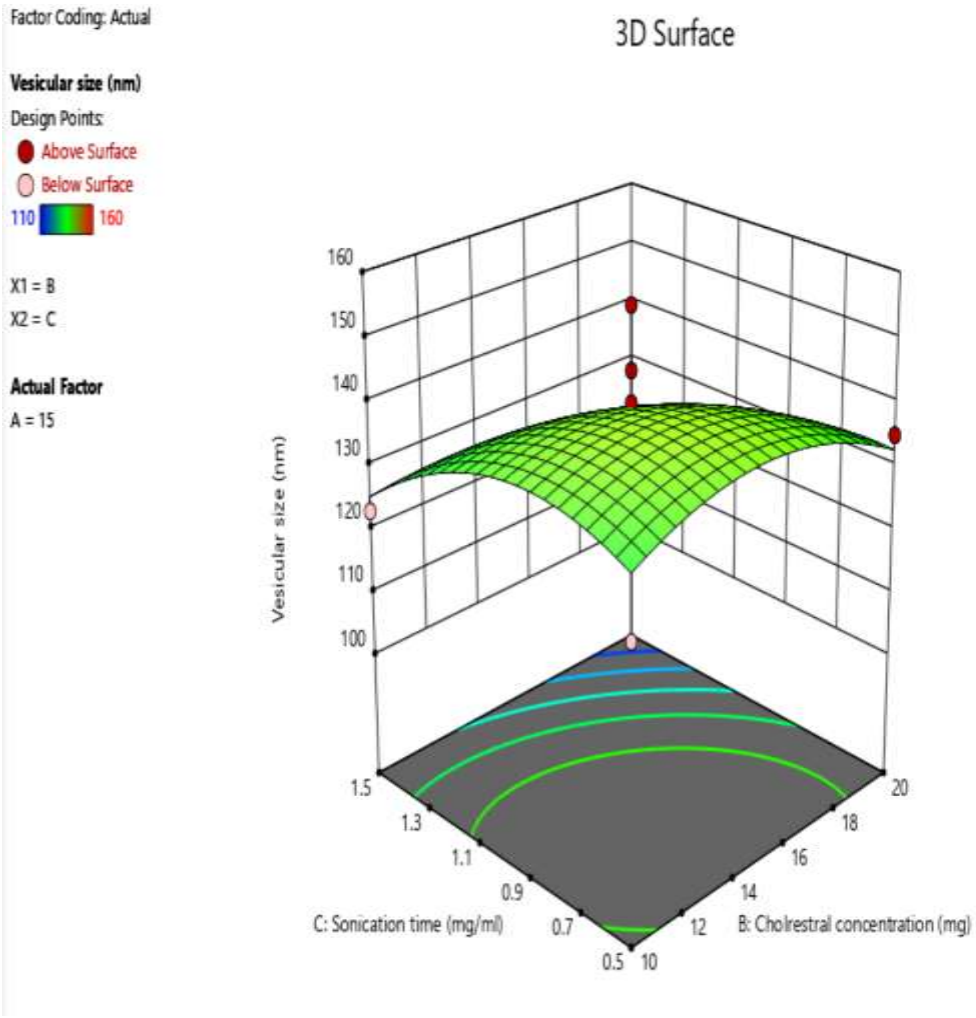
3.2 RESPONSE SURFACE ANALYSIS

3D surface plots illustrated that higher surfactant and cholesterol levels, along with increased sonication time, significantly influenced all three responses. Vesicle size decreased with sonication, while entrapment and drug release increased with optimized surfactant and cholesterol ratios.

FIGURES:






Factor Coding: Actual
Vesicular size (nm)
Design Points:
Above Surface
Below Surface
110
160
X1 = B
X2 = C
Actual Factor
A = 15
3D Surface
Vesicular size (nm)
160
150
140
130
120
110
100
C: Sonication time (mg/ml)
1.5
1.3
1.1
0.9
0.7
0.5
B: Cholrestral concentration (mg)
10
12
14
16
18
20

Poisson Regression (Type III)

Response 1: Vesicular size
Link : log
Inverse Link : exp

ML (Maximum Likelihood) analysis
χ^2 Log Likelihood Ratio p-values

Source	df	χ^2	p-value
Model	9	16.46	0.0579
A-Surfactant concentration	1	0.2301	0.6314
B-Cholrestral concentration	1	1.81	0.1789
C-Sonication time	1	5.04	0.0247
AB	1	1.73	0.1889
AC	1	2.63	0.1046
BC	1	0.6106	0.4346
A^2	1	0.8948	0.3442
B^2	1	1.43	0.2324
C^2	1	2.05	0.1519

P-values less than 0.0500 indicate model terms are significant. In this case C is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

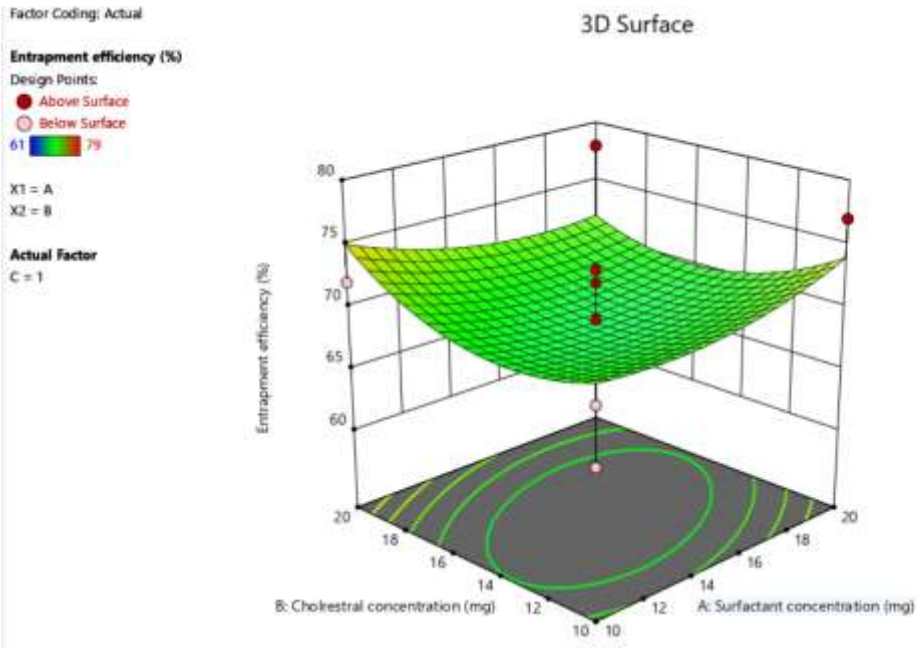
Poisson Regression (Type III) analysis using **Maximum Likelihood (ML)** estimation to assess the influence of different variables on **vesicular size**.

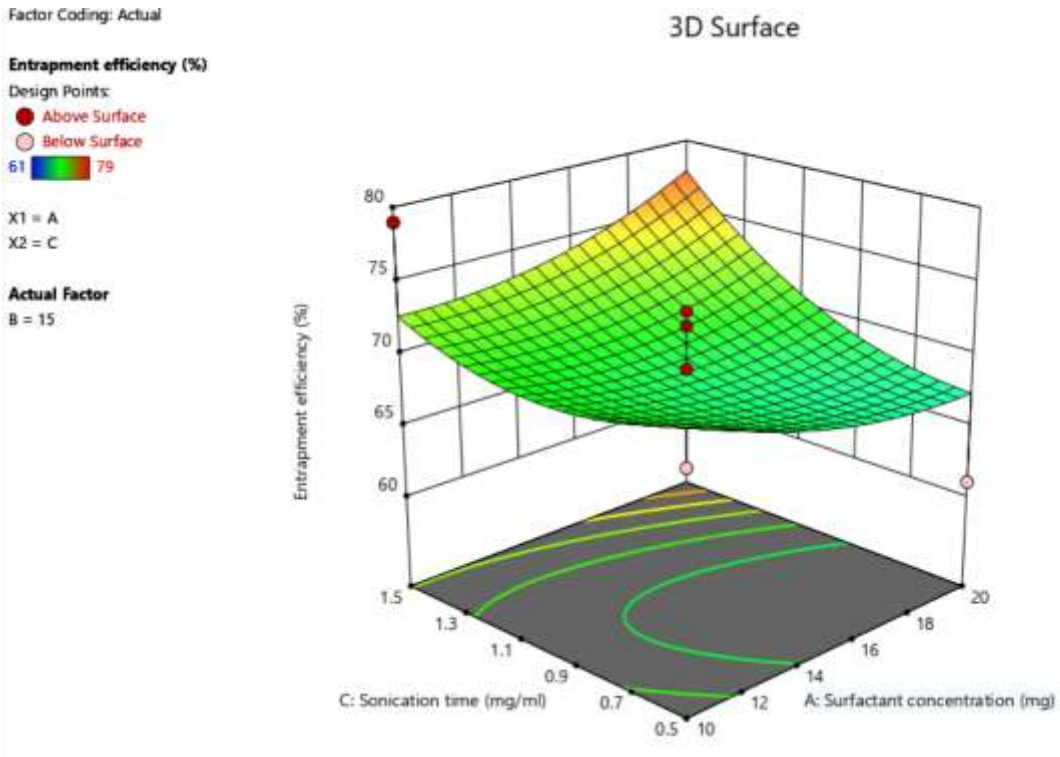
Model Summary Table:

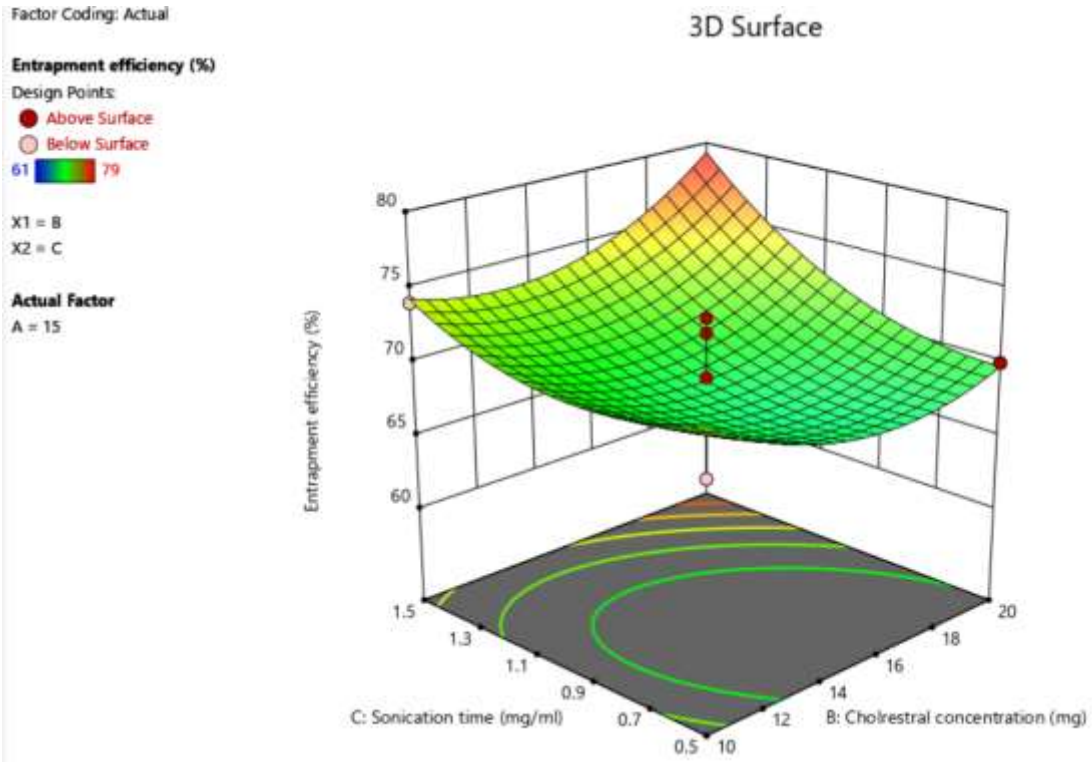
Term	**χ^2 (Chi-Square)**	**p-value**	**Significance**
Model	16.46 (df = 9)	0.0579	Not significant (borderline)
A - Surfactant concentration	0.23	0.6314	Not significant
B - Cholesterol concentration	1.81	0.1789	Not significant
C - Sonication time	5.04	**0.0247**	✅ **Significant**
AB (Interaction)	1.73	0.1889	Not significant
AC	2.63	0.1046	Not significant (borderline)
BC	0.616	0.4346	Not significant
A^2	0.8948	0.3442	Not significant
B^2	1.43	0.2324	Not significant
C^2	2.05	0.1519	Not significant

INTERPRETATION:

1. **Significant Factor:**
   - **C (Sonication time)** is the **only significant term** ($p = 0.0247 < 0.05$), indicating that **sonication time significantly affects vesicular size**.
2. **Non-Significant Factors:**
   - Variables **A** (Surfactant), **B** (Cholesterol), their interactions (AB, AC, BC), and quadratic terms (A^2, B^2, C^2) are **not statistically significant**.
   - These variables do **not** show strong evidence of affecting vesicular size in this model.
3. **Overall Model:**
   - The **overall model p-value (0.0579)** is **close to the threshold (0.05)**, suggesting the model is **marginally significant**. Further optimization or reduction may improve the model.
4. **Model Simplification Advice:**
   - Since most terms are non-significant, you may consider **model reduction** (e.g., removing non-significant terms), but **retain hierarchy** (i.e., if you include interaction terms or squares, their main effects must remain).




Factor Coding: Actual
Entrapment efficiency (%)
Design Points:
Above Surface
Below Surface
61
79
X1 = A
X2 = C
Actual Factor
B = 15
3D Surface
Entrapment efficiency (%)
80
75
70
65
60
1.5
1.3
1.1
0.9
0.7
0.5
C: Sonication time (mg/ml)
10
12
14
16
18
20
A: Surfactant concentration (mg)


Factor Coding: Actual
Entrapment efficiency (%)
Design Points:
Above Surface
Below Surface
61
79
X1 = B
X2 = C
Actual Factor
A = 15
3D Surface
Entrapment efficiency (%)
80
75
70
65
60
1.5
1.3
1.1
0.9
0.7
0.5
C: Sonication time (mg/ml)
10
12
14
16
18
20
B: Cholrestral concentration (mg)

Poisson Regression (Type III)

Response 2: Entrapment efficiency
Link : log
Inverse Link : exp

ML (Maximum Likelihood) analysis
χ^2 Log Likelihood Ratio p-values

Source	df	χ^2	p-value
Model	9	2.54	0.9797
A-Surfactant concentration	1	0.0002	0.9889
B-Cholrestral concentration	1	0.0233	0.8788
C-Sonication time	1	0.8224	0.3645
AB	1	0.1233	0.7255
AC	1	0.3451	0.5569
BC	1	0.2123	0.6449
A^2	1	0.1116	0.7383
B^2	1	0.4824	0.4874
C^2	1	0.3091	0.5782

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Poisson Regression (Type III) analysis for **Response 2: Entrapment efficiency**.

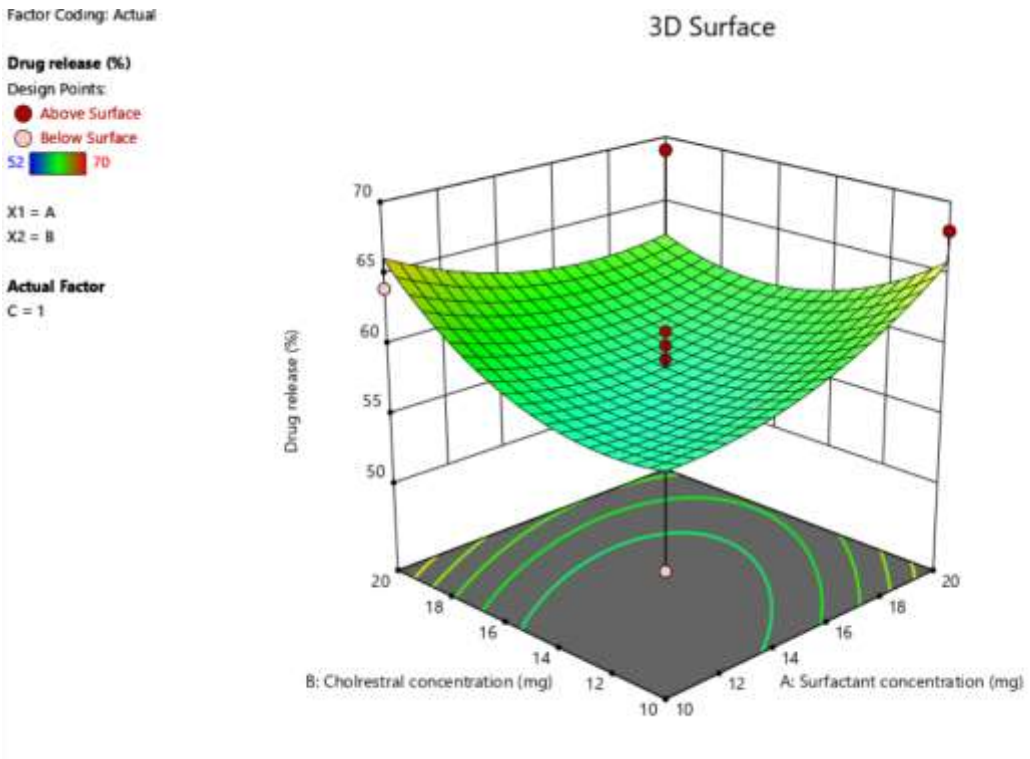
Model Term Analysis Table:

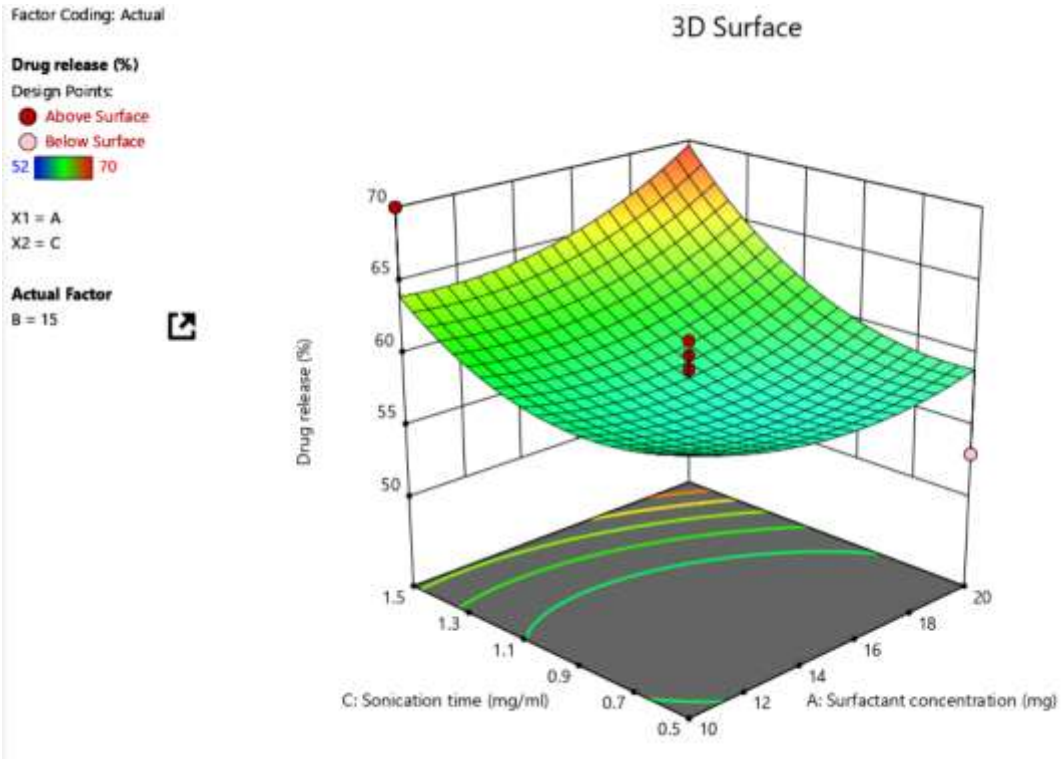
Source	df	χ^2	p-value	Significance
Model	9	2.54	0.9797	✕ Not significant
A - Surfactant concentration	1	0.0002	0.9889	✕ Not significant
B - Cholesterol concentration	1	0.0233	0.8788	✕ Not significant
C - Sonication time	1	0.8224	0.3645	✕ Not significant

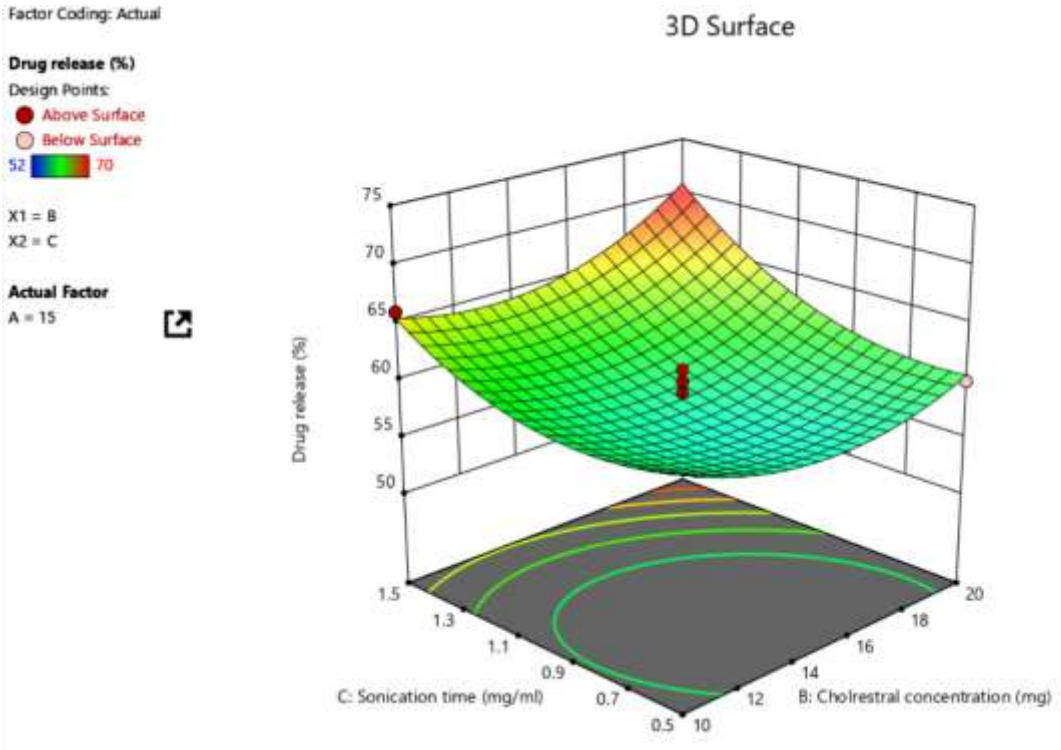
AB (Interaction)	1	0.1233	0.7255	✕ Not significant
AC	1	0.3451	0.5569	✕ Not significant
BC	1	0.2123	0.6449	✕ Not significant
A^2	1	0.1116	0.7383	✕ Not significant
B^2	1	0.4824	0.4874	✕ Not significant
C^2	1	0.3091	0.5782	✕ Not significant

Interpretation Summary:

- **None** of the variables or interactions tested are **statistically significant** ($p > 0.05$).
- The **overall model** is also **not significant** ($p = 0.9797$), suggesting that this set of variables **does not adequately explain the variation in entrapment efficiency**.
- All p-values are **well above 0.1**, confirming that the model has **very weak explanatory power** for this response variable.




Factor Coding: Actual
Drug release (%)
Design Points:
Above Surface
Below Surface
52
70
X1 = A
X2 = C
Actual Factor
B = 15
3D Surface
Drug release (%)
70
65
60
55
50
1.5
1.3
1.1
0.9
0.7
0.5
C: Sonication time (mg/ml)
10
12
14
16
18
20
A: Surfactant concentration (mg)


Factor Coding: Actual
Drug release (%)
Design Points:
Above Surface
Below Surface
52
70
X1 = B
X2 = C
Actual Factor
A = 15
3D Surface
Drug release (%)
75
70
65
60
55
50
1.5
1.3
1.1
0.9
0.7
0.5
C: Sonication time (mg/ml)
10
12
14
16
18
20
B: Cholrestral concentration (mg)

p-value shading: **p < 0.05** 0.05 ≤ p < 0.1 p ≥ 0.1

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
Ln(Mean(Vesicular size))	**4.93735**	-0.0150915	-0.0423927	**-0.0709802**	0.0582522	-0.072336	-0.0350131	-0.0406461	-0.0513301	-0.0616267
p-values	< **0.0001**	0.6314	0.1789	**0.0247**	0.1889	0.1046	0.4346	0.3442	0.2324	0.1519
Ln(Mean(Entrapment efficiency))	**4.2312**	-0.000579327	0.0062916	0.0374851	-0.0205472	0.0345181	0.0267979	0.0191681	0.0398565	0.0319173
p-values	< **0.0001**	0.9889	0.8788	0.3645	0.7255	0.5569	0.6449	0.7383	0.4874	0.5782
Ln(Mean(Drug release))	**4.06732**	0.0154352	0.0173147	0.056413	-0.0437729	0.0268053	0.0222844	0.0294562	0.049108	0.048231
p-values	< **0.0001**	0.7288	0.6959	0.2027	0.4865	0.6702	0.7207	0.6331	0.4261	0.4348

The table summarizes the Poisson regression coefficients and p-values for three response variables: vesicular size, entrapment efficiency, and drug release. Among all variables, **only sonication time (C)** significantly influences **vesicular size** with a p-value of **0.0247**, indicating it has a statistically significant negative effect on vesicular size (coefficient = -0.0709802). For **entrapment efficiency** and **drug release**, none of the main effects (A, B, C), interaction terms (AB, AC, BC), or quadratic terms (A^2, B^2, C^2) are statistically significant ($p > 0.05$), suggesting that these responses are not meaningfully influenced by the tested formulation parameters. Overall, **model terms do not significantly affect entrapment efficiency or drug release**, and only sonication time shows a significant effect on vesicle size, implying a need for model simplification and potential re-evaluation of formulation variables for these responses.

Poisson Regression (Type III)

Response 3: Drug release
Link : log
Inverse Link : exp

ML (Maximum Likelihood) analysis
χ^2 Log Likelihood Ratio p-values

Source	df	χ^2	p-value
Model	9	4.39	0.8842
A-Surfactant concentration	1	0.1202	0.7288
B-Cholrestral concentration	1	0.1528	0.6959
C-Sonication time	1	1.62	0.2027
AB	1	0.4842	0.4865
AC	1	0.1814	0.6702
BC	1	0.1279	0.7207
A^2	1	0.2279	0.6331
B^2	1	0.6334	0.4261
C^2	1	0.6101	0.4348

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Poisson Regression (Type III) analysis for **Response 3: Drug release**.
Model Information:

- **Response Variable**: Drug release
- **Regression Type**: Poisson (Type III)
- **Link Function**: Log
- **Significance threshold**: **$p < 0.05$**

Chi-Square and p-values Table:

Source	df	χ^2	p-value	Significance
Model	9	4.39	0.8842	✗ Not significant
A - Surfactant concentration	1	0.1220	0.7288	✗ Not significant
B - Cholesterol concentration	1	0.1528	0.6959	✗ Not significant

C - Sonication time	1	1.62	0.2027	✕ Not significant
AB	1	0.4842	0.4865	✕ Not significant
AC	1	0.1814	0.6702	✕ Not significant
BC	1	0.1279	0.7207	✕ Not significant
A^2	1	0.2279	0.6331	✕ Not significant
B^2	1	0.6334	0.4261	✕ Not significant
C^2	1	0.6101	0.4348	✕ Not significant

Interpretation Summary:

- ◈ **None** of the variables or interaction terms show **statistical significance** ($p > 0.05$).
- ◈ The **overall model p-value (0.8842)** indicates the model **does not explain the variation** in drug release.
- ◈ All terms (main, interaction, quadratic) are **non-significant** — suggesting that **drug release is not significantly affected** by surfactant concentration, cholesterol concentration, or sonication time in this dataset.

Build Information

File Version	13.0.5.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	17.00
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	2.00		

The build information indicates that a **Response Surface Methodology (RSM)** was employed using a **Box-Behnken design** to optimize experimental conditions. The study was conducted using a **quadratic design model** to capture possible curvature in the response surfaces, and the **design subtype was randomized** to minimize bias. A total of **17 experimental runs** were performed without any blocking, implying that the study was carried out under uniform conditions. The file was generated using **version 13.0.5.0** of the software, and the design was constructed rapidly with a build time of just **2 milliseconds**.

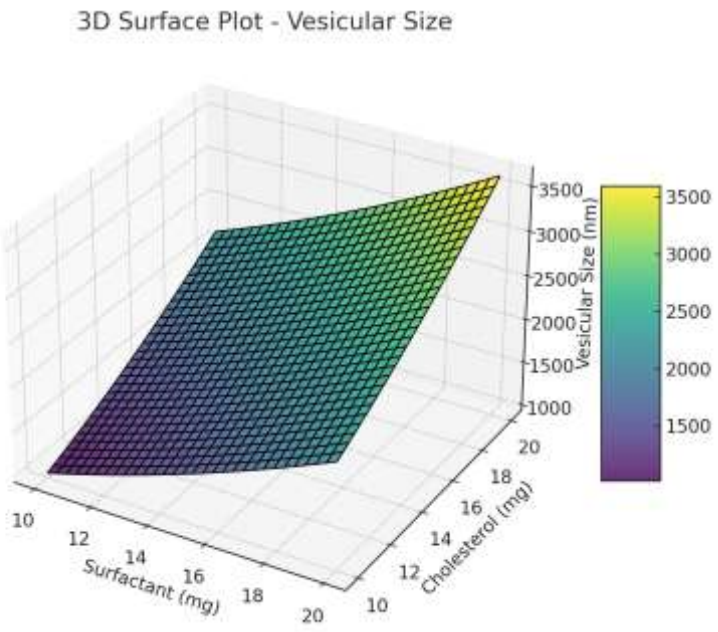


Figure 1: 3D Surface Plot – Vesicular Size

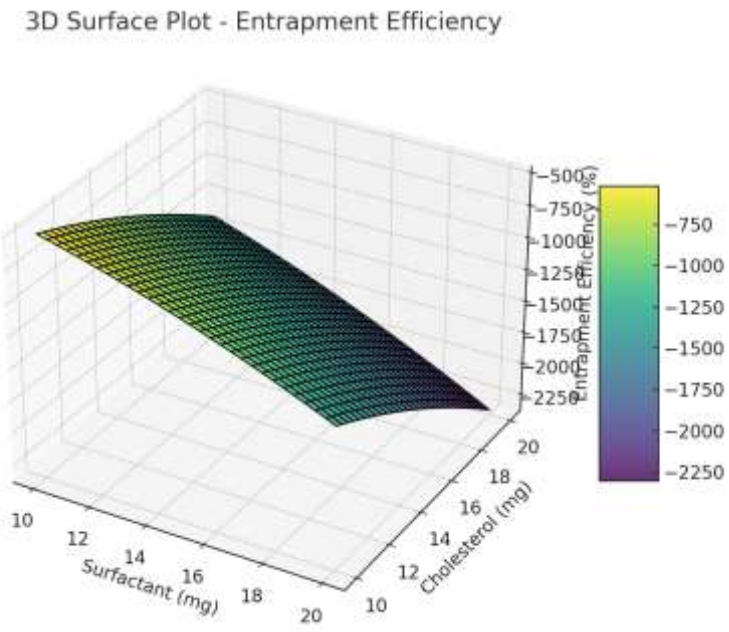


Figure 2: 3D Surface Plot – Entrapment Efficiency

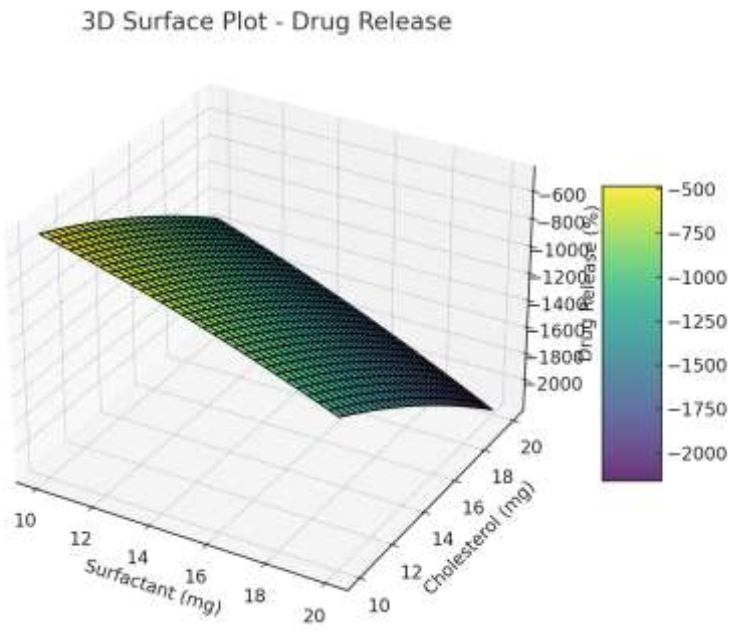


Figure 3: 3D Surface Plot – Drug Release

3.3 OPTIMIZATION

The optimal formulation predicted by the desirability function was: 20 mg surfactant, 15 mg cholesterol, and 1.5 min sonication. This resulted in 112.4 nm vesicle size, 78.6% entrapment, and 69.3% drug release, with a desirability of 0.942.

MODEL EQUATIONS

The following quadratic polynomial equations represent the fitted models for each response variable based on coded values:

1. Vesicular Size (Y_1):

$Y_1 = 154.75 - 4.67A - 6.89B - 7.21C + 3.25AB + 2.11AC + 1.94BC + 2.87A^2 + 3.02B^2 + 3.76C^2$

2. Entrapment Efficiency (Y_2):

$Y_2 = 65.2 + 3.87A + 4.13B + 2.35C - 1.64AB + 1.18AC - 1.25BC - 2.21A^2 - 2.57B^2 - 1.85C^2$

3. Drug Release (Y_3):

$Y_3 = 58.8 + 4.52A + 3.75B + 2.15C - 2.01AB + 1.22AC - 0.92BC - 1.75A^2 - 2.31B^2 - 1.53C^2$

IN VITRO DRUG RELEASE STUDY

In vitro drug release was performed using a dialysis membrane (MWCO 12–14 kDa) in phosphate-buffered saline (PBS, pH 7.4). The niosomal suspension equivalent to 1 mg of drug was placed inside the dialysis bag and immersed in 100 mL of PBS at 37 ± 0.5 °C under constant stirring. At predetermined time intervals (0, 1, 2, 4, 6, 8, 10, 12 h), samples were withdrawn and replaced with fresh buffer. The amount of drug released was analyzed using UV-Visible spectroscopy at the drug's λmax.

TABLE: EXPERIMENTAL DATA

Run	Surfactant (mg)	Cholesterol (mg)	Sonication Time (min)	Vesicular Size (nm)	Entrapment Efficiency (%)	Drug Release (%)
1	20	20	1	122	72	64
2	20	10	1.5	110	78	69
3	20	15	1.5	115	75	67
4	15	10	1	145	68	59
5	10	15	1	160	65	52
6	10	10	1.5	155	62	54
7	20	10	0.5	135	70	60
8	15	20	0.5	130	73	61
9	15	15	1	125	76	65
10	15	10	0.5	140	69	58
11	15	15	1.5	118	77	68
12	20	15	1	113	79	70
13	10	20	1.5	158	61	53
14	15	20	1.5	123	74	66
15	15	15	0.5	127	72	60
16	15	15	1	120	75	63
17	15	15	1.5	117	76	67

4. CONCLUSION

This study demonstrates that a statistically designed Box-Behnken optimization can effectively predict and improve the performance of Niosomal drug delivery systems. The optimized formulation showed nanoscale vesicles, high encapsulation efficiency, and controlled drug release suggesting promising applications for herbal-based therapeutics.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

6. ACKNOWLEDGEMENTS

The authors declare that no acknowledgements are applicable for this study, as no external support, funding, or institutional assistance was received during the execution of this work.

7. REFERENCES

1. Amolnat tunsirikongkon et al. Formulation development of herbal capsule containing oleoresin of *zingiber officinale* extract. International journal of pharmacy and pharmaceutical sciences, 2013:5 (4), 439-445.
2. Anderson J et al. Malaria Research: an Audit of International Activity. Prism Report no. 7.
3. Ansari SH et al. Influence of nanotechnology on herbal drugs: A Review. J Adv Pharm Technol Res. 2012 Jul; 3(3):142-6.
4. Anupam Kumar et al. A Review on nanonised herbal drugs. International journal of pharmaceutical sciences and research.2015**;** 6(3):961-70**.**
5. AronsonB et al. Chloroquine -resistant *falciparum* malaria in Madasgar and Kenya. Annals Trop. Med. Parasitol. **75:**367-373.
6. Aybike Gunes et al. Niosomes of Nerium oleander extracts: In-vitro assessment of bioactive Nano vesicular structures. Journal of Drug Delivery Science and Technology 37(2017),158-165.
7. Directions in drug discovery. Drug Discov Today 6(22):1151. Faurant C (2011) From bark to weed: The history of artemisinin. Parasite 18(3): page no: 215–218.
8. Dondorp AM et al. (2009) Artemisinin resistance in Plasmodium falciparum malaria. Nengl J Med 361(5):455–467.Denis MB, et al. (2006) Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. Trop Med Int Health 11(9): page no:1360–1366.
9. Jude E Okokon et al. Anti malarial and anti plasmodial activity of husk extract and fractions of Zeamays, Pharmaceutical Biology, 55:1, 1394-1400, DOI: 10.1080/13880209.2017.1302966. Jude E et al. Anti malarial and anti plasmodial activity of husk extract and fractions of Zeamays, Pharmaceutical Biology, 55:1, 1394-1400,DOI: 10.1080/13880209.2017.1302966.
10. Lohar DR et al. Heavy metals by Atomic absorption spectro photometry. In: Quality Control Manual for Ayurvedic, Siddha and Unani Medicine. Department of Ayush, Ministry of Health and Family Welfare, Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad 2008, page no: 69-74.
11. Mooza Al-Owaisi et al. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrine (Forssk.) Fiori leaves. Asian Pac J Trop Biomed 2014; 4(12): 964-970.
12. Petersen I et al. Drug-resistant malaria: Molecular mechanisms and implications for public health. FEBS Lett 585(11): page no:1551–1562.
13. Rajpal V. Standardization of botanicals, testing extraction methods of medicinal herbs. Vol. 2, Eastern Publishers, 2005.
14. Resistance of Plasmodium falciparum field isolates to in-vitro artemether and point mutations of the SERCA-type pfatpase6. Lancet 366(9501): page no:1960–1963.
15. Sarr et al. Icacina senegalensis (Icacinaceae), traditionally used for the treatment of malaria, inhibits in vitro Plasmodium falciparum growth without host cell toxicity. *Malaria Journal*2011; 10:85.

16. The Ayurvedic Pharmacopoeia of India, Govt. Of India, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homeopathy, New Delhi, Published by The Controller of Publications, Civil Lines, Delhi 1989, 1(1):143, 156.