International Journal of Engineering and Advanced Technology Studies

Vol. 9, No.2, pp.53-73, 2021

ISSN 20535783(Print)

ISSN 20535791(online)

# OPTIMIZED PRODUCTION OF SAPONINS FROM LOCALLY AVAILABLE PLANTS USING RESPONSE SURFACE METHODOLOGY

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**ABSTRACT:** *Saponins are biodegradable, surface active glycosides, commonly distributed in some indigenous plants were extracted using various solvents such as Methanol, Ethanol and Acetone. The relationship between the response (extract yield) and three independent process variables (mass, time and temperature) were optimized and evaluated using the response surface methodology (RSM) and statistical design. A three factor, five levels central composite design (CCD) were employed to determine the optimum extraction conditions. The fit model to describe the effects of mass (A), time (B), and temperature (C) for the extraction was quadratic. A, B, and C gave significant contribution to saponin (response) yield. The different plots of model adequacy recommended that the predicted values of saponin yield in the model were in conformity with the experimental values. The model developed to obtain the maximum yield of extract had a coefficient of determination ($R^2$) of 0.9997. The model adequacy was further checked using the adjusted (adj-$R^2$) which gave a value of 0.9994. Using the numerical optimization, the optimal extraction conditions of mass (2.895g), temperature (72.83$^oC$) and time (224.46mins), gave yield of 62.29% and mass (4.82g), temperature (52.85$^oC$) and time (152.55mins) gave the yield of 63.22% and for yellow yam and wild yam respectively.*

**KEYWORDS:** glycosides, yield, optimization

## INTRODUCTION

Saponins are usually derived from more renewable plant and animal sources. They are known to be major precursors for the preparation and synthesis of steroidal and modern drugs such as the progesterone due to their excellent functional (amphiphilic) properties. As a result of their lower toxicity, biodegradability and ecofriendly characteristics, they are more adaptable and are alternatives to other surfactant types (El-Aziz *et al.*, 2019; Samal *et al.*, 2017; Sahu *et al.*, 2018; Bachari *et al.*, 2019). This accounts for the growing interests in researches of biosurfactant-based material products and their applications. Celik *et al.*, 2021 reported that Biosurfactants are have the capacity to control microbial organisms (bacterial, viral and fungi) with strong ability of causing diseases, such as the severe acute respiratory syndrome, diarrhea, fever, etc. with can result to increase in illnesses and death rate. The dual nature of biosurfactants allows them to interface with the lipophilic (non-polar) molecule of the viral membrane which encloses the ribosomes (proteins and RNA) in the cytoplasm with a significant interest to disrupt cellular activities; this leads to the breakdown of its biochemical structure and consequently rendering it inactive (Smith *et al.*, 2020). The type of aglycones, carbohydrates and different attachment positions result in the several kinds of saponins. Also, in the course of extraction, processing and storage, the chemical structures of saponins due to their amorphous nature may undergo biotransformation due to hydrolysis, microbial and enzymatic reactions. The amorphous region of the saccharide units as well as chemical components of saponins may change due to fermentation, thereby modifying their physiological properties (Yuliana *et al.*, 2017; Deng *et al.*, 2013). Hossein *et al*., 2016 reported that appropriate extraction; processing and storage method is a major component of each efficient technology.

The elemental composition of saponins and their health benefits are evidenced in their roles as antioxidants, anticancer and anti-inflammatory micronutrients. Cardoso *et* al., 2014, Robberecht *et al.*, 2019, and Megan Ware, 2021 suggested that deficiency in Selenium may contribute to cognitive decline and impairments as a result of conditions such as Alzheimer's disease among aging people. Selenium (Se) which is an essential micromineral found in saponin, it is a powerful anti-oxidant. Anti-oxidant helps to reduce oxidative damage by keeping free radicals in check. It helps to reduce the risks of certain cancers. Studies by the office of Dietary Supplements (ODS) reported that Selenium helps to minimize asthma symptoms and protects the heart against diseases, prevents mental decline and is very essential in thyroid hormone metabolism, DNA synthesis, and protection of the body against oxidative stress and infections. Manganese (Mn) reduces disease risk and inflammation, it assists in the normal functions of the brain and the central nervous system. It promotes many other enzymatic and biochemical functions. Rubidium (Rb) aids the transport of defective cell membranes and suppresses the growth of tumour


ULR: https://doi.org/10.37745/ijeats.13

by restraining glucose carrying cancer cells. Strontium (Sr) has very useful application in medicine. It reduces pains in people with advanced bone cancer.

Strontium is similar to Calcium. It can be used to treat weak and brittle bones (osteoporosis). Calcium (Ca), Magnessium (Mg) and Zinc (Zn) play significant metabolic roles in the body, especially in bone formation, mineral absorption, brain development, improvement of body immunity and inflammation reduction. They help to combat infections and aid in wound healing processes. Nickel (Ni) increases hormonal activity and is actively involved in lipid metabolism. It promotes many other enzymatic functions. Bromine (Br) helps in tissue development in all animals. It helps to improve the health of patients in dialysis. Potassium (K) is an electrolyte since it is very reactive in aqueous solution. It performs several functions in the body. It reduces blood pressure and water retention in human cells by regulating body fluid thereby preventing formation of kidney stones. It helps to protect against stroke by sending nerve signals and regulating muscle contractions.

## MATERIALS AND METHODS

Some tubers of *Dioscorea cayenensis* (yellow yam) were sourced from the popular Uselu market in Benin City, Edo State. Wild yam (*Dioscorea villosa*) was harvested in a near bush, close to the University of Benin main gate, Ugbowo campus. Benin City, Edo State, Nigeria

The roots of the wild yam and tubers of yellow yam were washed to remove soil, peeled and cut into small sizes and were air dried (under shade). Air dried samples were ground with a mechanical grinder and sieved to smoothness using 250µm sieve to achieve constant particle sizes. The procedures employed in the study involved a two stage solvent extraction process which included dewaxing to remove residual oil, and reflux extraction. Dewaxing was done to remove the oil/wax from the sample. The experiment was performed in a 1000ml capacity distillation flask using n-hexane as dewaxing solvent. The reflux extraction setup consisted of a reflux condenser, thimble, distillation flask, heating mantle and a retort stand. The solvent (hexane) was heated to evaporate. The hexane traveled up a distillation arm and flooded into the thimble chamber housing the solids tied in a sack. The condenser ensured that any solvent (hexane) vapour was cooled and dripped back into the chamber housing the solid material. The chamber containing the solid material was slowly filled with warm hexane. Some of the undesired material (oil/wax) dissolved in the warm hexane. When the chamber was almost full, it was emptied by the siphon. The solvent was returned to the distillation flask with the oil/wax. The thimble ensured that the fast movement of the solvent up and down did not convey any solid material (material of interest) to the still pot. This cycle was repeated severally. After dewaxing process, the samples were withdrawn and oven dried at 60$^{o}$C for 3 hours to forestall any microbial/enzymatic degradation and kept in sample bottles for saponin

extraction. The central composite method was employed for the experimental design. Extract (saponins) yield would be chosen as the response for process optimization, using the response surface methodology (RSM). The response was studied at various parameters of Mass (g), Temperature ($^oC$) and time (mins).

These parameters to be optimized were coded at 5 different levels which gave the range for mass of sample (1-10g), Time (30-300mins) and Temperature (50-90$^o$c). Experimental observations from the extraction process of saponins were analyzed. The central composite design (CCD) was the design type with a total of 19 runs and 5 central points (Table 3). Quadratic design model (polynomial equation) was obtained from the experimental data to the second order. Design Expert software (version 11.0). Stat Ease Inc., Minneapolis, MN, USA) was used to perform the experimental design and statistical analyses. Correlation coefficient ($R^2$) adjusted determination coefficient (adj-$R^2$) and adequate precision were used to check the model adequacies and the goodness of fit of regression model. The ANOVA was used to establish the significance of the models. The means were tested for difference in statistical significance, using analyses of variance. These analyses included Fisher's F test (overall model significance), its associated probability p(F), correlation coefficient R. The model is termed adequate when P value < 0.05, lack of fit P value > 0.05, $R^2$ is >0.9 and adequate precision >4. The quadratic models were expressed as 3D surface plots to visualize the relationship between the response and experimental levels for each variable and to deduce the optimum conditions (Haloui *et al.*, 2018). The values of the independent variables for optimum response were determined using numerical optimization. The $R^2$ Model and regression coefficients were considered significant when the p-values were less than 0.05.

Table 2.0: Independent Variables for Central Composite Design (CCD)

| Independent variable | Symbols | -α | -1 | 0 | +1 | +α |
|---|---|---|---|---|---|---|
| Mass (g) | $X_1$ | 1.00 | 2.82 | 5.00 | 8.18 | 10.00 |
| Time (mins) | $X_3$ | 30.00 | 84.73 | 165.00 | 245.27 | 300.00 |
| Temperature $^o$c | $X_2$ | 50.00 | 58.11 | 70.00 | 81.89 | 90.00 |

**Solvent extraction method of Saponin**

Reflux extraction technique was adopted in the extraction of saponin from the different plant materials; wild yam and yellow yam. It involved the condensation of vapours and the

return of the condensate to the system from which it originated. A known mass of dewaxed sample was weighed in a round bottom flask containing a known volume of ethanol and the mixture was carefully stirred for 60 minutes. The extraction process was carried out according to the experimental design from the central composite design. The designs produced 19 runs (14 non-centre points and 5 centre points) for yellow yam and 20 runs for wild yam with independent variables of extraction time (30-300 minutes)**,** mass of wild yam (1-10gram) and with temperature of (50-90$^{o}$C) and a constant volume of 50ml of extraction solvent with a single response yield (%). Extraction was conducted by the reflux extraction method (Zhang *et al.,* 2018: Sharma *et al.,* 2014). Methanol and Ethanol were also used for the extractions differently.

The different extracts were filtered with a Buchner funnel and the filtrate was then concentrated by gentle evaporation with a temperature of 45$^{o}$C for several hours until dried solid extracts were obtained.

These were weighed and kept in a sealed plastic container for phytochemical screening.

$$\text{Extract Yield (\%)} = \frac{Wo}{Ws} X\ 100 \qquad (2.1)$$

where wo = mass of saponin extract; ws = mass of sample used

Quantitative determination of saponin was done following to the method by (Ejikeme *et al.,* 2014), the estimated amount of saponin contained in the yellow yam gave 7.8%, while the wild yam gave 6.6%.

**Test for saponins**

To test for the presence of saponin, about 5mls of distilled water was added to the Saponin plants extracts from the various biomaterials in test tubes and were shaken fervently. The formation of stable honey-comb foam indicated the presence of saponins. The lather when mixed with few drops of oil (olive) and shaken vigorously, led to the formation of emulsion as established by Gul *et al.,* 2017.

**Determination of Saponins**

Quantitative and qualitative determinations were carried out on the extracted samples. Total quantitative saponin determination was carried out using the method reported by Ejikeme *et al.,* 2014. Qualitative determination was done using the double beam Spectrophotometer (UV 6300PC) with scanning facility, Agilent 630 Cary and Empyrean X-Ray spectroscopy to determine the maximum wavelength, amorphous nature and functional/reactive groups in the samples.


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## RESULTS AND DISCUSSIONS

The relationship between the extract yield (response) and the three independent process variables (mass, temperature, time) were evaluated by using the response surface methodology (RSM) for building a second order (quadratic) model. The effect of mass, time and temperature on the yield were studied during the experiment. A total of 19runs were developed using the central composite design of experiment, the different ranges of process parameters, and the response extract yield values for yellow yam are shown in table 3.1. The result showed that extraction yield ranged from 7.70% to 61.17%.

Table 3.1 Response of yield value for saponin production from yellow yam

| | **Actual Values** | | | **Coded Values** | | | |
|---|---|---|---|---|---|---|---|
| Run | A- Mass (g) | B- Time (min) | C- Temp (oC) | A- Mass (g) | B- Time (min) | C- Temp (oC) | Extract Yield (%) |
| 1.00 | 5.50 | 165.00 | 70.00 | 0.00 | 0.00 | 0.00 | 51.09 |
| 2.00 | 8.18 | 245.27 | 81.89 | 1.00 | 1.00 | 1.00 | 39.12 |
| 3.00 | 10.00 | 165.00 | 70.00 | 1.68 | 0.00 | 0.00 | 20.50 |
| 4.00 | 8.18 | 245.27 | 58.11 | 1.00 | 1.00 | -1.00 | 30.45 |
| 5.00 | 5.50 | 165.00 | 70.00 | 0.00 | 0.00 | 0.00 | 51.80 |
| 6.00 | 8.18 | 84.73 | 81.89 | 1.00 | -1.00 | 1.00 | 7.70 |
| 7.00 | 2.82 | 84.73 | 58.11 | -1.00 | -1.00 | -1.00 | 47.16 |
| 8.00 | 5.50 | 165.00 | 70.00 | 0.00 | 0.00 | 0.00 | 51.09 |
| 9.00 | 5.50 | 165.00 | 70.00 | 0.00 | 0.00 | 0.00 | 51.18 |
| 10.00 | 5.50 | 165.00 | 90.00 | 0.00 | 0.00 | 1.68 | 34.30 |
| 11.00 | 2.82 | 84.73 | 81.89 | -1.00 | -1.00 | 1.00 | 22.13 |
| 12.00 | 1.00 | 165.00 | 70.00 | -1.68 | 0.00 | 0.00 | 59.97 |
| 13.00 | 5.50 | 165.00 | 50.00 | 0.00 | 0.00 | -1.68 | 46.36 |
| 14.00 | 2.82 | 245.27 | 58.11 | -1.00 | 1.00 | -1.00 | 61.17 |
| 15.00 | 5.50 | 30.00 | 70.00 | 0.00 | -1.68 | 0.00 | 8.55 |
| 16.00 | 2.82 | 245.27 | 81.89 | -1.00 | 1.00 | 1.00 | 56.37 |
| 17.00 | 8.18 | 84.73 | 58.11 | 1.00 | -1.00 | -1.00 | 18.55 |
| 18.00 | 5.50 | 300.00 | 70.00 | 0.00 | 1.68 | 0.00 | 47.89 |
| 19.00 | 5.50 | 165.00 | 70.00 | 0.00 | 0.00 | 0.00 | 51.50 |


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The maximum yield for yellow yam was 61.17%, under the mass of 2.82g at the time of 245.27mins and at a temperature of 58.11$^{o}$C. Maximum yield for wild yam was 60.79%, under mass of 5.5g time of 180mins and at temperature of 50.0$^{o}$C and respectively. The efficiency of the extraction of total saponin using reflux extraction method was evaluated. After extraction, the sample was analyzed by the response surface methodology and analysis of variance (ANOVA). The reflux extraction method adopted provided maximal amount of total saponin and the independent variables had some significant effects on the efficiency of the extraction process.

**Selection of Adequate Model for Extract Yield**

## Fit Summary

**Table 3.2: Yield Response**

| Source | Sequential p-value | Lack of Fit p-value | Adjusted $R^2$ | Predicted $R^2$ | |
|---|---|---|---|---|---|
| Linear | 0.0273 | < 0.0001 | 0.3191 | 0.0346 | |
| 2FI | 0.1166 | < 0.0001 | 0.4592 | 0.1674 | |
| **Quadratic** | **< 0.0001** | **0.7264** | **0.9950** | **0.9905** | **Suggested** |
| Cubic | 0.7264 | | 0.9936 | | Aliased |

Analyses of linear, cubic, two factor interaction and quadratic model were done to select the statistically important model for the determination of the affiliation between the response and the input (independent variable). From the sequential sum of squares, it was noticed that p-values were lower. The fit model to describe the effect of A, B, and C for the extraction is quadratic (table 3.2). A, B, and C are effects on increasing the extract yield.

The correlation between the response (yield) and three independent process variables (mass, temperature and time) were assessed by using the response surface methodology. The different ranges of process parameters, experimental and predicted yield values are shown in table 3.3. Correlation coefficient $R^2$, adjusted determination coefficient (adj-$R^2$) and adequate precision were used to determine the model adequacies. Model was found to be adequate. Aydar, 2018 established that the model is adequate when: P value < 0.05; Lack of fit P value > 0.05; R2 value is > 0.9; adequate precision > 4

**ANOVA for Quadratic model**

**Table 3.3: Table of Variance Analyses**

| Source | Sum of Squares | Df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 5225.50 | 9 | 580.61 | 3159.13 | < 0.0001 | significant |
| A-Mass | 1813.74 | 1 | 1813.74 | 9868.64 | < 0.0001 | |
| B-Time | 1821.52 | 1 | 1821.52 | 9910.95 | < 0.0001 | |
| C-Temp | 200.29 | 1 | 200.29 | 1089.77 | < 0.0001 | |
| AB | 3.04 | 1 | 3.04 | 16.55 | 0.0028 | |
| AC | 95.51 | 1 | 95.51 | 519.67 | < 0.0001 | |
| BC | 197.52 | 1 | 197.52 | 1074.74 | < 0.0001 | |
| $A^2$ | 210.47 | 1 | 210.47 | 1145.20 | < 0.0001 | |
| $B^2$ | 912.29 | 1 | 912.29 | 4963.82 | < 0.0001 | |
| $C^2$ | 206.87 | 1 | 206.87 | 1125.57 | < 0.0001 | |
| Residual | 1.65 | 9 | 0.1838 | | | |
| Lack of Fit | 1.27 | 5 | 0.2533 | 2.62 | 0.1864 | not significant |
| Pure Error | 0.3875 | 4 | 0.0969 | | | |
| Cor Total | 5227.16 | 18 | | | | |

From table 3.3; the **Model F-value** of 3159.13 indicated that the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise.

**P-values** less than 0.0500 indicated model terms were significant. A, B, C, AB, AC, BC, $A^2$, $B^2$, $C^2$ were all significant model terms. Values greater than 0.1000 indicated that the model terms were not significant. Many insignificant model terms (not counting those required to support hierarchy), would require model reduction, which may improve the model.

The **Lack of Fit F-value** of 2.62 implied that the Lack of Fit was not significant comparative to the pure error. There was a 18.64% chance that Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good – Which would enable the model to fit. This indicated that the model is suitable to describe and analyze the extraction of saponin. The developed model was adequate for predicting the yield (response). The model would be considered appropriate if lack of fit value model was not significantly different at the level of specific parameter.


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**Fit Statistics**

Table 3.4: Model Summary Statistics

| | | | |
|---|---|---|---|
| **Std. Dev.** | 0.4287 | **$R^2$** | 0.9997 |
| **Mean** | 39.84 | **Adjusted $R^2$** | 0.9994 |
| **C.V. %** | 1.08 | **Predicted $R^2$** | 0.9980 |
| | | **Adeq Precision** | 173.0000 |

In this study, CV obtained was 1.08%. (table 3.3). The coefficient of variance CV which is the ratio of estimated standard error to the mean value was considered reproducible once it was not greater than 10%.

The **Predicted $R^2$** of 0.9980 was in logical agreement with the **Adjusted $R^2$** of 0.9994; as the difference was less than 0.2. The model developed in this study had satisfactory fit the yield of saponin. This confirmed that the model was accurate and reliable to fit appropriately the interactions between the various independent variables.

**Adeq Precision** considered the signal to noise ratio. A ratio greater than 4 was desirable. Our ratio of 173.000 indicated a sufficient and acceptable signal. This model is adequate to navigate the design space. It could be used to predict and analyze the production of saponins. The mathematical model relating the production of saponin with the independent process variables A, B, C in actual units (table 3.1) was given by the quadratic equation in terms of coded factor and in terms of actual factor.

**Equation in Terms of Coded Factors**

Extract yield (Y) = (51.33 - 11.52A + 11.55B - 3.83C - 0.6167AB + 3.46AC + 4.97BC - $3.93A^2$ - $8.18B^2$ - $3.89C^2$) - - - 3.1

**Equation in Terms of Actual Factors**

Extract yield (Y) = (-12.86874 - 5.40135A + 0.213973B + 2.07566C – 0.002871AB + 0.108590AC + 0.005205BC - $0.548467A^2$ - $0.001269B^2$ - $0.027527C^2$) - - - - 3.2



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From this study, the high levels of the factors were coded as +α (1.68) and the low levels were coded as –α (-1.68). The equation (in terms of coded variables) was used to predict the response for given levels of each variable. The coded equation was useful in the identification of the relative impact of the variables by comparing the variable coefficients..

The equation in terms of actual variables was used to predict the response for given levels of each variable. Here, the levels should be specified in the original unit for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients were scaled to accommodate the units of each factor and the intercept was not at the center of the design space.

The results based of statistical analyses showed that the fit model used to describe the effect of A, B, C for the yield depicted a quadratic model. The independent variables with the largest effect on the yield were the linear terms of treatment (A, B, and C), the quadratic term of treatment ($A^2$, $B^2$ and $C^2$), followed by interaction between A and B, A and C, and B and C. P-values greater than 0.05 indicated that the model terms were not significant, so the interaction between A, B and C gave significant contributions to the yield (response).

**Diagnostics Plots of Model Adequacy**

The diagnostic plots generated below, using the experimental values, probability and residual values were used to check the adequacy of the model.

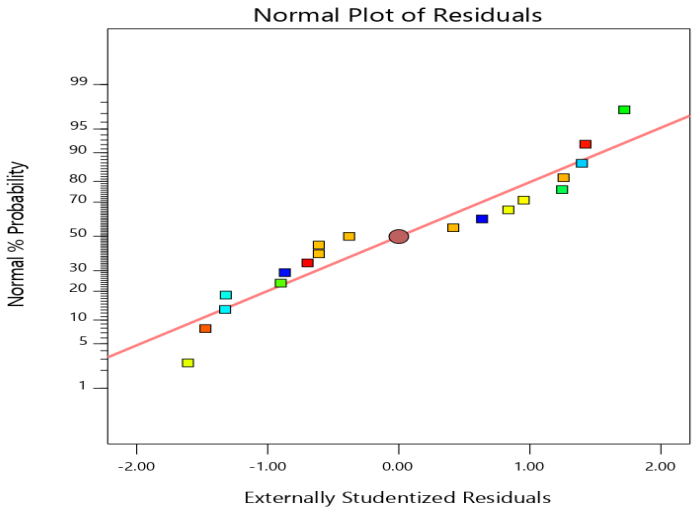


Fig. 3.1: Plot of Probability versus Residuals


ULR: https://doi.org/10.37745/ijeats.13

Vol. 9, No.2, pp.53-73, 2021

ISSN 20535783(Print)

ISSN 20535791(online)

As shown in figure 3.1: represented the normal % probability plot. Most of the plotted data were close to the straight line, meaning that the model was very robust, accurate and in conformity with normal distribution.

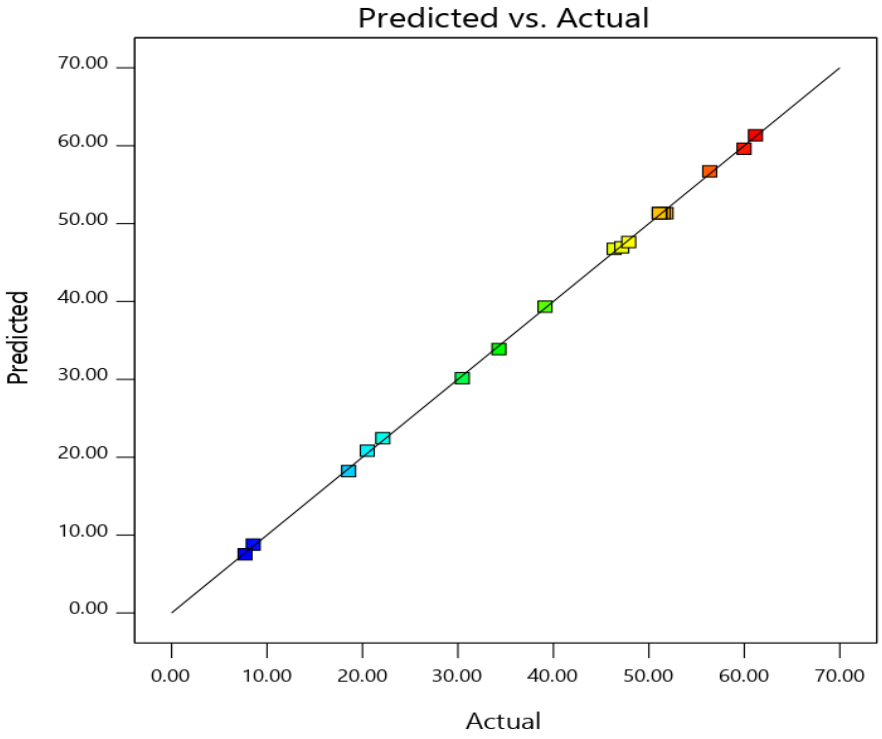


Fig. 3.2: Plot of Predicted versus Actual values of saponin yield

From figure 3.2: The data plots of predicted saponin yield and experimental values were reasonably aligned, meaning that the predicted values of saponin yield in the model were in agreement with the experimental values.


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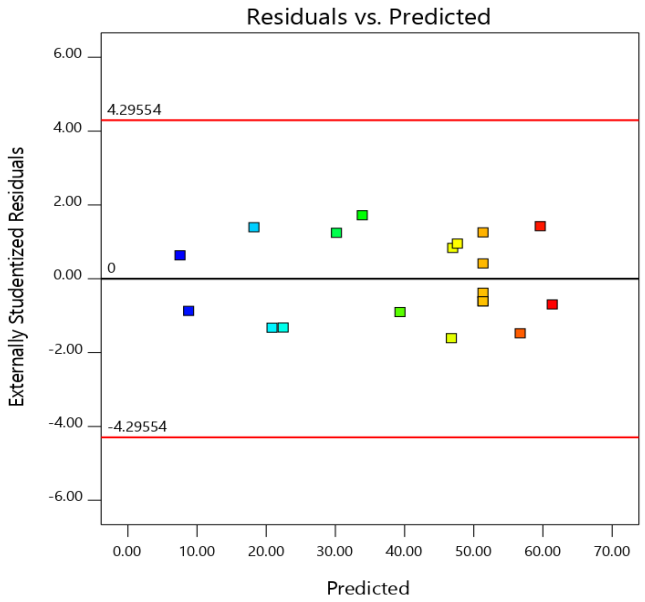


Fig. 3.3: Plot of Residuals versus Predicted

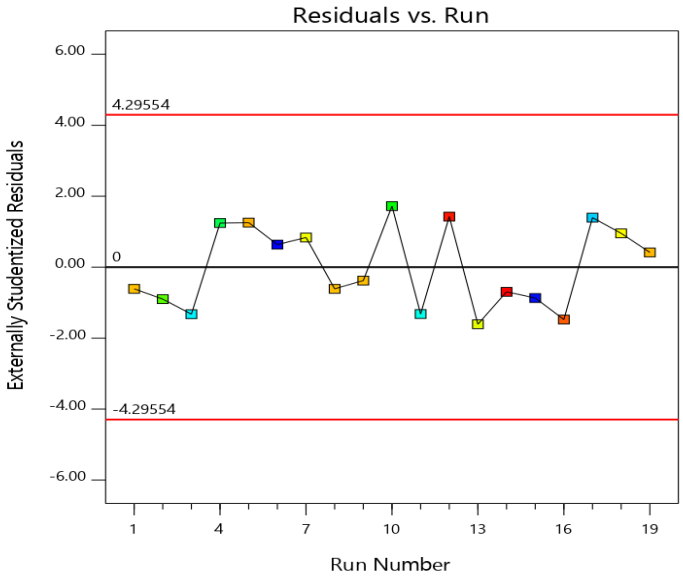


Fig. 3.4: Plot of Residuals versus Runs


ULR: https://doi.org/10.37745/ijeats.13

Vol. 9, No.2, pp.53-73, 2021

ISSN 20535783(Print)

ISSN 20535791(online)

Figures 3.3 and 3.4 represented the plots of the externally studentized residuals. The absolute values of each data point were less than three. This suggested that the model was adequate. The model developed in this study had satisfactory fits for the yield of saponin. Suggesting that the validation of the model was accurate and reliable to fit the interactions between the various independent variables.

**Mutual Factor Interaction Analysis**

The perturbation plot was used to estimate the effect and interactions of the various factors. This was done by moving each factor (variable) from a chosen reference value, while keeping the other variables at constant reference points. The changes in response were displayed using the perturbation plots. The Design Expert software (version 11.0) was used to analyze the effects of process parameters on saponins extraction (response) yield. A three factor, five levels central composite designs were employed to determine the optimum extraction conditions.

Figure 3.5.1 - 3.5.3 showed the relationship between the dependent variable (saponin extraction yield) and the independent variables (mass, temperature, and time) which were represented in a 3D response surface and contour plots. The data were generated by keeping one of the independent variables constant and varying the other two parameters within their experimental range.

Combined Effect of Time and Mass on Extraction Yield

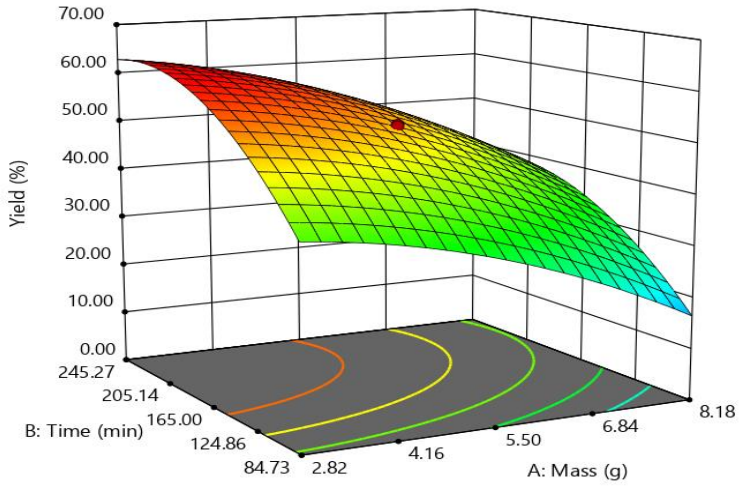


Fig. 3.5.1: Effect of Time and Mass on Saponin yield



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Vol. 9, No.2, pp.53-73, 2021

ISSN 20535783(Print)

ISSN 20535791(online)

Figure 3.5.1 is a representation of the 3-dimensional response surface plot which showed the effect of time and mass on the extraction (response) yield. Both factors (mass and time) significantly influenced the response (extract yield) as shown in the response surface plot. This is also clear from the analyses of variance (ANOVA) which gave p-value of 0.0028 for both mass and time. But, a closer observation showed that time had more profound effect on the extract yield compared to mass. From the plot, we could see that at constant time, the higher the mass the lower the yield from about 21 to 17%. Contrarily, at constant mass, increase in time increased yield from about 21 to 61%. Therefore, effect of both mass and time interaction on the yield had a significant impact on the yield of extraction.

Combined Effect of Mass and Temperature on Extraction Yield

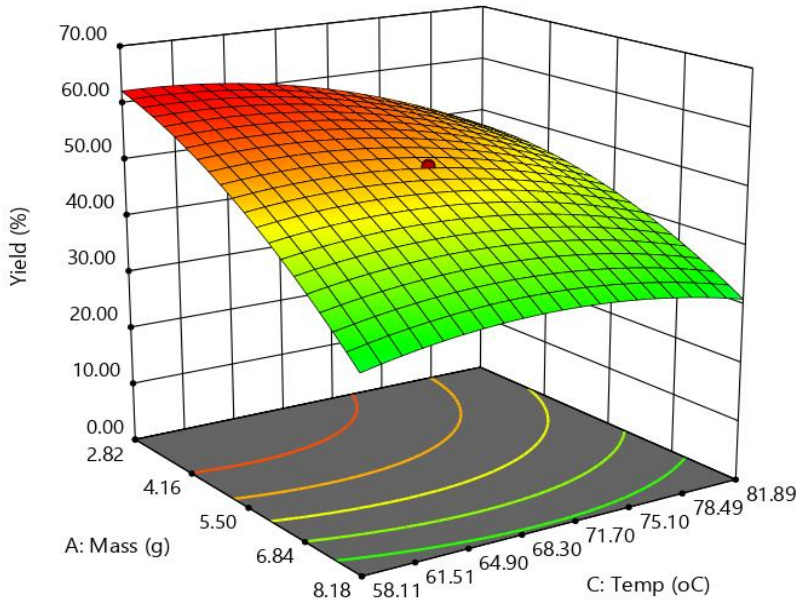


Fig. 3.5.2: Effect of Mass and Temperature on Saponin yield

Figure 3.5.2 shows the 3-dimensional response surface plot of the effect of mass and temperature on the saponins extraction yield. From the 3-dimensional plot, it could be deduced that changes in mass significantly influenced the extract yield, while temperature variation had almost inconsequential effect. The plot revealed that a reduction in mass resulted to an increase in extraction yield from 31% to 61% at steady temperature of 58.11$^{o}$C. Meanwhile, under a constant mass of 8.18g, the effect of temperature change was negligible which maintained a stable yield of about 31%. The saponins extraction yield increased when the temperature ranged from 58.11 to 71.70$^{o}$C, but decreased when


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temperature was higher than 71.70$^{o}$C. Elevated temperatures could result in activity loss, facilitate the degradation of thermo-sensitive compounds and increase the solubility rate of impurities (Similar work by Suleiman et al., 2017). Additionally, as both parameters increased simultaneously, the solid extract yield declined (Fig. 3.5.2). Therefore, regulating time and temperature at optimal levels would ensure good extraction performance.

It helped to protect the heat labile components of the extraction material and minimized losses due to evaporation for volatile solvents. It helped also to lower energy consumption (Morsli, et al., 2021).

Combined effect of Time and Temperature on the Extract Yield

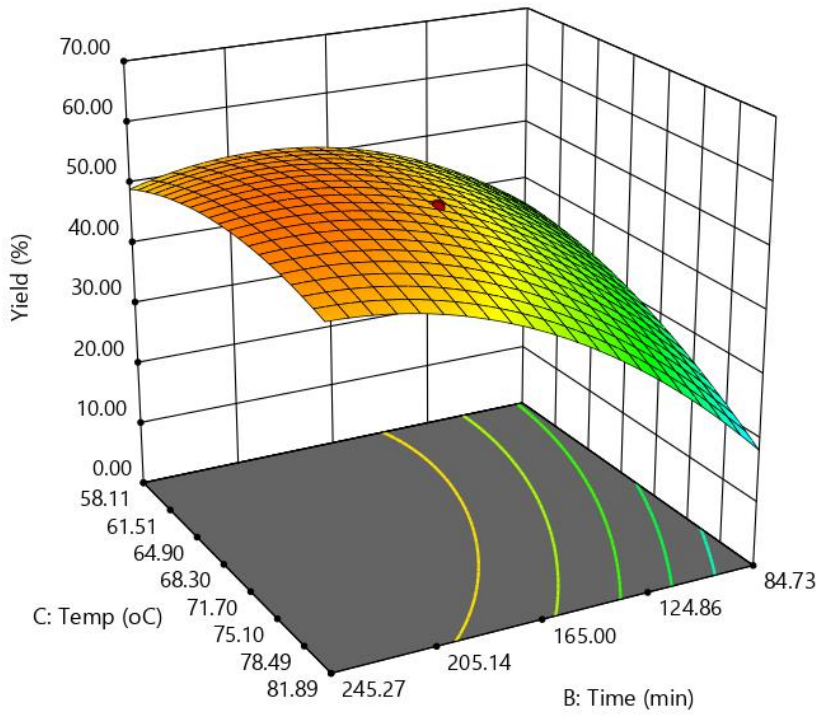


Fig. 3.5.3: Effect of Time and Temperature on Saponin yield

Figure 3.5.3 depicted the effects of time and temperature on the extraction yield, using the 3-dimensional response surface plot. From the plot, it could be established that both time and temperature significantly affected the extract yield. A closer observation revealed that time had more significant effect on the extract yield compared to temperature. An increase in time gave a corresponding increase in the extract yield (from 18% to 49%) from the plot under constant temperature of 81.89$^{o}$C. Sufficient time for the desired compounds to

diffuse into the solvent would be encouraged by prolonged exposure of the sample in the solvent (Suleiman *et al.*, 2017). On the other hand, an increase in temperature had a minimal effect as the extraction yield leveled around 49% at constant time of 245.27minutes. High temperature would cause the oxidation and degradation of the desired compounds (Silva *et al.*, 2007). Contrarily, keeping the temperature at 71.70$^{o}$C for maximum extraction time of 245.27minutes produced good yield. Furthermore, increase in both parameters led to a corresponding increase in the yield of extract (fig. 3.5.3).

**Optimization**

Optimization of saponin extraction from yellow yam was performed using numerical optimization.it requires that goals (minimum, maximum, target or in range) are set for the variables and response to find a set of conditions that will satisfy all the goals. Mass (A), Time (B) and Temperature (C) were set within range and the response (yield) was set at maximum. Tables 3.5 and 3.6 show the optimization factors and the response set at within range limits, corresponding to the required goals. Factors and response were given a criterion that was within the designed space represented in range.

Table3.5: Optimization criteria used in this study

| Name | Goal | Lower Limit | Upper Limit | Lower Weight | Upper Weight | Importance |
|---|---|---|---|---|---|---|
| A:Mass | in range | 2.82428 | 8.17572 | 1 | 1 | 3 |
| B:Time | in range | 84.7285 | 245.271 | 1 | 1 | 3 |
| C:Temp | in range | 58.1079 | 81.8921 | 1 | 1 | 3 |
| Yield | maximize | 7.7 | 61.167 | 1 | 1 | 3 |

Design Expert optimal solutions based on criteria and target on yield



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Table 3.6: Output of optimized results for ten (10) trials with a desirability factor of 1.000

| Number | Mass | Time | Temp | Yield | Desirability | |
|---|---|---|---|---|---|---|
| **1** | **2.895** | **224.463** | **72.830** | **62.289** | **1.000** | **Selected** |
| 2 | 3.877 | 229.979 | 68.387 | 61.360 | 1.000 | |
| 3 | 3.593 | 190.030 | 64.287 | 61.874 | 1.000 | |
| 4 | 2.852 | 244.518 | 58.250 | 61.413 | 1.000 | |
| 5 | 3.158 | 225.847 | 69.646 | 62.965 | 1.000 | |
| 6 | 2.837 | 244.916 | 67.434 | 63.238 | 1.000 | |
| 7 | 3.559 | 223.813 | 61.228 | 61.897 | 1.000 | |
| 8 | 3.378 | 210.253 | 59.717 | 62.539 | 1.000 | |
| 9 | 3.677 | 206.766 | 68.385 | 61.792 | 1.000 | |
| 10 | 3.130 | 240.878 | 74.140 | 61.352 | 1.000 | |

Using the optimal extraction conditions of mass (2.90g), temperature (72.83$^{o}$C) and time (224.46mins), yield was 62.29%. Other optimized results are shown in table 3.6 above. Using the numerical optimization and extraction conditions of mass (4.82g), time (152.53mins) and temperature of 52.85$^{o}$C wild yam gave the yield of 63.22% (table 3.8). These results show that our model was reasonable and feasible and could be used to predict and analyze the production of saponins from local plants. Optimization, evaluation and control of extraction process steps are very fundamental in chemical engineering and biotechnology applications for maximum response (yield), costs of energy and time

**Table 3.7: Optimization criteria for wild yam**

| Name | Goal | Lower Limit | Upper Limit | Lower Weight | Upper Weight | Importance |
|---|---|---|---|---|---|---|
| A:Mass | is in range | 3.22967 | 9.77033 | 1 | 1 | 3 |
| B:Time | is in range | 64.5935 | 195.407 | 1 | 1 | 3 |
| C:Temp | is in range | 48.1079 | 71.8921 | 1 | 1 | 3 |
| Yield | maximize | 5 | 60.79 | 1 | 1 | 3 |


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**Table 3.8: Output of optimized results for ten (10) trials with desirability factor of 1.000**

| Number | Mass | Time | Temp | Yield | Desirability | |
|---|---|---|---|---|---|---|
| **1** | **4.815** | **152.533** | **52.845** | **63.223** | **1.000** | **Selected** |
| 2 | 5.500 | 180.000 | 50.000 | 61.205 | 1.000 | |
| 3 | 7.666 | 104.343 | 53.934 | 63.720 | 1.000 | |
| 4 | 4.621 | 155.490 | 52.611 | 63.088 | 1.000 | |
| 5 | 6.996 | 87.679 | 57.048 | 61.685 | 1.000 | |
| 6 | 6.785 | 81.328 | 55.004 | 63.078 | 1.000 | |
| 7 | 7.790 | 77.255 | 52.878 | 64.984 | 1.000 | |
| 8 | 6.421 | 91.263 | 50.404 | 67.981 | 1.000 | |
| 9 | 8.177 | 84.109 | 54.027 | 63.491 | 1.000 | |
| 10 | 8.053 | 66.901 | 53.870 | 63.535 | 1.000 | |

**Frothing Characteristics of Saponins**

All samples gave significant froth (fig. 3.6), which stood for some reasonable time (above 30minutes). Development of stable foam showed the presence of saponins.

Fig. 3.6: Froth formation indicating the presence of saponin



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## CONCLUSIONS

From this study, suitability of response surface methodology (RSM) in the optimization of saponin production from locally sourced plant materials had been investigated successfully. A mathematical model was developed and queried using the analysis of variance (ANOVA) and projections were made. Results showed that the response (yield) of saponins had time and temperature as the utmost parameters in model terms, although other parameters such as mass also affected the effectiveness of the extraction process. Generally, the saponin yield was directly or indirectly influenced by the various independent variables of mass (g), temperature ($^{o}$C) and time (mins) as shown in the perturbation plots.

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