Comparative screening of dose-dependent and strain-specific antimicrobial efficacy of berberine against a representative library of broad-spectrum antibiotics

Stephanie Sun[1], Andrew Su[2], Simrun Sakhrani[3], Bhavesh Ashok[4], Sarah Su[5], Sarada Rajamanickam[4], *Edward Njoo*[6]

[1] BASIS Independent Silicon Valley, San Jose, CA; [2] Foothill High School, Pleasanton, CA; [3] The College Preparatory School, Oakland, CA; [4] Amador Valley High School, Pleasanton, CA; [5] Los Altos High School, Los Altos, CA; [6] Department of Chemistry, Biochemistry, & Physical Science, Aspiring Scholars Directed Research Program, Fremont, CA

KEYWORDS: Antibiotics, chemical biology, E. coli, alkaloids, natural products

ABSTRACT: Widespread use of antibiotics has resulted in the emergence of antibiotic-resistant bacteria strains, increasing the necessity in research towards the identification of novel antibiotic agents. Berberine, a natural alkaloid that is extracted from the roots and stems of plants in the genus Berberis, has been documented to have medicinal potential since 3000 BC, where it was used as an antibacterial agent in ancient Chinese medicine. Since then, berberine and synthesized analogs have been studied for a wide range of medicinal properties, including antimicrobial activity. Based on berberine's history, we hypothesize that berberine has broad-spectrum antibacterial properties, along with potency that is comparable to current broad-spectrum antibiotics that are commercially available. Here, we screened berberine against four strains of bacteria and evaluated its antimicrobial activity against five broad-spectrum antibiotics from different classes to better quantify berberine's antibacterial activity and compare its efficacy as an antibacterial agent to the broad-spectrum antibiotics. Our results indicated that berberine had strain-selective cytotoxic effects and was significantly less potent than most of the broad-spectrum antibiotics. A better understanding of the antimicrobial activity of the berberine may inform the design of future antimicrobial therapies.

INTRODUCTION

Bacterial resistance against antibiotics through evolution and selection has presented a global health epidemic that has created an urgency for the development of new antibiotics (1). The emergence of antibiotic-resistant bacteria can be attributed to the increasing use of antibiotics, including overuse and misuse. Every year, tens of thousands of people in America die from infections related to antibiotic-resistant bacteria (2). The issue of antibiotic resistance is one that concerns all parts of the world and presents the need for research towards the identification of novel antibacterial agents, including their strain specificity and dose dependency.

The start of the current age of antibiotic development is generally associated with Ehrlich, who developed the methodology behind systematic screening of drugs while trying to find a drug to treat syphilis in 1904, and Flemming, who accidentally discovered penicillin in 1929. Since then, hundreds of antimicrobial agents have been developed and clinically screened, the majority of which are derived from natural products (3). Current antibiotics lack diversity in their targets, with almost all antibiotics inhibiting DNA, RNA, protein synthesis, or cell wall synthesis. In fact, around half of all antibiotics target the cell wall. Antibiotics also lack diversity in scaffold design , with most of the novel antibiotics within the past forty years coming from five structural classes (4).

Berberine, an isoquinoline alkaloid extracted from plants in the genus Berberis, is of medicinal interest due to its long history in various ancient cultures, most notably Chinese and Ayurvedic medicine, where it was first documented as a therapeutic agent in 3000 BC (5). Berberine is now a supplement that is marketed to individuals with type II diabetes, high blood pressure, high cholesterol, or gastrointestinal infections. Studies on berberine suggest that it is effective against a wide range of diseases such as diabetes, metabolic syndrome, poly-

cystic ovary syndrome, cardiovascular diseases, bacterial infections, and cancer, serving as an indication of berberine's pharmaceutical potential (6-8). Alkaloids, the largest class of natural products, have also demonstrated remarkable biological activities. Berberine is classified as an isoquinoline alkaloid because of its biosynthetic route starting with tyrosine and its isoquinoline skeleton (9).

We are interested in the antibacterial effects of berberine due to its long history of use in ancient medicine and recent studies on berberine's antibacterial activity, including reports of berberine having cytotoxic effects against methicillin-resistant Staphylococcus aureus (MRSA) alone and in synergy with other antibiotics (10, 11). Many of these studies focused on one specific strain of bacteria, therefore focusing on one specific class of antibacterial agents. This led us to ask the question: is berberine's antibacterial effect consistent throughout all strains of bacteria, and how effective is berberine compared to other broad-spectrum antibiotics different structural classes? Based on previous studies and its reported use in ancient medicine, we hypothesized that berberine would result in cytotoxic effects against all strains of bacteria and have comparable potencies to the broad-spectrum antibiotics screened.

The antibiotics screened in this study are representative of five different structural classes, resulting in different biological targets within cells and interactions with bacteria (Table 1). Structural differences between the different classes of antibiotic agents can be seen in Table 1.

Ampicillin is semi-synthetic, beta-lactam penicillin; beta-lactams bind to penicillin-binding proteins (PBPs) located on the inner membrane of the bacterial cell wall (12). This binding inactivates PBP, interfering with the cross-linkage of peptidoglycan chains, which is essential to maintaining the shape of bacteria and allowing them to withstand osmotic pressure changes (13). The bacterial cell walls become weaker and less rigid, eventually leading to cell lysis.

Sulfanilamide is a sulfonamide antibiotic, which works by competing with para-aminobenzoic acid (PABA) for the enzyme dihydropteroate synthase, thus preventing PABA from being incorporated into dihydrofolic acid, which prevents the creation of folic acid (14). Within bacteria, folic acid is necessary for synthesizing DNA and bacterial growth, and a lack of folic acid results in bacterial death (15).

Enrofloxacin is a fluoroquinolone antibiotic that inhibits the A subunit of bacterial DNA gyrase, thus preventing the negative supercoiling of bacterial DNA, and DNA synthesis (16). DNA gyrase is an essential enzyme, a topoisomerase, that catalyzes the negative coiling of double-stranded DNA (17, 18). Without DNA gyrase, DNA replication isn't able to occur and cells are unable to replicate.

Nalidixic acid is a synthetic quinolone antibiotic, with a similar quinoline ring structure as enrofloxacin. Nalidixic acid binds to both DNA gyrase and Topo IV, both enzymes that affect the coiling of double-stranded DNA (19). While gyrase catalyzes the negative coiling of DNA, Topo IV relaxes positive supercoils (20). Topo IV is responsible for untangling daughter chromosomes by removing positive intertwinings and relaxing supercoils created during transcription (21). Binding to these targets inhibits DNA replication, affecting cell replication and growth. Though the targets and mechanisms of action between enrofloxacin and nalidixic acid are similar, nalidixic acid is only active against gram-negative strains of bacteria. The introduction of the fluorine into the structure of enrofloxacin increases its antibiotic spectrum to include some gram-positive strains of bacteria (22).

Kanamycin is an aminoglycoside bactericidal antibiotic, and it inhibits ribosomal proteins, thus inhibiting protein synthesis. Kanamycin binds to the A site of 16S rRNA in the 30S ribosomal subunit of bacteria (23). The binding of kanamycin into rRNA interferes with the interactions and translation of mRNA into tRNA, resulting in incorrect tRNA fragments (24). The peptide that is then synthesized contains incorrect amino acids, therefore inhibiting bacterial activity.

Berberine's mechanism of action within cells remains unknown, however putative methods of action have been studied. Berberine has intercalates into DNA and forms a complex with DNA (25). Upon photo-irradiation of the berberine-DNA complex, berberine oxidizes guanines, causing DNA damage and inhibiting cell replication (26).

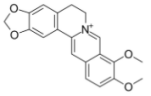
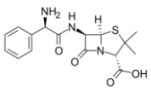
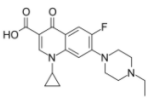
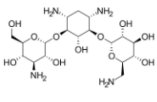
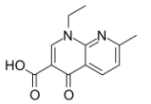
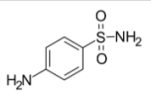
Class	Representative Antibiotic	Year In Commercial Use	Structure	Target
Isoquinoline Alkaloid	Berberine	N/A		Proposed Mechanism of Action - Oxidation of guanines within DNA when photoirridated
Beta-Lactam (Penicillin)	Ampicillin	1961		Transpeptidase-enzyme used to make bacterial cell wall
Fluoroquinolone	Enrofloxacin	1988		DNA gyrase- DNA and RNA synthesis
Aminoglycoside	Kanamycin	2003		30S ribosomal subunit- synthesis of proteins)
Quinolone	Nalidixic Acid	1967		DNA gyrase and topoisomerase IV-coiling of double stranded DNA
Sulfonamide	Sulfanilamide	1935		Dihydropteroate synthase-synthesizes folic acid from para-aminobenzoic acid

Table 1. An introduction into the antibiotics used in this study, including the class, year they went into commercial use, their struc-

tures, and biological target. Each biological target includes a description of the role they play (27-32).

Our selection of bacterial strains includes Bacillus cereus, Escherichia coli, Neisseria sicca, and Staphylococcus epidermidis, a combination of gram-positive and negative strains. Gram-positive bacteria have a thick peptidoglycan layer and no outer lipid membrane, while gram-negative bacteria have a thin peptidoglycan layer and an outer lipid membrane (33). The difference can help researchers determine selectivity within antibacterial agents. There are strains that belong to all four species that have been shown to be pathogenic. Details in Table 2 provide a better understanding of the differences between the strains of bacteria screened.

Strain	Gram Positive or Negative	Related Illnesses	Where they are found	Characteristics
Bacillus cereus	Gram Positive (34)	Food poisoning, vomiting, diarrhea (35)	Soil and vegetation; most commonly spread in meat, eggs, and dairy (36)	Facultative anaerobic - can produce ATP in the absence of oxygen (37)
Escherichia coli	Gram Negative (38)	Diarrheal diseases, bacteremia, infant mortality, and urinary tract infections (39)	In the gastrointestinal tract of humans and animals, typically not pathogenic (40)	Adaptable to many different environments, can survive in the harsh climate outside the gut. (41)
Neisseria sicca	Gram Negative (42)	Pneumonia, meningitis, endocarditis (43)	Human oropharynx, behind the oral cavity (44)	Oxidase-positive (aerobic) and produces cytochrome c oxidase, allowing oxygen to be used as an electron acceptor in the electron transport chain (45)
Staphylococcus epidermidis	Gram Positive (46)	Nosocomial infections (hospital related infections) (47)	Typically lives in symbiosis with skin, infections connected to medical devices (48)	Biofilm formation protects the bacteria from immune response and antibacterial agents (49)

Table 2. A comparison of the four strains of bacteria used in this study, including gram positive versus gram negative, illnesses that they cause, where they can typically be found, and some other properties essential to the bacteria's survival or pathogenic nature.

RESULTS

This work is important to understand the specificity of antibiotics which can help to address the globally pressing issue of antibiotic resistance. The average radius of inhibition (ROI) from the four duplicate experiments theand standard error were graphed (Figure 1A-D).

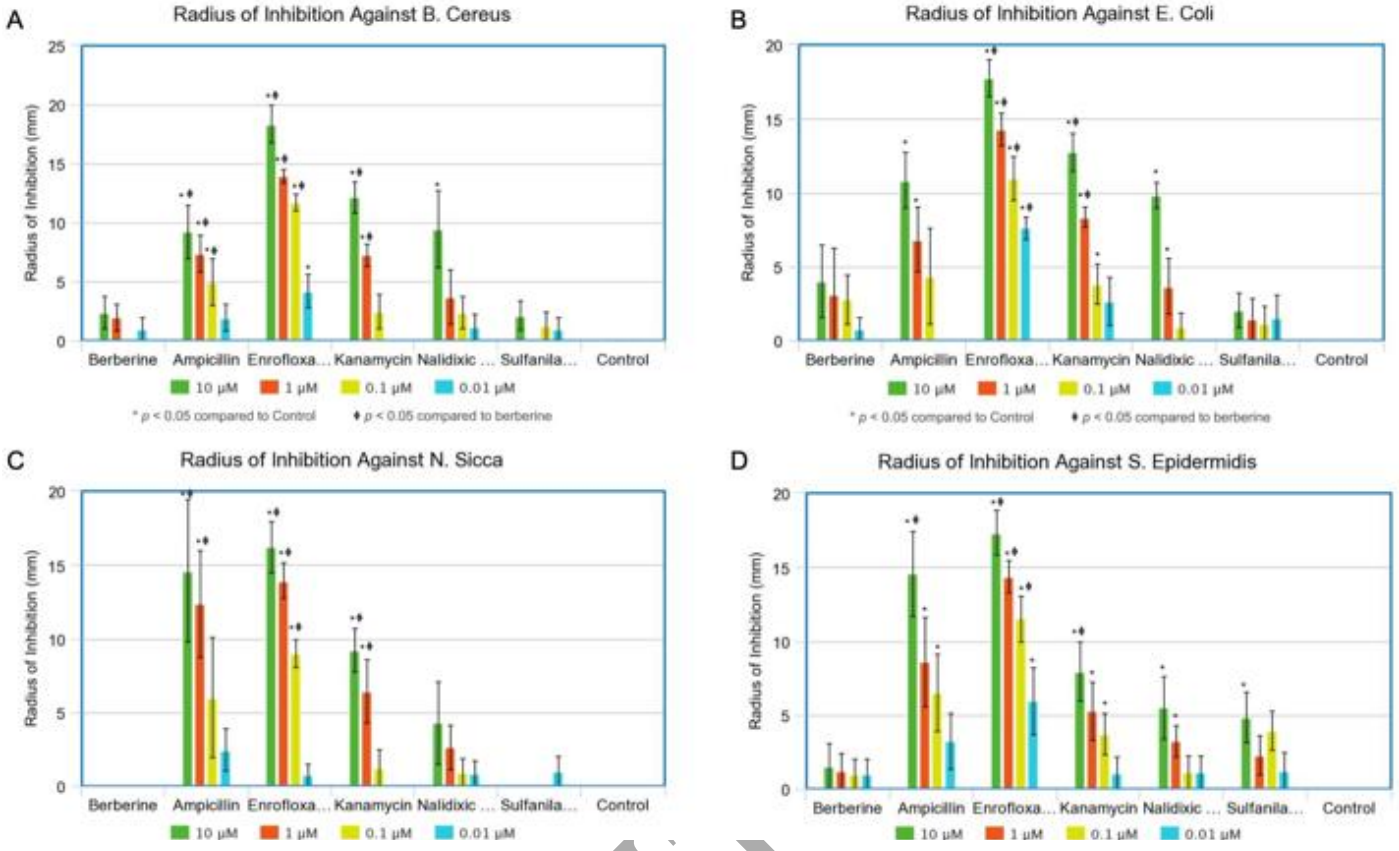


Figure 1. Radius of inhibition of different antibiotics compared to berberine against four strains of bacteria, reported in mm. (A) ROI against B. cereus (B) ROI against E. coli (C) ROI against N. sicca (D) ROI against S. Epidermidis. The * indicates that the p value is less than 0.05 compared to the control, and the ♦ indicates that the p value is less than 0.05 compared to the ROI of berberine. A p value of less than 0.05 indicates statistical significance.

Inhibition curves were then created to evaluate the effectiveness of the antibiotics and understand how concentration was related to inhibition. We observed dose-dependent cytotoxic effects of the various antibiotics, including berberine. A ten-fold increase in the concentration of the antibiotic solution resulted in a constant increase in the ROI of bacteria growth. R2 v alues from the linear fit of the data points throughout all four strains of bacteria for all the screened compounds indicate that the antibiotics and berberine were dose-dependent. Sulfanilamide consistently had R2 values that were significantly lower than the other screened compounds, with the lowest R2 against B. cereus (Figure 2A). Berberine had high R2 values when screened against E. coli (Figure 2B) and S. epidermidis (Figure 2D), but that trend did not hold against B. cereus (Figure 2A). All other broad-spectrum antibiotics consistently had high R2 values throughout all four strains of bacteria.

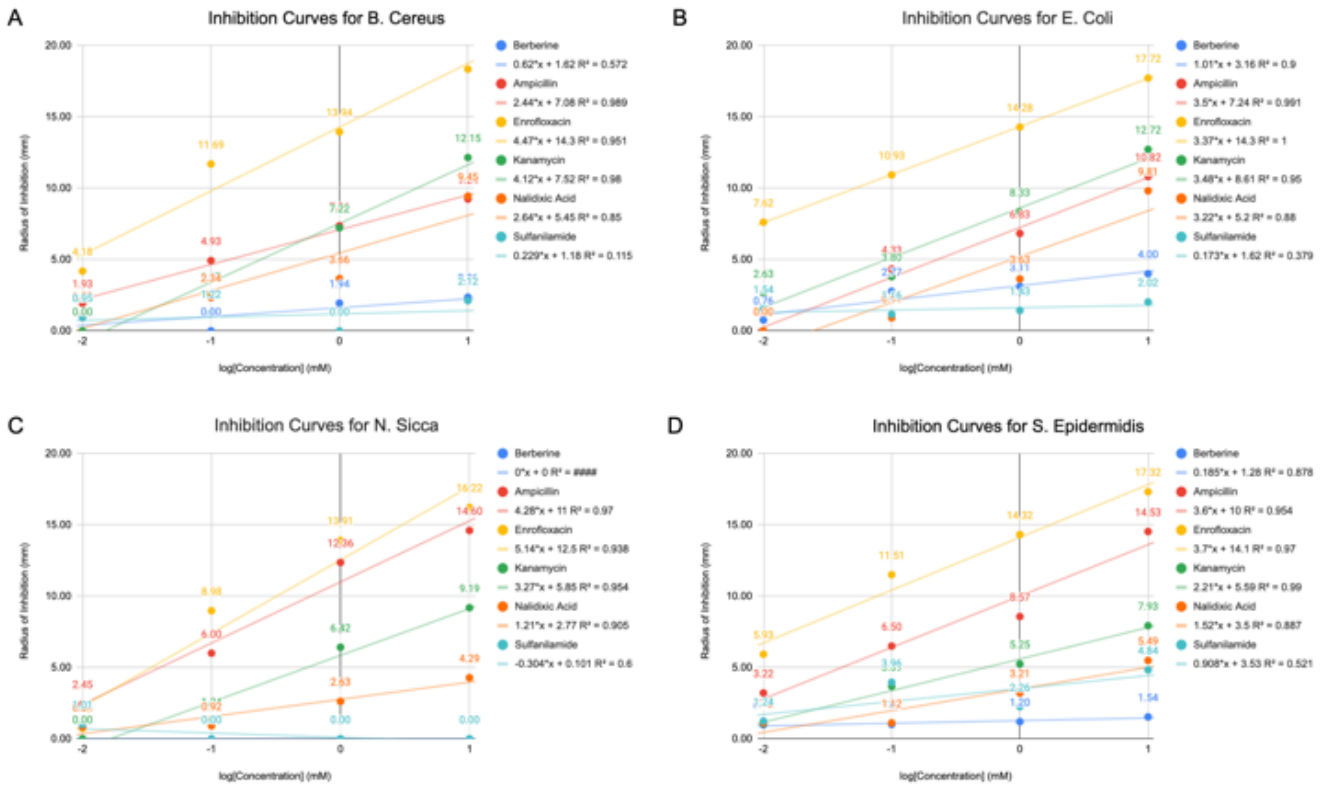


Figure 2. Inhibition curves for four strains of bacteria. All graphs are fit to a linear curve, with the equation of the line and R2 values reported. (A) Inhibition curve for B. cereus (B) Inhibition curves for E. coli (C) Inhibition curve for N. sicca (D) Inhibition curve for S. Epidermidis

B. cereus Berberine was ineffective against B. cereus compared to the other, broad-spectrum antibiotics screened. Enrofloxacin had the most potent effects against B. cereus, and ampicillin, kanamycin, and nalidixic acid had similar effects against B. cereus (Figure __). Results from unpaired t-testing, indicated that the ROI as a result of berberine was statistically insignificant compared to the control (Figure 1A). The ROI of bacteria growth from enrofloxacin, ampicillin, kanamycin, and nalidixic acid at a high concentration were significant compared to the control, however the ROI of nalidixic acid was not significant compared to berberine's ROI. Enrofloxacin, ampicillin, and kanamycin all have radii of inhibition that are statistically significant compared to berberine .

E. coli In the study with E. coli, berberine was not as potent as other antibiotics. Enrofloxacin, kanamycin, and ampicillin demonstrated inhibition against bacterial growth, with enrofloxacin being the most potent (Figure 1B). Unpaired t-testing revealed that the ROI from berberine and sulfanilamide are statistically insignificant compared to the control, while all other broad-spectrum antibiotics had radii of inhibition that were significant. Only enrofloxacin and kanamycin have ROI that are statistically significant compared to berberine, with enrofloxacin's ROI being significant at all concentrations.

N. sicca Berberine has no inhibition against the growth of N. sicca, demonstrating that berberine has strain-specific antibacterial effects. All broad-spectrum antibiotics except sulfanilamide have ROI that are nonzero, but the ROI from nalidixic acid are statistically insignificant compared to the control (Figure 1C). Sulfanilamide has an outlier at the lowest concentration, where the ROI is positive, however the value falls within the standard error. Enrofloxacin, ampicillin, and kanamycin have radii of inhibition that are statistically significant compared to berberine's ROI at higher concentrations, with ampicillin and enrofloxacin having the similar and the most potent effects.

S. epidermidis All broad-spectrum antibiotics except sulfanilamide have radii of inhibition that are statistically significant compared to the control, while the radii of inhibition as a result of berberine and sulfanilamide are statistically insignificant compared to the control (Figure 1D). Enrofloxacin had the most potent antibacterial effects, with all concentrations resulting in ROI that are statistically significant compared to berberine. At higher concentrations, kanamycin had ROI that are statistically significant compared to berberine, however both ampicillin and nalidixic acid are insignificant compared to berberine.

Enrofloxacin consistently had the most potent effects against all bacteria strains, with ampicillin possessing similar inhibitions against N. sicca and S. epidermidis. Against all strains of bacteria except N. sicca, kanamycin and nalidixic acid had comparable potencies, however they were less potent than enrofloxacin. The controls in our Kirby Bauer assay, a solution containing 5% DMSO,

all demonstrated no cytotoxic effects. The trends observed in our results, with antibiotic solutions at 10 mM concentrations having the greatest inhibition and the antibiotic solutions of 0.1 mM demonstrating the least inhibition, are expected.

Compared to the broad-spectrum antibiotics that were screened in this study, berberine consistently demonstrated less potent effects. Berberine and sulfanilamide had similar results with unpaired t-tests determining that the difference in inhibition compared to the control was insignificant. However, unlike the broad-spectrum antibiotics, berberine demonstrates strain-selective cytotoxic effects. Berberine presents no inhibition of bacterial growth in N. sicca and the radii of inhibition for S. epidermidis all fall within the standard error, while results against E. coli and B. cereus all have positive radii of inhibition. All the broad-spectrum antibiotics except sulfanilamide had cytotoxic effects against all strains of bacteria. Our results demonstrate that berberine is not a potent antibacterial agent compared to broad-spectrum antibiotics, however it does have strain-specific cytotoxic effects .

DISCUSSION

Although berberine has been used in ancient cultures as medicine for thousands of years, its antibacterial effects are not comparable to broad-spectrum antibiotics. The demand for new antibacterial agents as more and more drug-resistant bacteria emerge has led to increasing research in identifying novel antibacterial agents. Through screening berberine against four strains of bacteria, it was apparent that berberine had selective antibacterial effects, which demonstrates future pharmaceutical potential.

In order to further determine its effectiveness and its characteristics of selectivity as an antimicrobial compound, the same screening of antibiotics could be conducted against a larger variety of bacterial strains, allowing for an effective comparative analysis. This will allow us to gain a better understanding of which strains of bacteria berberine has cytotoxic effects against, and whether there is a pattern that is present.

Understanding the target in the cell of each antibacterial agent and bacterial strain screened would also allow for better drug design in the future. In particular, molecules like berberine that have already demonstrated biological activity can be synthetically modified to increase potency and cytotoxic effects. Though berberine's mechanism of action within cells is not well understood, berberine's strain-selective cytotoxic effects may provide more insight into its mechanism of action.

Using berberine as an antibacterial agent may also result in undesired side effects, as berberine has been previously demonstrated to affect eukaryotic cells. Berberine can bind to the G-quadruplex structures of DNA, where it is can then inhibit telomerase (50). Cytotoxic effects of berberine have also been attributed to its activity within the MAPK signal pathway where it specifically targets p38. The MAPK signal pathway ultimately affects DNA replication (51). These processes, which do not directly impact the antibacterial activity of berberine, may have other implications in the future and should be considered.

In this study, berberine's antibacterial effects are against four strains of bacteria, both gram-positive and gram-negative strains, are compared to cytotoxic effects of five, broad-spectrum antibacterial agents. Berberine demonstrated strain-selective antibacterial effects but was found to be significantly less potent than all the broad-spectrum antibiotics included in this study, except sulfanilamide. These results provide insight into the biological activity of berberine and its potential as a pharmaceutical agent.

MATERIALS AND METHODS

Berberine was compared to five broad-spectrum antibiotics: ampicillin, enrofloxacin, kanamycin, nalidixic acid, and sulfanilamide . Radius of inhibition (ROI) values were acquired through the Kirby Bauer assay (Figure 3).

Figure 3. Petri dishes inoculated with S. Epidermidis, with the radii of inhibition as the result of ampicillin (left) and berberine (right) at four different concentrations. Inhibition of bacterial growth can be seen surrounding the filter paper discs. Concentrations of the antibiotic solution of the filter paper discs are also shown.

Bacteria Live bacteria cultures of Bacillus cereus, Escherichia coli, Neisseria sicca, and Staphylococcus epidermidis were obtained from Carolina Biological. Overnight cultures of the bacteria were grown in falcon tubes of 10-15 mL of LB media (1% tryptone, 1% NaCl, 0.5% protein media, 97.5% water). The overnight cultures of the bacteria were incubated at 37°C for 12-14 hours.

Antibiotic Solutions Antibiotic solutions were made in four different concentrations (10 mM, 1 mM, 0.1 mM, 0.01 mM) in deionized water with 5% DMSO. The antibiotics were acquired from ACTGene, Aquaculture Antibiotics, Fisher BioReagants, or HiMedia. A solution of 5% DMSO in deionized water was used as the control. The antibiotic solutions were administered through filter paper discs that were saturated with the solution.

Inoculation Bacteria from the overnight cultures were inoculated on petri dishes plated with Mueller Hinton Agar acquired from HiMedia. Each Petri dish was first inoculated with one strain of bacteria. Four filter paper discs with the same antibiotic solution of varying concentrations were placed on the same petri dish. Petri dishes were incubated at 37°C for 12-18 hours in the absence of light. This was done in four different trials, in which the cultures used were biological replicates.

Statistical Analysis ROI measurements were acquired in millimeters using an electronic caliper. Results from all four experiments were averaged, and standard error was calculated using the follow equation: σ/√n,where σis equal to the standard deviation and n is equal to the number of values. Inhibition curves were created by graphing the logarithm of the concentrations of the antibiotics against the average ROI. The graphs were fit to a linear curve and R2 values were obtained.

Unpaired t-testing was completed using the following equation: t=(〖x̄〗 _1- 〖x̄〗 _2)/√(s^2 (1/n_1 -1/n_2)), where x̄ represents the population means, s2 represents the sample variances, and n the populations. The sample variance (s2) was calculated using the following equation: s^2=(∑_(i=1)^(n_1)(x_i- 〖x̄〗 _1)^2-∑_(i=1)^(n_2)▒((x_i- 〖x̄〗 _2)^2)/(n_1+n_2-2) , where the sum of the variances squared were subtracted and divided by the population. The t-distribution critical values table was then used to derive the statistic's corresponding critical value at the 95% confidence level, whereupon if the t-testing result was less than the critical value, the p-value was determined to be less than 0.05. Results with a p-value less than 0.05 were considered statistically significant.

ACKNOWLEDGEMENTS

We would like to thank the Center for Advanced Study at Olive Children Foundation and Biolink Depot for funding and material support. We would also like to thank Nanaylsis, Thermo Fisher Scientific, Bio-Rad, and Perkin-Elmer for instrumentation support

REFERENCES

(1) Davies, J.; Davies, D. "Origins and Evolution of Antibiotic Resistance. Microbiology and Molecular Biology Reviews, 74, 3, 2010, 417–433.

(2) Kim, Lindsay, et al. "Biological and Epidemiological Features of Antibiotic-Resistant Streptococcus Pneumoniae in Pre- and Post-Conjugate Vaccine Eras: a United States Perspective." Clinical Microbiology Reviews, vol. 29, no. 3, 2016, pp. 525–552., doi:10.1128/cmr.00058-15.

(3) Nicolaou, Kyriacos C, and Stephan Rigol. "A Brief History of Antibiotics and Select Advances in Their Synthesis." The Journal of Antibiotics, vol. 71, no. 2, May 2017, pp. 153–184., doi:10.1038/ja.2017.62.

(4) Aminov, Rustam I. "A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future." Frontiers in Microbiology, vol. 1, 2010, doi:10.3389/fmicb.2010.00134.

(5) Tillhon, Micol, et al. "Berberine: New Perspectives for Old Remedies." Biochemical Pharmacology, vol. 84, no. 10, 2012, pp. 1260–1267., doi:10.1016/j.bcp.2012.07.018.

(6) Stermitz, F. R., et al. "Synergy in a Medicinal Plant: Antimicrobial Action of Berberine Potentiated by 5-Methoxyhydnocarpin, a Multidrug Pump Inhibitor." Proceedings of the National Academy of Sciences, vol. 97, no. 4, Apr. 2000, pp. 1433–1437., doi:10.1073/pnas.030540597.

(7) Wang, Haoran, et al. "Metformin and Berberine, Two Versatile Drugs in Treatment of Common Metabolic Diseases." Oncotarget, vol. 9, no. 11, Nov. 2017, doi:10.18632/oncotarget.20807.

(8) Lau, Chi-Wai, et al. "Cardiovascular Actions of Berberine." Cardiovascular Drug Reviews, vol. 19, no. 3, July 2006, pp. 234–244., doi:10.1111/j.1527-3466.2001.tb00068.x.

(9) Grycová, Lenka, et al. "Quaternary Protoberberine Alkaloids." Phytochemistry, vol. 68, no. 2, 2007, pp. 150–175., doi:10.1016/j.phytochem.2006.10.004.

(10) Chu, Ming, et al. "Role of Berberine in the Treatment of Methicillin-Resistant Staphylococcus Aureus Infections." Scientific Reports, vol. 6, no. 1, 2016, doi:10.1038/srep24748.

(11) Wojtyczka, Robert, et al. "Berberine Enhances the Antibacterial Activity of Selected Antibiotics against Coagulase-Negative Staphylococcus Strains in Vitro." Molecules, vol. 19, no. 5, 2014, pp. 6583–6596., doi:10.3390/molecules19056583.

(12) Rafailidis, Petros I, et al. "Ampicillin/Sulbactam." Drugs, vol. 67, no. 13, 2007, pp. 1829–1849., doi:10.2165/00003495-200767130-00003.

(13) Buynak, John D. "Cutting and Stitching: The Cross-Linking of Peptidoglycan in the Assembly of the Bacterial Cell Wall." ACS Chemical Biology, vol. 2, no. 9, 2007, pp. 602–605., doi:10.1021/cb700182u.

(14) Benedetti, Pier G. De, et al. "Structure-Activity Relationships in Dihydropteroate Synthase Inhibition by Sulfanilamides. Comparison with the Antibacterial Activity." Journal of Medicinal Chemistry, vol. 24, no. 4, 1981, pp. 454–457., doi:10.1021/jm00136a015.

(15) Rossi, Maddalena, et al. "Folate Production by Probiotic Bacteria." Nutrients, vol. 3, no. 1, 2011, pp. 118–134., doi:10.3390/nu3010118.

(16) Reinhardt, A. K. "Fluoroquinolone Resistance in Mycoplasma Gallisepticum: DNA Gyrase as Primary Target of Enrofloxacin and Impact of Mutations in Topoisomerases on Resistance Level." Journal of Antimicrobial Chemotherapy, vol. 50, no. 4, June 2002, pp. 589–592., doi:10.1093/jac/dkf158.

(17) Reece, Richard J., and Anthony Maxwell. "DNA Gyrase: Structure and Function." Critical Reviews in Biochemistry and Molecular Biology, vol. 26, no. 3-4, 1991, pp. 335–375., doi:10.3109/10409239109114072.

(18) Witz, Guillaume, and Andrzej Stasiak. "DNA Supercoiling and Its Role in DNA Decatenation and Unknotting." Nucleic Acids Research, vol. 38, no. 7, 2009, pp. 2119–2133., doi:10.1093/nar/gkp1161.

(19) Sugino, A., et al. "Mechanism of Action of Nalidixic Acid: Purification of Escherichia Coli NalA Gene Product and Its Relationship to DNA Gyrase and a Novel Nicking-Closing Enzyme." Proceedings of the National Academy of Sciences, vol. 74, no. 11, Jan. 1977, pp. 4767–4771., doi:10.1073/pnas.74.11.4767.

(20) Ashley, Rachel E., et al. “Activities of Gyrase and Topoisomerase IV on Positively Supercoiled DNA.” Nucleic Acids Research, vol. 45, no. 16, 2017, pp. 9611–9624., doi:10.1093/nar/gkx649.

(21) Stone, M. D., et al. “Chirality Sensing by Escherichia Coli Topoisomerase IV and the Mechanism of Type II Topoisomerases.” Proceedings of the National Academy of Sciences, vol. 100, no. 15, Nov. 2003, pp. 8654–8659., doi:10.1073/pnas.1133178100.

(22) Pommier, Yves, et al. “DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs.” Chemistry & Biology, vol. 17, no. 5, 2010, pp. 421–433., doi:10.1016/j.chembiol.2010.04.012.

(23) “Kanamycin.” Tuberculosis, vol. 88, no. 2, 2008, pp. 117–118., doi:10.1016/s1472-9792(08)70012-x.

(24) Kotra, L. P., et al. “Aminoglycosides: Perspectives on Mechanisms of Action and Resistance and Strategies to Counter Resistance.” Antimicrobial Agents and Chemotherapy, vol. 44, no. 12, Jan. 2000, pp. 3249–3256., doi:10.1128/aac.44.12.3249-3256.2000.

(25) Krey, A. K., and F. E. Hahn. “Berberine: Complex with DNA.” Science, vol. 166, no. 3906, July 1969, pp. 755–757., doi:10.1126/science.166.3906.755.

(26) Hirakawa, Kazutaka, et al. “The Mechanism of Guanine Specific Photooxidation in the Presence of Berberine and Palmatine: Activation of Photosensitized Singlet Oxygen Generation through DNA-Binding Interaction.” Chemical Research in Toxicology, vol. 18, no. 10, 2005, pp. 1545–1552., doi:10.1021/tx0501740.

(27) Hahn, Fred E., and Jennie Ciak. “Berberine.” Mechanism of Action of Antimicrobial and Antitumor Agents, 1975, pp. 577–584., doi:10.1007/978-3-642-46304-4_38.

(28) Hatanaka, Minoru, and Toshiyasu Ishimaru. “Synthetic Penicillins. Heterocyclic Analogs of Ampicillin. Structure-Activity Relations.” Journal of Medicinal Chemistry, vol. 16, no. 9, 1973, pp. 978–984., doi:10.1021/jm00267a005.

(29) Efthimiadou, Eleni K., et al. “Neutral and Cationic Mononuclear Copper(II) Complexes with Enrofloxacin: Structure and Biological Activity.” Journal of Inorganic Biochemistry, vol. 100, no. 8, 2006, pp. 1378–1388., doi:10.1016/j.jinorgbio.2006.03.013.

(30) Cron, M. J., et al. “Kanamycin. V. The Structure Of Kanosamine.” Journal of the American Chemical Society, vol. 80, no. 17, 1958, pp. 4741–4742., doi:10.1021/ja01550a081.

(31) Achari, A., and S. Neidle. “Nalidixic Acid.” Acta Crystallographica Section B Structural Crystallography and Crystal Chemistry, vol. 32, no. 2, Jan. 1976, pp. 600–602., doi:10.1107/s056774087600349x.

(32) Kumler, W. D., and T. C. Daniels. “The Relation between Chemical Structure and Bacteriostatic Activity of Sulfanilamide Type Compounds.” Journal of the American Chemical Society, vol. 65, no. 11, 1943, pp. 2190–2196., doi:10.1021/ja01251a053.

(33) Hessle, Christina C., et al. “Gram-Positive and Gram-Negative Bacteria Elicit Different Patterns of pro-Inflammatory Cytokines in Human Monocytes.” Cytokine, vol. 30, no. 6, 2005, pp. 311–318., doi:10.1016/j.cyto.2004.05.008.

(34) Miller, J M, et al. “Fulminating Bacteremia and Pneumonia Due to Bacillus Cereus.” Journal of Clinical Microbiology, vol. 35, no. 2, 1997, pp. 504–507., doi:10.1128/jcm.35.2.504-507.1997.

(35) Bottone, E. J. “Bacillus Cereus, a Volatile Human Pathogen.” Clinical Microbiology Reviews, vol. 23, no. 2, Jan. 2010, pp. 382–398., doi:10.1128/cmr.00073-09.

(36) Granum, Per Einar, and Terje Lund. “Bacillus Cereus and Its Food Poisoning Toxins.” FEMS Microbiology Letters, vol. 157, no. 2, 2006, pp. 223–228., doi:10.1111/j.1574-6968.1997.tb12776.x.

(37) Duport, Catherine, et al. “Adaptation in Bacillus Cereus: From Stress to Disease.” Frontiers in Microbiology, vol. 7, Apr. 2016, doi:10.3389/fmicb.2016.01550.

(38) Schäfer, A, et al. “High-Frequency Conjugal Plasmid Transfer from Gram-Negative Escherichia Coli to Various Gram-Positive Coryneform Bacteria.” Journal of Bacteriology, vol. 172, no. 3, 1990, pp. 1663–1666., doi:10.1128/jb.172.3.1663-1666.1990.

(39) Kornacki, Jeffrey L., and Elmer H. Marth. “Foodborne Illness Caused by Escherichia Coli: A Review.” Journal of Food Protection, vol. 45, no. 11, Jan. 1982, pp. 1051–1067., doi:10.4315/0362-028x-45.11.1051.

(40) Lim, Ji Youn, et al. “A Brief Overview of Escherichia Coli O157:H7 and Its Plasmid O157.” Journal of Microbiology and Biotechnology, vol. 20, no. 1, 2010, pp. 5–14., doi:10.4014/jmb.0908.08007.

(41) Blount, Zachary D. “The Unexhausted Potential of E. Coli.” ELife, vol. 4, 2015, doi:10.7554/elife.05826.

(42) Bansmer, Charlotte, and Jacob Brem. “Acute Meningitis Caused By Neisseria Sicca.” New England Journal of Medicine, vol. 238, no. 17, 1948, pp. 596–597., doi:10.1056/nejm194804222381704.

(43) Entesari-Tatafi, Damoon, et al. “Iatrogenic Meningitis Caused By Neisseria Sicca/Subflava After Intrathecal Contrast Injection, Australia.” Emerging Infectious Diseases, vol. 20, no. 6, 2014, pp. 1023–1025., doi:10.3201/eid2006.131117.

(44) Gilrane, Timothy, et al. “Neisseria Sicca Pneumonia. Report of Two Cases and Review of the Literature.” The American Journal of Medicine, vol. 78, no. 6, 1985, pp. 1038–1040., doi:10.1016/0002-9343(85)90230-x.

(45) Liu, Guangyu, et al. “Non-Pathogenic Neisseria: Members of an Abundant, Multi-Habitat, Diverse Genus.” Microbiology, vol. 161, no. 7, Jan. 2015, pp. 1297–1312., doi:10.1099/mic.0.000086.

(46) Kaplan, Jeffrey B., et al. “Enzymatic Detachment of Staphylococcus Epidermidis Biofilms.” Antimicrobial Agents and Chemotherapy, vol. 48, no. 7, 2004, pp. 2633–2636., doi:10.1128/aac.48.7.2633-2636.2004.

(47) Ogara, James P., and Hilary Humphreys. “Staphylococcus Epidermidis Biofilms: Importance and Implications.” Journal of Medical Microbiology, vol. 50, no. 7, Jan. 2001, pp. 582–587., doi:10.1099/0022-1317-50-7-582.

(48) Otto, Michael. “Staphylococcus Epidermidis — the Accidental Pathogen.” Nature Reviews Microbiology, vol. 7, no. 8, 2009, pp. 555–567., doi:10.1038/nrmicro2182.

(49) Vuong, Cuong, and Michael Otto. “Staphylococcus Epidermidis Infections.” Microbes and Infection, vol. 4, no. 4, 2002, pp. 481–489., doi:10.1016/s1286-4579(02)01563-0.

(50) Franceschin, Marco, et al. “Natural and Synthetic G-Quadruplex Interactive Berberine Derivatives.” Bioorganic & Medicinal Chemistry Letters, vol. 16, no. 6, 2006, pp. 1707–1711., doi:10.1016/j.bmcl.2005.12.001.

(51) Zhou, Jiyin, et al. “Neuroprotective Effect of Berberine Is Mediated by MAPK Signaling Pathway in Experimental Diabetic Neuropathy in Rats.” European Journal of Pharmacology, vol. 774, 2016, pp. 87–94., doi:10.1016/j.ejphar.2016.02.007.