JOBIMB, 2021, Vol 9, No 2, 34-39
DOI: https://doi.org/10.54987/jobimb.v9i2.615

HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

The Effect of the plant *Adiantum philippense* Extracts on Biofilms Formation and Adhesion to *Shigella flexneri*: A Predictive Modelling Approach

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HISTORY

Received: 28th Oct 2021
Received in revised form: 23rd Nov 2021
Accepted: 21st Dec 2021

KEYWORDS

Shigella flexneri
Modified Gompertz
Adiantum philippense
Biofilm
modelling

ABSTRACT

In the quest for novel bioactive metabolites, which can also be used as therapeutic agents, *Adiantum philippense* (A. philippense), an ethnomedically important fern, has become a fascinating herb. In this study, the predictive mathematical modelling of *A. philippense* crude extract was tested against *Shigella flexneri*, a common food pathogen for its phytochemical constituents, antagonistic ability, and effect on bacterial adhesion and biofilm formation was calculated. Various kinetics models such as Von Bertalanffy, Baranyi-Roberts, modified Schnute, Modified Richards, Modified Gompertz, Modified Logistics and latest Huang were used to get values for the above kinetic constants or parameters. modified Gompertz of the entire model was found to be the best model with the highest adjusted R^2 value and lowest RMSE value. The accuracy and bias factors values were close to unity (1.0). The maximum specific growth rate (m_{max} (h^{-1}) for *S. flexneri* treatment with *A. philippense* extract was significantly much lower ($p<0.05$) with a value of 0.292 (95% confidence interval of 0.254 to 0.331) compared to control with a value of 0.540 (95% confidence interval of 0.481 to 0.599) indicating potential biofilm inhibition.

INTRODUCTION

Adiantum philippense is a small and delicate fern native to the Philippines. Rhizome is short and sub-erect, with scales covering the apex and being linear and a little wider at the base. Stipes are tufted, slender, dark brown, glossy, and glabrous, with lengths ranging from 5 to 21 cm. Fronds are tufted, herbaceous, and arching in shape. Stipe up to 20 cm long, dark brown to black in colour, glossy, and glabrous in appearance. The lamina is 36 11 cm long and linear to lance in shape, with the exception of a glabrous projection of the rhachis of varying length that often terminates in an inflorescence or proliferous bud or lamina with a terminal pinna. Rhachis, petiole, and pinnae are totally glabrous. Pinnings numerous (up to 15 pairs), persistent, lunulate, petiolate, alternating; 25-50 x 13-25 mm, decreased in size toward the apex, midrib forms the lower edge, outer border irregularly cut into wide lobes, apex decreased in size toward the midrib. The sori are oblong to linear in form and as long as the lobes are wide. [1–3].

The genus Shigella is designated after the Japanese physician Kiyoshi Shiga, who conducted study into the causes of dysentery. The year was 1892, and Shiga was a student at the Tokyo Imperial University School of Medicine, at which time he heard a lecture by Dr. Shibasburo Kitasato. Following graduation, Dr. Shiga was inspired by Dr. Kitasato's brilliance and boldness and decided to work for him as a research assistant at the Institute for Infectious Diseases. During the summer of 1897, Shiga concentrated his attention on what the Japanese called a "Sekiri" (dysentery) epidemic in the area. /These outbreaks, which were particularly harmful to the Japanese people in the late nineteenth century, happened on a regular basis. The sekiri pandemic of 1897 killed more than 91,000 people and resulted in a death rate of more than 20%. [3] Shiga conducted a study on 32 dysentery victims and utilised Koch's Postulates to successfully isolate and identify the bacteria that was responsible for the ailment. The bacterium was studied and characterized further, with the identification of the bacterium's toxin producing techniques, i.e.

Shiga Toxin, and he labored relentlessly to develop a vaccine against the sickness. *Shigella flexneri* is a Gram-negative bacterium that belongs to the *Shigella* genus that can cause diarrhea in humans when it infects them. *Shigella flexneri* is a member of the B serogroup of bacteria, which also comprises numerous other Shigella species. Certain strains of *S. flexneri*, on the other hand, have developed resistance to antibiotics that are frequently used. More serious problems can be treated more regularly because they become more resistive to treatment in the future. [1] *Shigella* can be distinguished from *E.coli* based on pathogenicity, physiology (inability to decarboxylate lysine or ferment lactose), and serology, among other factors.

Foodborne infections have the potential to cause long-term damage or death. Some examples of dangerous food include uncooked foods of animal origin, fruits and vegetables that have been infected with faeces, and raw shellfish that has been exposed to marine biotoxins[1]. The most main signs of these foodborne pathogens are diarrhoea, vomiting, stomach cramps, weakness, nausea, and fever. Vomiting and diarrhea are the most common side effects of these pathogenic organisms. These viruses have the potential to infect products at any moment throughout the production, distribution, and storage processes. It is consequently critical that we limit the establishment and development of food pathogens, despite the fact that removing these species is challenging due to their ability to firmly and adhesively adhere themselves to surfaces [5].

Microbes adhere evenly to surfaces and secrete extracellular polysaccharides, leading in the creation of biofilms on those surfaces. Increasing antimicrobial resistance among organisms associated with biofilms has resulted in biofilms becoming a substantial public health threat [4]. Additionally, biofilm bacterial cells are capable of protecting themselves against a number of physico-chemical aggressions, including acidity, salt, toxic metals, ultraviolet rays light, and phagocytosis (phagocytosis is the process by which bacteria ingest other bacteria) (Adnan et al., 2020a). 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 [4].

In addition to being immune to antibiotics, biofilm bacterial cells are also able to protect themselves against a variety of physico-chemical aggressions, including acidity, salinity, heavy metals, ultraviolet light and phagocytosis[5]. According to the facts, the formation of biofilm poses a significant global threat to the marine and oceanic sectors, as well as to the food and dairy industries, and, most importantly, to public health worldwide (Adnan et al., 2020a). Biofilm treatment is a global concern that necessitates the development of innovative natural bioactive compounds that are effective against harmful bacteria found in food. In comparison to chemically generated bioactive compounds, the requirement for naturally occurring bioactive compounds is a result of interactions with the food industry.

It has been discovered in prior investigations on traditional medicines that phytochemicals have antibacterial properties through the suppression of quorum sensing [6–8]. A variety of phytochemicals, including flavonols, flavonoid, phenolic compounds, and flavonones, are recognised and widely used as quorum-sensing inhibiters. Flavonols are the most well-known and widely used. Likewise, these types of phytochemicals are also well-known for their ability to block bacterial adhesion as well as gene suppression, both of which are linked with biofilm development [9–14]. Therefore, this analysis was aimed at studying the mathematical modeling of the effects of *A. philippense* phytochemicals on adhesive to biofilm formation with their antibacterial properties against the food pathogen *Shigella flexneri*.

MATERIALS AND METHODS

A previously published data [15] for *Shigella flexneri* was processed using the software Webplotdigitizer 2.5 [16].

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. For the purposes of modeling, the model that demonstrates the highest growth was adopted.

Table 1. Growth models used in modelling the growth curve of the bacterium.

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

DOI: https://doi.org/10.54987/jobimb.v9i2.615

Statistical analysis

An error function assessment, which included statistical parameters such as accuracy factor (AF), bias factor (BF), adjusted determination coefficient (R2), root-mean-square error (RMSE), and one relating to information theory, the AICc, was used in the choice of the appropriate models (corrected Akaike Information Criterion) [36].

RESULTS AND DISCUSSION

Prior to modelling, the growth curves were replotted and converted to log units (see **Fig. 1**) to ensure that they were comparable. During the modelling phase, the highest signal was utilised to determine which model was the best. It was clear that all of the models had a good match to the growth curve (**Figs 2** to **9**). The modified Gompertz model was used to find the optimal model, which had the lowest values for RMSE and AICc, as well as the highest value for adjusted R2, among other characteristics. The AF and BF values for the model were demonstrated to be excellent, with their values being the closest to unity. The modified logistic model had the lowest overall performance (**Table 2**). The lack of a significant lag phase for growth is most certainly the explanation for the superiority of the von Bertalanffy model over other growth models. These are the coefficients for the modified Gompertz model, which can be found in **Table 3**.

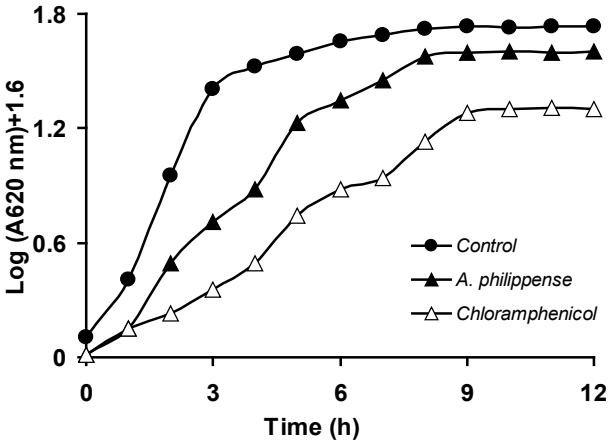

Fig. 1. Growth of *S. flexneri* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol).

Table 2. Statistical analysis of the various fitted models.

Model	*p*	RMSE	R^2	adR^2	AF	BF	AICc
Huang	4	0.068	0.989	0.983	1.036	1.020	-48.22
Baranyi-Roberts	4	0.043	0.995	0.993	1.025	1.006	-59.81
modified Gompertz	3	0.034	0.997	0.996	1.055	0.972	-72.13
Buchanan-3-phase	3	0.081	0.982	0.975	1.055	1.011	-49.76
modified Richards	4	0.036	0.997	0.995	1.040	0.983	-64.83
modified Schnute	3	0.036	0.997	0.995	1.040	0.983	-64.83
modified Logistics	3	0.046	0.994	0.992	1.048	1.026	-64.60
von Bertalanffy	4	0.041	0.996	0.994	1.148	0.892	-67.58

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

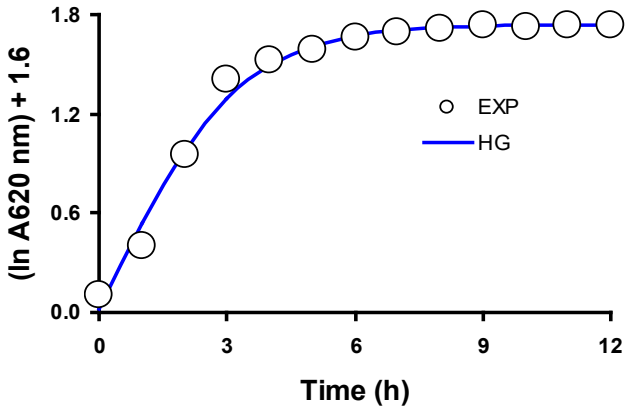

Fig. 2. Growth of *S. flexneri* biofilm (control) fitted to the Huang model.

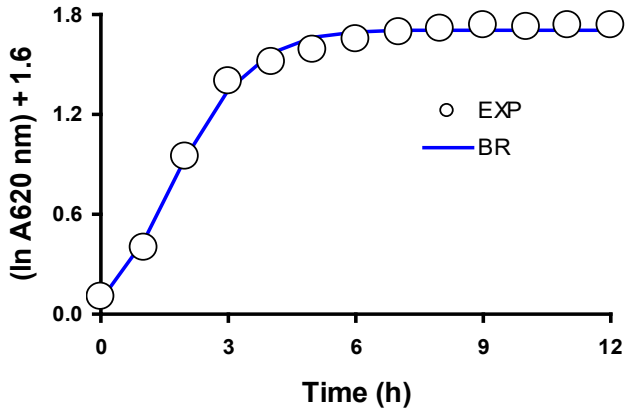

Fig. 3. Growth of *S. flexneri* biofilm (control) fitted to the Baranyi-Roberts model.

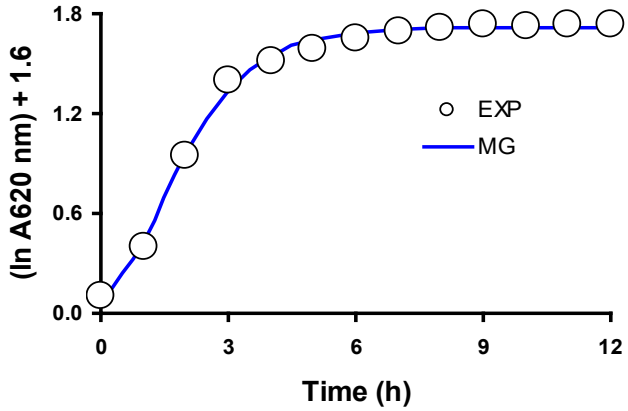

Fig. 4. Growth of *S. flexneri* biofilm (control) fitted to the modified Gompertz model.

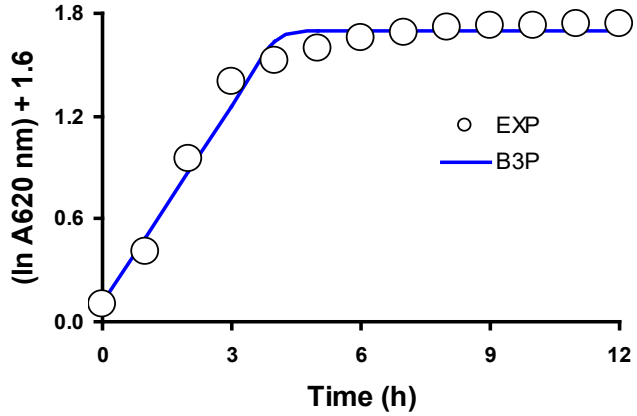

Fig. 5. Growth of *S. flexneri* biofilm (control) fitted to the Buchanan-3-phase model.

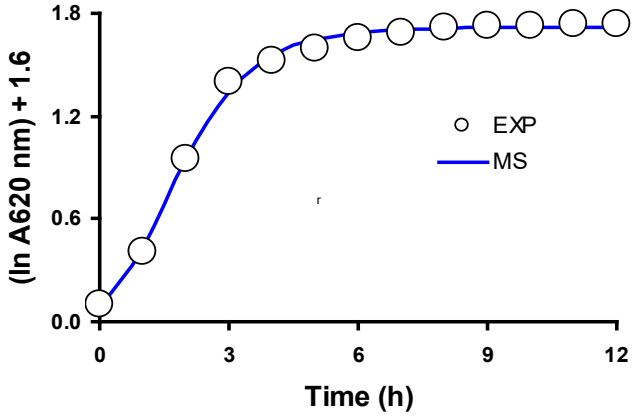

Fig. 8. Growth of *S. flexneri* biofilm (control) fitted to the modified Schnute model.

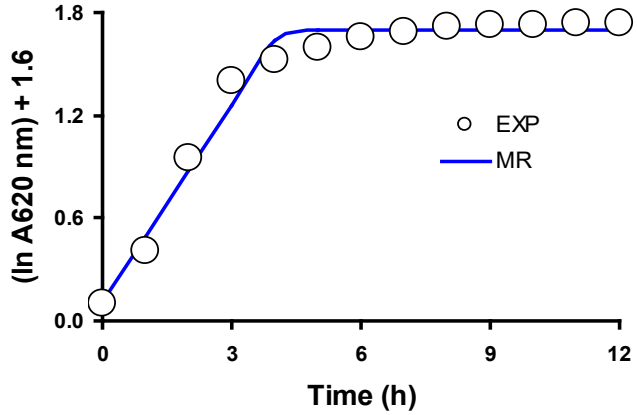

Fig. 6. Growth of *S. flexneri* biofilm (control) fitted to the modified Richards model.

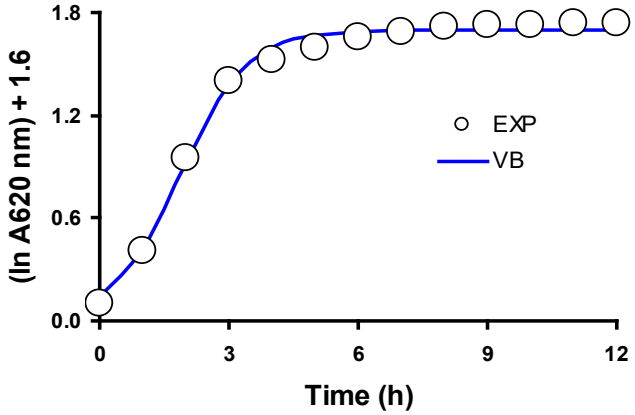

Fig. 9. Growth of *S. flexneri* biofilm (control) fitted to the von Bertalanffy model.

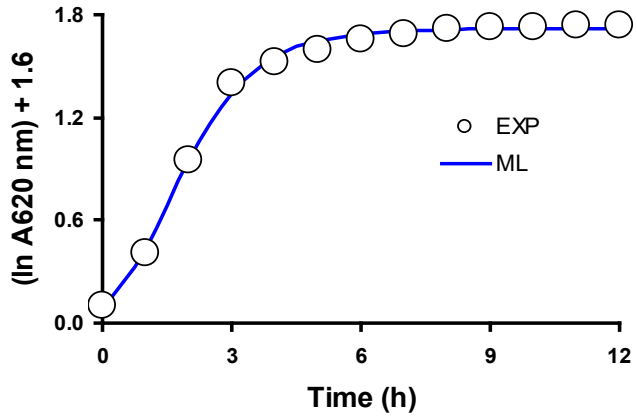

Fig. 7. Growth of *S. flexneri* biofilm (control) fitted to the modified logistics model.

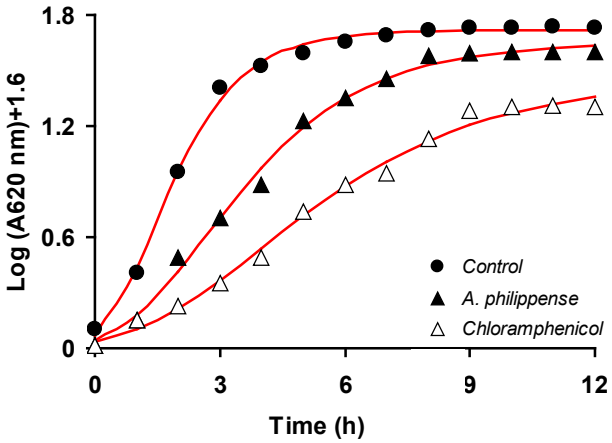

Figure 3. Growth of *S. flexneri* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol) fitted to the modified Gompertz model.

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

	Control		*S. flexneri*		Chloramphenicol	
	Value	(95% C.I.)	Value	(95% C.I.)	Value	(95% C.I.)
Y_{max}	1.719	1.689 to 1.748	1.650	1.585 to 1.715	1.455	1.329 to 1.580
μ_{max} (h^{-1})	0.540	0.481 to 0.599	0.292	0.254 to 0.331	0.177	0.152 to 0.203
lag (h)	0.204	0.019 to 0.388	0.588	0.220 to 0.956	0.954	0.431 to 1.476

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

The maximum specific growth rate (m_{max} (h^{-1}) for *S. flexneri* treatment with *A. philippense* extract was significantly much lower ($p<0.05$) with a value of 0.292 (95% confidence interval of 0.254 to 0.331) compared to control with a value of 0.540 (95% confidence interval of 0.481 to 0.599) indicating potential biofilm inhibition. The study carried out here attempt to optimize bacterial growth using mathematical models. Other growth models that are available including Baranyi-Roberts [17,18] and Logistic, modified Gompertz [19–25], Richards, Schnute [26,27], Von Bertalanffy [28,29], Buchanan three-phase [22,30–34] and more recently the Huang model [35]. The modified Gompertz model is the most popular model as it is the simplest (having three parameters). Previously, the mathematical modelling of the effect of *Adiantum philippensis* Extract and Adhesion to several bacteria have been studied with the best models for *P. aeruginosa*, *S. aureus* and *E. coli* was found to be von Bertalanffy [36], modified Gompertz [3] and von Bertalanffy [37] respectively. The advantage of mathematical modelling is statistical inferences can be made from differences of the parameters, especially specific growth rate. If the 95% confidence interval do not overlap, then there is a significant chance that the difference is significant at the 0.05 alpha value.The asymmetrical sigmoidal form of the modified Gompertz model, as opposed to the logistic model, provides greater flexibility than the logistic model. At the moment of inflection, sigmoidal models such as logistics and Gompertz differ primarily in the distance between the lower and higher asymptotes. There is a gap of 1/2 and 1/e between the lower and higher asymptotes of the logistics and Gompertz models, correspondingly, between both the lower and upper asymptotes of the logistics model [38]. In principle, many growth models feature a changeable inflection point between the lower and higher asymptotes, as well as a flexible function of the slope between them. Each of these functions is either a distinct function or a reduced version of a parent model. Examples of such models are the modified logistics, modified Gompertz, and von Bertalanffy growth models, all of which are derived from the parental Richard's model [27,38,39].

The model has its limitations with the most important being that in the static version, $y_{(t=0)}$ is not equal to y_o. Second, the sigmoidal curve has an intrinsic trait of having an inflection point, which causes the model to have a systematic problem expressing the exponential phase of the distribution (Baranyi et al., 1993). In the end, the model seems to overemphasize the significance of its parameters [40–42]. The modified Gompertz model, in spite of this, has been widely applied to the modelling of the evolution of bacteria and secondary bacterial products, such as biogas, methane, lactate, biodiesel, and bacterioricin, just to mention a few [43–47] including callus growth [48–50]. Its usage for modelling the effect of biological extracts to biofilm-forming bacteria has been reported for *S. aureus* [3], inhibitory effect of royal jelly on *Listeria monocytogenes* biofilm [51], inhibitory curves of Mexican Oregano (*Lippia berlandieri* Schauer) Essential Oil on biofilms of *Pseudomonas aeruginosa* and *Salmonella* Thyphimurium [13] and the inhibitory curves of two mangrove species, *Bruguiera cylindrica* and *Laguncularia racemose* on selected bacterium, yeast, and filamentous fungi biofilms [12]. For more secondary modeling, parameters derived from the fitting exercise may later be used. These mechanistic models aim to gain a better understanding of the processes of chemistry, physics and biology. Mechanistic models, such as the modified Gompertz, are more effective compared to purely empirical model, as mechanistic models tells us about the underlying mechanism or mechanisms that drive the effects of *Adiantum philippense* Extracts on Biofilms Formation and Adhesion to *Shigella flexneri* [52].

CONCLUSION

In concluding, the modified Gompertz model was found to be the most accurate model for modelling the inhibitory effects of *Adiantum philippensis* extracts on *S. flexneri* biofilm formation based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), accuracy factor (AF), bias factor (BF) and corrected AICC. Our data suggest that *A. philippense* extract was active against *S. flexneri,* and there is a potential usage of the plant for inhibiting bacterial biofilm.

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