

## Determination of the IC<sub>50</sub> Value of *Allium sativum* Methanolic Extract Against *Aeromonas hydrophila*

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### ABSTRACT

There are numerous studies done on the efficacy of plant extracts as inhibition agents of bacteria. Several of these studies, however, did not report on the benchmark the concentration of toxicant that causes 50% inhibition or IC<sub>50</sub> values, which would allow for comparison with other studies to be made. One of the most accurate ways to measure this value is through analysis of the data using nonlinear regression analysis, where the four parameter logistics equation is routinely employed by many researchers. The aim of this study is to use the four-parameter logistics equation to determine the IC<sub>50</sub> value and its confidence interval for the plant extract *Allium sativum* against the pathogen *Aeromonas hydrophila*. The result of the fitting gave an IC<sub>50</sub> value of 18.78 mg (95% confidence interval of 17.53 to 20.13) with a correlation coefficient value of 0.95, indicating acceptable fitting.

### INTRODUCTION

*Aeromonas hydrophila* is a bacilli-like bacterium, which is a Gram-negative rod-shaped from the family Aeromonadaceae. It features a solitary polar flagellum and it is extremely motile and it's also present in various environment such as soil, in sewage, and also brackish water. The bacterial virulence factors consist of its capability to produce a variety of tandem-like attack on the bacterial system, which include adhesions, the production of cytotoxins, enzymes like lipases, and the formation of an impenetrable biofilm.

It is known to become connected with motile aeromonad septicaemia (MAS) in numerous freshwater fishes and is also considered to be distributed by way of unintentional scrape [1]. Instances of this infection especially on fish species are documented in lots of nations around the world from the United States of America to south East Asia. fish species affected by the bacteria include cultured food fish like Tilapia (*Tilapia nilotica*), channel cat fish, hybrid striped bass, Goldfish (*Carassius auratus*), Snakehead fish (*Ophiocephalus striatus*), American eel (*Anguilla rostrata*), Carp (*Cyprinus carpio*), Rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*) to name a few [2]. The use of antibiotics like Oxytetracycline, Sulfadimethoxine and Florfenicol are effective but expensive. One of the most environmentally friendly chemicals is H<sub>2</sub>O<sub>2</sub> and is considered to be an effective agent for

this bacterium. Nevertheless, the extended use of this chemical can be damaging to the algae and zooplankton in the aquatic system [3]. Alternatives to the use of manmade chemicals are bioactive compounds from plants [4,5]. Numerous studies have shown that plant bioactive compounds exhibit antibacterial and antifungal properties. One of such studies demonstrated that thirty one methanolic extracts of Brazilian plant exhibited toxicity to *F. columnare* and *A. hydrophila* [6]. Common plant extract such as has been found to inhibit the growth of *Citrobacter freundii*, *Edwardsiella tarda*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio vulnificus*, and *Streptococcus agalactia* [7]. Even common spices like clove, coriander, onion, cumin and dill weed exhibit antibacterial activity [8].

In many cases, the determination of the efficacy or for comparative purposes of the plant or other organisms' extract is by measuring the concentration of toxicant that causes 50% inhibition (IC<sub>50</sub> or EC<sub>50</sub>) is done manually where the value is determined by half of the maximum inhibition [9–12]. Obviously, this method is not accurate, does not take into account of all of the data, and is unable to produce the value with uncertainty indicator, such as confidence interval. The most accurate way is through analysis of the data using nonlinear regression analysis. One of the most often used models is the four parameter logistics, and has been employed by many researchers to find the IC<sub>50</sub> values for various plant extracts against target

activity[13–22]. In a recent study, various common plant extracts have been tested against *A. hydrophila* including *Allium sativum*. This plant extract shows good antibacterial activity against the bacterium. However, the determination of the IC<sub>50</sub> was not determine [23].

Thus, the aim of this study is to use the four-parameter logistics equation to determine the IC<sub>50</sub> value and its confidence interval for this plant extract.

## 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 [24]. The software digitizes scanned figure into table of data with comma separated values [23]. The data acquired shows the effect of different concentrations of *Allium sativum* on the zone of inhibition of *A. hydrophila*.

### Fitting of the data

The inhibition curve was then fitted with a four-parameter logistics model available from PRISM non-linear regression analysis software from [www.graphpad.com](http://www.graphpad.com), where  $y$  is the zone of inhibition obtained (mm),  $bottom$  and  $top$  are the minimum and maximum inhibition (mm) of the calibration curve, IC<sub>50</sub> is the concentration of plant extract that produces a 50% inhibition and  $x$  is plant extract concentration (mg) in log value (Equation 1).

$$y = \frac{Bottom + (Top - Bottom)}{1 + 10^{(LogIC_{50} - x) \cdot Hill\ slope}} \quad (\text{Equation 1})$$

## RESULTS AND DISCUSSION

In the study by Ramena *et al* (2018), *Allium sativum* methanolic extract has shown a potential as an inhibitory agent against *A. hydrophila*. The determination of the IC<sub>50</sub> value via the four-parameter logistics equation requires the concentration of the extract to be log-transformed (Fig. 1). The result of the fitting shows visually good fitting (Fig. 2) and gave an IC<sub>50</sub> value of 18.78 (95% confidence interval of 17.53 to 20.13) with a correlation coefficient value of 0.95, indicating acceptable fitting [25].

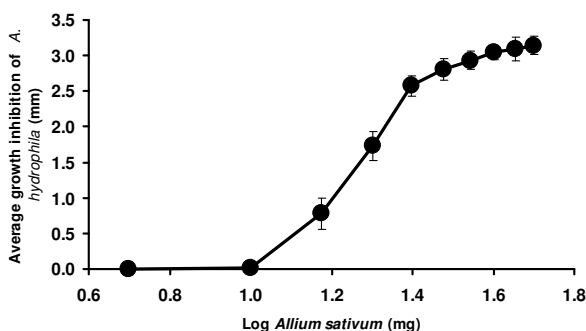


Fig. 1. The minimum inhibitory concentration of methanolic extract of *Allium sativum* plotted after log<sub>10</sub> transformation against the bacterium *Aeromonas hydrophila*. Error bars represent mean ± standard error (n=5).

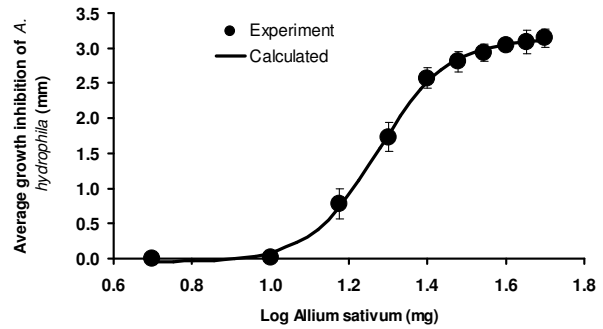


Fig. 2. Fitting of the curve of minimum inhibitory concentration of methanolic extract of *Allium sativum* against the bacterium *Aeromonas hydrophila* using the four-parameter logistic model. Error bars represent mean ± standard error (n=5).

The results of the fitting exercise shows the applicability of mathematical model in giving a numerical value to the IC<sub>50</sub> term with a statistical boundary as shown in the confidence interval. The results obtained can then be used for further comparative purposes to evaluate which essential oils is the most efficient as an inhibitory agent for the targeted pathogen. The results can also be used by other researchers for validation purposes. The final four parameter logistics equation in numerical form (Equation 2) is as follows;

$$y = \frac{3.177}{1 + 10^{(1.274 - x) \cdot 4.943}} \quad (\text{Equation 2})$$

## CONCLUSION

Many research papers on the inhibitory effect of plant extracts against bacterial pathogens do not utilize mathematical model or nonlinear regression to determine the inhibitory concentration causing 50% inhibition or IC<sub>50</sub> an important value for efficacy and validation works. In this study, the four parameter logistics model has been successfully used to model the inhibitory effect of the methanolic extract of *Allium sativum* against the pathogen *A. hydrophila*. The model gave a correlation coefficient value of 0.95, indicating acceptable fitting and the usefulness of the four-parameter logistics equation in finding the IC<sub>50</sub> value.

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