Title

Optimisation of extraction method for the plant *Stephania glabra* and its bioactive's through the implementation of Response surface methodology.

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Abstract

Stephania glabra (Roxb.) Miers, also known as *Stephania rotunda* and formerly referred to as *Cissampelos glabra* Roxb., belongs to the family Menispermaceae. Commonly known by vernacular names such as "Purha" and "Paahraa," this membrane-like, peltate-leaved climbing shrub is distributed widely across tropical and subtropical regions. In Indian traditional medicine systems, especially Ayurveda, S. glabra is recognized for its therapeutic applications in treating ailments such as diarrhea, fever, tuberculosis, asthma, and urinary and abdominal disorders. It possesses diverse pharmacological properties including anti-malarial, anti-bacterial, antiplasmodial, calcium antagonist, and wound-healing effects.

Phytochemical investigations have identified several alkaloids responsible for its medicinal efficacy, notably gindarine and stepharine (stephaglabrine), along with magnoflorine, menisperine, roemerine, palmatine, and tetrandrine. These compounds exhibit notable pharmacodynamic effects, including smooth muscle stimulation, cholinesterase inhibition, and suppression of locomotor activity in animal studies.

In the pharmaceutical industry, extraction plays a vital role in isolating and quantifying bioactive compounds. The efficiency of extraction is influenced by numerous parameters. The selection of an appropriate solvent is critical and should be guided by considerations of solubility, selectivity, cost, and safety, in accordance with the principles of intervisibility and chemical similarity. RSM thus provides a robust framework for optimizing extraction conditions to achieve maximum yield of plant *Stephania Glabra* and quality of desired phytoconstituents.

Key words: *Stephania glabra*, Alkaloids, Response Surface Methodology (RSM)

Introduction

Medicinal plants have been used as a natural, renewable supply of bioactive chemicals with a variety of therapeutic advantages as well as inexpensive therapies for a wide range of illnesses. Over the years, traditional medical systems have relied heavily on medicinal plants.[1] According to estimates from the World Health Organization (WHO), 80% of people who live in rural areas depend on herbal medicine to fulfill their medical requirements. All segments of the population in India use medicinal plants widely, either directly as traditional medicine, indirectly via pharmaceutical manufacture, or in many ancient medical systems. This is one of the reasons why a lot of individuals in industrialized nations have started using supplemental or alternative medicines, which include medicinal plants, through optimization. The Response surface methodology (RSM) is a widely used mathematical and statistical method for modeling and analyzing a process in which the response of interest is affected by various variables. Short period of time is required to test all the variables pertaining and evaluation of the process will become easy, making the laboratory test stage more efficient. RSM method is for the optimization of such process of experiment.[2] RSM has been applied in various experimental designs involving extraction process, food preservation, fermentation as well as other discipline of engineering. It comprises of different techniques; like Central Composite Design (CCD), Box Behken (BB), Full factorial design. It is useful for developing, improving, and optimizing the response variable like extraction yield on the extracted plant.[3]

In the pharmaceutical industry, extraction is a crucial procedure that usually serves to isolate and quantify substances of interest. However, it is recognized that several extraction parameters, including temperature, time, power, frequency, solvent concentrations, pH, solid-liquid proportion, particle size, etc., affect the extraction of different chemicals. For solvent extraction, the choice of solvent is essential. When choosing solvents, selectivity, solubility, cost, and safety should all be taken into consideration. Based on the principles of intermiscibility and resemblance.

Individual screening of these factors at a time is laborious and time taking job and need lots of experimental work. Therefore, establishment of optimization technique for extraction of various constituent like alkaloid, glycoside, tannins etc. is required. Two types of techniques generally used; the classical single factor experiments and the response-surface methodology RSM.[4] The present study is an attempt to determine the best solvent and optimal extraction conditions of independent variables (i.e. extraction, temperature, time, sample to solvent ratio, pH of the solvent, solvent concentration) Optimization of the extraction method by trial-and-error method is time consuming, tedious and leads to wastage of time and materials. Application of an experimental design helps in achieving the desired extract in an organized and logical manner - saving time, materials, and manpower.[4][5]

Stephania glabra (Roxb.) Miers (Family-Menispermaceae) *Stephania rotunda* is another name for this plant, which was previously referred to *Cissampelos glabra* Roxb. "Purha and Paahraa" are alternative vernacular names. The leaves of this climbing shrub are membrane-like and peltate. In India, *S. glabra* is a well-known plant that grows throughout tropical and subtropical parts of the globe. This plant primarily thrives in the lower Himalayan regions, particularly in tropical and temperate areas, at elevations of 2200 meters from Sindh eastward to the Khasia Hills and Pegu.[6] In India, Ayurveda and other traditional medical systems place a great importance on *Stephania glabra*. This plant is well-known in traditional medicine for treating a variety of illnesses, including diarrhea, pyrexia, and tuberculosis. In addition to its anti-malarial, anti-bacterial, antiplasmodial, and calcium antagonist properties, it was used to treat headaches, diarrhea, urinary tract infections, abdominal ailments, asthma, as well as ascariasis, dysmenorrhea, and wound healing. [7] Gindarine and stepharine (stephaglabrine), two alkaloids, are the primary contributors to *Stephania glabra's* pharmacological activity. In an animal investigation, gindarine inhibited both phentermine-induced and spontaneous locomotor activity. Stepharine stimulated animal smooth muscles, boosted organ sensitivity to ache, and reduced the action of real and false cholinesterase.[8] Stepharine, magnoflorine, menisperine, roemerine, palmatine, corydalmine, Nmethylcorydalmine, columbamine, tetrahydropalmatine, jatrorrhizine, and tetrandrine were among the eleven primary alkaloids that were found. These are this plant's and the genus's most significant components. [9][10] It has been demonstrated that the protoberberine alkaloid jatrorrhizine has antifungal properties. Palmatine, an isoquinoline alkaloid belonging to the class of proto-ber-berines, has been demonstrated to reduce the development of Magnoflorine, a quaternary aporphine alkaloid that is extensively distributed among various members of Magnoliaceae, Menispermaceae, Berberidaceae, or Papaveraceae species [11] and Palmatine an isoquinoline alkaloid from the class of proto-ber-berines, is shown to mitigate the development Stepharine, magnoflorine, menisperine, roemerine, palmatine, corydalmine, Nmethylcorydalmine, columbamine, tetrahydropalmatine, jatrorrhizine, and tetrandrine were among the eleven primary alkaloids that were found. These are this plant's and the genus's most significant components[12]

Material and method

Plant material:

Dried roots and rhizomes were collected and authenticated by Senior Scientist HRDI, Gopeshwar, Chamoli, Uttarakhand in January 2022. The sample was further confirmed by comparing with morphological and microscopical characteristics available in literature.

Experimental design

Design-Expert® (version 11) software was used for the experimental design based on the preliminary studies. Three factors were chosen as independent variables - pH (X1), Time(X2), concentration of Ethanol(X3). The effects of these factors on the properties of plant were evaluated Box-bahenkan. The experimental matrix for the experimental runs is provided in Table 1. %yield w/w (Y1), gm% of Alkaloid (Y2) were selected as dependent variables. Analysis of variance (ANOVA) was performed to determine the significance of each factor and their mutual interactions on response variables. The 3-D response surface plots were generated using the same software. In this research box-bahenkan Design was employed for optimization of extraction method of plant Stephania glabra. The three factors (Independent Variables) were selected pH (X1), Time(X2), Concentration of Ethanol(X3) Two factors were considered as dependent variables which was %yield (Y1) and %Alkaloid (Y2). Design expert software 11.0 was employed to find out the effect of the independent variables on dependent one. [13]

Preparation of extract for method optimization

For each experimental run 5gm powder of *Stephania glabra* roots tuber were extracted using Reflux with different concentration of Ethanol, different pH and time. The amount of ethanol (50 ml) remains same throughout the experiment. After performing the experiment the solution was filtered, marc was washed with alcohol; combined filtrate was collected in porcelain dish and evaporated to remove solvent. Concentrated semisolid extract was obtained. The %yield of the extract was calculated. Extraction performed by suggestive experimental run each run performed 3 times for accurate result.

Optimization of extraction method using response surface methodology

Experimental design [14]

Design-Expert® (version 11) software was used for the experimental design based on the preliminary studies. Three factors were chosen as independent variables - pH (X1), Time(X2), concentration of Ethanol(X3). The effects of these factors on the properties of plant was evaluated with Box-bahenkan design. The experimental matrix for the experimental runs is provided in Table 1. %yield w/w (Y1), gm% of Alkaloid (Y2) were selected as dependent variables. Analysis of variance (ANOVA) was performed to determine the significance of each factor and their mutual interactions on response variables. The 3-D response surface plots were generated using the same software.

Optimization Parameters of extraction method using box-bahenkan Design:

Selection of factors, Levels and Responses

Optimization of the extraction method by trial-and-error method is time consuming, tedious and leads to wastage of time and materials. Application of an experimental design helps in achieving the desired extract in an organized and logical manner - saving time, materials, and manpower. In this research box-bahenkan Design was employed for optimization of extraction method of plant *Stephania glabra*. The three factors (Independent Variables) selected were: pH (X1) , Time(X2), Concentration of Ethanol(X3)

Two factors were considered as dependent variables which is %yield (Y1) and %Alkaloid (Y2). Design expert software 11.0 was employed to find out the effect of the independent variables on dependent one.

- Layout of Box-Bahenkan designs

Experimental run and independent variables

Independent variables				**Dependent variables**	
Run	**pH (X1)**	**Minute (X2)**	**%of Ethanol (X3)**	**%yield w/w (Y1)**	**%Alkaloid (Y2)**
1	**4**	**35**	**75**		
2	**4**	**35**	**75**		
3	**3**	**50**	**75**		
4	**5**	**35**	**95**		
5	**4**	**20**	**95**		
6	**5**	**20**	**75**		
7	**4**	**50**	**55**		
8	**5**	**50**	**75**		
9	**4**	**35**	**75**		
10	**4**	**35**	**75**		
11	**4**	**50**	**95**		
12	**5**	**35**	**55**		
13	**4**	**20**	**55**		
14	**3**	**35**	**55**		
15	**3**	**20**	**75**		
16	**3**	**35**	**95**		
17	**4**	**35**	**75**		

Table no. 1 Layout of Box-Bahenkan design

Once selection of various factors and levels were done, all the batches of extraction were prepared by using above variables. The prepared extracts were evaluated for extractive value (%yield) and alkaloid content.

Optimization of the Extraction by square root method: A Box-Bahenkan Design was applied to determine the combined effect of the independent variables X1, X2 and X3 on the dependent variables Y1 and Y2 For this design. pH, time and concentrations of ethanol taken as X1, X2 and X3 respectively whereas Y1 and Y2 are % of yield of extract and gm % sof Alkaloid were respectively.

Preparation of extract for method optimization

For each experimental run 5gm powder of *Stephania glabra* roots tuber was extracted using Reflux with different concentration of Ethanol, different pH and time. The amount of ethanol (50 ml) remains same throughout the experiment. After performing the experiment the solution was filtered, marc was washed with alcohol; combined filtrate was collected in porcelain dish and evaporated to remove solvent. Concentrated semisolid extract was obtained. The %yield of the extract was calculated.

Extraction performed by suggestive experimental run each run performed 3 times.

Estimation of alkaloid [14]

There are many methods for determining individual alkaloid content in extract and formulation but there are few relatively methods for determination of total alkaloid content. When compared with other methods Dragendorff's reagent (DR) is very commonly used, very simple, rapid and efficient spectrophotometric method

Procedure for calibration curve

Calibration curve was obtained with the Berberine. Series dilution of stock solution were done by withdrawing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 and 1.2 ml in to 10 ml separate flask. A 1 ml of solution was taken and 5 ml thiourea solution added into that, yellow solution obtained which was measured at 435nm with the blank solution.

Preparation of plant Extract

20 mg plant extract was taken and upto 10 ml with 2% glacial acetic acid from that 5 ml of extract solution taken, 2ml (DR) were added into that, orange precipitate formed, the suspension was centrifuged to separate precipitates. The precipitates were then washed with alcohol for removal of excess reagent. The filtrate was discarded and residues were treated with 2ml disodium sulfide solution, Orange precipitates turned blackish brown which then treated with concentrated nitric acid for dissolution. The solution was diluted to 10 ml water, from that 1 ml was pipetted out and 5 ml thiourea was added and it turned yellow in color. Absorbance was measured at 435nm against blank solution [contained Nitric acid and Thiourea in distilled water].

Thin Layer Chromatography (TLC)

Sr. no.	Parameters	Condition
1	Stationary phase	Precoated with Silicagel G-254F
2	Mobile phase	Ethayl acetate: Formic Acid: Glacial acetic acid: Water (6.75:0.74:0.74:1.64)
3	Preparation of test solutions	10mg of ethanolic extract dissolved in 10ml volumetric flask and upto

		10ml with methanol.
4	Preparation of standard solution	5mg Palmatine was taken and dissolved in 5ml (1000µg/ml). 140ppm solution of Jatrorrhizine (140µg/ml). 100ppm solution of megnoflorine (100µg/ml).
5	Saturation time	30 min
6	Visualization	UV 254 and 366 nm

Table no. 2 Thin Layer Chromatography (TLC) parameter and condition

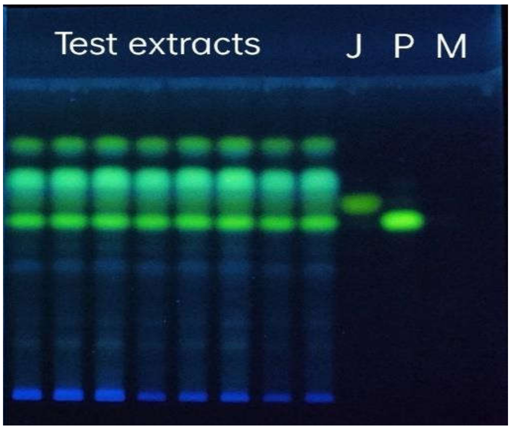


Fig. 1 TLC plate tests extract along with standard scanned at 366nm

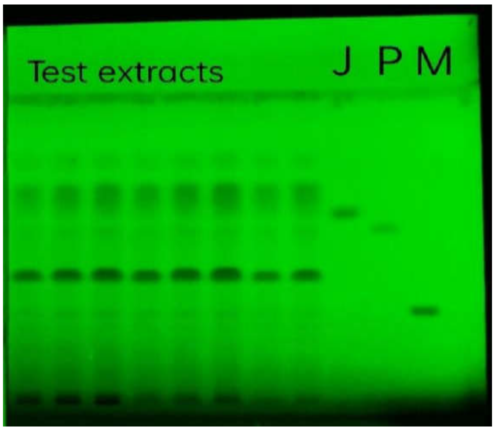


Fig. 2 TLC plate tests extract along with standard scanned at

Extractive value was calculated in all the experimental run and the highest extractive value was in the experimental run no. 7, 8, 14, 15 and 17.

Experimental run	**pH**	**Time (Min)**	**% of Ethanol**	**Extractive value (gm)**	**% yield w/w**
7	4	50	55	1.060	21.02%
8	5	50	75	1.011	20.22%
14	3	35	75	1.429	28.58%
15	3	20	75	1.021	20.92%
17	4	35	75	1.026	20.52%

Table no. 3 Results of Extractive value

Independent variables				Dependent variables	
Run	**pH (X1)**	**Minute (X2)**	**% of Ethanol (X3)**	**%Yield w/w (Y1)**	**%Alkaloid (Y2)**
1	4	35	75	19.12%	1.1320
2	4	35	75	19.44%	1.2542
3	3	50	75	19.22%	1.1772
4	5	35	95	13.8%	0.7914
5	4	20	95	6.78%	0.4844
6	5	20	75	19.54%	0.5527
7	4	50	55	21.2%	0.5253
8	5	50	75	20.22%	0.4550
9	4	35	75	19.52%	0.7384
10	4	35	75	18.82%	0.7274
11	4	50	95	16.88%	1.3622
12	5	35	55	14.78%	0.7362
13	4	20	55	24.38%	0.3297
14	3	35	55	28.58%	0.2357
15	3	20	75	20.92%	0.3338
16	3	35	95	12.02%	0.8386
17	4	35	75	20.52%	0.2357

Table no. 4 Result of optimization technique with extractive value and alkaloid content

Estimation of Alkaloid

The alkaloids were estimated in all the extracted samples and results indicated higher alkaloid in experimental run no 1, 2, 3, 11, and 16.

Experimental run	pH	Time (Min)	%of Ethanol	Alkaloid (gm)%w/w
1	4	35	75	1.1320
2	4	35	75	1.2542
3	3	50	75	1.1772
11	4	50	95	1.3622
16	3	35	95	0.8386

Table no. 5 Results indicated higher alkaloid by optimization technique

ANOVA for Quadratic model Response1: Yield %w/w

Transform: Square Root Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.44	9	0.8263	5.48	0.0177	Significant
A-pH	0.0063	1	0.0063	0.0421	0.8433	
B-Time	0.0317	1	0.0317	0.2103	0.6604	
C-ethanol %	4.13	1	4.13	27.37	0.0012	
AB	0.0264	1	0.0264	0.1750	0.6882	
AC	0.0683	1	0.0683	0.4534	0.5223	
BC	0.0472	1	0.0472	0.3132	0.5932	
A^2	0.5665	1	0.5665	3.76	0.0937	
B^2	0.1285	1	0.1285	0.8523	0.3866	
C^2	1.09	1	1.09	7.24	0.0310	
Residual	1.05	7	0.1507			
Lack of Fit	1.03	3	0.3447	65.88	0.0007	Significant
Pure Error	0.0209	4	0.0052			
Cor Total	8.49	16				

Table no. 6 ANOVA for Quadratic mode

Factor coding was **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 5.48 implies the model is significant. There is only a 1.77% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. 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.

The Lack of Fit F-value of 65.88 implies the Lack of Fit is significant. There is only a 0.07% chance that a Lack of Fit F-value this large could occur due to noise.

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.33	1	0.1692	3.93	4.73	
A-pH	-0.0341	1	0.1664	-0.4276	0.3593	1.27
B-Time	-0.0619	1	0.1350	-0.3810	0.2572	1.08
C-ethanol %	-0.8083	1	0.1545	-1.17	-0.4430	1.27

AB	0.0785	1	0.1876	-0.3650	0.5220	1.15
AC	0.1820	1	0.2703	-0.4571	0.8211	1.43
BC	0.1050	1	0.1876	-0.3385	0.5485	1.15
A^2	0.4297	1	0.2216	-0.0944	0.9538	1.34
B^2	-0.1936	1	0.2097	-0.6896	0.3023	1.24
C^2	-0.5408	1	0.2009	-1.02	-0.0657	1.13

Table no. 7 Coefficients in Terms of Coded Factor

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs if they are greater than 1 indicate multi-collinearity data, the higher the VIF the more severe the correlation of factors. If VIFs are less than 10 then they are tolerable.

ANOVA for Quadratic model Response2: Alkaloid%W/W

Transform: Square Root Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.41	9	0.8233	5.13	0.0443	Significant
A-pH	0.0072	1	0.0041	0.0851	0.5545	
B-Time	0.0885	1	0.0885	0.2142	0.1641	
C-ethanol %	2.37	1	2.37	22.36	0.4473	
AB	0.0253	1	0.0253	0.1450	0.1507	
AC	0.0616	1	0.0616	0.4276	0.5421	
BC	0.0462	1	0.0462	0.2159	0.5032	
A^2	0.5267	1	0.5267	3.25	0.0903	
B^2	0.1513	1	0.1513	0.6140	0.2751	
C^2	1.06	1	1.06	6.28	0.0216	
Residual	1.03	7	0.1471			
Lack of Fit	1.04	3	0.3466	65.88	0.0008	Significant
Pure Error	0.02481	4	0.0062			
Cor Total	13.84	16				

Table no. 8 ANOVA for Quadratic model Factor

Factor coding was **Coded**. Sum of squares is **Type III – Partial**

The **Model F-value** of 5.13 implies the model is significant relative to the noise. There is a 1.37% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case there were significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The **Lack of Fit F-value** of 65.88 implies the Lack of Fit is significant. There is only a 0.07% chance that a Lack of Fit F-value this large could occur due to noise.

- **Co-efficient in Terms of Coded Factors**

Factor	**Coefficient Estimate**	**df**	**Standard Error**	**95% CI Low**	**95% CI High**	**VIF**
Intercept	0.8848	1	0.0834	0.6876	1.08	
A-pH	-0.0509	1	0.0820	-0.2449	0.1431	1.27
B-Time	0.1034	1	0.0665	-0.0539	0.2607	1.08
C-ethanol %	0.0613	1	0.0762	-0.1188	0.2414	1.27

AB	-0.1492	1	0.0925	-0.3678	0.0695	1.15
AC	0.0275	1	0.1333	-0.2875	0.3426	1.43
BC	0.0117	1	0.0925	-0.2070	0.2303	1.15
A^2	-0.0028	1	0.1093	-0.2612	0.2556	1.34
B^2	-0.1224	1	0.1034	-0.3669	0.1221	1.24
C^2	-0.0297	1	0.0991	-0.2640	0.2045	1.13

Table no. 9 Coefficients in Terms of Coded Factors

When the factors were orthogonal the VIFs were 1; VIFs greater than 1 indicate multi- collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable

3-D Graph of highest extractive value 7, 8, 14, 15, and 17

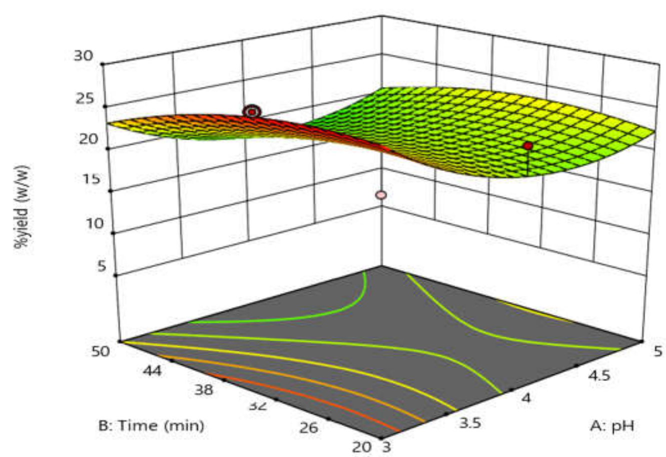


Figure no. 3 3-D Graph of highest extractive value Run

Run 7: The highest yield (~25–26%) is observed at a moderate pH (around 4.2) and intermediate time (around 38–42 minutes). At lower pH values (~3.0), the yield is generally lower regardless of the time. Yield increases as pH rises, peaking around 4.2–4.5, and slightly tapering off beyond that. Yield improves with time but tends to plateau or slightly decline beyond ~44 minutes, especially at non-optimal pH levels. There is a clear interaction between pH and time.

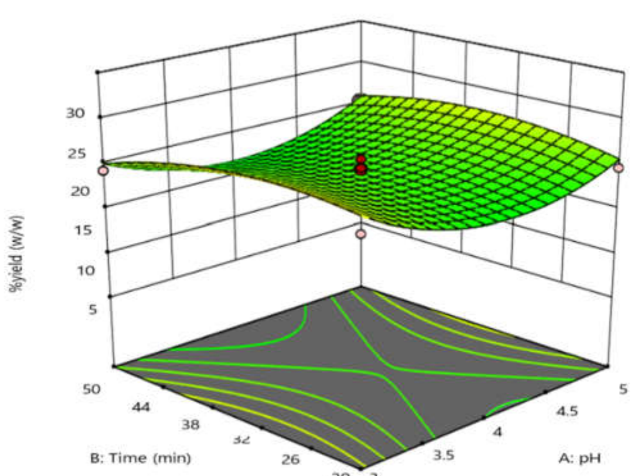


Figure no. 4 3-D Graph of highest extractive value Run 8

Run 8: Red and pink spheres represent actual experimental points used to build the surface model. The red dot at the peak suggests the maximum observed or predicted yield. Pink dots represent other experimental data points. The yield increases as pH increases from 3 to around 4.5, and as time increases to around 40–45 minutes, but plateaus or slightly decreases after that. Optimal conditions for maximum yield (red dot) appear to be around: pH ≈ 4.4, Time ≈ 42 min, Yield ≈ 30% (w/w)

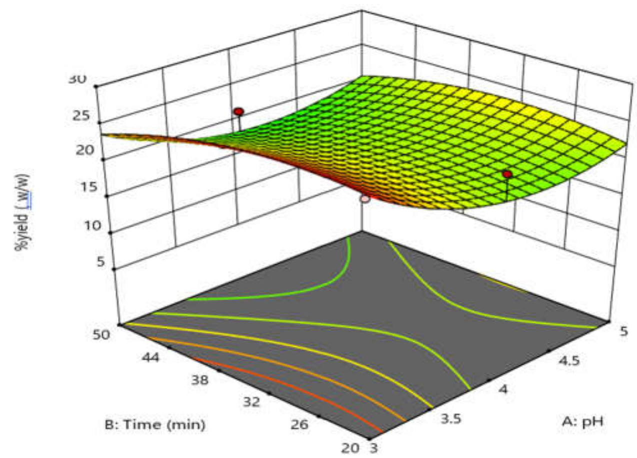


Figure no. 5 3-D Graph of highest extractive value Run 14

Run 14: Red dots represent experimental data points used to create or validate the model. The smooth surface is likely generated by interpolation. Higher pH values (around 4.5 to 5) and longer reaction times (above 35 minutes) favor higher % yield. At lower pH values and shorter reaction times, the yield significantly decreases. This model helps identify optimal conditions for maximizing yield, which would lie in the upper-right region of the graph. This gradient indicates that higher pH and longer time generally result in a higher yield. The highest yield appears to be in the region with higher pH (around 4.5-5) and moderate to higher time (above 35 mins).

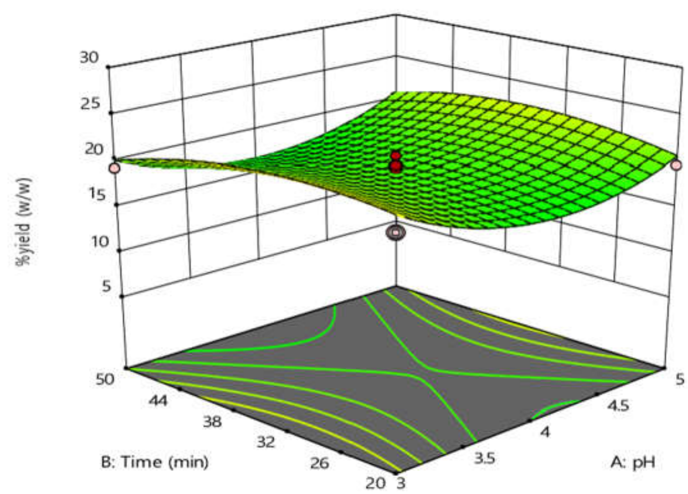


Figure no. 6 3-D Graph of highest extractive value Run 15

Run 15: The color gradient ranges from orange/red (low yield) to green/yellow (high yield). The surface is smoother compared to the first graph, indicating a less steep gradient in response over the range of inputs. Maximum yield (around 20–25%) occurs at higher pH (4–5) and moderate to high time (~35–45 minutes). The lowest yield (~10%) is at the lowest pH (~3) and lowest time (~20 minutes)—highlighted by the red circle on the surface.

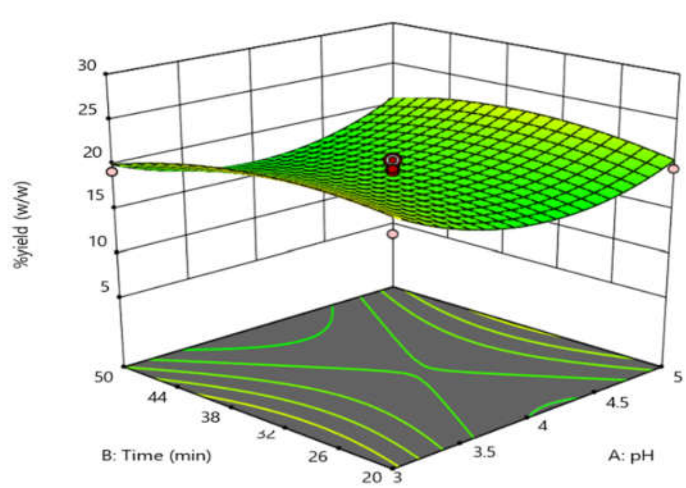


Figure no. 7 3-D Graph of highest extractive value Run 17

Run 17: The red dots represent experimental or simulated data points. The red circled dot at the peak marks the optimal condition for maximum yield (most likely determined through response surface methodology). Pink dots around the edges are boundary or factorial points. Achieved at moderate-high pH (4.0–4.5) and longer reaction times (35–40 min). This region gives maximum yield in the current design space.

3-D Graph of Higher alkaloid in experimental run no 1, 2, 3, 11, and 16.

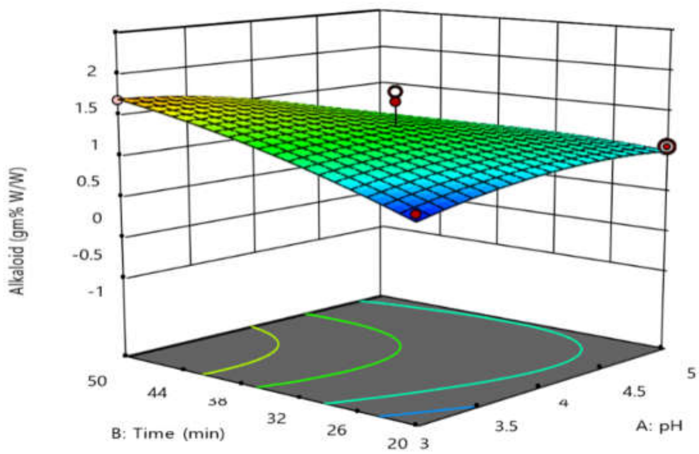


Figure no. 8 3-D Graph of highest alkaloid value in Run 1

Run 1: Blue areas: Lowest alkaloid yield, Green to Yellow: Increasing yield, Orange/yellow zone at top left: Highest alkaloid content. This gradient implies that high pH and longer extraction times result in higher alkaloid extraction. Maximum Alkaloid Yield Occurs at: High pH (~5) Long time (~50 min) Yield: Approaching or just above 1.5 gm% W/W This region is indicated by the light yellow-orange zone on the surface.

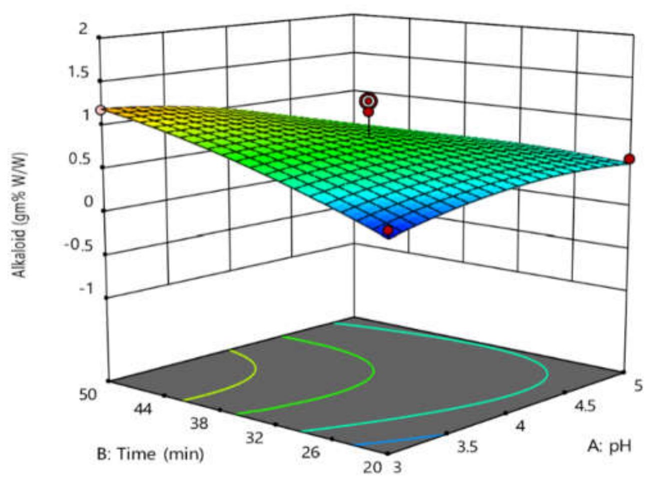


Figure no. 9 3-D Graph of highest alkaloid value in Run 2

Run 2: Red dots: Represent experimental or modeled values Encircled dot: Likely indicates a predicted or optimal value, Optimal condition for alkaloid extraction is at high pH and long time. At low pH and short time, the process is inefficient. Blue regions: Low alkaloid yield.
Green to Yellow: Increasing alkaloid yield, Orange/Yellow top corner: Highest yield Maximum yield is seen around: pH ~3, Time ~50 min, Yield ~1.1 gm% W/W (yellow region)

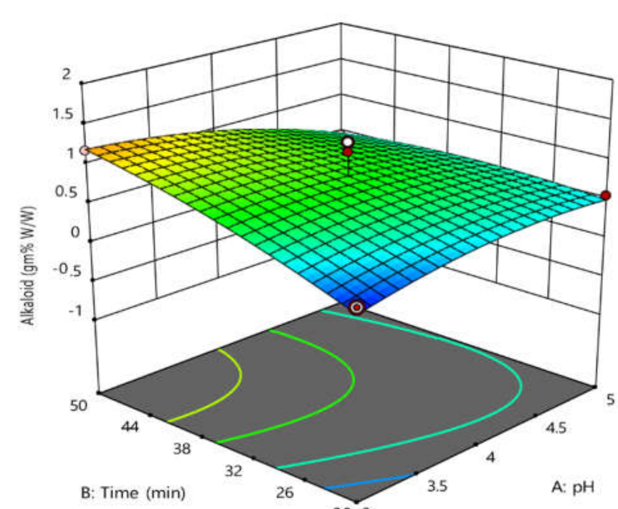
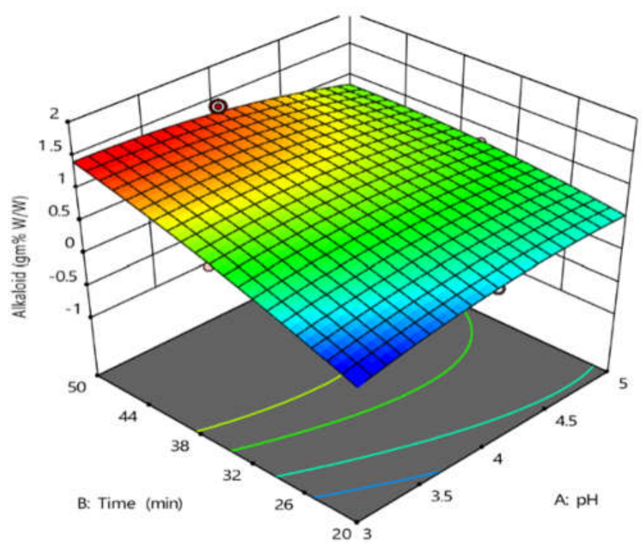


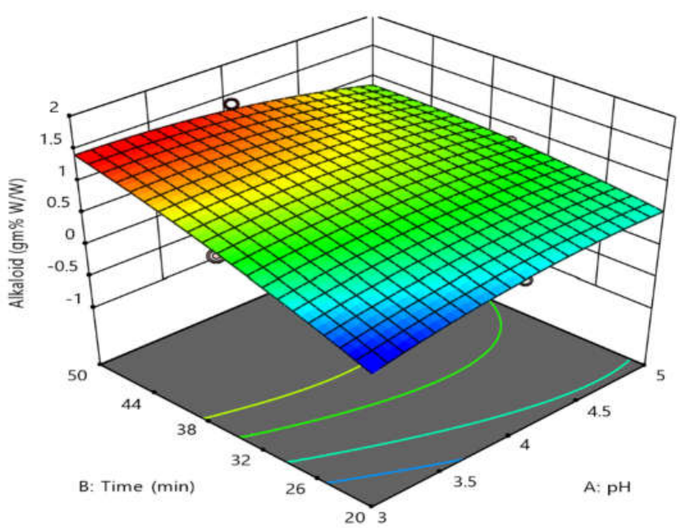
Figure no. 10 3-D Graph of highest alkaloid value in Run 3

Run 3 Blue: Low alkaloid yield, Green to Yellow/Orange: Higher yield, the color gradient visually indicates the alkaloid concentration across conditions. Higher alkaloid yield is observed at: Lower pH (~3) and longer time (~50 min) – indicated by the orange/yellow zone. Approximate yield: ~1.0 gm% W/W.



Run 11: Blue/Green areas: Low alkaloid yield, Yellow to Red: High alkaloid yield. The color distribution shows a clear increasing trend in alkaloid content with longer time and lower pH. Maximum alkaloid yield occurs at: Low pH (~3) and long time (~50 min), Alkaloid content here is above 1.5 gm%, reaching near 2 gm%, Marked with a red circular point at the top-left of the surface. Surface shape and model points hint this is part of a Response Surface Methodology (RSM) analysis, used for optimizing conditions.

Figure no. 11 3-D Graph of highest alkaloid value in Run 11



Run 16: The surface is colored from blue (low yield) to red (high yield). The color pattern indicates: Higher alkaloid yield at low pH (~3) and longer extraction time (~50 min). Lower yield at short time and low to mid pH, as time increases from 20 to 50 minutes, the alkaloid content consistently increases. This is particularly true at lower pH values, as seen in the red region. Best condition: pH ~3, Time ~50 min — gives maximum alkaloid yield (~1.8–2.0 gm%)

Figure no. 12 3-D Graph of highest alkaloid value in Run 16

Result and discussion

For *Stephania glabra*, a pH gradient technique was used to optimise the alkaloid extraction process, with particular emphasis on pH, ethanol concentration, and extraction period. The highest yields of *Stephania glabra*, about 1.429 gm% w/w, were found in Extract 14 out of the 17 extracts. The ideal extraction conditions were determined to be pH 3, 35 minutes, and 75% ethanol. The maximum alkaloid content (1.3622 gm% w/w) was discovered in experimental run 11, where pH was 4, extraction period was 50 minutes, and ethanol concentration was 95%. The ideal extraction conditions were determined to be pH 3, 35 minutes, and 75% ethanol. The potential commercial value of extracts from *Stephania glabra* is supported by this approach, which has been proven to be exact, accurate, cost-effective, and to guarantee high extractive value, total alkaloid content, and maximum phyto-constituent yield.

Conclusion

The improved extracted fraction of the *Staphania glabra* plant, which has several bioactive alkaloids known to exist, must be introduced to the market to demonstrate high yielding and effective results. Using three variables—pH, solvent percentage, and extraction time—the standard alkaloid extraction method using pH gradient methodology was selected for implementation. The model's significance in relation to the noise is indicated by its F-value of 5.13. The likelihood of an F-value this high occurring because of noise is 1.37%. (P-values less than 0.05 show that the model terms are important.) The model terms that were significant in this instance were p values less than 0.044. The model terms are not significant if the values are higher than 0.1000. With a minimum sum of squares of 1.04 and a Lack of Fit F-value of 65.88, the lack of fit is significant [< 0.0008]. A significant Lack of Fit F-value has a 0.07% probability of being caused by noise. Experimental runs 7, 8, 14, 15, 17, and 1, 2, 3, 11, and 16 have the highest extractive value and the highest total alkaloid content, respectively, according to the current extraction process improvement efforts using the Response Surface process. This indicates that the ideal extraction conditions would be pH 4 to 5, with a maximum extraction period of 35 minutes and an ethanol percentage 75.

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