THE ANTIBACTERIAL EFFICACY OF ETHANOLIC WHOLE-LEAF MORINGA OLEIFERA SUB-FRACTIONS ON ESCHERICHIA COLI

by

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Honors Thesis

Appalachian State University

Submitted to the Department of Biology
And The Honors College
in partial fulfillment of the requirements for the degree of

Bachelor of Science

May, 2018

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ABSTRACT

THE ANTIBACTERIAL EFFICACY OF ETHANOLIC WHOLE-LEAF MORINGA OLEIFERA SUB-FRACTIONS ON ESCHERICHIA COLI

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In an effort to provide safer alternative options to pharmaceutical-based antibacterial treatment for infection-induced preterm labor, we have previously used whole-leaf extracts and high performance liquid chromatography (HPLC)-isolated subfractions of hydroethanolic *Moringa oleifera* (MO) to screen the antibacterial efficacy of *MO* against *Escherichia coli (E. coli)*, a leading cause of urinary tract infections associated with preterm labor. These earlier studies found the ethanolic whole-leaf extract as well as three subfractions out of eight, to be the most potent. Here, we test the potency of each sub-fraction as well as investigate whether the antibacterial effects of the most potent leaf sub-fractions *act synergistically,* using *in vitro* liquid broth assays in a dose-dependent manner. Of the eight sub-fraction screened, sub-fraction five exerted the most antibacterial efficacy against *E. coli*. No significant synergistic antibacterial activity was observed between subfractions four, five, and six. We conclude that the anti-bacterial activity of whole leaf *M. oleifera* is exerted by the potent subfractions in a non-synergistic manner. Ongoing studies in our lab are testing the antibacterial potential of *MO* whole seed extracts.

ACKNOWLEDGMENTS

The findings of this study could not have been possible without the financial support provided by the Appalachian State University Office of Student Research, Appalachian State University Summer Ventures in Science and Mathematics Camp, and Appalachian State University Future Doctors and Scientist Camp. I would like to acknowledge Dr. Chishimba Nathan Mowa for his mentoring and friendship throughout my entire time at Appalachian State University. I would like to thank Ms. Lori Tyler, Director of Summer Ventures Camps, for providing me with the opportunity to attend the camp as a high school student, which ultimately led to my enrollment at Appalachian State University. I would like to especially acknowledge Brandon Edward Smith and Jacob Aaron Gordon for their wisdom and support as lab mentors and peers. I would like to thank Aubryana Payne and the students of the 2016 Future Doctors and Scientist Camp for their contributions to this study, as well as Dr. Nicholas Shaw for his commitment as a thesis committee member. I wish to thank my Mom, Dad, Ashton, and friends for the constant support and love that led me to the person I am today.

DEDICATION

I wish to dedicate this work to those whose have impacted my life in so many ways. To my father, Marcus Dean Orders, for being the foundation and pinnacle of my family, and in doing so teaching me the importance of dedication, perseverance, and a thirst for success. To my mother, Robin Hurdt Orders, for being the protective and loving emotional support that any young man needs. To my sister, Ashton Nichole Orders, for being someone in the medical field that I can look up to and aspire to be like. I love you all and appreciate all that you have done to shape the man I am.

I would also like to dedicate this work to Brandon Edward Smith, Matthew Robert Proctor, and Byron Lee Burrell for being the three best friends I could have asked for while at Appalachian State University. I thank each one of you for accepting me for who I am and pushing me to reach my goals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Preterm birth is defined as birth before the 37th week of gestation and affects approximately 15 million children annually (World Health Organization, 2017). It has been projected that if these trends continue to rise, more than 1 million preterm infants will not live to the age of five and those that do survive will likely have a life of perpetual ill health (Liu, 2016). These health challenges include respiratory problems, cerebral palsy and mental impairments (Centers for Disease Control and Prevention, 2017). Unfortunately, it is estimated globally that approximately three-fourths of all preterm births could be prevented with current medical practices (World Health Organization, 2017). The premature loss of cervical integrity, such as from a previous preterm birth, in the expecting mother is one of the key causes of preterm birth (Williams, 2015), which is largely triggered by either bacterial infection, inflammation and or a previous preterm birth history (American Pregnancy Association, 2017). The present study focuses on testing the effectiveness of a natural remedy in treating or preventing infection- and possibly inflammation-induced preterm birth.

Infection occurs when tissues are colonized by harmful bacterial as a result of the bacterial toxins released in the body, subsequently inducing an immune response (MedlinePlus, 2018). These immune responses will lead to immune cell tissue infiltration, vascular alterations, such as angiogenesis and lymphangiogenesis and notably inflammation (MedlinePlus, 2018). In the same vein, if maternal reproductive tissues were infected, i.e., either the cervical tissue directly or other nearby fluids or tissues, such as the amniotic fluid or placenta, the triggered inflammation could be enough to compromise cervical remodeling and barrier (Kirchner, 2000). Bacterial infections that

result in this form of preterm labor are caused by various microbes, including *Escherichia coli (E. coli)*, which descends from an infected urinary tract (Mayo Clinic, 2017).

The incidence of preterm birth has been notably greater in developing regions, such as Africa and Asia when compared to the west (Tielsch, 2015). It is likely that in these developing societies it is too costly to afford medical treatments and measures used to prevent preterm birth (Online Africa Renewal, 2017). Furthermore, bacterial-induced preterm births are ethically far more challenging to treat with antibiotics because of the teratogenic effects that these drugs pose to the growing fetus (Norwitz, 2009). Therefore, rather than using a prescription antibiotic that could be harmful to the fetus, a naturopathic option could be considered to prevent bacterial or inflammation-preterm labor.

Moringa oleifera (*M. oleifera*), otherwise known as The Miracle Tree, is a superfood and super medicinal plant indigenous to south east Asia and its oil has been used for both nutritional culinary and sun protectant for quite some time (Moringa The Miracle Tree, 2018). *M. oleifera* contains a plethora of nutrients, including vitamins A and C and calcium and potassium, and it has more protein per weight than cow's milk (Trees for Life International, 2011). *M. oleifera* also contains biologically active phytochemicals, such as glucosinolates, flavonoids, and phenolic acids (Saini, 2016), and importantly including those with anti-bacterial activity, such as 4-(α-L-rhamnopyranosyloxy)benzyl isothiocyanate and methyl N-4-(α-L-rhamnopyranosyloxy)benzyl carbamate (Wang, 2016). For instance, previous studies in our lab and others have shown, whole leaf ethanolic extract of *M. oleifera* inhibits the growth of *E. coli* comparable to common prescription antibiotics (Smith, 2016). One of

the advantages of *M. oleifera* is that it is readily accessible and grows in areas most affected by preterm labor, and thus making it readily available to pregnant mothers in these regions (Trees for Life, 2011). Furthermore, the economic cost of developing the plant products is not as costly as pharmaceuticals, making *M. oleifera* economically affordable in third world countries (Ezugwu, 2014).

In the present study, we sought to identify the whole leaf ethanolic extract of *M. oleifera* sub-fractions with anti-bacterial components, based on our recent studies (Smith, 2016). We hypothesized that the whole leaf ethanolic extract of *M. oleifera* likely has subfractions with varying degrees of anti-bacterial properties, which collectively could also have synergistic effects. The data generated from the present study could pave the way for the development of a safe and effective *M. oleifera* product for therapy of bacterial-induced preterm labor.

MATERIALS AND METHODS

Microbe and cell culture used

The present study used a non-pathogenic *E. coli*, D51α, which was generously provided by Dr. Ece Karatan (Department of Biology, Appalachian State University, Boone, NC). Various standard microbial tests were performed to assess the anti-bacterial activities of *M. oleifera* using standard LB agar and glycerol liquid media, including diffusion assays, lethal-dose 50s (LD_{50}), and minimum bactericidal amounts (MBA), as described below. Isolated colonies were inoculated and grown in liquid media for later experiments. To prevent contamination of the stock bacteria, as well as to prepare for further experiments, glycerol liquid media was prepared and inoculated with *E. coli.* A single colony from a petri dish streaked for isolation with *E. coli* was aseptically inoculated into 2 mL of LB broth (Ameresco, Solon, Ohio). The bacteria were cultured at 37°C for 24 hours, and stored at -80°C until needed. The bacterial concentration was consistently kept at 1500 CFU/μL.

Determination of an Effective M. oleifera Extract

Previous studies conducted by our research group determined that the most ideal *M. oleifera* extract for inhibiting E. coli was the ethanolic whole-leaf *M. oleifera* extract (Figure 1). We decided to divide the phytochemicals of this ethanolic whole-leaf M. oleifera extract into sub-fractions.

Stock M. oleifera Subfractions

M. oleifera was grown, processed and its sub-fractions from whole leaf ethanolic extracts were isolated using HPLC at North Carolina A&T University by Joshua Idassi and Jahangir Emrani (Table I). The residual ethanol from whole leaf extracts were removed by rotary evaporation and the extracts, as well as the sub-fractions were both suspended in 0.1 M PBS. Due to the varying concentrations, all subfractions were diluted to 1 mg/mL for consistency.

Diffusion Assay for Zones of Inhibition

All procedures were performed under high stringency for aseptic techniques. To begin, 100 μL of LB plus *E. coli* were added to each agar petri dish and spread evenly. The LB plus *E. coli* was allowed to dry completely before proceeding. Sterile disc (3) were evenly placed in a triangular formation on the agar petri dish and tweezers were used to lightly press the disc onto the agar. The intended amount of *M. oleifera* subfraction was added to each sterile disc. The diffusion assays were incubated at 37°C for 10 hours. Zones of inhibition exhibited by the *M. oleifera* extracts were measured to the nearest 0.5 mm.

Diffusion Assay for Determination of Minimum Inhibitory Amount

The protocol for disk diffusion assays, as mentioned above, was used to determine the minimum amount of *M. oleifera* subfraction needed to inhibit any growth of *E. coli*. Agar plates inoculated with *E. coli* were subjected to diminishing amounts of *M. oleifera* subfractions and incubated for 24 h at 37^{o}C. The minimum amount of *M. oleifera* needed

to inhibit *E. coli* was determined to the lowest amount of *M. oleifera* subfraction that produced a measurable zone of inhibition when compared to a 0.1 M PBS treated control.

Diffusion Assay for Determination of Dose-Dependency

The protocol for disk diffusion assays, as mentioned above, was used to determine the responsiveness of *M. oleifera* subfraction on *E. coli* growth inhibition. *E. coli* inoculated agar plates of the same *M. oleifera* subfraction at amounts of 20, 10, and 5 μg were incubated for 24 h at 37^{o}C. The diffusion assays were analyzed for differences in zones of inhibitions to the nearest 0.5 mm. As with the MIA diffusion assays, 0.1 M PBS treatment groups were used as controls for the dose-dependency tests.

Liquid Broth Assays for LD_{50}

All procedures required a high stringency for aseptic techniques. Test tubes were prepared with 1 mL of LB and 10 μL of LB plus *E. coli*. Once vortexed, *M. oleifera* subfractions were added to the test tubes. The inoculated test tubes were incubated for 24 h at 37^{o}C. Using LB as a baseline, anti-bacterial efficacy of the *M. oleifera* subfractions was measured by spectrophotometry at 600 nm. Concentrations of each *M. oleifera* subfraction were manipulated until half of the bacteria concentrations in the control groups were matched. Liquid assays treated with 0.1 M PBS were used as controls.

Liquid Broth Assays for MBA

All procedures required a high stringency for aseptic techniques. Test tubes were prepared with 1 mL of LB and 10 μL of LB plus *E. coli*. Once vortexed, *M. oleifera*

subfractions were added to the test tubes. The inoculated test tubes were incubated for 24 h at 37^{o}C. Using LB as a baseline, anti-bacterial efficacy of the *M. oleifera* subfractions was measured by spectrophotometry at 600 nm. Concentrations of each *M. oleifera* subfraction were manipulated until all of the bacteria in solution were killed. Again, liquid assays treated with 0.1 M PBS were used for control groups.

RESULTS

Ethanolic M. oleifera extract shows the most anti-bacterial properties by diffusion assay:

The phytochemicals of the five whole leaf *M. oleifera* extracts were tested to determine the anti-bacterial efficacy of each extract. The zones of inhibition from diffusion assays were used to assess the anti-bacterial properties of each extract against *E. coli* growth. Butanol and methanol extracts showed zones of inhibition similar to that of the control water extract (Figure 1). The 80/20 ethanol/water extract demonstrated zones of inhibition twice as large as the butanol, methanol and water extracts (Figure 1). The pure ethanol extract had the largest zones of inhibition, and thus the most anti-bacterial efficacy of the five *M. oleifera* extracts, with an average zone of inhibition significantly greater than all of the extracts (Figure 1).

HPLC of the Whole-Leaf Ethanolic M. oleifera Extract Results in Subfractions:

The HPLC of the whole-leaf ethanolic *M. oleifera* generated 8 sub-fractions, with varying concentrations (Table I). In order to compare the anti-bacterial properties and effectiveness of the sub-fractions, each sub-fraction was diluted in 0.1 M PBS to a final 1 mg/mL concentration.

M. oleifera sub-fraction 5 shows a significant anti-bacterial efficacy:

The seven HPLC sub-fractions of the ethanolic whole leaf *M. oleifera* extract (subfraction 1 was excluded based on its high water content or over dilution) were tested at the same concentration to determine the anti-bacterial efficacy of each sub-fraction.

The zones of inhibition from diffusion assays were used to assess the anti-bacterial properties of each sub-fraction against *E. coli* growth. Sub-fractions 6 and 7 showed inhibition of growth similar to the PBS control (Figure 2). Sub-fractions 2, 3, and 4 exhibited minimal inhibition to bacterial growth, with sub-fraction 8 exerting a two-fold inhibition when compared to the other sub-fractions (Figure 2). Sub-fraction 5 induced the most anti-bacterial efficacy that was significantly greater than any other sub-fraction (Figure 2).

M. oleifera subfractions 4, 5, and 6 inhibit bacterial growth with the least concentration:

Following the studies of the diffusion assay of each sub-fraction (Figure 2), studies on minimum inhibitory amount of each subfraction was conducted. Sub-fractions 3, 7, and 8 displayed the highest concentration to inhibit *E. coli* growth (Table II). In contrast, the concentrations required to inhibit bacterial growth for sub-fractions 2 and 4 was moderate (Table II), while sub-fractions 5 and 6 had the least concentration to exert inhibition of *E. coli* growth (Table II).

M. Oleifera subfraction 5 requires lowest amount for Lethal-Dose 50 (LD_{50}):

The lethal-dose 50 (LD_{50}) of sub-fraction 4, 5, and 6 was analyzed using spectrophotometer at OD_{600}. Although sub-fraction 6 had the highest concentration post HPLC, sub-fraction 6 required the highest amount to achieve LD_{50} (Table III). Sub-fractions 4 and 5 required far less quantities to inhibit bacterial growth, with sub-fraction 5 displaying the least amount for LD_{50} (Table III).

LD_{50} of sub-fractions 4, 5, and 6 also displayed minimum bactericidal amount (MBA):

In vitro methods similar to the LD_{50} analysis of subfractions 4, 5, and 6 were used to determine the MBA of sub-fractions 4, 5, and 6. The MBAs of sub-fractions 4, 5, and 6 were the same as the LD_{50}s for sub-fraction 4, 5, and 6 (Table IV).

Sub-fractions 4, 5, and 6 act in a dose-dependent manner:

The anti-bacterial efficacies of all sub-fractions were tested for inhibiting *E. coli* growth at 5, 10 and 20µg. The diffusion assays of sub-fraction 4 (Figure 3), sub-fraction 5 (Figure 4), and sub-fraction 6 (Figure 5) displayed dose-dependent responses in inhibiting *E. coli* growth. Sub-fractions 4, 5 and 6 all inhibited the most *E. coli* growth at 20 µg, some growth at 10 µg and did not inhibit any growth at 5 µg (Figures 3, 4, and 5).

Subfractions 4, 5, and 6 do not exhibit a synergistic effect:

Surprisingly, even though sub-fractions 4, 5 and 6 exhibited dose-dependent response (Figures 3, 4 and 5) the combined sub-fractions did not display any synergism in any significant way in the inhibition of *E. coli* growth (when compared to subfraction 5 (Figure 6).

DISCUSSION

In vitro experiments in the present study were utilized to determine the anti-bacterial efficacy of sub-fractions of HPLC-isolated whole leaf ethanolic *M. oleifera* extract *E. coli*. While these studies are preliminary, the evidence generated here lays the foundation for identifying the specific individual active compounds in each sub-fraction with anti-bacterial biological activities in *M. oleifera* in the future. The findings of the present study reveal that sub-fraction 5 exhibited the most potent anti-bacterial activity thus offering the most promising potential.

While the ethanolic, chloroform, and aqueous extracts of *M. oleifera* have all been shown to inhibit the growth of *E. coli* (Smith, 2016 & Abalaka, 2012), little is known about the identities of the specific phytochemicals that attribute to its antibacterial properties. By separating the *M. oleifera* whole leaf ethanolic extract into subfractions, further identification of individual compounds could be achieved. The initial screening of the present study indicated that subfractions 2, 3, 4, 5, and 8 were effective at inhibiting *E. coli* growth over the course of 24 hours; however, additional screenings for minimum inhibitory amounts showed that all subfractions 2-8 could prevent *E. coli* growth over the course of 10 hours. The variations in sub-fraction efficacy could be due to the effects of phytochemical identity, phytochemical concentration and bacteria susceptibility (Odenholt, 2003). While no *E. coli* strains resistant to *M. oleifera* have been identified, the separation of *M. oleifera* phytochemicals by HPLC could be the source of differing antibacterial properties between sub-fractions. HPLC separates compounds in a sample by using mobile and stationary phases to exploit differing polarities (Waters, 2018). It is possible that the HPLC separation of the whole-leaf M. oleifera ethanolic extract into

sub-fractions by HPLC divided the antibacterial phytochemicals into multiple sub-fractions, as seen in the present study.

The screening for minimum inhibitory amount showed that subfractions 5 and 6 required the least amount of extract to inhibit any *E. coli* growth, implying that they were the most potent. These preliminary screenings of the *M. oleifera* whole leaf ethanolic subfractions indicate that the phytochemicals with the highest antibacterial efficacy are likely to be concentrated in or around subfraction 5. This assumption led to further examination of subfractions 4, 5, and 6.

The determination of the most potent subfraction for inhibiting *E. coli* called for quantitative analysis through LD_{50}. Spectrophotometer data indicated that subfraction 5 required the least amount of extract to inhibit 50% of the cultures growth, followed by subfractions 4 and 6 respectively. Interestingly, the amount of subfraction needed to inhibit 50% of the *E. coli* growth was the same as the MBA needed to inhibit all *E. coli* growth. Such results indicate that *M. oleifera* whole leaf ethanolic extract subfraction 4, 5, and 6 act in an all-or-none threshold manner for inhibiting *E. coli* growth in liquid cultures. These findings would suggest that relatively low dosages of *M. oleifera* phytochemicals could be used as potential antibiotics. Even so, the all-or-none prescription dosage of *M. oleifera* as an antibiotic could be high, but *M. oleifera* has been shown to be safe when taken orally (Awodele, 2012).

Bacterial infections pose challenging symptoms unique to both the pathogen and the infected individual (Busch, 1998). Despite the individual-based need for antibacterial treatment and the increasing trend of antibacterial resistance, the development of novel antibiotics has declined rapidly since the late 1960's (Conly, 2005). In the present study,

dose-dependent assays were conducted to ensure that *M. oleifera* whole leaf ethanolic extract subfractions could be used in a case-by-case scenario. Subfractions 4, 5, and 6 all acted in a dose-dependent manner with *E. coli* inhibition proportional to the amount of subfraction added to the assay. These results suggest that the phytochemicals of subfractions 4, 5, and 6 can be strategically utilized in varying dosages to combat *E. coli* growth. Such dose-dependent qualities would be useful for devising individualistic medical treatments and preventing the likelihood of antibacterial resistance by over prescription. Furthermore, such aspects would be medically beneficial and economical in developing countries if *M. oleifera* were used as a naturopathic antibiotic.

Previous studies have shown that phytochemicals of various plants can act together in a synergistic way to lysis bacteria (Satyan, 2011). While these findings discussed the antibacterial efficacy of phytochemicals from multiple plant sources, such as *Allium cepa*, *Allium sativum*, etc., little is known about the relationship between phytochemicals from the same plant. The final screening of the present study sought to determine if any synergistic relationship existed between the phytochemicals of subfractions 4, 5, and 6 that could elicit more *E. coli* inhibition than just one subfraction alone. In comparison with the most potent subfraction, subfraction 5, the synergistic mixture of subfractions 4, 5, and 6, to our surprise, showed no difference in the inhibition of *E. coli* growth. These results in the present study indicate that no phytochemical synergy exist between subfractions 4, 5, and 6, and while any subfraction prevented *E. coli* growth to some degree, subfraction 5 contains the most potent phytochemical in regards to *E. coli* inhibition. It is possible that there may be some synergy that may exist between some other sub-fractions. Future studies should investigate this.

Previous proteomic studies in our lab have identified several possible pathways of antibacterial activity by the ethanolic whole-leaf *M. oleifera* extract against *E. coli* (Smith, 2016). Such modes of attack include affecting stress response, metabolic, and membrane instability, as well as disrupting many more regulatory processes in *E. coli*. While the present study did not investigate the identities of the phytochemicals responsible for the antibacterial properties of each sub-fraction, it is likely that these phytochemicals are inhibiting *E. coli* growth by similar mechanisms. Future studies should seek to examine the underlying antibacterial mechanisms of each sub-fraction by analyzing the proteomic data associated with each sub-fraction.

The present study suggests that ethanolic whole-leaf M. oleifera sub-fractions do possess phytochemicals effective for inhibiting *E. coli* growth. While more research is required to determine the molecular mechanisms behind each sub-fraction and accurately identify the phytochemicals responsible for its antibacterial properties, *M. oleifera* may one day be a natural and safe alternative to prescription antibiotics. A *M. olefiera* antibiotic would not only decrease the prevalence of antibacterial resistance, but will also provide an economic medical treatment for infection in underdeveloped regions of the globe. Most importantly, *M. oleifera* may one day save the millions of lives impacted by bacterial infection induced pre-term birth.

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TABLES

Table 1. Initial Concentrations of *M. oleifera* Sub-fractions

Sub-fraction	Concentration (mg/mL)
1	0.053
2	4.178
3	6.933
4	2.163
5	1.960
6	43.174
7	12.258
8	20.637

Table 2. Diffusion assay showing the minimum amount of subfraction needed to inhibit E. coli growth (n=3)

***Moringa oleifera* Sub-fraction**	**Minimum Inhibitory Amount (μg)**
2	18
3	20
4	18
5	9-10
6	10
7	20
8	20

Table 3. Lethal dose 50 (LD_{50}) results of M. oleifera subfractions 4, 5 and 6 (n=3)

***Moringa oleifera* Sub-fraction**	**LD_{50} (µg)**
4	220-230
5	150-156
6	1720-1849

Table 4. The MBA of M. oleifera subfractions 4, 5, and 6 (n=3)

***Moringa oleifera* Sub-fraction**	**MBC (μg)**
4	220-230
5	150-156
6	1720-1849

FIGURES

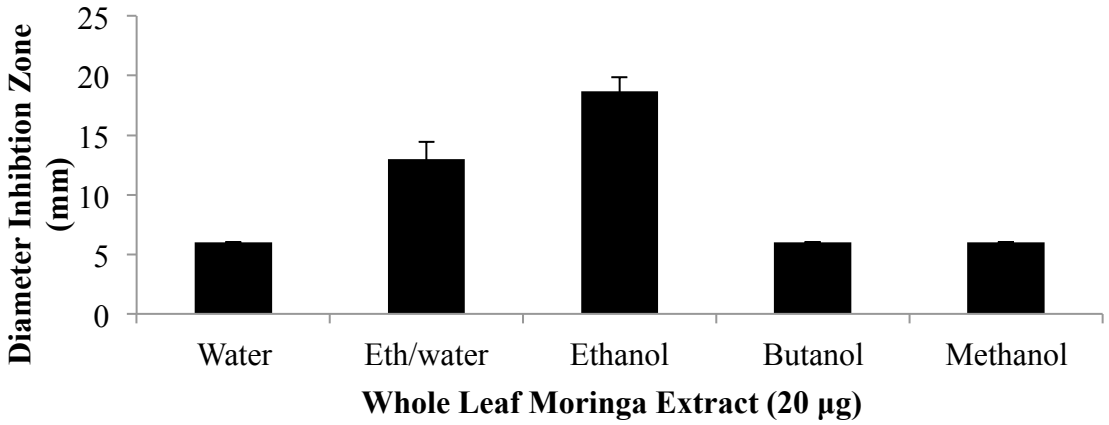

Figure 1. ***Diffusion assay showing the diameter inhibition of five different M. oleifera extracts at 20 µg:*** Data shows the most antibacterial performance by the ethanol extract, closely followed by the 80/20 ethanol/water extract.

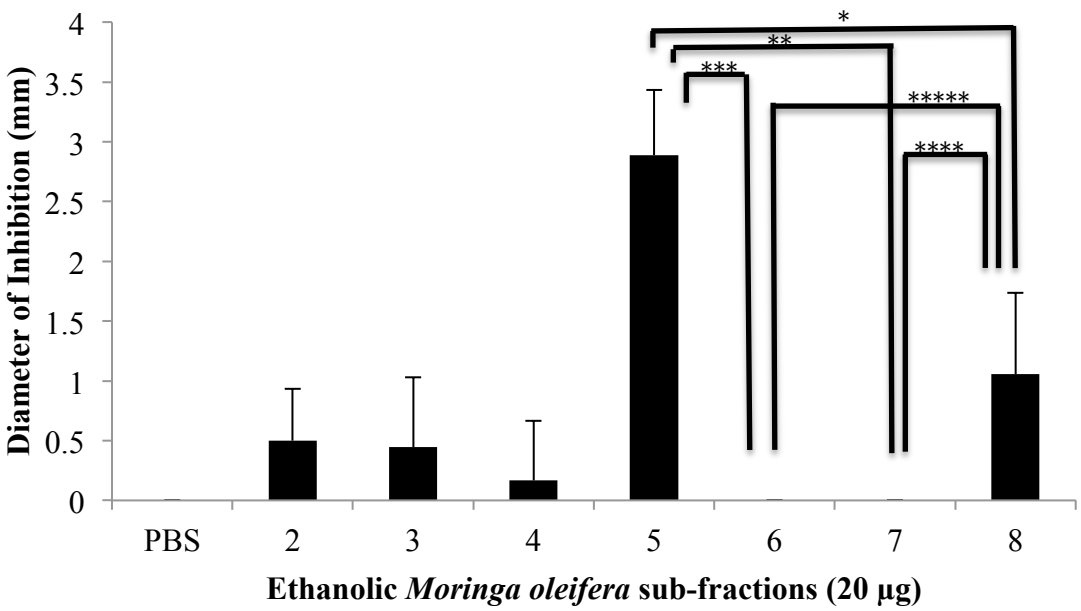

Figure 2. ***Diffusion assay showing diameter inhibition zone of seven M. oleifera sub-fractions at 20 µg:*** Data shows significant bacterial growth inhibition by sub-fractions 5 and 8. * $p \leq 0.05$ 5 vs. 8; ** $p \leq 0.05$ vs. 7; *** $p \leq 0.05$ vs. 6, **** $p \leq 0.05$ vs. 7, ***** $p \leq 0.05$ vs. 6 (n=3).

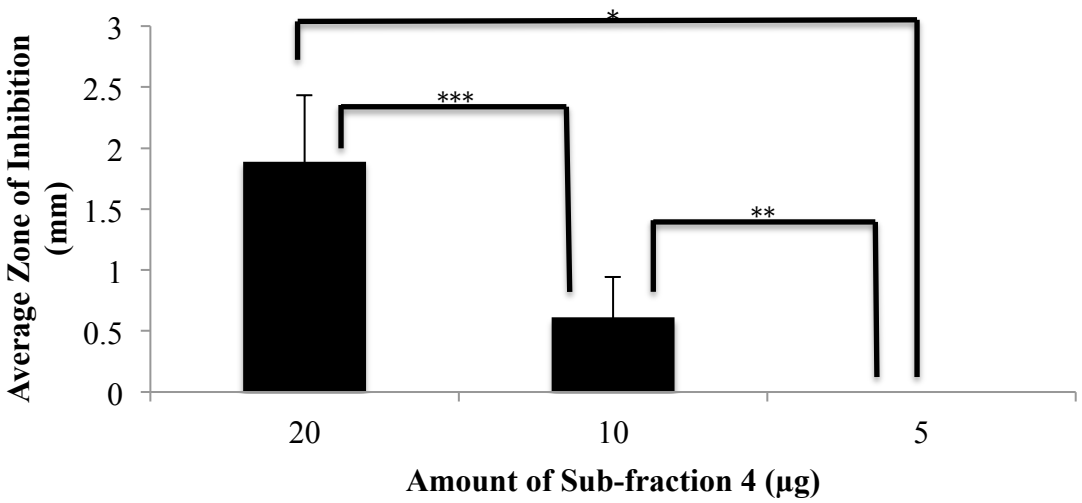

Figure 3. ***Dose-dependent diffusion assay showing the inhibition of E. coli growth by MO sub-fraction 4 at varying amounts:*** The concentration of sub-fraction 4 at which it inhibited the most bacterial growth was at 20 µg, with some limited inhibition at 10 µg, and none at 5 µg. * $p \leq 0.01$ vs. 5, ** $p \leq 0.01$ vs. 5, *** $p \leq 0.01$ vs. 10. (n=3).

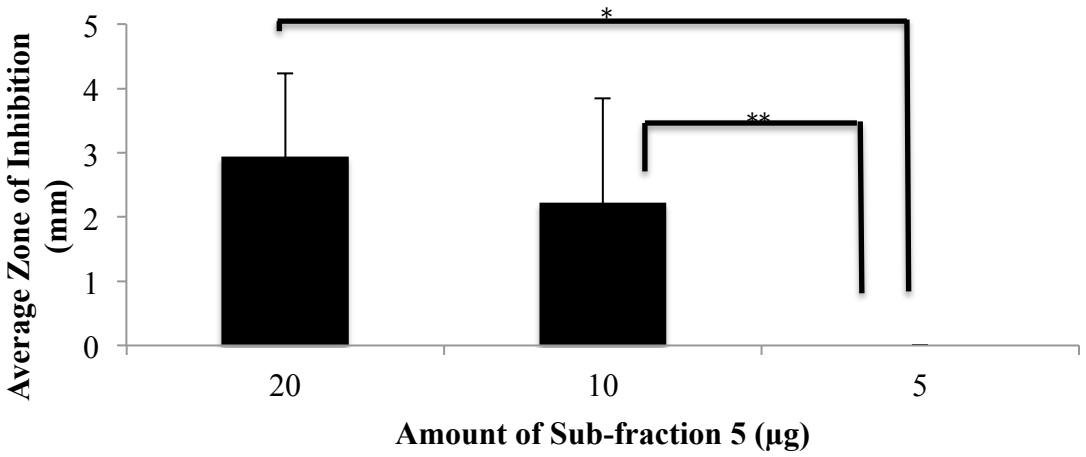

Figure 4. ***Dose-dependent diffusion assay showing the inhibition of E. coli growth by MO sub-fraction 5 at varying amounts:*** The concentration of sub-fraction 5 at which it inhibited the most bacterial growth was at 20 µg, with some limited inhibition at 10 µg, and none at 5 µg. * $p \leq 0.01$ vs. 5, ** $p \leq 0.01$ vs. 5. (n=3).

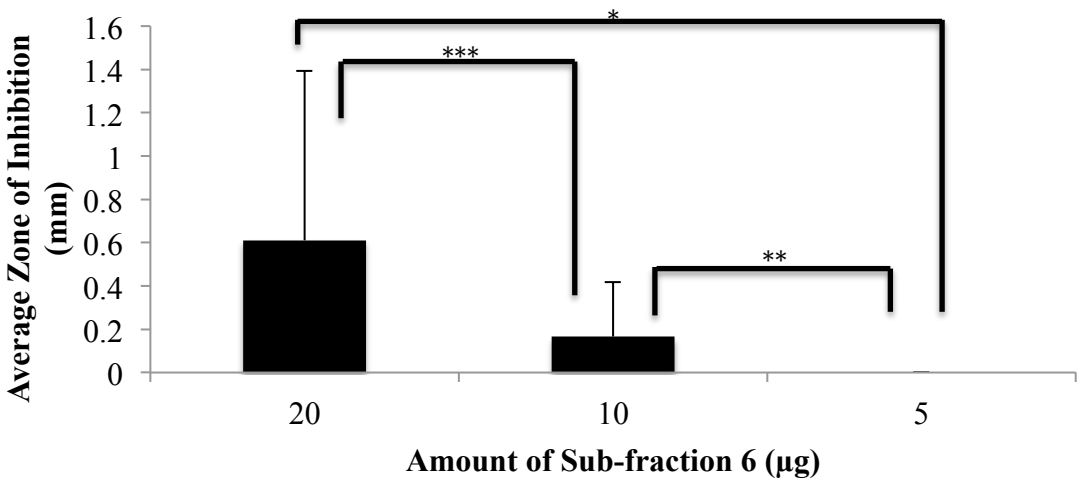

Figure 5. ***Dose-dependent diffusion assay showing the inhibition of E. coli growth by MO sub-fraction 6 at varying amounts:*** The concentration of sub-fraction 6 at which it inhibited the most bacterial growth was at 20 µg, with some limited inhibition at 10 µg, and none at 5 µg. * $p \leq 0.05$ vs. 5, ** $p \leq 0.05$ vs. 5, ***$p \leq 0.05$ (n=3).

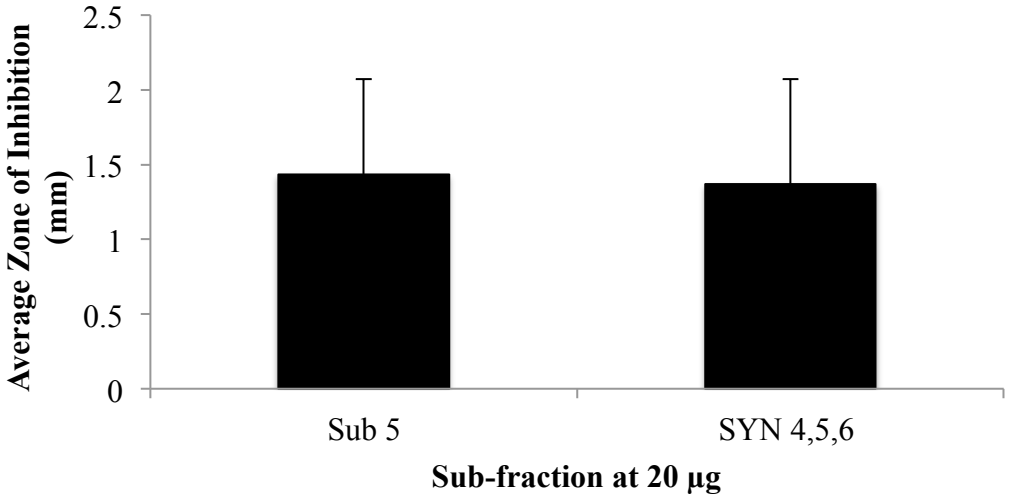

Figure 6*. Diffusion assay showing the antibacterial growth inhibition of combined sub-fractions 4, 5 and 6 at 20* µg*:* No significant synergistic effect in antibacterial growth inhibition was observed when sub-fractions 4, 5, and 6 when combined compared to the leading subfraction 5.

Vita

Tanner Marc Orders was born October 11, 1996 in Gastonia, North Carolina, to parents Marcus and Robin Orders with one older sister Ashton Orders. Tanner graduated from Kings Mountain High School in 2015 as president of the senior class and a member of both the National Honor Society and Beta Club. After attending Summer Ventures in Science and Mathematics, Tanner knew his collegiate home would be Appalachian State University. Upon his acceptance to Appalachian State University, Tanner was also accepted into the Honors College and Department of Biology Honors Program. After only three years, Tanner graduated from Appalachian State University in May of 2018 with summa cum laude honors and a Bachelor's Degree in Cellular/Molecular Biology with a minor in Chemistry.

Tanner hopes to attend medical school following a year after graduation. During that time, he will apply to medical schools across the state and country as he works as a Certified Nursing Assistant, Medication Aide, and college laboratory instructor. Tanner will also travel to Zambia in Africa to participate in a clinical shadowing experience. Tanner looks to further his knowledge and involvement in the fields of science and medicine by challenging his boundaries of experience. Tanner undoubtedly believes that hard work and determination, along with the support of his loving family and friends, will lead him to the successful and enriched life he hopes to obtain.