EVALUATION OF THE ANTI-BACTERIAL ACTIVITY OF BIOACTIVE FRACTIONS FROM ETHANOL PEEL EXTRACT OF *Ananascomosus*AGAINST*Escherichia coli*

BY

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A RESEARCH REPORT SUBMITTED TO PHARMACY SCHOOL IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A BACHELOR DEGREE IN PHARMACY BY KAMPALA INTERNATIONAL UNIVERSITY – WESTERN CAMPUS

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DECEMBER, 2017

DECLARATION

I,**Tumuhaise Vincent,**registration number **BPH/0032/133/DU**hereby declare that this research report has solely been compiled by me as a requirement for theaward of a bachelor degree in pharmacy by Kampala International University-Western Campus, and to the best of my knowledge I affirm that it has not been submitted fully or partially to any institution for any academic award.

However, I have acknowledged and referenced any piece of work where information has been obtained from different authors.

Researcher's Name: TUMUHAISE VINCENT

Researcher's Signature: .....

Date: 07th December 2017

APPROVAL

I certify that I have read and supervised the student undertaking this research study, by the names of **TUMUHAISE VINCENT (BPH/0032/133/DU)** and hereby recommend this research report for acceptance by Kampala International University – Western Campus for examination.

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Signature  **Date: 15th December, 2017.**

DEDICATION

I dedicate this research report toGod almighty for keeping me alive and healthy, my parents for their unending support and love, my supervisor Mrs. Mulkah O. Ajagun-Ogunleye,my fellow students and colleagues and all the lecturers at Kampala International University – Western Campus who guided me on the path to this academic excellence.

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ABBREVIATIONS

AMR:	Anti-Microbial Resistance
CDC:	Centers for Disease Control and Prevention
E. coli:	*Escherichia coli*
GARP:	Global Antibiotic Resistance Partnership
KIU:	Kampala International University
MBC:	Minimum Bacterial Concentration
MIC:	Minimum Inhibitory Concentration
MOH:	Ministry of Health
NGOs:	Non-Governmental Organizations
OTC:	Over-The-Counter services
TB:	Tuberculosis
UNAS:	Uganda National Academy of sciences
v/v:	Volume in volume
WHO:	World Health Organization
w/v	Weight in volume

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ABSTRACT

Antibacterial resistance is not only increasing the healthcare costs, but also the disease severity and death rates from certain infections that could have been avoided, prevented or treated by newer and more effective antibacterial agents. However, the antibacterial activity of bioactive constituents of the crude peel extract obtained from *Ananascomosus*is yet to be known. This is the research gap the present research wants to fill.

The general aim of the study was to isolate and identify the anti-bacterial bioactive constituents in the crude ethanol peel extract of *Ananascomosus*.

A suitable amount of*Ananascomosus*peels were obtained which were then grinded and pulverized with the help of ethanol solution to obtain a crude ethanol peel extract of *Ananascomosus*. A solvent extraction process on the crude extract was carried out to obtain five fractions which were then tested for antibacterial activity, phytochemical constituent screening plus MIC and MBC of the bio-active fractions. Of the five fractions obtained from the *Ananascomosus*peel extract, only three fractions showed antibacterial activity against *Escherichia coli*. The extraction of the pulverized *Ananascomosus* peels with 95% v/v ethanol gave a percentage yield of 8.9%. The preliminary phytochemical screening of the 5% w/v extract revealed the presence of alkaloids, flavonoids, saponins, tannins, and steroids constituents. Therefore *Ananascomosus*peel extract contains bio-active constituents. The ability of the bio-active fractions obtained from ethanol peel extract of *Ananascomosus* to inhibit the growth of *Escherichia coli* indicated the presence of chemical constituents of pharmacological importance.

In conclusion, *Ananascomosus*possesses antibacterial properties against the bacteria *Escherichia coli* which can be utilized in the cure and prevention of bacterial infectious diseases caused by *Escherichia coli* after standardization.

CHAPTER ONE: INTRODUCTION

1.1 Study Background

The efficacy of medicinal plants has been validated by scientists all over the world in the past few decades. Thanks to modern technology, researchers can now identify some of the specific properties and interactions of botanical constituents. With this scientific documentation, we now know why certain herbs are effective against certain conditions. Herbalists, however; consider that the power of a plant lies in the interaction of all its ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown. This review was design to provide information on the ethno-medicinal, pharmacological and phytochemical potentials of *Annonacomosus* Linn. Peel (Lawal D., 2013).

For centuries, mankind has survived on plants and plant products for nutrition and medicinal purposes. Ethno-pharmaceutical products are gaining a lot of advantages in the bid to resolve the problem of both communicable and non-communicable diseases. The development of herbal products has led to the innovation of various therapies for the management of different kinds of ailments and diseases (Ajagun-Ogunleye*et al*, 2015). *Ananascomosus (L) Merr*, popularly known as pineapple, is the third most abundant fruit crop in the tropical region of the world. It is usually enjoyed for its nutritional and refreshing value although there are lots of hidden medicinal properties in the plant. Majority of individuals usually see the pineapple peel as a waste as it is sometimes fed to the cows and goats. Interestingly, the peel should not be seen as a waste since it possesses some medicinal properties. It has a high antioxidant activity and antimicrobial activities. In Nigerian traditional medicine, the combination of *A. comosus*and *C. senensis*peels has been shown to have effect against typhoid fever and diarrhea. The extract of *Ananascomosus* peel has been shown to be rich in therapeutic phytochemicals (Lawal D., 2013). However, the bioactive constituent of the crude peel extract is yet to be known. This is the research gap the present research wants to fill.

There is an emerging challenge of drug resistance in the current trend in the science of infections and communicable diseases. More importantly to this research is anti-bacterial resistance. Bacteria have gained a lot of resistance to most available pharmaceutical drugs and treatments.

and this is posing a lot of threat to humanity across the globe. Even the therapeutic drug combinations are currently being abused, therefore, some disease causing bacteria are gaining resistance as well. Therefore there is need to develop potentially viable therapies to resolve this challenge of anti-bacterial drug resistance. Moreover, in most African countries where herbal medicines are still heavily relied upon because of the high cost of chemotherapeutic drugs, there is a need for scientific research to determine the biological activities of medicinal plants. The findings obtained from such research may lead to the validation of traditionally used and medicinally important plants which will consequently enable full usage of the properties of these plants (Akinyeye, Solanke, & Adebiyi, 2014).

Evidence from around the world indicates an overall decline in the total stock of antibiotic effectiveness: resistance to all first-line and last-resort antibiotics is rising. The patterns of which bacteria are resistant to specific antibiotics differ regionally and by country, mirroring patterns of infectious disease and antibiotic use(State & The, 2015). The U.S. Centers for Disease Control and Prevention (CDC) estimates that antibiotic resistance is responsible for more than 2 million infections and 23,000 deaths each year in the United States, at a direct cost of $20 billion and additional productivity losses of $35 billion (CDC 2013). In Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections, costing €1.5 billion annually in direct and indirect costs (EMA and ECDC 2009). Although reliable estimates of economic losses in the developing world are not available, it is estimated that 58,000 neonatal sepsis deaths are attributable to drug- resistant infections in India alone (State & The, 2015). Studies from Tanzania and Mozambique indicate that resistant infections result in increased mortality in neonates and children under five (State & The, 2015).

For instance, Tuberculosis,TB is a pertinent case in point because it is highly prevalent in many African and other developing countries but many other bacterial infections are severely compromised by resistance. These include diseases caused by to Gram-negative enteric rods, respiratory infections, bacterial meningitis, sexually transmitted diseases as well as hospital acquired infections. Due to the widespread distribution of penicillinase-producing Neisseria gonorrhea, penicillin or ampicillin can no longer be employed in the empiric management of gonorrhea. The prevalence of gonococcal resistance to affordable alternatives - such as tetracyclines, thiamphenicol and spectinomycin - continues to rise and resistance

tofluoroquinolones has emerged. The picture is similar with other organisms. Reports from different parts of Nigeria have observed temporal trends in the prevalence of resistance among enteric organisms, such as *Escherichia coli* and *Shigella*. In multiple studies, resistance to commonly used antimicrobials, including trimethoprim- sulfamethoxazole (TMP-SMX), also known as cotrimoxazole), ampicillin, tetracycline and chloramphenicol has shown increasing prevalence in the last 15 years. These studies have consistently found low prevalence of resistance to nalidixic acid and the fluoroquinolones, however, an upward trend has recently been observed with these agents. Studies in other parts of the world, where fluoroquinolones are more commonly employed, have revealed that routine use of these drugs is often associated with a rapid increase in the proportion of resistant strains (Okeke & Sosa, 2013).

Historically, resistance to antimicrobials has been seen, soon after their discovery. Not long after the discovery of penicillin, Nobel laureate Alexander Flemming identified Staphylococci that were resistant to the first 'wonder drug'. He correctly predicted that imprudent use of antibiotics could lead to clinical failures with these drugs in the future. Since Fleming's report, the valuable life span of virtually every antibiotic has been diminished by the acquisition of resistance in bacteria (Okeke & Sosa, 2013.).

1.2 Problem statement

Over the past centuries, the World Health Organization estimated that infections accounted for 45% of deaths in Africa and South-East Asia and that these diseases were responsible for 48% of premature deaths worldwide. Bacteria cause a significant proportion of infections in Africa. Unfortunately, in a remarkably short time, resistance to antibiotics has undermined the idealistic hope that bacterial infection would cease to be an important cause of death and disease. Indeed, antibiotic resistance increasingly compromises the outcome of many infections that were, until recently, treatable and remain the most common diseases in Africa. For example,multi-drug resistant tuberculosis (TB) infections are highly prevalent in some African countries. High resistance rates can often be correlated to the absence of properly implemented control programs such as directly observed treatment, short-course (DOTS) schemes. In a survey conducted in an area of Cameroon that lacks a fully functional TB control program, multi-drug resistance TB was observed in 27.6% of the patients with a previous history of treatment (Okeke & Sosa, 2013).

Furthermore, according to WHO; Global report on surveillance 2014; Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever increasing range of infections caused by bacteria, parasites, viruses and fungi. An increasing number of governments around the world are devoting efforts to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era in which common infections and minor injuries can kill far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century (Report, 2014). This WHO report, produced in collaboration with member states and other partners, provides for the first time a picture as is presently possible of the magnitude of AMR and the current state of surveillance globally (Report, 2014).

According to MOH, in Kampala, Uganda; Resistance to antibiotics among Ugandans is increasing, a new study has revealed (House, 2014). The Global Antibiotic Resistance Partnership (GARP-UG) study conducted by the Uganda National Academy of sciences (UNAS) shows worsening trends of resistance and diminishing effectiveness of antibiotics in the country (House, 2014). While presenting results of the study dubbed: "Antibiotic Resistance in Uganda: Situation analysis and recommendations," in Kampala, Prof. Denis Byaruhanga, the Chairperson of GARP working group, said that the resistance to Penicillin G and Cotrimoxazole is imminent or already at 100 percent. "Many health practitioners that may legally prescribe antibiotics are aware of antimicrobial resistance but don't know how to address it," said Prof. Byaruhanga. According to the report, the leading bacterial infections in Uganda are Pneumonia, Bacterial meningitis and Septicemia (House, 2014).

With this current challenge of anti-bacterial resistance, there is a need to look out for new products of therapeutic importance. Despite the known medicinal value of *Ananascomosus* peel as a potential anti-bacterial agent, the anti-bacterial bioactive fraction of the crude peel extract remains unknown.The bioactive constituent or fractions, could be a future active ingredient in developing new anti-bacterial drugs, therefore this research wants to evaluate the active fractions of the peel extract as a potential anti-bacterial regimen. This is the gap in this current study.

1.3General aim of the study

To isolate and identify the anti-bacterial bioactive constituents in the crude ethanol peel extract of *Ananascomosus*.

1.4Specific objectives

1. To obtain the different fractions in the crude ethanol peel extract of *Ananascomosus*with the aid of a separating funnel approach.
2. Phytochemical screening of both the crude ethanol peel extract and the fractions obtained from the crude ethanol peel extract of *Ananascomosus*.
3. To evaluate the antibacterial activity of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*.
4. To determine the MIC and MBC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*.

1.5 Research questions

1. How many fractions could be obtained from the crude ethanol peel extract of *Ananascomosus*?
2. What Phytochemical constituents could be obtained from both the crude ethanol peel extract and the fractions obtained from the crude ethanol peel extract of *Ananascomosus*?
3. What is the antibacterial activity of the fractions obtained from the crude ethanol peel extract of *Ananascomosus* with respect to Gentamicin?
4. What are the MIC and MBC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*with respect to Gentamicin?

1.6Research hypothesis

1.6.1 Alternative hypothesis

An ethanolcrude extract prepared from *Ananascomosus*peel hasspecific bioactive fractions possessing antibacterial activity comparable to that of Gentamicin.

1.6.2 Null hypothesis

An ethanol crude extract prepared from *Ananascomosus*does not contain any specific bioactive fractions possessing antibacterial activity comparable to that of Gentamicin.

1.7Justification of the study

The research led to a more scientific evaluation of antibacterial activity of *Ananascomosus*peel. thusindustries will be able toembark on the production of a pharmaceutical-grade formulation of

an antibiotic drug from this plant, which will help in curbing on the negative effects of antibacterial resistance in our community.

1.8 Feasibility of the study

The study was practicable and achievable because most of the reagents, equipment and materials were available at the pharmaceutics, biochemistry and microbiology laboratories at the study site i.e. KIU – western Campus.The bacteria used were safe for experimental purposes and the research project employed an experimental design approach which waspractically feasible to carry out.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter presents the literature review deemed important to guide and interpret the findings of the study. The review is extensive so as to enable the researcher to obtain detailed knowledge of the topic being studied and to reveal procedures, measuring instruments and strategies that will be useful in finding out or investigate the problem in question (Mugenda and Mugenda 1999). Literature has been obtained from journals, text books, dissertations, internet, government publications, NGOs' publications and research papers.

2.2 History of antimicrobials

Research has shown that the conception of exposure to antibiotics as being a modern "antibiotic era" is contrary to the findings, traces of tetracycline have been found in human skeleton remains from ancient Sudanese Nubiadating back to 350-550CE (Bassett, Keith, Armelagos, Martin, & Villanueva, 1980).

2.2.1 Natural and synthetic antibiotics

Not until1928 when Alexander Fleming found out that mould (*Penicilliumnotatum*) accidentally contaminated Staphylococcus culture plates did not have bacterial growth around it and had zones of inhibitionconsistent to the moulds' (*P. notatum*) growth ring (Bellis, 2013; Diggins F (institute of biomedical sciences), 2003). The actual search for antibiotics started as early as 1880s subsequent to the discovery of germ theory on the disease, theobjective was to find so-called "magic bullets" that would destroy microbes without toxicity to the person taking the drug(Aminov, Otto, & Sommer, 2010; Bellis, 2013). In 1942 the substance extracted from the fungus was named by Alexander Fleming as penicillin and it was successfully used to treat streptococcal infections and other infections like gonorrhea, strep throat, and pneumonia (Diggins F (institute of biomedical sciences), 2003; Wolfgang saxon, 1999).

New antibioties like streptomycin, chloramphenicol and tetracycline in the late 1940s and early 1950s were introduced, and the age of antibiotic chemotherapy started (Bellis, 2013). Synthetic antimicrobial agents such as the "sulfa drugs" (sulfonamides) Para-aminosalicylicacid and isoniazid, were also developed(Katakweba Selemani, 2014).

2.3 Use of antibiotics in humans

In human medicine antibiotics are approved for treatment and prevention of bacterial infections (Kagashea, Minzia, & Matoweb, 2010). Relevant factors such as geographical area, social circumstances and existing health care systems influence antibiotic use and misuse in various parts of the world. Research shows that many doctors prescribe broad spectrum antibiotics for patients they assume cannot wait for a full diagnosis or are unlikely to return because of transportation cost or time(Aryee, 2010). In many African countries there is free access to antimicrobials from the drug shops without any prescription(Kagashea *et al.*, 2010), this is assumed to be a cause of increased abuse of antibiotics as a result of the Over-The-Counter (OTC) services without prescription from qualified physicians (Aryee, 2010). This is contrary in developed countries where laws and by laws are more strictly adhered to, proper use and disposal of antimicrobials are practiced (DANMAP, 2008).

2.4 Antimicrobial resistance

2.4.1 Emergence and evolution of antibiotic resistance

The natural history of antibiotic resistance genes which can be studied through phylogenetic reconstruction suggest long term presence of resistant genes to several antibiotics in naturebefore the "antibiotic era" (Aminov& Mackie, 2007). Structural based phylogeny of serine and metallo-β-lactamases suggest that these ancient enzymes originated more than two billion years ago with β-lactamases being present on plasmid for millions of years (Hall & Barlow, 2004; Song *et al.*, 2005). Through phylogenetic analysis of β-lactamases in the metagenomic clones derived from 10,000 years old sediments also suggest that most of the diversity of these enzymes is not as a result of recent evolution but rather ancient evolution.Antibiotic resistance has therefore been considered to be an evolutional process driven by selection pressure of antibiotics. This leads to mutation asan immediate consequence of antibiotic challenge with subsequent development of resistance (J L Martinez & Baquero, 2000). Through horizontally mobile elements including conjugative plasmids, integrons, and transposons, bacteria may also acquire resistance determinants without direct exposure to an antimicrobial(Middleton & Ambrose, 2005).

2.4.2 Intrinsic resistance

Intrinsic resistance involves a complex mechanism exemplified by studies showing that large functional categories of genes contribute to intrinsic resistance(Breidenstein, Khaira, Wiegand,

Overhage, & Hancock, 2008; Fajardo *et al.*, 2008; Tamae *et al.*, 2008). The first reports on the presence of resistance determinants in *E.coli* from human and animal populations lacking selective antimicrobial pressure date back to the 1960s (Guenther, Ewers, & Wieler, 2011). Intrinsically resistant environmental bacteria are able to colonize rhizospheres, a habitat that ought to contain toxic compounds produced by plants or associated micro biota (Matilla, Espinosa-Urgel, Rodríguez-Herva, Ramos, & Ramos-González, 2007), probably they could also be able to resist several compounds including antibiotics(González-Pasayo & Martínez-Romero, 2000; Matilla *et al.*, 2007). Example is the presence of same chromosomally encoded MDR-pumps in all strains of a given species(Alonso & Martinez, 2001; Alonso, Rojo, & Martínez, 1999) capable of efflux of intracellular signal compounds(Evans *et al.*, 1998; Köhler, van Delden, Curty, Hamzehpour, & Pechere, 2001).This justifies that, opportunistic pathogens of environmental origin acquired the phenotype of intrinsic resistance during the evolution of these microorganisms in their natural habitats long before use of antibiotics(Jose L Martinez, 2009).

2.4.3 Acquiredresistance

Unlike intrinsic resistance which has been developed by bacterial population before use of antibiotics, acquired resistance is a recent phenomenon in the evolution of human pathogens in which the main selection pressure is the use of antibiotics(Alekshun *et al.*, 2007; Levy & Marshall, 2004).This is supported by the research in Naomi Datta which suggested that, plasmid families present in enterobacteria before and after the use of antibiotics are the same with differences being that the plasmids acquired resistant genes after the introduction of antibiotics for therapy (Guenther *et al.*, 2011).This attainment of resistant genes can be through mutation or Horizontal Gene Transfer (HGT). Mutation occurs during antibiotic treatment whereas HGT-acquired resistance requires a donor for resistance genes(Davies, 1994; J L Martinez & Baquero, 2000).For instance development of resistance to β-lactams by Gram positive bacteria(Reichmann *et al.*, 1997; Spratt, Bowler, Zhang, Zhou, & Smith, 1992) and polymicrobial infections as one way of DNA transfusion from commensals to pathogenic bacteria has been described(Martínez-Suárez*et al.*, 1987). Analysis of environmental microbiota demonstrates that there are vast number of potential resistance determinants in environmental bacteria but very few are present in human pathogens(Martínez, Baquero, & Andersson, 2007). This limitation is due to the following elements:

- Only those genes that coexist with human pathogens can contribute to resistance, for instance resistance genes in organisms found in Greenland ice core(Miteva, Sheridan, & Brenchley, 2004) are unlikely to contribute to resistance of human bacteria pathogens (Jose L Martinez, 2009).
- Only those genes recruited by gene transfer systems compatible with human pathogens will be transferred (Jose L Martinez, 2009) with barriers being host range (hosts in which plasmid can replicate), exclusion between plasmids and DNA restrictions (Thomas & Nielsen, 2005).
- Elements that will produce strong fitness costs will be counter selected(Andersson & Levin, 1999).

With presence of above elements, acquisition of first resistant determinants through Horizontal Gene Transfer will spread but the probability of acquiring of a new one will be low unless antibiotic selection pressure changes (Jose L Martinez, 2009; Jose Luis Martinez, 2009)

2.4.4 Global perspective of antibiotic resistance

During the period when the rate of discovery of novel antibiotics declined in 1970s, but the development of antibiotic resistance was escalating, it forced researchers to look into possibility of modifying the already existing novel antibiotics to confer improved activity, less side effects, less toxicity and less sensitive to resistant mechanisms(Aminov, 2010). Although this is still the successful means to combating this problem, acquisition of resistance to these modified antibiotics and molecular evolution of existing resistance to novel antibiotics is practically conceivable.This has become not only a complex problem to clinical microbiology but rather requires effort from ecologists, health care specialists, educationalists, policy makers, legislative bodies, agricultural and pharmaceutical industry workers, and the public to deal with(Aminov *et al.*, 2010). The concept of Global Public Goods for Health (GPGH) and their development to assist in preventing and containment of communicable diseases is growing in importance. Given there is no global government as final mediator, challenge may arise in determining how the containment of antimicrobial resistance as a GPGH can be implemented since collaborating on certain global initiatives, such as adhering to certain treatment protocols and surveillance may be significantly difficult to some countries due to deficiencies in their health care infrastructure(Surveillance, 2001).

2.5 Factors contributing to antibiotic resistance

Factors contributing to antimicrobial resistance according to (Surveillance, 2001)include: patient, prescriber, hospital factors, use of antibiotics in food producing animals, national governments and health systems, drug and vaccine development, pharmaceutical promotion and international aspects of containing antimicrobial resistance.

2.5.1 Patient

Patient misperceptions, self-medication, advertising and promotion and poor adherence to dosage regimens are relevant patient factors contributing to antibiotic resistance(Surveillance, 2001). In the study conducted by Branthwaite and Pechère on 3610 patients, 50% believed that antibiotics should be prescribed for all respiratory infections, 81% expected that improvement should be certain in three days and 87% believed that feeling better is the reason to cease the antibiotic therapy also patients thought that newer and expensive drugs are more efficacious, all of which encourage development of resistance(Surveillance, 2001)

2.5.2 Prescribers and dispensers

Lack of knowledge and training concerning infectious diseases, microbiology, differential diagnosis and choice of antimicrobials in addition to poor coverage of pharmacology of antimicrobial agents, their modes of action and spectrum of activity and issues relating to resistance that receive limited coverage in medical school curricula impose a great impact in the inappropriate use of antibiotics. One study in china showed that 63% of selected antimicrobials selected to treat a proven bacterial infection were found to be inappropriate, and a retrospective study in Vietnam showed that 70% of patients were prescribed inadequate dose(Surveillance, 2001). Other factors include lack of information, fear of bad results, lack of diagnostic support, perception of patients related to preferences and demand, peer pressure and social norms.

2.5.3 Use of antimicrobials in food-producing animals

Use of antimicrobials as growth promoters especially those that target gram positive bacteria are associated with an increase in the rate of animal growth when they are used in sub therapeutic quantities in stock feed to food-producing animals, however they have been found to alterantimicrobial flora of exposed animals such that the contained bacteria are resistant to the antibiotics used. With cross-resistance, such practice can expose humans to resistant bacteria

particularly growth promoter antibiotics that fall in the same class as antibiotics used in humans ("WHO | WHO Global Strategy for Containment of Antimicrobial Resistance," 2016).

Use of antimicrobials that affect food- borne pathogens such as *Salmonella typhimurium* DT104ubiquitous in many countries including UK, Germany and USA has become resistant to commonly used agents like ampicillin, tetracycline, streptomycin, chloramphenicol and sulfonamides(Glynn., 1998; "WHO | WHO Global Strategy for Containment of Antimicrobial Resistance," 2016).

2.6Pineapple(*Ananascomosus)*

Pineapple (*Ananascomosus*) is sometimes called the 'King of Fruit'. Pineapple is grown extensively in Hawaii, Philippines, Caribbean, Malaysia, Taiwan, Thailand, Australia, Mexico, Kenya and South Africa. Pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its attractive flavour and refreshing sugar-acid balance. It is available fresh, canned and as juice. 100 g pineapple contain 47-52 calories, 85.3-87.0g water, 0.4-0.7g protein, 0.2-0.3g fat, 11.6-13.7 g total carbohydrate, 0.4-0.5g fibre, 0.3-0.4g ash, 17-18 mg calcium, 8-12 mg phosphorus, 0.5 mg iron, 1-2 mg sodium and 125- 146mg potassium. Pineapple also contains 12-15% sugars of which two-third is in the form of sucrose and the rest are glucose and fructose. 0.6-1.2% of pineapple is acid of which 87% is citric acid and 13% is malic acid. The pH of pineapple is acidic, which is 3.71 and the acidity percentage is 53.5%. The composition of the juice varies with geographical, cultural and seasonal harvesting and processing (Sairi, Yih, & Sarmidi, 2004).

2.6.1Taxonomic classification of *Ananascomosus*

Kingdom: Plantae
Division: Spermatophyte
Sub-division: Angiospermae
Class: Dicotyledonae
Sub-class: Magnoliales
Order: Annonales
Family: Annonaceae
Genus: Annona
Species: comosus

2.6.2 Morphological description of *Ananascomosus*

Annonacomosus belongs to the Bromeliaceae family, from which one of its most important health promoting compounds, the enzyme bromelain, was named. The Spanish name for pineapple, pina, and the root of its English name, reflects the fruit's visual similarity to the pinecone. *A. comosus* are actually not just one fruit but a composite of many flowers whose individual fruit lets fuse together around a central core. Each fruit let can be identified by an 'eye,' the rough spiny marking on the *A. comosus* surface. *A. comosus* have a wide cylindrical shape, a scaly green, brown or yellow skin and a regal crown of spiny, blue green leaves. The fibrous flesh of *A. comosus* is yellow in color and has a vibrant tropical flavor that balances the testes of sweet and tart. The area closer to the base of the fruit has more sugar content and therefore a sweeter taste and more tender texture(Lawal D., 2013).

The *A. comosus* is known as the fruit of kings, and it was available only to natives of the tropics and to wealthy Europeans. Despite the fact that this fruit had become familiar to almost the whole world, it is still a true exotic, because it is a member of the bromeliad family, in which edible fruits are rare. *A. comosus* starts out as a stalk of a hundred or more flowers that shoots up from a plant about three feet tall. Each flower develops a fruit that forms one of the scales on the outside of the *A. comosus*.

The more scales or marks on *A. comosus*, the stronger the tropical taste will be. *A. comosus* with fewer and larger scales will have a milder but sweeter flavor and more juice. The fruit was spread from native Paraguay to throughout South and Central America when the Guarani Indians took *A. comosus* on sea voyages as provisions and to prevent scurvy. When Columbus found the fruit in 1493 called it pina, because he thought it looked like a pincone. The hybrids known today first appear, when the Dutch improved the fruit by crossbreeding, around 1700. The cuttings of the plants were sold to English, who grew them as pot-house plants(Lawal D., 2013).

The *A. comosus* is a tropical plant and fruit (multiple), native to Uruguay, Brazil, Puerto Rico, or Paraguay. It is a medium tall (1-1.5m) herbaceous perennial plant with 30 or more trough-shaped and pointed leaves 30-100cm long, surrounding a thick stem. *A. comosus* is an example of a multiple fruit: multiple, spirally arranged flowers along the axis each produce a fresh fruit that becomes pressed against the fruits of adjacent flowers, forming what appears to be a single fleshy fruit. *A. comosus* are the only bromelaid fruit in widespread cultivation. It is one of the most commercially important plants. The fruit lets of A.comosus are arranged in two

interlocking spirals, eight spirals direction, thirteen in the other; each being a Fibonacci number. Pollination is required for seed formation; the presence of seeds negatively affects the quality of the fruit(Lawal D., 2013).

2.6.3 Medicinal potential of *Ananascomosus*

Annonacomosus Peel: Is derived from the fruit of Annonacomosus, the plant is called pineapple (English) and is locally called Enanasi in Uganda. The root and fruit are either eaten or applied topically as an anti – inflammatory and as a proteolytic agent. It is traditionally used as an anthelminthic agent in the Philippine. A root decoction is used to treat diarrhea. This fruit also has anti-inflammatory and pro-digestive properties. The bromelain in *A. comosus* helps fight infections bydissolving layers of slough and bacteria-richsurfaces. This fruit can also be used to aid indigestion. It can clear bronchial passages in thosesuffering from pneumonia and bronchitis (Lawal, Yunusa, & Bala, 2013).

The anti-inflammatory properties in the fruit help reduce the symptoms of arthritis, and helpreduce pain after surgery and sport injuries. *A. comosus* is currently being studied for its effectivenessin preventing heart disease. *A. comosus* juice is takenas a diuretic and to expedite labour, also as a garglein cases of sore throat and as an antidote for seasickness. The flesh of very young (toxic) fruits isdeliberately ingested to achieve abortion (a little withhoney on 3 successive mornings); also to expelintestinal worms; and as a drastic treatment forvenereal diseases. In Africa the dried, powdered rootis a remedy for edema. The crushed rind is applied on fractures and the rind decoction with rosemary isapplied on hemorrhoids. Indians in panama use theleaf juice as a purgative, emmenogogue andvermifuge(Lawal *et al.*, 2013).

Previous report shows that the methanol extract of *A. comosus* peel was inactive to *B. subtilis*,*E. coli* at a concentration of 50mg/ml (50,000µg/ml),but it shows activity on *S. typhi* at a concentration of100mg/ml (100,000µg/ml) (Ishaii, *et al.*, 1984). Thejuice has antiviral activity and the undiluted juice wasfound to have activity on polio virus 1 (Lawal *et al.*, 2013).

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Introduction

This chapter presents and describes the approaches and techniques that were used to collect data and to investigate the research problem. The chapter is structured to include the study design, study area, materials and methods, quality control, safety profile, data analysis and ethical considerations that were employed during this research study.

3.2 Study design

This research was a controlled experimental study design investigating the antibacterial activity of constituent fractions obtained from a crude ethanol peel extract of *Ananascomosus*.

3.3 Study sites

The study was carried out in the Department of Pharmacy laboratories, Pharmacology department laboratories, Biochemistry department Laboratories and Microbiology department laboratories at Kampala International University – Western Campus.

3.4 Sample size

Sample size of the bacteria, *Escherichia coli* used was based on colonies of bacteria cultured on petri dishes.

3.5 Materials

3.5.1 Plant materials:

Pineapple (*Ananascomosus)* peels were used. About 15 of well sorted pineapple of *Ananascomosus* species yielded approximately 1000g of the fine granules of the peels.

3.5.2 Instruments and equipment:

These included the following;

- Separating Funnels
- Motor and Pestle
- Electric Mill
- Electric Blender
- Measuring Cylinders

- Hot air Oven
- Fractionating Column
- Beakers
- Plastic Bottles and Tops
- Petri Dishes
- Filter Paper discs
- Weighing Balance scale
- Refrigerator
- Knives
- Drying Oven
- Incubator
- Electric Shaker

3.5.3 Chemicals and reagents:

These included the following;

- 95% Ethanol
- Dilute Hydrochloric acid
- Chloroform Solution
- Sodium Bicarbonate
- Dragendroff's reagent
- Sodium hydroxide
- Distilled water
- 5% Iron Chloride
- 1% Lead acetate
- Conc. Sulfuric acid
- 50% Sulfuric acid
- Fehling's solution
- Acetic anhydride
- Alcoholic Potassium hydroxide
- Dilute Acetic acid
- Ninhydrin reagent

- Nutrient agar
- Muller-Hinton agar
- Gentamicin discs (Standard Drug as a Control)
- Distilled water

3.5.4 Microorganism:

*Escherichia coli*were utilized in this research study.

3.6 Data collection procedures

3.6.1 Source of test microorganism

The pure clinical isolates of *Escherichia coli* were obtained from KIU-Teaching Hospital laboratory. The cultures were maintained at 25°Con nutrient agar in an incubator.

The microorganisms wereisolated andpurified on Nutrient Agar Plates and characterized based on Biochemical methods of Collins *et al* 1995.

A confirmatory biochemical test for the *Escherichia coli* on the microorganism sample obtained from the hospital was carried out before the study i.e. Urase test was done to confirm that this was really *Escherichia coli.*

3.6.2 Collection of the plant material

Fresh fruits of *Ananascomosus* were collected from around the Campus vicinity, Bushenyi-IshakaMunicipality, Bushenyi district.

3.6.3Preparation of the plant extract

This method was done according to preparation of treatment samples and extraction protocols byLawal D., Yunusa I. and Bala I. (Lawal *et al.*, 2013).

*Ananascomosus*fruits were obtained from the locality of Kampala International University at Bushenyi and the peels were obtained by removing the pericarp. The peels were air-dried under the shade in the lab and grounded into powder with the aid of an electric blender. The powdered*Ananas* peel were thenweighed and placed in air tight containers in the laboratory.

Ananas peel crude extract were obtained after resolving the dried leaves powder in 95% ethanol in a beaker. The solution was allowed to stand for 48 hours, with intermittent shaking at regular

interval at room temperature, with the aid of a laboratory shaker. The percolates were then filtered off using Whatmann filter paper; thereafter the obtained liquid extract wasevaporated to dryness with the aid of a Rotatory evaporator (Lawal*et al*, 2013). On obtaining the crude extract, it was weighed and the percentage yield was calculated using the method of Fred *et al* 2013. The extract was then kept at 5°C until further analysis was started.Fractionation of the crude extract was carried out using a separating funnel approach according to the methods of Fred *et al*, 2013.

3.6.4 Phytochemical Screening of the Crude Extract and the fractions

The ethanol crude extract of *Ananascomosus*peel and the obtained fractions were analyzed separately for the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, triterpenoids, phytosterols and amino acids.

The peel extractof *Ananascomosus*and the obtained fractions wereanalyzed for the presence of alkaloids, flavonoids, saponins, tannins, steroids,glycosides, triterpenoids, phytosterols and amino acidsaccording to the methods of Lawal*et al* 2013 outlined below:

3.6.4.1 Test for alkaloids

A quantity (5 cm^3) of the extract was added to 2cm^3 of HCl. To this acidic medium, 1 cm3 ofDragendroff's reagent was added. A redprecipitate with turbidity was produced immediately to indicatethe presence of alkaloids.

3.6.4.2 Test for flavonoids

To 3cm^3 of the extract, 1cm^3 of NaOH was added. Ayellow colour produced indicated a positive test forflavonoids.

3.6.4.3 Test for saponins (Frothing test)

A quantity (2 cm^3) of the extract was placed in a testtube and then 2cm^3 of distilled water was added. Thetube was thenshaken vigorously. A persistent froththat lasted for at least 15-minutes indicated a positivetest for saponins.

3.6.4.4 Test for tannins

2 drops of 5% $FeCl_3$ was added to 1cm^3 of theextracts. A green precipitate produced indicated a positive testfor the presence of tannins.

3.6.4.5Salkwoski's test for steroids

To 1cm^3 of the extract, 5-drops of conc. H_2SO_4 wereadded. A red colourationproduced indicated a positive test forsteroids.

3.6.4.6 Fehling's test for glycosides

A quantity (10cm^3) of 50% $H_2SO_4$was added to 1cm^3 of the extract in a test tube. The mixture was heatedin a boiling water bath for 15 minute. A quantity (10cm^3) of Fehling's solution was added and the mixturewas boiled. Formation of brick red precipitatewould indicate a positive test for glycosides.

3.6.4.7 Test for triterpenoids

A quantity (10mg) of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition 2ml of conc. H_2SO_4. Formation of a reddish violet colourwould indicate the presence of triterpenoids.

3.6.4.8 Test for phytosterols

The extract was refluxed with a solution of alcoholic potassium hydroxide till complete saponification tookplace. The mixture was diluted and extracted with ether. The ether was evaporated and the residue was tested for the presence of phytosterols. The residue was dissolved in few drops of diluted acetic acid; 3ml of acetic anhydride were added followed by a few drops of conc. H_2S0_4. An appearance of bluish green colourwould show the presence of phytosterols.

3.6.4.9 Test for amino Acids

A quantity (1ml) of the extract was treated with a few drops of Ninhydrin reagent. Appearance of a purple colour would show the presence of amino acids.

3.7 Preparation of the Fractions from the Crude Extract

This was done by use of a separating funnel approach according to the methods of Fred *et al.* 2013.

3.8 Determination of Minimum inhibitory and bactericidal concentrations of the extracts

The MIC and MBC was determined for the different crude extract fractions separately. The following concentrations were prepared: 1000 micrograms/ml – 4000 micrograms/ml, 10,000 micrograms/ml – 40,000micrograms/ml, 100,000 micrograms/ml – 400,000 micrograms/ml

respectively. From the working inoculums 0.1 ml was inoculated into fresh nutrient broth tubes at different extract concentrations. The tubes were incubated at 37 °Cfor 18-24 hours. The lowest concentration of the extract that inhibited the growth of the test bacterium were noted and recorded as the MIC while the MBC were determined by sub-culturing 0.01ml of the highest concentration of the agent which showed no visible sign of growth in the MIC tube dilution test to fresh antibiotic free nutrient agar (oxoid). The plates were incubated at 37 °Cfor 18-24 hours after which they wereobserved for growth or otherwise of the test organism(Lawal *et al.*, 2013)

3.9 Data analysis

Data was processed using the ANOVA and SPSS statistical softwares. Data analysis involved subjecting our results to computerized statistical operations using Microsoft Excel and evaluation of different observations made during the research activities and data collection.P value i.e. $p< 0.05$ was considered as statistically significant.

3.10 Data presentation

Results were presented as mean ± standard deviation using graphs and tables. The results were presented in both tabular and graphic formats.

3.11 Ethical considerations

Permission was officially obtained through proper channels and protocols from both the department of Pharmacy and the University hierarchy of the Research and Ethics committee at Kampala International University - Western Campus to carry out the experiment and the entire research data collection from different laboratories located both within and outside the campus.

CHAPTER FOUR: RESULTS

4.1 INTRODUCTION

The results obtained are in line with the four specific objectives outlined above for this research project.

4.2 Fractions in the crude ethanol peel extract of *Ananascomosus*

The different fractions obtained in the crude ethanol peel extract are fraction A, fraction B, fraction C, fraction D and fraction E. The percentage yields of the different fractions obtained are presented in table 1 below.

Table 1: Percentage yield of the fractions obtained from 20g of the crude ethanol peel extract of *Ananascomosus*

Fractions	Solvents used	Weights (g)	Percentage Yield (%)
Fraction A	8% w/v NaHCO3 Solution	4	20
Fraction B	10% w/v NaOH Solution	8.3	41.5
Fraction C	Chloroform Solution	0.8	4
Fraction D	Chloroform Solution	0.4	2
Fraction E	10% NaOH Aqueous Solution	6.5	32.5

$$\text{Percentage yield} = \frac{\text{Weight of Extract obtained after drying/concentrating}}{\text{Weight of peels used in the extraction}} \times 100$$

(Fred *et al* 2013).

Total weight of the pineapple peels before extraction = 2000g

Total volume of the ethanol solution used to dissolve the grinded/blended pineapple peels = 6L

Total weight of the pineapple peel residues after drying under shade = 410.1g

Total weight of the dried/concentrated extract = 178g

Percentage yield of the Crude extract = $\frac{178g}{2000g} \times 100$

Percentage yield of the Crude extract = 8.9%

The percentage yield of the crude extract obtained during extraction was 8.9%

4.3 Phytochemical composition of both the crude ethanol peel extract and the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

The results obtained are in line with objective 2, phytochemical screening compositions are highlighted below.

Table 2: Phytochemicals in fractions: The phytochemicals obtained for both the crude ethanol peel extract and the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

Constituents	Crude Extract	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
Alkaloids	+++	++	-	-	+	++
Flavanoids	+	+	+	-	+	-
Saponins	++	-	-	-	-	++
Tannins	++	-	-	-	-	+
Steroids	+	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Phytosterols	-	-	-	-	-	-

Key: + = Presence of constituents; - = Absence of constituents

4.4 Antibacterial activity of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

The antibacterial activities of the obtained fractions are in line with objective 3 as presented below. The bioactive fractions are fractions A, fractions B and fraction E with respect to the positive control Gentamicin and the most active fraction is fraction B which gave the highest mean zone of inhibition i.e. 37.33mm.

Table 3: Zones of inhibition diameters (mm) of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

	Diameter of Zones of Inhibition (mm)			
Fractions	**1st Hole**	**2nd Hole**	**3rd Hole**	**Mean±SD**
Fraction A	28	27	25	26.67 ± 1.53^{a}
Fraction B	41	37	34	37.33 ± 3.51^{b}
Fraction C	0	0	0	0.00±0.00
Fraction D	0	0	0	0.00±0.00
Fraction E	27	24	25	25.33 ± 1.53^{a}
Control (CN 10)	14	15	15	14.67 ± 0.58^{c}

Key: Multiple Turkey's comparison test conducted and significant differences ($P < 0.05$) are represented by different superscripts (a, b, c).

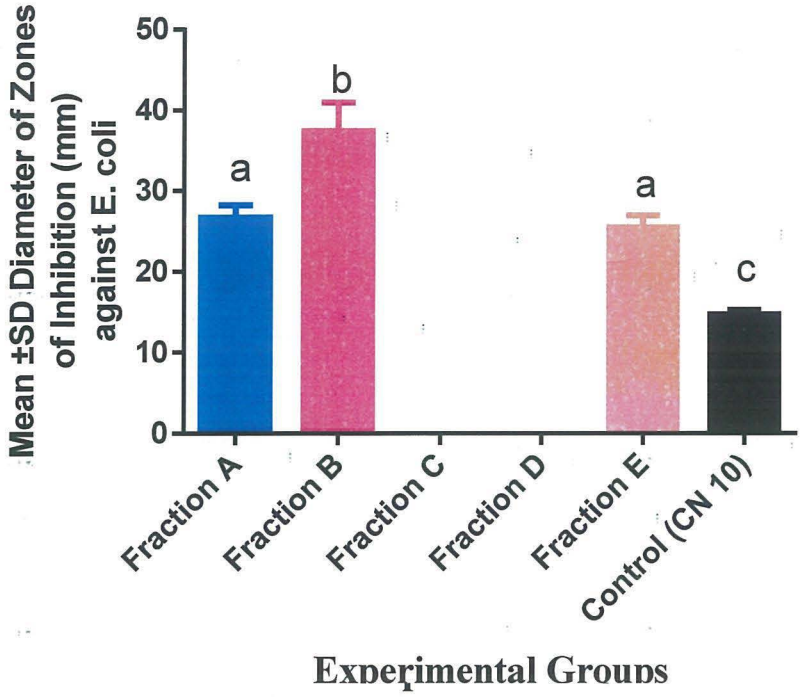

Figure 1: Zones of inhibition of fractions. Antibacterial bio-activity of the fractions obtained from the crude ethanol peel extract of *Ananascomosus.* The fractions A, B and E gave significant bioactivity with respect to the control.

4.5 MIC and MBC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

4.5.1 MIC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

The results obtained are in line with objective four after incubating the mixture of different fractions at different concentrations together with the *Escherichia coli* colonies for 12 hours. The Minimum inhibitory Concentrations (MIC) of the obtained fractions i.e. the minimum concentration of the fractions that inhibited bacterial growth are fraction A 0.4g/ml, fraction B 0.4g/ml and fraction E 0.04 g/ml.

Table 4: MIC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus* against 100µL of Escherichia coli

Experimental Groups (Concentration)	Growth	No Growth
Fraction A (g/ml)	0.04	0.4
Fraction B (g/ml)	0.04	0.4
Fraction E (g/ml)	0.004	0.04

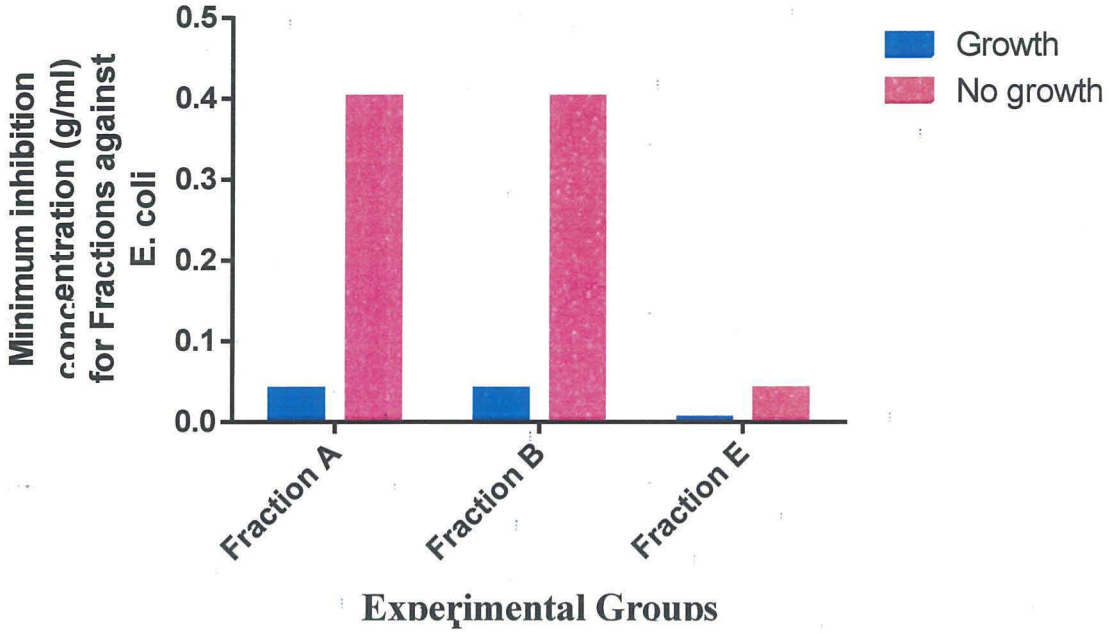

Figure 2: MIC values of fractions. The minimum inhibitory concentrations (MIC) for the fractions obtained from the crude ethanol peel extract against 100µL of *Escherichia coli.*

4.5.2 MBC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*

The results obtained are in line with objective 4. The minimum bactericidal showing the exposure of bacteria to the treatment of fractions for longer duration of time i.e. more than 24 hours at the same concentration as those of the MIC, revealed that MBC were as follows, fraction A 0.04g/ml, fraction B 0.04g/ml and fraction

Table 5: MBC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus* against 100µL Escherichia coli

Experimental Groups (Concentration)	Growth	No Growth
Fraction A (g/ml)	0.004	0.04
Fraction B (g/ml)	0.004	0.04
Fraction E (g/ml)	0.004	0.04

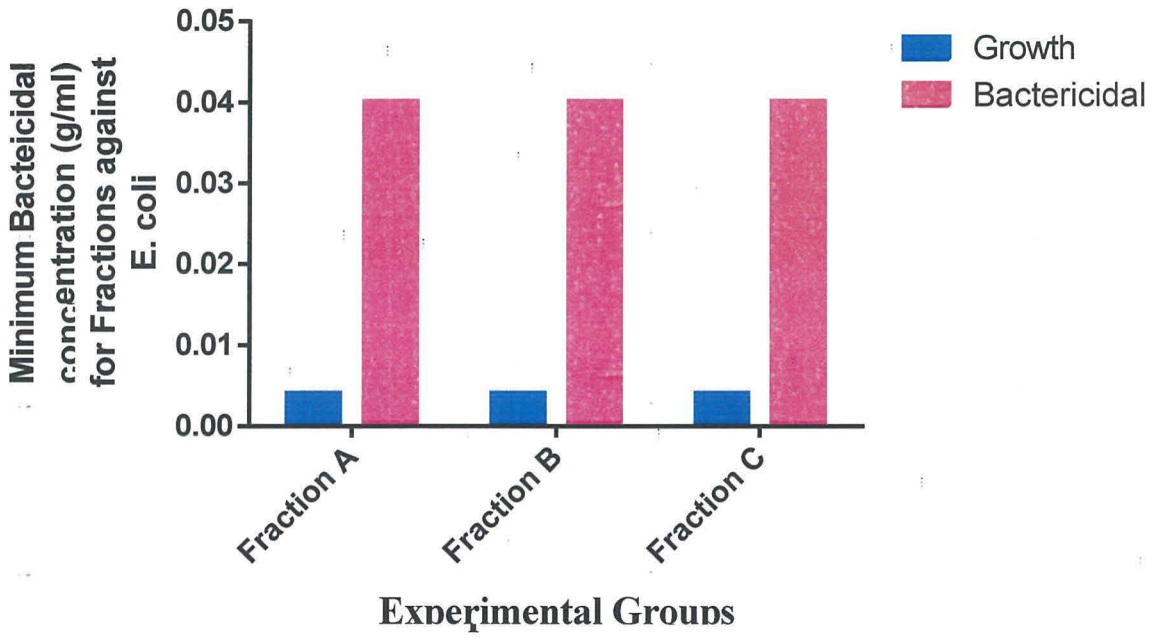

Figure 3: MBC of fractions. Minimum bactericidal concentrations of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*against *Escherichia coli.*

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

According to specific objective one; obtaining the different fractions in the crude ethanol peel extract of *Ananascomosus*with the aid of a separating funnel, different fractions were obtained from the crude ethanol peel extract i.e. fraction A, fraction B, fraction C, fraction D and fraction E. These fractions were obtained by solvent extraction (Stat-Otto Liquid-Liquid Separation) of the crude extract as was done by AnowiChinedu Fred (Theophilus U Onyekaba, Omojate Godstime Chinedu, 2013). In this project, we discovered the possibility of obtaining fractions from *Ananascomosus*peel crude extract which had not been employed before in related research project about antibacterial activity of *Ananascomosus*. Fraction B extracted with sodium hydroxide solution gave the highest percentage yield and fraction D extracted with chloroform solution produced the lowest percentage yield.

According to specific objective two; phytochemical screening of both the crude ethanol peel extract and the fractions obtained from the crude ethanol peel extract of *Ananascomosus*, different phytochemical characteristics were tested from composition screening carried out on both the *Ananascomosus*peel crude extract and the obtained fractions. The phytochemical constituents composition of the *Ananascomosus*crude extract obtained were comparable to the ones obtained by Lawal(Lawal, Yunusa, & Bala, 2013). More so, phytochemical composition screening was carried out on the fractions obtained from *Ananascomosus*peel crude extract to give more evidence to bio-active constituents present in the *Ananascomosus*peel.

According to specific objective three; evaluating the antibacterial activity of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*, the antibacterial activity against *Escherichia coli* was carried out and the bioactive fractions A, B and E with respect to the positive control Gentamicin were obtained and the most active fraction was fraction B which gave the highest mean zone of inhibition i.e. 37.33mm. According to the diameters of zones of inhibition obtained in table 3, antibacterial bio-activity of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*indicated varied activity. The fractions A, B and E gave significant bioactivity with respect to the control. Multiple Turkey's comparison test was conducted and significant differences ($P < 0.05$) obtained i.e. there was no significant difference in antibacterial activity between fraction A and fraction E but there was a significant difference

between fraction A and the control Gentamicin, there was a significant difference in antibacterial activity between fraction B and the rest of the fractions i.e. fraction A and fraction E plus the control Gentamicin and there was a significant difference in antibacterial activity between fraction E and the control Gentamicin. This was the novelity of my research study.

According to specific objective four; determining the MIC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*, the minimum inhibitory concentrations for the three bioactive fractions against *Escherichia coli* was carried out indicating varied inhibitory concentrations against *Escherichia coli* incubated overnight for 12 hours i.e. fraction A and fraction B both had MIC of 0.4mg/ml due to a short period of exposure while the MIC of fraction E was 0.04mg/ml.

According to specific objective four; determining the MBC values of the fractions obtained from the crude ethanol peel extract of *Ananascomosus*, the minimum bactericidal concentrations for the three bioactive fractions against *Escherichia coli* was carried out by incubating for 24 hours and all the fractions obtained i.e. fraction A, fraction B and fraction E all had MBC of 0.04mg/ml. The MBC is lower than the MIC due to a longer period of exposure for the bacteria to the extract fractions.

5.2 CONCLUSIONS

*Ananascomosus*peel extract contains bio-active constituents i.e. alkaloids, flavonoids, saponins, tannins and steroids.

The ability of the bio-active fractions obtained from ethanol peel extract of *Ananascomosus* to inhibit the growth of *Escherichia coli* indicated the presence of antibacterial bioactive constituents of pharmacological importance in the fractions of the ethanol peel extract of *Ananascomosus*.

Therefore, the fractions of *Ananascomosus*peel extract possess antibacterialbio-activity properties against the colony of bacteria *Escherichia coli*.

*Ananascomosus*peel fractions can be utilized in the cure and prevention of bacterial infectious diseases caused by *Escherichia coli* after standardization.

Locally, the peels of pineapple could be boiled in water, and the water obtained could be taken orally as a therapeutic herb to cure bacterial infections.

5.3 RECOMMENDATIONS

Further investigations to determine the molecular mechanism of action by which the *Ananascomosus*peel extract does its antibacterial activity against *Escherichia coli*need to be done.

Toxicity profile on both the *Ananascomosus*peel extract and the fractions obtained from this peel extract needs to be carried out using animal studies.

Quantitative analysis of phytochemicals and stability studies of both the *Ananascomosus*peels extract and the fractions obtained from this peel extract need to be carried out.

APPENDICES

Appendix I: Multiple comparisons on Antibacterial activity on fractions

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Fraction A vs. Fraction B	-10.67	-15.33 to -6.005	Yes	****	< 0.0001
Fraction A vs. Fraction C	26.67	22.01 to 31.33	Yes	****	< 0.0001
Fraction A vs. Fraction D	26.67	22.01 to 31.33	Yes	****	< 0.0001
Fraction A vs. Fraction E	1.333	-3.328 to 5.995	No	Ns	0.9219
Fraction A vs. Control (CN 10)	12.00	7.339 to 16.66	Yes	****	< 0.0001
Fraction B vs. Fraction C	37.33	32.67 to 41.99	Yes	****	< 0.0001
Fraction B vs. Fraction D	37.33	32.67 to 41.99	Yes	****	< 0.0001
Fraction B vs. Fraction E	12.00	7.339 to 16.66	Yes	****	< 0.0001
Fraction B vs. Control (CN 10)	22.67	18.01 to 27.33	Yes	****	< 0.0001
Fraction C vs. Fraction D	0.0	-4.661 to 4.661	No	Ns	> 0.9999
Fraction C vs. Fraction E	-25.33	-29.99 to -20.67	Yes	****	< 0.0001
Fraction C vs. Control (CN 10)	-14.67	-19.33 to -10.01	Yes	****	< 0.0001
Fraction D vs. Fraction E	-25.33	-29.99 to -20.67	Yes	****	< 0.0001
Fraction D vs. Control (CN 10)	-14.67	-19.33 to -10.01	Yes	****	< 0.0001
Fraction E vs. Control (CN 10)	10.67	6.005 to 15.33	Yes	****	< 0.0001

Appendix II: Multiple Sidak's comparison test for MIC on fractions

Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Growth - No growth				
Fraction A	-0.3600	-1.778 to 1.058	No	ns
Fraction B	-0.3600	-1.778 to 1.058	No	ns
Fraction E	-0.0360	-1.454 to 1.382	No	ns
Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**
Growth - No growth				
Fraction A	0.0400	0.4000	-0.3600	0.1871
Fraction B	0.0400	0.4000	-0.3600	0.1871
Fraction E	0.0040	0.0400	-0.0360	0.1871

Appendix III: Two-way ANOVA for Fractions on MBC

Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	0.0	> 0.9999	Ns	No	
Column Factor	100.0	< 0.0001	****	Yes	
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Row Factor	0.0	2	0.0	F (2, 2) =	P > 0.9999
Column Factor	0.001944	1	0.001944	F (1, 2) = +infinity	P < 0.0001
Residual	0.0	2	0.0		
Number of missing values	0				

Appendix IV: Research Budget

SN	ITEM	NEEDED QUATITY	UNIT COST (Ushs)	TOTAL AMOUNT (Ushs)
1.	Internet Surfing	10 Hours	2,000	20,000
2.	Stationary	4 Reams of Paper	15,000	60,000
3.	Lab. Reagents	Reagents	20,000	200,000
4.	Lab. Materials	5 Wares	10,000	50,000
5.	Proposal Booklets	2 Copies	20,000	40,000
6.	Report Booklets	4 Copies	30,000	120,000
7.	Contingency Fee	Single	50,000	50,000
		GRAND TOTAL COST:		540,000/=

Appendix V: Research Timeline

Month:	May	Jun.	Jul.	Aug.	Sept.	Oct.	Dec.
Activity:	2017	2017	2017	2017	2017	2017	2017
Proposal Writing							
Proposal Presentation							
Collection of Data							
Analysis of Data							
Report writing							
Result Presentation							
Research Report (Dissertation) Approval							

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