AJPB 2022, Vol 4, No 1, 11-14
https://doi.org/10.54987/ajpb.v4i1.697

HIBISCUS PUBLISHER

ASIAN JOURNAL OF PLANT BIOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/AJPB/index

AJPB VOL 4 NO 1 2022
3D model of OsTHIC

Gelam Honey Inhibition of Bacterial Pathogen: Determination of MIC and NIC Values Using the Lambert-Pearson Model

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

HISTORY

Received: 15th March 2022
Received in revised form: 24th June 2022
Accepted: 4th July 2022

KEYWORDS

Gelam honey
Antibacterial
MIC
NIC
Lambert-Pearson model

ABSTRACT

The curative effect of honey is due to the fact that it possesses antibacterial activity, it keeps wounds moist by retaining their natural moisture, and the honey's high viscosity contributes to the formation of a protective barrier that helps to keep infections at bay. All of these properties work together to make honey an effective wound healer. In addition, the immunomodulatory action that it possesses plays a part in the process of wounds healing. Honey's antibacterial efficacy against bacterial pathogens is frequently indicated by the lowest minimum inhibitory concentration (MIC) and the non-inhibitory concentration (NIC) values. The reality that all of the MIC and NIC techniques that are now being used are just semi-quantitative in nature is the primary issue that develops as a consequence of this circumstance. Use of data-driven nonlinear regression analysis as one of the ways to determine this value is one of the methods that has the highest degree of precision out of all of the methods that may be used. Using the Lambert-Pearson modified Gompertz model, it was feasible to successfully determine the MIC and NIC of the gelam honey against different bacterial pathogen remodels. According to the Shukor's chart for MIC values, *S. aureus* is the pathogen most sensitive to gelam honey followed by *E. coli* and *P. aeruginosa* while the MIC value for *B. cereus* overlaps with *P. aeruginosa* indicating that more research is required to determine where *B. cereus* falls in terms of sensitivity. There is a good match between the data and the Lambert-Pearson model, with values for the coefficient of determination (R^2) ranging from 0.94 to 0.97. Based on these findings, it appears that honey from gelam trees has the potential to inhibit the formation of bacterial infections.

INTRODUCTION

It is essential to stress, however, that antimicrobial resistance is also evident in other types of microorganisms, namely parasites, fungi, and viruses. This is one of the most significant aspects of the topic. In hospitals, the vast majority of antibiotics were being provided at the time, therefore it makes sense that these establishments were the first to report the emergence of drug-resistant bacteria. In military hospitals in the 1930s, researchers discovered the first penicillin-resistant strains of Streptococcus pyogenes. Not long after penicillin was initially administered in the 1940s5, the presence of Staphylococcus aureus became an issue in the civilian hospitals of London. In a similar manner, streptomycin-resistant forms of Mycobacterium tuberculosis emerged in the population not long after this drug was initially identified. These strains of M. tuberculosis were able to withstand treatment with streptomycin. Antimicrobial resistance has been around for a considerable amount of time; despite this fact, the number of resistant species, the geographical locations affected by antibiotic resistance, and the breadth of resistance in single organisms are all at unprecedented levels and are continuing to increase. Antibiotic resistance is increasing, which implies that illnesses and disease agents that were formerly thought to be under control by antibiotics are making a comeback in new forms. This is because medicines are becoming less effective with time. Within the context of this review, the major attention is given on the fundamental ideas as well as the ecological elements that are connected with drug resistance in

bacteria. Antibiotics differentiate out from other sorts of medical treatments owing to the influence they have on distinct ecosystems. People refer to these substances as "societal drugs" since their usage has an effect on individuals who share their environment. Antibiotic therapy for acne, for example, was discovered to generate a multiple drug resistance (MDR) skin flora not only in the individual with acne but also in other members of the household who shared the same environment as the individual with acne. This was discovered after conducting study on the subject.

Despite the fact that none of the participants investigated had ever received an antibiotic, it was shown that the gut flora of ambulatory people in certain regions of the world included significant percentages of MDR bacteria. It was discovered that the total use of antibiotics in a community has a larger association with individual resistance rates than the person's own use of antibiotics. This was an unexpected discovery. This was discovered by scholars all throughout the world. Antimicrobials may still be found in their natural habitats, and for the most part, these antimicrobials have not been changed, which helps to guarantee that the selection process that leads to resistance continues.

The presence of antimicrobials in waste streams is becoming more widespread, and these antimicrobials have the potential to play an essential role in the environmental selection of antibiotic-resistant microbes. According to the study's results, one method to addressing the problem of antibiotic resistance might be the development of medications that, when administered, force the organisms to whom they are administered to commit suicide. As a result, one of the factors that lead to the spread of resistance would be abolished. Honey's historical use to cure wounds is owing to its antibacterial characteristics, and with the advent of honey production from diverse nations having varied antibacterial capabilities, new research has been done to analyze individual honey against significant human infections. The MIC and NIC are significant metrics for comparing the effectiveness of different honey origins.

IC_{50} levels are one of the important measures used to assess antibiotic effectiveness. Other important criteria include the lowest non-inhibitory concentration (NIC) and the minimum inhibitory concentration (MIC). In vitro, the lowest feasible concentration of antibiotic (typically measured in g/mL) inhibits growth. MIC The lower an antibiotic's minimum inhibitory concentration, the more efficient it is at suppressing bacterial growth (MIC). When administered in the smallest amount feasible, antibiotics "totally" suppress bacterial growth at the lowest concentration. Bacterial growth is inhibited by NIC antibiotics. Growth is equivalent to the control at concentrations lower than the NIC. It appears that defining phrases like "totally retarding bacterial growth" and "slowing bacteriological growth" is a nonexact procedure [1].

A semi-quantitative approach is used to estimate the lowest antibiotic concentration necessary to inhibit bacterial growth in order to calculate the MIC. To evaluate the efficiency of the preservative, microorganisms were placed in growth broth containing a little amount of it. In this test, the lowest antimicrobial concentration that created a clear solution, signifying no observable growth, was determined as a final step [2,3]. Microtiter plates are now used instead of the standard tubes that were previously used. When the turbidity of a test material interferes with a test, one approach is to employ end-point indicators. Microtiter plates are now used instead of the standard tubes that were previously used. When the turbidity of a test material interferes with a test, one approach is to employ end-point indicators. These include resazurin [1] and fluorescein diacetate [4]. Even though there was no growth seen in one of the wells, it was still considered to be the MIC [5]. The lack of a quantifiable standard technique has stymied a large number of antibiotic research studies [6,7]. The fundamental difficulty that arises, however, is that all of the MIC techniques that are presently being used are only semi-quantitative. Lambert and Pearson used a technique called as nonlinear regression to calculate the NIC and MIC. The slope and point of inflection are utilized in the modified Gompertz model that was just given to compute the MIC and NIC [8]. Nonlinear regression is beneficial because the MIC and NIC 95 percent confidence intervals may now be determined.

MATERIALS AND METHODS

Acquisition of Data

Data from the works of Zainol et al. [9], from figure 2a was obtained by scanning the graph processing electronically the image using Webplotdigitizer 2.5 [10]. Using the software, data from scanned images is converted into a table with comma-separated columns [11].

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

The model requires log concentration of data as well as a y response that has been translated into a fractional unit, such as fractional area or another fraction of unity, to do statistical analysis with a modified version of the Gompertz equation (**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 [8].

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

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

RESULTS AND DISCUSSION

An important microbiological criterion is the antibacterial agent's minimum inhibitory concentration (MIC). It has received broad acclaim for quite some time. There have only been a few instances when this finding has been made over the years, but it has lately begun to appear more regularly in the results of routine checkups. However, its ability to be used for effective and optimum therapy remains limited, and there are times when it is completely worthless despite the fact that the costs involved are significantly more than those connected with qualitative techniques. The calculated MIC and NIC values are shown in **Table 1**, and the fitted curve to the inhibition data shows that it gives an acceptable match to the data, with coefficient of determination (R^2) values ranging from 0.95 to 0.97. (**Fig. 1**).

Table 1. MIC and NIC values (%w/v) of gelam honey against various bacterial pathogens.

Bacteria		95% C.I.			
S aureus	MIC	12.24	11.08	to	14.57
	NIC	9.33	8.44	to	11.10
E. coli	MIC	25.32	23.01	to	27.86
	NIC	17.71	16.09	to	19.48
P. aeruginosa	MIC	31.07	28.92	to	33.38
	NIC	14.55	13.55	to	15.63
B. cereus	MIC	34.09	32.87	to	35.35
	NIC	20.83	20.09	to	21.61

According to the Shukor's chart for MIC values (**Fig. 2**), *S. aureus* is the pathogen most sensitive to gelam honey followed by *E. coli* and *P. aeruginosa* while the MIC value for *B. cereus* overlaps with *P. aeruginosa* indicate that additional data is required to demonstrate their real sensitivity rank. ANOVA analysis of the MIC values and their standard error taking degrees of freedom into account is another approach for comparison, although this comparison will be carried out in future studies to examine similarity and difference to the Shukor's chart method.

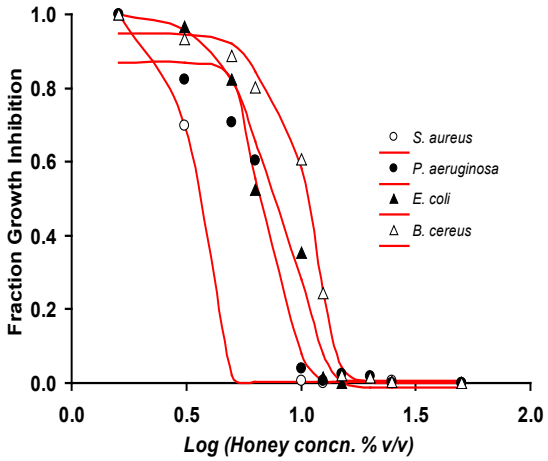

Fig. 1. Curve fitting of the inhibitory effect of gelam honey against several pathogenic bacteria using the Lamber-Pearson model.

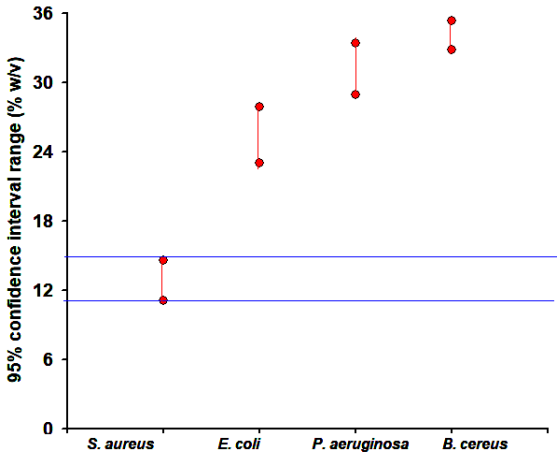

Fig. 2. Shukor's graph comparing the confidence intervals (95 percent) for the MIC values of gelam honey against several bacterial infections. Values of nonoverlap confidence intervals are regarded statistically significant ($p<0.05$).

Honey, bee pollen, and propolis have antibacterial qualities due to their capacity to break down the cytoplasm membrane of bacteria. This causes a depletion of potassium ions, which causes damage and eventually leads to cell autolysis. Both honey and propolis include a flavonoid called quercetin, which has the ability to increase bacterial membrane permeability while decreasing bacterial potency [12]. As a result, the bacteria are unable to migrate, synthesize adenosine triphosphate, or transmit adenosine across their membranes (ATP). According to the findings of a study that examined Indonesian honey and Brazilian propolis, bee products appear to be more efficient than Gram-negative bacteria in blocking Gram-positive bacteria [13,14]. Statistical discrimination, on the other hand, is a considerably more accurate result and is often based on ANOVA analysis of inhibitive characteristics such as MIC [8]. Bee pollen, which is similar to honey, has bacterial pathogen inhibitory effects. In one investigation, for example, it was discovered that bee pollen from Portuguese Natural Parks produced the MIC value of 0.21% (w/v) for *S. aureus*, 0.17% (w/v) for *B. cereus*, and <5% (w/v) for *E. coli* [15]. In another study a crude honey from Nigeria shows MIC value against *S. aureus* was 5% (w/v), *E. coli* at 20%, *P. aeruginosa* at 50%, and *P. mirabilis* at 100% (Bunza et al. 2019). A Thai study using longan honey shows MIC values of 680 mg/mL (68% w/v) for *E. coli* and 340 mg/mL (34% w/v) for *S. aureus* and *B. cereus* [14]. The wide range of MIC values observed for honey against bacterial pathogens appears to be hone-specific and dependent on the honey's complex bioactive components.

CONCLUSION

It is observed that mathematical models or nonlinear regression that would be required to obtain the MIC and NIC values have not been implemented in many of the studies on plant extracts and bacterial pathogens. These values are required for investigations of comparison, effectiveness, and validation. The mathematical models or nonlinear regression that would be required to obtain the MIC and NIC values have not been implemented in many of the studies on plant extracts and bacterial pathogens. These values are required for investigations of comparison, effectiveness, and validation. The modified Gompertz model proposed by Lambert and Pearson proved successful in establishing the minimum inhibitory concentration (MIC) and maximum inhibitory concentration (NIC) values of gelam honey to bacterial pathogens in the current study. Based on the overlapped confidence interval chart for MIC values. According to the Shukor's chart for MIC values, *S. aureus* is the pathogen most sensitive to gelam honey followed by *E. coli* and *P. aeruginosa* while the MIC value for *B. cereus* overlaps with *P. aeruginosa* indicating that additional data is required to demonstrate their real sensitivity rank. The Lambert-Pearson model fits well, with coefficient of determination (R^2) values ranging from 0.94 to 0.97 confirming the nonlinear regression model's appropriateness. The Lambert-Pearson model allows for the consistent determination of MIC values, which allows for model comparison using statistical discriminatory functions like as ANOVA.

REFERENCES

1. Mann CM, Markham JL. A new method for determining the minimum inhibitory concentration of essential oils. J Appl Microbiol. 1998;84(4):538–44.
2. Collins CH. Antibiotics and antibacterial substances. Microbiol Methods. 1964;296–305.
3. Davidson PM, Parish ME. Methods for testing the efficacy of food antimicrobials. Food Technol. 1989;43(1):148–55.

4. Chand S, Lusunzi I, Williams LR, Karuso P, Veal DA. Rapid screening of the antimicrobial activity of extracts and natural products. J Antibiot (Tokyo). 1994;47(11):1295–304.
5. Sommers HM. Drug susceptibility testing in vitro: Monitoring of antimicrobial therapy. Biol Clin Basis Infect Dis. 1980;782–804.
6. Janssen AM, Scheffer JJC, Baerheim Svendsen A. Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods. Planta Med. 1987;53(5):395–8.
7. Manou I, Bouillard L, Devleeschouwer MJ, Barel AO. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. J Appl Microbiol. 1998;84(3):368–76.
8. 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.
9. Zainol MI, Mohd Yusoff K, Mohd Yusof MY. Antibacterial activity of selected Malaysian honey. BMC Complement Altern Med. 2013 Jun 10;13(1):129.
10. Rohatgi A. WebPlotDigitizer. http://arohatgi.info/WebPlotDigitizer/app/ Accessed June 2 2014.; 2015.
11. 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.
12. Mirzoeva OK, Grishanin RN, Calder PC. Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria. Microbiol Res. 1997 Sep;152(3):239–46.
13. Schmidt EM, Stock D, Chada FJG, Finger D, Christine Helena Frankland Sawaya A, Eberlin MN, et al. A Comparison between Characterization and Biological Properties of Brazilian Fresh and Aged Propolis. BioMed Res Int. 2014;2014:257617.
14. Suriyatem R, Auras RA, Intipunya P, Rachtanapun P. Predictive mathematical modeling for EC_{50} calculation of antioxidant activity and antibacterial ability of Thai bee products. J Appl Pharm Sci. 2017;7(9):122–33.
15. Morais M, Moreira L, Feás X, Estevinho LM. Honeybee-collected pollen from five Portuguese Natural Parks: palynological origin, phenolic content, antioxidant properties and antimicrobial activity. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc. 2011 May;49(5):1096–101.