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XENOBIOTICS-DEGRADING BACTERIUM

Determination of MIC and NIC values of *Allivum sativum* methanolic extract against *Aeromonas hydrophila*

Ahmad Syazwan Ismail[1], Mohd Yunus Shukor[2] and Noor Azlina Masdor[1]*

[1]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, D.E, Malaysia.

*Corresponding author:
Dr. Noor Azlina Masdor,
Biotechnology and Nanotechnology Research Centre,
Malaysian Agricultural Research and Development Institute (MARDI),
Persiaran MARDI-UPM,
43400 Serdang,
Selangor,
Malaysia.
Email: azlina@mardi.gov.my

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ABSTRACT

Plant extracts have been studied extensively for their ability to suppress the growth of bacteria. Because these findings were not reported on in the benchmark values, it was not possible to compare the results of this study to other investigations. Nonlinear regression analysis of the data is one of the most accurate methods of determining this value. The minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values of the methanolic extract of *Allium sativum* against the pathogen A*eromonas hydrophila* were successfully determined using Lambert and Pearson's modified Gompertz model. As shown by the model's high correlation coefficient (0.995), garlic's MIC and NIC values of 28.02 mg/mL (95% Confidence Interval from 26.42 to 29.71) and 12.60 mg/mL (95% C.I., 11.88 to 13.37) suggest that it could be used as an inhibitory agent against this essential fish pathogen.

INTRODUCTION

Aeromonas hydrophila, a Gram-negative rod-shaped bacterium from the family Aeromonadaceae, is a bacilli-like bacteria. In addition to being exceedingly mobile, it can be found in soil, sewage, and brackish water. It contains a single polar flagellum. Adhesion, cytotoxins, lipases, and biofilm development are some of the virulence factors of bacteria, which involve their potential to produce a variety of simultaneous attacks on the bacterial system. Motile aeromonad septicaemia (MAS) has been linked to MAS in a number of freshwater fish species, and it is also thought to be spread through inadvertent scrapes [1]. Several countries throughout the world have documented cases of this virus, particularly affecting fish species, including the United States. Affected species include channel cat fish, hybrid striped bass, Tilapia (*Tilapia nilotica*), Snakehead fish (*Ophiocephallus striatus*), Goldfish (*Carassius auratus*), American eel (*Anguilla Rostrata*), Carp (*Cyprinus carpio*), Chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (Oncorhynchus mykiss), to name a few [2]. Several popular plant extracts, including Allium sativum or garlic, were evaluated against A. hydrophila in a recent study. In terms of medical value, garlic is the most frequently cited herb (Agarwal, 1996). The use of garlic as a folk medicine has been documented in Ayurvedic and related Indian and Chinese ancient systems of medicine. There has been a resurgence in the use of herbal medicines in recent years, and eco-friendly phytoproducts are being researched as effective antifungal agents for human therapy. The bacterium responds well to this plant extract's antimicrobial properties [2]. The determination of the IC_{50} which was not determined in the publication but was later determined [3]. However, IC_{50} values are one of several important parameters of efficacy of an antibiotic agent against pathogens.

Two other important parameters are non-inhibitory concentration (NIC) and minimum inhibitory concentration (MIC), respectively. It is the lowest concentration of antibiotic (typically in g/mL) that inhibits growth in vitro. MIC An antimicrobial's ability to suppress bacterial growth increases with a lower minimum inhibitory concentration (MIC). It is the smallest amount of an antibiotic that "fully" inhibits bacterial growth at the lowest concentration. Antibiotics that limit bacterial growth can be found in the NIC form. Growth is equal to the control at concentrations below the NIC. When defining "totally retarding bacterial growth" and "slowing [bacteriological] growth," it appears that the definitions were made on the fly. [4].

A semi-quantitative test approach is used to estimate the lowest concentration of antimicrobial necessary to suppress bacteria growth in order to arrive at the MIC. To evaluate the preservative, microorganisms were inoculated into growth broth that included a small amount of preservative. Finally, the least concentration of antimicrobial that generated a clear solution, meaning no visible growth was found in this test [5,6]. Currently, microtiter plates are being used in place of the old-fashioned tubes. End-point indicators can be utilized when turbidity of a test substance interferes with a test. These include resazurin [4] and fluorescein diacetate [7]. Even though no increase was observed in one well, it was regarded as the MIC [8]. Numerous antibiotic investigations have been hindered by the lack of a quantifiable standard procedure. [9,10]. However, the principal problem encountered is that all MIC techniques currently used are semi-quantitative. Nonlinear regression was used by Lambert and Pearson to determine the NIC and MIC. The MIC and NIC are derived from the slope and inflection point using the modified Gompertz model above. [11]. Using nonlinear regression is useful since the 95% confidence interval of the MIC and NIC can now be estimated.

MATERIALS AND METHODS

Acquisition of Data

Data from the works of Ramena et al. [2], from figure 3 graphs were scanned and electronically processed using Webplotdigitizer 2.5 [12]. Using the software, data from scanned images is converted into a table with comma-separated columns. [13].

Measurement of NIC and MIC: Fitting of a modified Gompertz function

A modified Gompertz equation requires log concentration data and a y response that has been translated into a fractional unit, such as fractional area or another fraction of unity, in order to be used for data analysis (Eqn. 1).

$$y = A + Ce^{-3^{B(x-M)}} \qquad \text{(Eqn. 1)}$$

where A, B, C and M represents the y lower asymptote with a value of approximately zero, slope parameter, distance from the upper and lower asymptote (with a value of approximately one) and log concentration of the inflexion point, respectively. The NIC and MIC (Eqns. 2 and 3) values are obtained through the intersection of the lines y=A+C and y=A, with the equation of the line tangential to the point (M, (A, A+Ce^{-1})), respectively [11].

$$MIC = 10^{\left(M+\frac{1}{B}\right)}10 \qquad \text{(Eqn. 2)}$$

$$NIC = 10^{\left(M-\frac{1.718}{B}\right)} \qquad \text{(Eqn. 3)}$$

RESULTS AND DISCUSSION

The antimicrobial's minimum inhibitory concentration (MIC) is one of these microbiological parameters. It has been widely accepted for a long time. There have been very few instances of this finding over the years, but it is now showing up more regularly in the results of routine tests. However, the ability to use it for effective and optimal therapy is still limited and sometimes, despite much higher costs incurred than in qualitative methods, it is completely unuse. The calculated MIC and NIC values are shown in **Table 1** whilst the fitted curve to the inhibition data show good fitting (R^2=0.995) (**Fig. 1**). The MIC and NIC values

Table 1. MIC and NIC values of methanolic extract of *A. sativum* against *A. hydrophila*.

	mg/mL	95% Confidence interval
MIC	28.02	26.42 to 29.71
NIC	12.60	11.88 to 13.37

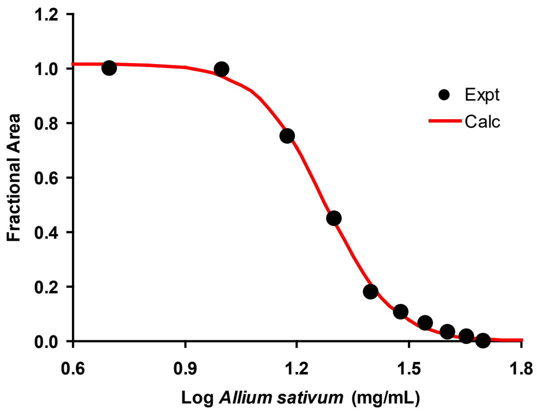

Fig. 1. Fitting of the inhibitory effect of *A. sativum* against *A. hydrophila* using the Lambert-Pearson model.

Allium sativum is a potent antipathogen. In one study, the effect of the fresh extracts of *A sativum* as an anticryptococcal agent in broth showed an MIC values between 125 and 250 µg/mL [14]. *A. hydrophila* is a fish pathogen which can cause fish production to be affected. Numerous studies have been conducted to find potential inhibition agents from plants, animal extracts, synthetic chemicals and also nanomaterials. In one such study, the methanolic gray nail extract (*Nephrolepis biserrata*) shows an MIC value for *A. hydrophila* at 50 mg/mL [15]. Cinnamaldehyde, a plant-derived ingredient shows an MIC value against *A. hydrophila* of 256 µg/mL [16]. Peppers are also known as a traditional bacterial growth inhibitory agent. In one study, five Brazilian Piper species (Piper aduncum, Piper callosum, Piper hispidinervum, Piper hispidum and Piper marginatum) exhibits MIC values ranging from 0.23 to 30 mg/mL [17]. Nanoparticles are also emerging antibiotics for pathogens. In one study, titanium dioxide (TiO2) nanoparticle (NP) shows an MIC value against Aeromonas hydrophila at 20 µg/mL [16] paving the way for the use of nanometals in aquaculture as antibiotics.

Antibiotics like oxytetracycline, sulfadimethoxine, and florfenicol, which are effective yet expensive, have prompted researchers to conduct exploratory searches for new antibiotics. H2O2, a chemical that is both safe for the environment and powerful against this bacterium, is one such option. However, prolonged usage of this chemical can harm aquatic organisms such as algae and zooplankton [18]. Plant-derived bioactive molecules can be used as an alternative to synthetic chemicals.[19,20]. Plant bioactive chemicals have been proven in numerous research to have antibacterial and antifungal activities. Thirty-one Brazilian plant methanolic extracts were found poisonous to F. columnare and A. hydrophila in one such study [21]. *Citrobacter freundii, Vibrio parahaemolyticus, Edwardsiella tarda, Staphylococcus aureus, Vibrio vulnificus,*

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Escherichia coli, and Streptococcus agalactia have been discovered to be inhibited by common plant extracts. [22]. The antibacterial activity of common spices like clove, garlic and dill is demonstrated even by the more popular ones like coriander, onion and cumin. [23] and is likely to be explored for their antibiotic properties against this important fish pathogen in the near future.

CONCLUSION

There is a lack of mathematical models or nonlinear regression used to obtain the MIC and NIC values, which are critical for comparison, efficacy, and validation studies, in many studies on plant extracts and bacterial pathogens. There is a lack of mathematical models or nonlinear regression used to obtain the MIC and NIC values, which are critical for comparison, efficacy, and validation studies, in many studies on plant extracts and bacterial pathogens.In this study, the modified Gompertz model introduced by Lambert and Pearson has been successfully used to determine the MIC and NIC values of the methanolic extract of *Allium sativum* against the pathogen *A. hydrophila.* The model gave a correlation coefficient value of 0.995, indicating acceptable fitting and MIC and NIC values of 28.02 and 12.60 mg/mL, respectively, indicating the potential utility of garlic as inhibitory agent for this important fish pathogen.

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