Antibacterial Properties of Plant Extracts Used in Traditional Chinese Medicine for *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*

Kelly Saine

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Kelly Saine

APPROVED:

Dr. Mary B. Farone
Biology

Dr. Lynn Boyd
Biology

Honor's rep??
Department type

Dr. John R. Vile
University Honors College Dean

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Abstract

The overuse of antibiotics has led to multi-drug resistant (MDR) organisms of which few antibiotics can kill. Due to the need for novel antibiotics, pharmaceutical developers are looking at ancient homeopathic remedies for answers. The purpose of this study was to determine if any traditional Chinese medicine (TCM) crude extracts had antibacterial properties, with the larger goal of identifying antibacterial drug candidates. A total of 120 extracts from 22 plants used in traditional Chinese medicine (TCM) from Guangxi Botanical Garden of Medicinal Plants (GBGMP) in Nanning, China were assayed against *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*. This was done following CLSI guidelines for aerobacteria using the broth microdilution assay and the disk diffusion assay, respectively. The broth microdilution assayed revealed the minimum inhibitory concentration (MIC) and the MIC_{50}. Select extracts exhibiting greater than 80% bacterial inhibition and less than 10% toxicity against mammalian cells underwent a dose response to determine the minimum bactericidal concentration (MBC). Of the 120 extracts assayed, none were found to inhibit *N. gonorrhoeae*. Overall, 28 extracts exhibited inhibition within the above parameters against *S. pneumoniae*. Three of the extracts had bactericidal potential, 21 had bacteriostatic potential, and four extracts had confounding results and need to be retested. Further testing is needed to identify pure compounds from the crude extracts.

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I. INTRODUCTION

Background

The use of plants as pharmaceuticals is not a novel concept. The peoples of ancient China used various plants to cure aches, pains, and even fevers. It was not until the early 1900s when Paul Ehrlich discovered Arsphenamine, and coined the term *magic bullet,* that the idea of a compound that could selectively target a pathogenic organism was created. Ehrlich's discovery inclined other scientists to search for *magic bullets* that would cure other prevalent illnesses (Bosch and Rosich 2008). One such scientist was Alexander Fleming who discovered and developed penicillin, the first antibiotic, in the 1920s from a Penicillium fungus.

Over the last nine decades, the overuse of and misuse of antibiotics has severely hindered the efficacy of present-day antibiotics, and has led to a drastic increase in antibiotic resistant bacteria for which the medical community has no treatment (CDC 2014a). Currently, there are hundreds of drugs including antibiotics, anti-tumors, anti-fungals, and anti-inflammatories that are either entirely derived from plants or are inspired by specific plant compounds. Qinghao is listed in the traditional Chinese text *Fifty-two remedies* circa 200 B.C.E. as an anti-fever agent and in the traditional Chinese text *Compendium of Materia Medica* circa 1500s as an anti-malarial tea (Weiyuan 2009). In 2009, Artemisinin, a compound derived from qinghao, was created as an artemisinin-based therapy for malaria and is currently on the market as Coartem. Due to the success of traditional Chinese medicine (TCM) and a lack of novel antibiotics, researchers are beginning to focus their efforts

towards finding a modern day *magic bullet* from extracts obtained from plants used in traditional Chinese medicine.

Drug Resistance

From 1995-1998, penicillin resistance in *Streptococcus pneumoniae* rose form 21% to 25%, and multidrug resistance increased from 9% to 14% (Whitney *et al.* 2001), implying that pneumococcal resistance to penicillin is strongly correlated with resistance to additional drugs (Schrag *et al.* 2004). The PCV13 vaccine was introduced in 2010 to combat the emergence of six new, pathogenic serotypes of *S. pneumoniae*; however, only one year later, resistance to PCV13 had already been documented (Azzari 2014). A major concern for the treatment of *S. pneumoniae* is the increasing antibiotic resistance, particularly with serotypes for which vaccines do not exist (Reynolds *et al.* 2012). A similar situation exists for *Neisseria gonorrhoeae* which is resistant to penicillin, as well as to a host of other drugs.

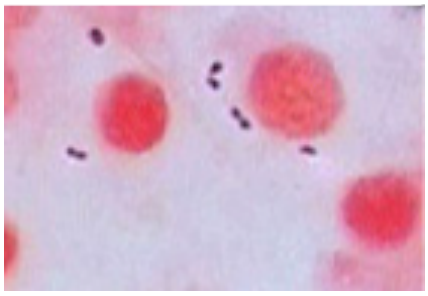
N. gonorrhoeae has been shown to exhibit resistance to penicillins and tetracyclines; however, third-generation cephalosporins like ceftriaxone, have yet to display resistance (Tapsall 2001). Unfortunately, third-generation cephalosporins are expensive, limiting their availability to areas with the highest rates of gonorrhea, the impoverished regions of developing countries. Due to the lack of vaccines or new antibiotics for *N. gonorrhoeae*, gonococcal diseases are on the path to becoming untreatable (WHO 2015).

Bacterial Characteristics

S. pneumoniae and *N. gonorrhoeae* are classified as fastidious bacteria.

Fastidious bacteria require additional nutrients in their culture medium and environment in order to grow. Due to these factors, fastidious bacteria can be difficult to culture in a laboratory setting; however, these microorganisms are abundant, and some are even part of the indigenous microbiota. *S. pneumoniae* is one such organism and is typically found in the oronasopharyngeal membranes, yet is known to be pathogenic (Todar 2012). *S. pneumoniae* is a gram-positive, lancet-shaped diplococcus, with each independent cell measuring between 0.5 to 1.25 μm in length (Figure 1). Illnesses caused by *S. pneumoniae* fall under the umbrella term Pneumococcal Disease. *S. pneumoniae* is known to be the leading cause of community-acquired pneumonia, as well as a host of other maladies including otitis media, sinusitis, bacteremia, and bacterial meningitis (CDC 2014b).

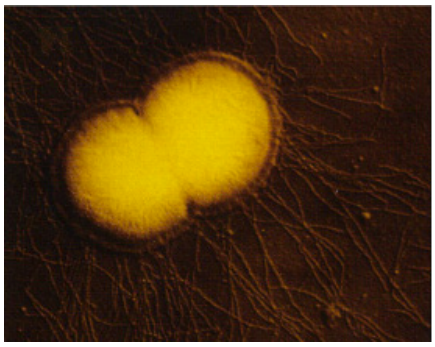
Pneumococcal diseases can be transmitted through the air and through close, non-sexual contact. Despite vaccination efforts in the young and old, this bacterium is still responsible for over four million cases of illness in the U.S. (Reynolds *et al* 2012), and together with influenza, is ranked as the eighth leading cause of death in the United States. (Assaad *et al.* 2014).



CDC http://www.cdc.gov/meningitis/lab- manual/chpt06-culture-id.html

Figure 1: Magnified gram stain of *S. pneumoniae*

Unlike *S. pneumoniae, N. gonorrhoeae* do not constitute part of the indigenous microbiota. The fastidious bacterium *N. gonorrhoeae* is a gram-negative, diplococcus bacterium ranging in size of 0.6 to 1.0 μm in diameter with multiple fimbriae extending from the soma (Figure 2). These bacteria are exclusively found on or near mucus membranes in the conjunctiva, oropharynx, anorectal area, and genitalia. *N. gonorrhoeae* is responsible for, neonatal conjunctivitis, the sexually transmitted infection gonorrhea, and other disorders (Tille 2014). The existence of antimicrobial resistance significantly threatens successful treatments of both *S. pneumoniae* and *N. gonorrhoeae.*



http://textbookofbacteriology.net/neisseria.html

Figure 2: Scanning electron microscopy image of *Neisseria gonorrhoeae* with fimbriae.

Current Study

The plant extracts provided by Guangxi Botanical Garden of Medicinal Plants (GBGMP) in Nanning, China have been used for thousands of years to treat a variety of diseases in China, including bacterial infections related to both pneumonias and sexually-transmitted infections (Phillips 2012, Matsuoka 1989). The overall goal of

this study was to identify extracts from plants with antibacterial activity for *S. pneumoniae* and *N. gonorrhoeae* using methods outlined by the clinical microbiology laboratories to measure resistance to known antibiotics. For this study, a cell viability assay using the reagent PrestoBlue was also developed for *S. pneumoniae*, as it has not been traditionally used due to the presence of lysed horse blood in the culture medium.

Streptococcus pneumoniae

S. pneumoniae was screened against 120 plant extracts from 22 plants grown in the GBGMP to assess their potential antibacterial properties. Promising candidates were then assayed using a broth microdilution assay to determine the minimum inhibitory concentration (MIC). Using the same data, the MIC_{50} was calculated.

Neisseria gonorrhoeae

N. gonorrhoeae was screened against 120 plant extracts from 22 plants grown in the GBGMP to assess their potential antibacterial properties. No promising anti-*Neisseria* candidates were found.

II. METHODS

The culturing of bacteria, preparation of extracts and controls, and incubation times were the same for both assays performed.

Procedures for *Streptococcus pneumoniae*

Culturing

S. pneumoniae was purchased from the American Type Culture Collection (*S. pneumoniae* ATCC 49136). *S. pneumoniae* was cultured on trypticase soy agar containing 5% sheep blood (BAP; Becton-Dickinson), and incubated for 18-24 h at 37°C and 5% carbon dioxide. *S. pneumoniae* was subcultured weekly.

Preparation for Assay

Bacteria. Antibacterial testing for *S. pneumoniae* was done following the Clinical Laboratories Standard Institute guidelines for aerobic bacteria (CLSI 2009) using a 96-well, black walled plate. *S. pneumoniae* was cultured 18-24 h before each assay as outlined above.

To prepare *S. pneumoniae* for assay, colonies from the overnight BAP were resuspended in trypticase soy broth (TSB; Becton Dickinson). The turbidity, or optical density, of the suspension was assessed using a GeneQuant UV/visible spectrum spectrophotometer (GE Healthcare) at a wavelength of 600 nm. The optical density, OD_{600}, was adjusted to 0.1 ± 0.05 by further diluting the suspension in TSB. Once a solution of the desired OD_{600} was obtained, the solution was diluted to a 1:20 ratio in Cation-Adjusted Mueller Hinton broth (CAMHB; Becton-Dickinson). The suspension was then adjusted to contain 2.5% lysed horse blood (LHB;Remel) (CLSI 2009).

Extracts. Plant extracts for both *S. pneumoniae* and *N. gonorrhoeae* were provided by GBGMP. The extracts were prepared in Nanning, China by grinding whole plants and part of plants and then fractionating out crude extracts using a host of solvents: water, petroleum ether, ethyl acetate, ethanol, and chloroform. The crude extracts were then resuspended to a concentration of 10 mg/mL in pure dismethylsulfoxide (DMSO).

Plant extracts were prepared by diluting a 10 mg/mL stock solution of plant extract using a 1:100 ratio in a CAMHB and bacteria mixture. To achieve this ratio, 3 μL of plant extract were added to 267 μL of CAMHB. Then, 30 μL of a 1:20 dilution of bacteria in CAMHB + 2.5% LHB were added to the 270 μL for a final plant extract concentration of 100 μg/mL, and a final bacterial concentration of 5×10^5 CFU/mL. A volume of 90 μL of the extract solution was added to each individual well of a set of triplicate wells on a 96-well black walled plate. Each well contained 9 μg of extract solution and 4.5×10^4 CFU/mL (Figure 3). This entire process was repeated for each extract (CLSI 2009) in at least two independent experiments.

Controls. Appropriate controls were set up similarly to the plant extracts, substituting 3 μL of the control solution for the extract. A DMSO control was implemented to observe any effect on bacterial growth. Tetracycline was used as a positive killing control. To quantitatively measure inhibition, a bacterial control was made of 30 μL of the 1:20 dilution of bacteria suspended in CAMHB + 2.5% LHB, 270 μL of CAMHB, and no extract. A media control was implemented to negate

background fluorescence and turbidity of CAMHB + 2.5% LHB. The media control constituted of 270 µL of CAMHB and 30 µL CAMHB + 2.5% LHB with no bacteria.

Initial Screening Assay

The inhibitory activity of extracts against *S. pneumoniae* was assessed using a modified PrestoBlue cell viability assay. A volume of 90 µL of extract solution plus prepared bacteria was placed in an individual well of a series of triplicate wells. Each plate contained a row of controls as outlined in Figure 3. The plate was quick spun in a Sorvall Legend X1 centrifuge to mix all reagents together before being placed in a humidified incubator for 18-24 h at 37°C with 5% carbon dioxide. After the initial incubation, the plate was removed and the optical density was measured from 555 – 585 nm. A volume of 9 µL of PrestoBlue (Life Technologies) was added to each well, and the plate was returned to the incubator for 1 h. After the second incubation, the plate was removed and data was collected using a PrestoBlue cell viability assay (described below).

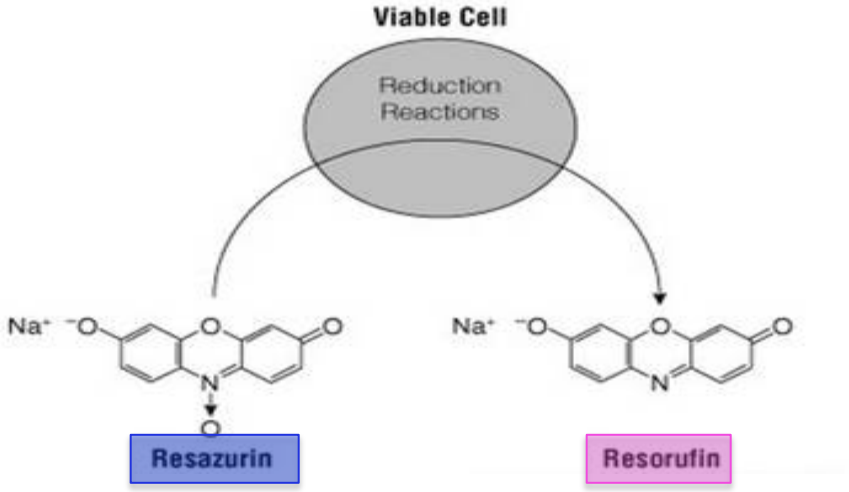
	1	2	3	4	5	6	7	8	9	10	11	12
A	← Extract A →			← Extract B →			← Extract C →			← Extract D →		
B	← Extract E →			← Extract F →			← Extract G →			← Extract H →		
C	← Extract I →			← Extract J →			← Extract K →			← Extract L →		
D	← Extract M →			← Extract N →			← Extract O →			← Extract P →		
E	← Extract Q →			← Extract R →			← Extract S →			← Extract T →		
F	← Extract U →			← Extract V →			← Extract W →			← Extract X →		
G	← Extract Y →			← Extract Z →			← Extract AA →			← Extract BB →		
H	← BACTERIA →			← DMSO →			← TETRACYCLINE →			← MEDIA →		

Figure 3: Sample 96-well plate set up for initial screening of multiple plant extracts against *S. pneumoniae.*

Cell Viability Assay

The PrestoBlue assay was used to determine the metabolic activity of the treated bacteria. Metabolically active cells reduce resazurin, a non-fluorescent blue dye, to resorufin, a fluorescent, pink dye using redox reactions in various cellular metabolic processes (Figure 4). The PrestoBlue assay was performed following the turbidometric measurement.

After the initial 18- 24 h incubation and measuring the turbiodmetric measurement at 625nm, 10 µL of PrestoBlue reagent were added to each well. The plate was then returned to the incubator for 1 h to allow time for the cells to incorporated and reduce the reagent. After 1 h, the absorbance was measured using a spectrophotometer set at a wavelength span of 555 – 585 nm, the wavelength at which resorufin fluoresces.



http://www.pharmacelsus.de/resazurin_assay

Figure 4: Reduction of resazurin dye (non-fluorescent) to resorufin dye (fluorescent).

Broth Microdilution Method

The purpose of this assay was to determine the lowest dose of extract to yield at least 50% inhibition of *S. pneumoniae.* For this assay, four extracts were tested per plate. The volume of the first row was double to 180 µL. Rows B-G were initially filled with 90 µL of media (CAMHB). A volume of 90 µL was taken from each successive row and mixed with the following row until a concentration of 1.563 µL/mL was obtained (Figure 4). The plate was then treated in a similar manner as for the Initial Screening Assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	← Extract A 180 µL/mL →			← Extract B 180 µL/mL →			← Extract C 180 µL/mL →			← Extract D 180 µL/mL →		
B	← 50 µL/mL →			← 50 µL/mL →			← 50 µL/mL →			← 50 µL/mL →		
C	← 25 µL/mL →			← 25 µL/mL →			← 25 µL/mL →			← 25 µL/mL →		
D	← 12.5 µL/mL →			← 12.5 µL/mL →			← 12.5 µL/mL →			← 12.5 µL/mL →		
E	← 6.25 µL/mL →			← 6.25 µL/mL →			← 6.25 µL/mL →			← 6.25 µL/mL →		
F	← 3.125 µL/mL →			← 3.125 µL/mL →			← 3.125 µL/mL →			← 3.125 µL/mL →		
G	← 1.563 µL/mL →			← 1.563 µL/mL →			← 1.563 µL/mL →			← 1.563 µL/mL →		
H	← BACTERIA →			← DMSO →			← TETRACYCLINE →			← MEDIA →		

Figure 5: Sample 96-well plate set up for screening of plant extracts in a Broth Microdilution Assay.

Data Analysis

All calculated data were at capped at 100% and -100% inhibition. Negative inhibitions reflect extracts that aided in bacterial growth.

Turbidometric Measurement. The purpose of this measurement was to exploit the red pigmentation of the LHB and observe any effects the extracts had on bacterial

growth. After the initial 18-24 h incubation of *S. pneumoniae,* a turbiodmetric was measured with a spectrophotometer at 625 nm, the wavelength of the color red.

Minimum Inhibitory Concentration. The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after an 18-24h incubation (Andrews 2001). In this experiment, the extracts served as the antimicrobial agent. MICs were determined for extracts exhibiting greater than 80% inhibition and less than 10% toxicity against an L6 mammalian cell model (Newsome lab, MTSU, personal communication). In most cases, the exact MIC was not determined, but can be assumed to be less than 1.563 μg/mL, which was the lowest concentration of extract used for this study.

Minimum Bactericidal Concentration. The minimum bactericidal concentration (MBC) is the lowest dose exhibiting 99.9% inhibition of the bacteria inoculum (Levison and Levison 2009). Drugs with an MBC less than four-fold of the MIC are considered bactericidal and kill the microorganism (Levison and Levison 2009). Conversely, drugs with MBCs far greater, more than four-fold than their MICs, are considered bacteriostatic (Levison and Levison 2009). The MBC was determined for extracts exhibiting greater than 80% inhibition and less than 10% toxicity (for L6 cells) using the percent inhibition of *S. pneumoniae* calculated from the turbiodmetric measurements. When MBCs could not be determined or if the MBC was much larger than the MIC, the extract was considered bacteriostatic.

Statistics

Standard Deviation. The standard deviation (SD) between each individual well in triplicate was calculated to ensure that each individual well of the triplicate wells were relatively equal.

Standard Error of the Mean. The standard error of the mean (SEM) was calculated to assess how the mean of a triplicate well would vary in sequential experiments using the same initial concentrations of media and extract.

Relative Standard Error. The relative standard error (RSE) was calculated to determine how likely each triplicate well was to deviate from the actual bacterial population. Large RSEs have greater sampling error. RSEs greater than 0.2 were used with caution

Dixon Quotient Minimum and Dixon Quotient Max Test. The Dixon Q Min and Dixon Q Max were calculated to mathematically identify and reject an outlier within the triplicate wells. The calculated value was compared to a 95% confidence level (CL). Individual wells flagged at 0.95 or greater were eliminated from the data set.

Z-Factor. The Z-factor measures the magnitude of the effect size between the positive killing control (tetracycline) and the negative control (bacteria plus media). The Z-factor acts as a quality control and offers a means to validate the results. Z-factor values do not exceed 1.0. Values between 0.5 – 1 represent quality results,

while scores less than 0.5 indicate inaccurate readings. Assays with initial Z-scores less than 0.5 were evaluated and optimized by removing outliers as appropriate to their Dixon Q Min and Dixon Q Max scores. Data with Z-factors of 0.5 or below were not used.

Percent Inhibition. The percent inhibition was calculated using plate data in units of relative fluorescence units (RFUs) for the cell viability assay. The mean of the triplicate wells was calculated and then the mean from the plate blank was subtracted from the controls and extract means. The adjusted mean RFUs for the extract were subtracted from the adjusted mean RFUs emitted from the bacterial control, divided by the RFUs from the bacterial control, and then multiplied by 100.

Procedures for *Neisseria gonorrhoeae*

Culturing

N. gonorrhoeae was purchased from the American Type Culture Collection (*N. gonorrhoeae* ATCC 49226). *N. gonorrhoeae* was cultured on chocolate agar (Becton-Dickinson) and incubated for 18-24 h at 37°C and 5% carbon dioxide. *N. gonorrhoeae* was subcultured weekly.

Preparation for Antibacterial Testing Assay

Bacteria. Antibacterial testing for *N. gonorrhoeae* was done following the Clinical Laboratories Standard Institute guidelines for the disk diffusion method (CLSI 2007). *N. gonorrhoeae* was cultured 18-24 h before each assay as outlined above. To prepare

N. gonorrhoeae for the assay, 3-5 colonies were resuspended in CAMHB and adjusted to an OD_{600}, of 08 – 1.15 in a similar manner as done for *S. pneumoniae*.

Extracts and Controls. Plant extracts and controls were prepared on sterile 6 mm filter paper disks (Fisher Scientific) in 1:10 CAMHB. A 1 µL volume of extract was added to the paper disk followed by 9 µL of CAMHB. The filter paper disks were allowed to dry for 10 min in a covered, sterile, petri-dish. The DMSO control was prepared similarly. A tetracycline disk impregnated with 30 µL of tetracycline (Becton-Dickinson) served as a positive killing control. A disk with 10 µL of CAMHB served as the media control.

Antibacterial Testing Assay

Disk Diffusion Method. The purpose of this assay was to qualitatively determine if extracts inhibited the growth of *N. gonorrhoeae*. A volume of 0.1 L of the correctly prepared OD_{600} solution was added to each chocolate agar plate. The prepared disks were then laid facedown onto the GC agar. The plates were incubated 18-24 h and then observed for zones of inhibition (Figure 6).

Plates were checked for a zone of inhibition of bacterial growth around the filter paper disk impregnated with the extract. Extracts inhibiting the growth of *N. gonorrhoeae* with zones greater than or equal to 15 mm in diameter (including the 6 mm disk) would have been tested further using two-fold dilutions of the extract. The decision for the acceptable size of the zone of inhibition was derived from the minimal zone of inhibition described for antibiotic sensitivity testing of *N.*

gonorrheae by the disk diffusion method to consider *N. gonorrheae* susceptible (CLSI 2007).

http://www.cram.com/flashcards/microbiology-1553744

Figure 6: Disk diffusion method with no inhibition (left) and inhibition (right).

Data Analysis

III. RESULTS

Streptococcus pneumoniae

In this study, 120 extracts from 22 plants used in TCM were assayed for activity against *S. pneumoniae.* Prior to determining the MIC of plant extracts, parameters were established at 80% bacterial inhibition and less than 10% toxicity. Extracts were initially categorized based upon inhibitory effects and toxicity against a mammalian L6 cell model (immortalized rat, *Rattus norvegicus* skeletal muscle cells, ATCC CRL-1458). Extracts exhibiting less than 80% inhibition at 100 µg/mL were not assayed further. Select extracts exhibiting greater than 10% toxicity were further tested using the broth microdilution assay to determine the MIC. Figure 7 is a

summary of the amount of inhibitory activity of the 120 extracts at 100 µg/mL. Numerical data can be found in Appendix I.

Of the 120 extracts, 54 were found to have greater than 80% inhibition and less than 10% toxicity in the cell viability assay. Before the Z-factor calculation was done, 28 of the 54 extracts were chosen for further testing. After the Z-factor was performed, 26 additional extracts were found in this category. Based upon the ratio of MIC to MBC, the extracts were tentatively categorized as either bactericidal or bacteriostatic. Figure 8 summarizes the assaying and categorization of the 120 extracts.

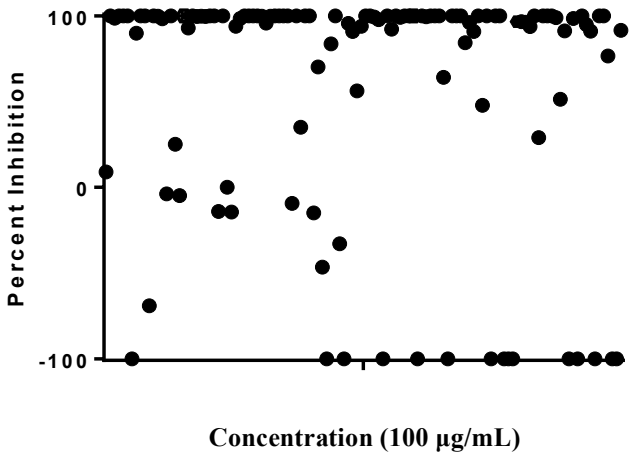


Figure 7: Percent inhibition of the 120 TCM plant extracts. Negative percentages represent extracts that aided in bacterial growth.

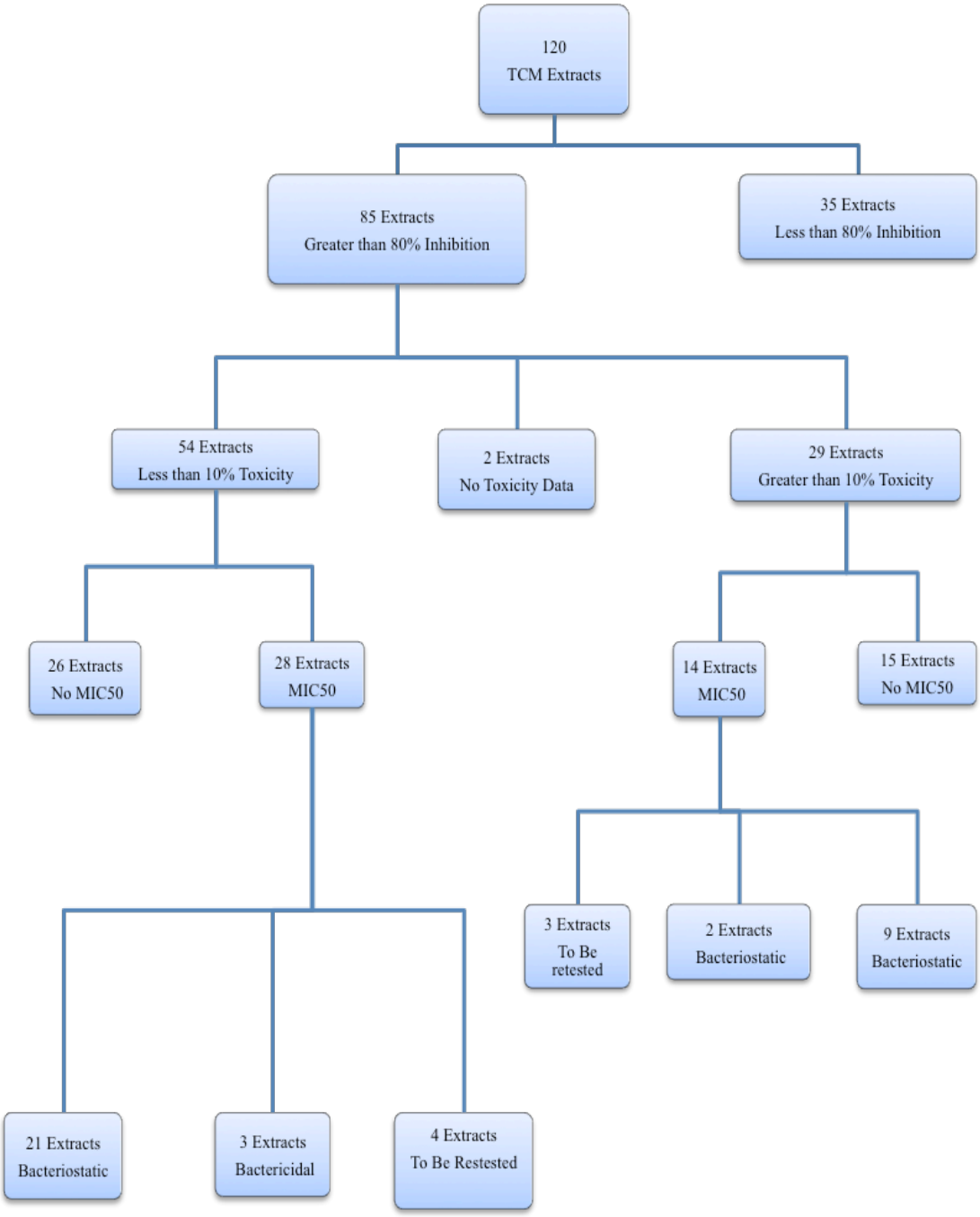


Figure 8: Summary of how the 120 extracts were categorized and assayed. Extracts that exhibited confounding results at 100 µg/mL were labeled as to be retested.

The 28 extracts chosen before the Z-factor calculation were further tested using the microdilution assay. Table 1 shows the percent inhibition and toxicity data at 100 μg/mL. The percent inhibitions were rounded to the nearest whole number. Negative percent toxicities are representative of extracts that aided in mammalian cell growth and were not harmful to the mammalian cell. The specific 28 extracts are presented in Table 2.

Extracts with greater than 10% toxicity were assumed to be too toxic to purpose as a drug candidate or an antiseptic. However, these extracts may contain a compound(s) that can be used as a disinfectant. Of the extracts, 29 had greater than 80% inhibition and greater than 10% toxicity (Figure 8). The specific extracts are presented in Table 3.

Table 1: 28 Extracts with greater than 80% and less than 10% during the cell viability assay. These extracts were chosen to undergo a microdilution assay to determine the MIC. Negative percent toxicities indicate extracts that aided in mammalian cell growth.

Extract	Percent Inhibition 100 µg/mL	Percent Toxicity 100 µg/mL
6A	99	-0.12
9A	>100	-0.19
15A	>100	-1.66
19A	100	0.59
21A	99	-2.65
12B	100	5.24
17B	100	1.46
2C	>100	3.91
12C	>100	-4.85
13C	>100	-2.72
14C	>100	4.03
15D	84	4.03
ANB	94	-3.06
ANC	>100	-1.56
BEB	98	1.9
CP1	>100	6.74
CP4	>100	4.34
CT1	>100	3.1
CT2	100	-2.29
DEB	>100	-2.32
DEC	>100	4.34
FLB	>100	-8.58
LP3	>100	-3.53
MAA	97	-2.2
PAD	99	-1.78
RIB	>100	-5.5
RID	91	-7.43
SB2	>100	4.35

Table 2: The 28 extracts tentatively categorized into three categories based upon the ratio of the MIC to the MBC. Numerical data can be found in Appendix I.

Bacteriostatic	Bactericidal	To Be Retested
6A	CP4	2C
9A	CT2	13C
15A	LP3	15D
19A		ANB
21A		
12B		
17B		
12C		
14C		
ANC		
BEB		
CP1		
CT1		
DEB		
DEC		
FLB		
MAA		
PAD		
RIB		
RID		
SB2		

Table 3: The 14 extracts with greater than 80% inhibition and 10% toxicity. These extracts were assayed using the microdilution assay to determine the MIC and MBC. Classifications are tentative. Numerical data can be found in Appendix I.

Bacteriostatic	Bactericidal	To Be Retested
10A	CT4	19D
10B	CT5	20D
AND		BED
CT3		
DED		
LP1		
MAD		
MDZ1		
RIC		

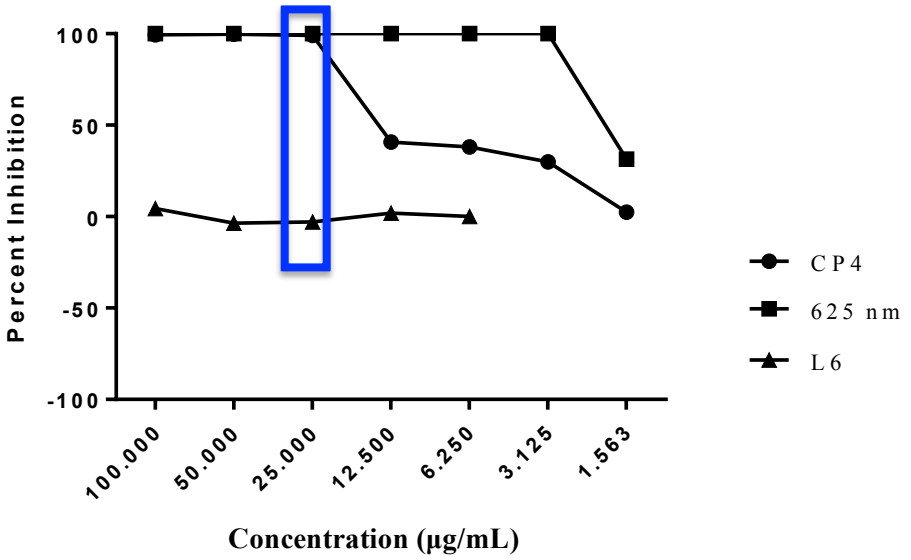
There were a total of 39 extracts that need to be tested using the microdilution assay. After the Z-factor was calculated and outliers were removed, 24 extracts with greater than 80% inhibition and less than 10% toxicity were added to the previous list of extracts before the Z-factor calculation. Similarly, after the Z-factors were calculated, 15 extracts with greater than 80% inhibition and greater than 10% toxicity were added. The exact extracts are listed in Table 4.

Table 4: Extracts with greater than 80% inhibition and either less than or greater than 10% toxicity to be tested. These extracts were found to have higher inhibition than initially thought after the Z-factor was calculated and outliers were eliminated.

Less than 10% Toxicity	Greater than 10% Toxicity
7A	4A
12A	14A
11B	18A
1C	2B
2C	7B
17C	9B
18C	6C
20C	11C
1D	16C
2D	19C
4D	21C
6D	16D
8D	CP3
BEA	MDZ4
CP2	MDZ5
CP5	
EUA	
EUB	
EUC	
EUD	
MAB	
MAC	
MDZ3	
MDZ6	
PAB	
SB3	

Figures 9-11 show the cell viability, turbidometric measurement, and the toxicity data. In the legend, the extract name is graphically shown as circles and represents the cell viability assay. The squares are titled 625nm, the wavelength at which the spectrophotometer was set, and represent the turbidometric measurement. The toxicity data is shown by triangles and is titled L6. The cell viability and turbidometric data were measured using a series of two-fold dilutions starting at 100 μg/mL and ending at 1.563 μg/mL. The toxicity data was measured from 100 μg/mL to 6.25 μg/mL in a similar manner. It is unknown why the toxicity data was not diluted down to a concentration of 1.563 μg/mL, as the data was obtained through a personal contact.

The average percent inhibition of extract CP4 at 100 μg/mL, over four different experiments was 102.33 ± 3.09% with a standard error of the mean of 1.55%. The average MIC was found at the concentration 6.25 μg/mL. The MIC_{50} was 12.50 μg/mL. The positive killing control, tetracycline, had a 99.76 ± 0.28% percent inhibition, showing that the bacteria could be inhibited (Figure 9b). The MBC was found to be at a concentration of 25 μg/mL with a 99.51 ± 0.60% inhibition.



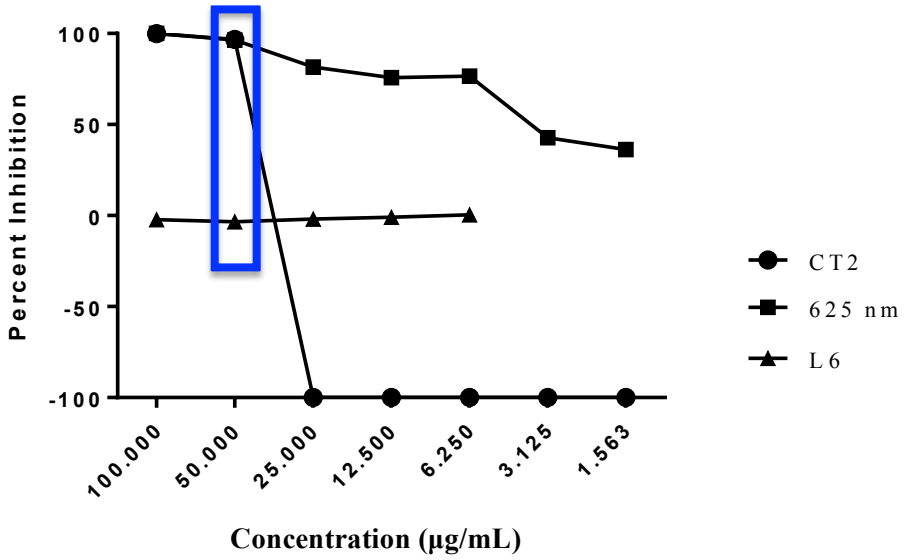
a)

CP4											
DILUTIONS ug/mL	100	50	25	12.5	6.25	3.125	1.5625	BACTERIA	DMSO	TETRACYCLINE	MEDIA
TRIALS											
Trial 1	106.67										
Trial 2	101.83										
Trial 1*	99.35	99.83	99.09	40.77	38.10	29.97	2.40	0.00	76.73	99.95	97.16
Trial 2*	101.47	100.81	99.94	98.03	52.33	-219.44	-230.29	0.00	95.41	99.56	88.32
Standard Deviation	3.09	0.69	0.60	40.49	10.06	176.36	164.54	0.00	13.21	0.28	6.25
Standard Error of the Mean	1.55	0.49	0.42	28.63	7.11	124.71	116.35	0.00	9.34	0.20	4.42
Average Percent Inhibition	102.33	100.32	99.51	69.40	45.21	-94.74	-113.94	0.00	86.07	99.76	92.74

b)

Figure 9: a) Percent inhibition of *S. pneumoniae* using two-fold dilutions of extract CP4 from 100 to 1.563 µg/mL. Percent inhibitions were calculated from the average of three replicate wells and the average of three replicate control wells, thus no error bars are depicted on the graph. The blue bar represents the MBC. b) Trials 1 and 2 are the initial assay and Trials 1* and 2* are the microdilution data.

The average percent inhibition of extract CT2 at 100 μg/mL, over four different experiments was 104.27± 7.84% with a standard error of the mean of 3.92%. The average MIC was found at the concentration 50 μg/mL. The MIC_{50} was also 50 μg/mL. Tetracycline had a percent inhibition of 108.55 ± 10.08%. The MBC was found to be at a concentration of 50 μg/mL with 106.47 ± 13.63% inhibition (Figure 10).



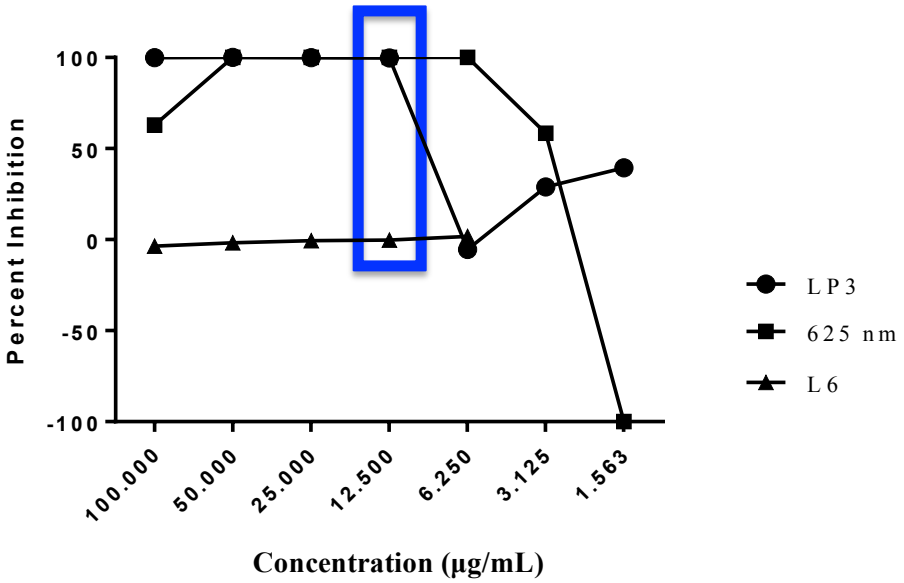
a)

CT2											
DILUTIONS ug/mL	100	50	25	12.5	6.25	3.125	1.5625	BACTERIA	DMSO	TETRACYCLINE	MEDIA
TRIALS											
Trial 1	101.71										
Trial 2	99.54										
Trial 1*	99.89	96.83	-388.41	-384.24	-343.52	-182.38	-217.32	0.00	-11.96	101.42	82.49
Trial 2*	115.94	116.11	103.72	73.97	40.17	20.74	16.69	0.00	6.40	115.67	76.37
Standard Deviation	7.84	13.63	347.99	324.00	271.31	143.63	165.47	0.00	12.98	10.08	4.33
Standard Error of the Mean	3.92	9.64	246.06	229.11	191.84	101.56	117.00	0.00	9.18	7.13	3.06
Average Percent Inhibition	104.27	106.47	-142.35	-155.14	-151.68	-80.82	-100.32	0.00	-2.78	108.55	79.43

b)

Figure 10: a) Percent inhibition of *S. pneumoniae* using two-fold dilutions of extract CT2 from 100 to 1.563 µg/mL. Percent inhibitions were calculated from the average of three replicate wells and the average of three replicate control wells, thus no error bars are depicted on the graph. The blue bar represents the MBC. b) Trials 1 and 2 are the initial assay and Trials 1* and 2* are the microdilution data.

The average percent inhibition of extract LP3 at 100 μg/mL, over three different experiments was 101.15± 1.04% with a standard error of the mean of 0.52%. The average MIC was found at the concentration 25 μg/mL. The MIC_{50} was at the concentration of 12.50 μg/mL. Tetracycline had a percent inhibition of 99.76 ± 0.28%. The MBC was found to be at a concentration of 12.50 μg/mL with 94.38 ± 7.52% inhibition (Figure 11).



a)

LP3											
DILUTIONS ug/mL	100	50	25	12.5	6.25	3.125	1.5625	BACTERIA	DMSO	TETRACYCLINE	MEDIA
TRIALS											
Trial 1	101.89										
Trial 2	---										
Trial 1*	99.95	100.20	99.93	99.70	-5.43	28.90	39.40	0.00	76.73	99.95	97.16
Trial 2*	101.60	100.06	97.66	89.06	-150.68	-239.56	-138.37	0.00	95.41	99.56	88.32
Standard Deviation	1.04	0.10	1.60	7.52	102.71	189.83	125.70	0.00	13.21	0.28	6.25
Standard Error of the Mean	0.52	0.07	1.13	5.32	72.62	134.23	88.89	0.00	9.34	0.20	4.42
Average Percent Inhibition	101.15	100.13	98.80	94.38	-78.05	-105.33	-49.48	0.00	86.07	99.76	92.74

b)

Figure 11: a) Percent inhibition of *S. pneumoniae* using two-fold dilutions of extract LP3 from 100 to 1.563 µg/mL. Percent inhibitions were calculated from the average of three replicate wells and the average of three replicate control wells, thus no error bars are depicted on the graph. The blue bar represents the MBC. b) Trials 1 and 2 are the initial assay and Trials 1* and 2* are the microdilution data.

Neisseria gonorrhoeae

No extracts were found to inhibit *N. gonorrhoeae*. Possible explanations are offered in the discussion section.

IV. Discussion

Streptococcus pneumoniae

There exists a strong, positive correlation between resistance and the overuse of antibiotics. In 2000, Tennessee ranked 20% higher than the national average in its distribution of antibiotics (Briles *et al* 2005). The effects of the continued misuse of antibiotics in Tennessee are shown in Figure 12, and as of 2015, *S. pneumoniae* multi-drug resistance to common antibiotics, such as erythromycin, clindamycin, tetracycline and penicillin remain on the rise and have been reported as high as 95.9%, 94.5%, 87.7%, and 45.2 %, respectively (Lu *et al* 2015). The alarming resistance rates are a call for novel antibiotics.

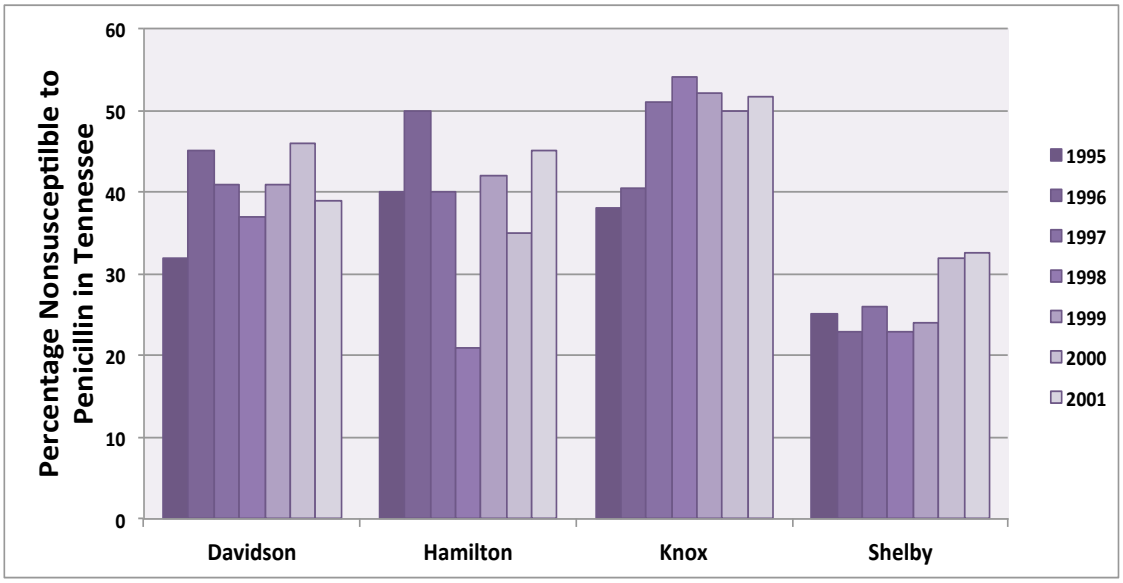


Figure 12: Percentage of invasive, non-susceptible *S. pneumoniae* isolates for four metropolitan counties in Tennessee from 1995 -2001.

Of the 120 extracts assayed, 54 extracts of plants used in traditional Chinese medicine exhibited greater than 80% inhibition of *S. pneumoniae* and less than 10% toxicity against the mammalian cell model, L6, at the concentration of 100 µg/mL. Of those extracts, 28 were selected for further testing using the broth microdilution assay to determine the MIC or MIC_{50} (Table 1).

Effective antibacterial drugs are categorized as either bactericidal or bacteriostatic. Bacteriostatic drugs require the implementation of host defenses to clear an infection. If an area is void of host defenses, lower in defense concentrations, as is the case for cerebral spinal fluid, or if the host is unable to fully clear the infection, then the microorganism will return as soon as the antibiotics are stopped. Conversely bactericidal antibiotics do not rely on host defenses and are able to fight an infection independently. Based upon the MICs and MBCs, the extracts were tentatively divided into two classifications, bacteriostatic or bactericidal (Table 2).

The plants that the three extracts were tentatively identified as bactericidal (Table 2) are known to have flavonoids. It has been found that flavonoids, plant metabolites that have known health benefits, can cause bacterial cells to aggregate. The high inhibitions obtained may be wholly due to actual inhibitory effects or may be, in part, due to the aggregatory effect of potentially present flavonoids (Cushine *et al* 2007).

Bactericidal Extracts

Extracts CP4, CT2, and LP3 all have flavonoid compounds, which are polyphenolic phytochemicals found in various plants that are responsible for yellow, red, and blue floral pigmentation. Flavonoids have been previously reported to bactericidal or bacteriostatic inhibit by inducing damage to the cytoplasmic membrane, blocking metabolism, and/or preventing nucleic acid synthesis (Ahmad *et al.* 2015). Further testing should consist of at least three replicates of the microdilution assay to ensure consistency and bioassay guided fractionation to identify pure compounds.

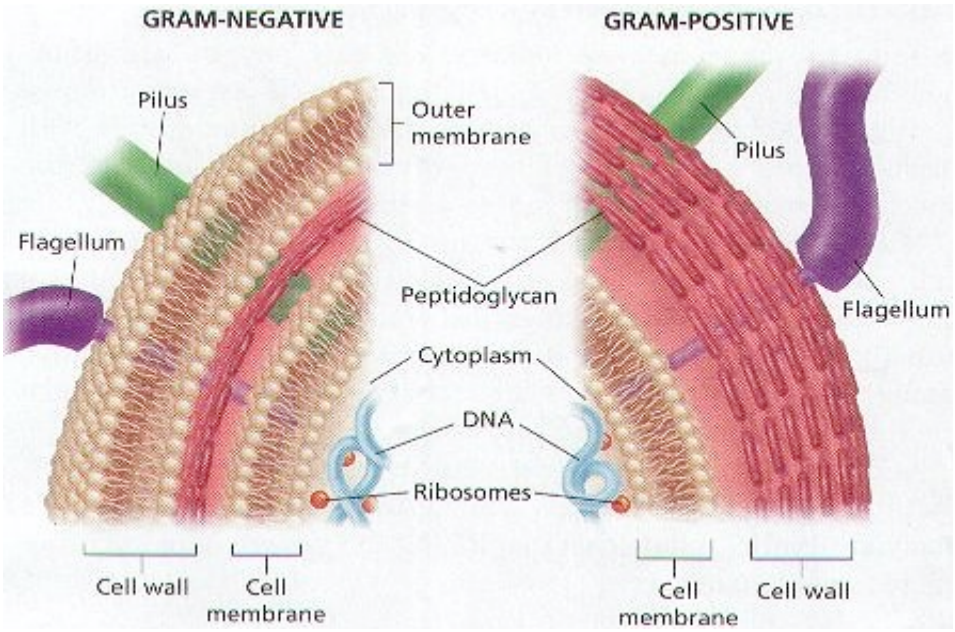
Currently, no data exists for the bioactivity of the plant from which CP4 was extracted against *S. pneumoniae.* No antibacterial activity has been noted for extract LP3, however, CT2 has already been published for antibacterial properties against *Pseudomonas aeruginosa*, *E.coli*, and *Staphylococcus aureus* (Sripriya 2014).

Bacteriostatic Extracts

In *in vitro* studies, bactericidal drugs are preferred over bacteriostatic drugs. However, the dichotomy is of little importance in the actual treatment of gram-positive bacteria in otherwise healthy individuals (Pankey and Sabath 2003). A total of 30 extracts were found to be bacteriostatic against *S. pneumoniae*, 21 of which had less than 10% toxicity. The other nine extracts had greater than 10% toxicity (Figure 8).

Neisseria gonorrhoeae

Of the 120 extracts assayed, none showed inhibition against *N. gonorrhoeae* using the disk diffusion assay. One hypothesis for this result lies within the differing chemical composition of gram-positive and gram-negative bacteria. Gram-positive bacteria have a thick peptidoglycan wall, which can absorb a large amount of foreign material. Conversely, gram-negative bacteria have a thinner peptidoglycan wall sandwiched between two membranes, the outer membrane and the cytoplasmic membrane (Figure13). The difference in cell walls and their strengths are analogous to concrete and Kevlar (Schaalije 2013). Concrete, though dense and thick, is easily cracked, while Kevlar is thin and nearly impenetrable. Gram-negative cell walls are tough, elastic, and strong (Beveridge 1999) and are consequently more difficult to destroy. The extracts may not have been able to destroy or penetrate the gram-negative wall of *N. gonorrhoeae.*



http://www.mdpi.com/1424-8247/6/12/1451/htm

Figure 13: Gram-negative and gram-positive bacteria cell wall.

Additionally, *N. gonorrhoeae* is not culturable in liquid media, thus microdilution assays are not possible as with *S. pneumoniae.* For testing of *N. gonorrhoeae,* the plant extracts were all dissolved in DMSO and added to filter paper disks. The compounds in the extract may be too large or nonpolar to readily diffuse across the agar surface and inhibit the *N. gonorrhoeae*. A broth or semisolid method to measure antibacterial activity of plant extracts may have to be developed to test the extracts against *N. gonorrhoeae.*

Summary

Although no extracts from plants used in traditional Chinese medicine were identified as having antibacterial activity for *N. gonorrhoeae*, 28 of the extracts were antibacterial for *S. pneumoniae*. Three of the extracts had bactericidal potential, 21 had bacteriostatic potential, and four extracts had confounding results and need to be retested. Further testing should include bioassay guided fractionation to identify pure compounds from the crude extracts.

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APPENDICES

Appendix I: All Assayed Extracts with Microdilution Assay and Toxicity Data

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L 6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
2A	9.02	-1.98	-	-	-	-	-	-	-	**2A**	-1.98	-0.87	-0.47	0.64	1.64
4A	103.33	62.05	-	-	-	-	-	-	-	**4A**	62.05	-5.36	-1.54	0.18	-0.44
6A	98.77	-0.12	116.18	115.80	115.03	89.11	54.64	18.75	16.00	**6A**	-0.12	-1.26	1.78	-0.85	-1.15
7A	103.46	-2.38	-	-	-	-	-	-	-	**7A**	-2.38	-5.13	-5.18	3.21	-0.65
9A	103.41	-0.19	99.99	100.05	100.14	47.92	47.88	49.01	52.82	**9A**	-0.19	3.06	5.02	3.57	2.25
10A	111.71	36.02	99.78	99.60	70.55	-13.09	-8.26	-7.76	6.17	**10A**	36.02	7.23	5.97	4.95	3.76
11A	-1156.85	-2.85	-	-	-	-	-	-	-	**11A**	-2.85	-3.61	1.72	2.08	1.01
12A	89.93	-1.35	-	-	-	-	-	-	-	**12A**	-1.35	-1.31	0.29	-0.41	-2.50
14A	101.35	93.78	-	-	-	-	-	-	-	**14A**	93.78	77.09	6.48	1.59	2.35
15A	103.21	-5.11	98.92	99.07	98.64	22.21	16.15	37.31	38.63	**15A**	-5.11	-2.50	6.36	-1.85	-3.02
17A	-69.15	-0.49	22.03	6.25	8.16	19.51	20.30	34.14	29.41	**17A**	-0.49	2.26	5.32	3.94	2.80
18A	100.99	10.12	-	-	-	-	-	-	-	**18A**	10.12	1.99	-1.82	-2.80	-0.67
19A	99.90	-1.66	99.63	99.91	99.78	94.64	18.60	1.75	1.91	**19A**	-1.66	-9.42	-9.65	-9.63	-4.87
21A	98.39	0.59	99.38	99.38	99.24	58.11	98.46	20.52	7.31	**21A**	0.59	10.86	-5.15	-6.25	-4.30
1B	-3.84	67.94	-	-	-	-	-	-	-	**1B**	67.94	34.31	1.97	4.82	2.43
2B	103.57	95.47	-	-	-	-	-	-	-	**2B**	95.47	2.99	0.21	1.13	2.22
5B	25.18	-3.28	-	-	-	-	-	-	-	**5B**	-3.28	-1.79	-1.43	0.13	3.03
6B	-4.80	19.56	-	-	-	-	-	-	-	**6B**	19.56	-0.58	-1.33	-0.84	1.44
7B	103.47	42.99	-	-	-	-	-	-	-	**7B**	42.99	10.57	0.82	0.54	1.91
9B	92.95	28.09	-	-	-	-	-	-	-	**9B**	28.09	4.90	4.52	-6.22	-6.31
10B	100.53	99.76	99.49	99.57	36.12	25.91	9.78	23.77	16.24	**10B**	99.76	99.08	4.43	3.61	4.47
11B	99.76	-0.44	-	-	-	-	-	-	-	**11B**	-0.44	-3.52	-2.01	1.71	-0.76
12B	99.99	-2.65	99.50	21.76	9.33	8.50	48.76	57.18	43.01	**12B**	-2.65	-2.71	-2.79	-1.46	0.48
17B	99.82	5.14	100.09	99.32	96.22	54.13	12.09	18.38	21.07	**17B**	5.14	-3.00	-1.00	1.33	-0.88
1C	103.51	-3.66	-	-	-	-	-	-	-	**1C**	-3.66	-1.88	0.37	0.31	1.61
2C	103.45	1.46	62.70	71.89	18.25	14.42	7.36	49.98	22.15	**2C**	1.46	1.89	2.34	3.94	5.89
3C	-14.13	5.78	-	-	-	-	-	-	-	**3C**	5.78	-0.13	-1.38	-2.16	-0.85
6C	103.57	11.70	-	-	-	-	-	-	-	**6C**	11.70	4.16	1.61	1.86	-2.14
7C	0.08	-1.63	-	-	-	-	-	-	-	**7C**	-1.63	0.73	-0.32	1.15	2.81
9C	-14.37	10.99	-	-	-	-	-	-	-	**9C**	10.99	3.62	3.26	5.39	7.50
10C	93.97	-1.57	-	-	-	-	-	-	-	**10C**	-1.57	0.26	0.56	1.64	1.67
11C	98.63	100.20	-	-	-	-	-	-	-	**11C**	100.20	4.26	-1.79	-1.84	-0.26
12C	100.32	3.91	98.11	43.18	42.27	18.11	66.47	1.01	30.79	**12C**	3.91	-0.01	0.83	1.09	3.60
13C	101.70	-4.85	20.52	21.38	21.49	57.73	69.31	16.23	34.56	**13C**	-4.85	-2.53	-0.65	0.52	2.44
14C	100.91	-2.72	100.08	98.81	98.86	60.73	10.27	12.11	33.33	**14C**	-2.72	-1.19	0.16	-0.68	2.65
16C	101.58	27.43	-	-	-	-	-	-	-	**16C**	27.43	16.06	9.60	9.83	6.95
17C	99.91	4.40	-	-	-	-	-	-	-	**17C**	4.40	-2.00	-1.10	-1.20	0.70
18C	95.87	-2.05	-	-	-	-	-	-	-	**18C**	-2.05	-2.46	-0.75	0.49	1.04
19C	99.96	23.02	-	-	-	-	-	-	-	**19C**	23.02	11.78	7.84	4.44	4.67
20C	102.10	3.77	-	-	-	-	-	-	-	**20C**	3.77	-1.10	-5.00	-5.37	-3.91
21C	100.39	11.56	-	-	-	-	-	-	-	**21C**	11.56	-0.77	-4.01	8.44	-3.46
1D	103.48	1.03	-	-	-	-	-	-	-	**1D**	1.03	1.70	2.08	-3.33	-5.83
2D	100.29	-7.08	-	-	-	-	-	-	-	**2D**	-7.08	-2.73	-0.40	0.86	2.63
3D	-9.41	11.71	-	-	-	-	-	-	-	**3D**	11.71	8.56	7.22	6.55	5.00
4D	103.43	3.24	-	-	-	-	-	-	-	**4D**	3.24	1.23	4.28	4.02	2.43
5D	35.17	1.17	-	-	-	-	-	-	-	**5D**	1.17	-4.15	-3.41	-2.98	-2.57
6D	103.54	9.17	-	-	-	-	-	-	-	**6D**	9.17	2.36	2.10	-1.35	1.37
8D	103.61	3.39	-	-	-	-	-	-	-	**8D**	3.39	0.45	3.23	-4.36	-4.37
9D	-14.98	1.62	-	-	-	-	-	-	-	**9D**	1.62	-1.97	0.03	-2.96	-3.33
10D	70.23	-0.44	-	-	-	-	-	-	-	**10D**	-0.44	-0.24	0.54	-1.63	-0.88
11D	-46.57	73.37	-	-	-	-	-	-	-	**11D**	73.37	46.42	23.81	7.10	-0.39
13D	-235.46	0.83	-	-	-	-	-	-	-	**13D**	0.83	-1.54	0.55	1.15	2.30
15D	83.80	4.03	17.61	-2.86	31.45	36.61	15.98	-1.97	4.03	**15D**	4.03	2.10	3.43	3.00	2.78
16D	103.47	27.43	-	-	-	-	-	-	-	**16D**	27.43	16.06	9.60	9.83	6.95
17D	-32.98	15.58	-	-	-	-	-	-	-	**17D**	15.58	6.59	5.06	-3.88	-0.28
18D	-131.34	-3.13	-	-	-	-	-	-	-	**18D**	-3.13	-3.07	-1.43	-1.21	0.43
19D	95.70	19.47	26.33	1.84	7.38	14.45	15.26	15.90	19.47	**19D**	19.47	0.01	-6.01	7.40	-0.75
20D	90.99	41.32	35.47	23.40	42.27	23.15	22.35	16.21	41.32	**20D**	41.32	-2.67	-4.79	8.52	4.93

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
ANA	56.31	-1.36	-	-	-	-	-	-	-	ANA	-1.36	-1.73	0.11	0.51	-3.06
ANB	94.00	-3.06	100.04	100.53	-69.39	-20.45	32.04	92.63	93.61	ANB	-3.06	-3.77	-1.86	-1.78	0.41
ANC	102.71	-1.56	99.69	2.15	-42.32	-56.43	-26.92	-0.04	23.12	ANC	-1.56	-1.53	1.00	-1.07	-3.82
AND	117.14	16.32	101.26	101.10	101.25	100.79	-44.98	-54.45	-54.25	AND	16.32	7.32	4.15	5.66	5.45
BEA	99.48	0.54	-	-	-	-	-	-	-	BEA	0.54	-5.73	-5.12	-4.27	-3.13
BEB	97.96	1.90	99.89	-39.29	-49.08	34.91	68.26	-5.10	-16.85	BEB	1.90	-3.75	-2.58	-1.48	3.48
BEC	-2579.07	13.86	-	-	-	-	-	-	-	BEC	13.86	3.65	1.61	-0.62	-5.65
BED	123.95	10.25	64.47	43.72	11.25	8.50	14.61	8.78	16.73	BED	10.25	-0.40	0.74	1.92	3.96
CLA	92.36	-	-243.75	-208.80	-377.83	-73.67	-310.37	-333.73	-306.29	CLA	-	-	-	-	-
CP1	106.19	6.74	99.73	99.48	99.87	99.79	99.52	99.57	28.16	CP1	6.74	-3.39	-2.49	2.98	0.41
CP2	99.20	2.41	-	-	-	-	-	-	-	CP2	2.41	2.06	2.28	2.24	4.27
CP3	109.05	53.56	-	-	-	-	-	-	-	CP3	53.56	-0.03	-1.61	-2.27	-1.48
CP4	106.67	4.34	99.35	99.83	99.09	40.77	38.10	29.97	2.40	CP4	4.34	-3.57	-2.85	1.84	0.10
CP5	102.01	2.58	-	-	-	-	-	-	-	CP5	2.58	-2.37	-4.23	-4.15	-1.83
CP6	-967.35	-2.25	-	-	-	-	-	-	-	CP6	-2.25	-2.96	-1.77	-1.17	-4.66
CT1	100.75	3.10	100.94	93.32	-356.41	-287.98	-127.25	-87.87	-103.99	CT1	3.10	0.66	-0.17	0.02	4.20
CT2	99.54	-2.29	99.89	96.83	-388.41	-384.24	-343.52	-182.38	-217.32	CT2	-2.29	-3.45	-1.94	-0.86	0.41
CT3	112.69	52.20	116.72	116.12	114.43	98.25	44.86	22.50	22.66	CT3	52.20	20.81	3.43	1.40	3.17
CT4	111.25	78.92	99.93	61.45	18.85	26.88	14.77	21.09	16.95	CT4	78.92	20.51	4.63	-1.90	-4.35
CT5	108.48	13.73	100.00	50.36	38.01	12.96	16.31	9.03	14.77	CT5	13.73	1.47	1.15	2.16	3.29
CT6	64.17	20.03	-	-	-	-	-	-	-	CT6	20.03	9.64	4.63	-1.97	-3.75
DEA	-2151.89	-2.54	-	-	-	-	-	-	-	DEA	-2.54	-0.47	0.02	1.51	3.11
DEB	103.26	-2.32	99.58	99.80	99.66	98.17	18.98	25.13	26.49	DEB	-2.32	-12.88	1.56	-9.41	-8.23
DEC	132.48	4.34	99.38	97.46	4.95	2.84	6.99	30.79	5.38	DEC	4.34	-4.92	-2.42	0.05	-5.84
DED	127.39	50.53	99.34	98.86	63.73	11.82	6.62	12.59	12.88	DED	50.53	12.52	6.68	-0.65	-1.05
EUA	84.41	-9.74	-	-	-	-	-	-	-	EUA	-9.74	-18.48	-16.02	16.80	14.82
EUB	96.26	-22.30	-	-	-	-	-	-	-	EUB	-22.30	-20.88	-18.61	-19.38	-15.39
EUC	91.05	-2.25	-	-	-	-	-	-	-	EUC	-2.25	-1.24	-0.17	-0.53	2.44
EUD	100.39	7.10	-	-	-	-	-	-	-	EUD	7.10	-6.99	-11.84	8.11	6.23
FLA	47.91	-2.79	-	-	-	-	-	-	-	FLA	-2.79	-1.32	0.86	1.15	1.66
FLB	104.53	-8.58	99.30	99.19	98.10	7.78	30.06	22.79	8.69	FLB	-8.58	-8.32	-5.18	3.63	-2.02
FLC	-1474.84	45.69	-	-	-	-	-	-	-	FLC	45.69	19.60	11.40	8.43	12.72
LP1	102.25	11.21	100.07	100.08	98.54	96.97	9.16	6.17	33.57	LP1	11.21	1.97	3.22	1.55	4.31
LP3	101.89	-3.53	99.95	100.20	99.93	99.70	-5.43	28.90	39.40	LP3	-3.53	-1.73	-0.59	-0.17	1.74
LP4	-165.58	11.87	-	-	-	-	-	-	-	LP4	11.87	5.04	0.32	-2.77	-6.10
LP5	-162.18	15.18	-	-	-	-	-	-	-	LP5	15.18	9.85	7.15	5.23	5.75
LP6	-123.02	-2.70	-	-	-	-	-	-	-	LP6	-2.70	-1.51	0.34	0.06	1.68
MAA	96.76	-2.20	98.37	14.76	23.00	3.61	5.38	6.54	10.78	MAA	-2.20	-0.66	1.22	0.82	3.35
MAB	96.78	-1.63	-	-	-	-	-	-	-	MAB	-1.63	-1.91	-0.06	0.59	1.85
MAC	96.44	4.08	-	-	-	-	-	-	-	MAC	4.08	5.50	-0.07	2.15	3.25
MAD	93.79	93.13	100.07	100.00	100.00	100.02	99.24	51.68	57.32	MAD	93.13	36.88	0.80	1.85	2.61
MDZ1	100.07	61.50	100.01	99.45	95.13	99.13	99.16	98.81	61.16	MDZ1	61.50	39.98	30.04	-20.55	-12.72
MDZ2	29.06	29.01	-	-	-	-	-	-	-	MDZ2	29.01	13.52	9.01	6.33	4.68
MDZ3	100.19	-2.14	-	-	-	-	-	-	-	MDZ3	-2.14	-2.79	-1.14	1.05	-1.97
MDZ4	101.76	34.53	-	-	-	-	-	-	-	MDZ4	34.53	19.73	7.75	2.23	3.54
MDZ5	102.22	79.18	-	-	-	-	-	-	-	MDZ5	79.18	46.51	32.53	-17.21	-15.09
MDZ6	99.05	8.49	-	-	-	-	-	-	-	MDZ6	8.49	-0.22	-1.77	-2.23	0.28
PAA	51.50	-1.05	-	-	-	-	-	-	-	PAA	-1.05	0.39	0.74	1.31	3.13
PAB	91.35	-11.01	-	-	-	-	-	-	-	PAB	-11.01	-11.47	-9.76	3.36	1.06
PAC	-1663.21	-0.56	-	-	-	-	-	-	-	PAC	-0.56	-0.46	2.05	2.97	2.34
PAD	98.57	-1.78	99.67	96.57	15.74	8.29	16.77	17.28	19.36	PAD	-1.78	-0.82	-0.44	0.78	2.35
RIA	-1195.56	4.56	-	-	-	-	-	-	-	RIA	4.56	0.59	-1.69	3.97	-3.14
RIB	103.50	-5.50	99.89	99.81	99.82	98.69	50.32	14.22	17.14	RIB	-5.50	-2.44	-2.07	-0.80	-1.54
RIC	94.97	23.31	99.67	46.06	18.59	12.21	16.91	20.59	16.88	RIC	23.31	9.15	7.26	6.78	11.63
RID	91.20	-7.43	99.61	98.67	29.14	0.34	20.32	5.25	5.04	RID	-7.43	-5.74	-2.79	-2.36	0.77
SB1	-1192.38	-2.49	-	-	-	-	-	-	-	SB1	-2.49	-1.28	0.53	1.32	2.86
SB2	101.56	4.35	99.67	78.09	16.86	20.65	0.26	3.59	2.81	SB2	4.35	-1.09	-2.32	0.22	0.27
SB3	108.80	-0.57	-	-	-	-	-	-	-	SB3	-0.57	1.48	1.65	2.07	2.67
SB5	76.63	-1.49	-	-	-	-	-	-	-	SB5	-1.49	0.13	0.86	1.95	3.56
SB6	-791.29	-1.98	-	-	-	-	-	-	-	SB6	-1.98	0.08	0.06	1.37	2.25
TBU-46	-1368.13	-	-	-	-	-	-	-	-	TBU-46	-	-	-	-	-
TBU-72	91.60	-	99.96	98.43	99.33	96.96	92.18	76.38	41.05	TBU-72	-	-	-	-	-

Appendix II. Extracts With Less than 80% Inhibition

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L 6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
2A	9.02	-1.98	-	-	-	-	-	-	-	**2A**	-1.98	-0.87	-0.47	0.64	1.64
11A	-1156.85	-2.85	-	-	-	-	-	-	-	**11A**	-2.85	-3.61	1.72	2.08	1.01
17A	-69.15	-0.49	22.03	6.25	8.16	19.51	20.30	34.14	29.41	**17A**	-0.49	2.26	5.32	3.94	2.80
1B	-3.84	67.94	-	-	-	-	-	-	-	**1B**	67.94	34.31	1.97	4.82	2.43
5B	25.18	-3.28	-	-	-	-	-	-	-	**5B**	-3.28	-1.79	-1.43	0.13	3.03
6B	-4.80	19.56	-	-	-	-	-	-	-	**6B**	19.56	-0.58	-1.33	-0.84	1.44
3C	-14.13	5.78	-	-	-	-	-	-	-	**3C**	5.78	-0.13	-1.38	-2.16	-0.85
7C	0.08	-1.63	-	-	-	-	-	-	-	**7C**	-1.63	0.73	-0.32	1.15	2.81
9C	-14.37	10.99	-	-	-	-	-	-	-	**9C**	10.99	3.62	3.26	5.39	7.50
3D	-9.41	11.71	-	-	-	-	-	-	-	**3D**	11.71	8.56	7.22	6.55	5.00
5D	35.17	1.17	-	-	-	-	-	-	-	**5D**	1.17	-4.15	-3.41	-2.98	-2.57
9D	-14.98	1.62	-	-	-	-	-	-	-	**9D**	1.62	-1.97	0.03	-2.96	-3.33
10D	70.23	-0.44	-	-	-	-	-	-	-	**10D**	-0.44	-0.24	0.54	-1.63	-0.88
11D	-46.57	73.37	-	-	-	-	-	-	-	**11D**	73.37	46.42	23.81	7.10	-0.39
13D	-235.46	0.83	-	-	-	-	-	-	-	**13D**	0.83	-1.54	0.55	1.15	2.30
17D	-32.98	15.58	-	-	-	-	-	-	-	**17D**	15.58	6.59	5.06	-3.88	-0.28
18D	-131.34	-3.13	-	-	-	-	-	-	-	**18D**	-3.13	-3.07	-1.43	-1.21	0.43
ANA	56.31	-1.36	-	-	-	-	-	-	-	**ANA**	-1.36	-1.73	0.11	0.51	-3.06
BEC	-2579.07	13.86	-	-	-	-	-	-	-	**BEC**	13.86	3.65	1.61	-0.62	-5.65
CP6	-967.35	-2.25	-	-	-	-	-	-	-	**CP6**	-2.25	-2.96	-1.77	-1.17	-4.66
CT6	64.17	20.03	-	-	-	-	-	-	-	**CT6**	20.03	9.64	4.63	-1.97	-3.75
DEA	-2151.89	-2.54	-	-	-	-	-	-	-	**DEA**	-2.54	-0.47	0.02	1.51	3.11
FLA	47.91	-2.79	-	-	-	-	-	-	-	**FLA**	-2.79	-1.32	0.86	1.15	1.66
FLC	-1474.84	45.69	-	-	-	-	-	-	-	**FLC**	45.69	19.60	11.40	8.43	12.72
LP4	-165.58	11.87	-	-	-	-	-	-	-	**LP4**	11.87	5.04	0.32	-2.77	-6.10
LP5	-162.18	15.18	-	-	-	-	-	-	-	**LP5**	15.18	9.85	7.15	5.23	5.75
LP6	-123.02	-2.70	-	-	-	-	-	-	-	**LP6**	-2.70	-1.51	0.34	0.06	1.68
MDZ2	29.06	29.01	-	-	-	-	-	-	-	**MDZ2**	29.01	13.52	9.01	6.33	4.68
PAA	51.50	-1.05	-	-	-	-	-	-	-	**PAA**	-1.05	0.39	0.74	1.31	3.13
PAC	-1663.21	-0.56	-	-	-	-	-	-	-	**PAC**	-0.56	-0.46	2.05	2.97	2.34
RIA	-1195.56	4.56	-	-	-	-	-	-	-	**RIA**	4.56	0.59	-1.69	3.97	-3.14
SB1	-1192.38	-2.49	-	-	-	-	-	-	-	**SB1**	-2.49	-1.28	0.53	1.32	2.86
SB5	76.63	-1.49	-	-	-	-	-	-	-	**SB5**	-1.49	0.13	0.86	1.95	3.56
SB6	-791.29	-1.98	-	-	-	-	-	-	-	**SB6**	-1.98	0.08	0.06	1.37	2.25
TBU-46	-1368.13	-	-	-	-	-	-	-	-	**TBU-46**	-	-	-	-	-

Appendix III: Extracts With Greater than 80% Inhibition

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L 6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
4A	103.33	62.05	-	-	-	-	-	-	-	**4A**	62.05	-5.36	-1.54	0.18	-0.44
6A	98.77	-0.12	116.18	115.80	115.03	89.11	54.64	18.75	16.00	**6A**	-0.12	-1.26	1.78	-0.85	-1.15
7A	103.46	-2.38	-	-	-	-	-	-	-	**7A**	-2.38	-5.13	-5.18	3.21	-0.65
9A	103.41	-0.19	99.99	100.05	100.14	47.92	47.88	49.01	52.82	**9A**	-0.19	3.06	5.02	3.57	2.25
10A	111.71	36.02	99.78	99.60	70.55	-13.09	-8.26	-7.76	6.17	**10A**	36.02	7.23	5.97	4.95	3.76
12A	89.93	-1.35	-	-	-	-	-	-	-	**12A**	-1.35	-1.31	0.29	-0.41	-2.50
14A	101.35	93.78	-	-	-	-	-	-	-	**14A**	93.78	77.09	6.48	1.59	2.35
15A	103.21	-5.11	98.92	99.07	98.64	22.21	16.15	37.31	38.63	**15A**	-5.11	-2.50	6.36	-1.85	-3.02
18A	100.99	10.12	-	-	-	-	-	-	-	**18A**	10.12	1.99	-1.82	-2.80	-0.67
19A	99.90	-1.66	99.63	99.91	99.78	94.64	18.60	1.75	1.91	**19A**	-1.66	-9.42	-9.65	-9.63	-4.87
21A	98.39	0.59	99.38	99.38	99.24	58.11	98.46	20.52	7.31	**21A**	0.59	10.86	-5.15	-6.25	-4.30
2B	103.57	95.47	-	-	-	-	-	-	-	**2B**	95.47	2.99	0.21	1.13	2.22
7B	103.47	42.99	-	-	-	-	-	-	-	**7B**	42.99	10.57	0.82	0.54	1.91
9B	92.95	28.09	-	-	-	-	-	-	-	**9B**	28.09	4.90	4.52	-6.22	-6.31
10B	100.53	99.76	99.49	99.57	36.12	25.91	9.78	23.77	16.24	**10B**	99.76	99.08	4.43	3.61	4.47
11B	99.76	-0.44	-	-	-	-	-	-	-	**11B**	-0.44	-3.52	-2.01	1.71	-0.76
12B	99.99	-2.65	99.50	21.76	9.33	8.50	48.76	57.18	43.01	**12B**	-2.65	-2.71	-2.79	-1.46	0.48
17B	99.82	5.14	100.09	99.32	96.22	54.13	12.09	18.38	21.07	**17B**	5.14	-3.00	-1.00	1.33	-0.88
1C	103.51	-3.66	-	-	-	-	-	-	-	**1C**	-3.66	-1.88	0.37	0.31	1.61
2C	103.45	1.46	62.70	71.89	18.25	14.42	7.36	49.98	22.15	**2C**	1.46	1.89	2.34	3.94	5.89
6C	103.57	11.70	-	-	-	-	-	-	-	**6C**	11.70	4.16	1.61	1.86	-2.14
10C	93.97	-1.57	-	-	-	-	-	-	-	**10C**	-1.57	0.26	0.56	1.64	1.67
11C	98.63	100.20	-	-	-	-	-	-	-	**11C**	100.20	4.26	-1.79	-1.84	-0.26
12C	100.32	3.91	98.11	43.18	42.27	18.11	66.47	1.01	30.79	**12C**	3.91	-0.01	0.83	1.09	3.60
13C	101.70	-4.85	20.52	21.38	21.49	57.73	69.31	16.23	34.56	**13C**	-4.85	-2.53	-0.65	0.52	2.44
14C	100.91	-2.72	100.08	98.81	98.86	60.73	10.27	12.11	33.33	**14C**	-2.72	-1.19	0.16	-0.68	2.65
16C	101.58	27.43	-	-	-	-	-	-	-	**16C**	27.43	16.06	9.60	9.83	6.95
17C	99.91	4.40	-	-	-	-	-	-	-	**17C**	4.40	-2.00	-1.10	-1.20	0.70
18C	95.87	-2.05	-	-	-	-	-	-	-	**18C**	-2.05	-2.46	-0.75	0.49	1.04
19C	99.96	23.02	-	-	-	-	-	-	-	**19C**	23.02	11.78	7.84	4.44	4.67
20C	102.10	3.77	-	-	-	-	-	-	-	**20C**	3.77	-1.10	-5.00	-5.37	-3.91
21C	100.39	11.56	-	-	-	-	-	-	-	**21C**	11.56	-0.77	-4.01	8.44	-3.46
1D	103.48	1.03	-	-	-	-	-	-	-	**1D**	1.03	1.70	2.08	-3.33	-5.83
2D	100.29	-7.08	-	-	-	-	-	-	-	**2D**	-7.08	-2.73	-0.40	0.86	2.63
4D	103.43	3.24	-	-	-	-	-	-	-	**4D**	3.24	1.23	4.28	4.02	2.43
6D	103.54	9.17	-	-	-	-	-	-	-	**6D**	9.17	2.36	2.10	-1.35	1.37
8D	103.61	3.39	-	-	-	-	-	-	-	**8D**	3.39	0.45	3.23	-4.36	-4.37
15D	83.80	4.03	17.61	-2.86	31.45	36.61	15.98	-1.97	4.03	**15D**	4.03	2.10	3.43	3.00	2.78
16D	103.47	27.43	-	-	-	-	-	-	-	**16D**	27.43	16.06	9.60	9.83	6.95
19D	95.70	19.47	26.33	1.84	7.38	14.45	15.26	15.90	19.47	**19D**	19.47	0.01	-6.01	7.40	-0.75
20D	90.99	41.32	35.47	23.40	42.27	23.15	22.35	16.21	41.32	**20D**	41.32	-2.67	-4.79	8.52	4.93
ANB	94.00	-3.06	100.04	100.53	-69.39	-20.45	32.04	92.63	93.61	**ANB**	-3.06	-3.77	-1.86	-1.78	0.41
ANC	102.71	-1.56	99.69	2.15	-42.32	-56.43	-26.92	-0.04	23.12	**ANC**	-1.56	-1.53	1.00	-1.07	-3.82
AND	117.14	16.32	101.26	101.10	101.25	100.79	-44.98	-54.45	-54.25	**AND**	16.32	7.32	4.15	5.66	5.45
BEA	99.48	0.54	-	-	-	-	-	-	-	**BEA**	0.54	-5.73	-5.12	-4.27	-3.13
BEB	97.96	1.90	99.89	-39.29	-49.08	34.91	68.26	-5.10	-16.85	**BEB**	1.90	-3.75	-2.58	-1.48	3.48
BED	123.95	10.25	64.47	43.72	11.25	8.50	14.61	8.78	16.73	**BED**	10.25	-0.40	0.74	1.92	3.96
CLA	92.36	-	-243.75	-208.80	-377.83	-73.67	-310.37	-333.73	-306.29	**CLA**	-	-	-	-	-
CP1	106.19	6.74	99.73	99.48	99.87	99.79	99.52	99.57	28.16	**CP1**	6.74	-3.39	-2.49	2.98	0.41
CP2	99.20	2.41	-	-	-	-	-	-	-	**CP2**	2.41	2.06	2.28	2.24	4.27
CP3	109.05	53.56	-	-	-	-	-	-	-	**CP3**	53.56	-0.03	-1.61	-2.27	-1.48
CP4	106.67	4.34	99.35	99.83	99.09	40.77	38.10	29.97	2.40	**CP4**	4.34	-3.57	-2.85	1.84	0.10
CP5	102.01	2.58	-	-	-	-	-	-	-	**CP5**	2.58	-2.37	-4.23	-4.15	-1.83
CT1	100.75	3.10	100.94	93.32	-356.41	-287.98	-127.25	-87.87	-103.99	**CT1**	3.10	0.66	-0.17	0.02	4.20
CT2	99.54	-2.29	99.89	96.83	-388.41	-384.24	-343.52	-182.38	-217.32	**CT2**	-2.29	-3.45	-1.94	-0.86	0.41
CT3	112.69	52.20	116.72	116.12	114.43	98.25	44.86	22.50	22.66	**CT3**	52.20	20.81	3.43	1.40	3.17
CT4	111.25	78.92	99.93	61.45	18.85	26.88	14.77	21.09	16.95	**CT4**	78.92	20.51	4.63	-1.90	-4.35
CT5	108.48	13.73	100.00	50.36	38.01	12.96	16.31	9.03	14.77	**CT5**	13.73	1.47	1.15	2.16	3.29
DEB	103.26	-2.32	99.58	99.80	99.66	98.17	18.98	25.13	26.49	**DEB**	-2.32	-12.88	1.56	-9.41	-8.23
DEC	132.48	4.34	99.38	97.46	4.95	2.84	6.99	30.79	5.38	**DEC**	4.34	-4.92	-2.42	0.05	-5.84
DED	127.39	50.53	99.34	98.86	63.73	11.82	6.62	12.59	12.88	**DED**	50.53	12.52	6.68	-0.65	-1.05
EUA	84.41	-9.74	-	-	-	-	-	-	-	**EUA**	-9.74	-18.48	-16.02	16.80	14.82
EUB	96.26	-22.30	-	-	-	-	-	-	-	**EUB**	-22.30	-20.88	-18.61	-19.38	-15.39
EUC	91.05	-2.25	-	-	-	-	-	-	-	**EUC**	-2.25	-1.24	-0.17	-0.53	2.44
EUD	100.39	7.10	-	-	-	-	-	-	-	**EUD**	7.10	-6.99	-11.84	8.11	6.23
FLB	104.53	-8.58	99.30	99.19	98.10	7.78	30.06	22.79	8.69	**FLB**	-8.58	-8.32	-5.18	3.63	-2.02
LP1	102.25	11.21	100.07	100.08	98.54	96.97	9.16	6.17	33.57	**LP1**	11.21	1.97	3.22	1.55	4.31
LP3	101.89	-3.53	99.95	100.20	99.93	99.70	-5.43	28.90	39.40	**LP3**	-3.53	-1.73	-0.59	-0.17	1.74
MAA	96.76	-2.20	98.37	14.76	23.00	3.61	5.38	6.54	10.78	**MAA**	-2.20	-0.66	1.22	0.82	3.35
MAB	96.78	-1.63	-	-	-	-	-	-	-	**MAB**	-1.63	-1.91	-0.06	0.59	1.85
MAC	96.44	4.08	-	-	-	-	-	-	-	**MAC**	4.08	5.50	-0.07	2.15	3.25
MAD	93.79	93.13	100.07	100.00	100.00	100.02	99.24	51.68	57.32	**MAD**	93.13	36.88	0.80	1.85	2.61
MDZ1	100.07	61.50	100.01	99.45	95.13	99.13	99.16	98.81	61.16	**MDZ1**	61.50	39.98	30.04	-20.55	-12.72
MDZ3	100.19	-2.14	-	-	-	-	-	-	-	**MDZ3**	-2.14	-2.79	-1.14	1.05	-1.97
MDZ4	101.76	34.53	-	-	-	-	-	-	-	**MDZ4**	34.53	19.73	7.75	2.23	3.54
MDZ5	102.22	79.18	-	-	-	-	-	-	-	**MDZ5**	79.18	46.51	32.53	-17.21	-15.09
MDZ6	99.05	8.49	-	-	-	-	-	-	-	**MDZ6**	8.49	-0.22	-1.77	-2.23	0.28
PAB	91.35	-11.01	-	-	-	-	-	-	-	**PAB**	-11.01	-11.47	-9.76	3.36	1.06
PAD	98.57	-1.78	99.67	96.57	15.74	8.29	16.77	17.28	19.36	**PAD**	-1.78	-0.82	-0.44	0.78	2.35
RIB	103.50	-5.50	99.89	99.81	99.82	98.69	50.32	14.22	17.14	**RIB**	-5.50	-2.44	-2.07	-0.80	-1.54
RIC	94.97	23.31	99.67	46.06	18.59	12.21	16.91	20.59	16.88	**RIC**	23.31	9.15	7.26	6.78	11.63
RID	91.20	-7.43	99.61	98.67	29.14	0.34	20.32	5.25	5.04	**RID**	-7.43	-5.74	-2.79	-2.36	0.77
SB2	101.56	4.35	99.67	78.09	16.86	20.65	0.26	3.59	2.81	**SB2**	4.35	-1.09	-2.32	0.22	0.27
SB3	108.80	-0.57	-	-	-	-	-	-	-	**SB3**	-0.57	1.48	1.65	2.07	2.67
TBU-72	91.60	-	99.96	98.43	99.33	96.96	92.18	76.38	41.05	**TBU-72**	-	-	-	-	-

Appendix IV: Extracts with Greater than 80% Inhibition and Less than 10% Toxicity

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L 6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
6A	98.77	-0.12	116.18	115.80	115.03	89.11	54.64	18.75	16.00	**6A**	-0.12	-1.26	1.78	-0.85	-1.15
7A	103.46	-2.38	-	-	-	-	-	-	-	**7A**	-2.38	-5.13	-5.18	3.21	-0.65
9A	103.41	-0.19	99.99	100.05	100.14	47.92	47.88	49.01	52.82	**9A**	-0.19	3.06	5.02	3.57	2.25
12A	89.93	-1.35	-	-	-	-	-	-	-	**12A**	-1.35	-1.31	0.29	-0.41	-2.50
15A	103.21	-5.11	98.92	99.07	98.64	22.21	16.15	37.31	38.63	**15A**	-5.11	-2.50	6.36	-1.85	-3.02
19A	99.90	-1.66	99.63	99.91	99.78	94.64	18.60	1.75	1.91	**19A**	-1.66	-9.42	-9.65	-9.63	-4.87
21A	98.39	0.59	99.38	99.38	99.24	58.11	98.46	20.52	7.31	**21A**	0.59	10.86	-5.15	-6.25	-4.30
11B	99.76	-0.44	-	-	-	-	-	-	-	**11B**	-0.44	-3.52	-2.01	1.71	-0.76
12B	99.99	-2.65	99.50	21.76	9.33	8.50	48.76	57.18	43.01	**12B**	-2.65	-2.71	-2.79	-1.46	0.48
17B	99.82	5.14	100.09	99.32	96.22	54.13	12.09	18.38	21.07	**17B**	5.14	-3.00	-1.00	1.33	-0.88
1C	103.51	-3.66	-	-	-	-	-	-	-	**1C**	-3.66	-1.88	0.37	0.31	1.61
2C	103.45	1.46	62.70	71.89	18.25	14.42	7.36	49.98	22.15	**2C**	1.46	1.89	2.34	3.94	5.89
10C	93.97	-1.57	-	-	-	-	-	-	-	**10C**	-1.57	0.26	0.56	1.64	1.67
12C	100.32	3.91	98.11	43.18	42.27	18.11	66.47	1.01	30.79	**12C**	3.91	-0.01	0.83	1.09	3.60
13C	101.70	-4.85	20.52	21.38	21.49	57.73	69.31	16.23	34.56	**13C**	-4.85	-2.53	-0.65	0.52	2.44
14C	100.91	-2.72	100.08	98.81	98.86	60.73	10.27	12.11	33.33	**14C**	-2.72	-1.19	0.16	-0.68	2.65
17C	99.91	4.40	-	-	-	-	-	-	-	**17C**	4.40	-2.00	-1.10	-1.20	0.70
18C	95.87	-2.05	-	-	-	-	-	-	-	**18C**	-2.05	-2.46	-0.75	0.49	1.04
20C	102.10	3.77	-	-	-	-	-	-	-	**20C**	3.77	-1.10	-5.00	-5.37	-3.91
1D	103.48	1.03	-	-	-	-	-	-	-	**1D**	1.03	1.70	2.08	-3.33	-5.83
2D	100.29	-7.08	-	-	-	-	-	-	-	**2D**	-7.08	-2.73	-0.40	0.86	2.63
4D	103.43	3.24	-	-	-	-	-	-	-	**4D**	3.24	1.23	4.28	4.02	2.43
6D	103.54	9.17	-	-	-	-	-	-	-	**6D**	9.17	2.36	2.10	-1.35	1.37
8D	103.61	3.39	-	-	-	-	-	-	-	**8D**	3.39	0.45	3.23	-4.36	-4.37
15D	83.80	4.03	17.61	-2.86	31.45	36.61	15.98	-1.97	4.03	**15D**	4.03	2.10	3.43	3.00	2.78
ANB	94.00	-3.06	100.04	100.53	-69.39	-20.45	32.04	92.63	93.61	**ANB**	-3.06	-3.77	-1.86	-1.78	0.41
ANC	102.71	-1.56	99.69	2.15	-42.32	-56.43	-26.92	-0.04	23.12	**ANC**	-1.56	-1.53	1.00	-1.07	-3.82
BEA	99.48	0.54	-	-	-	-	-	-	-	**BEA**	0.54	-5.73	-5.12	-4.27	-3.13
BEB	97.96	1.90	99.89	-39.29	-49.08	34.91	68.26	-5.10	-16.85	**BEB**	1.90	-3.75	-2.58	-1.48	3.48
CP1	106.19	6.74	99.73	99.48	99.87	99.79	99.52	99.57	28.16	**CP1**	6.74	-3.39	-2.49	2.98	0.41
CP2	99.20	2.41	-	-	-	-	-	-	-	**CP2**	2.41	2.06	2.28	2.24	4.27
CP4	106.67	4.34	99.35	99.83	99.09	40.77	38.10	29.97	2.40	**CP4**	4.34	-3.57	-2.85	1.84	0.10
CP5	102.01	2.58	-	-	-	-	-	-	-	**CP5**	2.58	-2.37	-4.23	-4.15	-1.83
CT1	100.75	3.10	100.94	93.32	-356.41	-287.98	-127.25	-87.87	-103.99	**CT1**	3.10	0.66	-0.17	0.02	4.20
CT2	99.54	-2.29	99.89	96.83	-388.41	-384.24	-343.52	-182.38	-217.32	**CT2**	-2.29	-3.45	-1.94	-0.86	0.41
DEB	103.26	-2.32	99.58	99.80	99.66	98.17	18.98	25.13	26.49	**DEB**	-2.32	-12.88	1.56	-9.41	-8.23
DEC	132.48	4.34	99.38	97.46	4.95	2.84	6.99	30.79	5.38	**DEC**	4.34	-4.92	-2.42	0.05	-5.84
EUA	84.41	-9.74	-	-	-	-	-	-	-	**EUA**	-9.74	-18.48	-16.02	16.80	14.82
EUB	96.26	-22.30	-	-	-	-	-	-	-	**EUB**	-22.30	-20.88	-18.61	-19.38	-15.39
EUC	91.05	-2.25	-	-	-	-	-	-	-	**EUC**	-2.25	-1.24	-0.17	-0.53	2.44
EUD	100.39	7.10	-	-	-	-	-	-	-	**EUD**	7.10	-6.99	-11.84	8.11	6.23
FLB	104.53	-8.58	99.30	99.19	98.10	7.78	30.06	22.79	8.69	**FLB**	-8.58	-8.32	-5.18	3.63	-2.02
LP3	101.89	-3.53	99.95	100.20	99.93	99.70	-5.43	28.90	39.40	**LP3**	-3.53	-1.73	-0.59	-0.17	1.74
MAA	96.76	-2.20	98.37	14.76	23.00	3.61	5.38	6.54	10.78	**MAA**	-2.20	-0.66	1.22	0.82	3.35
MAB	96.78	-1.63	-	-	-	-	-	-	-	**MAB**	-1.63	-1.91	-0.06	0.59	1.85
MAC	96.44	4.08	-	-	-	-	-	-	-	**MAC**	4.08	5.50	-0.07	2.15	3.25
MDZ3	100.19	-2.14	-	-	-	-	-	-	-	**MDZ3**	-2.14	-2.79	-1.14	1.05	-1.97
MDZ6	99.05	8.49	-	-	-	-	-	-	-	**MDZ6**	8.49	-0.22	-1.77	-2.23	0.28
PAB	91.35	-11.01	-	-	-	-	-	-	-	**PAB**	-11.01	-11.47	-9.76	3.36	1.06
PAD	98.57	-1.78	99.67	96.57	15.74	8.29	16.77	17.28	19.36	**PAD**	-1.78	-0.82	-0.44	0.78	2.35
RIB	103.50	-5.50	99.89	99.81	99.82	98.69	50.32	14.22	17.14	**RIB**	-5.50	-2.44	-2.07	-0.80	-1.54
RID	91.20	-7.43	99.61	98.67	29.14	0.34	20.32	5.25	5.04	**RID**	-7.43	-5.74	-2.79	-2.36	0.77
SB2	101.56	4.35	99.67	78.09	16.86	20.65	0.26	3.59	2.81	**SB2**	4.35	-1.09	-2.32	0.22	0.27
SB3	108.80	-0.57	-	-	-	-	-	-	-	**SB3**	-0.57	1.48	1.65	2.07	2.67

Appendix V: Extracts With Greater than 80% Inhibition and Less than 10% Toxicity Microdilution Data

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L 6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
6A	98.77	-0.12	116.18	115.80	115.03	89.11	54.64	18.75	16.00	**6A**	-0.12	-1.26	1.78	-0.85	-1.15
9A	103.41	-0.19	99.99	100.05	100.14	47.92	47.88	49.01	52.82	**9A**	-0.19	3.06	5.02	3.57	2.25
15A	103.21	-5.11	98.92	99.07	98.64	22.21	16.15	37.31	38.63	**15A**	-5.11	-2.50	6.36	-1.85	-3.02
19A	99.90	-1.66	99.63	99.91	99.78	94.64	18.60	1.75	1.91	**19A**	-1.66	-9.42	-9.65	-9.63	-4.87
21A	98.39	0.59	99.38	99.38	99.24	58.11	98.46	20.52	7.31	**21A**	0.59	10.86	-5.15	-6.25	-4.30
12B	99.99	-2.65	99.50	21.76	9.33	8.50	48.76	57.18	43.01	**12B**	-2.65	-2.71	-2.79	-1.46	0.48
17B	99.82	5.14	100.09	99.32	96.22	54.13	12.09	18.38	21.07	**17B**	5.14	-3.00	-1.00	1.33	-0.88
2C	103.45	1.46	62.70	71.89	18.25	14.42	7.36	49.98	22.15	**2C**	1.46	1.89	2.34	3.94	5.89
12C	100.32	3.91	98.11	43.18	42.27	18.11	66.47	1.01	30.79	**12C**	3.91	-0.01	0.83	1.09	3.60
13C	101.70	-4.85	20.52	21.38	21.49	57.73	69.31	16.23	34.56	**13C**	-4.85	-2.53	-0.65	0.52	2.44
14C	100.91	-2.72	100.08	98.81	98.86	60.73	10.27	12.11	33.33	**14C**	-2.72	-1.19	0.16	-0.68	2.65
15D	83.80	4.03	17.61	-2.86	31.45	36.61	15.98	-1.97	4.03	**15D**	4.03	2.10	3.43	3.00	2.78
ANB	94.00	-3.06	100.04	100.53	-69.39	-20.45	32.04	92.63	93.61	**ANB**	-3.06	-3.77	-1.86	-1.78	0.41
ANC	102.71	-1.56	99.69	2.15	-42.32	-56.43	-26.92	-0.04	23.12	**ANC**	-1.56	-1.53	1.00	-1.07	-3.82
BEB	97.96	1.90	99.89	-39.29	-49.08	34.91	68.26	-5.10	-16.85	**BEB**	1.90	-3.75	-2.58	-1.48	3.48
CP1	106.19	6.74	99.73	99.48	99.87	99.79	99.52	99.57	28.16	**CP1**	6.74	-3.39	-2.49	2.98	0.41
CP4	106.67	4.34	99.35	99.83	99.09	40.77	38.10	29.97	2.40	**CP4**	4.34	-3.57	-2.85	1.84	0.10
CT1	100.75	3.10	100.94	93.32	-356.41	-287.98	-127.25	-87.87	-103.99	**CT1**	3.10	0.66	-0.17	0.02	4.20
CT2	99.54	-2.29	99.89	96.83	-388.41	-384.24	-343.52	-182.38	-217.32	**CT2**	-2.29	-3.45	-1.94	-0.86	0.41
DEB	103.26	-2.32	99.58	99.80	99.66	98.17	18.98	25.13	26.49	**DEB**	-2.32	-12.88	1.56	-9.41	-8.23
DEC	132.48	4.34	99.38	97.46	4.95	2.84	6.99	30.79	5.38	**DEC**	4.34	-4.92	-2.42	0.05	-5.84
FLB	104.53	-8.58	99.30	99.19	98.10	7.78	30.06	22.79	8.69	**FLB**	-8.58	-8.32	-5.18	3.63	-2.02
LP3	101.89	-3.53	99.95	100.20	99.93	99.70	-5.43	28.90	39.40	**LP3**	-3.53	-1.73	-0.59	-0.17	1.74
MAA	96.76	-2.20	98.37	14.76	23.00	3.61	5.38	6.54	10.78	**MAA**	-2.20	-0.66	1.22	0.82	3.35
PAD	98.57	-1.78	99.67	96.57	15.74	8.29	16.77	17.28	19.36	**PAD**	-1.78	-0.82	-0.44	0.78	2.35
RIB	103.50	-5.50	99.89	99.81	99.82	98.69	50.32	14.22	17.14	**RIB**	-5.50	-2.44	-2.07	-0.80	-1.54
RID	91.20	-7.43	99.61	98.67	29.14	0.34	20.32	5.25	5.04	**RID**	-7.43	-5.74	-2.79	-2.36	0.77
SB2	101.56	4.35	99.67	78.09	16.86	20.65	0.26	3.59	2.81	**SB2**	4.35	-1.09	-2.32	0.22	0.27

Appendix VI: All Extracts With Greater than 80% Inhibition and Greater than 10% Toxicity

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L 6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
4A	103.33	62.05	-	-	-	-	-	-	-	**4A**	62.05	-5.36	-1.54	0.18	-0.44
10A	111.71	36.02	99.78	99.60	70.55	-13.09	-8.26	-7.76	6.17	**10A**	36.02	7.23	5.97	4.95	3.76
14A	101.35	93.78	-	-	-	-	-	-	-	**14A**	93.78	77.09	6.48	1.59	2.35
18A	100.99	10.12	-	-	-	-	-	-	-	**18A**	10.12	1.99	-1.82	-2.80	-0.67
2B	103.57	95.47	-	-	-	-	-	-	-	**2B**	95.47	2.99	0.21	1.13	2.22
7B	103.47	42.99	-	-	-	-	-	-	-	**7B**	42.99	10.57	0.82	0.54	1.91
9B	92.95	28.09	-	-	-	-	-	-	-	**9B**	28.09	4.90	4.52	-6.22	-6.31
10B	100.53	99.76	99.49	99.57	36.12	25.91	9.78	23.77	16.24	**10B**	99.76	99.08	4.43	3.61	4.47
6C	103.57	11.70	-	-	-	-	-	-	-	**6C**	11.70	4.16	1.61	1.86	-2.14
11C	98.63	100.20	-	-	-	-	-	-	-	**11C**	100.20	4.26	-1.79	-1.84	-0.26
16C	101.58	27.43	-	-	-	-	-	-	-	**16C**	27.43	16.06	9.60	9.83	6.95
19C	99.96	23.02	-	-	-	-	-	-	-	**19C**	23.02	11.78	7.84	4.44	4.67
21C	100.39	11.56	-	-	-	-	-	-	-	**21C**	11.56	-0.77	-4.01	8.44	-3.46
16D	103.47	27.43	-	-	-	-	-	-	-	**16D**	27.43	16.06	9.60	9.83	6.95
19D	95.70	19.47	26.33	1.84	7.38	14.45	15.26	15.90	19.47	**19D**	19.47	0.01	-6.01	7.40	-0.75
20D	90.99	41.32	35.47	23.40	42.27	23.15	22.35	16.21	41.32	**20D**	41.32	-2.67	-4.79	8.52	4.93
AND	117.14	16.32	101.26	101.10	101.25	100.79	-44.98	-54.45	-54.25	**AND**	16.32	7.32	4.15	5.66	5.45
BED	123.95	10.25	64.47	43.72	11.25	8.50	14.61	8.78	16.73	**BED**	10.25	-0.40	0.74	1.92	3.96
CP3	109.05	53.56	-	-	-	-	-	-	-	**CP3**	53.56	-0.03	-1.61	-2.27	-1.48
CT3	112.69	52.20	116.72	116.12	114.43	98.25	44.86	22.50	22.66	**CT3**	52.20	20.81	3.43	1.40	3.17
CT4	111.25	78.92	99.93	61.45	18.85	26.88	14.77	21.09	16.95	**CT4**	78.92	20.51	4.63	-1.90	-4.35
CT5	108.48	13.73	100.00	50.36	38.01	12.96	16.31	9.03	14.77	**CT5**	13.73	1.47	1.15	2.16	3.29
DED	127.39	50.53	99.34	98.86	63.73	11.82	6.62	12.59	12.88	**DED**	50.53	12.52	6.68	-0.65	-1.05
LP1	102.25	11.21	100.07	100.08	98.54	96.97	9.16	6.17	33.57	**LP1**	11.21	1.97	3.22	1.55	4.31
MAD	93.79	93.13	100.07	100.00	100.00	100.02	99.24	51.68	57.32	**MAD**	93.13	36.88	0.80	1.85	2.61
MDZ1	100.07	61.50	100.01	99.45	95.13	99.13	99.16	98.81	61.16	**MDZ1**	61.50	39.98	30.04	-20.55	-12.72
MDZ4	101.76	34.53	-	-	-	-	-	-	-	**MDZ4**	34.53	19.73	7.75	2.23	3.54
MDZ5	102.22	79.18	-	-	-	-	-	-	-	**MDZ5**	79.18	46.51	32.53	-17.21	-15.09
RIC	94.97	23.31	99.67	46.06	18.59	12.21	16.91	20.59	16.88	**RIC**	23.31	9.15	7.26	6.78	11.63

Appendix VII: Extracts With Greater than 80% Inhibition and Greater than 10% Toxicity Microdilution Data

Extract	Inhibition 100ug/mL	L6 100ug/mL	Microdilution Assay							L6 Data					
			100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL	3.125ug/mL	1.563ug/mL		100ug/mL	50ug/mL	25ug/mL	12.50ug/mL	6.25ug/mL
10A	111.71	36.02	99.78	99.60	70.55	-13.09	-8.26	-7.76	6.17	**10A**	36.02	7.23	5.97	4.95	3.76
10B	100.53	99.76	99.49	99.57	36.12	25.91	9.78	23.77	16.24	**10B**	99.76	99.08	4.43	3.61	4.47
19D	95.70	19.47	26.33	1.84	7.38	14.45	15.26	15.90	19.47	**19D**	19.47	0.01	-6.01	7.40	-0.75
20D	90.99	41.32	35.47	23.40	42.27	23.15	22.35	16.21	41.32	**20D**	41.32	-2.67	-4.79	8.52	4.93
AND	117.14	16.32	101.26	101.10	101.25	100.79	-44.98	-54.45	-54.25	**AND**	16.32	7.32	4.15	5.66	5.45
BED	123.95	10.25	64.47	43.72	11.25	8.50	14.61	8.78	16.73	**BED**	10.25	-0.40	0.74	1.92	3.96
CT3	112.69	52.20	116.72	116.12	114.43	98.25	44.86	22.50	22.66	**CT3**	52.20	20.81	3.43	1.40	3.17
CT4	111.25	78.92	99.93	61.45	18.85	26.88	14.77	21.09	16.95	**CT4**	78.92	20.51	4.63	-1.90	-4.35
CT5	108.48	13.73	100.00	50.36	38.01	12.96	16.31	9.03	14.77	**CT5**	13.73	1.47	1.15	2.16	3.29
DED	127.39	50.53	99.34	98.86	63.73	11.82	6.62	12.59	12.88	**DED**	50.53	12.52	6.68	-0.65	-1.05
LP1	102.25	11.21	100.07	100.08	98.54	96.97	9.16	6.17	33.57	**LP1**	11.21	1.97	3.22	1.55	4.31
MAD	93.79	93.13	100.07	100.00	100.00	100.02	99.24	51.68	57.32	**MAD**	93.13	36.88	0.80	1.85	2.61
MDZ1	100.07	61.50	100.01	99.45	95.13	99.13	99.16	98.81	61.16	**MDZ1**	61.50	39.98	30.04	-20.55	-12.72
RIC	94.97	23.31	99.67	46.06	18.59	12.21	16.91	20.59	16.88	**RIC**	23.31	9.15	7.26	6.78	11.63

Appendix VIII: Possible Drug Candidates – 28 Extracts With Known Medicinal Properties

Extract	Toxicity 100ug/mL	L6 100ug/mL	Part of plant	Solvent	Amount (mg)	Note
6A	98.77	-0.12	stem	petroleum ether	40	anti-microbial, anti-tumor, anti-inflammation
9A	103.41	-0.19	the whole plant	etroleum ethe	60	anti-microbial, anti-tumor, anti-inflammation
15A	103.21	-5.11	the whole plant	etroleum ethe	40	anti-microbial, anti-tumor, anti-inflammation
19A	99.90	-1.66	the whole plant	etroleum ethe	30	anti-microbial, anti-tumor, anti-inflammation
21A	98.39	0.59	aerial parts	etroleum ethe	70	anti-microbial, anti-tumor, anti-inflammation
12B	99.99	-2.65	stem	ethyl acetate	60	anti-microbial, anti-tumor, anti-inflammation
17B	99.82	5.14	the whole plant	petroleum ether	40	anti-microbial, anti-tumor, anti-inflammation
2C	103.45	1.46	branch, leaf	95% ethanol	50	anti-microbial, anti-tumor, anti-inflammation
12C	100.32	3.91	stem	95% ethanol	110	anti-microbial, anti-tumor, anti-inflammation
13C	101.70	-4.85	aerial parts	95% ethanol	80	anti-microbial, anti-tumor, anti-inflammation
14C	100.91	-2.72	the whole plant	95% ethanol	50	anti-microbial, anti-tumor, anti-inflammation
15D	83.80	4.03	the whole plant	water	60	anti-microbial, anti-tumor, anti-inflammation
ANB	94.00	-3.06	the whole plant	Petroleum ether	37.82	---
ANC	102.71	-1.56	the whole plant	Ethanal	47.71	---
BEB	97.96	1.90	root	Petroleum ether	62.19	antitumor, anti-inflammatory and analgesic
CP1	106.19	6.74	---	Ethanol	---	anti-bacteria, tumor, inflammation, oxidation reduce blood sugar (hypodlycemic activity), cholesterol lower blood pressure
CP4	106.67	4.34	---	Ethyl acetate	---	anti-bacteria, tumor, inflammation, oxidation reduce blood sugar (hypodlycemic activity), cholesterol lower blood pressure
CT1	100.75	3.10	---	Ethanol	---	reduce cholesterol, lower blood pressure
CT2	99.54	-2.29	---	Petroleum ether	---	reduce cholesterol, lower blood pressure
DEB	103.26	-2.32	the whole plant	Petroleum ether	15.94	Tooth pain, pestcide, antibacterial, antifungus
DEC	132.48	4.34	the whole plant	Ethanal	94.27	Tooth pain, pestcide, antibacterial, antifungus
FLB	104.53	-8.58	stem	Petroleum ether	16.59	---
LP3	101.89	-3.53	---	Chloroform	---	reduce blood sugar, reduce cholesterol, lower blood pressure,, anti-bacteria
MAA	96.76	-2.20	the whole plant	Water	28.18	---
PAD	98.57	-1.78	the whole plant	Ethyl acetate	19.4	diarrhea
RIB	103.50	-5.50	the whole plant	Petroleum ether	19.06	---
RID	91.20	-7.43	the whole plant	Ethyl acetate	42.85	---
SB2	101.56	4.35	---	Petroleum ether	---	anti-microbial, anti-tumor, anti-inflammation