

Kinetic Study of Fresh and Fermented *Vernonia amygdalina* Extracts Using Water as an Extraction Solvent

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Abstract—The extraction of bioactive compounds from medicinal plants is influenced by several factors, including solvent polarity, plant pre-treatment, temperature, and extraction time. *Vernonia amygdalina* (bitter leaf) is widely used in African traditional medicine due to its pharmacologically active compounds such as saponins, alkaloids, flavonoids, terpenoids, and glycosides. This study investigates the kinetics of aqueous extraction of fresh and fermented *Vernonia amygdalina* leaves using the Soxhlet method at temperatures ranging from 100°C to 110°C. Extraction yield was monitored at regular intervals, and the kinetic behaviour was analyzed using the Power-law model, from which the diffusion index and kinetic rate constant were evaluated. Results show that fresh leaves produced a significantly higher percentage yield (7.58%–11.79%) than fermented leaves (6.76%–8.03%), indicating that fermentation reduces solute availability. Kinetic parameters further demonstrate that fresh leaves exhibited a greater rate constant ($k = 146.54 \text{ min}^{-1}$) and a more negative diffusion index ($n = -0.5735$), reflecting faster and more sensitive solute transfer. Fermented leaves gave lower values of $k = 22.515 \text{ min}^{-1}$ and $n = -0.2368$. These results show that fresh leaves release solutes more rapidly, while fermentation decreases extraction efficiency due to cellular degradation. This research provides essential baseline data for optimizing industrial extraction of *Vernonia amygdalina* using water as a low-cost, environmentally friendly solvent.

Keywords— *Vernonia Amagydalina*, Solvent (water), Extract (chemicals), Kinetic (Power) Model.

I. INTRODUCTION

Medicinal plants remain a major source of bioactive compounds used in traditional medicine, pharmaceutical development, nutraceutical formulations, and dietary supplements worldwide. Among these, *Vernonia amygdalina* commonly known as bitter leaf is one of the most widely utilized species in West and Central Africa due to its diverse therapeutic properties, including antimalarial, antidiabetic, anticancer, anti-inflammatory, and antimicrobial activities (Azmir et al. 2013). Its leaves contain a rich profile of phytochemicals such as saponins, alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, all of which contribute to its pharmacological relevance (Bian et al. 2016). Because the concentration, bioavailability, and functional efficiency of these compounds depend strongly on extraction conditions, optimizing extraction processes for *V. amygdalina* remains an important focus in natural products research. Extraction is the primary step in isolating plant-derived

bioactive compounds, and the efficiency of this process depends on several factors including solvent polarity, temperature, particle size, extraction duration, and the pre-treatment of plant materials (Browne, 2010). Water, despite being a less selective solvent compared to organic alternatives, remains widely used because it is safe, inexpensive, non-toxic, and suitable for food and pharmaceutical applications (Allison, 2001). Additionally, hot-water and Soxhlet extractions remain effective methods for liberating thermally stable phytochemicals from plant matrices. However, the extraction behaviour of fresh versus pretreated (e.g., dried, fermented, blanched) plant materials can vary significantly due to structural and chemical modifications affecting solute accessibility and diffusion (Azwanida, 2015).

Fermentation in particular is known to alter phytochemical composition, degrade certain thermolabile compounds, break down plant cell walls, and modify the matrix porosity, thereby influencing extraction yield and kinetics (Byron, 2008). While fermentation may enhance the concentration of certain bioactive compounds in some plant species, it may also reduce extractable phytochemicals in others depending on microbial activity, enzymatic degradation, and leaching effects (David, 2021). Limited studies, however, have explored how fermentation influences the aqueous extraction behaviour of *V. amygdalina*, especially under controlled thermal conditions. In evaluating extraction behaviour, kinetic modelling plays a crucial role in understanding mass-transfer mechanisms, predicting solute release profiles, and optimizing process parameters for scale-up (Egedigwe, 2010). Models such as the Power-law (or empirical diffusion model) provide insights into extraction rate constants, diffusion indices, and the time dependence of solute transfer. These parameters allow researchers to determine whether extraction is governed primarily by washing, diffusion, or degradation mechanisms (Ifeoluwa et al. 2018). Understanding extraction kinetics is particularly important for medicinal plants whose therapeutic properties are strongly tied to solute concentration and extraction efficiency.

Despite the therapeutic importance of *V. amygdalina*, there is a notable gap in literature regarding the kinetic comparison of aqueous extraction from fresh versus fermented leaves. Most existing studies focus on phytochemical analysis rather than extraction behaviour under controlled kinetic conditions. This

study therefore aims to quantify and model the extraction kinetics of fresh and fermented *V. amygdalina* leaves using Soxhlet extraction with water as the solvent. By evaluating extraction yield, solute concentration, and kinetic parameters such as rate constants and diffusion indices, this research provides a mechanistic understanding of how fermentation alters mass transfer and extraction efficiency. These insights are essential for optimizing processing conditions in herbal medicine, functional foods, and pharmaceutical applications where *V. amygdalina* extracts are used.

II. MATERIALS AND METHODS

Sample Collection

The sample used for this research work is *Vernonia Amagydalina* (bitter leaf). The *Vernonia Amagydalina* was obtained from Agba-Ndele Community, in Emohua Local Government Area of Rivers State and then transported to the Rivers State University Chemical/Petrochemical Engineering laboratory where the sample went through some treatments before the extraction process.

Method of Experimental Measurement in the Extraction of Chemicals from the Natural Plant- *Vernonia Amagydalina*

1. Experimental Set Up

The sample for extraction - *Vernonia Amagydalina* (bitter leaf) was considered in two different forms that is fresh and fermented.

2. Fresh Sample

The fresh leaf sample of *Vernonia Amagydalina* (bitter leaf) was thoroughly washed under tap water to remove all contaminants and then shade dried for two hours. After the drying, the sample was subjected to size reduction by crushing to a nano particle size of 300nm. The size reduction was basically to ensure adequate penetration of the extraction solvent (water) into the plant matrix. And after the size reduction, 30g of the nano particle size of 300nm was each time weighed using the weighing balance and fed into a thimble whose weight had already been noted. Similarly, the soxhlet extractor was washed, cleaned and dried and the thimble together with the 30g sample fixed into extraction unit of the soxhlet extractor already connected to a distillation flask. Then, the distillation flask containing 350ml extraction solvent (water) was inserted on the heating mantle connected to a contactor to maintain the extraction temperature. Also, a stopwatch was used in order to determine the time taken for each extraction to be completed. Thus, the temperature range for the plant extraction was between 100°C to 110°C while the extraction time was between 32 minutes (0.533hr) to 180 minutes (3hrs) with the extraction time increasing as the temperature reduces for all sample extractions.

3. Fermented Sample

In obtaining the fermented *Vernonia Amagydalina* sample, the fresh sample was firstly and thoroughly washed under tap water and rinsed with distilled water to remove all contaminants before preserving in a reactor using tap water for seven (7) consecutive days with the readings of P^H, temperature and weight loss accordingly taken each day under an aerobic condition. After the fermentation, 30g of the fermented sample

was each time weighed using the weighing balance and fed into a thimble whose weight had already been noted. Similarly, the soxhlet extractor was washed, cleaned and dried and the thimble together with the 30g sample fixed into extraction unit of the soxhlet extractor already connected to a distillation flask. Then, the distillation flask containing 350ml extraction solvent (water) was inserted on the heating mantle connected to a contactor to maintain the extraction temperature. Also, a stop watch was used in order to determine the time taken for each extraction to be completed. As well, the temperature range for the plant extraction was between 100°C to 110°C while the extraction time was between 68 minutes (1hr 8minutes) to 180 minutes (3hrs) with the extraction time increasing as the temperature reduces.

Isolation of individual Phytochemical Constituents using Fractionating Column

The individual phytochemical constituents of the various extracts were separately isolated and obtained from the raw extracts using the fractionating column. Before the isolation step, the initial extracts were first obtained from the *vernonia amagydalina* leave through a soxhlet extraction method. Then after, the extract was subjected to a distillation process in order to remove every trace of the extraction solvent from the real extract. And finally, the extract further subjected to a moisture test, where every trace of moisture (water) from the leave was similarly eliminated to have the raw extract for fractionation process by feeding the raw extract into the distillation flask of the fractionating column which was then inserted into the heating mantle. And as the temperature increased, the individual phytochemical components were obtained in the receiving flask using their respective boiling points. Some of the phytochemical constituent's isolated include the myrcene, the anabasine, the copaene, the pelletierine, the 3-furaldehyde, etc.

Physical Properties of the Isolated Phytochemical Constituents

The physical properties of the isolated phytochemical compositions were obtained from the Chemical Engineering laboratory of the Rivers State University. The essence of this was to ensure the conformity of the isolated phytochemical compositions with other conventional chemicals particularly in the pharmaceutical industry. Some of the physical parameters obtained include the density of the individual phytochemical compositions, the viscosity, and the refractive index. The density was obtained using a beaker, measuring cylinder, weighing balance, etc. Also, the viscosity was obtained using the Viscometer Apparatus while the refractive index was obtained using the Refractometer.

Model Equation for Extraction of Solute from the Solid Matrix

A Pseudo Second Order Equation of the form (1) has been developed to characterize solute extraction from solid matrix

$$\frac{dC_t}{dt} = K(C_s - C_t)^2 \quad (1)$$

where:

C_t = concentration of solute (extract) in the solution at time (t).

C_s = extraction capacity

$\frac{dC_t}{dt}$ = extraction rate

K = extraction constant

Applying Arrhenius equation

$$\ln(K) = \ln(K_o) + \left(\frac{-E_a}{R} \times \frac{1}{T} \right)$$

$$K = K_o \ell^{\frac{-E_a}{RT}} \tag{2}$$

From equation (1),

$$C_t = \frac{C_t^2 K t}{1 + C_s K t} \tag{3}$$

Simplifying equation (3)

$$C_t = K(C_t^2 t - C_t C_s t) \tag{4}$$

Hence, rearranging equation (4), we obtain

$$K = \frac{1}{C_t t} - \frac{1}{C_s t} \tag{5}$$

Using equation (2) in (5) gives

$$K_o \ell^{\frac{-E_a}{RT}} = \frac{1}{C_t t} - \frac{1}{C_s t} \tag{6}$$

$$\ell^{\frac{-E_a}{RT}} = \frac{1}{K_o t} \left(\frac{1}{C_t} - \frac{1}{C_s} \right) \tag{7}$$

$$\frac{-E_a}{RT} = \ln \left[\frac{1}{K_o t} \left(\frac{1}{C_t} - \frac{1}{C_s} \right) \right] \tag{8}$$

$$RT = -E_a \ln \left[\frac{1}{K_o t} \left(\frac{1}{C_t} - \frac{1}{C_s} \right) \right] \tag{9}$$

$$C_A = \frac{1}{K t} (1 + C_s K t) \tag{10}$$

$$C_t = \frac{1}{K_o t \ell^{\frac{-E_a}{RT}}} \left[1 + t C_s K_o \ell^{\frac{-E_a}{RT}} \right] \tag{11}$$

But

$$\frac{M_{Solute}}{V_{Solute}} = C_t \tag{12}$$

$$\frac{M_{Solid}}{V_{Solid}} = C_s \tag{13}$$

Rewriting equation (13) in differential form,

$$\frac{dC_t}{dt} = \frac{1}{K_o t \ell^{\frac{-E_a}{RT}}} \left[1 + t C_s K_o \ell^{\frac{-E_a}{RT}} \right] \tag{14}$$

$$\therefore \frac{dC_t}{dt} = \frac{1}{K_o t \ell^{\frac{-E_a}{RT}}} \left[1 + \frac{t M_s}{V_s} K_o \ell^{\frac{-E_a}{RT}} \right] \tag{15}$$

Hence, equation (15) can be used to simulate how the amount of extract will vary with time at different temperatures.

Kinetic Model for Chemical Extraction from Natural Plants/Leaves

Various Models can be used in the determination of the Kinetics of Chemical Extracts using different extraction solvents. One of the models include the Power Model

Power Model

The Power Model can be expressed as

$$Y = K t^n \tag{16}$$

where:

Y= Percentage yield of chemical/extract (%)

K= Extraction rate constant (min⁻¹)

t = Extraction time (min)

n= Power index

The rate constant and power index were calculated by linearizing the power model expression in equation (16) to obtain the expression

$$\ln Y = n \ln t + \ln K \tag{17}$$

Then, by plotting ln y against ln t will give a slope equivalent to n and intercept equivalent to k.

where k is the characteristic constant combining the active coefficient, where-as the power index n, is the diffusion rate which implies the transport medium of the extract.

III. RESULTS AND DISCUSSION

Results of yield and temperature variation

From Figure 1, it is seen that the yield of *Vernonia Amagdalina* increased with an increase in temperature using water as an extraction solvent in a soxhlet extraction apparatus. Due to the extent of solvent transfer from the bulk solution to the surface of the solid, an increase in the extraction temperature from 100°C to 104°C enhanced the extraction yield relatively from 7.5820% to 8.4489%. Afterward, the extraction yield increased significantly from 8.4489% to 11.7600% following a temperature increment from 104°C to 108°C. And subsequently, the yield finally increased from 11.7600% to 11.7903% as the extraction temperature increased between 108°C to 110°C. In general, it can be shown that the percentage (%) yield of *vernonia amagdalina* conspicuously increased from 7.5820% to 11.7903% between temperatures 100°C to 110°C.

From Figure 2, the relationship between the percentage yields of *vernonia amagdalina* and the extraction temperature using water as an extraction solvent in a soxhlet extraction apparatus is depicted. The extent in increase of the yield can be attributed to the increased minimum temperature of the water

solvent. By this, the yield sharply increased from 6.7577% to 7.7913% between temperatures 100°C to 104°C. Then after, the yield slightly increased from 7.7913% to 7.8113% between temperatures 104°C to 106°C. Lastly, the increase in the yield was observed consistently from 7.8113% to 8.0317% between temperatures 106°C to 110°C following the penetrability of the water solvent into the plant membrane. It is seen that between temperatures 100°C to 110°C, the yield of *vernonia amagyalina* increased from 6.7577% to 8.0317%.

From Figure 3, the relationship between the percentage yield of fresh and fermented *vernonia amagyalina* extract and temperature using water as an extraction solvent in a soxhlet extraction apparatus is illustrated. From Figure 3, the yield of the fresh *vernonia amagyalina* increased moderately from

7.58% to 8.45% between temperatures 100°C to 104°C and further increased severely from 8.45% to 11.77% between temperatures 104°C to 108°C. And finally, it increased insignificantly from 11.77% to 11.79% as the extraction temperature also increased from 108°C to 110°C. In a similar behavior, the yield of the fermented *vernonia amagyalina* also increased moderately from 6.76% to 7.79% between temperatures 100°C to 104°C before further increasing slowly from 7.79% to 8.03% between temperatures 104 to 110°C. Hence, it is established that with water as an extraction solvent, the fresh *vernonia amagyalina* had a higher yield than the fermented *vernonia amagyalina* consequent to the denaturing of the plant's nutrient during the temperature extraction.

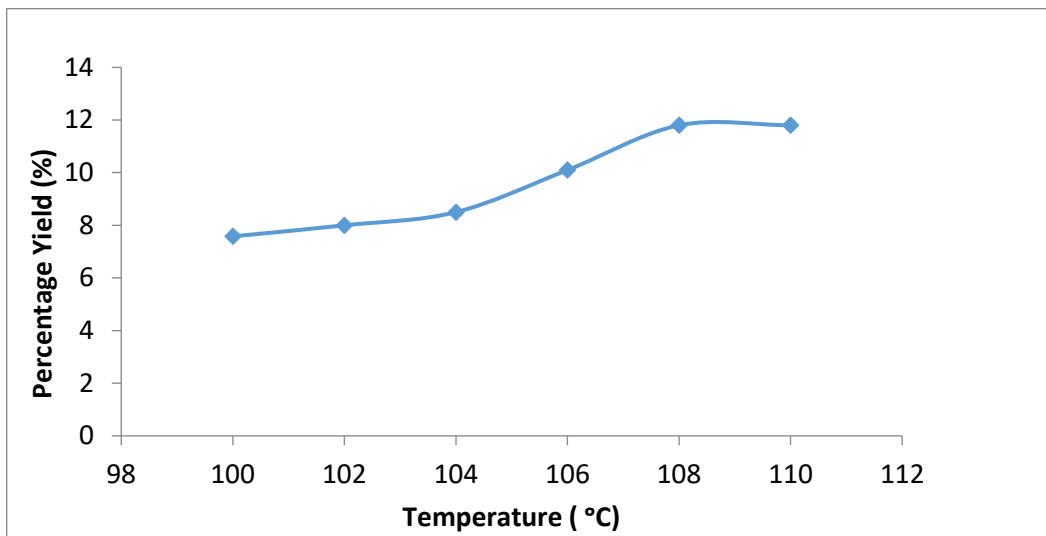


Figure 1: Variation of Percentage Yield of Fresh *Vernonia Amagyalina* Extract with Temperature

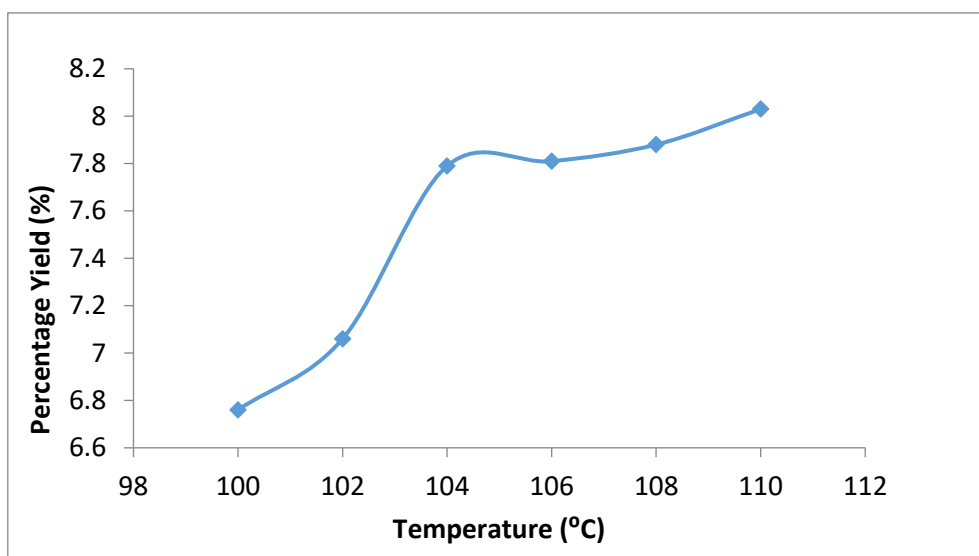


Figure 2: Variation of Percentage Yield of Fermented *Vernonia Amagyalina* Extract with Temperature

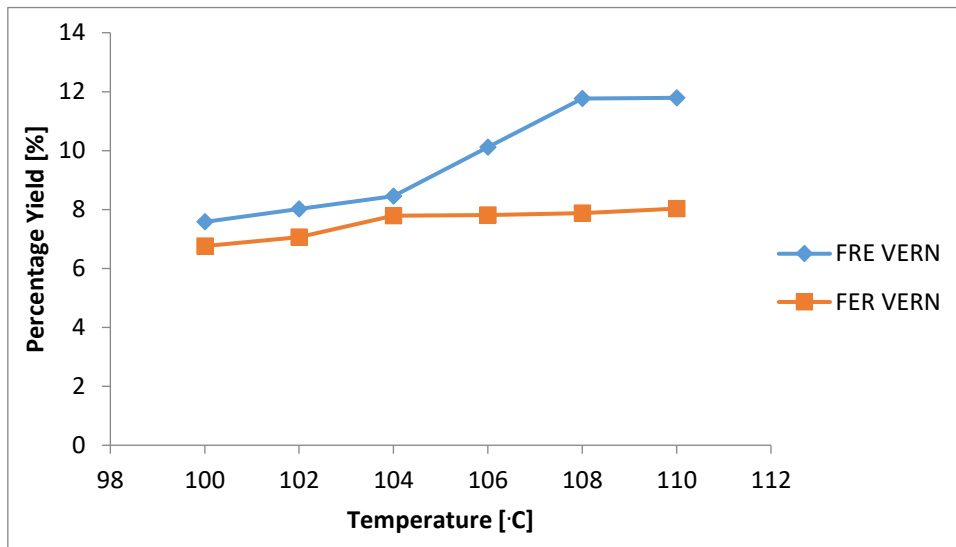


Figure 3: Comparison of Percentage Yield of Fresh and Fermented *Vernonia Amagdalina* Extracts against Temperature

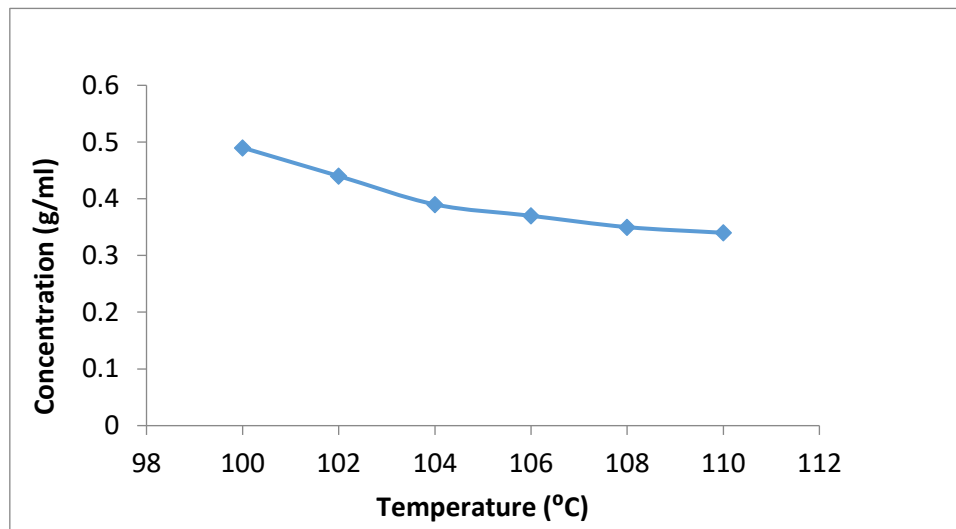


Figure 4: Variation of Concentration of Fresh *Vernonia Amagdalina* Extract with Temperature

From Figure 4, it is seen that the concentration of *vernonia amagdalina* decreased with increase in temperature using water as the extraction solvent in a soxhlet extraction apparatus. From this illustration, the concentration of the *vernonia amagdalina* extract reduces as the extraction temperature increased consequent to the increase in the amount of solvent which necessitated a reduction in the solute concentration. Thus, the increase in the extraction temperature from 100°C to 104°C led to the reduction of the concentration significantly from 0.4912g/ml to 0.3901g/ml. Similarly, the increase in temperature from 104°C to 108°C showed a gradual reduction in the concentration of the extract from 0.3901g/ml to 0.3509g/ml following the solute dissolvability into the solvent. Finally, as a result of mixability of the solute with water, the concentration further decreased from 0.3509g/ml to 0.3423g/ml upon a temperature decrease from 108°C to 110°C. Generally, it is obvious in this study that the concentration of *vernonia*

amagdalina extract decreased from 0.4912g/ml to 0.3423g/ml between temperatures 100°C to 110°C.

Figure 5 demonstrates the relationship between the concentrations of *vernonia amagdalina* with temperature using water as an extraction solvent in a soxhlet extraction apparatus. In view of this, the concentration of the extract reduces as the extraction temperature increased following an increase in the amount of solvent leading to a considerable reduction in the solute concentration. The *vernonia amagdalina* witnessed a decrease in concentration from 0.3531g/ml to 0.3490g/ml between temperatures 100°C to 104°C. Going further, the *vernonia amagdalina* had its concentration decreased drastically from 0.3490g/ml to 0.3384g/ml between temperatures 104°C to 106°C due to its degree of dissolvability into the solvent. And finally, between temperatures 106°C to 110°C, the concentration decreased from 0.3384g/ml to 0.3322g/ml. indisputably, it is noted in this work that the concentration of fermented *vernonia amagdalina*

decreased from 0.3531g/ml to 0.3322g/ml when the temperature increased between 100°C to 110°C.

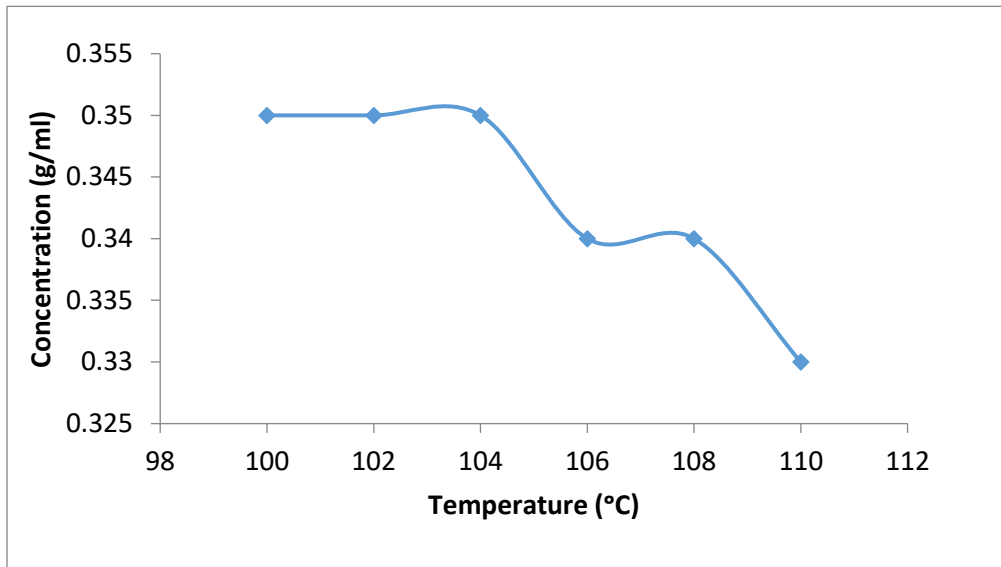


Figure 5: Variation of Concentration of Fermented *Vernonia Amagdalina* Extract with Temperature

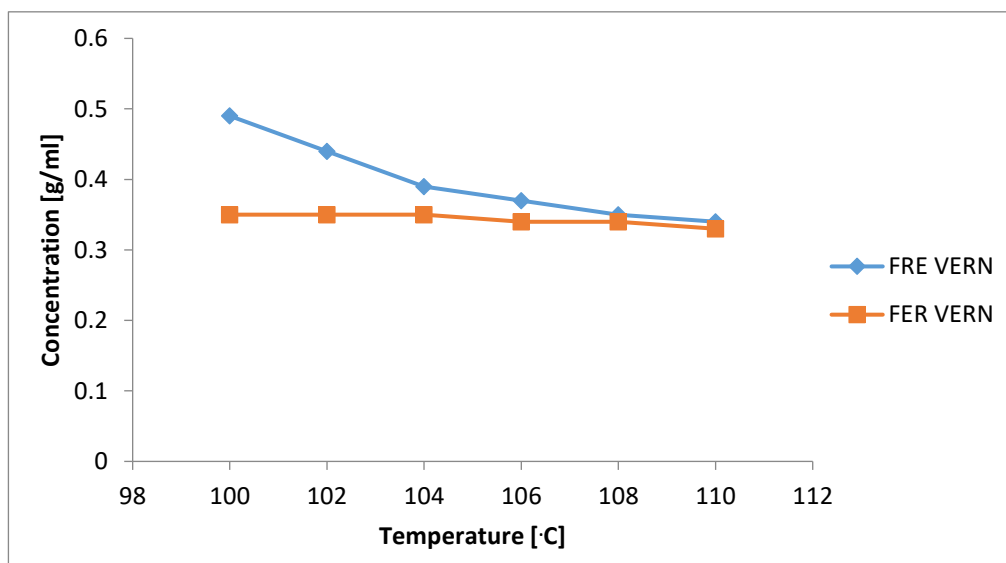


Figure 6: Comparison of Concentration variation of Fresh and Fermented *Vernonia Amagdalina* Extracts with Temperature

Figure 6 reveals the relationship between the concentration of fresh and fermented *vernonia amagdalina* extract with temperature using water as an extraction solvent in a soxhlet extraction apparatus. From Figure 6, it is shown that the concentration of the fresh *vernonia amagdalina* decreased sharply from 0.49g/ml to 0.39g/ml between temperatures 100°C to 104°C. Furthermore, the concentration decreased averagely from 0.39g/ml to 0.34g/ml between temperatures 104°C to 110°C. On the other hand, the concentration of the fermented *vernonia amagdalina* maintained stability at 0.35g/ml between temperatures 100°C to 104°C and then decreased finally from 0.35g/ml to 0.33g/ml between temperatures 104°C to 110°C. Thus, the comparism institutes that in using water as an extraction solvent, the fresh *vernonia amagdalina* had the

greatest concentration when compared to the concentration of the fermented *vernonia amagdalina* due to the decrease in the amount of phytaye in the fermented plant.

Kinetic Analysis of Chemical [Extracts] from Fresh and Fermented Vernonia Amagdalina Plants/Leaves

The extraction of chemicals from natural plants/leaves has been investigated using some mathematical models. And to apply this model, the constant coefficients have to be evaluated. *Evaluation of Power Model Constants for Fresh and Fermented Vernonia Amagdalina Plants/Leaves*

Both the power index (n) and the constant coefficient (k) are to be determined from the Figures representing the different plant leaves extracts for fresh and fermented *Vernonia*

Amagydalina. Each Figure is obtained by plotting the natural logarithm of yield versus natural logarithm of extraction time. The power index (n) and the constant coefficient (k) are obtained by comparing the linear regression equation in each Figure with equation (16).

From the linear regression equation in Figure 7, the power index, n and the constant coefficient, k have been obtained as -0.5735 and 146.54023min⁻¹ respectively. Hence, by putting the values into equation (16), the percentage yield of extract [chemical] from the fresh *Vernonia Amagydalina* can be obtained as $Y = 146.54023t^{-0.5735}$.

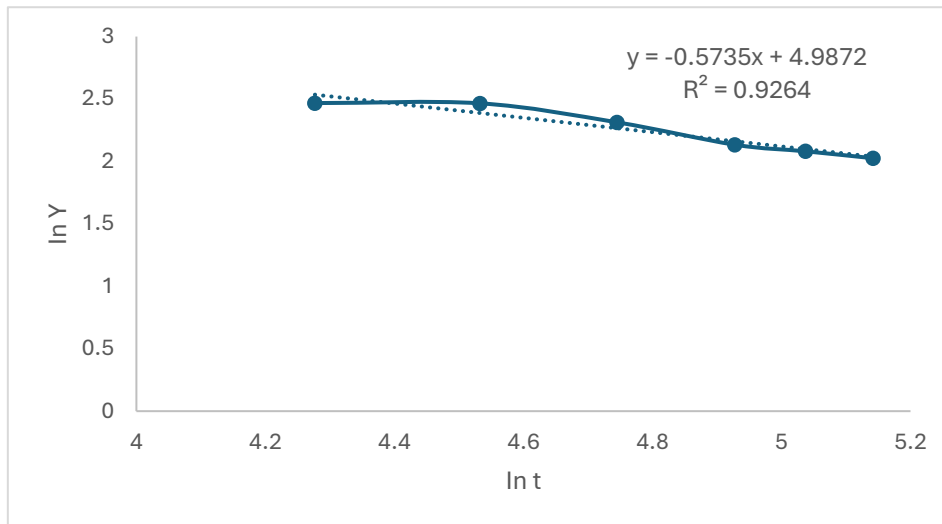


Figure 7: Evaluation of Power Index and Rate Constant of fresh *Vernonia Amagydalina*

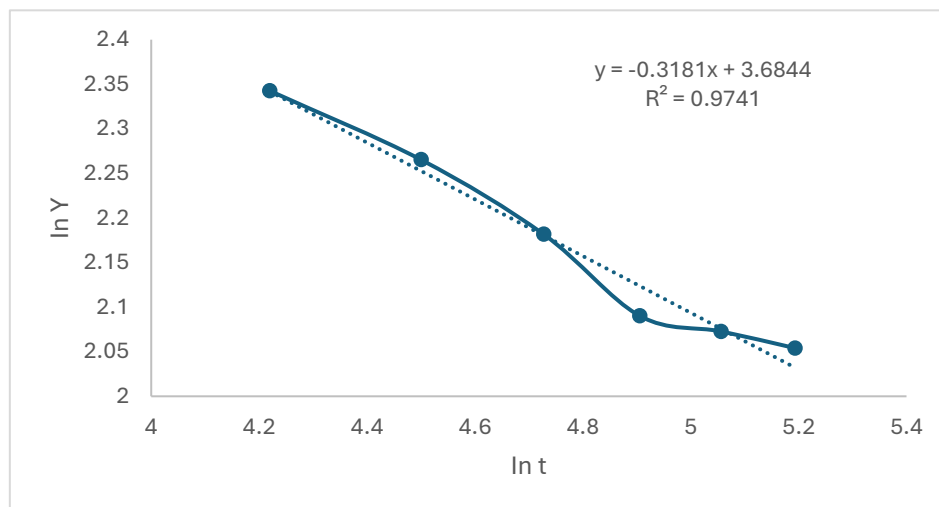


Figure 8: Evaluation of Power Index and Rate Constant of fermented *Vernonia Amagydalina*

Table 1: Comparison of Values of Kinetic Model for Fresh and Fermented *Vernonia Amagydalina* leaves

Fresh <i>Vernonia Amagydalian</i>	Fermented <i>Vernonia Amagydalina</i>
<i>Kinetic Model (Y = ktⁿ)</i>	<i>Kinetic Model (Y = ktⁿ)</i>
Power Model (n) = -0.5735	Power Model (n) = -0.2368
Constant Coefficient (k) = 146.54023min ⁻¹	Constant Coefficient (k) = 22.5154min ⁻¹
Percent Yield (Y) = 146.54023t ^{-0.5735}	Percent Yield (Y) = 22.5154t ^{-0.3181}

From the linear regression equation in Figure 8, the power index, n and the constant coefficient, k have been obtained as -0.3181 and 22.5154min⁻¹ respectively. Hence, by putting the values into equation (16) the percentage yield of extract

[chemical] from the fermented *Vernonia Amagydalina* can be obtained as $Y = 22.5154t^{-0.3181}$.

IV. CONCLUSION

Extraction kinetics of fresh and fermented *Vernonia amygdalina* leaves using water as a low-cost, environmentally benign solvent under Soxhlet extraction conditions was successfully carried out in this work. The results clearly demonstrate that extraction temperature and plant pre-treatment significantly affect solute yield, concentration, and kinetic behaviour. Fresh leaves consistently produced higher percentage yields (7.58–11.79%) compared to fermented leaves

(6.76–8.03%), indicating greater solute availability and less structural degradation. Similarly, kinetic analysis using the Power-law model shows that fresh leaves exhibited a higher extraction rate constant ($k = 146.54 \text{ min}^{-1}$) and a more negative diffusion index ($n = -0.5735$), signifying faster solute transfer and stronger sensitivity to extraction time. Fermented leaves, with lower rate constant ($k = 22.515 \text{ min}^{-1}$) and diffusion index ($n = -0.2368$), showed slower solute release due to fermentation-induced changes to cellular morphology and phytochemical integrity. The findings confirm that fermentation reduces extraction efficiency in aqueous systems and alters the diffusion-controlled dynamics governing solute release. The results provide a quantitative baseline for optimizing extraction of *Vernonia amygdalina* in herbal, nutraceutical, and pharmaceutical applications where water is used as a solvent. More importantly, the study offers kinetic parameters that can guide scale-up, process modelling, and comparative evaluation of alternative solvents or extraction techniques.

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