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Modeling the Effect of Natural Substances and Antibiotics Concentrations on the Variation of Inhibition Zone Diameter in Disc Diffusion Susceptibility Test

Gislaine Aurelie Kemegne[1], Ghislain Seuyim Kouogueu[1], Maximilienne Ascension Nyegue[1], Sylvain Leroy Sado Kamdem[1*], Chantal Menut[2] and Francois-Xavier Etoa[1]

[1]Laboratory of Microbiology, Department of Microbiology, University of Yaounde I, P.O.Box 812, Yaounde, Cameroon.
[2]Equipe de Recherche « Glyco et Nanovecteurs Pour le Ciblage Thérapeutique » Institut des Biomolécules Max Mousseron (IBMM), Université de Montpellier, BP 14491 34093 Montpellier, France.

Authors' contributions

This work was carried out in collaboration between all authors. Authors GAK, GSK, MAN and SLSK designed the study and wrote the protocol. Authors GAK and GSK did the bench work and author GAK wrote the first draft of the manuscript. Author SLSK managed the statistical analyses of the study. Authors GAK, SLSK, MAN and FXE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to propose a model describing the relationship between the antimicrobial concentration and the inhibition zone diameter during antimicrobial susceptibility testing.

**Corresponding author: E-mail: sadosylvain@hotmail.com;*

Place and Duration of Study: Department of Microbiology of the University of Yaoundé I between June 2016 and December 2016 (6 months).
Methodology: Water, ethanol extracts and essential oils from plants as well as antibiotics were tested at different concentrations against five bacteria including both Gram+ and Gram-. The inhibition diameters obtained were plotted against the quantity of substance loaded on the disc. Data were divided into two groups and one was used for model construction (test data) while the other was combined to literature data and used for validation. The model construction begun with CurveExpert 1.4 software in order to search for the most suitable equation family fitting the data. The model family selected was modified accordingly to reduce the number of parameters.
Results: We propose a model where the inhibition diameter (Id) = aX^b where "a" and "b" are parameters estimated using nonlinear regression, X is the quantity of antimicrobial deposited on the disc. Validation of the model on new set data gave R^2 values ranging between 0.96 and 0.98. Moreover the proposed model described three inhibition patterns already discussed theoretically in literature.
Conclusion: This work proposes for the first time a simple and direct mathematical relationship between the antimicrobial concentration and inhibition diameter in the disc diffusion susceptibility test. This may be used in comparing the level of activity of antimicrobials and contribute in classifying natural substances according to their activity pattern.

Keywords: Inhibition zone diameter; plant derived antimicrobials; antibiotics; mathematical model.

1. INTRODUCTION

Antimicrobial susceptibility test can be done using a variety of methods grouped into 3 categories, including diffusion, dilution and bioautography methods [1,2,3,4]. Diffusion methods are widely used to investigate the antibacterial activity of active compounds. These assays are qualitative and are based on the use of a reservoir containing the substance to be examined, which is brought into contact with an inoculated agar medium; after incubation, the clear zone of inhibition around the reservoir is measured [4,5]. Different types of reservoirs have been used, with the main two being filter paper discs placed on the surface of the solid media [2,5,6,7] and holes punched in the medium [4,8,9].

Diffusion methods hold a number of advantages over quantitative methods used to determine the MIC (Minimum Inhibitory Concentration). They are less labor intensive, use a smaller amount of the test agent, and allow up to 5 or 6 substances to be tested against a single microorganism in one Petri dish [4]. It is well established that diffusion methods are not the best choice for testing non polar or other samples that do not diffuse in the media well, resulting in no correlation between diffusion power and antimicrobial power [10].

Most reports use a single diffusion method at only one concentration to draw definite conclusions from results obtained [4,5,6,11,12]. Most of the time in disc diffusion assay, there is no justification on the choice of the concentration tested. Concentration is always observed to influence the diameter of inhibition [13,14,15]. It has also been demonstrated that the compound diffusion creates a gradient of concentration which is one of the reason of the low inhibiting effect at the edges of the inhibiting zone [16,17]. Chandrasekar et al. [16] used a finite element computational model based on Fick's second law of diffusion to predict the radius of the inhibition zone in a biodiffusion bioassay and applied it to nisin. The model obtained was based on the diffusivity of nisin through the agar, the concentration of nisin, the initial concentration of microorganism inoculated and the time of visible appearance. This approach which tries to be mechanistic has the limit to be very specific to the condition of the experiment performed. However, it is already an initial solution to the conclusion of King and Dykes [18] who stated that there is no linear or logarithmic relation between inhibition zone size and the concentration of the agent.

There are some reasons behind the search of predictive models in antimicrobial susceptibility testing. One is the possibility of easy bioprospection of molecules and the second is the need of an international method of expressing and comparing the level of activity of a substance. In this regard, few works have attempted to propose simple mathematical approaches to relate antimicrobial substances and inhibition diameters. Antimicrobial substances are used in this work as a generic term for any compound or mixture of compound with antimicrobial properties.

The aim of this study was to develop an empirical and deterministic model able to express the concentration dependent-activity of antimicrobial substances. For this purpose, a disc diffusion assay was used to assess the activity of different antimicrobial substances obtained from hydrodistillation (essential oil), or by aqueous and ethanol extraction from four Cameroonian plants namely *Psidium guajava*, *Mangifera indica*, *Sida corymbosa* and *Tristemma incompletum* and three antibiotics (Amoxicillin, Gentamicin and Ampicillin) against five bacterial species (Gram- and Gram+) of relevance due to their roles in food intoxication and infectious diarrhea.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Sample of fresh and air-dried barks of *Mangifera indica* (from the University of Yaounde I, Cameroon, July 2015), fresh and air-dried leaves of *Psidium guajava* (from the University of Yaounde I, Cameroon, July 2015), air-dried leaves of *Sida corymbosa* and *Tristemma incompletum* (from Mbalmayo, Cameroon in December 2015) were collected in the morning. The botanical identification and authentication was carried out at the National Herbarium of Cameroon (Yaounde) where voucher specimens are kept: 18646/SRF Cam, 2885/SRFK, 33890/HNC and 44543/HNC respectively. Fresh samples were used for the extraction of essential oil (EO) and samples air-dried at 30°C under a shell were used for aqueous and ethanol extracts.

2.2 Extraction of Essential Oil

Fresh leaves of *Psidium guajava* and fresh barks of *Mangifera indica* were chopped into small pieces and EO was obtained by hydrodistillation using a Clevenger-type apparatus for 6-8 h. The oils were dried after decantation over anhydrous sodium sulfate. The oil yield was 0.06% and 0.04% w/w for *P. guajava* and *M. indica* respectively with respect to fresh plant weight, and the densities were 0.88.

2.3 Extraction of Aqueous and Ethanol Extract

The extraction was carried out by macerating the dried powdered barks with water for aqueous extract and with ethanol 96% (Sigma Aldrich) for ethanol extract. After filtration, the filtrates were lyophilized to give aqueous and ethanol extract. The aqueous yield was 7.43% w/w and 5.80% w/w for *M. indica* and *S. corymbosa* respectively while the ethanol yield was 3.81% w/w and 8.32% w/w for *P. guajava* and *T. incompletum* respectively. All yield were calculated with respect to the dried matter.

2.4 Antibiotics and Microorganisms

Ampicillin (AMP), Gentamicin (GEN) and Amoxicillin (AMOX), (Sigma-Aldrich, St Quentin Fallavier, France) were used as reference antibiotics.

Microorganisms included in this study for antimicrobial activity screening were five bacteria amongst which three Gram-: *Escherichia coli* ATCC 25922, *Salmonella enteritidis* 155A, *Shigella* spp and two Gram+: *Staphylococcus aureus* NCTC 10652, *Bacillus cereus* ATCC 11966 kindly offered by the Laboratory of Food Microbiology University of Bologna-Italy. *Shigella* spp was a clinical isolate obtained from the University Hospital Center of Yaounde-Cameroon. Strains stored at -80°C were cultured twice at 37°C for 24h in Brain Hearth Infusion (BHI; Biolife Italiana, Milano-Italy) broth before being used in the tests.

2.5 *In vitro* Antimicrobial Activity: Disc Diffusion Assay

The essential oil, aqueous, ethanol extracts and antibiotics were dissolved in 10% DMSO to 5 final concentrations of 1200, 600, 300, 150 and 75ppm (µl/L for essential oils and µg/mL for extracts). Sterile paper discs (6mm of diameter) prepared from Whatman filter paper (Whatman N°1) were sterily impregnated with 30 µl of each solution at different concentration in triplicate. Antimicrobial tests were then carried out by disc diffusion method [19]. 200 µl of the suspension of the tested microorganisms cultured as indicated previously containing 10^6 Cells/ml prepared from an overnight BHI broth culture was used to seed each prepared and dried Mueller Hinton agar medium. Subsequently, impregnated discs were arranged and firmly pressed on the agar surface of each seeded plate. These plates were stored at ambient temperature for 1 h before incubation at 37°C for 24 h. Negative control was also prepared using the same solvent employed to dissolve the extracts and essential oil. Microbial sensitivity was evaluated by measuring the zone of inhibition of the tested microorganisms (expressed in mm). Only essential oils or extracts

who gave inhibition zones for at least three consecutive concentrations were selected for modeling purposes.

2.6 Elaboration of a Mathematical Model Relating Substances Concentration with Inhibition Diameter

Experimental data obtained were recorded as mm inhibition diameter for a certain antimicrobial concentration expressed in nanograms (ng) per mm^2 of the filter disks. These data were used to elaborate a mathematical model describing the variation of inhibition zone diameter as a function of the antimicrobial substance concentration. For this purpose, the first step was done by using CurveExpert 1.4 to have the best family equation or Model fitting the experimental data. The best family equation was then modified to a more suitable and flexible form. Subsequently, STATISTICA 10 StatSoft was used to compare the performance of the proposed Model and Harris Model on new experimental and literature data from Daniyan et al. [14] obtained via the web site http://bioactifplantbase.com freely available database (Table 1).

Table 1. Literature Inhibition zone diameters (mm) from Daniyan et al., 2013

ng/mm^2	PA_KP	EH_EC	AMO_ST
106.10	15	18	16
84.88	/	17	17
63.66	12	15.5	16.5
42.44	12	11.5	15
21.22	6	11	12
0.00	0	0	0

PA= Phyllanthus amarus methanol extract, KP=Klebsiella pneumonia methanol extract, EH= Euphorbia hirta methanol extract, EC= Escherichia coli, ST= Salmonella typhi, AMO= Amoxicillin; /: no inhibition zone diameter observed; ng/mm^2= antimicrobial quantity per mm^2

3. RESULTS AND DISCUSSION

3.1 Inhibition Zone Diameter Values of Tested Antimicrobials on Selected Bacteria

The experimental inhibition zone diameters expressed in mm are presented in Table 2. Only data presenting enough experimental points to be modeled was considered. In general, it can be observed that for plant antimicrobials, there is a non constant pattern of the increase of inhibition diameter with concentration. The aqueous extract of *Mangifera indica* barks was the one giving high proportionality and correlation between the concentration and the inhibition zone diameter. Regarding the overall results, it can be observed that depending on the antimicrobial concentration, the classification of the antimicrobials efficacy towards a strain is not the same, stressing the need of a comparison method dependent of the concentration. These data were used to elaborate a mathematical model describing the variation of inhibition zone diameter as a function of the test agent concentration.

3.2 Elaboration of a Mathematical Model

Table 3 indicates the different model parameters estimation as well as their R^2 and SE for the different susceptibility test of different extracts and antibiotics towards Gram- and Gram+ bacteria. The different family Models that were proposed by CurveExpert 1.4 software to fit the experimental data gave quite good results.

Equation 1: Harris model

$$Y = \frac{1}{(a+\mathrm{b}*X^{\mathrm{c}})} \quad (1)$$

Equation 2: MMF Model

$$Y = \frac{a*b+\mathrm{c}*X^{\mathrm{d}})}{(\mathrm{b}+X^{\mathrm{d}})} \quad (2)$$

Equation 3: Exponential association model

$$Y = a(1 - e^{-bX}) \quad (3)$$

Equation 4: Third degree polynomial fit model

$$Y = a + bX + CX^2 + dX^3 + \ldots \quad (4)$$

Equation 5: Rational function model

$$Y = \frac{a+\mathrm{b}*\mathrm{X}}{1+\mathrm{c}\mathrm{X}+d^2)} \quad (5)$$

As seen in Table 3, the rational function and third degree polynomial function could only fit two experimental data each. On the contrast, Harris model proof to well fit the data obtained.

One of the characteristics of a good model is it simplicity and low number of parameters to be estimated. Harris type Model and the exponential association Model proved to perform well and had 3 and 2 parameters to be estimated respectively. Harris type Model was the most frequently proposed type of Model for the data fitted. We hence tried to propose a model obtained from the reparametization of the Harris Model.

Table 2. Experimental Inhibition zone diameters (mm) of tested bacteria by selected extracts and antibiotics using agar disc diffusion

	Extracts										Antibiotics			
ng/mm^2	**1H_EC**	**1H_BC**	**2A_SE**	**2A_Shi**	**2H_SA**	**2A_SA**	**5A_SA**	**1E_SE**	**6E_SA**	**ng/mm^2**	**AMO_BC**	**AMP_Shi**	**AMO_Shi**	**GEN_BC**
1273.24	/	/	27.66	28.5	/	31	10.5	13	/	106.10	17.5	38.25	37	15.33
636.62	10.75	19	18.25	18.5	/	17	10.5	/	11.75	53.05	14	32.75	33.25	13.33
318.31	10	13	17.5	17.75	29	12.25	9.83	10.66	11.5	26.53	12	26.25	28.5	11.5
159.15	7	7	10	12	23	9.5	8	9.5	10	13.26	11.25	23.6	23.5	8
79.58	7	6.66	9	7.5	18.66	9.33	7	7	9	6.63	/	20	20	8
0.00	0	0	0	0	0	0	0	0	0	0.00	0	0	0	0

1H= Psidium guajava leaves essential oil, 1E= Leaves of Psidium guajava ethanol extract, 2H= Mangifera indica barks essential oil, 2A= Mangifera indica barks aqueous extract, 5A= Sida corymbosa aerial parts aqueous extract, 6E= Tristemma incompletum leaves ethanol extract, AMO= Amoxicillin, AMP= Ampicillin, Gen= Gentamicin, EC= Escherichia coli, SE= Salmonella enteritidis, Shi= Shigella spp, BC= Bacillus cereus, SA= Staphylococcus aureus, ng/mm^2= antimicrobial quantity per mm^2, /: no inhibition zone diameter observed

Table 3. Best family models fitting experimental data as proposed by CurveExpert 1.4 Software, ranged in order of decreasing suitability

	Model types	Harris					MMF						Exponential association				3rd degree polynomial fit						Rational function					
1H_EC	Rang	**1**					**2**						**3**				/						/					
	Parameters	a	b	c	R^2	SE	a	b	c	d	R^2	SE	a	b	R^2	SE	/	/	/	/	/	/	/	/	/	/	/	/
	values	3.594	-3.332	7.728	0.984	0.552	1.709	25.365	54.868	0.285	0.988	0.706	10.348	0.01	0.969	0.857	/	/	/	/	/	/	/	/	/	/	/	/
1H_BC	Rang	**1**					**3**						**2**				/						/					
	Parameters	a	b	c	R^2	SE	a	b	c	d	R^2	SE	a	b	R^2	SE	/	/	/	/	/	/	/	/	/	/	/	/
	values	1.87	-1.526	0.027	0.984	1.464	0.065	-45E5.4	-18E5.4	0.593	0.988	1.613	22.56	0.003	0.978	1.513	/	/	/	/	/	/	/	/	/	/	/	/
2A_SE	Rang	**2**					**3**						/				**1**						/					
	Parameters	a	b	c	R^2	SE	a	b	c	d	R^2	SE	/	/	/	/	a	b	C	d	R^2	SE	/	/	/	/	/	/
	values	1.161	-0.964	-0.022	0.974	3.725	0.0298	6680	9088	0.418	0.998	3.96	/	/	/	/	0.393	0.092	-0.0002	7.1E-08	0.988	2.592	/	/	/	/	/	/
2A_Shi	Rang	**3**					**2**						/				**1**						/					
	Parameters	a	b	c	R^2	SE	a	b	c	d	R^2	SE	/	/	/	/	a	b	C	d	R^2	SE	/	/	/	/	/	/
	values	1.145	-0.951	0.022	0.973	4.448	-0.036	399.11	51.139	0.439	0.981	4.046	/	/	/	/	0.128	0.099	-0.0002	7.844E-08	0.999	0.102	/	/	/	/	/	/
AMO_BC	Rang	**2**					/						**3**				/						**1**					
	Parameters	a	b	c	R^2	SE	/	/	/	/	/	/	a	b	R^2	SE	/	/	/	/	/	/	a	b	c	d	R^2	SE
	values	1.24	-1.18	0.006	0.994	1.987	/	/	/	/	/	/	33.957	0.101	0.974	6.698	/	/	/	/	/	/	0.026	7.04	0.216	-0.0003	0.998	0.752
AMP_Shi	Rang	**2**					**3**						/				/						**1**					
	Parameters	a	b	c	R^2	SE	a	b	c	d	R^2	SE	/	/	/	/	/	/	/	/	/	/	a	b	c	d	R^2	SE
	values	1.215	-1.155	0.006	0.991	2.948	0.005	67E4.01	93E5.23	0.216	0.993	3.341	/	/	/	/	/	/	/	/	/	/	-0.008	9.936	0.336	-0.008	0.996	1.662

a,b,c,d= Different parameters obtained by the Model, SE= Standard Error, EC= Escherichia coli, Shi= Shigella spp, SE= Salmonella enteritidis, BC= Bacillus cereus, 1H= Psidium guajava leaves essential oil, 2A= Mangifera indica barks aqueous extract, AMO= Amoxicillin, AMP= Ampicillin, /: no inhibition zone diameter observed

Equation 6: reparametization of Harris model

$$Id = \frac{1}{\alpha+\beta*X^{\gamma}} \quad (6)$$

A starting point is to consider that, for an antimicrobial concentration X equal to 0, the Id should be equal to 0, then α should be equal to 0 in regard to the condition. Hence,

$$Id = \frac{1}{\beta * X^{\gamma}} \Longrightarrow \quad Id = \frac{1}{\beta} X^{-\gamma} \quad (7)$$

with a=1/β and b=(-γ). We can then simplify equation 7 as follows:

$$Id = a * X^{b} \quad (8)$$

where "a" and "b" are parameters to be estimated, X is the quantity of antimicrobial in ng/mm^2 of disc and Id the inhibition zone diameter in mm.

This Model suitability was validated on new experimental data and literature data in comparison with the Harris type Model, equation 1.

The Harris Model used in comparison has 3 parameters and hence a low degree of freedom since literature data has a number of records which are in general less than 7. The proposed model has the advantages to have 2 parameters and is easier to be adapted. In general (Table 4), the proposed model showed fitting characteristics not too different to Harris type model (R^2 and MMSE) but only 69.60% of Harris parameters estimated were statistically significant ($P<0.05$) compared to 86.36% of the proposed model parameters. Both experimental and literature data were fitted with good R^2.

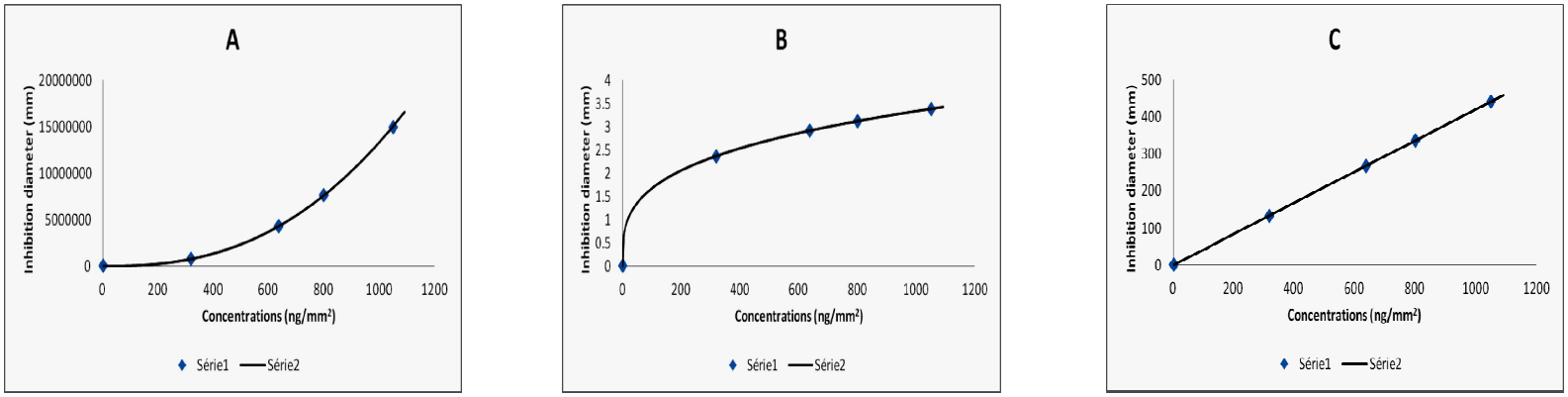
From the model proposed, it was theoretically possible to clearly define tree patterns of variation of the inhibition diameter with the antimicrobial concentration used (Fig. 1). In fact for b<1, the pattern of variation is convex meaning that the variation of antimicrobial concentration at lower values gives higher changes in inhibition diameters than variation at high concentrations (Fig. 1B). For b>1, the pattern of variation is concave meaning that the variation of antimicrobial concentration at lower values gives lower changes in inhibition diameters than variation at high concentrations (Fig. 1A). For b=1, the variation of antimicrobial concentration is directly proportional to the variation of inhibition diameter (Fig. 1C). All the experimental and validation data gave "b" values lower than 1. This shows a reduction of the influence of concentration on inhibition diameter that starts at a breaking point. As it can be observed in Fig. 2, on the same strain, the higher the "b" value the more proportional is the concentration to the Id. Moreover, the higher the "a" value, the more efficient are the lower concentrations (Fig. 2).

The *in vitro* antimicrobial activity of drugs is usually assessed by the determination of MIC and MBC in low protein medium. These conditions offer an environment where cells are in constant contact with the total amount of the drug whereas in *in vivo* conditions the exposure to the antimicrobial varies depending on the pharmacokinetics of the drug. The disc diffusion assay offers an advantage to simulate an activity depending on the diffusion of the compound in the medium. But as indicated by Bonev et al. [20], the assumption of the free diffusion of antimicrobial substances on solid nutrient medium always lead to significant deviation of the predicted behavior. Chandrasekar et al. [16] proposed a model based on the Fick second law to take into account the diffusion of the antimicrobial compound and the creation of a concentration gradient. This model was aimed at predicting by computational approaches the inhibition diameter of a compound. Bonev et al. [20] on the other hand proposed a model for the prediction of the MIC based on the antibiotic concentration, the inhibition diameter and a modified diffusion coefficient. The model proposed here in our study allies simplicity to a description of the different relations between the antimicrobial compounds and microorganisms. Unlike the other models presented, the genesis of equation 4 is based on data from antibiotics and plants derived substances that are currently intensively used as substitutes of antibiotics. The three patterns of activity described in Fig. 1 by the proposed model can be explained by already consolidated mechanism of action of antimicrobial. In fact, two primary patterns of antimicrobial activity are commonly proposed [21]. The concentration-dependent activity where higher doses in a wide range of concentration result in a greater rate of inhibition/killing. This pattern corresponds to the case of b=1 in our model. The second pattern, the minimal concentration-dependent activity defines the case where the saturation of the killing/inhibiting rate occurs at a certain concentration and above that concentration the increase of the dose do not increase the rate of the activity.

Table 4. Comparison between the proposed model and Harris model when fitting the experimental and litteraturdata

Proposed model		Experimental data								Literature data		
		1H_BC	2H_SA**	2A_SA	5A_SA	1E_SE**	6E_SA**	AMO_Shi	GEN_BC	PA_KP**	EH_EC**	AMO_ST**
a	a	0.422	4.564*	0.527	3.960*	3.377*	5.166*	13.501*	4.654*	1.810	3.431*	7.269*
	SE	0.177	0.161*	0.300	0.714*	0.627*	0.628*	0.623*	0.528*	0.871	0.826*	1.497*
	p-value	0.097	0.001*	0.154	0.005*	0.013*	0.004*	0.000*	0.001*	0.129	0.014*	0.008*
b	b	0.589*	0.320*	0.562*	0.144*	0.192*	0.131*	0.220*	0.259*	0.459*	0.356*	0.184*
	SE	0.069*	0.007*	0.085*	0.029*	0.030*	0.022*	0.012*	0.029*	0.114*	0.057*	0.050*
	p-value	0.003*	0.000*	0.003*	0.008*	0.008*	0.009*	0.000*	0.001*	0.027*	0.003*	0.020*
Model	R^2	0.980	1.000	0.958	0.983	0.987	0.996	0.997	0.987	0.961	0.985	0.985
	MSSE	1.624	0.022	7.524	0.454	0.650	0.185	0.711	0.632	2.797	1.106	1.078
Harris model		**Experimental data**								**Literature data**		
		1H_BC	2H_SA**	2A_SA	5A_SA	1E_SE**	6E_SA**	AMO_Shi	GEN_BC	PA_KP**	EH_EC**	AMO_ST**
a	a	-0.044	0.009	-0.396	0.089*	0.071*	0.077*	0.019*	0.033	0.065*	-0.008	0.059*
	SE	0.162	0.013	0.844	0.006*	0.004*	0.009*	0.002*	0.032	0.009*	0.081	0.003*
	p-value	0.813	0.616	0.671	0.001*	0.004*	0.014*	0.004*	0.379	0.020*	0.928	0.000*
b	b	1.145	0.269	0.737	3.569	3.277	1.011	0.083*	0.231*	40.239	0.288	11.370
	SE	1.757	0.112	0.718	5.058	2.670	1.773	0.008*	0.070*	112.162	0.099	31.661
	p-value	0.581	0.251	0.380	0.531	0.345	0.626	0.002*	0.045*	0.754	0.061	0.743
c	c	-0.384	-0.407	-0.076	-0.945	-0.882*	-0.770	-0.498*	-0.427	-1.975	-0.326	-2.011
	SE	0.495	0.157	0.140	0.328	0.186*	0.448	0.075*	0.264	0.884	0.328	0.927
	p-value	0.519	0.234	0.625	0.064	0.042*	0.228	0.007*	0.204	0.155	0.394	0.119
Model	R^2	0.986	1.000	0.989	0.995	0.998	0.998	0.999	0.987	0.984	0.984	0.996
	MSSE	2.972	0.160	2.847	0.180	0.177	0.162	0.322	0.949	2.324	1.691	0.443

*a,b,c,d= Different parameters obtained by the Model, SE= Standard Error, MSSE=Mean Sum of Standard Error, *= Significant estimated parameters (P< 0.05),**= data used for validation, PA= Phyllanthus amarus, EH= Euphorbia hirta EC= Escherichia coli, SA= Staphylococcus aureus, BC= Bacillus cereus, SE= Salmonella enteritidis, EC= Escherichia coli, Shi= Shigella spp, KP=Klebsiella pneumonia, ST= Salmonella typhi, AMO= Amoxicillin, AMP= Ampicillin, 1H= Psidium guajava leaves essential oil, 2A= Mangifera indica barks aqueous extract*



b>1 b<1 b=1

Fig. 1. Example of theoretical variation of the inhibition diameter with the antimicrobial concentration according to our model

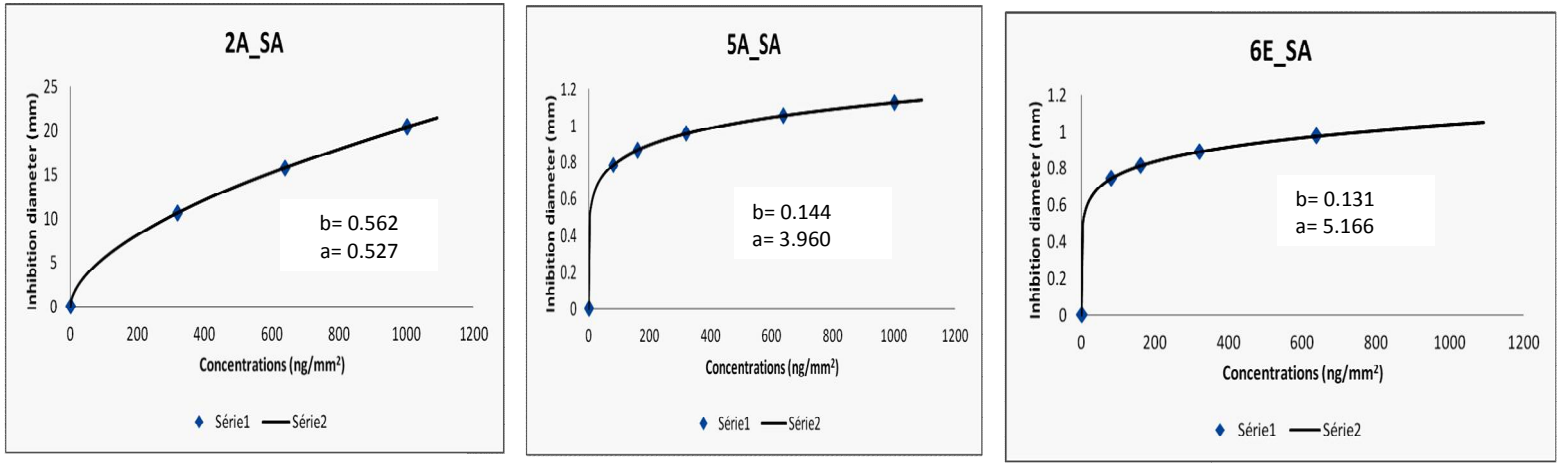


Fig. 2. Pattern variation of the inhibition diameter on *Staphylococcus aureus* (SA) with the antimicrobial concentrations where b<1 obtained in our work. 2A is *Mangifera indica* barks aqueous extract, 5A is *Sida* corymbosa aerial parts aqueous extract and 6E is *Tristemma incompletum* leaves ethanol extract. Points are experimental data and lines the adaptation curves of the proposed model to experimental data

This pattern which is also called the time dependent activity describes the pattern of the proposed model where b<1. According to Graig [21], pattern 1 is for example observed with aminoglycosides, fluoroquinolones, ketolides while pattern 2 is observed with beta-lactamides antibiotics, macrolides, tetracyclines, trimethopsin. The population density is also an important factor along side with antimicrobial concentration and time of exposition, affecting the outcome of the antimicrobial activity. According to Karslake et al. [22], the population density-dependent growth inhibition is pervasive for commonly used antibiotics, with some drug showing increase inhibition and others decrease inhibitions at high population densities. Our hypothesis is that, increasing the concentration of the antimicrobial can mitigate its density-inhibition dependency. This can help explain the third pattern of the proposed model (equation 8) represented in Fig. 1A. This pattern which was not observed during our experiment can represent a case where a certain antimicrobial concentration is necessary to cover the needs of the entire population and after that critical value, the concentration increase follows a concentration depending activity. Moreover, while comparing fitting parameters for plant extracts and antibiotics, it can be observed that parameter “a” can be used as an indication of the potency of activity of an antimicrobial and the parameter “b” can be considered as an indicator of the pattern of inhibition of a substance.

4. CONCLUSION

This work has provided a simple mathematical relationship between the antimicrobial concentration and microbial growth inhibition diameter in the disc diffusion susceptibility test. The patterns of activity described by the models are in accordance with theoretical patterns of antibiotic activity described in literature. These results can contribute in classifying natural substances according to their activity pattern.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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