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Hydrocarbon-Utilizing Bacteria in a Nigerian Soil

Predictive Mathematical Modeling Biofilm Potential of Phytochemicals from *Adantum philippensis* Extract and Adhesion with *P. aeruginosa* Activities

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ABSTRACT

A predictive mathematical modeling of the biofilm potentials of phytochemicals from *A. philippense* extract and adhesion *with P. aeruginosa* was studied for the very first time. Eight different kinetic models Von Bertalanffy, Baranyi-Roberts, modified Schnute, modified Richards, modified Gompertz, Modified Logistics and latest Huang were used to get values for the kinetic constants. Von Bertalanffy of the entire model was found to be the best model with the highest adjusted R^2 value with the lowest RMSE value. The accuracy and bias factors values were close to one (1.0). The parameters obtained from von Bertalanffy model *for P. aeruginosa* and chloramphenicol were K 1.551 (95% C.I 1.385 to 1.718) and 1.617 (95% C.I 1.204 to 2.031), A -1.055(95% C.I -1.492 to -0.61) and -1.142 (95% C.I -1.612 to -0.67), U_m 1.041 (95% C.I 0.740 to 1.342) and 0.744 (95% C.I 0.399 to 1.089) respectively. This finding shows the influence of von Bertalanffy model in the roles of phytochemicals from *Adantum philippense* in the biofilm potentials and adhesion with *P. aeruginosa* against foodborne pathogens.

INTRODUCTION

Phytochemicals are substances formed primarily by plants and have biological activity on these substances. In the pharmaceutical industry, the primary source for the manufacture of different active ingredients is plants. They show pharmacological effects beneficial to the treatment of infections of bacteria and fungi, as well as chronic degenerative diseases such as cancer and diabetes [1]. Current bacterial multi-resistance as well as biofilm issues resistance not only to traditional therapies, but also to modern ones. The screening and development of new active products and new enhanced alternative methods for biofilm control have stimulated the creation of drugs and toxicity to some of the existing antimicrobials used [2].

Biofilms are three-dimensional microbial communities that are surface-attached, compact, organized and embedded in a matrix of proteins, polysaccharides and other molecules of self-produced extracellular polymeric substances [3]. Usually, foodborne pathogens are proficient in sticking to different surface types (inert or living) and forming biologic films. The bacteria within are less susceptible to antibiotics and other chemical substances than their counterparts, planktonic cells, once the biofilm is formed [4]. In accordance with the Centre for Disease Control and Prevention, (CDC), *P. aeruginosa, Staphylococcus aureus*, *Escherichia coli, Shigella flexneri, Listeria spp., Clostridium perfringens, Campylobacter spp., and Salmonella spp*. A few of the pathogens causing food poisoning [3,4]. Diarrhea, vomiting, weakness from stomach cramps, nausea, and fever are the most common symptoms of these food pathogens. At any point during production, distribution, and storage, these pathogens may contaminate foodstuffs. It is therefore extremely necessary that we monitor the production and development of food pathogens, although it is difficult to eradicate these species since they are capable of forming biofilms on a variety of planes [5]. *A. Philippense* is a fern with many curative properties that is medicinally treasured. Plant-derived extracts are highly regarded these days because of their lack of side effects, and many are actually traditionally used as

ethnomedicine to prevent and treat various forms of infections [3]. The presence of phenols, terpenoids, flavonoids, and carbohydrates was found, and this was due to the phytochemical analysis of this plant. Such kinds of phytochemicals are also considered to resist bacterial inhibition adhesion to, and repression of genes associated with biofilm formation. Therefore, the availability of these compounds provides this fern with the capacity to behave as a healer, however, there is a lack of research into the detailed role of phytochemicals in antibiofilm potentials [6,7]. Therefore, this analysis was aimed at assessing the modeling effects of *A. Philippense* phytochemicals, with their antibacterial properties; adhere to biofilm formation against common food pathogens

MATERIALS AND METHODS

A previously published data [3] was processed using the software Webplotdigitizer 2.5 [8].

Statistical analysis

Statistical analysis or error function analysis was carried out using discriminatory factors such as accuracy factor (AF), bias factor (BF), adjusted determination coefficient (R^2), root-mean-square error (RMSE) and one based on information theory, the AICc (corrected Akaike Information Criterion). In this analysis, several common growth models were used (**Table 1**).

Table 1. Growth models used in modelling the growth curve of *P. aeruginosa.*

Model	p	Equation
Modified Logistic	3	$y=\frac{A}{\left\{1+\exp\left[\frac{4\mu_m}{A}(\lambda-t)+2\right]\right\}}$
Modified Gompertz	3	$y=A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda-t)+1\right]\right\}$
Modified Richards	4	$y=A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda-t)\right]\right\}^{\left(\frac{-1}{v}\right)}$
Modified Schnute	4	$y=\left(\mu_m\frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Robert	4	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)$ $-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{max}-A)}}\right)$
Von Bertalanffy	3	$y=K\left\{1-\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(\mu_m e/3K^{\frac{1}{3}}\right)t}\right\}^3$
Huang	4	$y=A+y_{\max}-\ln\left(e^A+\left(e^{Y_{\max}}-e^A\right)e^{-\mu_m B(x)}\right)$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, IF X < LAG Y=A + K(X−λ), IF λ ≤ X ≥ X_{MAX} Y = Y_{MAX}, IF X ≥ X_{MAX}

Note:
A= growth lower asymptote;
y_{max}= growth upper asymptote;
μ_{max}= maximum specific growth rate;
v= affects near which asymptote maximum growth occurs.
l=lag time
e = exponent (2.718281828)
t = sampling time
a,b, k = curve fitting parameters
h_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process. The lag time (h^{-1}) or (d^{-1}) can be calculated as $h_0=m_{max}$

Fitting of the data

Nonlinear regression was conducted using tools from CurveExpert Professional (Version 1.6). The μmax of the estimation was carried out by the curve's steepest ascent rifle, while the x-axis crossing of that line is an estimate of λ. The model that shows the highest growth was adopted for the purposes of modeling.

RESULTS AND DISCUSSION

The growth curves were replotted and converted to log units (**Fig. 1**) prior to modeling. The highest signal was used in the modeling process to select the best model. The required adaptation of all models to the growth curve was apparent (**Figs 2 to 9**). Using the von Bertalanffy model with the least value for RMSE, AICc and the uppermost value for modified R^2, the best model was found. The AF and BF values were shown to be outstanding for the model and their values were nearest to unity. The least performance was the modified logistic model (**Table 2**). The near absence of lag period for growth is likely the reason for the superiority of the von Bertalanffy model.

The coefficients for the von Bertalanffy model are shown in **Table 3**. The growth and death can be studied by the bacterial growth curve over a wide variety of antibacterial concentrations of bacteria and has been used frequently to assess the impact of over time, antibacterial. When the concentration of antibacterial agents exceeds MIC for the bacteria, a time-dependent bactericidal effect occurs [4,9]. **Table 4** shows different medicinal plants and their biofilms and adhesion ability.

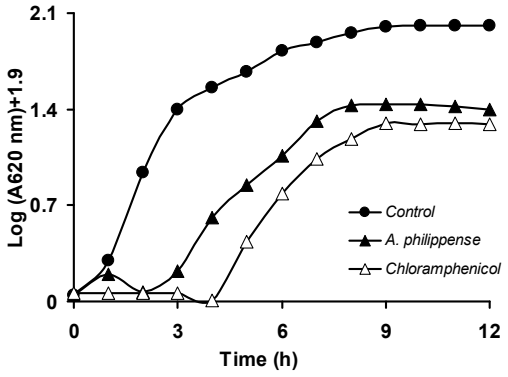

Fig. 1. Growth of *P. aeruginosa* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol).

Table 2. Statistical analysis of the various fitted models.

Model	p	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.062	0.994	0.990	1.040	1.010	-50.60
Baranyi-Roberts	4	0.076	0.990	0.985	1.054	1.024	-45.09
Modified Gompertz	3	0.067	0.991	0.989	1.166	1.054	-54.75
Buchanan-3-phase	3	0.132	0.967	0.955	1.166	1.087	-37.01
Modified Richards	4	0.070	0.991	0.987	1.090	1.054	-47.17
Modified Schnute	3	0.054	0.995	0.993	10.185	0.103	-53.86
Modified Logistics	3	0.103	0.978	0.971	1.182	1.123	-43.51
Von Bertalanffy	4	0.056	0.994	0.992	1.093	0.965	-59.47

Note:
p no of parameters
adR^2 Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor

Few studies published previously have revealed that Phytochemicals have been involved in biofilm prevention by means of Inhibiting adhesion through various pathways. Plant

extracts have been shown to have the exceptional ability to prevent six bacterial strains from the first stage of biofilm growth by interfering with attachment forces such as Lifshitz-Van der Waals, Brownian, sedimentation, and electrostatic interaction forces, facilitating bacterial attachment to different surface types [3,10].

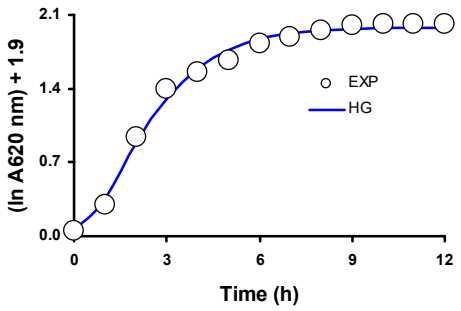

Fig. 2. Growth of *P. aeruginosa* biofilm (control) fitted to the Huang model.

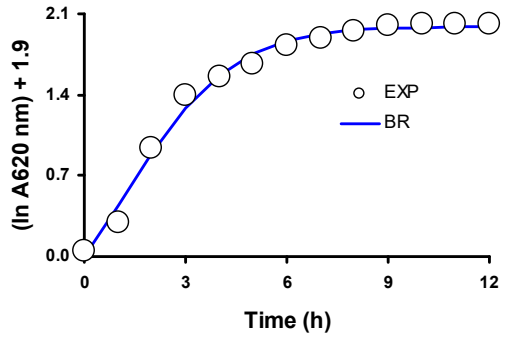

Fig. 3. Growth of *P. aeruginosa* biofilm (control) fitted to the Baranyi-Roberts model.

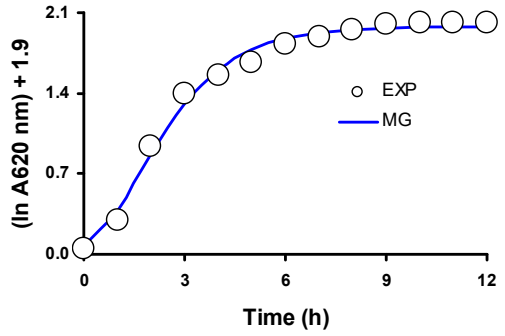

Fig. 4. Growth of *P. aeruginosa* biofilm (control) fitted to the modified Gompertz model.

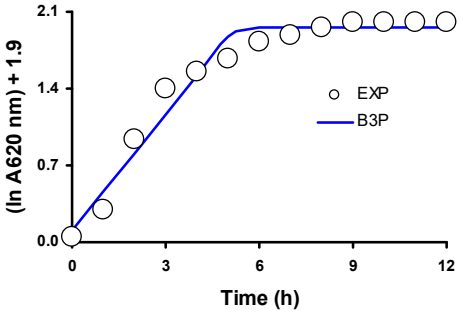

Fig. 5. Growth of *P. aeruginosa* biofilm (control) fitted to the Buchanan-3-phase model.

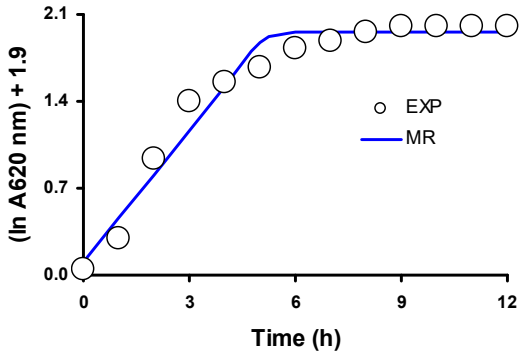

Fig. 6. Growth of *P. aeruginosa* biofilm (control) fitted to the modified Richards model.

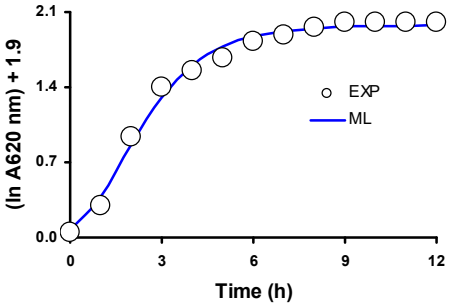

Fig. 7. Growth of *P. aeruginosa* biofilm (control) fitted to the modified logistics model.

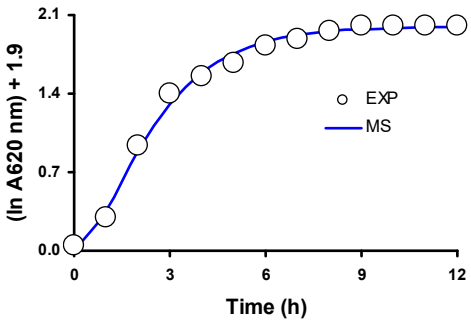

Fig. 8. Growth of *P. aeruginosa* biofilm (control) fitted to the modified Schnute model.

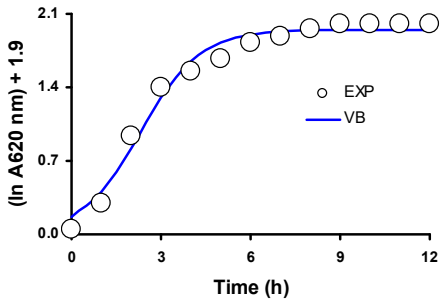

Fig. 9. Growth of *P. aeruginosa* biofilm (control) fitted to the von Bertalanffy model.

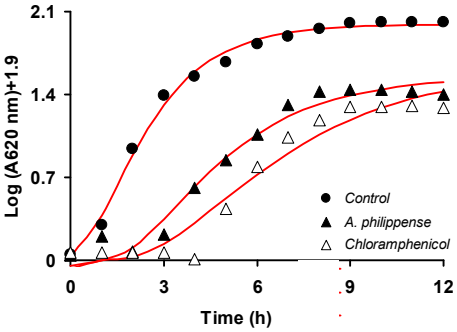

Figure 3. Growth of *P. aeruginosa* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol) fitted to the von Bertalanffy model.

Table 3. Coefficients of *P. aeruginosa* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol) fitted to the von Bertalanffy model.

	Control		*P. aeruginosa*		Chloramphenicol	
	Value (95% C.I.)		Value (95% C.I.)		Value (95% C.I.)	
K	1.993	1.934 to 2.051	1.551	1.385 to 1.718	1.617	1.204 to 2.031
A	1.219	0.943 to 1.495	-1.055	-1.492 to -0.617	-1.142	-1.612 to -0.671
μ_m (h^{-1})	1.412	1.175 to 1.649	1.041	0.740 to 1.342	0.744	0.399 to 1.089

Note: 95% C.I. denotes 95% confidence interval.

The Von Bertalanffy model assumes that there is no change in the growth of bacterial cells or species over time or through invariance [11]. The formula has historically been used to model fish weight growth for the first time [12] and is rooted in the Bernoulli differential equation [6].

$$\frac{dA}{dt}=\mu_m A^{\frac{2}{3}}\left[1-\left(\frac{A}{K}\right)^{\frac{1}{3}}\right]$$

Upon integration of the equation the following solution is obtained;

$$y=K\left[1-\left[1-\left(\frac{A}{K}\right)^{3}\right]\exp^{-\left(\mu_m x/3K^{\frac{1}{3}}\right)}\right]^{3}$$

Where A is the population size at time t=0, K is the carrying capacity, μ_m is the intrinsic growth rate and represents growth rate per capita [11]. as t (x) tends to infinity, the population size stabilizes to carrying capacity K.

Although the von Bertalanffy model has historically been used to model the increase in fish weight, other species, such as chicken, tumour and cancer growth, *Daphnia magna*, kelp and microbial growth [12-15], have been used to model growth.

Parameters obtained from the fitting exercise were maximum biofilm production rate (μ_m), lag time (λ) and maximal biofilm formation and adhesion (Y_{max}) Biologically important coefficients, such as the two-parameter Monod model or other more complex secondary models, such as Haldane, Aiba, Yano and others, will later be used for secondary modeling. In basic science, these mechanistic models are used to achieve a deeper understanding of the physical, chemical and biological mechanisms that relate to the growth profile that is observed. Mechanistic models are more efficient, all other things being equivalent, when they teach you about the fundamental mechanisms that drive trends. When extrapolating outside the observable parameters, they are more likely to function right. [12].

Table 4. MIC and percentage inhibition of biofilm for different plant extract with antimicrobial potentials.

Plant exract	Bacteria	MIC µg/m	% Biofilm inhibition	Reference
P. granatum	*L. monocytogen*	78 – 625	80-60	[13]
R. coriacia	*S. aureus*		90-80	
	E. coli	312- 125		
	P. aeruginosa			
Adiantum philippense	*E. coli*	31.25	54.73	[3]
	S. aureus	500	60.92	
	S. flexneri	62.5	37.34	
	P. aeruginosa	250	50.26	
Pepper mint	*P. aeruginosa*	0.75 – 2.5-		[14]
	C. albican	mg/ml		
E. agustifolia	*P. aeruginosa*	0.38 – 1.		
	C. albican	mg/ml		
R. officinalis	*P. aeruginosa*	0.75 – 1.		
	C. albican	mg/ml		
Coriandrum sativum L.	*S. aureus*	2-4 mg/m	0.08%	[5]
Pimpinella anisum L.)	*E. coli*		0.63%	
Eugenia erythrophylla	*Bacillus cereus*	0.04– 0.08 mg/ml		[15]

CONCLUSION

In conclusion, the von Bertalanffy model was the best model in modelling the antibiofilm potential of phytochemicals from *Adantum philippense* extract with the *P. aeruginosa* based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), and accuracy factor (AF) and corrected AICc (Akaike Information Criterion). This indicate the best fits of Von bertalaffy in modeling the role of phytochemicals from *A. philippense* extract as antibiofilm against *P. aeruginosa.*

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