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

Ahmad Syazwan Ismail¹, Mohd Yunus Shukor² and Noor Azlina Masdor¹*

¹Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
²Department 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

INTRODUCTION

Most essential oils are composed of terpenes, which include various hydrocarbons, alcohols, aldehydes, ketones, esters and others 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 (*Ophiocephalus striatus*), Goldfish (*Carassius auratus*), American eel (*Anguilla Rostrata*),

ABSTRACT

Extracts 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.

KEYWORDS

*Aeromonas hydrophila*, *Salvia officinalis*, MIC, NIC, Lambert-Pearson model

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).
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 IC50 of sage which was not determined in Eqn. 1 (fractional unit, such as fractional area or another fraction of unity data as well as a y response that has been converted into a Gompertz equation, you will need to have log concentration in order to perform statistical analysis with a modified version of Gompertz function 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 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 find 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).

\[ y = A + Ce^{-(x-M)} \]  
(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 (\( M, (A, A + Ce^{-M}) \)), respectively [20].

\[ \text{MIC} = 10^{(M + \frac{1}{2})} \]  
(Eqn. 2)

\[ \text{NIC} = 10^{(M + \frac{1}{2})} \]  
(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).

| 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.](https://example.com/f1.png)

*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

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).
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 biserata) shows an MIC value for A. hydrophila at 50 μg/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 nanomets 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 (H2O2), 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 paraahaemolyticus, Edwardsiella tarda, Staphylococcus aureus, Peptostreptococcus sp., and Streptococcus agalactiai 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 the 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.

ACKNOWLEDGEMENT

This project was financed by RMK-12 Development Project Fund (PRB-502), MARDI, Selangor.

REFERENCES

1. Rana IS, Rana AS, Rajak RC. Evaluation of antifungal activity in essential oil of the Syzygium aromaticum (L.) by extraction, purification and analysis of its main component eugenol. Braz J Microbiol. 2011 Dec;42(6):1269–77.
2. Reyes-Jurado F, Munguía-Pérez R, Cid-Pérez TS, Hernández-Carranza P, Ochoa-Velasco CE, Avila-Sosa R. Inhibitory Effect of Mexican Oregano (Lippia berlandieri Schauer) Essential Oil on Pseudomonas aeruginosa and Salmonella Thphymurium Biofilm Formation. Front Sustain Food Syst. 2020;4:36.
3. Das S, Singh VK, Dwiivedy AK, Chaudhari AK, Dubey NK. Myristica fragrans essential oil nanoemulsion as novel green preservative agent against fungal and aflatoxin contamination of food commodities with emphasis on bioactive mode of action and molecular docking of major components. LWT. 2020 Aug 1;130:109495.
4. Perumal AB, Li X, Su Z, He Y. Preparation and characterization of a novel green tea essential oil nanoemulsion and its antifungal mechanism of action against Magnaporthe oryzae. Ultrasanum 1981;13:105-109.
5. Nose NP e, Dalcin MS, Dias BL, Toloy RS, Mourao DSC, Giongo M, et al. Noni essential oil associated with adjuvants in the production of phytoalexins and in the control of soybean anthracnose. J Med Plants Res. 2022 Jan 31;16(1):1–10.
6. Canto–Tejero M, Paseua–Villalobos MJ, Guirao P. Anised essential oil botanical insecticides for the management of the currant–lettuce aphid. Ind Crops Prod. 2022 Jul 1;181:114804.
7. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J Agric Food Chem. 2007 Sep 15;55(19):7879–85.
8. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesarino A. From traditional European medicine to discovery of new drug candidates for the treatment of dementia and Alzheimer’s disease: acetycholinesterase inhibitors. Curr Med Chem. 2013;20(8):976–83.
9. Beheshti-Royou M, Azarsina M, Rezaei-Soufi L, Alikhani MY, Roshanaie G, Komaki S. The antibacterial effect of sage extract (Salvia officinalis) mouthwash against Streptococcus mutans in dental plaque: a randomized clinical trial. Iran J Microbiol. 2015 Jun;7(3):173–7.
10. Hoque F. Biocontrol of β-haemolytic Aeromonas hydrophila infection in Labeo Rohita using antagonistic bacterium Pseudomonas aeruginosa FARP72. Int J Pharm Sci Res. 2014;5(2):490–501.
11. Ramana G, Ramana Y, Challa N. Identification and determination of minimum inhibitory concentrations of plant extracts having antimicrobial activity as potential alternative therapeutics to treat Aeromonas hydrophila infections. J Microb Pathog. 2018 Jan 27;2(1):1–9.
12. Rusnami. Bacterial inhibition activity of methanolic extract from salvia officinalis: determination of the IC_{50} value by nonlinear regression. Bioremediacation Sci Technol Res. 2018 Jul 31;6(1):23–5.
13. Mann CM, Markham JL. A new method for determining the minimum inhibitory concentration of essential oils. J Appl Microbiol. 1998;84(4):538–44.
14. Collins CH. Antibiotics and antibacterial substances. Microbiol Methods. 1964;296–305.
15. Davidson PM, Parish ME. Methods for testing the efficacy of food antimicrobials. Food Technol. 1989;43(1):148–55.
16. Chand S, Lusunzi I, Williams LR, Karuso P, Veal DA. Rapid screening of the antimicrobial activity of extracts and natural products. J Antimicrob. Tokyo). 1994;47(11):1295–304.
17. Sommers HM. Drug susceptibility testing in vitro: Monitoring of antimicrobial therapy. J Clin Blood Infec Dis. 1980;782–804.
18. Janssen AM, Scheffer JJC, Baerheim Svendsen A. Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of Microbiol. 2011 Dec;42:1269–77.
19. Manou I, Bouillard L, Devleeschouwer MJ, Barel AO. Evaluation of the preservative properties of Thymus vulgaris essential oil in aquatic environments, such as algae and zooplankton [27].
topically applied formulations under a challenge test. J Appl Microbiol. 1998;84(3):368–76.

20. Lambert RJ, Pearson J. Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. J Appl Microbiol. 2000 May;88(5):784–90.

21. Rohatgi A. WebPlotDigitizer.
http://arohatgi.info/WebPlotDigitizer/app/ Accessed June 2 2014.; 2015.

22. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Mathematical modelling of the degradation kinetics of Bacillus cereus grown on phenol. J Environ Bioremediation Toxicol. 2014;2(1):1–5.

23. Ali M. Antimicrobial Activities of Aqueous and Methanolic Extracts from Salvia officinalis and Salix acmophylla Used in the treatment of wound infection isolates. Ibn Al-Haytham J Pure Appl Sci. 2017;23(3):25–39.

24. Maulianawati D, Suharni S. Antibacterial activity of Nephrolepis biserrata extract against Aeromonas hydrophila and Vibrio parahaemolyticus. In 2022.

25. Yin L, Chen J, Wang K, Geng Y, Lai W, Huang X, et al. Study the antibacterial mechanism of cinnamaldehyde against drug-resistant Aeromonas hydrophila in vitro. Microb Pathog. 2020;145.

26. Majolo C, Monteiro PC, Nascimento AVPD, Chaves FCM, Gama PE, Bizzo HR, et al. Essential Oils from Five Brazilian Piper Species as Antimicrobials Against Strains of Aeromonas hydrophila. J Essent Oil-Bear Plants. 2019;22(3):746–61.

27. Pridgeon JW, Klesius PH, Mu X, Song L. An in vitro screening method to evaluate chemicals as potential chemotherapeutants to control Aeromonas hydrophila infection in channel catfish. J Appl Microbiol. 2011;111(1):114–24.

28. Mahyuni S, Radzali M, Marziah M, Johari R, Mohd Aspollah S. Studies on the production of flavonoids (quercetin and hesperitin) from callus culture of Citrus aurantifolia (Christm and Panzer) swingle. In: Proceedings of the Seminar Medicinal Plants: Quality Herbal Products for Healthy Living. Forest Research Institute Malaysia (FRIM); 2000.

29. Ong SL, Kiong ALP, Poospooragi R, Hussein S. Production of Flavonoid compounds in cell cultures of Ficus deltoidea as influenced by medium composition. Int J Med Aromat Plants. 2011;1(2):62–74.

30. Castro SBR, Leal CAG, Freire FR, Carvalho DA, Oliveira DF, Figueiredo HCP. Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. Braz J Microbiol. 2008;39(4):756–60.

31. Lee S, Najiah M, Wee W. In vitro antimicrobial activities of Colocasia esculenta extract against Vibrio spp. short communication. Agric Slov. 2010;7(1):5–7.

32. Mahmoud AM, El-Baky RMA, Ahmed ABF, Gad GFM. Antibacterial activity of essential oils and in combination with some standard antimicrobials against different pathogens isolated from some clinical specimens. Am J Microbiol Res. 2016;4(1):16–25.