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Bacterial Inhibition Activity of Methanolic Extract from *Salvia officinalis*: Determination of the IC_{50} value by Nonlinear Regression

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ABSTRACT

Quite a few scientific studies carried out on the effectiveness of solvent-extracts from plant as inhibitory agents for microorganisms. A number of these studies, nevertheless, failed to report on the standard IC_{50} values, that would permit assessment along with other studies. Probably the most correct methods to calculate this value is via analysis of the data using nonlinear regression analysis, in which the four-parameter logistics (4PL) equation is regularly utilized by numerous scientists. In a previous study carried out from solvent extracts from *Salvia officinalis*, the extract was able to inhibit the bacterium *Aeromonas hydrophila,* a well-known fish pathogen. However, the study did not report the IC50 value for the extract. Thus, the aim of this research is to apply the four-parameter logistics equation to determine the IC_{50} value and its confidence interval for the plant extract. The model predicts an IC_{50} value of 21.92 (mg/ml) with a 95% confidence interval from 20.86 to 23.03, with a correlation coefficient value of 0.96. The result from this study allow future comparative studies to be made with other plant extracts or with other antibacterial activity using this plant extract.

INTRODUCTION

One of the most damaging fish pathogens is *Aeromonas hydrophila*, a well cause of motile aeromonad septicaemia (MAS) in several river fishes and is particularly regarded as distributed by means of unintended scratches [1]. Cases of this disease particularly on fish species are recorded in some large amount international locations from the United states to south East Asia. Species of fish impacted by the bacteria consist of cultured food fish like channel cat fish, hybrid striped bass, Tilapia (*Tilapia nilotica*), Goldfish (*Carassius auratus*), Snakehead fish (*Ophiocephalus striatus*), Carp (*Cyprinus carpio*), American eel (*Anguilla rostrata*), Rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*) to name a few [2].

Aeromonas hydrophila is a Gram-negative rod-shaped bacilli-like bacterium from the family Aeromonadaceae. It possesses a great individual polar flagellum which is incredibly motile, and it's also contained in different surroundings for example soil, in sewage, as well as brackish water. The bacterial virulence elements include its power to create a number of tandem-like assault on the bacterial system, including adhesions, the creation of cytotoxins, enzymes like lipases, and the development of a dense biofilm.

Some studies report on the potential antibacterial activity of plant extracts without reporting the concentration of toxicant that causes 50% inhibition (IC_{50} or EC_{50}) [2–5]. The most often used models to determine the IC_{50} is the four parameter logistics, which has been used by many researchers [6–11]. In one new study, various common plant extracts have been tested against *A. hydrophila* including *Salvia officinalis*, with extract from this plant showing good antibacterial activity against the bacterium. However, the IC_{50} value was not reported [2]. Thus, the purpose of this research is to apply the four-parameter logistics equation to figure out the IC50 value as well as the confidence interval.

MATERIALS AND METHODS

Acquisition of Data

Data from the works of Ramena et al. [2], from figure 3 showing the effect of different concentrations of *Salvia officinalis* on the zone of inhibition of *A. hydrophila*. The data was processed using the software Webplotdigitizer 2.5 [12] which digitizes the scanned figure into comma separated data [13].

Data Fitting

The four-parameter logistics model is available from the PRISM non-linear regression analysis software from www.graphpad.com, as follows (**Eqn. 1**);

$$y = \frac{Bottom + (Top - Bottom)}{1 + 10^{(LogIC_{50} - x) * Hill\ slope}} \qquad \textbf{(Eqn. 1)}$$

where IC_{50} is the concentration of plant extract that produces a 50% inhibition, y is the zone of inhibition obtained (mm), *bottom* and *top* are the minimum and maximum inhibition (mm) of the calibration curve and x is plant extract concentration (mg/ml) in log value

RESULTS AND DISCUSSION

Plant solvent extracts have been known to exhibit antibacterial and antifungal characteristics. Examples include 31 methanolic extracts from Brazilian plant that shows antibacterial property to the bacteria *F. columnare* and *A. hydrophila* [14]. Other plant extract have been demonstrated to inhibit a diverse microorganism such as *Vibrio parahaemolyticus, Citrobacter freundii, Edwardsiella tarda, Escherichia coli*, *Staphylococcus aureus*, *Vibrio vulnificus*, and *Streptococcus agalactia* [15]. The screening of inhibitory properties of more plant extract against pathogenic bacteria would someday yield potent new antibiotics including *Salvia officinalis*.

Salvia is the biggest genus from the Lamiaceae family, consists of approximately nine hundred species, spread all through the globe, most of which are financially essential given that they possess utility as herbs and flavour components in perfumery and makeup products [16–19]. 1,8-cineole (eucalyptol), and borneol have been recognized are the main compounds from this plant [19]. 1,8-cineole, thujone and camphor have been reported as the main compounds responsible for the antimicrobial activity of *Salvia officinalis* [20]. In the study by Ramena *et al* (2018), *Salvia officinalis* methanolic extract has shown a potential as an inhibitory agent against *A. hydrophila*.

The determination of the IC_{50} 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_{50} value of 21.92 (95% confidence interval of 20.86 to 23.03) with a correlation coefficient value of 0.96, indicating acceptable fitting [21].

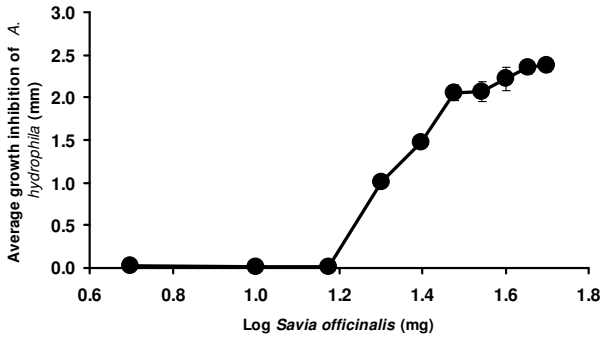

Fig. 1. The minimum inhibitory concentration of methanolic extract of *Salvia officinalis* plotted after log_{10} transformation against the bacterium *Aeromonas hydrophila*. Error bars represent mean ± standard error (five replicates).

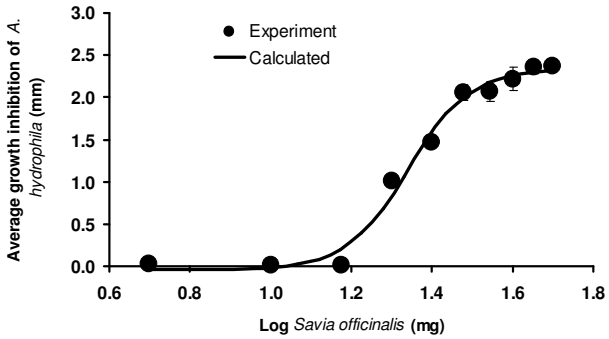

Fig. 2. Fitting of the curve of minimum inhibitory concentration of methanolic extract of *Salvia officinalis* against the bacterium *Aeromonas hydrophila* using the four-parameter logistic model. Error bars represent mean ± standard error (five replicates).

The results of the fitting exercise yield an IC_{50} term with the 95% 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 (**Eqn. 2**) is as follows;

$$y = \frac{2.381}{1 + 10^{(1.341 - x) * 5.677}} \qquad \textbf{(Eqn. 2)}$$

One of the reason scientists kept on searching for new antibiotics from plants is that the use of antibiotics like oxytetracycline, sulfadimethoxine and florfenicol for the control of *A. hydrophila* are effective but expensive. Chemical effective against this bacterium including H_2O_2 can damage nontarget organisms including algae and zooplankton [22]. The isolation of bioactive compounds from plants including this plant can alleviate major problems attributed to the above issues [23,24].

CONCLUSION

The IC_{50} determination from antibacterial assay is one of the most important data aside from Minimum Inhibitory Concentration (MIC), but many research papers do not report this value which prevent a comparative process for efficacy and validation works to be carried out. In this study, the 4PL model has been successfully used to model the inhibitory effect of the methanolic extract of *Salvia officinalis* against the pathogen *A. hydrophila.* A correlation coefficient value of 0.96 for the model indicates acceptable fitting and the usefulness of the four-parameter logistics equation in finding the IC_{50} value.

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