# HUMULUS LUPUS ALPHA ACID ISO-DERIVATIVES AS A NOVEL ANTIMICROBIAL APPROACH AGAINST STAPHYLOCOCCUS AUREUS

MARC T. SMITH

# *HUMULUS LUPUS* ALPHA ACID ISO-DERIVATIVES AS A NOVEL ANTIMICROBIAL APPROACH AGAINST *STAPHYLOCOCCUS AUREUS*

A Thesis

Presented to the College of Graduate Studies

In Partial Fulfillment of the Requirements for

Master's Degree

Marc T. Smith

May 2009

TO THE GRADUATE COUNCIL:

I am submitting herewith a thesis written by Marc T. Smith entitled Humulus Lupus Alpha Acid Iso-Derivatives as a Novel Antimicrobial Approach Against Staphylococcus Aureus. I have examined the final paper copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science of Biology.

Dr. Chad Brooks, Major Professor

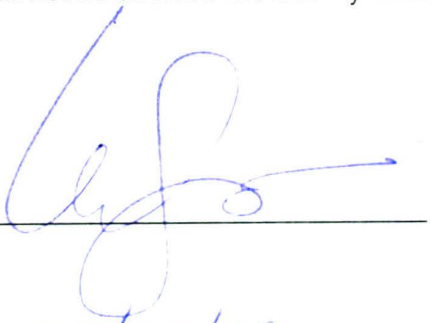
Accepted for the Council:

Dean, College of Graduate Studies

# STATEMENT OF PERMISSION TO USE

In presenting this (thesis/field study) in partial fulfillment of the requirements for the Master's degree at Austin Peay State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this (thesis/field study) are allowable without special permission, provided that accurate acknowledgement of the source is made.

Permission for extensive quotation from or reproduction of this (thesis/field study) may be granted by my major professor, or in his/her absence, by the Head of Interlibrary Services when in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this (thesis/field study) for financial gain shall not be allowed without my written permission.

Signature 

Date 05/07/09

**Acknowledgments**

The physical completion of this thesis was truly a testament to inspiration, discipline, and self-sacrifice. I wish *first* to thank my family. **Thank you** for promoting creative expression, believing in my abilities, and providing exhaustive amounts of encouragement and love throughout graduate school; *you* made this possible. Dr. Chad Stanton Brooks, your infectious zeal for life and brilliant execution as mentor was only surpassed by your capacity to befriend and uplift; **thank you** for enabling my maturation not only as a student, but also as a young man. Dr. Perry Scanlan, **thank you** for your attention and kind words throughout this arduous process; it did not go unnoticed and greatly affected my well-being. To my committee consisting of Dr. Don Dailey and Dr. Karen Meisch, your selection afforded me the privilege of honoring the influence you have had on my experience here at Austin Peay State University. I share this feeling of respect and adoration with the countless students you encounter on a daily basis and **thank you** for playing a crucial role in my graduate career. Lastly, the friendships I have developed throughout the years with the gifted students that have passed through the graduate student research laboratory is something I will always cherish. Although I may never get to fully express my thanks on an individual basis, without you folks, none of this would have made sense—**Thank you**!

*Now to Him who is able to do immeasurably more than we ask or imagine, according to His power that is at work within us. Ephesians 3:20*

**Abstract**

It is inevitable that modern societies will soon be forced to address the imminent threat of multi-resistant infectious microbes on account of indiscriminant use of contemporary antibiotics and various other control methods. Currently, conventional treatment options for multi-resistant bacteria are limited or nonexistent. These infections, for the most part, are being treated empirically (broad spectrum) owing to the lag time experienced in culturing and the aggressive nature of these organisms. Until the development and implementation of rapid diagnostic techniques, empirical antibiotic treatment is the only preemptive strategy available to combat infection, yet, ironically enables further resistance. This catch22 has forced microbiologists to think abstractly with respect to novel antimicrobial approaches and *organic* methodologies are currently in development. This study advocates for the use of *Humulus lupus* (hop plant) as a selective Gram-positive biocide.

Crude hop flower extracts were isomerized and assayed for their bactericidal potential against *Staphylococcus aureus*, a Gram-positive organism with multi-resistance potential. A working relationship was established for alpha acid (4% and 12%), the chemical responsible for Gram-positive membrane disruption, with respect to the *minimum* concentration required to exhibit maximum biocidal effect against the test organism. Minimum inhibitory concentration for 12% alpha acid hop strains was determined to be 6mg and 2.33mg for 4% strains. In order to more completely understand the nature of alpha acid, alternative variables were evaluated for their prospective impact on

the chemicals mode of action including death over time, pH augmentation, extreme temperature resilience, and metabolic analysis. Experimentation revealed that time *was* a limiting factor with respect to alpha acid induced lysis with 99.9% death observed within 30 minutes of exposure. The resting pH of the hop solution (pH 6) was acidified (pH 2) and alkalized (pH 11) and significantly enhanced the chemicals overall killing capacity. Various concentrations of hop solution were subjected to temperatures normally reserved for sporocidal sterilization (121°C) at 15lbs pressure for 15 minutes and displayed reduced killing capacity attributed to possible destabilization of the alpha acid. Buildup of toxic metabolites in *S. aureus* due to alpha acid influence was investigated and determined *not* to be a contributing factor to the death of the cell. Evidence derived from this study highlights alpha acid as a novel advancement in Gram-positive disinfection.

## Table of Contents

Page

Acknowledgements....ii

Abstract....iii

Lists of Figures....vi

Introduction....1

Materials and Methods....14

Results....24

Discussion....34

Literature Cited....42

Vita....46

## List of Figures


Figure Page

1. Female Hop Cone Anatomy.....7
2. Molecular Structure of Humulone.....8
3. The Effects of pH Augmentation on the Bactericidal Effect of Hops.....12
4. Serial Dilution Alpha Acid Death Curve Established vs. *S.aureus*.....25
5. The Bactericidal Effect Exhibited Over Time by 0.48% Alpha Acid.....27
6. Possible Deleterious Effects of Toxic Metabolic Accumulation.....28
7. The Bactericidal Effect Over Time by 0.6% Alpha Acid.....29
8. The Bactericidal Effect Over Time by 0.3% Alpha Acid.....30
9. The Effects of Autoclaving on Alpha Acid Efficacy.....32
10. Initial pH Determination.....38

# Introduction

## Brief Microbial History

Archeological record indicates that microbes made their first appearance on planet Earth approximately 3.5 billion years ago and since the dawn of man, they have proved themselves to be quite enigmatic. Their insidious presence has shaped the history of mankind with an eerie indifference and perplexed early societies and scholars attempting to understand and curtail their infectious manifestations. Hippocrates (460–370 BC) may have been the first to support the concept that mitigation of the biological expressions of infection somehow promoted survival centuries before microbes were even linked to infectious disease. He asserted that the formation of pus was *not* a natural component in the healing process and open wounds should receive treatment to avoid suppuration. His recommendations for managing lesions were similar to those employed by the ancient Sumerians (2900 – 1800 BC) who were known to cleanse wounds with wine, apply bandages, and then saturate the bandages with additional wine (8). Microbial infection was the driving force behind ambitious innovations including lavatories dating roughly 2800 BC in Pakistani homes and documented toilet paper usage by the Chinese as early as 589 AD (8). These novel inventions served as early indications that ancient civilizations recognized that certain unfavorable conditions were communicable, unbeknownst to its true origin, and may be prevented and/or controlled. This basic understanding of the

possible spread of disease and the need for aseptic adoption ultimately contributed to the exclusion of individuals and ushered societal segregation and class warfare. This phenomenon echoed throughout history with the shunning of lepers in biblical times and was the catalyst that influenced the rich to flee to their country homes in the Middle Ages in order to escape the small pox pandemic (10). A dismal biological accolade, known as the Bubonic Plague, crippled medieval-mankind while claiming over 200 million lives (1/3 of the world population). This flea-borne lymphatic infection all but eradicated the human species and shaped the course of early history with epic proportion by exploiting man's inability to contest the microbial apparition to blame. Early societies were surely acquainted *visually* with fungal and bacterial development commonly associated with the spoilage of food before the advent of preservation techniques, but overlooked the fact that they indeed were living, infectious entities and made no attempt at correlation. Sadly, this elementary awareness that humans were prey to an unseen relic did not enhance the survivability of our ancestors and, more often than not, life was cut short due to their inability to visualize the problem. The mid-1600's brought promise in the form of Anton van Leeuwenhoek, a cloth merchant from Holland, with respect to the long awaited, and unexpected, visualization of bacterial life. Known as the "Father of Microbiology," Mr. van Leeuwenhoek stumbled upon bacterial life by sheer accident as his original intent was to better inspect the quality of the weaves he produced and purchased with his newly revamped microscope. This microcosm

of microbial life, described as "tiny animalcules" by Leeuwenhoek, was not fully appreciated until some 200 years later when Ignaz Semmelweis discovered that an "invisible agent" must be responsible for the transmittance of pathogenic disease (1). Semmelweis made this correlation after witnessing the death of a close friend who cut himself during an autopsy of a patient whom had recently succumbed to sepsis. He inferred that the same agent responsible for the death of the patient had also played a role in the demise of his colleague. This notion was supported when hand washing and the utilization of clean lab coats were implemented in his hospital maternity ward and fatalities were subsequently reduced by 66.6% (4). Scientific research rapidly progressed towards the realization that the etiological agent responsible for infection indeed was the microbe through various ingenious studies performed in the mid-1800's. Louis Pasteur's swan neck flask experiment debunked the dogma of spontaneous generation by demonstrating that microbial life did not spawn at random or without reason (12). Robert Koch, a country doctor in the 1870's, established the Germ Theory of disease by isolating the suspected infectious agent from a deceased animal, growing it in pure culture, infecting a healthy, competent host with pure organism, observing clinical symptoms, and then re-isolating the same organism upon death. These steps later came to be known as Koch's Postulates and appropriately suggested that microorganisms *were* the etiological agents directly responsible for the signs and symptoms of what we call infection (1).

## Controlling microbes

Building upon historic scientific data correlating infection with agent, contemporary science has been focused on exactly *how* to eliminate and/or control microbes by chemical means. Recently, much effort has been directed towards the development of novel, safe antimicrobial agents to ensure disinfection of devices in the medical and food industries to combat contaminants like *Escherichia coli* and *Staphylococcus aureus*, just to name a few. On a smaller scale, a proliferation of personal, alcohol-based antiseptics such as Purell and GermX has spawned from mounting concerns over the potential for microbial contamination by way of daily interactions. Disinfectants can be defined as any substance applied to inanimate objects designed to kill microorganisms. Disinfectants and antiseptics are alike in that both are germicidal, but antiseptics are applied primarily to living tissue (20). The ideal disinfectant rapidly destroys bacteria, fungi, viruses, and protozoans, and would not corrode surgical instruments or discolor surfaces. A wide variety of active chemical agents (or "biocides") are found in disinfectants and antiseptics, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation (5). Common chemical disinfectants can be broadly classified as alcohols, chlorine compounds, ammonium compounds, aldehydes, phenolic compounds, halogenated tertiary amines, hydrogen peroxide and ethylene oxide gas. These familiar chemical classes demonstrate broad spectrum effectiveness against bacteria, as well as some fungi, but are expensive to produce and in

some cases are abrasive to the user due to the harshness of the active chemical agent. The unappealing, caustic nature of harsh chemicals used as disinfectants has pioneered an effort to deviate from their persistent use and allowed for the exploration of organic equivalents. This naturistic approach to antimicrobial development has aided in the emergence of a novel facet of research focused solely on the use of organic antiseptics against pathogenic and non-pathogenic bacteria such as tea tree oil, eucalyptus, birch tar, cinnamon, oregano, lemon grass, thyme, coriander, balsam of tolu and cedar leaf oils, to name a few (18). The antibacterial activity of plant oils and their constituents have been known for many years but have just recently gained in popularity and attracted novel approaches for their use (16).

**The History of Hops**

Hops were first used as a vegetable by ancient Roman civilizations in salads and played an integral role in their daily nutrition. As early as the eighth century, French and German monks cultivated hops for medicinal purposes and the Hebrews, recognizing the flower's intrinsic medical value, used hop extracts to help ward off plague. In North America, several Native American tribes independently discovered the healing properties of hops and used them as a sleep inducer, pain reliever, and digestive aid; these applications are now attributed to lupulin, a mild sedative secreted from the lupulin gland of the hop cone (6). Earliest written evidence of hop cultivation appears to be that concerning a hop garden near Geisenfeld in the Hallertau region of Germany in

736 AD. Additional documentary evidence from the 9th - 12th centuries shows that hop cultivation centered around Bohemia, Slovenia and Bavaria, and the use of hops as a flavor and aroma additive in beer in the Netherlands by the 11th century has been recorded (2). Hops were probably first added to beer as a *preservative* by Germans in the twelfth century. The bitter hop flavor was not particularly well received, but brewers used hops, despite its taste, because the bactericidal properties kept brew from spoiling and afforded manufacturers the luxury of transport and allowed marketing further from the production site. This may have eventually led to early large-scale brewing (6). The United States currently produces about 25% of the world's hops, and is second only to Germany in worldwide production. In 1997 US hop growers harvested 74.87 million pounds of hops valued at 117.9 million dollars and in 1998 the US produced 27,010 metric tons of hops on 14,830 hectares, according to data from the Food and Agriculture Organization. Germany, the world leader in hop production, harvested 34,200 metric tons of hops on 19,789 hectares in 1998. The average price per pound received by growers from 1995 to 1998 has ranged from $1.71 in 1995 to $1.59 per pound in 1998 (13).

**Hops**

The hop plant, a member of the Cannabaceae family, thrives in temperate climates and is native to Europe, North America and regions of Asia (9, 23). This dioecious species (separate male and female plants) is a tall bine (similar to a vine) that can reach heights of 16 to 30 feet (17). The female fruiting body,

known as the cone, is an essential ingredient in the beer-brewing process. Male plants do not produce cones and are not typically grown in hop gardens; instead, their function is simply to fertilize the female flower which leads to the development of seeds in the hops cones. The simplistic anatomy of the hop cone consists of a series of petal-like bracteoles radiating around an inner stem structure known as the strig. Small, yellow lupulin glands found at the base of these bracteoles house the acids and resins which were assayed for their antimicrobial potential in this study.

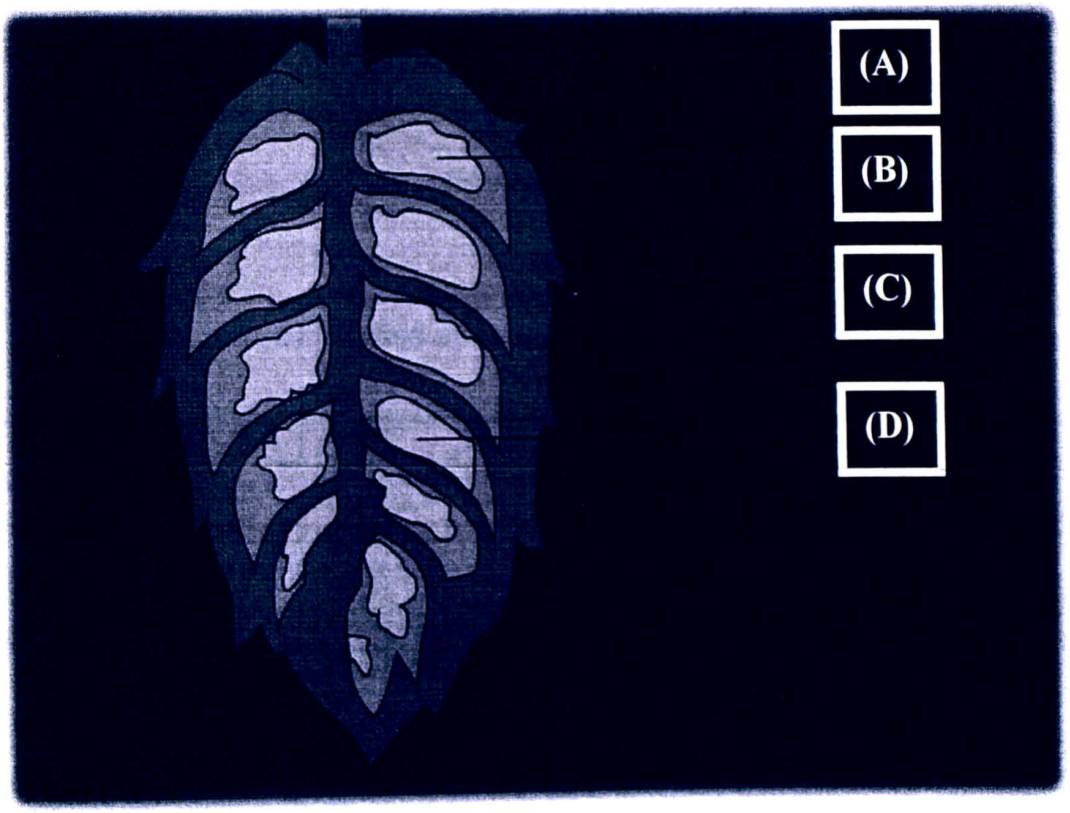



Figure 1. Female hop cone anatomy. (A) The strig, (B) bracteole, (C) bract, (D) lupulin gland (containing resins and essential oils).

**Alpha acid**

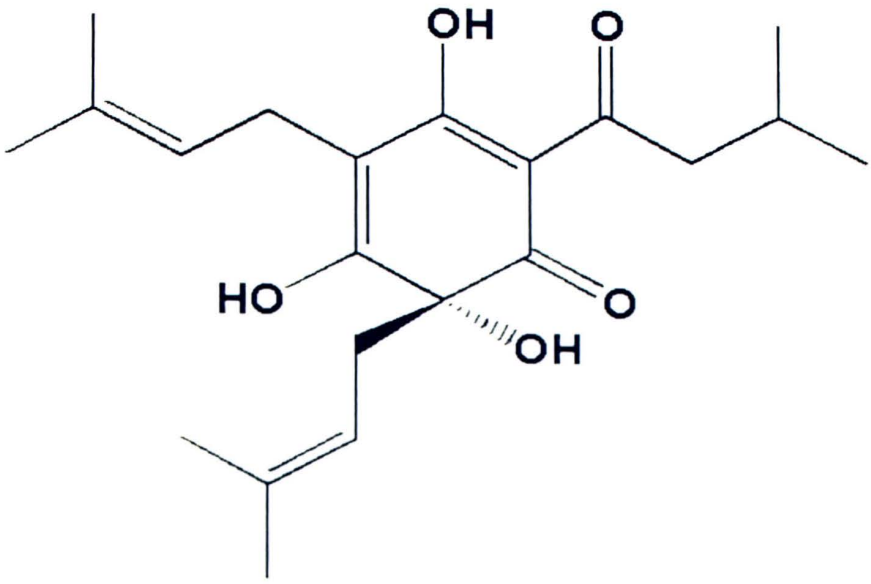



Figure 2. Molecular structure of humulone (alpha acid), $C_{21}H_{30}O_5$

Hop strains are classified by their intrinsic concentrations of alpha acid within their soft resin which is normally comprised of about 20-50% humulone, 20-50% co-humulone and 15% ad-humulone, respectively (3). Alpha acid, a major constituent of the plant *Humulus lupus*, is released from the soft resin of the hop flower when exposed to boiling temperatures for a marked period of time (3). Primary alpha acids, known as humulones, are isomerized during the boil into three secondary iso-analogs known as humulone, ad-humulone, and co-humulone differing slightly due to their specific R-groups and cis- or trans-conformations. These analogs exhibit bactericidal qualities that can be attributed

to their prenyl R-group (3-methyl-2-buten-1-yl) interfering with the plasma membrane of gram-positive microbes (35, 3, 31).

The gram positive cell wall of staphylococci is composed essentially of peptidoglycan and teichoic acid. Neither of which appears to act as an effective barrier preventing the entry of antiseptics and disinfectants. Since high molecular-weight substances can readily traverse the cell wall of staphylococci and vegetative *Bacillus* spp., this may explain the sensitivity of these organisms to antibacterial agents such as humulone and its iso-derivatives (31, 25, 27, 28, 26). The outermost layers of microbial cells can thus have a significant effect on their susceptibility (or insusceptibility) to antiseptics and disinfectants; it is disappointing how little is still known about the passage of these antimicrobial agents into different types of microorganisms.

**Significance**

Hop alpha acid displays great potential as an organic form of antimicrobial which can be attributed to its mode of action enabling the selective targeting of gram positive organisms and some fungi commonly associated with human infection (36). MRSA (methicillin-resistant *S. aureus*), an aggressive bacterium responsible for difficult-to-treat infections in humans, displays susceptibility to alpha acid residues in clinical trials, yet fails to be adequately controlled by modern beta-lactam antibiotics commonly prescribed to combat similar infections (34). Alpha acid also demonstrates effectiveness against *Mycobacterium tuberculosis*, a pathogenic, acid-fast bacterial species first discovered by Robert

Koch in 1882 (32). One third of the world's population has been infected with *M. tuberculosis* and new infections occur at a rate of one per second according to the World Health Organization (38). Inevitable bacterial resistance to modern antibiotics highlights the merit of implementing novel antimicrobial approaches, like the usage of alpha acid to treat mycobacterial infections, in attempts to retard the development of "super-organisms" we will surely face in the future. Alpha acid is a mild, non-toxic chemical that is well tolerated topically and internally due to its near neutral pH. Hops can be grown for personal use and are extremely cost-effective in comparison to antibiotics prescribed by a physician (13). Fresh and dried hops have different properties and are used to treat different symptoms. They are used in pillows to help ease a restless or anxious person into sleep and can be made into a tea that is taken to combat insomnia (23). Hops aid in digestion, stimulate appetite, function in relieving colic and have been documented to relieve tooth aches when placed between the tooth and the cheek. Hops belong to the same family of herbs as marijuana, and some claim it produces a mild, relaxed, euphoric feeling when smoked although there is no scientific evidence to support this currently (23).

**Practical Use**

The antimicrobial properties of the hop plant, specifically the alpha acid constituents, could prove useful on a practical level. Mouthwashes, toothpastes and hand soaps are designed to combat the buildup of biofilms orally and on the epidermal layer of the skin and would profit directly from the addition of alpha

acid. Results from this study indicate that the alkaline pH of handsoaps and the acidic pH of mouthwashes would enhance the antimicrobial activity of alpha acid by co-action and therefore supports the possible use of humulone-infused products such as these (Figure 3). Humulone iso-derivatives could also be useful additions to hygienic products such as tampons, diapers and deodorant. Prolonged use of tampons can promote the development of a condition known as Toxic Shock Syndrome (TSS) in women caused by *S. aureus*. This deadly infection may be averted by incorporating humulone into the absorbent material of the tampon which harbors the nutrients required to initialize the cascade effect of TSS. The mild sedative effects of hop acid may, in turn, assist with mitigating the symptoms of irritability and anxiety associated with pre-menstral syndrome as well. Diaper dermatitis, commonly known as bacterial diaper rash, is a condition caused by superficial *S. aureus* infection associated with pustules and crusted lesions. This condition is normally treated topically with Mupiricin or Neosporin but could benefit from implementing humulone either into the topical agent itself or to the absorbent material of the diaper directly to assist in bacterial prevention. Deodorants are commonly employed to alleviate malodorous expressions of microbial contamination of sweat and sebum upon excretion from the apocrine glands in the arm pit. Antiperspirants are designed to impede the physical flow of sweat altogether and therefore deny colonized bacteria the opportunity to prey upon its contents, thus, accomplishing the same task of odor prevention (15). Both methods of odor deterrence currently enlist the help of germicides such as

# The effects of pH augmentation on the bactericidal effect of hops

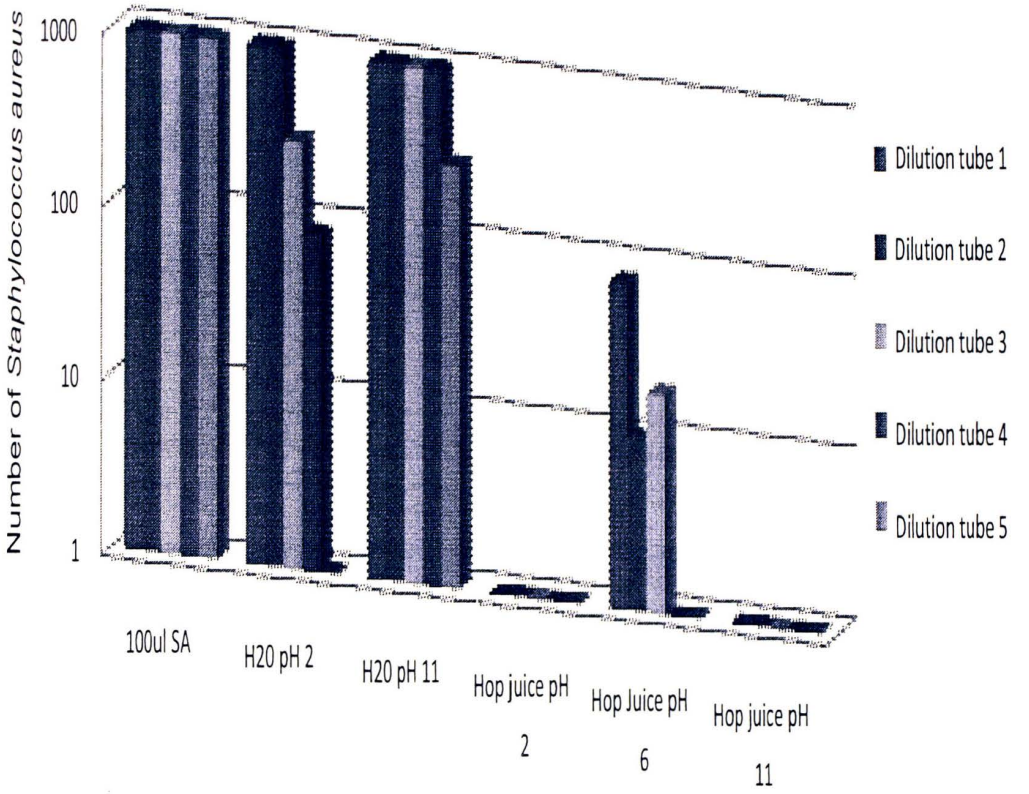



Figure 3. pH augmentation enhances the killing capacity of alpha acid against *S. aureus*. Serially diluted tubes 1-5 were subjected to various pH treatments and, when acidified and alkalized, 3.5g/100ml hops demonstrated 100% efficacy over a 2 hour incubation.

quaternary ammonium chlorides, phenolics, or Triclosan as the active ingredient designed to control gram positive bacterial loads responsible for undesirable effects. These chemicals, as mentioned previously, are not well tolerated in some cases due to their abrasive nature. Humulone incorporation would prove to be an effective addition to the chemical composition of these products, or provide a mild alternative for users negatively affected by reactions to the harsh chemicals in use. The dairy industry, normally sanitizing the teats of cows with diluted bleach solutions, could instead use sufficient concentrations of humulone in water to eliminate pathogens such as *Lactobacillus*, a commonly encountered milk contaminant. The application of humulone as the primary sanitizing agent would not only inhibit pathogenic presence but also provide a noticeable improvement in the condition of the teat itself due to its much milder chemical disposition. Humulone could even be grown on site for indefinite usage and would surely prove to be cost-effective. Alpha acid could be incorporated into the daily diets of dairy and beef cattle to inhibit the presence of gram positive organisms in the rumen that rob essential nutrition and produce byproducts such as methane that contribute to the destruction of our atmosphere. Antibiotic co-action is a topic of current research that focuses on the use of lesser amounts of antibiotics in conjunction with sufficient amounts of antibacterial phytochemicals (chemicals derived from plants) (21). In addition to the problem of resistance development, factors such as toxicity and cost also limit the use the antibiotics. The combination of antibiotic with hop acid could effectively reduce the quantity

of antibiotic necessary, thus, reducing its resistance potential while synergistically neutralizing bacteria on a level comparable to conventional regiments prescribed. This method could demonstrate broad-spectrum activity simply by focusing the antibiotic in use towards Gram-negative bacteria while exploiting the inherent Gram-positive selection of the alpha acids (21). Designer antibiotics could readily be developed and put into use based upon this concept to target specific infections more effectively.

## Methods and Materials

### Hop selection.

Twelve varieties of *Humulus lupus*, the hop flower, were purchased for this study from www.monsterbrew.com; each variety contained differing concentrations of alpha acid. These varieties included: Columbus (12.0%), Simcoe (11.9%), Centennial (9.5%), Cluster (7.9%), Glacier (6.0%), Cascade (5.4%), Fuggle (4.6%), Ahtanum (4.5%) and Mt. Hood (4.0%) in descending alpha acid concentration. Traditionally, the hop is harvested in whole-flower form, but this study utilized the pellet-form. Hop pellets are ground up whole hops that are then dried and pressed. The act of pressing creates a hop product with significantly less surface area and superior storage. The hop pellet contains roughly 25% more flower than the average whole hop, thus, allowing for optimization of the flower to liquid ratio. The Columbus and Mt. Hood strains were intentionally selected to be the primary representatives in this study based

upon the fact that they embodied the highest and lowest ranges of alpha acid concentration within the set.

**Hop preservation.**

The hop flower is photo-sensitive (16). When attacked by either visible or ultraviolet light, it converts the alpha acid, humulone, into reactive intermediates known as free radicals, thus, impairing its antimicrobial function (16). This adverse reaction to light is responsible for the "skunky" sentiment imparted to spoiled batches in the beer brewing process. In order to mitigate this phenomenon, the Columbus and Mt. Hood pellets were transferred into 50mL screwcap conical tubes and covered with aluminum foil to ensure minimal external light exposure. The hop strains were subjected to indefinite refrigeration at 4°C to ensure proper quality between uses.

**Crude hop extract preparation.**

Preliminary concentration troubleshooting dictated that the minimum antimicrobial properties of humulone are observed between the range of 2.5% to 5.0% in solutions of hop and water. In order to test these parameters, three hop stock solutions, 2.5g/100mL, 3.5g/100mL and 5.0g/100mL were employed in this study and obtained by means of simple filtration. Upon weighing out the necessary amount of hop, for example 2.5g, the pellets were aseptically transferred into a 250mL Erlenmeyer flask containing 100mL of deionized water. Contact with the water caused the pellets to quickly disassociated from pellet form and expand during rehydration. This phenomenon limited the sheer amount

of pellet that could be used in 100mL and still provide a workable aqueous portion, containing humulone, to be filtered away from the flower. The flask was transferred to a ceramic stir plate which was set to maximum heat and the hop and water mixture was boiled vigorously for 10 minutes. After the boiling time elapsed, the green, pulpy solution was crudely filtered into an autoclaved Erlenmeyer flask through a kimwipe in order to free the aqueous portion from the solid phase. This protocol was followed for all stock solutions utilized in this study and proved to be a useful means of obtaining isomerized humulone derivatives from the flower.

**Media preparation.**

Difco Nutrient Agar was prepared in house for use in this study as a growth medium allowing for the uninhibited cultivation of the microorganism *Staphylococcus aureus* ATCC 25923. Nutrient Agar consists of peptone, beef extract and agar and provides the nutrients necessary for the replication of a large number of microorganisms that are not excessively fastidious. The beef extract contains water soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptones are the principle sources of organic nitrogen, particularly amino acids and long-chained peptides, and agar acts as the solidifying agent. Nutrient Agar powder (12g) was aseptically transferred into a 2L Erlenmeyer flask containing 1L of deionized water and allowed to reach a vigorous boil for 10 minutes atop a heat plate to promote homogenization. After satisfactory mixing, the boiling liquid was autoclaved at

121°C and 15lbs of pressure for 15 minutes in order to ensure adequate sterilization. Upon removal, the flask's contents were allowed to cool for 20 minutes then poured into individual Petri plates totaling roughly 45 plates per liter of solution. After solidification of the Nutrient Agar, the plates were inverted to minimize condensing moisture from settling on the agars surface and were either stored at 4°C or used the same day in the study.

**Test organism selection.**

The test organism, *Staphylococcus aureus* ATCC 25923, was selected based upon primary literature that suggested it's susceptibility to the alpha acid humulone derived from hop resin (34). *S. aureus* is a gram-positive, cluster-forming coccus that inhabits the human nasal passages, skin and mucous membranes as a commensal organism. It is nonmotile, nonsporeforming, catalase positive, coagulase positive and readily ferments mannitol, a slightly sweet, crystalline alcohol. *S. aureus* is also known to be highly pathogenic; the most noteworthy of these being MRSA (methicillin-resistant *Staphylococcus aureus*). MRSA is a strain of *S. aureus* that is resistant to the broad-spectrum antibiotics commonly prescribed to treat infection, including penicillin of the beta-lactam family, and can prove to be fatal in some cases (34). This inborn resistance is attributed to a genetic element known as staphylococcal cassette chromosome mec element (34). *S. aureus* is also the causative agent responsible for Toxic Shock Syndrome which can cause death by irreversibly impairing kidney and liver function quite rapidly. Aside from the presence of the

virulence cassette found in MRSA isolates, *S. aureus* ATCC 25923 used in this study is genetically identical and much less virulent. This slight disparity enabled a model for representation of pathogenic presence while mitigating the potential dangers involved in working with such a powerful organism.

**Serial Dilutions.**

*S. aureus ATCC 29523* was streaked for isolation on Nutrient Agar and allowed to incubate for 48 hours at 37°C. One medium-sized colony was picked from the surface of the media and added to 1ml of Nutrient Broth in a 1.5mL microcentrifuge tube. Nutrient Broth is simply Nutrient Agar without the solidifying agent, agar powder. The tube containing a *S. aureus* colony, known as the "neat," was vortexed vigorously for 2 minutes to ensure that the entire colony was uniformly distributed throughout the tube. The concentration of organism in the neat, roughly $1x10^{8}$ bacteria, was serially diluted 1:10 in logarithmic fashion. Briefly, 100µl of the neat containing the *S. aureus* was aseptically pipetted into 900µl Nutrient Broth (1:10 dilution) and repeated over a span of 11 tubes with the eleventh tube representing $1x10^{-10}$ organism. This dilution scheme would subsequently be used to provide information pertaining to the minimum concentration of organism that can be effectively inhibited by varying alpha acid concentrations.

**Treatment of Dilution Scheme.**

Initial testing of the bactericidal effects of varying alpha acid concentrations was performed on the eleven serial dilutions of *S. aureus* in order

to provide quantifiable data. The eleven serially-diluted control tubes were divided into three sets containing 300µl of corresponding organism and denoted Set 1, Set 2, and Control. Each tube in Set 1 was treated with 300µl of Mt. Hood 4% alpha acid hop solution (2.5g/100mL) providing a 1:1 ratio of hop-solution to organism-solution. Set 2 was treated in the same fashion except with a Columbus 12% alpha acid hop solution (2.5g/100mL) and the third set acted as a control and went untreated. The treated sets were allowed to incubate at room temperature for 1 hour. After a short incubation to promote interaction of alpha acid and organism, 100µl of each tube from Set 1, 2, and Control was pipetted onto Nutrient Agar surface and diffused uniformly using a cell spreader rinsed with 80% ethanol between plates. These plates were then allowed to incubate at 37°C for 24 hours after which time the extent of bactericidal effect was determined by the number of countable colonies present on treated plates compared to the number present in the corresponding control. This method can also be carried out by spectrophotometric means reliably, but colony-counting is still considered the gold standard in growth inhibition studies (28).

**Timed Study.**

Results from the treatment of the initial dilution scheme indicated that the amount of time that the organism was exposed to alpha acids may also be an unexpected variable with respect to its efficacy. As stated earlier, it is believed that the prenyl R-group binds the membrane of the microbe, thus, inhibiting its ability to undergo binary fission and disrupting the membrane itself (3, 31). Does

this mode of action require a certain amount of time to fully exhibit inhibitory effects? In order to adequately test this phenomenon, specific tubes in the dilution scheme were selected for a timed assay based upon initial growth values when treated with 12% alpha acid. The tubes selected were 3–6 because they covered the spectrum that best represented the diminution of growth patterns and would ensure countable numbers of colonies. This also conveniently allowed for conservation of resources and time. The basis of this study was to test the minimum amount of alpha acid necessary to provide maximum effect against the inhibition of bacterial growth. Therefore, the 12% alpha acid Columbus strain was selected for the rest of the study due to the fact that we established previously there was a proportional relationship between the ability to inhibit growth and the amount of alpha acid present in the resin of the flower. For example, 12% alpha acid Columbus hop extract proved to be roughly three times more efficacious than 4% alpha acid Mt. Hood. To treat tubes 3 through 6, 2.5g/100mL stock solution of Columbus 12% alpha acid was used on our initial dilution scheme of *S. aureus*. At time zero, the representative tubes, each containing 300µl organism, were treated with 300µl 12% alpha acid extract and allowed to incubate at room temperature for 30 minutes. Following this period 100µl was immediately plated from the dilutions along with 100µl of positive controls using the protocol previously discussed in this section. This process of extracting and plating 100µl of the treated tubes along with 100µl of positive control was repeated in 30 minute intervals for three hours in order to determine

if there is a lag stage involved in the process of growth inhibition of *S. aureus*. To assess possible survivability of the organism, 100µl of the treated tubes and positive control was saved to be tested 24 hours later. The results of this study at 2.5g/100mL concentration provoked assaying of different concentrations of 12% Columbus alpha acid including 3.5g/100mL and 5.0g/100mL following the same protocol. These timed studies were performed in triplicate to ensure accuracy and precision.

**Toxic Metabolite?**

Results concluded from the timed studies indicated that prolonged exposure to alpha acid indeed plays a role in growth inhibition. Could this be due to the buildup of a toxic metabolite induced by uptake of hop solution by *S. aureus*? In order to demonstrate and test this idea, 300µl *S. aureus* from dilution scheme tubes 3-6 was treated with 300µl 3.5g/100mL Columbus 12% alpha acid and incubated for 90 minutes. Previous data in this study suggested that 90 minutes be used to give the organism enough time to produce toxic metabolites that could possibly inhibit its growth. After the incubation period the solution was centrifuged at maximum speed for 10 minutes to ensure the formation of a strong pellet of organism. The supernatant, or supe, was removed after centrifugation and added to a fresh 300µl of *S. aureus* then allowed to incubate for 90 minutes. After the 90 minutes elapsed, 100µl was plated from each tube and incubated for 24 hours. This method was employed to each tube, 3-6, in triplicate to ensure accuracy and precision.

**Autoclaving Hop Extract.**

To demonstrate the extent of heat lability on the inhibitory growth effect of the solution, 600µl of Columbus 12% alpha (3.5g/100mL) was filtered and used. Two sets of 300µl were derived from the 600µl stock and one of the sets was subjected to autoclaving at 121°C at 15 lbs pressure for 15 minutes. The outcome had noticeable visible effects on the solution by changing its normal color of mint green to a dark brown hue. Next, 300µl of tubes 3-6 in the primary dilution scheme were treated with 300µl autoclaved and non-autoclaved solutions and allowed to incubate at room temperature for 90 minutes before plating. The plates were incubated at 37°C overnight and assessed for growth pattern changes against the control set the following day, roughly 24 hours post-treatment.

**pH Determination.**

Fresh 3.5g/100mL Columbus 12% alpha acid hop solution was obtained by the protocol previously stated in the crude hop extract preparation section and pH was determined in triplicate by litmus paper. This simple test confirmed the resting pH of isomerized hop solution to be slightly acidic at pH 6 on a normal scale of 1 (most acidic) to 14 (most basic). The testing thus far in the experiment has been performed with unaltered isomerized hop solution at a pH of 6. Does pH have an enhancing effect on the process of *S.aureus* growth inhibition? If so, does it favor acidic or basic conditions? Two additional 3.5g/100mL Columbus 12% alpha acid solutions were produced following identical protocol and their pH

was amended to a pH of 2 for the acidic, and pH of 11 for the basic to roughly span the extent of the scale. These test solutions, originally pH 6, were modified by addition of 1M HCl for the acidic representative and 1M NaOH for the basic by adding 1 drop of reagent at a time from a disposable pipette and litmus tested between drops until desired pH was achieved. As negative controls, 200mL of deionized water was obtained and autoclaved for 15 minutes and divided into two 100mL solutions. One control was acidified with 1M HCl to a pH of 2 while 1M NaOH was added to the other in similar fashion until a pH of 11 was achieved. A fresh dilution scheme was obtained from a medium-sized colony of stock *S. aureus* following the protocol previously mentioned in the Serial Dilutions section. Also, 600µl of each tube represented in the dilution set, 1 through 5, was extracted and divided equally to create six 100µl sets of diluted organism in order to test the variable pH solutions. These sets were labeled pH 2, pH 6, pH 11, $H_20$ 2, $H_20$ 11 and positive control (i.e., original conditions). Each of these sets was treated accordingly with 100µl of the desired pH set in tubes 1 through 5 and allowed to incubate at room temperature for 1 hour. After this incubation period, the total volume, 200µl per tube, was plated to Nutrient Agar and allowed to incubate for 24 hours at 37°C. Growth was recorded the following day.

## Results

To initially evaluate the bactericidal properties of humulone iso-derivatives, different concentrations of alpha acids were added to serial 1:10 dilutions of *S. aureus*. The growth inhibiting potential of differing percentages of alpha acid derived from the hop resin was performed using 4% Mt. Hood hop strain at a concentration of 3.5g/100mL, or 0.1% total alpha acid and 12% Columbus hop strain, or 0.3% total alpha acid with the same concentration. A serial dilution death curve was established after a period of incubation with the alpha acid for 1 hour in 1:10 dilutions over the span of 11 dilution tubes (Figure 4). In the 4% (i.e., 0.1% alpha acid) trial, 127 countable colonies were recorded in the $1x10^{-5}$ dilution and showed substantial bactericidal effect against *S.* aureus, killing approximately 86% (773/900 cfu) of the organisms. In subsequent dilutions after $1x10^{-5}$, 4% hop cleared all *S.* aureus organisms (Figure 4). The 12% (i.e., 0.3% alpha acid) trial displayed a countable number of 115 colonies in the $1x10^{-3}$ dilution and showed significant bactericidal effect, killing 99.89% (99,885/100,000 cfu) of the *S* aureus organisms (Figure 4). This data indicates that hop alpha acids were bactericidal to *S. aureus* and dependent on alpha acid concentration in 24 hours.

To better characterize the bactericidal effects of hop alpha acids, a time course study was developed. *S. aureus* organisms were serially 1:10 diluted and 3.5g/100mL of Columbus 12% (0.3% alpha acid) hop strain was used to treat dilution tubes 3 through 6 (i.e., $1x10^{-2}$ through $1x10^{-5}$ dilutions) at time point

## Serial dilution alpha acid death curve established vs. *S. aureus*

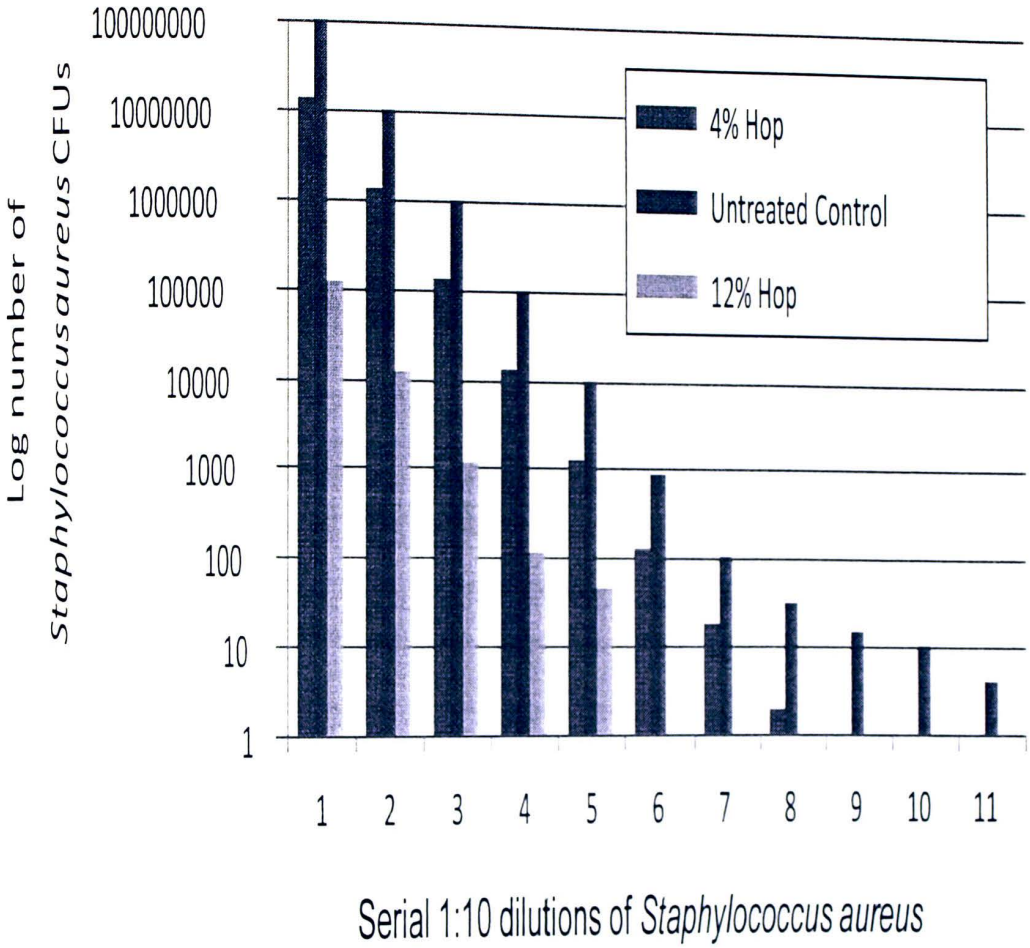



Figure 4. 1:10 Serial Dilution Death Curve displaying 4% alpha acid and 12% alpha acid vs. *Staphylococcus aureus* . 4% MIC value determined to be 2.33mg/20ml and 12% MIC value was 6.0mg/20ml.

"zero." At 30 minute intervals over the course of 3 hours, samples from the *S. aureus* bacteria treated with 0.48% alpha acid were plated to determine death curves (Figure 5). These data shows that hop alpha acids kill approximately 99.0% of *S. aureus* bacteria in the first 30 minutes, but requires up to 24 hours to exhibit 100% bactericidal effect.

It was interesting to note that in Figure 5, every *S. aureus* dilution, when treated with 0.3% alpha acid resulted in an initial profound killing of *S. aureus* but the data "plateaus" for at least three hours before the last time point taken. One proposed explanation for the abrupt death, followed by plateau phenomenon, was that the organism was emitting a toxic metabolic substance that built up until ultimately causing death at some point post the last time point taken at three hours. To better understand this plateau phenomenon, *S. aureus* organisms were treated identically above and supernatants were collected to evaluate any deleterious metabolic byproducts which could have been generated by alpha acid exposure (Figure 6). Solutions of 2.5g and 5.0g/100mL 12% Columbus hops were used to test this trend to determine if it in fact was concentration dependent. The same plateau effect was observed when the concentration of the hop solution was reduced to 2.5g/100mL and likewise when increased to 5.0g/100mL in a relative manner (Figure 7, 8). This suggested that the concentration of hop in the solution was *not* directly responsible for the abrupt death curve followed by a period of stagnation. To further test this variable, the prospective toxic metabolite was harvested from previously treated *S. aureus*

after 1.5 hour incubation by centrifugation.

## The bactericidal effect exhibited overtime by 0.48% alpha acid

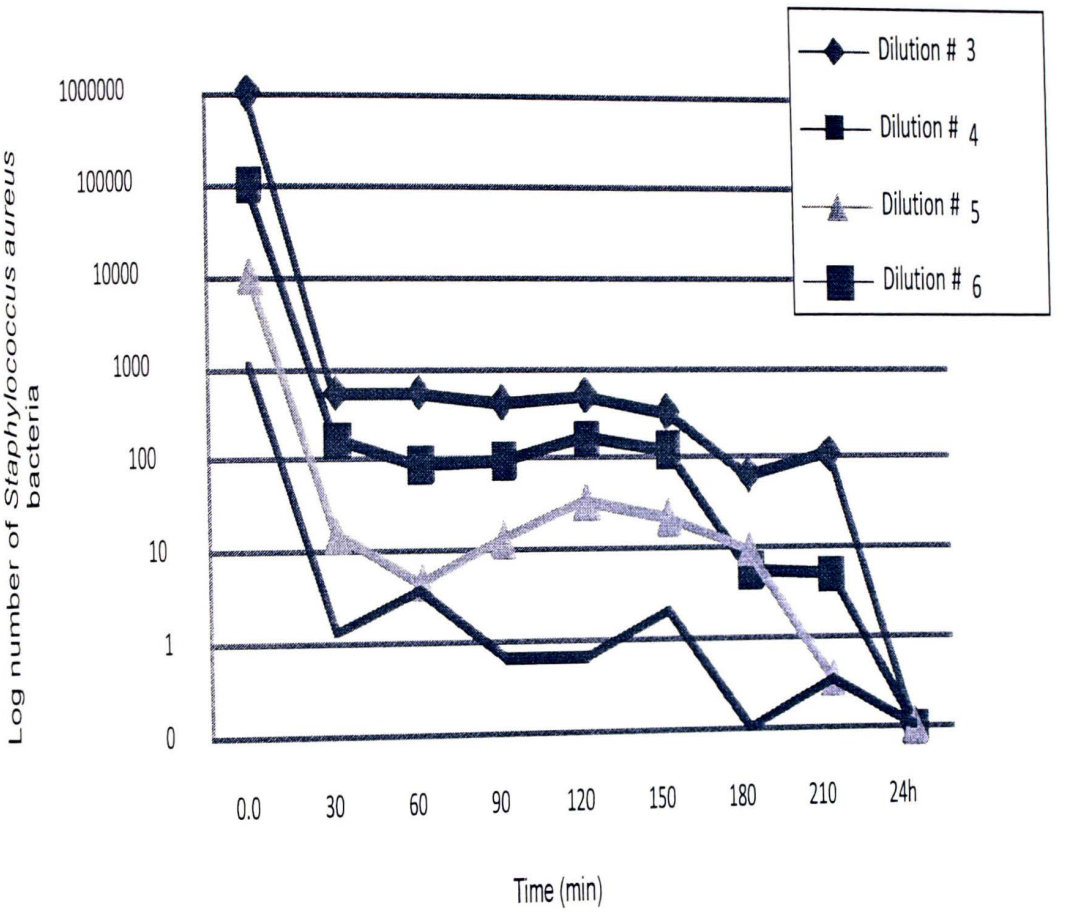



Figure 5. 12% Columbus hops (0.48% aa) 3.5g/100ml vs. *S. aureus* over a period of 3 hours in serially diluted tubes 3-6. 99% death is shown to occur within the first 30 minute interval and 100% displayed 24 hours post treatment.

## Possible deleterious effects of toxic metabolic accumulation

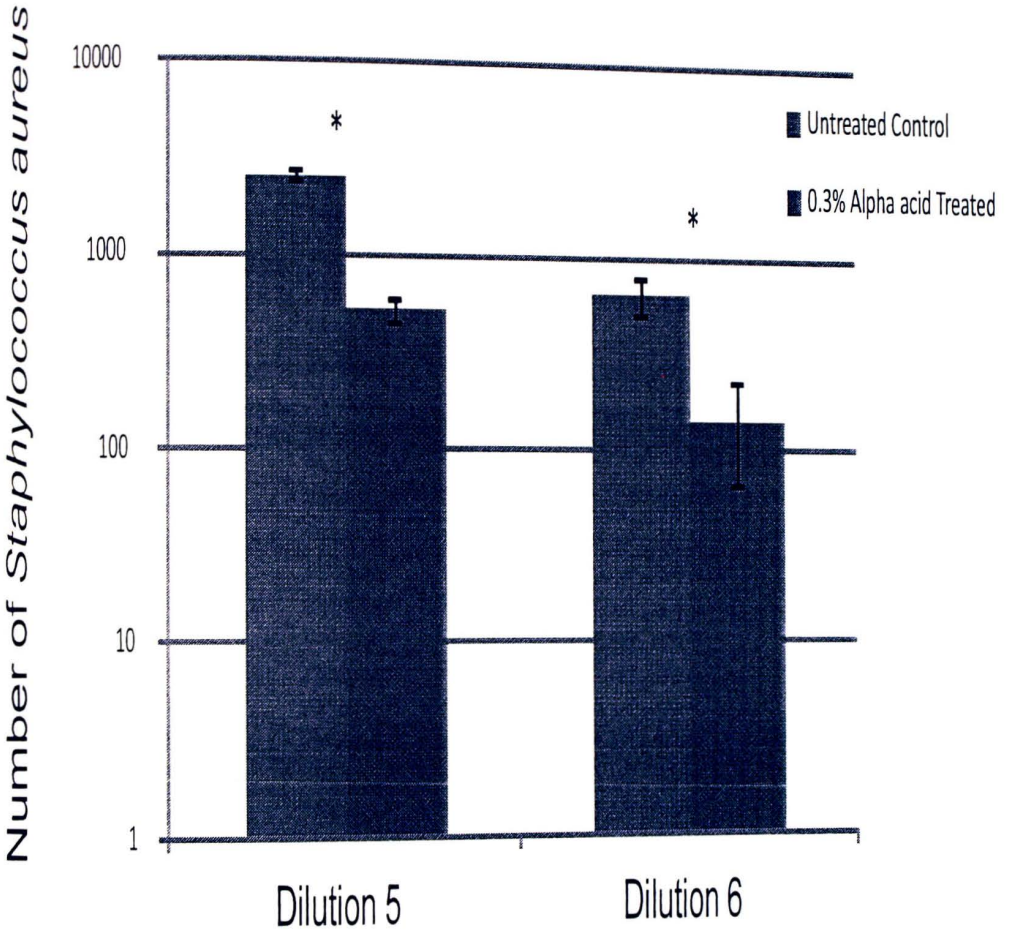



Figure 6. Alpha acids do not induce the production of toxic metabolites contributing to *Staphylococcus aureus* cell death. However, dilution 5 and 6 shows a residual killing effect of unbound (free) humulone on *S. aureus* which was still significant at P=0.05.

## The bactericidal effect exhibited overtime by 0.6% alpha acid

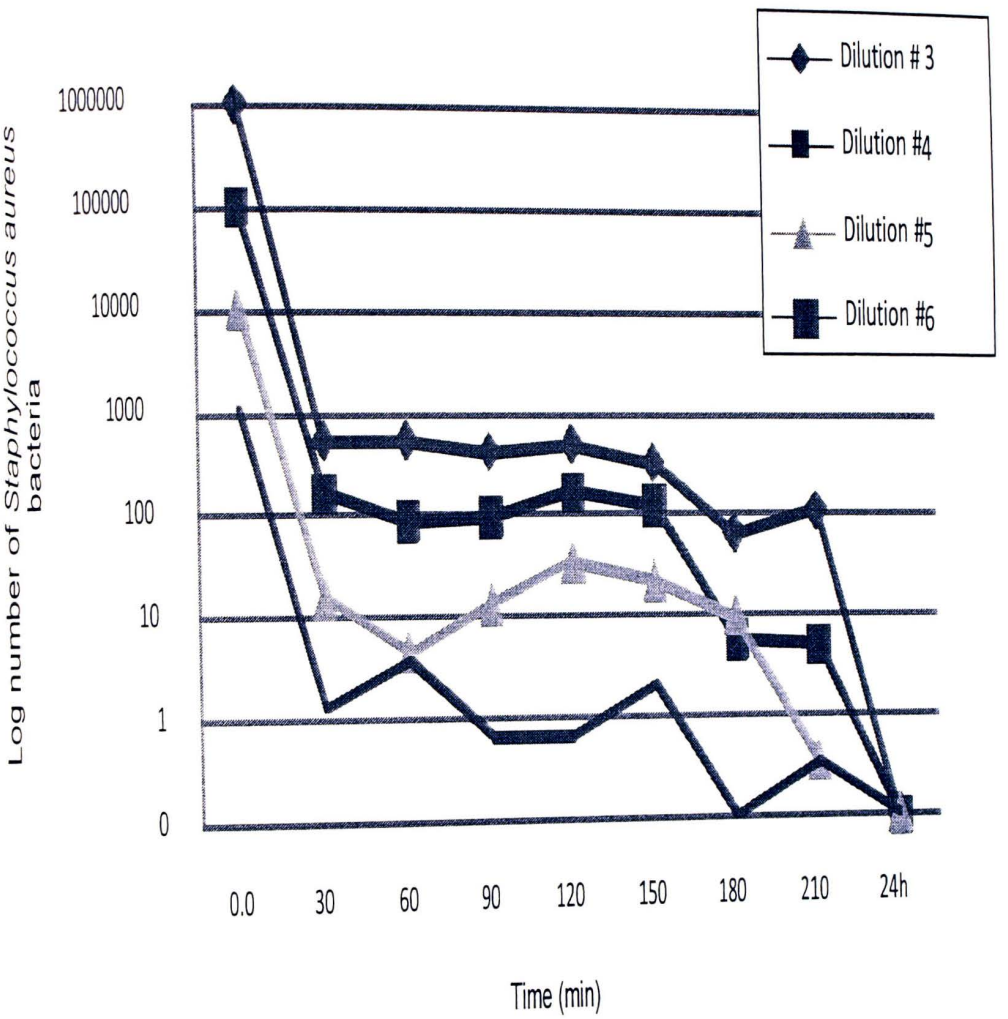



Figure 7. 12% Columbus hops (0.6% aa) 5.0g/100ml vs. *S. aureus* over a period of 3 hours in serially diluted tubes 3-6. 99.0% death is shown to occur within the first 30 minute interval and 100% displayed 24 hours post treatment.

## The bactericidal effect exhibited overtime by 0.3% alpha acid

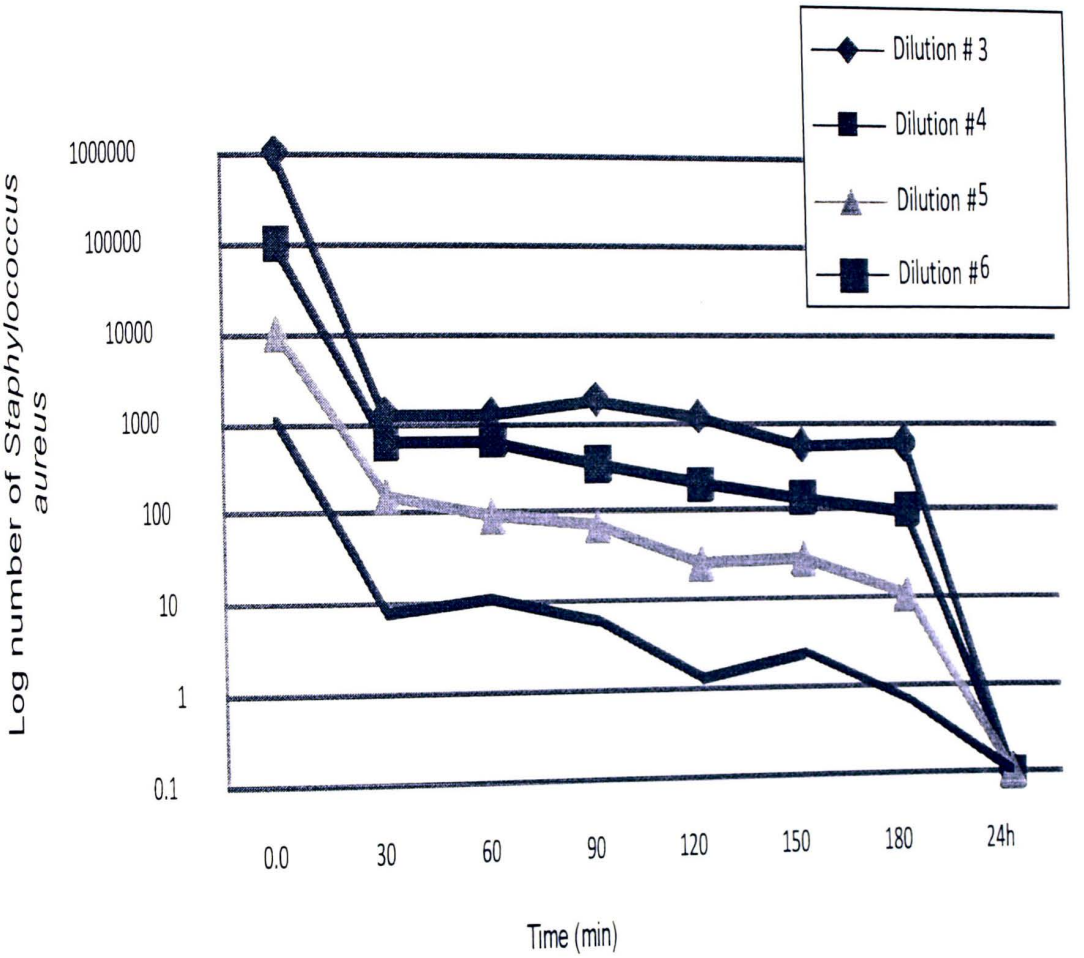



Figure 8. 12% Columbus hops (0.3% aa) 2.5g/100ml vs. *S. aureus* over a period of 3 hours in serially diluted tubes 3-6. 99% death is shown to occur within the first 30 minute interval and 100% displayed 24 hours post treatment.

The supe, thought to contain the metabolite, was subsequently used to treat fresh, untreated *S. aureus* and assayed for effectiveness. Tubes 3 through 6 from the original dilutions were tested in triplicate against a control. Tubes 3 and 4 did show slight signs of inhibition but were too numerous to count when plated. Tube 5 displayed 75.3% death and tube 6 supported with 72.6% (Figure 6). These numbers were slightly less than anticipated and were not able to definitively support the concept of a toxic metabolite being responsible for the steep death curve witnessed in the beginning interval of the timed studies. Autoclaving was employed as a way to test the heat lability of 3.5g/100mL 12% Columbus hop solution and proved effective at significantly *lessening* the bacteriocidal effect. Tubes 3 through 6 from the original dilutions of *S. aureus* were treated with autoclaved hop solution and showed significant resilience when compared to the non-autoclaved control after 1.5 hours of incubation at room temperature. Non-autoclaved hop solution killed 99.9% of organism originally present in each of the respective dilution tubes while autoclaved hop solution killed 72.2% in tube 5 and 58.4% in tube 6 (Figure 9). Tubes 3 and 4 in the autoclaved set were too numerous to count and demonstrated little killing effect (Figure 9). The antibacterial effect of weak acids derived from the hop plant was enhanced significantly with decreasing and increasing pH (31). In preliminary treatment trials of modified hop solution, 100% death was observed with an adjusted pH of 2 in each of the 11 (1:10) dilutions of *S. aureus*.

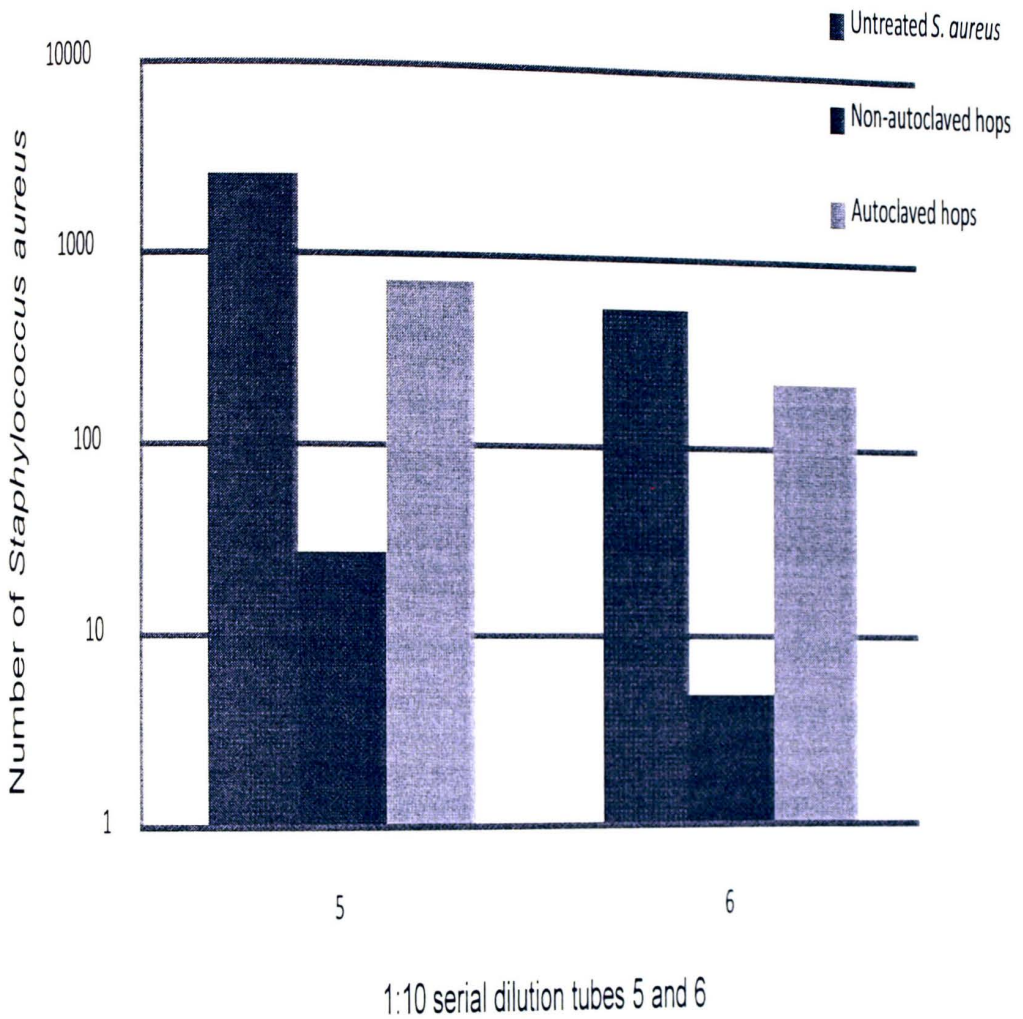



Figure 9. 3.5g/100ml 12% Columbus hops overall killing capacity is negatively affected by exposure to sporocidal temperatures of 121°C at 15lbs pressure for 15 minutes.

Amending the solution to pH 11 reproduced this effect when treated with a 1:1 ratio of hop solution to organism with 100% efficacy in each of the dilution tubes (Figure 3). These results were observed after an incubation period of 2 hours in hop solution before plating. This assay was repeated on tubes 1 through 5 with an incubation period of 1 hour and identical results were observed in the pH 2 and pH 11 trials as no growth was observed. Water controls (pH 2 and pH 11) were implemented and displayed significantly reduced killing capacity although favoring acidification. The lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, known as the minimum inhibitory concentration (MIC), was extrapolated from this study for 12% Columbus and 4% Mt. Hood hop varieties against *S. aureus*. MIC for 12% Columbus hops was mathematically determined to be 6mg/20ml, with 20ml being the total volume of the Petri plate used for culture. MIC for 4% Mt. Hood hops was subsequently determined to be 2.33mg/20ml. The dose of an antimicrobial required to kill *half* the members of a tested population, known as the median lethal dose (LD 50), is a general indicator of a substance's acute toxicity. The LD 50 of 12% Columbus hops was mathematically determined to be 0.75mg (750µg). If 100,000 ($10^5$) organisms were treated with 750µg of 12% hops, it would result in 50% death, for example. Likewise, 4% Mt. Hood hops LD 50 was determined to be 0.873mg (873µg) when used to treat 1000 organisms according to data extrapolated from Figure 4.

## Discussion

Crude hop-resin extract has shown great potential as a biocide in a controlled laboratory environment against *S. aureus*. Low concentrations (2.5g/100ml) of two different strains, Mt. Hood (4%) and Columbus (12%), were shown to dramatically impair bacterial growth (~99.98%) within 30 minutes or less. This finding highlights its potential for use as a stand-alone, or supplemental, organic disinfectant. Unfortunately, resource conservation and time limited the opportunity to study the biocidal effects exhibited between time zero and 30 minutes in greater detail and as a result they are not well understood. Substantial death occurs within this interval and the extensive breakdown and analysis (minute by minute) may illustrate that death occurs much earlier than this study indicates. More detailed analysis of the 30 minute interval should be carried out spectrophotometrically rather than employing the colony counting technique for obvious efficiency purposes and to promote increased sensitivity. This piece of information would refine and further validate the use of humulone and its iso-derivatives as an effective, affordable method of gram-positive disinfection.

Treatment of *S. aureus* was carried out in a 1:1 ratio of hop solution to organism in varying concentrations for the purpose of understanding the minimum amount of physical hop necessary to deliver maximum effect. Dipping below the 1:1 ratio significantly reduced killing capacity in preliminary experimentation and, conversely, it was determined that increasing the ratio significantly increased its ability to kill. It was determined, then, that a 1:1 ratio of

treatment closely represented the minimum volume necessary to obtain the desired effect of 99.9% death and was used throughout the study. Although this variable could be further tested for accuracy it should provide a practical understanding of the concentration vs. volume necessary for development of standardized hop solutions used as commercial disinfectants.

Hop alpha acid has limited, if any, effectiveness, according to the literature, against Gram-negative bacteria due to the intrinsic differences in their cell wall makeup (3). The prenyl-group present on the humulone molecule functions in membrane attachment and is ultimately responsible for the disruption of gram positive cell walls and inducing lysis (3). This mode of action could be exploited and used to target Gram-positives that colonize the body by infusing everyday items such as deodorants or tampons with hop-resin extract. Gram-positives, for the most part, are responsible for unpleasant conditions including tooth decay, food poisoning, body odor and more severe conditions such as TSS. It seems only logical, then, to infuse items designed to combat these organisms with a chemical designed specifically to screen for them. Gram-positive bacteria parasitize the gastrointestinal tract of farm animals by robbing essential nutrients from food intake and are responsible for much of the methane gas emitted from cows into the environment. Incorporating humulone into an animal's diet should, in turn, allow for more efficient utilization of food stuffs by maximizing nutrient uptake and subsequently cut down on methane emission. Incorporating humulone in toothpastes may play a useful role in biofilm reduction

and promote overall oral health. The results from this study should enable production of effective concentrations of humulone derivatives to be made available for use in topical disinfectants designed to prevent Gram-positive infections such as MRSA. Modern acne treatments are designed, in part, to target Gram-positive bacteria responsible for oil production and inflammation of pores such as *Propionibacterium acnes*. Integration of humulone derivatives into modern topical treatment methods could prove to be an effective, safe enhancement.

An appealing advantage of the use of hops as an organic form of disinfection is that it is non-toxic and can be readily ingested or applied to objects without the risk of adverse noxious effects as is the case in most commercial disinfectants that use harsh chemicals like bleach, chlorine, alcohol or aldehydes, for example. Hop resin has been given "generally recognized as safe" (GRAS) status by the FDA, however; the use of medicinal quantities of hops may pose more risk than common levels of exposure in food use. Studies indicate that dogs appear to be somewhat sensitive to hop compounds. Malignant hyperthermic reaction, a potentially fatal biochemical chain reaction response "triggered" by commonly used general anesthetics, was observed in five dogs who consumed boiled hops residues used in home beer brewing (11). A subchronic toxicity study of the hops alpha-acids was conducted in dogs; while high doses induced vomiting, lower doses were well tolerated without ill effects. A wide safety margin for humans was extrapolated from this experiment (7).

100µl of 12% Columbus (3.5g/100ml) was ingested orally on two separate occasions by the experimenter in this study and potential physiological effects were assayed. The resin extract proved to be extremely bitter and induced a sense of stomach discomfort lasting roughly 30 minutes which may explain the vomiting observed in the canine studies. Although generally unpleasant, no adverse effects were deemed significant.

Whether pH has an enhancing effect on the process of gram positive growth was an interesting find throughout the experimentation process. The isomerized hop extract has a natural resting pH of 6 and demonstrated significant killing capacity without augmenting the pH balance of the treated solution (Figure 10). This information would be beneficial for possible incorporation into topical and oral treatment methods due to the relaxed pH being easy on the epidermal and dermal layers of the skin which carry a pH of 5.5 when factoring in excretion of oil and sweat from the pores. The normally alkaline pH of the oral cavity at 7.2 should also provide a conducive environment for humulone treatment where Gram-positive organisms such as staphylococci, streptococci, and lactobacilli normally colonize. *Streptococcus mutans* is an important colonizer on the surface of teeth and their function includes the breakdown of sugar molecules to assist in adherence to the tooth. The breakdown of sugar produces lactic acid, lowers the overall pH, and ultimately leads to degradation of enamel and tooth decay. It has been shown that the germicidal effectiveness of humulone derivatives is enhanced by increasing or decreasing pH conditions (Figure 3).

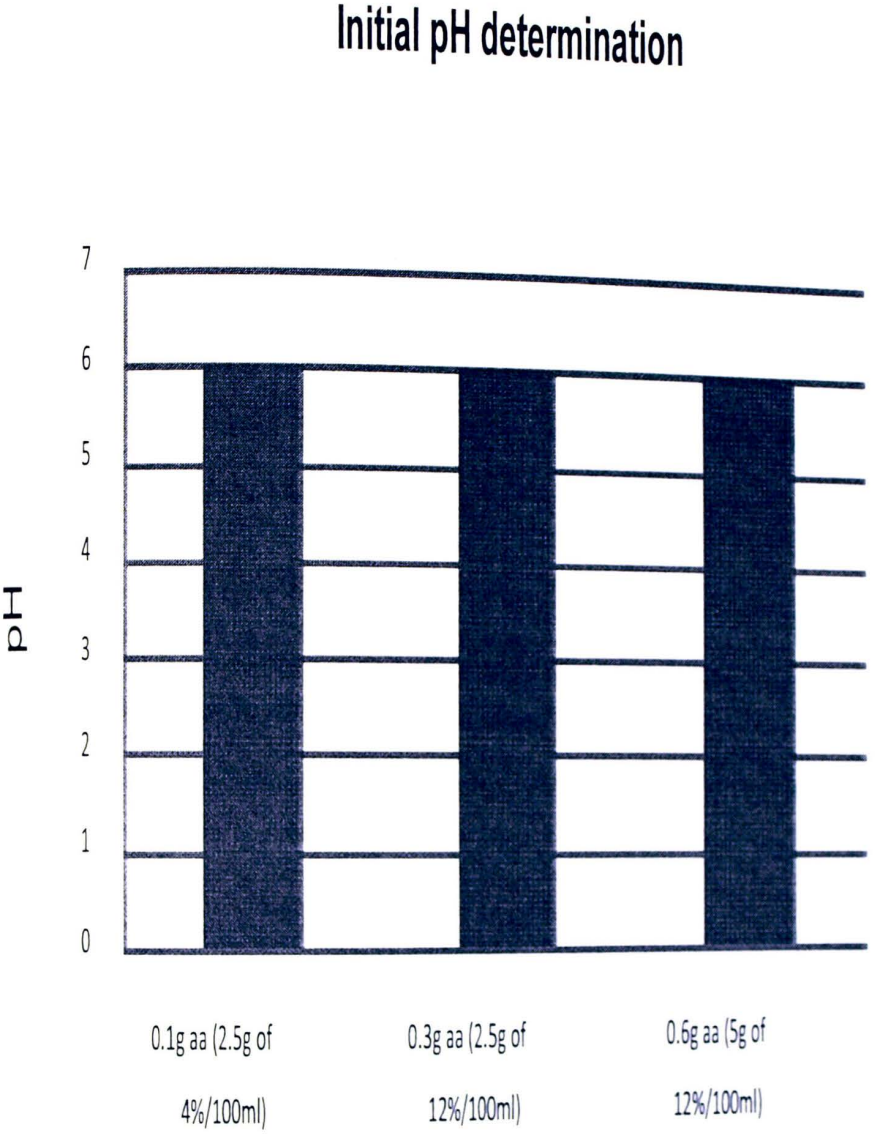



Figure 10. Various concentrations of 12% Columbus hops and 2.5g/100ml 4% Mt. Hood display the same pH of 6.0.

Results from this study indicate that oral antiseptics including toothpastes or mouth rinses should benefit from the addition of hop residues and should be further tested in a laboratory setting (29).

Although testing was not conducted on humulones ability to survive pasteurization, a low-temperature commercial sterilization method used in the food industry, the chemicals ability to withstand autoclaving at 121°C under pressure for 15 minutes was assayed. Results indicated that high temperatures reserved for sporocidal sterilization had diminishing effects on the chemicals overall killing capacity, although not entirely (Figure 9). This phenomenon may be due, in part, to the denaturing effect that extreme temperatures have on molecular stability and appears to render its mode of action of membrane disruption less effective. The literature states that when humulone is boiled in water at 100°C the rate of destruction of preservative value is significant and there is a mechanical loss owing to the adhesion of humulone aggregates on the sides of the vessel (37). Even though the overall ability to carry out cellular death is effected by autoclaving, results indicate that humulone is still 58.4 – 72.2% efficacious and therefore would still provide remarkable preservative value to an already sterile solution or object with respect to possible Gram-positive contamination. The post-autoclave survivability of humulone derivatives may be a useful attribute that could assist in maintaining sterile medical instruments or machinery used in the food industry.

This study used nutrient agar as the primary nutrient substrate for growth of the test organism. Although purity of culture was assured both visually and physically based upon the absence of obvious contaminants and rigorous subculturing, it would be more fitting for future studies to incorporate a better indication method to ensure *S. aureus* was indeed the sole organism present on the plate during colony counting. One proposed resolution would be to use a media that is not only selective for Gram-positive bacteria but also differential to ensure that only test organism is represented on the plate. MSA (mannitol-salt agar) inhibits Gram-negative contamination and provides a differential color indicator by exploiting *S. aureus'* natural fermentation of mannitol and ability to withstand increased salt concentrations. Although purity was assured throughout the study, a selective, indicating growth media would alleviate the potential for contamination issues that may affect the outcome of similar studies.

*S. aureus* ATCC 25923 was selected as the test organism for this study based upon availability of pure strain and primary literature supporting its susceptibility to *Humulus lupus* soft resin extracts including humulone iso-derivatives (30). Although not mentioned in this study, the literature confirms that humulone iso-derivatives demonstrate effectiveness against many gram positive organisms including *Micrococcus spp.*, *Staphylococcus aureus, Bacillus subtilis*, *Streptococcus spp., Mycobacterium tuberculosis, Lactobacillus* brevis; the fungus *Trichophyton mentagrophytes*, and parasitic members of the genus *Plasmodium* (33, 16).

Deionized (DI) water, devoid of ionic content, was exclusively used throughout this study. Monovalent cations such as $Na^+$, $K^+$, and $NH_4^+$ are integral components found in natural water and demonstrate enhancing effects on the antimicrobial potential of hop extract (37). Future studies may be inclined to incorporate the use of natural water during the isomerization process instead of DI water to test its potential enhancing effects while using the data obtained in this study as a control. In a practical sense, ammonium-based, all-purpose glass cleaners, like Windex, for example, would benefit directly by the addition of humulone iso-derivatives. The addition of hop extract may, in fact, broaden its usage and the co-action of alpha acid and $NH_4^+$ would grant superior antimicrobial properties to any ammonium-based product. Alkaline pH, shown to improve the antimicrobial potential of humulone iso-derivatives, was achieved by the addition of NaOH in this study (Figure 3). It is only logical to assume that substituting $NH_4^+$ in the place of NaOH to offset pH would further endorse its antimicrobial potential. The potassium ion is necessary for the function of all living cells (14). Future in vitro studies could focus on intracellular Gram-positive infection and the co-action of humulone iso-derivatives with internal monovalent cations, such as $K^+$. These results could subsequently support in vivo studies pertaining to internal administration of concentrated humulone iso-derivatives to mitigate infection.

## Vita

Marc T. Smith graduated from Livingston Academy in Livingston, TN in May 2000. He began his undergraduate degree at Tennessee Technological University in August 2000 and received a bachelor's degree in Microbiology in May 2005. Upon graduating, he was admitted to the Microbiology graduate program at Austin Peay State University in 2006. During his studies at APSU he worked in the biology dept. media kitchen at a graduate assistant for 3 years. His research involved DNA-capture assays utilizing protein-coated, magnetic microbeads to enhance PCR-assays and ultimately focused on the usage of *Humulus lupus* (hop plant) alpha acid extracts as a novel form of *S. aureus* disinfection. Simultaneously while completing his Master's degree he entered into the Medical Technologist program at APSU in June 2008, attended Cookeville Regional Medical Center for clinical rotations, and was ASCP certified in June 2009.