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Mathematical models

Mathematical Modeling of the Effect of *Adiantum philippense* Extracts on Biofilms Formation, Adhesion with *E. coli* Activities Against Foodborne Pathogens

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ABSTRACT

In the quest for novel bioactive metabolites, which can also be used as therapeutic agents, *Adiantum philippense* (*A. philippense*), an ethnomedical important fern, has become a fascinating herb. In this study, the predictive mathematical modelling of *A. philippense* crude extract was tested against *E. coli*, a common food pathogen for its phytochemical constituents, antagonistic ability, and effect on bacterial adhesion and biofilm formation was calculated. For the first time in this paper we present various kinetics models such as von Bertalanffy, Baranyi-Roberts, modified Schnute, Modified Richards, Modified Gompertz, Modified Logistics and Huang were used to get values for the above kinetic constants or parameters. 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 unity (1.0). The parameters obtained from Von Bertalanffy model for *E. coli* and chloramphenicol when compared with control values were the K 1.146 (95% C.I. 1.050 - 1.241) and 0.912 (95% C.I. 0.783 – 1.041), A 0.831 (95% C.I. 0.669 – 0.994) and 0.699 (95% C.I. 0.519 – 0.880) K_m 1.146 (95% C.I. 0.746 – 1.546) and 1.210 (95% C.I. 0.478 – 1.942) respectively. This shows that *A. philippense* was active against *E. coli*.

INTRODUCTION

The association between the consumption of food and human diseases was recognized very early and it was Hippocrates (460 B.C.) who reported that there is a strong connection between food consumed and human illness [1]. food borne illnesses, after the conception of human society, it was a problem for humanity. The prime cause of food poisoning and food borne diseases are food borne pathogens that pose a significant risk to the safety of food [2]. The number of diseases that have been caused in the last couple of years due to this has ultimately become a significant and important public health concern. [3]. Food-contaminating diseases have been given major concern, as they reportedly cause exceptional mortality and morbidity statistics at a rate of 420,000 fatalities annually (World Health Organization). As per the data from the disease control and prevention centre (CDC), *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, *Clostridium perfringens*, *Listeria spp*., *Campylobacter* spp., and *Salmonella* spp. A few of the pathogens that cause food are toxicity [3].

Cancers and inflammatory disease are acute and chronic toxicity or long-term illnesses that may be caused by complications from chemical pollution [4]. Uncooked foods of agricultural origin can lead to long-lasting injury and death and can come from infected fruits and vegetables. On another note, crude shellfish containing marine biotoxins are examples of unhealthy foods [1]. The most normal symptoms of these food infections include diarrhea, vomiting, stomach cramps, fatigue, nausea, and fever. These pathogens can contaminate foodstuffs at any point during processing, distribution, and storage. It is also highly critical that we control the growth and production of food pathogens, but it is difficult to eradicate these organisms since they are capable of binding themselves securely and strongly to foodstuffs and packagings [5]. Microorganisms bind to surfaces uniformly and produce extracellular

polysaccharides, resulting in biofilm formation. Due to the increased resistance of biofilm-associated species to antimicrobial agents, biofilms present a significant public health problem [6]. In addition to being immune to antibiotics, Bacterial biofilm cells may also defend themselves against a range of physical and chemical aggressions, including acidity, salinity, heavy metals, ultraviolet light and phagocytosis [3].

In view of the facts, the creation of biofilm presents a great global danger to the marine and oceanic industries, to the food and dairy industries and above all, to public health [3]. Biofilm treatment is a global challenge that involves the invention of novel natural bioactive molecules against pathogenic foodborne bacteria. In comparison to the chemically synthesized, the need for natural bioactive compounds is due to encounters with food industries. The antibacterial and antibiofilm ability of *A. philippense* crude extract are untapped potential of this plant. Traditionally, this plant is used as an herbal medicine, with many functions such as anti-inflammatory, antipurgative, anticoagulant, anthelmintic, antipyretic, anticancer, analgesic and antimicrobial to combat leucoderma, leprosy, ulcers, spleen, liver, tumors, and intestinal diseases [7].

The antibiotic potential of phytochemicals via repression of quorum sensing was identified in previous studies on medicinal plants [8]. Various forms of phytochemicals, such as flavonols, flavonoids, phenols, and flavonones, are known and common quorum-sensing inhibitors [9–11]. Similarly, these forms of phytochemicals are also known for their inhibition of bacterial adhesion and gene repression associated with biofilm formation. Therefore, this analysis was aimed at studying the mathematical modeling of the effects of *A. philippense* phytochemicals are adhesive to biofilm formation with their antibacterial properties against food pathogens *Escherichia coli* (E. *coli*).

Materials and methods

A previously published data [34] was processed using the software Webplotdigitizer 2.5 [35].

Statistical analysis

In the selection for the best models, statistical analysis or error function analysis was carried out using discriminatory factors such as accuracy factor (AF), bias factor (BF), adjusted determination coefficient (adjR^2), root-mean - square error (RMSE) and one based on information theory which is the AICC (Corrected Akaike Information Criterion) [36].

Fitting of the data

Nonlinear regression was carried out using the Curveexpert professional software (version 1.6). Several popular growth models were utilized in this study (Table 1). The μmax of the estimation was performed by the steepest ascent rifle of the curve, whereas the x-axis crossing of this line is an estimate of λ. The model that shows a high growth was adopted for the purpose of modelling.

Table 1. Growth models used in modelling the growth curve of E. coli Activities.

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	$y=A\left\{1+v\exp(1+v)\exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda-t)\right]\right\}$
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-Roberts	4	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_o}\right)$ $-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)}-1}{e^{(y_{\max}-A)}}\right)$
Von Bertalanffy	3	$y=K\left[1-\left[1-\left(\frac{\lambda}{K}\right)^3\right]\exp^{-\left(\mu_m x/3K^{\frac{1}{3}}\right)}\right]^3$
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;
M_{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 $h_0=m_{max}$

RESULTS AND DISCUSSION

The growth curves were replotted and converted to log units (**fig. 1**) prior to modeling. The highest signal was utilized in the modelling process to select the best model. All of the curves tested display visually satisfactory fitting (**figs 2** to **9**). The finest growth was found using the von Bertalanffy model with the best values (smallest) for RMSE, AICc and the uppermost value for adjusted R^2. The AF and BF values were seen to be excellent for the model and their values were nearer to 1.0. 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**.

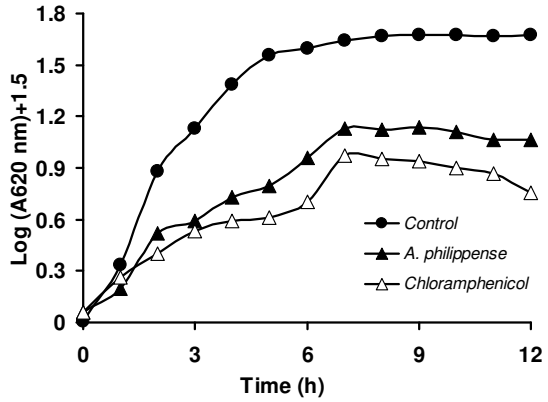

Fig. 1. Growth of *E. coli* biofilm (control) in the presence of *adiantum philippense* and a positive control (chloramphenicol).

Although several methods are available for estimating the effects of plant extracts on biofils formation, adhesion with microbial activities against food borne pathogens in addition to laboratory findings. There is need therefore, for accurate and precise estimates of prediction method [9].

The crude extract of A*diantum philippense* was sufficiently capable of distorting the preformed biofilms, have an impact on their adhesion capacity and at the microbial inhibitory concentration stage. The results obtained showed that *Adiantum philippense* had an affinity to prevent biofilms from developing and preforming by hindering their capacity for adhesion at Microbial inhibition concentration.

The inhibition of preformed biofilms by *Adiantum philippense* for *E. coli* was around 62.72 percent. *A. philippense* decreases the adhesion ability of biofilms with percentage of inhibition for *E. coli* at 54.73% [3]. This finding was similar to [10] who reported that the tolerance and virulence of pathogenic bacteria, such as Salmonella, are often correlated with their ability to form biofilms that are sessile structures found on different surfaces and whose production is regarded as a universal mechanism of stress response. In a similar finding by [11] reported that two plants extracts, *P. granatum* L. and *R. coriaria* L. demonstrated best antibacterial activity with a minimum inhibitory concentration (MIC) of 78-625 µg/mL for *Listeria monocytogenes and Staphylococcus aureus* and 312-1250 µg/mL for *Escherichia coli* and *Pseudomonas aeruginosa*.

Table 2. Statistical analysis of the various fitted models.

Model	*p*	RMSE	Adr^2	AF	BF	AICc
Huang	4	0.03	1.00	1.02	1.00	-71.75
Baranyi-roberts	4	0.03	1.00	1.02	1.01	-65.76
Modified gompertz	3	0.04	0.99	1.22	1.23	-69.15
Buchanan-3-phase	3	0.06	0.99	1.22	1.17	-55.69
Modified richards	4	0.04	0.99	1.25	1.23	-61.57
Modified schnute	3	0.03	1.00	8.55	0.12	-70.80
Modified logistics	3	0.07	0.98	1.36	1.31	-53.84
Von bertalanffy	4	0.03	1.00	1.14	1.12	-76.66

Note:
P no of parameters
Adr^2 adjusted coefficient of determination
Bf bias factor
Af accuracy factor

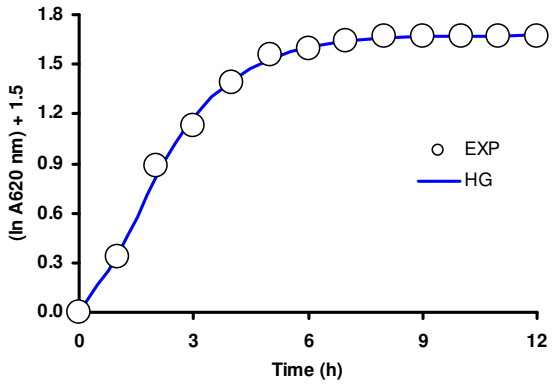

Fig. 2. Growth of *E. coli* biofilm (control) fitted to the Huang model.

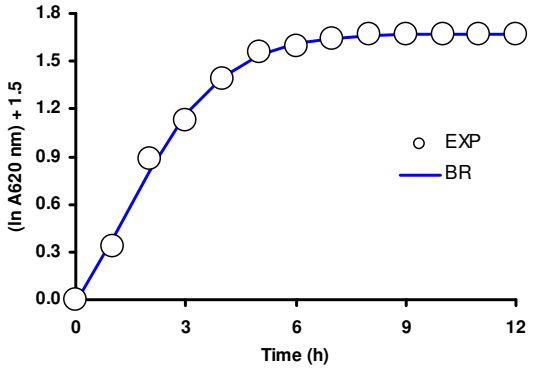

Fig. 3. Growth of *E. coli* biofilm (control) fitted to the Baranyi-Roberts model.

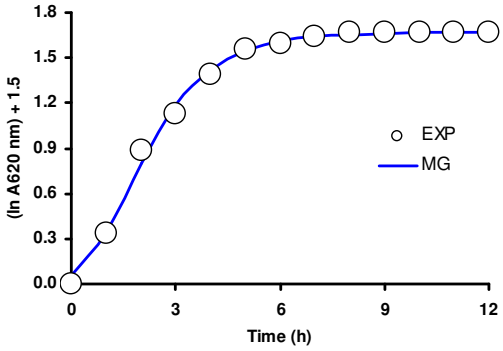

Fig. 4. Growth of *E. coli* biofilm (control) fitted to the Modified Gompertz model.

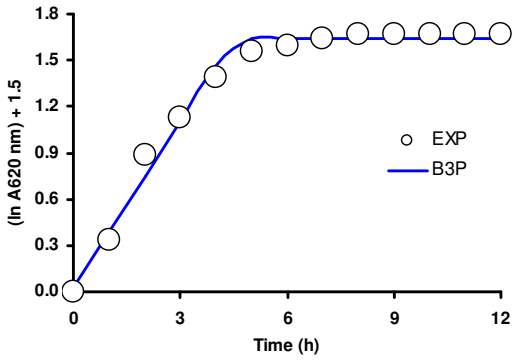

Fig. 5. Growth of *E. coli* biofilm (control) fitted to the Buchanan-3-phase model.

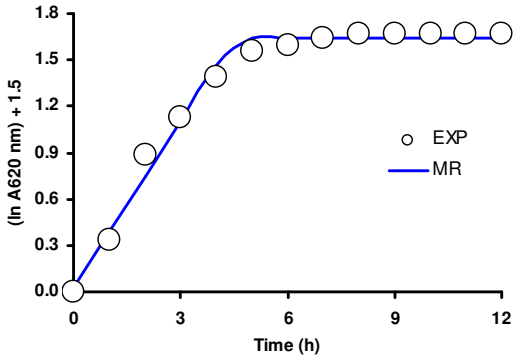

Fig. 6. Growth of *E. coli* biofilm (control) fitted to the Modified Richards model.

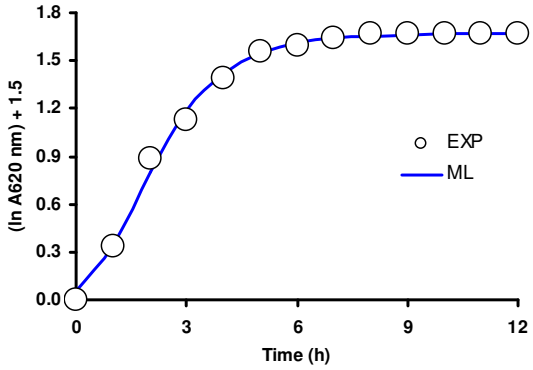

Fig. 7. Growth of *E. coli* biofilm (control) fitted to the Modified Logistics model.

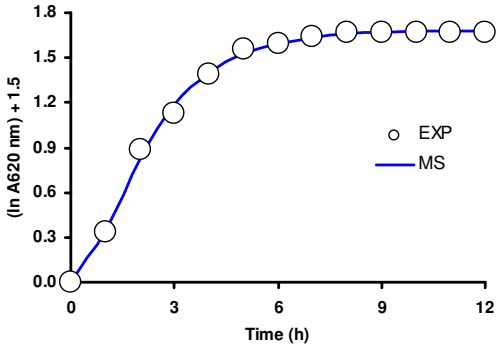

Fig. 8. Growth of *E. coli* biofilm (control) fitted to the Modified Schnute model.

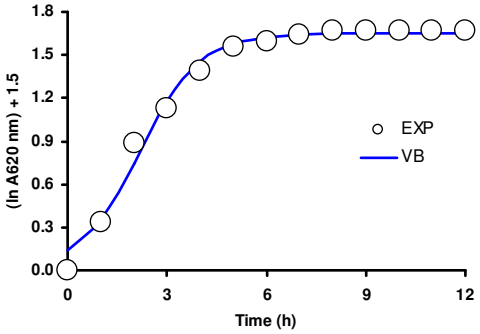

Fig. 9. Growth of *E. coli* biofilm (control) fitted to the Von Bertalanffy model.

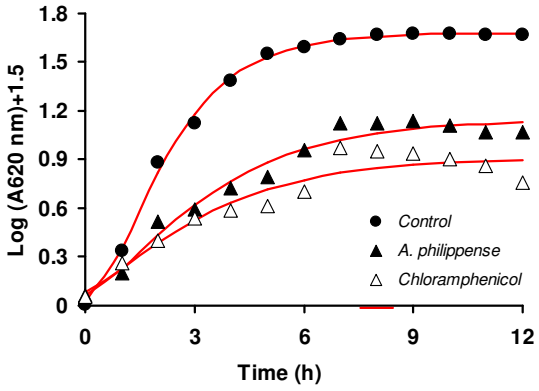

Figure 3. Growth of *E. coli* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol) fitted to the von Bertalanffy model.

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

	Control		*E. Coli*		Chloramphenicol	
	Value	(95% C.I.)	Value	(95% C.I.)	Value	(95% C.I.)
K	1.677	1.650 to 1.705	1.146	1.050 to 1.241	0.912	0.783 to 1.041
A	1.006	0.850 to 1.161	0.831	0.669 to 0.994	0.699	0.519 to 0.880
K_m (h^{-1})	1.652	1.482 to 1.822	1.146	0.746 to 1.546	1.210	0.478 to 1.942

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

The von Bertalanffy model assumes that growth of bacterial cells or organisms does not change with time or invariant [12]. Traditionally, the model was first used to model fish weight growth [13] and originates from the Bernoulli differential equation [14];

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

The following solution is obtained upon integration of the equation;

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

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 [15]. The population size stabilizes to the carrying capacity k as *t* (*x*) inclines to infinity,

Although the von Bertalanffy model has been traditionally used for modelling the increase in fish weight [16], it has found use in modelling the growth in other organisms such as chicken, tumour and cancer growth [17], *Daphnia magna*[18], seaweed [19] and microorganims' growth [20–25].

Parameters obtained from the fitting exercise were maximum growth rate of A. *Philippense* (μ_{max}), lag time (L) and maximal effect of *A. Philippense* (y_{max}) of *E. coli* (h^{-1}). Such biologically important coefficients would later be used for secondary modeling of the impact of the effect of *Adiantum philippense* extracts on biofilms formation, adhesion with *E. coli* activities against food borne pathogens using model such as the two-parameter Monod model or other more complex models "secondary models" such as Haldane, Aiba, Yano and others.

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. [25].

CONCLUSION

In conclusion, the Von Bertalanffy model was the best model in modelling the effects of *Adiantum philippensis* extracts on biofilms formation, adhesion with E. *coli* activities based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and corrected AICC (akaike information criterion). Our findings reveal that *A. Philippense* was active against *E. coli* and von Bertalanffy best describe the rapid time dependent kinetics of bacterial (E. coli) killing. *A. philippense* crude extract also impedes the biofilm matrix by reducing the total content of exopolysaccharide

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