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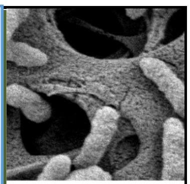

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SEM of *Aeromonas hydrophila*

Minimum Inhibitory Concentration (MIC) and the Non-Inhibitory Concentration (NIC) Values of *Salvia officinalis* Methanolic Extract Against *Aeromonas hydrophila*

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ABSTRACT

Extractions from plants have been the subject of considerable research because of their potential to inhibit the growth of bacteria. It was not possible to compare the outcomes of this study to those of previous investigations because these discoveries were not reported on in the benchmark values. The use of data-driven nonlinear regression analysis as one of the approaches to finding this value is among the approaches with the highest degree of precision. Using Lambert and Pearson's modified Gompertz model, it was possible to successfully determine the minimum inhibitory concentration (MIC) and the non-inhibitory concentration (NIC) of the methanolic extract of *Salvia officinalis* (sage) against the pathogen *Aeromonas hydrophila*. Sage's MIC and NIC values, which come in at 31.92 (95% Confidence Interval from 29.85 to 34.28) and 15.56 mg/mL (95% C.I. from14.39 to 16.71), respectively, suggest that it has the potential to be utilized as an inhibitory drug against this critical fish pathogen. This is demonstrated by the model's strong correlation coefficient, which was 0.995.

INTRODUCTION

Most essential oils are composed of terpenes, which include various hydrocarbons, alcohols, aldehydes, ketone, esters and ethers as well as sulfur compounds. On a commercial scale, more than 300 essential oils (EO) are manufactured and have numerous therapeutic applications [1–6]. *Salvia officinalis* (sage) essential oil has considerable antibacterial activity due to active ketones and alcohols in sage EO (SEO), which include camphor (2-46 percent), thujene (2.5-30 percent), and 1-8-cyneol (2.5-30 percent) (2-18 percent). It is one of the most often utilized herbs in traditional medicine. Sage" is a common name for this plant. Sage has been shown to have a variety of medicinal properties, including antibacterial, antiviral, antifungal, and antioxidant properties. SEO's antibacterial qualities have been studied and found to be effective against a wide range of microbes. such as *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Shigella flexneri, Yersinia enterocolitica, Listeria monocytogenes, Staphylococcus epidermidis, Staphylococcus aureus, Salmonella spp., Enterococcus faecalis, Aspergillus niger* and *Bacillus subtilis* [7–9].

'Bacilli-like' Gram-negative rod-shaped *Aeromonas hydrophila* is from the Aeromonadaceae family. It can be found in dirt, sewage, and brackish water in addition to being extremely mobile. A solitary polar flagellum is present. Some of the virulence factors of bacteria include adhesion, cytotoxins, lipases, and biofilm growth, all of which are capable of simultaneously attacking the bacterial system. MAS has been linked to a number of freshwater fish species, and it is also believed to be transferred by unintentional scrapes [10]. Aeromonas hydrophila can cause a wide range of illnesses in humans, including meningitis, septicemia, and endocarditis, in immunocompromised individuals. 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*),

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Carp (*Cyprinus carpio*), Chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (Oncorhynchus mykiss), to name a few [11].

Herbal medications have recently been revived, and eco-friendly phytoproducts are being studied as efficient antifungal agents for human treatment. Since the antibacterial activities of this plant extract are well-tolerated by the bacterium [11]. The determination of the IC_{50} of sage which was not determined in the publication but was later determined [12]. Antibiotic efficacy is measured by IC_{50} values, which are one of several significant metrics. The minimum non-inhibitory concentration (NIC) and the minimum inhibitory concentration (MIC) are two other significant factors. In vitro, growth is inhibited by the lowest possible concentration of antibiotic (usually measured in g/mL). MIC The lower the minimum inhibitory concentration of an antibiotic, the more effective it is at suppressing bacterial growth (MIC). Antibiotics "completely" suppress bacterial growth at the lowest concentration when used in the smallest amount possible. There are NIC antibiotics that inhibit bacterial growth. At concentrations below the NIC, growth is equal to the control. Defining terms like "completely retarding bacterial growth" and "slowing [bacteriological] growth" appears to be a haphazard process [13].

To determine the MIC, a semi-quantitative technique is used to estimate the lowest antibiotic concentration required to suppress bacterial growth. Microorganisms were put into growth broth containing a modest dose of preservative to test the preservative's effectiveness. As a final step, the lowest concentration of antimicrobial that produced a clear solution, implying no visible growth was identified in this test [14,15]. In place of the traditional tubes that were once utilized, microtiter plates are currently being utilized. When the turbidity of a test substance causes problems for a test, one option available is to use end-point indicators. These include resazurin [13] and fluorescein diacetate [16]. Even though there was no growth seen in one of the wells, it was still considered to be the MIC [17]. The absence of a quantified standard approach has been a roadblock for a great number of antibiotic research projects [18,19]. However, the primary issue that arises is the fact that all of the MIC procedures that are now being utilized are just semi-quantitative. Lambert and Pearson employed a method known as nonlinear regression in order to figure out the NIC and MIC. The slope and the point of inflection are used in the modified Gompertz model that was just presented so that the MIC and NIC may be calculated [20]. Using nonlinear regression is useful since the 95% confidence interval of the MIC and NIC can now be estimated.

METHODS

Acquisition of Data

Data from the works of Ramena et al. [11], from figure 3 graphs was scanned and electronically processed using Webplotdigitizer 2.5 [21]. Using the software, data from scanned images is converted into a table with comma-separated values [22].

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

In order to perform statistical analysis with a modified version of the Gompertz equation, you will need to have log concentration data as well as a y response that has been converted into a fractional unit, such as fractional area or another fraction of unity (**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 [20].

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

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

RESULTS AND DISCUSSION

One of these microbiological criteria is referred to as the minimum inhibitory concentration (MIC) of the antimicrobial agent. Since quite some time ago, it has garnered widespread approval. Over the course of the years, there have only been a handful of occasions in which this finding has been made, but recently, it has started turning up more frequently in the outcomes of routine examinations. However, the capacity to use it for effective and optimal therapy is still restricted, and there are occasions when it is utterly useless despite the fact that the expenses involved are far more than those associated with qualitative approaches. **Table 1** displays the estimated MIC and NIC values, and the fitted curve to the inhibition data demonstrates that it provides a satisfactory fit to the data (R^2=0.995) (Fig. 1).

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

	mg/mL	95% Confidence interval
MIC	31.92	29.85 to 34.28
NIC	15.56	14.39 to 16.71

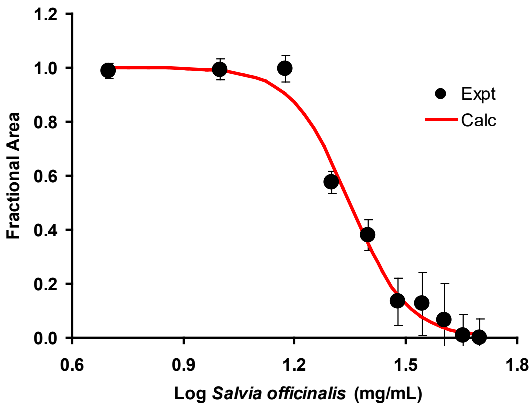

Fig. 1. Fitting of the inhibitory effect of *Salvia officinalis* against *A. hydrophila* using the Lambert-Pearson model.

Salvia officinalis is a potent general purpose antibacterial as demonstrated in one study where the ethanol extract shows MIC values of between 25 -50 mg/mL on several Gram positive- and negative bacteria [23]. *A. hydrophila* is a fish and human 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 [24]. Cinnamaldehyde, a plant-derived ingredient shows an MIC value against *A. hydrophila* of 256 µg/mL [25]. Peppers have been used for centuries as a traditional agent that inhibits the growth of bacteria. One study found that the MIC values of five different Brazilian Piper species (*Piper callosum, Piper aduncum, Piper hispidum, Piper hispidinervum, and Piper marginatum*) ranged from 0.23 to 30 mg/mL [26]. Nanoparticles are also a new class of antibiotics that are effective against infections. In one study, titanium dioxide (TiO2) nanoparticle (NP) shows an MIC value against Aeromonas hydrophila at 20 µg/mL [25] paving the way for the use of nanometals in aquaculture as antibiotics.

Because medicines such as oxytetracycline, sulfadimethoxine, and florfenicol are efficient but costly, researchers have been motivated to perform exploratory searches for novel antibiotics. One such alternative is the use of hydrogen peroxide (H_2O_2), which is a chemical that is not harmful to the environment but is effective against this bacterium. However, extended exposure to this chemical can be harmful to creatures found in aquatic environments, such as algae and zooplankton [27]. Plant-derived bioactive molecules can be used as an alternative to synthetic chemicals.[28,29]. Numerous studies have conclusively demonstrated that the bioactive compounds found in plants possess antibacterial and antifungal properties. In one of these studies, there were thirty-one Brazilian plant methanolic extracts that were determined to be harmful to *F. columnare* and *A. hydrophila* [30]. *Citrobacter freundii, Vibrio parahaemolyticus, Edwardsiella tarda, Staphylococcus aureus, Vibrio vulnificus, Escherichia coli, and Streptococcus agalactia* have been discovered to be inhibited by common plant extracts [31]. Even more popular spices like coriander, onion, and cumin show evidence of their antibacterial effect, in addition to more common ones like clove, garlic, and dill [32] and in the not-too-distant future, is likely going to be investigated for the antibiotic qualities they possess against this significant fish infection.

CONCLUSION

In many of the investigations that have been conducted on plant extracts and bacterial pathogens, the mathematical models or nonlinear regression that would be necessary to acquire the MIC and NIC values have not been implemented. These values are essential for comparison, efficacy, and validation studies. In many of the investigations that have been conducted on plant extracts and bacterial pathogens, the mathematical models or nonlinear regression that would be necessary to acquire the MIC and NIC values have not been implemented. These values are essential for comparison, efficacy, and validation studies. In the present investigation, the modified Gompertz model that was developed by Lambert and Pearson proved to be effective in determining the minimum inhibitory concentration (MIC) and the maximum inhibitory concentration (NIC) values of the methanolic extract of *Salvia officinalis* in relation to the pathogen *A. hydrophila*. The model produced a correlation coefficient value of 0.982, which indicated that the fitting was satisfactory. It also produced MIC and NIC values of 31.92 and 15.56 mg/mL, respectively, which indicated the potential efficacy of sage as an inhibitory agent for this significant fish pathogen.

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