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Optimization of the whole extract of *Zarawand Mudaharaj (Aristolochia rotunda* L.) root by Response Surface Methodology (RSM)

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ABSTRACT

The chemical constitution of a drug has been accepted as an important basis for pharmacological action in Unani medicine. Various dosage forms have been developed on this concept, such as decoctions *(Joshanda)*, infusions *(Khesanda)*, extract *(Rub / Usara)*, and syrup. *Zarawand Mudaharaj* (*ZM*.) / *Aristolochia rotunda* L. root was subjected to extraction process using Soxhlet's apparatus by using Response Surface Methodology (RSM) to design the number of random runs of the extracts with variation in the factors of temperature, the concentration of ethanol in water, time for extraction, for optimizing and maximizing the yield concentration. The data obtained, was analyzed with regression equation and ANOVA two-way summary to interpret the interaction of the factors for yield maximization. Minitab version 18 was used to design and analyze data. Validation of the optimum conditions for maximum yield of the whole extract of ZM. Root was carried out by re-run of the extract using the optimized conditions. The maximum yield percentage thus obtained using RSM was 20.87% whereas using these optimum conditions 21.35 % yield was obtained thereby validating the method. The association between the response functions and the process variables was identified by a three-factor recorded Box-Behnken design. In the present study RSM is used because itis a cheap and affordable method to optimize maximum yield percentage which may be reliably used by researchers. The study set in the surface conditions for *ZM*. root extraction by the Soxhlet apparatus for maximizing the yield percentage.

Keywords Response Surface Methodology, Soxhlet extraction, *Zarawand Mudaharaj* root

1. INTRODUCTION

Medicinal plants have been utilized in the treatment of diseases for a long. Plant-based medicines have been observed to be safe for utilization with lesser side effects compared to synthetic drugs (Dakshayini *et al.,* 2016). A lot of medicinal compounds are originated from plants and constantly play an important role to conserve human health since antique.

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Plant extracts or their active constituents are also used in traditional therapies. Moreover, 80% of the drugs used throughout the world are of natural origin (Amenu, 2014; Amenu *et al*., 2014). *Aristolochia* - a large plant genus with over 500 species, collectively known as birthworts, pipevines, or Dutchman's pipes - belongs to the family *Aristolochiaceae.* They are found quite commonly across diverse climates, but they are not native to Australia. Some species, like *Aristolochia utriformis* and *Aristolochia westlandii* are threatened with extinction. VU/R (Vulnerable / Rare) status was assigned, according to the species compiled in the Red Data List of South Indian medicinal plants (Dey & De, 2011). The plants emit aroma and their strong scent attracts the insects. The inner part of the perianth tube is hairy, which can attract a fly. These hairs then wither away to release the fly, covered with pollen (http://shodhganga.inflibnet.ac.in).

The common names "Dutchman's Pipe" and "Pipevine" refer to old-fashioned meerschaum pipes, it was, common at one time in Northern Germany and Netherlands. "Birthwort" (e.g. European Birthwort, *Aristolochia clematitis*) refers to these species due to the shape of the flower, which resembles the birth canal. It has been widely used as a folk medicine to cure wounds, snake bites, fever, and, as the name itself implies, in childbirth (aristos=excellent, lokia=birth) (Carboni *et al.*, 1966).

Aristolochia rotunda L., commonly known as smear wort or round-leaved birthwort is an herbaceous perennial plant native to Southern Europe. Its' Unani Name is *Zarawand Mudaharaj* (ZM) (Nafiu & Adeyemi, 2017).

Extraction and isolation of phytoconstituents from the crude drug is an initial phase in the analysis of the constituents of medicinal plants. It helps in assessing individual constituents for their pharmacological value. The process of separation of medicinally beneficial parts of the plant or animal tissues from the inactive components by using appropriate solvents in standard extraction methods is called extraction in pharmaceutical sciences. There are a host of methods of extraction i.e., maceration, hot continuous extraction, percolation, decoction, ultrasound extraction, supercritical fluid extraction, microwave-assisted extraction, etc. (Altemimi *et al.*, 2017). There are classes of preparations as extracts known as decoctions, infusions, fluid extracts, and powdered extracts. These preparations are widely called galenicals, named after the second-century Greek physician, Galen (Handa *et al.*, 2008).

The goal of extraction procedures for crude drugs is to attain the therapeutically active portion and to eliminate the inactive material by treating it with an appropriate solvent. The extract obtained thus may be used as a medicinal substitute in the form of extracts. It may be further processed to manufacture in different dosage forms or fractionated to separate individual chemical entities. Standardization of these extraction procedures enhances the final quality of the herbal drug (Jyothi *et al*., 2010). If the criterion to select the plant was based on its' traditional use, then the need is to prepare the extract as described by the traditional healer to come as close as possible to the traditional drug in question. The choice of a solvent system to a great extent depends largely on the particular idea of the bioactive compound being focused on (Handa *et al*., 2008).

Unani system of medicine uses crude forms of drugs regularly except in the case of decoctions, syrups, *Arqiyaat* (distillates), and some salts obtained from the burning of various plant drug parts. When it comes to these forms the whole extract is emphasized more compared to the isolated extracts. Even processing a crude plant drug part is regarded as a change in its *Surat-e-Nu'iea* (specific form) and it's *Taseer* (effect) may change, such as the extent of powdering can also do so *(*Tan *et al.,* 2012*)*. The chemical constitution of a drug has also been accepted as an important basis for pharmacological action in Unani medicine. Unani scholars have developed various dosage forms on this concept, such as decoctions (*Joshanda*), infusions (*Khesanda*), extract (*Rub* / *Usara*), and even syrups, which includes an element of separating the soluble chemicals in water (Husain *et al.*, 2010).

Since whole extracts are needed for the necessary *Taseer (Effect)* as described in Unani medicine, so whole extract optimization of these drugs is needed. No study is in sight for the optimization of the whole extract of *ZM* as yet. *Zarawand Mudaharaj* (*ZM.)* root is an important herb in the Unani and other indigenous medical systems. *ZM.* root has been prescribed to be taken in decoction form as well as in powder form. So, the drug, *ZM*. has been selected for the present study by the hot percolation (Soxhlet extraction) method to optimize various conditions of temperature and water, alcohol solvent ratio, and time to maximize the yield of the whole extract using RSM.

Soxhlet extraction is a useful tool for preparative purposes, in which the analyte is concentrated from the menstruum as a whole or separated from other unwanted substances. Sample preparations of environmental samples have been developed for a long using different methods or techniques (Bukya & Vijay, 2015). Soxhlet extraction is needed where the compound has limited solubility in a solvent, and the unwanted substance is insoluble. The benefit of this system is that in place of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as continuous heating can lead to the degradation of compounds (Tewari *et al*., 2015). Several works have been performed in the past to extract for maximum yield.

Since whole extracts are needed for the necessary *Taseer* as described in Unani medicine, so whole extract optimization of these drugs is needed. No study is in sight for the optimization of the whole extract of *ZM* as yet. *Zarawand Mudaharaj (ZM.)* root is an important herb in the Unani and other indigenous medical systems. *ZM.* root has been prescribed to be taken in decoction form. So, the drug, *ZM*. has been selected for the present study by the hot percolation (Soxhlet extraction) method to optimize various conditions of temperature and water, alcohol solvent ratio, and time to maximize the yield of the whole extract using RSM.

In statistics, RSM studies the relationships between several surface variables and response variable(s). The method was introduced by GEP Box and KB Wilson in 1951 (Levey, 1959). The basic principle underlying RSM is to use a sequence of designed experiments to obtain an optimal response (Levey, 1959). When many variables and interactions affect desired response, RSM comes in handy for optimizing the process and it is the reduced number of experimental trials needed to optimize the parameters with the reduced number of experimental trials needed to optimize the parameters (Worthen, 2006). RSM is a statistical technique in which many types of response surface designs are used for optimization like Central composite, Doehlert, and Box–Behnken. Box–Behnken design is preferable to the Central composite and Doehlert designs as it requires fewer test runs and is rotatable. Box-Behnken design is of importance because it does not contain any points at the extremes of the cubic region created by the two-level factorial level combinations (Bolanle *et al*., 2014). In the present investigation, Box- Behnken design was selected and used to optimize the extraction of *Zarawand Mudaharaj* root with hydro-alcoholic solvent in different extraction conditions (Temp, Conc. and Time) by RSM, as the design provides three levels for each factor and requires fewer runs in the three-factor case than Central composite and Doehlert design. The drug *ZM*. is used in the form of powders or decoctions.

2. MATERIALS AND METHODS

To achieve the aims of the present study entitled "Whole extraction and optimization of *Zarawand Mudaharaj* (*Aristolochia rotunda* L.) root by response surface methodology (RSM)", and some conventional and instrumental methods were applied. In addition to these methods, a software Minitab version

18 was used to obtain the experimental design. The response variable (yield % age) was optimized by RSM using Minitab version 18. All the experiments were carried out in the Department of *Ilmul Advia*, NIUM Bangalore.

Plant material

The roots of *Zarawand Mudaharaj* (*Aristolochia rotunda*) were oven-dried at 45^{0}C. The dried roots were powdered using a grinder. The powder thus obtained, was extracted in a hydro-alcoholic solution. For the extraction, 40gram of sample powdered drug with 1:8 (sample: solvent ratio) was used at the desired concentration of hydroalcoholic, temperature, and solvent-to-liquid ratio as per the experimental plan. The extraction was carried out using Soxhlet Extraction Apparatus.

Method for extraction

All the extractions were carried out according to randomized design (Table 4) generated through RSM by Minitab 18 for yield response with temperature and concentration variation. The extract was cooled, filtered through Whatman filter paper No 1. After that water bath was used to concentrate the extract then calculated yield in grams (g) for every extraction.

Plant extract yield (EY)

The yield of the extraction was calculated from the equation (W1/W2) x 100. Where W_1 is the weight of extract after evaporation of the solvent and W_2 is the dry weight of the plant sample.

Statistical Analysis

Statistical analysis was carried out by using RSM by software Minitab 18. After generating the random design, the yield obtained in various conditions of temperature and concentration was analyzed for optimization. The regression equation was generated and the optimization of the surface factors for obtaining maximum yield was noted. The results were generated in the form of numerical tables and graphs. The data obtained, was analyzed with regression equation and ANOVA two-way summary to interpret the interaction of the factors for yield maximization. Minitab version 18 was used to design and analyze data in both graphical and numerical form. The association between the response functions and the process variables was identified by three-factor recorded Box-Behnken design.

Validation of the data obtained through RSM

The optimized values for the response variable of yield were used for extraction and the yield percentage was observed and compared with the yield as generated from RSM. If the yield was similar to the value obtained from the RSM then the process stands validated.

3. RESULTS AND OBSERVATION

The whole extraction and optimization of *Zarawand Mudaharaj* root by RSM was carried out. Using Soxhlet apparatus after designing the procedure using Minitab 18. The design created for the factors of temperature, concentration, and time that influenced the yield % were randomly carried out. The random design was also charted out through the Minitab software. The input conditions were initially set as for temperature was 60 ^{0}C to 80 ^{0}C, Concentration of Ethanol in water was from 30% to 70% and time 6 hrs to 8 hrs. The solvent and drug ratio was kept constant for all the runs for extraction as 1: 8. (Table 2-7)

Experimental design for Extraction of ***Zarawand Mudaharaj*** **root**

RSM was used for designing the experimental combinations. The variables used were Temperature in degree centigrade (^{0}C), Concentration of ethanol (%), i.e.; % age of alcohol in the hydro-alcoholic solvent, and Time (hrs) (Table 1, 2, 3 and 4). The tables were generated using Minitab version 18 which were followed to run the extracts with specified conditions. From the tables, it is evident that 15 runs were needed for the extraction process for each drug to optimize the factors giving maximum yield. The random runs were also created for each drug (Table 4).

Table 1. Boundaries of the Experimental Domain and Spacing of the Compositional Variable levels

Independent Variables	Symbol code	Low variables	High variables
Temperature (^{0}C)	A	60	80
Concentration (%)	B	30	70
Time (hrs.)	C	6	8

Table 2. Randomized Design for running Extract of *Zarawand Mudaharaj root*

Run	Block	A	B	C
1	1	1	1	0
2	1	0	0	0
3	1	0	1	1
4	1	-1	0	-1
5	1	1	-1	0
6	1	0	0	0
7	1	0	-1	1
8	1	-1	0	1
9	1	-1	1	0
10	1	1	0	1
11	1	0	-1	-1
12	1	0	1	-1
13	1	0	0	0
14	1	-1	-1	0
15	1	1	0	-1

Table 3. Design executed for surface factors of Temperature, Conc. and Time for yield percentage

Std. Order	Run Order	Point Type	Blocks	Temp.	Conc.	Time
4	1	2	1	80	70	7
13	2	0	1	70	50	7
12	3	2	1	70	70	8
5	4	2	1	60	50	6
2	5	2	1	80	30	7
14	6	0	1	70	50	7
11	7	2	1	70	30	8
7	8	2	1	60	50	8
3	9	2	1	60	70	7
8	10	2	1	80	50	8
9	11	2	1	70	30	6
10	12	2	1	70	70	6
15	13	0	1	70	50	7
1	14	2	1	60	30	7
6	15	2	1	80	50	6

Std.= Standard , Pt= Point, Temp= Temperature , Conc. = Concentration

Table 4. Yield percentage for Zarawand Mudaharaj root after Soxhlet extraction

Std. Order	Run Order	Pt. Type	Blocks	Temp.	Conc.	Time	Yield
4	1	2	1	80	70	7	16.98
13	2	0	1	70	50	7	18.75
12	3	2	1	70	70	8	11.65
5	4	2	1	60	50	6	17.30
2	5	2	1	80	30	7	20.38
14	6	0	1	70	50	7	18.75
11	7	2	1	70	30	8	17.55
7	8	2	1	60	50	8	18.78
3	9	2	1	60	70	7	17.23
8	10	2	1	80	50	8	18.68
9	11	2	1	70	30	6	13.36
10	12	2	1	70	70	6	15.85
15	13	0	1	70	50	7	13.88
1	14	2	1	60	30	7	17.32
6	15	2	1	80	50	6	20.43

Std.= Standard , Pt= Point, Temp= Temperature , Conc. = Concentration

Extract Yield percentage after Soxhlet extraction

The yield percentage for extracts of each of the drugs is depicted in Table 5. The yield % is given against each of the runs.

Table 5. Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.77913	82.13%	49.95%	59.55%

Table 6A. Response Optimization: Yield Parameters

Response	Goal	Lower	Target	Upper	Weight	Importance
Yield	Maximum	11.65	20.43	-	1	1

Table 6B. Solution

Solution	Temp.	Conc.	Time	Yield Fit	Composite Desirability
1	80	32.0202	7.61616	20.8668	1

For prediction Minitab 18 used for RSM for optimizing the extract conditions to get the highest yield regression equations were generated as follows:

Yield = -4.9 - 2.69 Temp. + 1.399 Conc. + 22.8 Time + 0.02523 Temp.*Temp.
- 0.00418 Conc.*Conc. - 0.852 Time*Time - 0.00414 Temp.*Conc. - 0.0808 Temp.*Time
- 0.1049 Conc.*Time

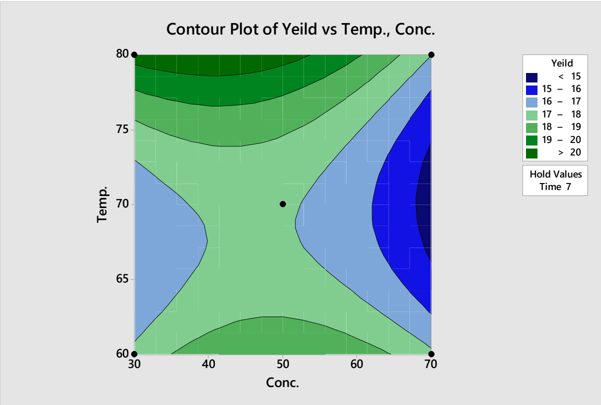

Fig. 1. Contour plot of Yield v/s Temperature and Concentration

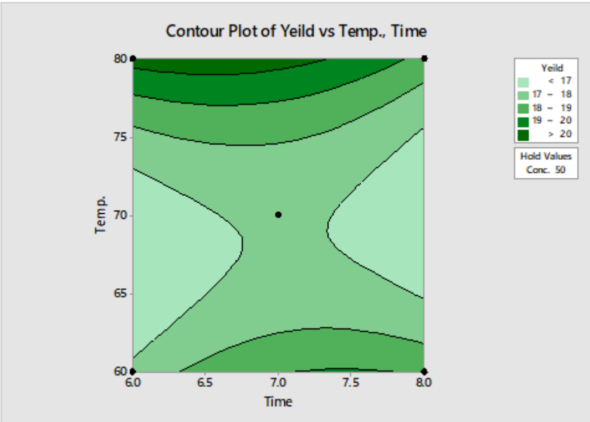

Fig. 2. Contour plot of Yield v/s Temperature and Time

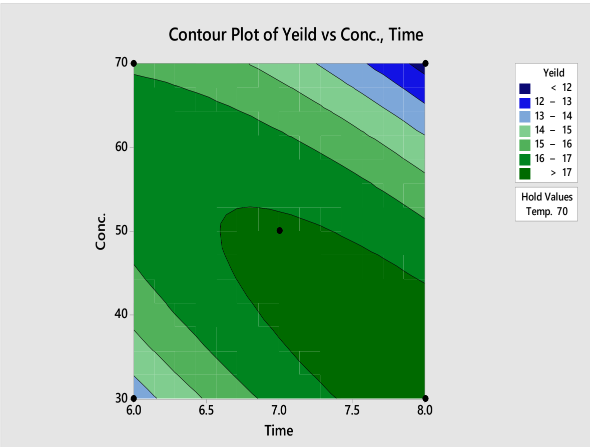

Fig. 3. Contour plot of Yield v/s

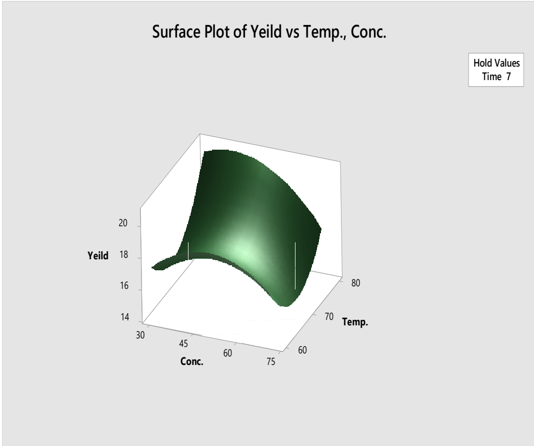

Fig. 4. Surface plot of Yield v/s Temperature and Concentration

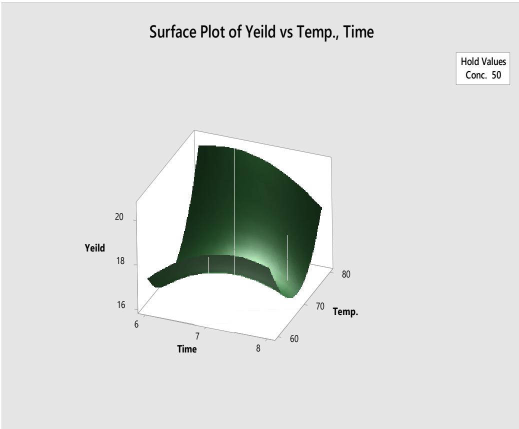

Fig. 5. Surface plot of Yield v/s Temperature and Time

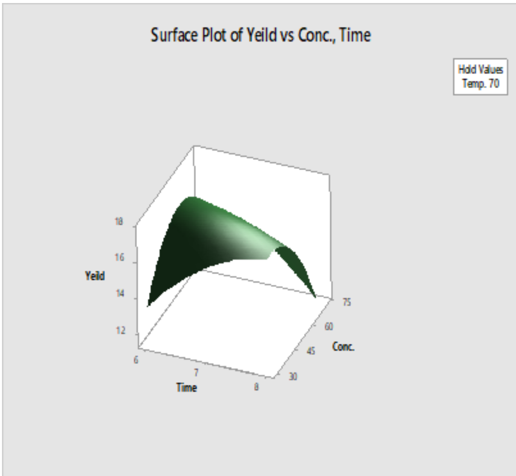

Fig. 6. Surface plot of Yield v/s Concentration and Time

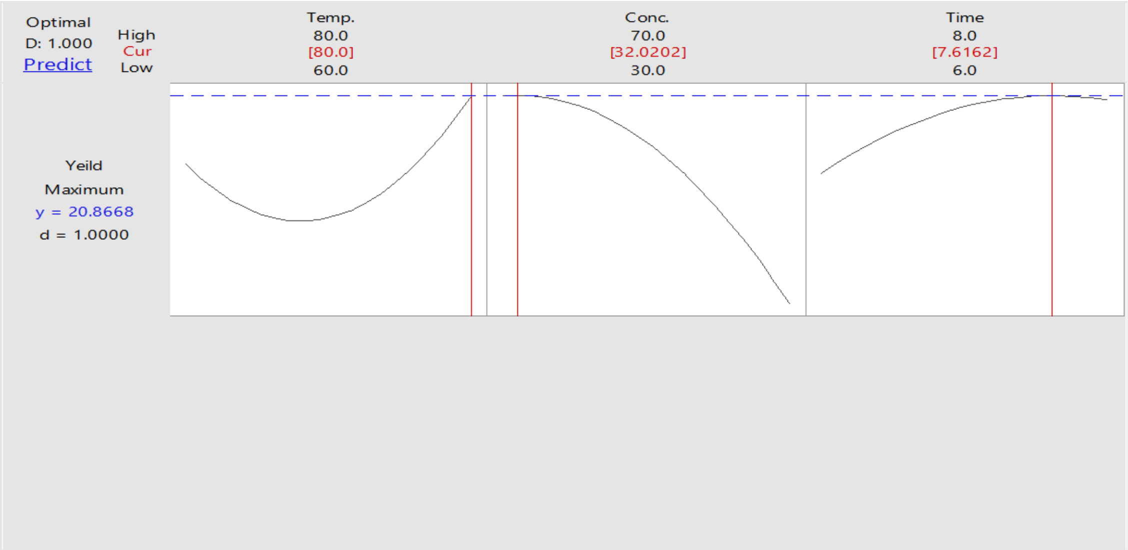

Fig. 7. Optimization Plot for Yield V/s Temperature, Concentration and time for *Zarawand Mudaharaj root* Root Extract

Regression Equation Units for extract

$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta\ X\ ^2 + €$

For prediction Minitab 18 used for RSM for optimizing the extract conditions to get the highest yield regression equations were generated.

$$\text{Yield} = -4.9 - 2.69\,X + 1.399\,Y + 22.8\,Z + 0.02523\,X^2 - 0.00418\,Y^2 - 0.852\,Z^2 - 0.00414\,XY - 0.0808\,XZ - 0.1049\,YZ$$

Where X is Temperature, Y is Concentration and Z is Time

Optimization conditions for the yield % age

When the individual yield extracts were analyzed for optimum yield it was found that the following conditions for the factors gave maximum yield. The optimum conditions predicted response factors need to be 80^0C Temperature, 32.02% Concentration, and 7.6 hrs (Time). The maximum yield of 20.87% was obtained with 95% confidence interval (16.78 – 24.96) and SE 1.5 (Table-6B).

Validation of the data obtained through RSM

The optimized values for the response variable of yield were used for extraction and the yield percentage was observed and compared with the yield as generated from RSM. If the yield was similar (21.3) to the value obtained from the RSM (20.87) then the process stands validated.

4. DISCUSSION

In the present study, the association between the response functions and the process variables were identified by three-factor recorded Box-Behnken design. Further, the extraction conditions of the response variable were optimized. Active constituents were extracted by subjecting the plant material to acid hydrolysis to release the bound particles from their complex matrix, and then selectively separated with hydro alcohol, usually, a helpful solvent for preliminary extraction of chemical constituents from the plants (Bolanle *et al.*, 2014).

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) that is influenced by several independent variables (input variables). An experiment is a series of tests, called runs, in which changes are made in the input variables to identify the reasons for changes in the output response. Originally, RSM was developed to model experimental responses (Box and Draper, 1987), and then migrated into the modelling of numerical experiments. The difference is in the type of error generated by the response. In physical experiments, inaccuracy can be due, for example, to measurement errors while, in computer experiments, numerical noise is a result of incomplete convergence of iterative processes, round-off errors, or the discrete representation of continuous physical phenomena. In RSM, the errors are assumed to be random.

The application of RSM to design optimization is aimed at reducing the cost of expensive analysis methods (e.g. finite element method or CFD analysis) and their associated numerical noise. Venter *et al.*, (1996) have discussed the advantages of using RSM for design optimization applications. For example, in the case of the optimization of the extraction, we want to find the temperature (x_1), solvent concentration (x_2), and time (x_3) that maximize the yield % (y) of the extract. The yield % is a function of the levels of temperature time and solvent concentration, as $y = f(x_1, x_2, x_3) + \varepsilon$, Where ε represents the noise or error observed in the response y.

The surface represented by $f(x_1, x_2, x_3)$ is called a response surface. The response can be represented graphically, either in the three-dimensional space or as *contour plots* that help visualize the shape of the response surface (Morshedi & Akbarian, 2014).

Contours are curves of constant response drawn in the x_i, x_j plane keeping all other variables fixed. Each contour corresponds to a particular height of the response surface, as shown in Figures 1, 2 & 3 cited from the present study.

Table 5 shows the results of the predicted and experimentally measured responses for the 15 runs according to the experimental design. The yield % age ranged from 11.65%

to 20.43%. The maximum yield % age was obtained for the 15th run under the experimental conditions of A = 80 ^{0}C; B = 50%; C = 6hrs. The lowest activity was observed for 8th run under the experimental conditions of A = 70^{0}C; B = 70%; C = 8hrs. Based on these data, the extraction process was optimized for obtaining desirable responses at maximum.

There was fair interaction between surface factors of temperature and concentration, temperature and time, and time and concentration. The summary table for ANOVA shows the p values of the same as 0.298, 0.229, and 0.958 (Table 13) for *Zarawand Mudaharaj* extract. The interaction though found is there but not significant ($p>0.05$). This employs that the three factors are independently acting on the yield. The surface plot is shown in the graph tends to exhibit the parabolic shape that opens on the X-axis signifies that there lie optimum conditions that result in the maximum or optimized yield. This is also evident from the uncoded regression equation obtained for these factors against yield. It is a quadratic equation with a positive sign for the 2nd degree terms. Equation, $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta\ X\ ^2 + €$ as seen from the regression equation generated through Minitab 18.

The yield percentage as a response variable was seen that the optimum yield was at the conditions of temp 80^{0}C, conc. 48.18% and time 8 hrs.

It was 20.87 for *Zarawand* root at the conditions of temp 80^{0}C, conc. 32.02% and time 7.61 hrs. The optimum yield at these conditions was validated by conducting the practical trial for extraction at these conditions and it was found to be for 21.35 for *Zarawand* root. The yield thus obtained was almost similar to the yield suggested by the RSM validating the procedure applied.

A lot of work has been carried out to describe an optimum method to extract for maximum yield of isolated phytoconstituents like Optimization of Subcritical Water Extraction of Bioactive Compounds from fruits (*Berberis vulgaris*) (Sharif *et al*., 2013), Withanolides from roots of *Ashwgandaha* (*Withania somnifera*), optimization for essential oils production from *Citrus latifolia. E*xtraction of bioactive compounds from *Feronia limonia* (wood apple) fruit (Likhith *et al*., 2015). Optimization of conditions to isolate protein from germinated green gram (*Vigna radiata L.*). Microwave-assisted extraction of inulin from Chicory roots, Effect of Temperature, Alkali concentration, Mixing Time and Meal/Solvent ratio on the Extraction of Watermelon Seed Proteins (Wani *et al*., 2012), Extraction of Peanut Proteins with Water by Response Surface Methodology (Rustom *et al.,* 1991). These studies have used response variables as various useful (medicinal or pharmaceutical) chemical constituents. The present study aimed to optimize yield for the whole extract. The reason behind it was that Unani medicine uses these drugs as decoctions, concoctions, or powders. For exclusive concoctions or decoctions water would have been the ideal solvent, but for powders or powder-based dosage forms hydro alcohol was used as maximum ingredients are likely soluble in it. Thus, hydroalcoholic solvent may fulfill the need for all three conditions. The drugs selected for the study were of the nature that they have an insignificant amount of volatile content. Apart from taking these precautions more of the surface factors may be chosen in future studies to fine-tune and better-set parameters for the extraction process. Despite this, it seems that the study will save a lot of solvents, drug material, time, and cost if the drugs are selected for future studies for analyzing constituents. It will help pharmacies to adopt the set in conditions for maximum extract yield for the drugs to use.

The study observed that temperature has a definite effect on the extraction process. With increased temperature from 60 to 80 ^{0}C it was found that there was a consistent increase in the yield which saturated towards the higher end. The temperature enhancement, therefore, does not show any maximization trend of the yield after which it would tend to decrease the yield. Saturation or the maximum solubility of the extraction affects on the increased temperature. It may be explained as the boiling point of alcohol is 80 ^{0}C while water is 100 ^{0}C. The constituents soluble seem to be alcohol soluble more than the water-soluble ones.

The concentration of alcohol and water has got a sure optimizing effect on the extract as seen from the results. The optimization concentration is 32.02 % for *Zarawand* root extract whereas concentrations ranged from 30% to 70%. In the case of time factor, the optimizing condition was 7 hours for *Zarawand Mudaharaj* root, whereas the temperature variation used was 5 hrs to 8 hrs.

ZM roots contain polar and nonpolar are. Hydro-alcohol extract is used as *Ethanol* is a good solvent for polyphenol extraction and water can extract extremely *polar* components. Both hydro alcohol extract can dissolve polar and non-polar component

From the ANOVA summary the p values for interaction variables of temperature and Concentration, Temperature and time, and Concentration and Time, were 0.298, 0.162, and 0.693, respectively. Though they were not significant at $p <0.05$, at80% significant level the results may be interpreted to have fair interaction except time and concentration as the temperature is vital in the method of extraction adopted. The observations were made from the present study viz. RSM using Box-Behnken design is an easy and reliable method to optimize factors affecting yield during extraction, the optimum conditions for *Zarawand* root 80^{0}C, conc. 32.o2% and time 7.61 hrs. Respectively to obtain a yield of 20.87%. Optimized conditions may be used in researches involving the test drugs for the starting procedure for extraction for analysis of the samples.

RSM being an effective tool for accounting surface factors for an outcome has been used here to save labour cost, and standard procedure for the starting drug material for analysis. There are no documented references for the exact starting conditions to get maximum yield in the extraction process. The present study is helpful as a reference for getting maximum yield when extraction of the tested drug is carried out through the Soxhlet apparatus. This apparatus has remained as an indispensable tool for extraction of the plant drugs especially those which are used in decoction or concoction form and have insignificant heat-labile ingredients. Moreover, the traditional system pharmacies when manufacture solid dosage forms like tablets need to minimize the size of the tablet for better compliance. Extractable used as fillers and accommodation of the dosage of drug can go a far to accomplish the same. Similar means have been adopted by the Himalaya Company and may be a way forward for others.

5. CONCLUSION

The effect of extraction conditions on yield % age in *Zarawand* root was studied by response surface methodology.

The results showed that ethanol concentration and temperature and time affected the measured responses significantly. Under the optimal condition of 32.02% ethanol, 80^0 C temperatures, and 7.61 Hrs. time predicted values were found were 20.87%. Overall, our study suggests that the model obtained in the present study can be applied for large-scale production of extract for further use in the pharmacy/food industries. This may encourage the supplementation of bioactive compounds in various food formulations planned to fight oxidative-stress mediated health problems by their biochemical and physiological processes.

RSM using Box-Behnken design is an easy and reliable method to optimize factors affecting yield during extraction. Optimized conditions may be used in researches involving the test drugs for the starting procedure for extraction for analysis of the samples. RSM being an effective tool for accounting surface factors for an outcome has been used here to save labour, cost, and standard procedure for the starting drug material for analysis.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

REFERENCES

Akbar S. *Cuscuta reflexa* Roxb (*Convolvulaceae*). In Altemimi A, Lakhssassi N, Baharlouei A, Watson D & Lightfoot D. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants.* 2017; 6(4): 42.

Amenu D. Antimicrobial Activity of Medicinal Plant Extracts and their Synergistic Effect on Some Selected Pathogens, Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *American Journal of Ethno medicine*. 2014; 1: 018-029.

Amenu S, Jan A, Bahadar K, Marwat & Khan MA. Phytochemistry and Medicinal Properties of *Ammi Visnaga (Apiacae). Pak. J. Bot*. 2014; 46: 861-867.

Bolanle AO, Funmilola AS & Adedayo A. Proximate analysis, mineral contents, amino acid composition, anti-nutrients and phytochemical screening of *Brachystegia Eurycoma* Harms and *Pipper Guineense Schum* and *Thonn*. *Am. J. Food Nutr.* 2014; 2: 11-7.

Box GEP & Draper NR. Wiley series in probability and mathematical statistics. Empirical model-building and response surfaces. (John Wiley & Sons, 1987.

Bukya A & Vijay KTP. Optimization of Conditions to Isolate Protein from Germinated Green Gram (*Vigna radiata* L.) using Response Surface Methodology. *Int. j. adv. res. comput. sci. softw. eng.* 2015; 5: 430-434.

Dakshayini CG, Louella CR & Ujwal P. Optimization of shoot regeneration using leaf explants from *Cichrium intybus* by Box- Behnken Design. *Indian J Adv Chem Sci.* 2016; 179-182.

Handa SS, Khanuja SPS, Longo G & Rakesh DD. An Overview of Extraction Techniques for Medicinal and Aromatic Plants. Aromatic Plants, International centre for science and high technology, Trieste. 2008: 20-25.

Husain A, Sofi GD, Tajuddin, Dang R & Kumar N. Unani system of medicine-introduction and challenges. *Med. j. islamic world acad. sci.* 2010; 18:1, 27-30.

Jyothi D, Khanam S & Sultana R. Optimization of Microwave Assisted Extraction Of Withanolides from Roots of *Ashwagandha* and its Comparison With Conventional Extraction Method. *Int. J. Pharm. Pharm. Sci.* 2010; 4.

Levey M. Chemistry and Chemical Technology in Ancient Mesopotamia, Elsevier, Amsterdam. The Netherlands 1959.

Likhith KR, Babu GRS & Khanum F. Optimization of extraction of bioactive compounds from *Feronia limonia* (wood apple) fruit using response surface methodology (RSM). *Food Chem.* 2015; 173-354.

Morshedi A & Akbarian M. Application of response surface methodology: design of experiment and optimization: a mini review. *J Fund Appl Life Sci.* 2014; 54: 2434-9.

Nafiu MO & Adeyemi SB. Preparation, Standardization, and Quality Control of Medicinal Plants in Africa. *Medicinal Spices and Vegetables from Africa*. 2017; 171-204.

Rustom IYS, ópez-leiva MH & Nair BM. Optimization of extraction of peanut proteins with water by response surface methodology. *J. Food Sci.,* 1991.

Sharifi A, Mortazavi SA, Maskooki A, Niakousari M & Elhamirad AH. Optimization of Subcritical Water Extraction of Bioactive Compounds from Barberry Fruit (*Berberis vulgaris)* by using Response Surface Methodology. *Int. j. agric. crop sci.* 2013; 289-96.

Simon Y, Mills. The Essential Book of Herbal Medicine, Arkana Penguin group London. 380.

Tan QLP, Kieu XNT, Kim NHT & Hong XNT. Application of response surface methodology (RSM) in condition optimization for essential oil production from *Citrus latifolia*. Emir. *J. Food Agric.* 2012; 24: 25-30.

Tewari S, Ramalakshmi K, Methre L & Rao LJM. Microwave-Assisted Extraction of Inulin from Chicory Roots Using Response Surface Methodology. *J Nutr Food Sci*. 2015; 5:1-6.

Wani TA, Ahmad A, Zargar S, khalil NY & Darwish IA. Use of response surface methodology for development of new micro well-based spectrophotometric method for determination of atrovastatin calcium in tablets. *Chem Cent J*. 2012; 6:134.

Worthen DB, Fincher JH, Fuhrman LC, Krypel LL, Lawson KA, Pray WS *et al*. Dictionary of Pharmacy, New age International Publishers, New Delhi, 2006;1993:132.