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Utilisation of 5-Methylcoumarin-4 β Glucoside Isolated From
vernonia Glaberrima Leaves for The Production Of 4-Hydroxy-5-
Methylcoumarin Via Alkali Hydrolysis

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ABSTRACT

Various literature reports had highlighted the significance of coumarins as a potential source for manufacturing of drugs. *Vernonia glaberrima* is one of the major plants that possess 5-methylcoumarin-4 β glucoside in abundance. This study was aimed at utilizing 5-methylcoumarin-4 β glucoside isolated from *V. glaberrima* to produce 4-hydroxy-5-methylcoumarin through alkali hydrolysis. Crude extract was fractionated and subjected to column chromatography. The isolated compound was hydrolyzed and characterized using FTIR and NMR spectroscopy. Optimization of process parameters for the hydrolysis of 5-methylcoumarin-4 β glucosides such as (reaction time, temperature, and NaOH concentration) was determined using Response Surface based on Box-Beinkhen experimental design on Minitab 17 statistical software. The optimum yield of 4-hydroxy-5-methylcoumarin (17%) was achieved at the temperature of 90°C, NaOH Concentration of 1M, and 1 hour reaction time. From this study it can be concluded that 4-Hydroxy-5-Methylcoumarin can be obtained from 5-methylcoumarin-4 β glucoside albeit with maximum yield of 17%.
Keywords: Alkali hydrolysis; Optimization; Coumarin; *Vernonia glaberrima*; Box-beinkhen

1.0 INTRODUCTION

Coumarin is a chemical compound present in many plants, specifically in high concentration in the tonka bean, vanilla grass, woodruff, mullein, sweet grass, *Vernonia glaberrima* and sweet clover. The name derives from French word, *coumarou*. It has a sweet smelling scent and has been used industrially since 1882. Sweet grass and sweet clover in particular are named for their sweet smell, which is due to their high content of this substance. It is a bitter tasting appetite suppressant, and is probably produced by plants as a defense chemical to discourage predation (Ali *et al* 2020). Coumarins have a number of diverse biological and clinical activities, including plant growth regulation, insecticidal, antioxidation, anticoagulation, anti-tumor activity, and antibacterial activity, but the insolubility of coumarins in the active range of concentrations rules out many practical direct

applications, especially as clinical pharmaceuticals (Usman *et al.*, 2021). Recently There has been a great deal of interest, in the use of traditional medicines, particularly those obtained from plants sources for the treatment of diseases (Ugbogu *et al.*, 2021). Phytochemical investigation carried out on the leaves of *V. glaberrima* revealed that it contains 5-methylcoumarin-4- β -glucoside and this compound was reported to be present in very substantial amount in the leaves of this plant (Anifowose *et al.*, 2022). This finding implies that *V. glaberrima* leaves can serve as a clean and renewable source of 5-methylcoumarin-4- β -glucoside. Considering its chemical structure; this compound can serve as a platform chemical for the production of variety of coumarin based chemicals for pharmaceutical and other industrial applications.

The purpose of this research was to determine the best process conditions (time, temperature and NaOH concentration) to obtain 4-hydroxy-5-methylcoumarin from basic hydrolysis of 5-methylcoumarin-4 β glucoside as well as isolation of the compounds from *V.glaberrima*.

2.0 MATERIALS AND METHODS

2.1 Materials

Methanol with percentage purity of 99.5% was provided by Sigma-Aldrich (Merck), Chloroform (99.0%), Butanol (85.0%), Hexane (99.0%), Sodium hydroxide (98.0%), Ethyleacetate (95.0%) and Hydrochloric acid (36.0%) were of analytical grades from British Drug house and Sigma-Aldrich (Merck).

2.2 Methods

2.3 Plant collection and Authentication

The leaves of *V.glaberrima* were collected in October, 2019 in Nasarawa LGA of Nasarawa State. The leaves were authenticated by Dr Ibrahim Malami of Department of Pharmacology and ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. The authentication number given was PCG/udus/Aste/003. The dried leaves were grinded using pestle and mortar and then sieved through a mesh (>60 mesh). Then the powdered sample was stored in separate clean, air-tight polythene bag until required for use.

2.4 Preparation of methanol extract of *Vernonia glaberrima* Leaves

The Powdered dried leaves of *V.glaberrima* (400g) were and transferred into maceration tank with methanol (2L) was soaked for 48hrs at room temperature. The extract was collected, filtered and concentrated at 40°C under reduced pressure using rotary evaporator to obtain methanol extract. The process was repeated twice to ensure maximum extraction. The residual solvent was allowed to evaporate in an open tray for five days. The combined concentrated extracts produced dried crude methanol extract (Anifowose *et al.*, 2022).

2.5 Isolation and Characterization of Phytoconstituents

The dried aqueous insoluble fraction (5g) was weighed and transferred into 100 ml of conical flask and 50ml of ethanol was added. The mixture was heated at 90°C using water bath until it dissolved to form a saturated solution. The solution was filtered immediately and 5 ml of distilled water was added to the solution and allowed to cool and crystallized for 2-3 hours. The crystals formed were filtered and dried under room temperature. The process was repeated twice to obtain pure compound. The purity of the compound was ascertained with the use of TLC and melting point as stated by Anifowose *et al.*, (2022).

2.6 Alkali Hydrolysis of isolated compound

The isolated product (3g) was placed into 250ml One Neck Round Bottom Flask and 20ml of 1M sodium hydroxide aqueous solution was added into the reaction bottle. The reaction was then heated under reflux with water bath at temperature 90°C for about 1hr. There after the solution was allowed to cool and the alkali solution was neutralized by adding excess concentrated HCl and the reaction was allowed to cool which resulted to the formation of precipitate. The precipitate was then filtered and washed with excess distilled water and dried. The experiment was repeated under different experimental reaction conditions (i.e. Temperature 60-90°C, Time 1-3hrs and Concentration 1-5M).

2.7 Data Analysis

The hydrolyzed product yield obtained from the experiments conducted were analyzed on MINITAB 17 Statistical Software base on Box Beikhen design to estimate the main interaction effects on the reaction variables (i.e.; temperature, time and Concentrations), on the hydrolyzed products yield. The percentage products yield was fitted with a full quadratic polynomial model using regression analysis. The fitness of the model was evaluated by the coefficient of determination (R^2) and the effects of terms were evaluated using analysis of variance (ANOVA) at 95% confidence level.

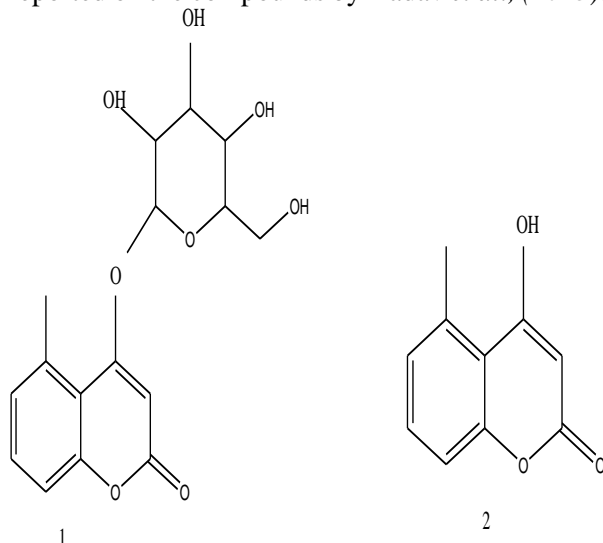
Contour plots were developed using the fitted quadratic polynomial equation. Optimizer on the MINITAB 17 was used to optimize the factors and

the optimal level obtained was experimentally validated (Mustafa *et al.*, 2019).

3.0 RESULTS AND DISCUSSION

3.1 Structural elucidation of isolated compounds

The separation of the dried insoluble aqueous portion from *Vernonia glabberina* using crystallization afforded the isolation of one compound (1) namely 5-Methylcoumarin-4- β -glucoside and alkali hydrolysis of 5-Methylcoumarin-4- β -glucoside under reflux resulted to the production of compound (2) namely 4-hydroxy-5-methylcoumarin. The structures of these compounds are shown in scheme 1 and structures of the compounds were elucidated using FTIR, ^1H and ^{13}C NMR spectroscopy. The results obtained were also compared with the data earlier reported on the compounds by Yadav *et al.*, (2023).



3.2 Scheme 1; Structure of Isolated Compounds 5-Methylcoumarin-4- β -glucoside (1)

The FT-IR spectrum shows the presence of broad band at 3365 cm^{-1} which can be attributed to the stretching O-H vibration groups. The absorption band at around 2883 cm^{-1} was attributed to aromatic C-H stretching (alkyl) group (Narayanan *et al.*, 2021). Another band at about 2940 cm^{-1} was associated to the presence of (=C-H) in benzene ring similar observation was made by (Mustafa *et al.* 2019). A sharp band at around 1270 cm^{-1} was attributed to the presence of group (Saktura *et al.*, 2020). Also another sharp band was observed at

around 1595 cm^{-1} which indicate the present of C=C vibration possibly in aromatic compound (Saktura *et al.*, 2020). The bands at about 1680 cm^{-1} can be assigned to C=O of ketone groups which is equally peak was observed by (Saktura *et al.*, 2020).

The ^1H NMR spectrum shows 3 aromatic protons at $\delta_{\text{H}} 7.52\text{ ppm}$ (1H, t, $J = 7.8\text{ Hz}$), $\delta_{\text{H}} 7.24\text{ ppm}$ (1H, d, $j = 8.4\text{ Hz}$) and $\delta_{\text{H}} 7.15\text{ ppm}$ (1H, d, $j = 7.8$) a characteristic of substituted benzene assignable to H-6, H-7, and H-8 respectively. Similarly, another proton at $\delta_{\text{H}} 5.98\text{ ppm}$ (1H, s) assignable to H-3 was observed which suggest the presence of coumarin nucleus (Phoofolo *et al.*, 2019). The spectrum also revealed 1 anomeric proton at $\delta_{\text{H}} 5.21\text{ ppm}$ (1H, d, $j = 7.8\text{ Hz}$) and a cluster of 5 sugar proton at $\delta_{\text{H}} 3.76\text{ ppm} - 3.20\text{ ppm}$ which revealed the present of sugar moiety as a glucose due to an oxygenated methylene carbon (CH_2) coupled to 3.74 (H-6') (Esra *et al.*, 2020). The $^1\text{H}-^1\text{H}$ COSY revealed the correlation especially between adjacent protons; Major correlations include: proton at $\delta_{\text{H}} 7.52$ (H-7) correlated with $\delta_{\text{H}} 7.15$ (H-6) and proton at $\delta_{\text{H}} 7.24$ (H-8) which confirmed tri substituted benzene ring. Other correlations appeared in the sugar region including: Anomeric proton at $\delta_{\text{H}} 5.21$ (H-1'') correlated with the proton at $\delta_{\text{H}} 3.41\text{ ppm}$ (H-2') and 3.33 ppm (H-3'), also another correlation was observed at proton $\delta_{\text{H}} 3.50\text{ ppm}$ (H-5'') correlated with the proton at $\delta_{\text{H}} 3.50\text{ ppm}$ (H-5'') and 3.22 ppm (H-4''). APT analysis revealed the presence of 16 carbon atom, 9 aromatic methine carbons, 5 quaternary carbons, 1 methylene carbon and 1 methyl carbon. cluster of carbon at $\delta_{\text{C}} 60.5\text{ ppm}$ to 77.3 suggest the present of sugar and anomeric carbon at $\delta_{\text{C}} 99.9\text{ ppm}$ indicated the sugar is to be glucose. The quaternary aromatic oxygenated carbons among others suggesting a chromene nucleus (Mabry *et al.*, 2020). The HSQC analysis was used to establish the correlation of various protons with their respective carbon atoms. These include $\delta_{\text{H}} 7.15$ (H-6) was attached to carbon at $\delta_{\text{C}} 127.7$ (C-6) also a proton at $\delta_{\text{H}} 7.52$ (H-7) was correlated with carbon at $\delta_{\text{C}} 131.9$ (C-7) and proton at $\delta_{\text{H}} 7.24\text{ ppm}$ (H-8) was correlated to carbon at $\delta_{\text{C}} 114.8$ (C-8) in ring A and $\delta_{\text{H}} 5.98$ (H-3) correlated with $\delta_{\text{C}} 92.9$ (C-3), further confirmed the presence of ring B and possibly a chromene nucleus derivatives. The anomeric protons at $\delta_{\text{H}} 5.21$ (H-1') was correlated

with anomeric carbon at $\delta_C 99.9$ (C-1'). Sugar protons correlated with their respective carbon atoms which include; a proton at $\delta_H 3.22$ (H-4') correlated to carbon at their anomeric carbon via correlation between; $\delta_H 5.21$ (H-1'') and $\delta_C 99.9$ ppm (C-1'') and proton $\delta_H 3.41$ ppm (H-2'') with carbon $\delta_C 73$. (C-2''). Sugar protons correlated with their respective carbon atoms which include; a proton at $\delta_H 3.22$ ppm (H-4'') correlated with carbon at $\delta_C 69.5$ ppm (C-4'') a proton at $\delta_H 3.74$ ppm (H-6') was correlated to carbon at $\delta_C 60.5$ ppm (C-6') among other this was also in the same agreement with (Anifowose *et al.*, 2022). The HMBC displayed signals correlating various protons to their neighboring carbon atoms and linked various fragments to their respective positions in the molecule. Major correlations in the aromatic region include; a proton at $\delta_H 7.52$ ppm (H-7) was correlated with carbon at $\delta_C 137.0$ (C-5), and $\delta_C 154.7$ (C-8a), a proton at $\delta_H 5.98$ (H-3) correlated with carbon at $\delta_C 161.2$ (C-2) and $\delta_C 166.5$ (C-4) which confirmed the present of coumarin nucleus (Flores *et al.*, 2022). In the sugar moiety there is correlation between protons and their neighbouring carbon atoms which include; proton at $\delta_H 3.74$ ppm correlated with carbon at $\delta_C 77.3$ ppm (C-3') and 99.9 ppm (C-1'), further more a proton at $\delta_H 3.74$ ppm (H-6') correlated with carbon at $\delta_C 69.5$ (C-4'). A long correlation was observed between an anomeric proton at $\delta_H 5.21$ ppm (H-1') and quaternary carbon in the coumarin nucleus at $\delta_C 166.5$ ppm (C-4) which confirmed the linkage of sugar moiety (glycon) to a coumarin nucleus (Zhang *et al.*, 2022) based on this elucidation and in comparison with data published by (Flores *et al.*, 2020) confirmed that this compound is 5-methylcoumarin-4 β -glucoside.

3.3 4-Hydroxy-5-methylcoumarin (2)

The FT-IR spectrum shows a clear absorption band at 3237 cm^{-1} that is characteristic of O-H stretching. Absorption at 2920 cm^{-1} is due to aromatic C-H while 1595 cm^{-1} was a result stretching band of C=C bond typical of a aromatic Compounds. The band at about 1680 cm^{-1} can be assigned to C-O of esters group. The result of $^1\text{H NMR}$ spectrum shows 3 aromatic protons at $\delta_H 7.46$ ppm (1H, t, J= 7.5 Hz), $\delta_H 7.16$ ppm (1H, d, j=8Hz) and $\delta_H 7.11$ ppm (1H, d, j=7.5Hz) which is attributed to substituted

benzene assignable to H-6, H-7, and H-8 respectively. Similarly, another proton was observed at $\delta_H 5.59$ ppm which was assignable to H-3 and suggest the coumarin nucleus (Saha *et al.*, 2023). The $^1\text{H-}^1\text{H}$ COSY revealed the correlation especially between adjacent protons; Major correlations include: proton at $\delta_H 7.45$ (H-7) correlated with proton at $\delta_H 7.11$ (H-6) and proton at $\delta_H 7.16$ (H-8) which confirmed substituted benzene ring. Other correlations appeared at proton $\delta_H 2.72$ correlated with proton at $\delta_H 7.11$ ppm. The HSQC analysis was used to establish the correlation of various protons with their respective carbon atoms. These include $\delta_H 7.11$ (H-6) was attached to carbon at $\delta_C 127.31$ (C-6) also a proton at $\delta_H 7.46$ (H-7) was correlated with carbon at $\delta_C 131.86$ (C-7) and proton at $\delta_H 7.16$ ppm (H-8) was correlated to carbon at $\delta_C 114.62$ (C-8) in aromatic ring and $\delta_H 5.59$ (H-3) correlated with $\delta_C 90.47$ (C-3), further confirmed the presence of ring B and possibly a chromen nucleus derivatives (Zhang *et al.*, 2022). The HMBC analysis of hydrolyzed compound displayed signals correlating various protons to their neighboring carbon atoms and linked various fragments to their respective positions in the molecule. Major correlations in the aromatic region include; a proton at $\delta_H 7.46$ ppm (H-7) was correlated with carbon at $\delta_C 137.78$ (C-5), and $\delta_C 155.23$ (C-8a), another major correlation exist in the aromatic ring between a proton at $\delta_H 7.11$ ppm (H-6) and carbon at $\delta_C 114.32$ (C-4a) and carbon at $\delta_C 21.77$ ppm. Also, proton at $\delta_H 5.59$ (H-3) correlated with carbon at $\delta_C 170.07$ (C-2) and $\delta_C 114.32$ (C-4a) which confirmed the present of coumarin nucleus (Zhang *et al.*, 2022). With this data and in comparison, with data elucidated by Alhassan *et al.*, 2018 confirmed this compound as pure 4-hydroxy-5-methylcoumarin.

3.4 Effect of the Process Variables on the Yield of Hydrolysis Reaction 4-Hydroxy-5-Methylcoumarin

The experiments performed on 4-Hydroxy-5-Methylcoumarin production from 5-methylcoumarin-4 β -glucoside. Table 1 shows the experimental conditions and percentage yield of 4-Hydroxy-5-Methylcoumarin from various experimental runs. From the result observed that the 4-Hydroxy-5-Methylcoumarin yield obtains ranges

from 9% to 17% lower and highest value respectively. The highest yield of 4-hydroxy-5-methylcoumarin 17% was obtained at a temperature

of 90 °C, 1 hour reaction time, and 1M NaOH Concentration.

Table 1: Optimization process for 4-Hydroxy-5-Methylcoumarin production and the yield obtained

StdOrder	RunOrder	ptType	Blocks	Temp(°C)	Time(hrs)	NaOH Conc.(M)	Yield%
1	21	2	1	60	1	3	9
2	27	2	1	90	1	3	16
3	6	2	1	60	3	3	13
4	9	2	1	90	3	3	10
5	7	2	1	60	2	1	14
6	28	2	1	90	1	1	17
7	3	2	1	60	2	5	9
8	29	2	1	90	2	5	11
9	22	2	1	75	1	1	13
10	16	2	1	75	3	1	16
11	15	2	1	75	1	5	10
12	30	2	1	75	3	5	9
13	11	0	1	75	2	3	11
14	4	0	1	75	2	3	10
15	19	0	1	75	2	3	11
16	14	2	1	60	1	3	10
17	8	2	1	90	1	3	14
18	17	2	1	60	3	3	13
19	24	2	1	90	3	3	11
20	1	2	1	60	2	1	15
21	5	2	1	90	2	1	16
22	18	2	1	60	2	5	10
23	10	2	1	90	2	5	10
24	26	2	1	75	1	1	12
25	2	2	1	75	3	1	15
26	23	2	1	75	1	5	9
27	20	2	1	75	3	5	11
28	13	0	1	75	2	3	10
29	25	0	1	75	2	3	11
30	12	0	1	75	2	3	10

The analysis of variance (ANOVA) Table 2, show that the model F-value for 4-Hydroxy-5-Methylcoumarin yield is significant. The significance of the model is confirmed by relatively

higher Fischer's 'F-statistics' value and lower value of probability ('P' value) (Ajala *et al.*, 2021). The results of analysis of variance (ANOVA) revealed that all the linear interactions terms of the

process variables with exception of time are statistically significant ($p < 0.05$, at $\alpha = 0.05$), It also shows that all the interactions of square terms with the exception of time are statistically significant. The ANOVA results also indicates that with the exception of temperature and NaOH Concentration ratio interaction, all other 2-way interaction terms of the process variables are statistically insignificant ($p > 0.05$, at $\alpha = 0.05$), the “lack of fit” (F -value = 4.75) and (p -value = 0.014) implies that the lack of fit of the data is statistically significant ($p < 0.05$, at $\alpha = 0.05$). The high coefficient variation ($R^2 = 90.62\%$) shows that the model adequately accounts for the empirical relationship between the 4-Hydroxy-5-Methylcoumarin yield and the process variables.

Table 2: Result Analysis of Variance (ANOVA) for 4-Hydroxy-5-Methylcoumarin yield

Source	D F	Adj SS	Adj MS	F- Val ue	P- Val ue
Model	9	177.4	19.72	21.4	0.00
Linear	3	118.8	39.62	43.4	0.00
Temp.(⁰ C)	1	12.25	12.25	13.3	0.00
Time (hr)	1	1.562	1.562	1.70	0.20
NaOH Conc.(M)	1	105.0	105.0	114.	0.00
Square	3	21.49	7.164	7.80	0.00
Temp. (⁰ C)*Temp. (⁰ C)	1	12.72	12.72	13.8	0.00
Time(hr)*Tim e(hr)	1	0.260	0.260	0.28	0.60
NaOH Conc.(M)* NaOH Conc.(M)	1	10.41	10.41	11.3	0.00
2-Way interaction	3	37.12	12.37	13.4	0.00
Temp.(⁰ C)*Ti me(hr)	1	32.00	32.00	34.8	0.00
Temp. (⁰ C)* NaOH Conc.(M)	1	2.00	2.00	2.18	0.15
Time(hr)* NaOH Conc.(M)	1	3.125	3.125	3.40	0.08

Error	2	18.37	0.919		
Lack-of-fit	3	8.375	2.792	4.75	0.01
Pure Error	1	8.375	2.792	4.75	0.01
Total	2	195.8			
	9	67			

Key: DF= degree of freedom, Adj SS= adjusted sum of squares, Adj MS= adjusted mean squares, F-Value = F-statistic values

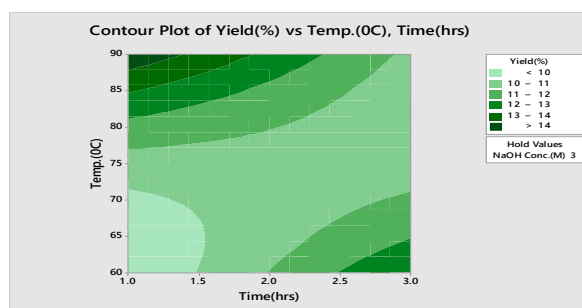


Figure1: Effects of reaction time and temperature on the yield of 4-Hydroxy-5-Methylcoumarin

i. Effect of reaction time on the yield of 4-Hydroxy-5-Methylcoumarin

Figure 1; shows the yield of 4-Hydroxy-5-Methylcoumarin from alkali hydrolysis of methylcoumarin-4 β -glucoside in different reaction time from 1 to 3 hours. As shown in the figure 1, time has significant effect on the yield of the 4-Hydroxy-5-Methylcoumarin, the hydrolyzed product yield decreased with the increase in reaction time at about 60-72⁰C.. However, at 72-90⁰C the hydrolyzed product yield increased with increase in reaction time. The optimum yield of above 14% was achieved at about 1 hour reaction time when the value of temperature is about 90⁰C. In a similar experiment, (Nasab *et al.*, 2023) also reported on the conversion of coumarin to coumarin acid shows that appropriate time to obtain the best yield is 1 hour.

ii. Effect of reaction temperature on the yield of 4-Hydroxy-5-Methylcoumarin

Hydrolysis reaction of 5-methylcoumarin-4 β -glucoside to produce 4-Hydroxy-5-Methylcoumarin is an equilibrium reaction. Therefore, reaction variables such as temperature, time and Concentration can shift the equilibrium

towards the formation of the products. A temperature range of 60-90°C was used in this study. As shown in Figure 1, temperature has significant effect on the yield of 4-Hydroxy-5-Methylcoumarin. The maximum yield of above 14% was achieved at about temperature of 90°C. As the temperature decreases to 85°C, the yield obtained also decreases to 12 %; so therefore, the increases in temperature from 88 to 90°C the yield obtained also increases. However, as the

temperature decreases further from 70-60 °C, the 4-Hydroxy-5-Methylcoumarin yield also decreases abruptly to 9% which is the lowest yield obtained in this study. Decrease in the yield as the temperature decreases to 90 °C is attributed to the fact that, increase in temperature result to the increase in the hydrolyzed yield of the product. Also according to Nasab *et al.*, (2023) increase in reaction temperature result to increase in coumaric acid yield vise visa.

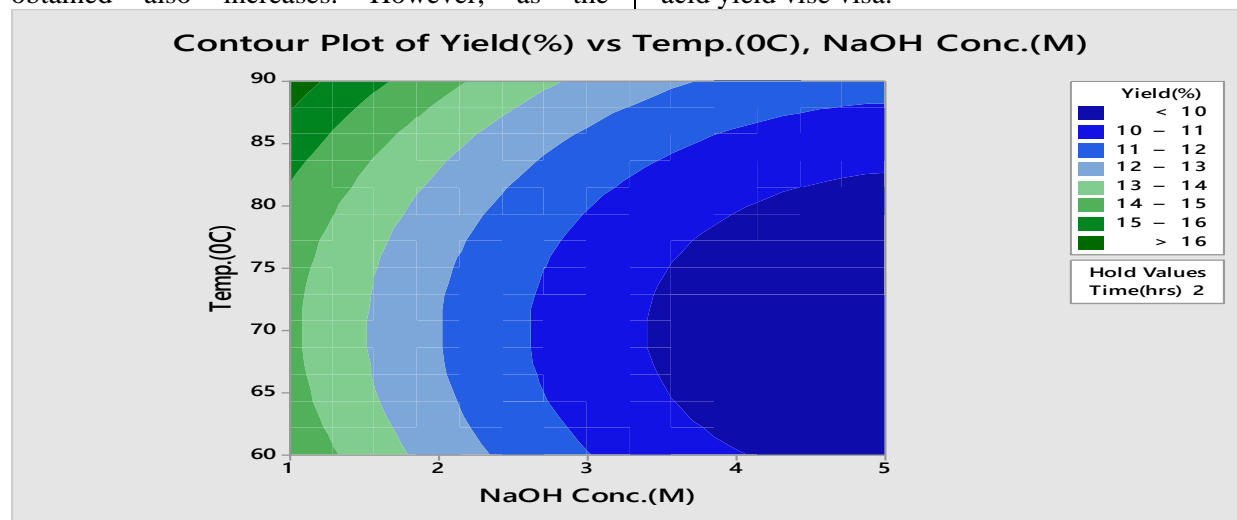


Figure 2: Effects of temperature and NaOH concentration on the yield of 4-Hydroxy-5-Methylcoumarin yield

Table 3: Experimental Result of Optimization and Validation of 4-Hydroxy-5-Methylcoumarin obtained from the hydrolysis of 5- methylcoumarin-4 β -glucoside.

Solution	Temp. (°C)	NaOH conc. (M)	Expected yield	Time(hr)	Experimental yield (%)	Desirability
1	90	1	16.8	1	17	1

The contour plot as shown in figure 2 indicates that NaOH Concentration ratio has significant impact on the yield of 4-Hydroxy-5-Methylcoumarin produced. The effect of NaOH concentration on the hydrolysis of 5-methylcoumarin-4 β -glucoside to 4-Hydroxy-5-Methylcoumarin yield was studied using different concentrations within the range of 1M – 5M, at a different temperature range from 60-90°C, the optimal yield of 4-Hydroxy-5-Methylcoumarin was determined at concentration 1M at 90°C which was above 16%. However, the minimum yield of the product was observed below

10% at Concentration of 5M from 60-70°C. The 5-methylcoumarin-4 β -glucoside conversion to 4-Hydroxy-5-Methylcoumarin is found to decrease as the Concentration increases from 1 to 5M. This was also in the same agreement with (Nasab *et al.*, 2023) where he used sodium hydroxide and revealed that an increase in the concentration content lead to produce a variation in the yield, since some by-products such as phenol may appear and complicate the purification process.

3.5 Response Optimization and Validation

Table 3 shows the predicted results for optimal solution obtained from the optimization. The solution predicted maximum 4-Hydroxy-5-Methylcoumarin yield of 17% and desirability of one (1) for the solution with optimal process conditions of temperature (90°C,) time (1hours) and concentration (1M) respectively. 4-Hydroxy-5-Methylcoumarin yield of 17% was obtained from validation of experiment carried out at the levels of the process variables predicted in solutions 1. It was observed that the optimized predicted and experimental validated result for solution was 17%. Thus, with predicted yields of 17% which is similarly closer to the experimental yields of 17%, it can be concluded that the optimization model is from 5-methylcoumarin-4 β glucoside with the conditions established in this paper.

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reliable and may therefore be more attractive for larger scale 4-Hydroxy-5-Methylcoumarin production from 5-methylcoumarin-4 β -glucoside obtained from *V.glaberrima*.

4.0 CONCLUSION

This study demonstrated the production of 4-hydroxy-5-methylcoumarin from 5-methylcoumarin-4 β glucoside obtained from *Vernonia glaberrima* leaves through basic hydrolysis. An optimum yield of 4-hydroxy-5-methylcoumarin was achieved at 1hour reaction time, 90°C temperature and 1M concentration. However, from this study it has been proven that 4-Hydroxy-5-Methylcoumarin can be obtained

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