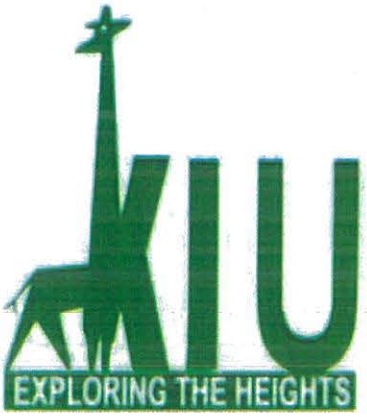

IN VITRO ANTIBACTERIAL ACTIVITY OF ETHANOIC AND DIETHYL ETHER CRUDE STEM BARK EXTRACTOF *Maesa lanceolata* Forssk AGAINST GASTROENTERITIS CAUSING BACTERIA ISOLATES

BY

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DECEMBER, 2018

DECLARATION

I **SSEREMBA ANDREW LWANGA** hereby declare that the work in this dissertation is an original compilation and sole result of my efforts. And that it has never been presented anywhere either partially or in total for any degree award.

Signature:..... Date: 3rd December, 2018

SUPERVISOR'S APPROVAL

I hereby declare that this research report has been produced under my supervision and submitted for examination with my approval.

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DEDICATION

I dedicate this dissertation report with deepest love, affection and honour to my dearest beloved parents Mr. and Mrs. Edward Lwanga, to Mummy Ruth and my siblings: Teddy, Maurice, Leticia, George, and Anna whose consistent love and support has seen me come this far.

And with heartfelt appreciation for the kind timely professional support and consistent guidance during the whole process of this research, from its inception to its completion, I further dedicate this report to Mr. Ntulume Ibrahim.

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ACRONYMS AND ABBREVIATIONS

AMR	Antimicrobial resistance
AIS	Autoinducers
WHO	World health organization
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
KIUTH	Kampala international university teaching hospital
KIU-WC	Kampala international university western campus
LPS	Lipopolysaccharide
ANOVA	Analysis of variance
BN	Billion
CDC	Centre for disease control
CF	Cystic fibrosis
COPD	Chronic obstructve pulmonary disease
DMSO	Dimethyly sulphoxide
DNA	Dinucleotide adenosine
BHI	Brain heart infusion broth
AMPS	Antimicrobial peptides
CF	Cystic fibrosis
AIS	Autoinducers
PAIS	Pathogenicity islands
NCRI	Natural chemotherapeutics research institute
SEM	Standard error mean

ZOIS	Zones of inhibition
BHI	Brain heart infusion broth
MIHA	Mueller hinton agar
PIA	Polysaccharide intercellular adhesion
PNAG	Poly-n-acetyl glucosamine
et al	And others

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ABSTRACT

Introduction: The burden of gastrointestinal (GI) illness remains significantly high in developing countries mostly among children below five years in East African countries. Gastroenteritis is further worsened by antimicrobial resistance evolving and spreading among bacterial pathogens as a public health problem of increasing magnitude.

Objectives: To evaluate the in vitro antibacterial activity of ethanolic and diethyl ether crude stem bark extracts of *Maesa lanceolata* Forssk against gastroenteritis causing bacteria such as; *Staphylococcus aureus* ATCC® 25923™, Methicillin-resistant *Staphylococcus epidermidis*, Methicillin-resistant *Staphylococcus aureus*, *Proteus mirabilis* NCTC® 10975™, *Shigella dysentery* ATCC® 13313™, *Pseudomonas aeruginosa* ATCC® 27853™, *Salmonella typhimuriuum* ATCC® 14028™ and *Escherichia coli* ATCC® 25922™.

Methods: The study was carried out in the microbiology laboratory and the Pharmacognosy laboratory at Kampala International University western campus. The cold maceration method was used for extraction of *Maesa lanceolata* stem bark using 80% ethanol and 99.9% diethyl ether solvents separately. The agar well diffusion and broth dilution methods were used to determine the in vitro antibacterial activity of the extracts. Data for the zones of inhibition was analysed using SPSS-16 by one-way Analysis Of Variance (ANOVA). The statistical significance of the comparisons was considered at 95% level of confidence interval ($p<0.05$).

Results: The stem bark crude ethanolic extract had a higher antibacterial activity against the test bacterial isolates known to cause gastroenteritis compared to diethyl ether extract. Both extracts didn't have activity against MRSA, *S. typhimurium* and *E. coli*. The lowest recorded MIC for ethanolic extract was 3.125 mg/mL against both MRSE and MSSA whereas the least obtained MIC for diethyl ether extract was 6.25 mg/mL against MSSA. The ethanolic extract exhibited the lowest MBC of 1.5625 mg/mL against MRSE compared to diethyl ether extract which had 12.5 mg/mL against MSSA. The study showed that the gram-positive bacteria were significantly more susceptible to the plant extract than gram negative bacteria.

Conclusion: *Maesa lanceolata* stem back is a potential source for development of new antibacterial drugs to combat bacterial gastroenteritis and also overcome the burden of emerging antimicrobial resistance.

CHAPTER ONE

INTRODUCTION

1.0 Background of the study

Gastroenteritis is the inflammation of the membrane lining the stomach, large and small intestines characterized especially by vomiting nausea, cramps, and diarrhea when caused by either viral or bacterial pathogens (Schlossberg, 2015). According to a report by the World Health Organization (WHO) in 2013, the mortality and global health burden of gastroenteritis infections was 16million deaths in 1990, and 15 million deaths in 2010 (WHO,2013). Additionally, WHO reported that the substantial worldwide disease burden from contaminated food occurring in children <5 years of age who represent 9% of the global population was estimated to be at 43% of the burden in this group (Torgerson *et al.*, 2015).

A cohort of results showed that 20% of England population experienced acute gastroenteritis each year with approximately 9.4 million cases with non-typhoidal *Salmonella* species accounting for 106,800 cases. In a standardized community-incidence of acute gastroenteritis for Netherlands was 283 cases per 1000 person-years approximately 4.5 million cases (Flint *et al.*, 2005). Flint *et al.* (2005) futher reported the high rates of acute gastroenteritis in Northern Republic of Ireland at 0.60 episodes per person per year, which, if extrapolated to include the total population, amounts to 3.2 million episodes of gastroenteritis each year. Furthermore, the burden of gastroenteritis has been reported in 5.4 million cases per year in Australia while in Canada, studies demonstrated that 3.4% of patients who were examined by physicians received diagnosed with acute gastroenteritis each month.

In developing countries, the burden of gastrointestinal (GI) illness remains significantly high despite a marked decrease in mortality rates from 4.6 million in 1980 to about 1.5 million annually in 1999 (Fletcher *et al.*, 2011). It was also estimated that approximately 1.87 million children die from diarrhea before reaching their fifth birthday (Fletcher *et al.*, 2011). The diarrheal diseases accounted for 1/5 of all child deaths, 78% of which were concentrated in the African and South East Asian regions (Fletcher *et al.*, 2011). Recently, increased diarrhea rates over the period between 1967 to 1997 in East African countries was reported in Kenya (6%-18%), Uganda (16-21%) and Tanzania (11-8%) (Fletcher *et al.*, 2011).

According to review and meta-analysis studies, several enteric micro-organisms are responsible for gastroenteritis illnesses and are bacterial, viral or parasitic in nature (Fletcher *et al.*, 2011; Ntulume *et al.*, 2017). The majority of gastroenteritis illnesses in developing countries have been reported by several studies to be caused by pathogenic bacteria such as; diarrhoeagenic *E. coli spp*, *Shigella spp, Salmonella spp.*, *Staphylococcus aureus, Pseudomonas* spp and *Campylobacter spp* among others (Fletcher *et al.*, 2011).

However, gastroenteritis has been further worsened by occurrence of antimicrobial resistance among the causative bacteria thus elevating the current global health problem (Radhouani *et al.*, 2014). The high threat of resistance has been registered among carbapenems antibiotics which are used as drugs of last resort in treatment of resistant pathogens of which most are involved in causing chronic gastroenteritis(Livermore *et al.*, 2011; Manenzhe*et al.*, 2014; Papp-Wallace*et al.*, 2011). In a recent study conducted in Uganda at Mulago National Referral Hospital, Kampala by Kateete*et al.*(2017) and Okoche*et al.* (2015) reported a high prevalence of carbapenem resistant bacteria isolated from admitted patients.

Thus in the light of addressing the antimicrobial resistance, the use of plant extracts and phytochemical could be of great significance in therapeutic treatments (Weckesser *et al.*, 2007; Nascimento *et al.*, 2000). This can be based on the WHO estimates that 80% of the population in developing countries employ the usage of herbal medicine to treat several aspects of diseases (Timothy*et al.*, 2018). These medicines also serve as precursor for 20 to 25% of modern medicine as these herbal medicines are considered to be economical, safer and less toxic (Al-Daihan *et al.*, 2013).

Maesa lanceolata is a medicinal plant that belongs to the primulaceae family is traditionally used as a herbal remedy in the treatment of various bacterial infections, dermatophytic conditions, helminthic, malaria and dermatophytic infections (Mukungu *et al.*, 2016; Kokwaro, 2009; Ngwenya *et al.*, 2003; Timothy *et al.*, 2018). Similar studies have validated the antibacterial potential of *M. lanceolata* however, among the extraction solvents worked on, ethanolic and diethyl ether crude of stem bark against specifically resistant isolates has never been reported. It is against this background that this study seeks to evaluate the antibacterial activity of ethanolic and diethyl ether crude stem bark extract against gastroenteritis causing bacteria.

1.1 Problem statement

The burden of gastroenteritis illness remains significantly high in developing countries, despite a marked decrease in mortality rates from 4.6 million in 1980 to about 1.5 million annually in 1999 (Fletcher *et al.,* 2011). Furthermore, the same study also estimated that approximately 1.87 million children die from diarrhea before reaching their fifth birthday which accounted for 1/5 of all child deaths, 78% of which were concentrated in the African children. There has been reported increases of this burden among the East African countries, that is; in Kenya (6%-18%), Uganda (16-21%) and a slightly lower prevalence Tanzania (11-8%) (Fletcher *et al.,* 2011).

This disease burden has been complicated by the rising occurrence of antibiotic resistance among the reported gastroenteritis-causing bacteria which has diminished the effectiveness of antibiotics in currently used in Uganda as reported by the Global Antibiotic Resistance Partnership the Uganda National Academy of Sciences. Such resistances have been reported among some affordable drugs, such as penicillin G and cotrimoxazole at resistance at or near 100%. Furthermore, multidrug resistant strains of *P. aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MRSA) were reported to be the most notorious causative pathogens of gastroenteritis (Ramakant*et al.,* 2011). Similarly, resistant strains acquired through food poisoning such as; *Salmonella typhimurium, Proteus mirabilis, Escherichia coli, Shigella dysentery* and Methicillin-resistant *Staphylococcus epidermidis* were also reported by several studies as causative agents of both acute and chronic gastroenteritis illness (Kirk *et al.*, 2015; Fletcher *et al.,* 2011). These are currently associated with significant morbidity and mortality owing to the treatment challenges faced during their management worldwide (Bindu*et al.,* 2017; Kateete *et al.,* 2017; Okoche *et al.,* 2015).

It is against this background that there urgent need to develop new drugs from alternative sources such as herbal plants to address this current challenge of bacterial gastroenteritis. Therefore, *M. Lanceolata* extracts were explored in the present study to validate their claimed usage towards combating this global challenge.

1.2 General objective

To evaluate the in vitro antibacterial activity of ethanolic and diethyl ether crude stem bark extracts of *Maesa lanceolata* Forssk against gastroenteritis causing bacteria.

1.3 Specific objectives

1. To determine the secondary phytochemical constituents, present in the ethanolic and diethyl ether crude stem bark extracts of *Maesa lanceolata* Forssk.
2. To determine the antibacterial activity of the ethanolic and diethyl ether crude extracts of *Maesa lanceolata* Forssk against gastroenteritis causing bacteria.
3. To determine the Minimum inhibitory concentration of ethanolic and diethyl ether crude stem bark extracts of *Maesa lanceolata* Forssk against gastroenteritis causing bacteria.
4. To determine Minimum Bactericidal Concentration of *Maesa lanceolata* Forssk ethanolic and diethyl ether crude stem bark extract against gastroenteritis causing bacteria.

1.4 Research questions

1. What are the phytochemical constituents present in the ethanolic and diethyl ether stem bark extracts of *Maesa lanceolata*?
2. What is the susceptibility of the gastroenteritis causing bacteria to ethanolic and diethyl ether solvent extracts of *Maesa lanceolata*?
3. What is the Minimum Inhibitory Concentration of the ethanolic and diethyl ether of the stem bark extracts of *Maesa lanceolata Forssk*?
4. What is the Minimum Bactericidal Concentration of the ethanolic and diethyl ether crude stem bark extract of *Maesa lanceolata* Forssk?

1.5 Significance of study/ Justification of study

This study will lead to better understanding of the antibacterial effect of the phytochemicals that will be extracted using ethanol and diethyl ether from the stem back of *Maesa lanceolata* Forssk which are associated with treatment of bacterial pathogens that cause gastroenteritis illness. This will provide research data which will present ground for the development of alternative herbal medicine for the treatment of gastroenteritis. This will in turn improve the mortality and global health estimates from 15 million deaths caused by bacterial infections and will help in hitting the targeted reduction to 13 million deaths by 2050 as estimated by the WHO. Especially given that that approximately 1.87 million children in developing countries die from diarrhea before reaching their fifth birthday (Fletcher *et al.*, 2011). With diarrheal diseases accounting for 1/5 of all child deaths, 78% of which are concentrated in the African

especially children (Fletcher *et al.*, 2011). This efficacious alternative medicine will also lead to reduction in the burden of AMR. As new medical knowledge about the effectiveness of this plant, this will lead to healthier lives of patients suffering from gastroenteritis illness.

1.6 Scope of study

A voucher specimen was taken to the taxonomist at Natural Chemotherapeutics Research Institute (NCRI) for proper identification and authentication. The voucher number of Lwanga 001/19/08/18 to the specimen and it was then kept in the herbarium at the Pharmacognosy unit of NCRI. Collection of the plant leaves and stem bark from Maesa lanceolata was carried out from Samaliya village in Masaka district. The collection was done using a knife, collecting bags and wearing protective gears like gumboots and gloves was ensured. The stem bark was dried under shade on dry table in the Pharmacognosy laboratory of Kampala International University until constant weight for two weeks.

The antibacterial susceptibility tests was done using Agar well diffusion test for the crude plant extracts against standards of antibiotics test that was carried out at Kampala International University microbiology laboratory where antimicrobial sensitivity study was done against the test bacteria of; *Staphylococcus aureus ATCC 25923, Salmonella typhimuriuum ATCC 14028, Proteus mirabilis NCTCC1075, Resistant Pseudomonas aeruginosa, Pseudomonas Aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Resistant Escherichia coli, Shigella dysentery ATCC 13313,* Methicillin-resistant *Staphylococcus aureus*, and Methicillin-resistant *Staphylococcus epidermidis* compared to Gentamicin as a positive control and DMSO as a negative control.

Antibiotic Susceptibility test was done for the plant crude extract from *Maesa lanceolata* stem bark against all of the above mentioned bacterial strains according to standard microbiological protocol (Parekh *et al.*, 2005) on Mueller-Hinton agar. The minimum inhibitory concentration and minimum bactericidal concentration for the crude plant extract was done using broth macro-dilution method according to (Ogutu *et al.*, 2012) and (Ugoh *et al.*, 2013) respectively. The antibiotic standards were got from the Microbiology Laboratory Standards of Kampala International University and the bacteria preparations were got from the standard preparations of bacterial in the microbiology laboratory at Kampala International University. Extraction of the plant crude extract from the *Maesa lanceolata* stem bark was carried out at the Pharmacognosy laboratory of Kampala International University. The Reagents for extraction were obtained from the Pharmacognosy laboratory of Kampala International University. And

the phytochemical screening for the secondary metabolites in the crude plant extracts was carried out at the Pharmacognosy laboratory of Kampala International University.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Specifies of *Maesa lanceolata*

2.1.1 Classification of *Maesa lanceolata*

According to *African Plant Database*, *Maesa lanceolata* was classified as follow;

Infraspecific taxon:	*Maesa lanceolata* subsp. lanceolata
Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Ericales
Family:	Primulaceae
Genus:	Maesa
Botanical name:	*Maesa lanceolata*
English name:	False assegai
Local name:	Ekiwondowondo (Luganda)

2.1.2 Description

Maesa lanceolata is a very variable species as it can be a straggling evergreen shrub, 2 to 3 m tall, or a small tree with a single stem up to 9 m tall, or a rounded bushy tree with branches almost at ground level (Ngwenya*et al.*, 2003). Leaves spirally arranged, lance-shaped, 40-180 x 25-130 mm, tapering to pointed tips, sometimes with blunt or rounded tips, and simple, pale or dark green, with toothed margins. The midrib and lateral veins are conspicuously raised below. Sometimes the leaves are large and thick and resemble those of Curtisiadentata, the assegai, and people often mistake them, but the leaves of C. dentata are opposite, not alternate, as those of the false assegai are. The bark is usually smooth and brown. Flowers are minute, bisexual, sweetly scented, white or yellow, in many-flowered sprays in the axils of leaves and at the ends of branches. Flowering in spring (peak in October in KwaZulu-Natal) but it has

been observed flowering on and off throughout the year. Matured fruit are usually found in March, usually crowded in dense sprays, are creamy-white, edible berries that have remnants of calyx lobes at the top, 3-6 mm in diameter(http://pza.sanbi.org/maesa-lanceolata).

2.1.3 Distribution and habitat

The False assegai (*Maesa lanceolata*) is a tree species that is widespread in the Afro tropics, including Madagascar. It occurs from the southern Arabian Peninsula, southwards to the Eastern Cape, South Africa. It grows on stream verges, river banks and forest verges, where it is often a pioneer plant (Mabberley, 1997).

2.1.4 Ecology

Although the fruits of this tree are poisonous to humans and other mammals, they are eaten by birds. Plants are therefore bird-dispersed. The white, sweetly scented flowers are typical of those which are pollinated by night-flying insects such as moths. It seems reasonable to assume that they are pollinated by small moths (Ngwenya *et al.*, 2003).

2.1.5 Ethnobotanical uses of *M. Lanceolata*

Its uses and cultural aspects in South Africa include are; - Ground-up fruits in milk are given to a child at night as a treatment for ringworm. The roots are used for steaming and as a purgative to remove pimples (Mabberley, 1997). In Kenya, the plant is traditionally used for the therapy of helminthic and bacterial infections (Kokwaro, 1993). In Market community, the stem bark is sliced to pieces, boiled and used to treat dermatophytic conditions (Kipkore*et al.*, 2014). An ethnobotanical study at Luhya community of Kakamega East sub-County, Kenya showed that about 2% frequency use of plant family of primulaceae where *Maesa lanceolata* belongs for the management of malaria (Mukungu *et al.*, 2016). It was also reported that *M. lanceolata* extracts had been used for the treatment of helminthes, fungal and bacterial infections (Kokwaro, 2009).

2.1.6 Phytochemical constituents of *M. Lanceolata*

The antibacterial activity of *M. Lanceolata* has been attributed to the presence of its chemotherapeutic compounds that can serve to protect other plants against microbial infections (Okemo *et al.*, 2003). And these could have included flavonoids, tannins, phenols, saponins, terpenoids, alkaloid and anthraquinones that were tested from the leaves of *Maesa lanceolata* (Timothy *et al.*, 2018).

2.2 Gastroenteritis illness

Gastroenteritis is the inflammation of the membrane lining the stomach, large and small intestines characterized especially by vomiting nausea, cramps, and diarrhea when caused by either viral or bacterial pathogens (Schlossberg, 2015). Because of the various causes, variable symptomology, and numerous different terms used to describe disease of the gastrointestinal tract, no standard definition of *gastrointestinal diseases* has been presented in the medical literature (Hennessy *et al.,* 2004).

Gastroenteritis can be caused by diarrheal illness which is usually divided into three main categories based on its clinical presentation and according to Benenson*et al.,* (1995) and these include Acute watery diarrhea that is associated with several pathogens such as *Vibrio cholera* and rotavirus, usually last for several days, and poses a high risk for dehydration which can be fatal especially in young children, the elderly and immuno-compromised persons (Fletcher *et al.,* 2011). Acute bloody diarrhea or dysentery is also usually evidence of infection with *Campylobacter, Salmonella, Shigella,* enterohaemorrhagic *E.coli* pathotypes, *Entamoeba histolytica* and other organisms, and can lead to dehydration. And the major risks associated with this presentation include intestinal damage, sepsis and malnutrition. Persistent diarrhea lasts for 14 or more days and is usually associated with parasitic aetiology but can be due to non-infectious causes such as inflammatory bowel disease(Fletcher *et al.,* 2011). Due to the prolonged duration of persistent diarrhea there is a risk of nutrient deficiency, extra intestinal infection and dehydration especially in children, the elderly and immuno-compromised.

Pathogenesis of gastroenteritis illness

Infectious gastrointestinal illnesses are transmitted through a variety of routes including contaminated food or water borne, the fecal oral route, and person-to-person (Fletcher *et al.,* 2011). A significant proportion, about 36%, of gastrointestinal illnesses is attributable to food borne transmission and among all illnesses attributable to food borne transmission,30% are caused by bacteria(Fletcher *et al.,* 2011). Infections with pathogenic *E. coli* strains are usually considered an indication of poor hygiene with up to 63% of children with persistent diarrhea in low and middle income countries have tested positive for *E. coli* strains(Fletcher *et al.,* 2011). Travel-associated diarrhea has been described and travel to a developing region is a risk factor among patients presenting with diarrhea in developed settings(Fletcher *et al.,* 2011).

2.3 Antibacterial medicines.

The first antimicrobial agent in the world was salvarsan, a remedy for syphilis that was synthesized by Ehrlich in 1910. In 1928, Fleming discovered penicillin. He found that the growth of *Staphylococcus aureus* was inhibited in a zone surrounding a contaminated blue mold (a fungus from the *Penicillium* genus) in culture dishes. *S. aureus* is the resistant bacterium most familiar in the clinical setting. That rapidly acquired resistance to sulfonamides when they were in use. Penicillin was initially effective to this microorganism, but resistant strains that produce penicillinase increased in the 1950s.

Therefore, penicillinase-stable methicillin was developed in 1960, as mentioned previously. However, as early as the following year, 1961, methicillin-resistant *S. aureus* (MRSA) was isolated in the UK.3. In genetic lineage analysis of nosocomial MRSA strains, major nosocomial clones throughout the world would converge on only seven types.4 On the other hand, community associated methicillin-resistant *S. aureus* (CAMRSA), which was noticed in the US around1997, is of a different type from nosocomial MRSA. Fortunately, MRSA so far in Japan have responded to glycopeptide antibiotics such as vancomycin.

However, in the latter half of the1990s, vancomycin-intermediate *S. aureus* (VISA)was reported in this country. The vancomycin-resistant *S. aureus* (VRSA) was also reported in the US. Although *P. aeruginosa* are intrinsically resistant to many antimicrobial agents, the emergence of *P. aeruginosa* strains resistant to all of three classes of antimicrobials, i.e., carbapenems, quinolones, and aminoglycosides is a recent concern. These multidrug resistant *P. aeruginosa* (MDRP)sometimes seems to cause an outbreak in some institutions (Saga & Yamaguchi, 2009).

2.4 Study Bacteria

2.4.1 Gram positive bacteria

These are bacteria that give a positive result in the Gram stain test they take up the crystal violet stain used in the test, and then appear to be purple-coloured when seen through a microscope. This is because the thick peptidoglycan layer in the bacterial cell wall retains the stain after it is washed away from the rest of the sample, in the decolorization stage of the test. Their cell walls have virtually no lipopolysacharide content, low lipid content and low lipopolysacharide content (Gibbons & Murray, 1978).

2.4.1.1 *Staphylococcus aureus*

Staphylococcus aureus was discovered by rosenbch in 1884 and has since become one of the most studied bacteria. It's a gram-positive coccus forming spherical to ovoid cells about 1μm in diameter usually appearing in a cluster form. *Staphylococcus aureus* is a gram-positive coccus forming spherical to ovoid cells about 1 μm diameter with tendency to form cluster cells. Staphylococci are coagulase–positive, oxidase–negative, facultative anaerobes but grow best in aerobic conditions. The bacterium is non-motile and most strains grow well at temperature between 35-40 (Steinka & Kukulowicz, 2011). *S. aureus* is a gram-positive, facultative anaerobic, coccal bacterium that is a permanent part of the normal flora of the nostrils of approximately 20% of the population and is carried intermittently by approximately 30% of the population (Wertheim *et al.*, 2005).

Staphylococcus aureus causes infections such as skin infections such as folliculitis, carbuncle, impetigo, cellulitis, abcess, it also deep infections of blood stream infections and toxin-mediated diseases. Examples of Blood stream infections include arthritis, osteomyelitis bacteremia, sepsis, metastatic infections: endocarditis, meningitis, osteomyelitis, arthritis, pericarditis, lung abscess. Whereas the toxin-mediated diseases include food poisoning, scaled skin syndrome, toxic shock syndrome with multiple organ failure (Lemaire, 2008).

Methicillin-resistant *Staphylococcus aureus* (MRSA) refers to a group of gram-positive bacteria that are genetically distinct from other strains of *Staphylococcus aureus*. MRSA is responsible for several difficult-to-treat infections in humans. MRSA is any strain of *S. aureus* that has developed, through horizontal gene transfer and natural selection, multiple drug resistance to beta-lactam antibiotics. β-lactam antibiotics are a broad spectrum group which includes some penams – penicillin derivatives such as methicillin and oxacillin, and cephems such as the cephalosporins. Strains unable to resist these antibiotics are classified as methicillin-susceptible *S. aureus*, or MSSA.

Pathogenesis of *Staphylococcus aureus*

S. aureus is one of the main causes of hospital and community-acquired infections which can result in serious consequences (Diekema*etal.*, 2001). Nosocomial *S. aureus* infections affect the bloodstream, skin, soft tissues and lower respiratory tracts. *S. aureus* can be a cause of central venous catheter-associated bacteremia and ventilator assisted pneumonia(Plata, Rosato, & Węgrzyn, 2009). It also causes serious deep-seated infections, such as endocarditis and

osteomyelitis (Schito, 2006). In addition to the infections listed above, *S. aureus* is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome and staphylococcal food borne diseases (SFD). Hospitalized patients are particularly exposed to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections (Lindsay & Holden, 2004). Immuno-compromised people for example those on cancer chemotherapy and other immunosuppressant medications, diabetic patients, vascular diseases and lung diseases are at the biggest risk of contraction of infection through direct contact with the pus.

2.4.1.2 *Staphylococcus epidermidis*

It's a Gram-positive facultative anaerobic bacterium, and one of over 40 species belonging to the genus *Staphylococcus*. And it's part of the normal human flora, typically the skin flora, and less commonly the mucosal flora.

Pathogenesis of *Staphylococcus epidermidis*.

According to Fey, (1998)the pathogenesis of S. Epidermidis are bacteria that are ingested by phagocytosis and then killed by antimicrobial peptides (AMPs) and proteins, as well as reactive oxygen species, in the neutrophil phagosome. S. epidermidis infection over comes this through a number of mechanisms as described below through its pathogenesis. With the formation of biofilms is the most important mechanism in S. Epidermidis infection and contributes to resistance of phagocytosis. It's also arguable that S. epidermidis colonization of the human skin involves a biofilm-like state formation. Furthermore, S. epidermidis produces protective surface polymers that inhibit phagocytosis, such as biofilm exopolysaccharide polysaccharide intercellular adhesin (PIA, or poly-N-acetyl glucosamine, PNAG) and poly-γ-glutamic acid. Secreted proteases such as S. epidermidis SepA may degrade bactericidal antimicrobial peptides (AMPs). Accordingly, SepA has a significant impact on resistance to elimination by human neutrophils. Finally, S. epidermidis can sense the presence of AMPs by a system called Aps, which upon activation up-regulates AMP-protective mechanisms, including the VraFG transporter, the D-alanylation of teichoic acids, and the lysylation of phospholipids. The latter two mechanisms increase the positive charge of the cell surface, thereby increasing repulsion of the usually positively charged AMPs.

2.4.2 Gram negative bacteria

These are bacteria that do not retain the crystal violet stain used in the gram-staining method of bacterial differentiation. They are characterized by their cell envelopes, which are composed of a thin peptidoglycan cell sandwiched between an inner cytoplasmic cell and a bacterial outer membrane. They are an important medical challenge, as their outer membrane protects them from many antibiotics (including penicillin); detergents that would normally damage the peptidoglycans of the (inner) cell membrane; and lysozyme, an antimicrobial enzyme produced by animals that forms part of the innate immune system. Additionally, the outer leaflet of this membrane comprises a complex lipopolysaccharide (LPS) whose lipid A component can cause a toxic reaction when these bacteria are lysed by immune cells.

2.4.2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative rod-shaped and ubiquitous environmental bacterium. It is an opportunistic human pathogen capable of causing a wide array of life-threatening acute and chronic infections, particularly in patients with compromised immune defence. It has been of particular importance since it is the main cause of morbidity and mortality in cystic fibrosis (CF) patients and one of the leading nosocomial pathogens affecting hospitalized patients while being intrinsically resistant to a wide range of antibiotics. *P. aeruginosa* strains possess large genomes. Their metabolic capacity is thus extensive as exemplified by their ability to produce multiple secondary metabolites and polymers as well as their ability to use various carbon sources and electron acceptors. The repertoire of *P. aeruginosa* genes which are substantially conserved suggest the highest proportion of regulatory genes and networks observed in known bacterial genomes and is foundational to respond and adapt to diverse environments (Frimmersdorf*et al.*,2010; Mathee *et al.*, 2008; Stover *et al.*, 2000). The ubiquitous presence of *P. aeruginosa* as well as its prevalence and persistence in clinical settings including intrinsic resistance to therapeutics are attributed to its extraordinary capability of survival by recruiting an arsenal of responsive mechanisms (Moradali*et al.*, 2017).

Pathogenesis of *Pseudomonas aeruginosa*

Communication between individual cells i.e. quorum sensing (QS) allows bacterial communities to regulate a variety of biological processes important for bacterial adaptation and survival. And it relies on regulating the expression of specific sets of genes in response to a critical threshold of signalling molecules known as auto inducers (AIs). QS will mediate

population density dependent collective responses and is therefore beneficial for community survival. A study showed that cells' responses to QS signals and the corresponding gene expression profile is heterogeneous within a given community leading to increasing fitness and chance of survival (Grote*et al.*, 2015). During pathogenesis P. Aeruginosa QS plays a critical role for survival and colonization by coordinating phenotypic alterations at early stages of infection i.e., after attachment (González & Keshavan, 2006). The progress of acute to chronic infection is critically influenced by QS-dependent gene expression. More than 10% of *P. Aeruginosa* genes are regulated by QS. These genes are mainly involved in virulence factor production, motility, motility-sessility switch and biofilm development, antibiotic resistance mechanisms and the adjustment of metabolic pathways for stress responses(Barr *et al.*, 2015; Venturi, 2005; Williams & Cámara, 2009).

2.4.2.2 *Escherichia coli (E. coli)*

According to Bhunia, (2018) Escherichia coli is a Gram-negative , short-rod (1--2 μm in length), facultative anaerobe and is motile. It expresses peritrichous flagella, fimbriae or pili, and curli. Some strains express capsules. The optimum growth temperature is 35–37 °C. They are lactose fermenter and produce pink colonies when grown on MacConkey agar plate. Some pathogenic strains are also acid tolerant. The genome size of a pathogenic strain could be 1 Mb larger than the non-pathogenic strains because of the presence of virulence genes. In pathogenic strains, the virulence genes are clustered in pathogenicity islands (PAIs), plasmids, or prophages. The PAIs are flanked by mobile genetic elements – bacteriophages, insertion sequences, or transposons. Horizontal gene transfer facilitates host adaptation and emergence of new pathogenic strains such as enterohemorrhagic E. coli (EHEC), Shiga-toxin producing E. coli (STEC), and enteroaggregative E. coli (EAEC) pathovars

Pathogenesis of *Escherichia coli* (E. coli)

Pathogenic E. coli are classified broadly into two groups: gastrointestinal tract-infecting E. coli that cause diarrhoea and extraintestinal tract-infecting E. coli that affect the kidney, urinary tract, brain, and circulatory system leading to septicemia. These E. coli strains are designated septicemic E. coli (SepEC), uropathogenic E. coli (UPEC), and neonatal meningitis-causing E. coli (NMEC). Diarrheagenic *E. coli* are divided into six pathotypes: enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), Shiga toxin-producing E. coli (STEC), enterohemorrhagic E. coli (EHEC), enteroaggregative E. coli (EAEC), enter invasive E. coli (EIEC), and diffusely adhering E. coli (DAEC).

Enterotoxigenic E. coli (ETEC) adhere to epithelial cells and produce several toxins including heat-labile (LT) and/or heat-stable toxins (ST), but they do not invade epithelial cells. Enteropathogenic E. coli (EPEC) adhere to epithelial cells intimately, produce attachment/effacement (A/E) lesion, and are non-invasive. They do not produce any heat-labile (LT), heat-stable (ST), or Shiga toxins (Stx). Shiga toxin-producing E. coli (STEC) and their most pathogenic subset enterohemorrhagic E. coli (EHEC): EHEC bind strongly to the epithelial cells, produce attachment/effacement lesions, and produce Stx. Enteroaggregative E. coli (EAEC) adhere to epithelial cells; form aggregates, appearing like "stacked brick;" and produce toxin but do not invade. Enteroinvasive E. coli (EIEC) cells also adhere, invade cells, and move from cell to cell but do not produce toxin. The pathogenicity of EIEC resembles infection caused by Shigella spp., and the predominant symptom is dysentery. The diffusely adhering E. coli (DAEC) adhere to epithelial cells, but they neither invade nor produce toxin.

2.4.2.3 *Proteus mirablis*

Proteus mirablis, is a member of the *Enterobacteriaceae* family and it's a small gram-negative bacillus and a facultative anaerobe which is characterised by its swarming motility, its ability to ferment maltose and its inability to ferment lactose. *P. Mirablis* has the ability to elongate itself and secrete a polysaccharide when in contact with solid surfaces, making it extremely motile on items such as medical equipmenthttp://web.uconn.edu/mcbstaff/graf. According Hamilton *et al.,* (2018) *Proteus spps* are usually considered commensals in the gut and are most commonly recognized clinically as a cause of urinary tract infections. With the recent identification of *Proteus* spp. as potential pathogens in Crohn's disease recurrence after intestinal resection which serves as a stimulus to examine their potential role as gut pathogens. Gastrointestinal conditions that have been linked to *Proteus* include gastroenteritis (spontaneous and foodborne), nosocomial infections, appendicitis, colonization of devices such as nasogastric tubes, and Crohn's disease. The association of *Proteus* species with Crohn's disease was particularly strong. *Proteus* species are low-abundance commensals of the human gut that harbour significant pathogenic potential; further investigation is needed.

Pathogenesis of *Proteus mirablis*

The flagellum of *P. Mirabilis* is crucial to its mortality, a characteristic that helps the organism colonize. The flagellum has also been linked to the ability of the *P. mirabilis* to form biofilms, aiding in the bacteria's resistance to defences of the host and select antibiotics. *P. mirabilis* also relies on on the pilli for adhesion to avoid being flushed out of the urinary tract system. Important to *Proteus mirabilis* is urease, responsible for raising the pH and consequently making it easier to thrive. Increased pH allows stone formation to take place. On occasion the stones fill the entire renal pelvis. Also present are endotoxins, responsible for induction of the inflammatory response system and pore forming haemolysins (Armbruster, Mobley, & Pearson, 2018).

4.2.4 *Salmonella typhimurium*

According to Robert, (1996) Salmonellae are Gram-negative, flagellated, facultatively anaerobic bacilli possessing three major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen (possessed by only a few serovars). As with other Gram-negative bacilli, the cell envelope of salmonellae contains a complex lipopolysaccharide (LPS) structure that is liberated on lysis of the cell and, to some extent, during culture. The lipopolysaccharide moiety may function as an endotoxin and may be important in determining virulence of the organisms. This macromolecular endotoxin complex consists of three components, an outer O-polysaccharide coat, a middle portion (the R core), and an inner lipid A coat.

Pathogenesis of *Salmonella typhimurium*

Salmonellosis includes several syndromes such as gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state. Particular serovars show a strong propensity to produce a particular syndrome i.e. *S typhi*, *S paratyphi-A*, and *S schottmuelleri* produce enteric fever; *S choleraesuis* produces septicemia or focal infections; *S typhimurium* and *S enteritidis* produce gastroenteritis; however, on occasion, any serotype can produce any of the syndromes. Pathogenic salmonellae must possess a variety of attributes called virulence factors such as; the ability to invade cells, a complete lipopolysaccharide coat, the ability to replicate intracellularly, and possibly the elaboration of toxins. These enable it to spread from one person to another. Salmonellae get to infect through ingestion of food and gets to survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of proinflammatory cytokines which induce an inflammatory reaction. The acute inflammatory

response causes diarrhoea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease

2.4.2.5 *Shigella dysentery*

According to Robert, (1996) organisms of the genus *Shigella* belong to the tribe Escherichia in the family Enterobacteriaceae. The genus *Shigella* is differentiated into four species: *S dysenteriae* (serogroup A, consisting of 12 serotypes); *S flexneri* (serogroup B, consisting of 6 serotypes); *S. boydii* (serogroup C, consisting of 18 serotypes); and *S sonnei* (serogroup D, consisting of a single serotype). The identification of shigellae by species in the clinical laboratory is usually accomplished by slide agglutination using commercially available, absorbed rabbit antisera.

Pathogenesis of *Shigella dysentery*

Infection is initiated by ingestion of shigella usually via faecal-oral contamination. An early symptom, diarrhoea could be possibly elicited by enterotoxins and/or cytotoxin, may occur as the organisms pass through the small intestine. The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: scanty, unformed stools tinged with blood and mucus

2.5 Antimicrobial resistance and evolution

Bacterial infections are becoming harder due to antimicrobial resistance which is a challenge to the health sector. It is a natural phenomenon that bacteria and other microorganisms will always develop a mechanism of evading the antibacterial systems applied against them. The emergency of antibacterial resistance was predicted by Alexander flemming during his speech on receiving his noble prize in 1928 after discovering penicillin. Bacteria including staphylococcus aureus and streptococcus pneumonia have devised mechanisms such as production of enzymes that destroy the antibacterial agent before it reaches the target site, acquire impermeability to the drug, and the bacteria efflux pump against the drug (Sternberg *et al.,* 1881).

Methicillin resistant staph aureus is among the common resistant strains of *staphylococcus aureus* but a recent and alarming emergency of intermediately resistant vancomycin resistant *staphylococcus aureus* was isolated from two patients in Johannesburg, South Africa rose a

new worry in the aspect antimicrobial resistance. This could be wide spread across the rest of Africa but due to poor surveillance thus lack of reports owing to several factors there is an information gap (Hal *et al.*, 2012). *Pseudomonas aeruginosa* has got an extraordinary capacity of developing resistance against a wide range of antimicrobials through various molecular mechanisms which are often simultaneously present in clinical isolates.

Although each resistance mechanism is related to a specific class of antibiotics, multiple mechanisms mediate variably resistance to each class of antibiotics (Potron*et al.*, 2015). Furthermore, the contribution of each mechanism varies from country to country. Loss or reduced copy numbers of OprD and overproduction of active efflux pumps, AmpC b-lactamase and extended-spectrum b-lactamases have been mainly reported as main contributors to multi-drug resistance phenotypes of *P. aeruginosa* isolates (Moradali *et al.*, 2017).

2.6 Antibacterial susceptibility testing

This is the process of determining the degree to which a microorganism is affected by a given drug. AST is important when confirming the treatment that is best against a given organism by comparing the organisms' susceptibility against certain different drugs. Dosages and dosage patterns can be determined and potential adverse effects can be lowered or predicted.

AST can reduce the expenses and client disappointment that usually would be more likely with bind trials, can reduce the potential of antibacterial resistance and improving the patient's chance of recovery. During the process, Minimum Inhibitory Concentration which is the lowest antibiotic concentration in µg/ml of the antibiotic which will stop growth of a given bacteria strain and Minimum Bactericidal Concentration which is the lowest antibiotic concentration in µg/ml which kills a given bacterial strain are determined. These are used to reflect how much of the drug will be required to produce the desired therapeutic effect when administering the drug.

2.7 Evaluation of the antibacterial activity

2.7.1 Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants microbial extracts (Balouiri *et al.*, 2016). Similarly, to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 mL) of the antimicrobial agent or

extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

2.7.2 Antibiotic susceptibility

The determination of antibiotic susceptibility of a pathogen is important in selecting the most appropriate one for treating a disease. There are several different procedures used by clinical microbiologists to determine the sensitivity of microorganisms to antibiotics. Two such procedures are described below. The first one (the Kirby-Bauer Disc Method) is used to determine which antibiotic is the most effective against a certain pathogen. The second (MIC) is used to determine the lowest concentration that is needed to kill the pathogen at the site of infection.

2.7.3 Kirby-Bauer Disc Method

According to Das *et al.,* (2010), this method is also called the agar diffusion method/ the disk diffusion method. The procedure followed is simply that a filter disk impregnated with an antibiotic is applied to the surface of an agar plate containing the organism to be tested and the plate is incubated at 37°C for 24-48 hours. As the test substance diffuses from the filter paper into the agar, the concentration decreases as a function of the square of the distance of diffusion. At some particular distance from each disk, the antibiotic is diluted to the point that it no longer inhibits microbial growth. The effectiveness of a particular antibiotic is shown by the presence of growth-inhibition zones. These zones of inhibition (ZOIs) appear as clear areas surrounding the disk from which the substances with antimicrobial activity diffused. The diameter of the ZOI can be measured with a pair of compass, a ruler and the results of such an experiment constitute an antibiogram. The agar diffusion method uses commercially available filter paper disks, each containing a defined concentration of a specific antibiotic.

The relative effectiveness of different antibiotics provides the basis for a sensitivity spectrum of the organism. This information, together with various pharmacological considerations, is used in the selection of an antibiotic for treatment. It should be emphasized that chemotherapeutic agents are not chosen simply on the basis of the drug producing the widest ZOI. This is because of the nature of the growth-inhibition substances. The size of the zone may be affected by the density or viscosity of the culture medium, the rate of diffusion of the antibiotic, the concentration of the antibiotic on the filter disc, the sensitivity of the organism to the antibiotic, and the interaction between the antibiotic and the medium. In addition, an

agent that has been found to have a significant antibiotic effect may not be therapeutically useful because it may also have significant adverse effects in the system for which it is intended. The disk diffusion method represents a simple procedure for screening substances to determine if they have significant antibiotic activity.

2.7.4 Minimum Inhibitory Concentration (MIC) Method

The minimum inhibitory concentration (MIC), according to Balouiri *et al.*, (2016) which is the lowest concentration that still inhibits the growth of a particular organism, can be determined using serial dilution methods. This procedure establishes the concentration of an antibiotic that is effective in preventing the growth of the pathogen and gives an indication of the dosage of that antibiotic that should be effective in controlling the infection in the patient. A standardized microbial inoculum is added to the tubes containing serial dilutions of an antibiotic, and the growth of the microorganism is monitored as a change in turbidity. In this way, the break point, titre, or minimum inhibitory concentration (MIC) of the antibiotic that prevents growth of the microorganism at the site of infection can be determined. By knowing the MIC and the theoretical levels of the antibiotics that may be achieved in body fluids, such as blood and urine, the physician can select an appropriate antibiotic, the dosage schedule, and the route of administration. Generally, a margin of safety of ten times the MIC is desirable to ensure successful treatment of the disease.

The use of microtiter pipette and automated for inoculation and reading systems makes the determination of MIC feasible for use in the clinical laboratory. MIC can even be performed on normally sterile body fluids without isolating and identifying the pathogenic microorganisms. For example, blood or cerebrospinal fluid containing an infecting microorganism can be added to tubes containing various dilutions of an antibiotic and a suitable growth medium. An increase in turbidity would indicate that the microorganism is growing and that the antibiotic at that concentration was ineffective in inhibiting microbial growth. Conversely, a lack of growth would indicate that the pathogenic microorganisms were susceptible to the antibiotic at the given concentration.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

The study was an experimental quantitative study that determined the phytochemical constituents, the antibacterial susceptibility, MIC and MBC of the crude plant extract of *Maesa lanceolata* from September to November 2018.

3.2 Study Site.

The study was conducted in the KIU microbiology laboratory and the Pharmacognosy laboratory at the School of Pharmacy.

The crude plant extraction from the plant stem bark was carried out at the pharmacognosy laboratory at KIU. Whereas the in-vitro experimental study for the determination of the phytochemical constituents, the antibacterial susceptibility, the MIC and MBC with gentamicin as positive control and Dimethyl sulfoxide (DMSO) as negative control were carried in the microbiology laboratory at KIU.

3.3 Collection and identification of plant material

The stem back of *Maesa lanceolate* Forssk was collected from Samaliya village in Masaka district. The coordinates of location where the plant parts were collected from were recorded. The plant was then authenticated by a Botanist at Natural Chemotherapeutics Research Institute (NCRI), who then issued out a voucher number to the collected plant leaves and stem back of the collected plant. And there after a voucher number was given to the collected plant sample and was stored into the NCRI Herbarium for future reference.

3.4 Apparatus

A mortar and pestle (PS4494 W German), Light Microscopic, refrigerator, rotary evaporator , Whatman filter paper (No. 1), Electric crusher, Separating funnel, Water bath setup, round bottomed flask, pipette, Conical flask, Beakers, conical flasks, boiling flasks, Test tubes, tongs, and racks, Watch glasses, Crucibles, Funnels, Graduated cylinders, Volumetric flasks, Droppers, Pipettes, Burettes, Ring stands, rings, and clamps, Tongs and forceps, Spatulas, Thermometer, Bunsen Burner, Weighing balance.

3.5 Storage, drying and pulverization.

The collected plant stem bark was dried under shade at room temperature with occasional changing to prevent fungal infection on the dry floor in the Pharmacognosy laboratory of

Kampala International University-Western Campus. The dried samples were crushed using a mortar and pestle to get a fine powder according to method by (Sanghavi *et al.*, 2014) with slight modification where instead of using a pulverizer, a mortar and pestle was used for crushing the stem bark.

3.5.1 Extraction

The collected fresh plant stem bark was washed under cold running tap water and gently brushed to remove soil and other debris. The stem bark was air-dried in a shaded region at room temperature (25 °C) for 2 weeks and was then pulverized to an appropriate size by grinding with an electric mill to yield 700g of the powdered stem bark. Using 100g of the stem bark powder, extraction using 80% ethanol and 99.9% diethyl ether was done respectively and the percentage crude extract yield was calculated. Each 100 grams of the powdered stem bark was extracted using 500mLof ethanol and diethyl ether respectively for 72 hours. And the organic solvents evaporated to dryness by vacuum evaporation using a rotary evaporator that was rotated intermittently.

The percentage yield was calculated as follows:

$$\% \text{ yield} = \frac{\text{Weight of extract obtained} \times 100\ \%}{\text{Weight of powdered material}}$$

3.5.2 Chemical reagents and materials

Distilled water, plant extract, cold running tap water, 1 % ferric chloride , concentrated sulphuric acid , acetic anhydride, concentrated HCl, 10% ferric chloride, distilled water, 1 % aqueous HCl, 1 ml, 1%ferric chloride, 10% ammonium hydroxide , concentrated amyl alcohol, ethanol, Wagner's reagent Ferric Chloride reagent, Ammonia solution, Acetic Anhdride, Chloroform solution, DMSO, Muller Hinton Agar (MHA), Brain Heart Infusion Broth (BHI), Cotton Swabs, Positive Cotton- Gentamicin, Negative control:-DMSO, ethanol and diethyl ether.

3.6 Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents of the *Maesa lanceolata* ethanolic and diethyl ether plant extracts were done using the standard procedures as described by Bankole *et al.* (2016).

3.6.1 Qualitative determination of phytochemical constituents.

Test for tannins

About 0.5 g of each plant extract was stirred with about 10 ml of distilled water and then was filtered. Few drops of 1 % ferric chloride solution were added to 2 ml of the filtrate, and occurrence of a blue-black, green, or blue- green precipitate indicated the presence of tannins.

Test for terpenoids

A little of each plant extract was dissolved in ethanol. And to it, 1 ml of acetic anhydride was added followed by the addition of concentrated sulphuric acid. A change in colour from pink to violet showed the presence of terpenoids.

Test for saponins

1g of each plant extract was boiled with 5 ml of distilled water and was then filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 min. Any frothing which was persistent on warming showed the presence of saponins.

Ferric chloride test for flavonoids

About 0.5 g of each plant extract was boiled with distilled water and was then filtered. To 2 ml of the filtrate, a few drops of 10% ferric chloride solution were then added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group.

Test for alkaloids

About 0.2 g of each plant extract was stirred with 5 ml of 1 % aqueous HCl on a water bath and was then filtered. From the filtrate, 1 ml was taken into two test tubes. And to 1 ml of the filtrate, a few drops of Wagner's reagent were then added; the occurrence of red-brown precipitate was taken as a positive.

Anthraquinone Borntrager's test

About 0.5 g of each plant extract was shaken with chloroform layer and was then allowed to separate. Then half of the test volume of 10%ammonia solution was added. A pink, red, or violet coloration in the ammoniacal phase indicated the presence of anthraquinone.

3.7 Sample collection, preparation of bacterial standards and controls.

3.7.1 Micro-organism sample collection

The study experiment included standard bacterial isolates of; *Staphylococcus aureus* ATCC® 25923™,*Salmonella typhimuriuum* ATCC® 14028™, *Proteus mirabilis* NCTC® 10975™, *Pseudomonas aeruginosa* ATCC® 27853™, *Escherichia coli* ATCC® 25922™, *Shigella dysentery* ATCC® 13313™ and resistant clinical isolates of; Methicillin-resistant *Staphylococcus aureus*(MRSA)and Methicillin-resistant *Staphylococcus epidermidis* (MRSE) that were obtained from the microbiology laboratory of Kampala International University Western Campus.

3.7.1 Preparation of bacterial standards.

The standard bacterial inoculum were sub-cultured on pre-prepared Mueller Hinton agar and incubated at 37°C for 24hrs to obtain visible bacteria inoculum.

3.7.2 Preparation of positive controls.

Dilution with sterilized distilled water was done to make different concentrations for the positive control which was gentamicin that was used as a positive control in the Agar well diffusion method. The following method according to a report by Uddhav *et al.*, (2016) was used for reconstitution as follows: [1000/P x V x C= W]

Where P=Potency given by the manufacturer in relation to the base, V= Volume in ml required,

C=Final concentration of solution (multiples of 1000), W= Weight of the antimicrobial to be dissolved in the volume V.

3.8 Preparation of media, inoculum and McFarland standard

3.8.1 Preparation of Mueller Hinton Agar (MHA)

Mueller Hinton Agar media (HiMediaM173, India) was prepared according to the manufacturer's instructions. In which 11.4g of the powdered Mueller Hinton agar media was be dissolved in 300ml of distilled water and was sterilized in an autoclave at a temperature of 121°C at 15 Ibs pressure for 15 minutes. The hot media was then cooled to 45- 50°C. The sterilized agar was then stirred gently to form a vortex and which was then be heated on the top of the conical flask with heat from a Bunsen burner. The media was poured on sterilized culture plates and was then allowed to solidify. The plates were covered and were incubated for 24hrs to in an incubator.

3.8.2 Preparation of Brain Heart Infusion Broth (BHI)

The media was prepared according to the manufacturer's instructions. In which 2.22grams of the powdered Brain Heart Broth (BHI) (Oxoid-CM1135, UK) was dissolved in 60ml of distilled water. And was then dispensed into 100 test tubes each with a volume of 200µL using a micropipette. The test tubes were then covered with pieces of cotton cool and they were then sterilized by an autoclave at a pressure of 15 (Ibs), and at a temperature of 121°C for 15 minutes. The sterilized BHI media was used on the day it was prepared.

3.8.4 Preparation of the McFarland standard

The following procedure describes a method for preparing the desired 0.5 McFarland standard (Andrews, 2001). 0.5 ml of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.2H2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring. The standard was distributed into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. The tubes were sealed tightly to prevent loss by evaporation and were then stored and protected from light at room temperature. The standard was vigorously agitated to get turbidity standard on a vortex mixer before it could be used (Andrews, 2001).

3.8.5 Preparation of inoculum

Preparation was according to Andrews, (2001). The inoculum was adjusted so that 1.5×10^8cfu/ml was equivalent to 0.5 Mc Farland standard.

Direct colony suspension method

Colonies were taken directly from the plate and were added into distilled water. And the density of the organism suspension was prepared and adjusted to equal that of the 0.5 McFarland standard by adding the organism to the sterile distilled water. The comparison between the test and standard was aided by comparing them against a white background with a contrasting black line.

3.9 Evaluation of the antibacterial activity

3.9.1 Determination of the antimicrobial activity of the ethanolic and diethyl ether solvent extracts of *Maesa lanceolata* Forssk against gastroenteritis causing bacteria

Agar well diffusion method was used (Parekh *et al.,* 2005). The overnight broth cultures of susceptible and resistant bacterial isolates were reconstituted using sterilized distilled water to adjust turbidity equivalent to 0.5 McFarland solution and were agitated with a vortex mixture

immediately before use. A sterile swab was used to remove the bacteria by pressing and rotating inside the tube above the culture level. Using sterile a swab, the bacteria isolates were uniformly distributed on the culture by streaking the swab over the entire surface of the Mueller Hinton Agar (HiMediaM173, India) plate into three directions to obtain uniform inoculum.6 wells of 6mm in diameter and 4mm in depth were made equidistant on the medium surface using a sterile cork bore and the wells were labeled with marker after inverting the agar plates upside down. These assigned labels constituted D50 mg/mL, D100 mg/mL, E50 mg/mL, E100 mg/mL, -ve DMSO, and +ve GEN. The two extracts of ethanol and diethyl ether were then reconstituted using sterilized10% DMSO to acquire a concentration of 50 mg/mL and 100mg/ml each.

A concentration of 10 µg/mL of intravenous gentamicin was prepared from the stock. Then using sterile automatic pipettes, 50µl of the prepared solutions of diethyl ether and ethanolic extract concentrations each at 50 and 100 mg/mL, 10% DMSO, and 10 µg/mL of gentamicin were poured into the respectively labeled wells. The negative control was 10% DMSO and the positive control was10µg/mL of gentamicin. The added solutions were allowed to diffuse at room temperature for 2 hours and the plates then incubated at 37°C for 24hours.Using a pair of compasses and a ruler, the diameters of inhibition were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) +SEM (standard Error Mean).

3.9.2 Determination of minimum inhibitory concentration of each extract.

The Minimum Inhibitory Concentration (MIC) of the crude extracts was determined according to the method described by (Ogutu *et al.*, 2012). A twofold serial dilution of the extracts was carried out in a series of 10 tubes labeled 1 to 10 that were filled with 200µL each of the prepared sterilized Brain Heart infusion broth (BHI), (Oxoid-CM1135, UK). The starting concentration was 200mg/ml which was added into the first test tube to form the preceding concentrations of; 100, 50, 25, 12.5, 6.25, 3.1 25, 1.563, 0.781, 0.391, and 0.195 mg/ml respectively. Then 200 µL of the prepared bacteria at concentration of 1×10^6cfu/mL was added into each of the test tube containing BHI. This gave a final inoculum density of 5×10^5 cfu/mL of the prepared test bacteria in each test tube. This method was modified by preparing two sets of serial tubes for each of the bacteria, one set of tubes contained only the broth and the extract without the test bacteria that served as negative control and also used to check the sterility of broth media used. The other set of tubes contained both BHI broth and test bacteria only purposely to check the viability of the test bacteria used after incubation (positive control) by

plating out a loop full of broth suspension onto the sterile BHI agar. All test tubes were incubated overnight at 37°C for 24 hours. After incubation, the turbidity from each diluted tube was compared with that of the negative control tubes and the highest dilution without turbidity was considered as MIC and interpreted in mg/ml.

3.9.3 Determination of minimum bactericidal concentration of the extracts

After performing MIC, the series of test tubes without visible growth were used to determine minimum bactericidal concentration (MBC) by plating out on the prepared Mueller Hinton agar as described by Ugoh *et al.* (2013). The streaked plates were incubated at 37°C for 24 hours. After incubation, the minimum concentration at which no visible growth was seen was taken as the MBC in mg/mL

3.9.4 Data analysis

The results mean zone of inhibition were recorded as mean SEM. The data was collected and was entered into computer using Microsoft Office Excel. It was then exported to SPSS-16 and analyzed by one-way ANOVA (Analysis of Variance). The inhibition zone diameters were obtained as mean ± SEM. Multiple comparison using Tukey's post hoc test was used to assess and compare the difference between the individual means zones of inhibition of the extracts at varying concentrations and controls. The statistical significance of the comparisons was considered at 95% level of confidence interval ($p \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage yield

The ethanolic solvent extract had a mass of 8.1725 mg with a percentage yield of 4.086% whereas the diethyl ether solvent stem bark extract had a mass yield of 5.89 mg with a percentage yield of 2.9945%.

4.2 Phytochemical screening

Phytochemical screening of the extracts revealed that ethanolic extract contained: tannins, alkaloids, flavonoids, saponins, terpenoids and anthraquinones while the diethyl ether extracts recorded presence of secondary metabolites of: tannins, flavonoids and terpenoids as shown in table 1.

Table 1; Phytochemical screening of stembark crude extracts of *Maesa lanceolata* Forssk

Secondary Metabolite	Ethanolic Extract	Diethyl ether extract
Tannins	Present	Present
Alkaloids	Present	Absent
Flavonoids	Present	Present
Saponins	Present	Absent
Terpenoids	Present	Present
Anthraquinones	Present	Absent

4.3 Antibacterial Susceptibility study of the crude plant extract against the test bacteria

4.3.1 Ethanolic extract

From table 2, the antibacterial susceptibility Mean Zones of Inhibition of the ethanolic crude plant extract was registered against MSSA, MRSE, *P. mirabilis* and *S. dysentery* (figure 1 and 2). The ethanolic crude plant extract did not show any antibacterial susceptibility against MRSA, *P. aeruginosa, S. typhimurium,* and *E. coli.*

The antibacterial susceptibility at 50mg/ mL of the ethanolic extract against MRSE and MSSA was not significantly different ($p = 0.9184$). The antibacterial susceptibility of the ethanolic

extract at 50mg/ mL of MRSE was significantly different from that *P. mirabilis* ($p < 0.0001$) *and S. dysentery* ($p = 0.006$). The antibacterial susceptibility of the ethanolic extract at 50mg/ mL of *S. dysentery* and *P. mirabilis* was not significantly different ($p = 0.1160$)

The effect of ethanolic extract at 50mg/ mL against MRSA was not significantly different ($p > 0.9999$) from *S. typhimurium* and *E. coli*. The highest potent antibacterial susceptibility against the selected bacteria was observed against MSSA with inhibition zone of 14.50±0.50 mm at 50mg/mL of the extract. Greatest antibacterial susceptibility was observed in MRSE at 100 mg/mL with 14.75±0.25mm (figure 1) whereas the least antibacterial susceptibility was observed in *P. mirabilis* at 50mg/mL with 9.50±0.5 mm (figure 2).

There was no significant difference in the antibacterial activity of the ethanolic plant extract between the concentrations of 50mg/ mL and 100mg/ mL against the susceptible bacteria of MSSA ($p = 0.9999$), MRSE ($p = 0.3617$), *P. mirabilis* ($p = 0.6016$), and *S. dysentery* ($p = 0.8378$) as shown in appendix.

The positive control of gentamicin (10µg/mL) showed antibacterial susceptibility against all the bacteria isolates with ethanolic solvent extract. The antibacterial susceptibility of the ethanolic extract at 50mg/ mL and gentamicin (10µg/mL) was significant for MSSA ($p < 0.0001$), MRSE ($p < 0.0001$), *P. mirabilis* ($p < 0.0001$), and *S. dysentery* ($p < 0.0001$) as shown in figure 1 and 2 The negative control of DMSO did not show any antibacterial susceptibility against all the bacteria isolates with ethanolic solvent extract. The antibacterial susceptibility of the ethanolic extract at 50mg/ mL and DMSO (10%) was significant for MSSA ($p < 0.0001$), MRSE ($p < 0.0001$), *P. mirabilis* ($p < 0.0001$), *S. dysentery* ($p < 0.0001$) (Appendix).

From table 3, the antibacterial susceptibility Mean Zones of Inhibition of the diethyl ether crude plant extract was registered against MSSA, MRSE, *P. mirabilis*, *S. dysentery* and *P. aeruginosa* (figure 2) The ethanolic crude plant extract did not show any antibacterial susceptibility against MRSA, *S. typhimurium*, and *E. coli*.

The antibacterial susceptibility at 50mg/ mL of the diethyl ether extract was not significantly different when MRSE was compared with MSSA ($p=0.2977$). Furthermore, when the antibacterial susceptibility was not significantly different when *P. mirabilis* was compared with MRSE ($p > 0.9999$) and MSSA ($p>0.1457$).

The antibacterial susceptibility at 50mg/ mL of the diethyl ether extract for *S. dysentery* and *P. aeruginosa* was not significantly different (p= 0.9328). The antibacterial susceptibility of the diethyl ether extract at 100mg/ mL of the plant extract between *S. dysentery* and MSSA was significant (p =0.0092) whereas at 50mg/ mL it was not significantly different (p= 0.2977). The antibacterial susceptibility at 50mg/ mL of the diethyl ether extract was not significantly different when MRSA was compared with *S. typhimurium* (p= 0.9999), *E. coli* (p = 0.9999). There was a significant difference in the antibacterial activity of the diethyl ether plant extract between the concentrations of 50mg/ mL and 100mg/ mL against the susceptible bacteria of MSSA (P= 0.0038), MRSE (p= 0.0290), *P. mirabilis* (p= 0.0038), and *P. aeruginosa* (p= 0.0038). However, there were no significant results for *S. dysentery* (p= 0.1668) as shown in appendix.

The positive control of gentamicin (10µg/mL) showed antibacterial susceptibility against all the bacteria isolates. The antibacterial susceptibility of the diethyl ether extract at 50mg/ mL and gentamicin (10µg/mL) was significant for MSSA (p< 0.0001), MRSE (p = 0.0001), *P. mirabilis* (p< 0.0001), *S. dysentery* (P< 0.0001) and *P. aeruginosa* (p = 0.0108) as shown in appendix. The negative control of DMSO did not show any antibacterial susceptibility against all the bacteria isolates with ethanolic solvent extract. The antibacterial susceptibility of the ethanolic extract at 50mg/ mL and DMSO (10%) was significant for MSSA (p< 0.0001), MRSE (p< 0.0001), *P. mirabilis* (p< 0.0001), *S. dysentery* (P< 0.0001) as shown in table 3 and appendix.

Table 2: Mean and standard deviation of inhibition zone diameters of ethanolic stem bark crude extracts of *Maesa lanceolata* Forssk against the test bacteria

est bacteria	Mean and standard deviation of diameters of inhibition zones (mm)			
	Ethanolic extract		Controls	
	50 mg/ mL	100 mg/mL	Gen (10µg/mL)	10% DMSO
SSA	14.50±0.50[a]	14.50±0.50	24.00±0.50	0.00±0.00
RSA	0.00±0.00[c]	0.00±0.00	13.75±1.25	0.00±0.00
RSE	13.75±0.25[a]	14.75±0.25	26.25±0.25	0.00±0.00
mirabilis NCTC® 10975™	9.50±0.5[b]	10.25±0.25	19.50±0.50	0.00±0.00
dysentery ATCC® 13313™	11.25±0.25[b]	11.75±0.25	20.50±0.50	0.00±0.00
aeruginosa ATCC® 27853™	0.00±0.00[c]	0.00±0.00	10.25±0.25	0.00±0.00
typhimurium ATCC® 14028™	0.00±0.00[c]	0.00±0.00	10.50±0.5	0.00±0.00
coli ATCC® 25922™	0.00±0.00[c]	0.00±0.00	14.50±1.50	0.00±0.00

Table 3: Mean and standard deviation of inhibition zone diameters of ethanolic stem bark crude extracts of *Maesa lanceolata* Forssk against the test bacteria

Test bacteria	Mean and standard deviation of diameters of inhibition zones (mm)			
	Diethyl ether extract		Controls	
	50 mg/ mL	100 mg/mL	Gen(10µg/mL)	10% DMSO
MSSA	10.5±0.50[a]	12.75±0.75	24.00±0.50	0.00±0.0
MRSA	0.00±0.0[c]	0.00±0.00	13.75±1.25	0.00±0.0
MRSE	12.00±0.50[a]	13.75±0.25	26.25±0.25	0.00±0.0
P. mirabilis NCTC® 10975™	12.25±0.25a	14.50±0.00	19.50±0.50	0.00±0.0
S. dysentery ATCC® 13313™	9.00±0.0[a,b]	10.25±0.25	20.50±0.50	0.00±0.0
P. aeruginosa ATCC® 27853™	8.25±0.25[b]	10.50±0.50	10.25±0.25	0.00±0.0
S. typhimurium ATCC® 14028™	0.00±0.0[c]	0.00±0.00	10.50±0.5	0.00±0.0
E. coli ATCC® 25922™	0.00±0.0[c]	0.00±0.00	14.50±1.50	0.00±0.0

MSSA: *Staphylococcus aureus* ATCC® 25923™, *MRSE*: Methicillin-resistant *Staphylococcus epidermidis*, MRSA: Methicillin-resistant *Staphylococcus aureus*. *P mirabilis*: *Proteus mirabilis* NCTC® 10975™, *S. dysentery*: *Shigella dysentery* ATCC® 13313™, *P. aeruginosa*: *Pseudomonas aeruginosa* ATCC® 27853™, *S. typhimurium*: *Salmonella typhimuriuum* ATCC® 14028™, and *E. coli*: *Escherichia coli* ATCC® 25922™, Gen; Gentamicin

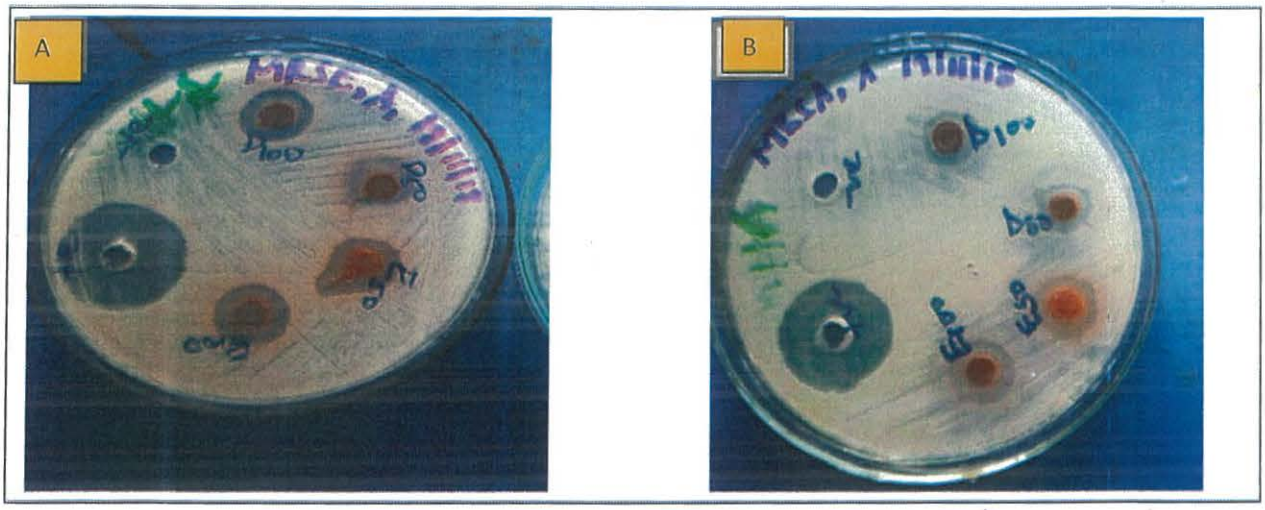

Figure 1; Susceptibility of gram positive bacteria to stem bark crude extracts of *M. lanceolata* F

A: *Staphylococcus aureus ATCC 25923*, B: *MRSE*: Methicillin-resistant *Staphylococcus epidermidis*. D50 and D100: Diethyl ether extract concentrations at 50 and 100 mg/mL, E50 and E100: Ethanolic extract concentrations at 50 and 100 mg/mL, +ve: Positive control, -ve: Negative control

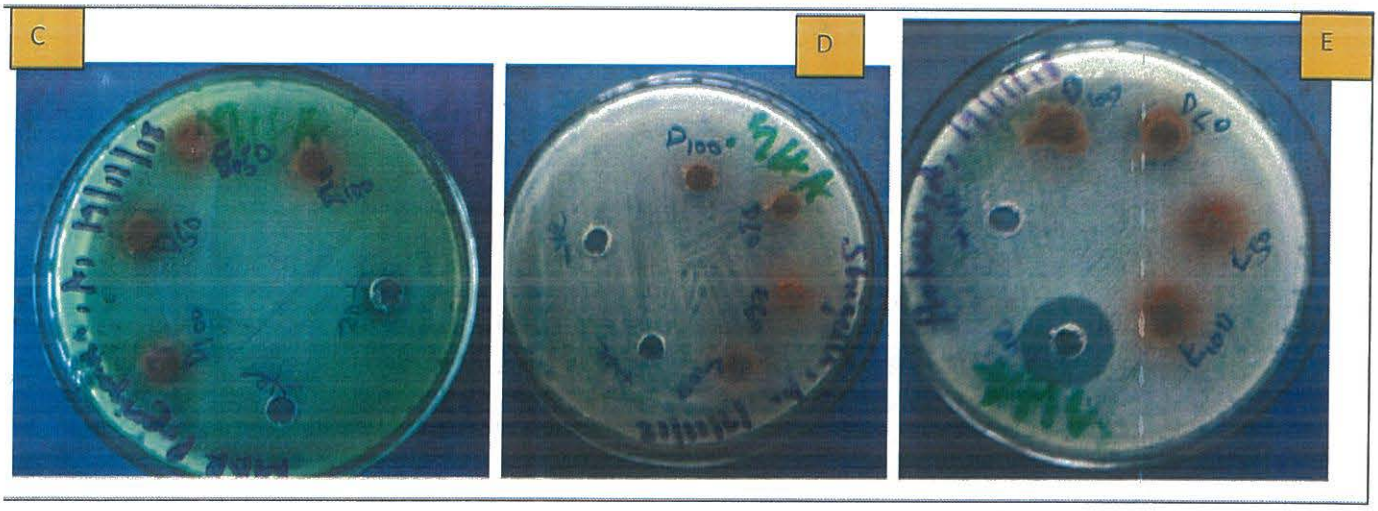

Figure 2; Susceptibility of gram negative bacteria to stem bark crude extracts of *M. lanceolata* F. C: *Pseudomonas aeruginosa* ATCC® 27853™, D: *Shigella dysentery* ATCC® 13313™, E: *Proteus mirabilis* NCTC® 10975™. D50 and D100: Diethyl ether extract concentrations at 50 and 100 mg/mL, E50 and E100: Ethanolic extract concentrations at 50 and 100 mg/mL, +ve: Positive control, -ve: Negative control

4.4 Minimum Inhibitory Concentration (MIC) stem bark extracts of *M. lanceolata*

The lowest recorded MIC for ethanolic extract was 3.125 mg/mL against both MR *Staph epidermidis* and Staph aureus ATCC® whereas the least recorded MIC for diethyl ether extract was against *Staph aureus ATCC*® at 6.25 mg/mL .as shown in table 4

Table 4: Minimum Inhibitory Concentration of stem bark crude extracts of *Maesa lanceolata* Forssk against the test bacteria.

	Minimum Inhibitory Concentration, (mg/mL)	
Test bacteria	**Ethanolic extract**	**Diethyl ether extract**
MRSE	3.125	50
MSSA	3.125	6.25
P. mirabilis NCTC® 10975™	25	25
S. dysentery ATCC® 13313™	25	50

MSSA: *Staphylococcus aureus ATCC 25923, MRSE*: Methicillin-resistant *Staphylococcus epidermidis*, *Proteus mirabilis*: *Proteus mirabilis NCTCC1075*, *Shigella dysentery*: *Shigella dysentery ATCC 13313.*

4.5 Minimum Bacterialcidal Concentration (MBC) stem bark extracts of *M. lanceolata*

The least recorded MBC for ethanolic extract was 1.5625 mg/mL against*MR Staph epidermidis* whereas the least recorded MBC for diethyl ether extract was 12.5 mg/mL against *Staph aureus* ATCC® as shown in table 5

Table 5: Minimum Bacterialcidal Concentration (MBC) of stem bark crude extracts of *Maesa lanceolata* Forssk against the test bacteria

	Minimum Bactericidal Concentration, (mg/mL)	
Test bacteria	**Ethanolic extract**	**Diethyl ether extract**
MRSE	1.5625	100
MSSA	6.25	12.5
P. mirabilis NCTC® 10975™	>100	>100
S. dysentery ATCC® 13313™	>100	>100

MSSA: *Staphylococcus aureus ATCC 25923, MRSE*: Methicillin-resistant *Staphylococcus epidermidis*, *Proteus mirabilis*: *Proteus mirabilis NCTCC1075*, *Shigella dysentery*: *Shigella dysentery ATCC 13313.*

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion of the zone of inhibition

5.1.1Ethanolic extract

This study revealed that not all extracts showed antibacterial activity against the selected bacteria which was contrary to a study conducted by Timothy*et al.*, (2018) that made a study using different solvents and identified that all extracts had showed antibacterial activity against the selected bacteria at varying levels of concentration. This antibacterial activity could have been attributed to the presence of secondary metabolites of the plant extract that included; Tannins, Alkaloids, Flavonoids, Saponins, Terpenoids and Anthraquinones. Similar studies showed that medicinal plants have been known to produce an array of phytochemicals with recognized antibacterial activity belonging to chemical structural classes: phenolic, terpenoids alkaloids, lectins, polypeptides, and polyacetylenes (Ho *et al.*, 2016).

Upon comparison, significant results based on p-values showed that the greatest antibacterial susceptibility was observed against MRSE. This could be due to the presence of flavonoids known to have a cidal effect against most bacterial as reported by Silvia *et al.*, (2013) and Singh & Kumar, (2013) where the activity of the flavonoid fraction crude extract was shown to have high activity against both MRSE and standard *S. aureus*. Furthermore, this could probably explain the antibacterial activity of the extract against MSSA, *P. mirabilis* and *P. aeruginosa*.

The antibacterial susceptibility of the ethanolic extract reduced when the concentration was halved to 50 mg/mL, however most of the gram-negative bacteria showed resistance which could be attributed by the nature of cell wall peptidoglycan layer which could be destroyed by extract phytochemicals. Additionally, Ogueke *et al.*, (2007) reported similar results when the extract concentration was reduced for most gram-negative bacteria. These results also showed that susceptibility of MRSA was affected by lower extract concentrations. This was due to the presence of MecA gene which contributed to resistance to the extract.

The inability of the extract to inhibit *S. typhimurium* may have been because of its mechanism for detoxifying the active principles in the extract. Some bacteria are known to posses' mechanisms by which they convert substances that inhibit their growth in to non-toxic

compounds such as; *S. aureus* which produces the enzyme penicillinase (Ogueke *et al.*, 2007). The high level of resistance of *P. aeruginosa* towards the extract could be attributed by increased efflux pump and mutation phenotypes through QS-dependent gene expression (González & Keshavan, 2006).

The gram positive bacteria (MSSA, MRSE) were the most susceptible organisms to the plant extract compared to the gram negative bacteria (*P. aeruginosa* and *S. dysentery*). This susceptibility difference could be due to differences in cell wall structures of the test bacteria in which the gram negative bacteria have a thick lipopolyssacharide layer hindering penetration of the extract into the bacterial cell (Singh & Kumar, 2013).

5.1.2 Diethyl ether Extract

The antibacterial activity of diethyl ether extract against the test bacteria increased slightly when the extract was doubled. The greatest antibacterial activity was observed against *P. mirabilis* at 100mg/mL. The extract showed no antibacterial activity against gram negative bacteria of *S. typhimurium* and *E. coli* which could be attributed by their thick lipopolyssacharide layer hindering penetration of the extract into the bacterial cell (Singh & Kumar, 2013). However, among the gram-positive bacteria only MRSA showed complete resistance due to the presence of MecA gene which probably contributed to the efflux pumping of the extract.

5.1.3 General discussions about the ZOIs

The findings the study showed that plant crude extracts possessed varying antibacterial potencies with greater antibacterial activity against gram positive bacteria being registered by the ethanolic extract whereas the gram-negative bacteria were more susceptible to diethyl ether crude extract. This finding was consistent with the findings reported by Timothy *et al.* (2018). The higher ethanolic extract activity against the gram-positive bacteria could be attributed to the amphiphilic nature and relatively high polarity index of ethanol thereby many phytoconstituents dissolve in it at a greater degree.

However, the diethyl ether solvent only took up the non-polar phytochemicals and less extracted the polar phytochemicals such as: saponins, anthraquinolones and alkaloids which are known to contribute to antibacterial activity of crude plant extracts, and thus the lower antibacterial activity reported (Ho *et al.*, 2016).

The study also showed that Gentamicin (positive control) exhibited a significantly higher antibacterial activity against the selected bacteria compared to the crude ethanolic extract and 10% DMSO (negative control) which showed no activity. This was also in agreement with the studies done by Timothy *et al.* (2018).

5.2 Discussion of the minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration was the lowest concentration of the plant extract inhibited the visible growth of test bacteria after overnight incubation (Andrews, 2001). The lowest MIC value (3.125 mg/mL) was exhibited by ethanolic extract against both MRSE and MSSA whereas the least recorded MIC for diethyl ether extract was 6.25 mg/mL against MSSA. This could be explained by the presence of more polar phytochemicals of; alkaloids, tannins, saponins, anthraquinones and flavonoids in ethanolic extract with antibacterial activity as compared to the diethyl ether extract thus exhibiting a higher MIC. The study also showed that both extracts exhibited a lower MIC values against the gram positive bacteria as compared to the gram negative bacteria. This was attributed by the large impermeable cell wall possessed by the gram negative bacteria which excluded the extract from the bacterial cells.

5.3 Discussion of the minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration was the lowest concentration of antimicrobials that prevented the growth of test bacteria after subculture on the antibiotic free media. (Andrews, 2001). The ethanolic extract had a lower MBC value compared to the diethyl ether extract against MRSE and MSSA respectively. This could have been due to the presence of more phytochemicals in the ethanolic extract compared to the diethyl ether extract. *S. typhimurium* and *Pseudomonas aeruginosa* required a higher MBC concentration above 100 mg/mL which was consistent with resistance patterns reported by Moradali *et al.* (2017) and Potron *et al.* (2015) of these gram negative bacteria.

5.4 Conclusions and Recommendations

5.4.1 Conclusions

The *Maesa lanceolata* Forssk extract was found to have a composition of secondary phytochemicals that include tannins, alkaloids, flavonoids, saponins, terpenoids, and anthraquinones.

The crude plant extracts of *Maesa lanceolata* Forssk were found to have antibacterial properties against some Gram positive and Gram-negative gastroenteritis causing bacteria but it did not show any antibacterial activity against MRSA, *S. typhimurium* and *E. coli.*

The ethanolic extract was more effective against gram positives with low MIC and MBC of 3.125 mg/mL against MSSA and MRSE as compared to the diethyl ether crude extract.

5.4.2 Recommendations

The results exhibited by the *Maesa lanceolata* Forssk extract against the test bacteria could be of great significance in the development of an alternative remedy in the treatment of gastroenteritis caused by antibiotic resistant pathogens

There is need for further studies to isolate and characterize phytochemical constituents of *Maesa lanceolata* Forssk bark extract and perform their antibacterial assays.

There is need to perform in vivo studies to ascertain its toxicity effects *Maesa lanceolata* Forssk bark extracts and required therapeutic doses in humans

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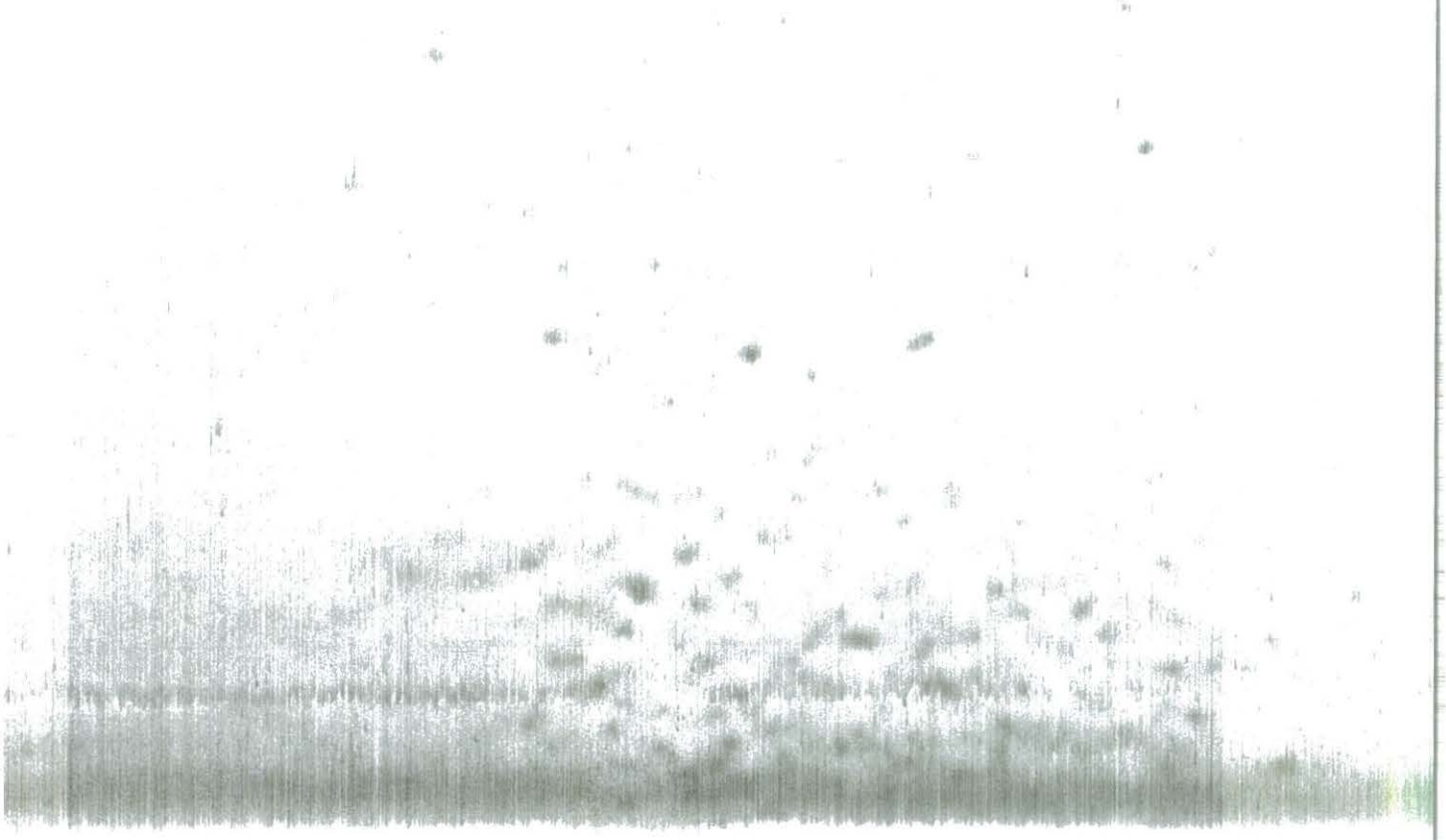
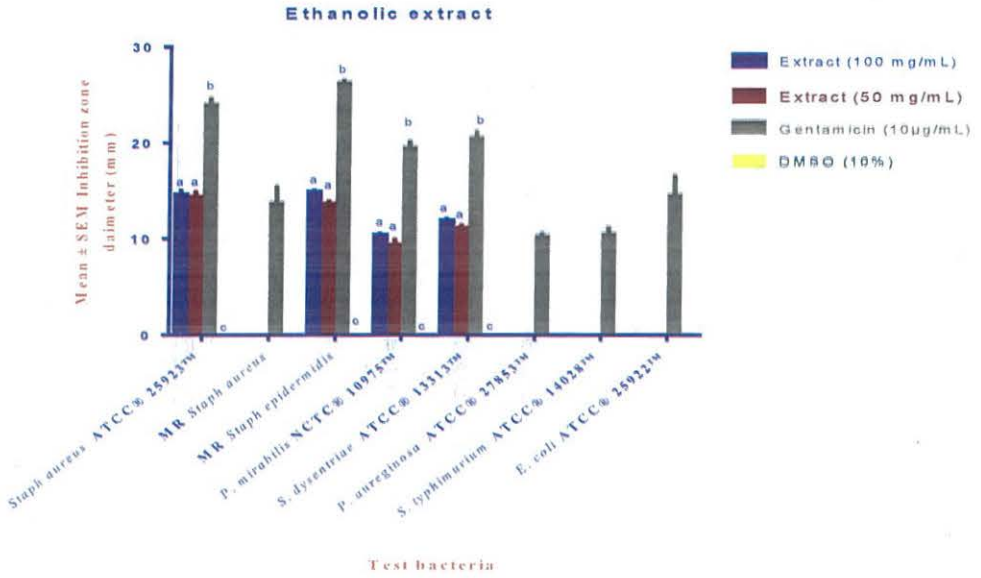


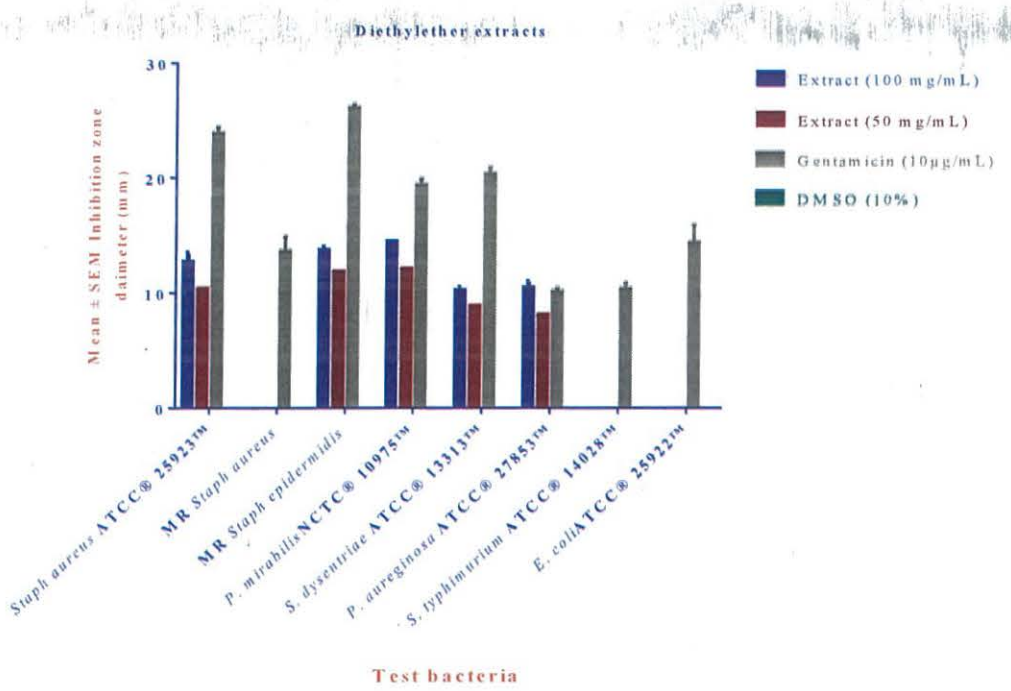
APPENDICES

Appendix I:

G.1A graph showing the antimicrobial activity of the ethanolic plant extract.



G.2 A graph showing the antimicrobial activity of the diethyl ether plant extract.



Appendix II: Statistical Data analysis

1. TABLES SHOWING ZOIS FOLLOWING ANTIMICROBIAL ACTIVITIES OF THE PLANT EXTRACTS AND CONTROLS AGAINST THE SELECTED BACTERIA.

Table 6: Table showing the P- values and the Confidence intervals of the plant extracts against the susceptible gastroenteritis bacteria

Tukey's multiple comparisons test	Mean Diff	95% CI of diff.	Significant ?	Adjusted P Value
ETHANOLIC EXTRACT				
***Staph aureus* ATCC® 25923™**				
Extract (50 mg/mL) vs. DMSO (10%)	14.50	12.85 to 16.15	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-9.500	-11.15 to -7.846	Yes	< 0.0001
MR *Staph epidermidis*				
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-12.50	-14.15 to -10.85	Yes	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	13.75	12.10 to 15.40	Yes	< 0.0001
***P. mirabilis* NCTC® 10975™**				
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-10.00	-11.65 to -8.346	Yes	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	9.500	7.846 to 11.15	Yes	< 0.0001
***S. dysentriae* ATCC® 13313™**				
Extract (50 mg/mL) vs. DMSO (10%)	11.25	9.596 to 12.90	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-9.250	-10.90 to -7.596	Yes	< 0.0001

DIETHYL ETHER EXTRACT				
***Staph aureus* ATCC® 25923™**				
Extract (50 mg/mL) vs. DMSO (10%)	10.50	8.895 to 12.11	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-13.50	-15.11 to -11.89	Yes	< 0.0001
MR *Staph epidermidis*				
Extract (50 mg/mL) vs. DMSO (10%)	12.00	10.39 to 13.61	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-14.25	-15.86 to -12.64	Yes	< 0.0001
P. mirabilis NCTC® 10975™				
Extract (50 mg/mL) vs. DMSO (10%)	12.25	10.64 to 13.86	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-7.250	-8.855 to -5.645	Yes	< 0.0001
S. dysentriae ATCC® 13313™				
Extract (50 mg/mL) vs. DMSO (10%)	9.000	7.395 to 10.61	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-11.50	-13.11 to -9.895	Yes	< 0.0001
P. aureginosa ATCC® 27853™				
Extract (50 mg/mL) vs. DMSO (10%)	8.250	6.645 to 9.855	Yes	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-2.000	-3.605 to -0.3949	Yes	<0.0108

Table 7: Table showing the P- values and the Confidence intervals of the analyzed dose effect of the plant extracts against the susceptible gastroenteritis bacteria

Turkey's multiple comparisons test	Mean Diff	95% CI of diff.	Significant?	Adjusted P Value
ETHANOLIC EXTRACT				
Staph aureus ATCC® 25923™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.654 to 1.654	No	> 0.9999
MR Staph epidermidis				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	1.000	-0.6537 to 2.654	No	0.3617
P. mirabilis NCTC® 10975™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.7500	-0.9037 to 2.404	No	0.6016
S. dysentriae ATCC® 13313™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.5000	-1.154 to 2.154	No	0.8378
DIETHYL ETHER EXTRACT				
Staph aureus ATCC® 25923™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	2.250	0.6449 to 3.855	Yes	0.0038
MR Staph epidermidis				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	1.750	0.1449 to 3.355	Yes	0.0290

P. mirabilis NCTC® 10975™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	2.250	0.6449 to 3.855	Yes	0.0038
S. dysentriae ATCC® 13313™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	1.250	-0.3551 to 2.855	No	0.1668
P. aureginosa ATCC® 27853™				
Extract (100 mg/mL) vs. Extract (50 mg/mL)	2.250	0.6449 to 3.855	Yes	0.0038

2. TABLES SHOWING COMPARISONS OF ZONES OF INHIBITION (ZOIS) BETWEEN THE BACTERIA AND COMPARISONS AT VARIOUS CONCENTRATIONS FOR EACH SOLVENT EXTRACT.

a) Ethanolic extracts

Table 8: Table showing the p- values and the confidence intervals of the comparison between bacteria that cause gastroenteritis

Tukey's multiple comparisons test	Mean	Diff 95% CI of diff	Significan
Extract (100 mg/mL)			
Staph aureus ATCC® 25923™ vs. MR Staph aureus	14.50	12.52 to 16.48	Yes
Staph aureus ATCC® 25923™ vs. MR Staph epidermidis	-0.2500	-2.234 to 1.734	No
Staph aureus ATCC® 25923™ vs. P. mirabilis NCTC® 10975™	4.250	2.266 to 6.234	Yes
Staph aureus ATCC® 25923™ vs. S. dysentriae ATCC® 13313™	2.750	0.7663 to 4.734	Yes
Staph aureus ATCC® 25923™ vs. P. aureginosa ATCC® 27853™	14.50	12.52 to 16.48	Yes
Staph aureus ATCC® 25923™ vs. S. typhimurium ATCC® 14028™	14.50	12.52 to 16.48	Yes
Staph aureus ATCC® 25923™ vs. E. coli ATCC® 25922™	14.50	12.52 to 16.48	Yes
MR Staph aureus vs. MR Staph epidermidis	-14.75	-16.73 to -12.77	Yes
MR Staph aureus vs. P. mirabilis NCTC® 10975™	-10.25	-12.23 to -8.266	Yes
MR Staph aureus vs. S. dysentriae ATCC® 13313™	-11.75	-13.73 to -9.766	Yes
MR Staph aureus vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. P. mirabilis NCTC® 10975™	4.500	2.516 to 6.484	Yes
MR Staph epidermidis vs. S. dysentriae ATCC® 13313™	3.000	1.016 to 4.984	Yes
MR Staph epidermidis vs. P. aureginosa ATCC® 27853™	14.75	12.77 to 16.73	Yes
MR Staph epidermidis vs. S. typhimurium ATCC® 14028™	14.75	12.77 to 16.73	Yes
MR Staph epidermidis vs. E. coli ATCC® 25922™	14.75	12.77 to 16.73	Yes
P. mirabilis NCTC® 10975™ vs. S. dysentriae ATCC® 13313™	-1.500	-3.484 to 0.4837	No

P. mirabilis NCTC® 10975™ vs. P. aureginosa ATCC® 27853™	10.25	8.266 to 12.23	Yes
P. mirabilis NCTC® 10975™ vs. S. typhimurium ATCC® 14028™	10.25	8.266 to 12.23	Yes
P. mirabilis NCTC® 10975™ vs. E. coli ATCC® 25922™	10.25	8.266 to 12.23	Yes
S. dysentriae ATCC® 13313™ vs. P. aureginosa ATCC® 27853™	11.75	9.766 to 13.73	Yes
S. dysentriae ATCC® 13313™ vs. S. typhimurium ATCC® 14028™	11.75	9.766 to 13.73	Yes
S. dysentriae ATCC® 13313™ vs. E. coli ATCC® 25922™	11.75	9.766 to 13.73	Yes
P. aureginosa ATCC® 27853™ vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
P. aureginosa ATCC® 27853™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
S. typhimurium ATCC® 14028™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
Extract (50 mg/mL)			
Staph aureus ATCC® 25923™ vs. MR Staph aureus	14.50	12.52 to 16.48	Yes
Staph aureus ATCC® 25923™ vs. MR Staph epidermidis	0.7500	-1.234 to 2.734	No
Staph aureus ATCC® 25923™ vs. P. mirabilis NCTC® 10975™	5.000	3.016 to 6.984	Yes
Staph aureus ATCC® 25923™ vs. S. dysentriae ATCC® 13313™	3.250	1.266 to 5.234	Yes
Staph aureus ATCC® 25923™ vs. P. aureginosa ATCC® 27853™	14.50	12.52 to 16.48	Yes
Staph aureus ATCC® 25923™ vs. S. typhimurium ATCC® 14028™	14.50	12.52 to 16.48	Yes
Staph aureus ATCC® 25923™ vs. E. coli ATCC® 25922™	14.50	12.52 to 16.48	Yes
MR Staph aureus vs. MR Staph epidermidis	-13.75	-15.73 to -11.77	Yes
MR Staph aureus vs. P. mirabilis NCTC® 10975™	-9.500	-11.48 to -7.516	Yes
MR Staph aureus vs. S. dysentriae ATCC® 13313™	-11.25	-13.23 to -9.266	Yes
MR Staph aureus vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No

MR Staph aureus vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. P. mirabilis NCTC® 10975™	4.250	2.266 to 6.234	Yes
MR Staph epidermidis vs. S. dysentriae ATCC® 13313™	2.500	0.5163 to 4.484	Yes
MR Staph epidermidis vs. P. aureginosa ATCC® 27853™	13.75	11.77 to 15.73	Yes
MR Staph epidermidis vs. S. typhimurium ATCC® 14028™	13.75	11.77 to 15.73	Yes
MR Staph epidermidis vs. E. coli ATCC® 25922™	13.75	11.77 to 15.73	Yes
P. mirabilis NCTC® 10975™ vs. S. dysentriae ATCC® 13313™	-1.750	-3.734 to 0.2337	No
P. mirabilis NCTC® 10975™ vs. P. aureginosa ATCC® 27853™	9.500	7.516 to 11.48	Yes
P. mirabilis NCTC® 10975™ vs. S. typhimurium ATCC® 14028™	9.500	7.516 to 11.48	Yes
P. mirabilis NCTC® 10975™ vs. E. coli ATCC® 25922™	9.500	7.516 to 11.48	Yes
S. dysentriae ATCC® 13313™ vs. P. aureginosa ATCC® 27853™	11.25	9.266 to 13.23	Yes
S. dysentriae ATCC® 13313™ vs. S. typhimurium ATCC® 14028™	11.25	9.266 to 13.23	Yes
S. dysentriae ATCC® 13313™ vs. E. coli ATCC® 25922™	11.25	9.266 to 13.23	Yes
P. aureginosa ATCC® 27853™ vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
P. aureginosa ATCC® 27853™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
S. typhimurium ATCC® 14028™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
Gentamicin (10µg/mL)			
Staph aureus ATCC® 25923™ vs. MR Staph aureus	10.25	8.266 to 12.23	Yes
Staph aureus ATCC® 25923™ vs. MR Staph epidermidis	-2.250	-4.234 to -0.2663	Yes
Staph aureus ATCC® 25923™ vs. P. mirabilis NCTC® 10975™	4.500	2.516 to 6.484	Yes
Staph aureus ATCC® 25923™ vs. S. dysentriae ATCC® 13313™	3.500	1.516 to 5.484	Yes
Staph aureus ATCC® 25923™ vs. P. aureginosa ATCC® 27853™	13.75	11.77 to 15.73	Yes

Staph aureus ATCC® 25923™ vs. S. typhimurium ATCC® 14028™	13.50	11.52 to 15.48	Yes
Staph aureus ATCC® 25923™ vs. E. coli ATCC® 25922™	9.500	7.516 to 11.48	Yes
MR Staph aureus vs. MR Staph epidermidis	-12.50	-14.48 to -10.52	Yes
MR Staph aureus vs. P. mirabilis NCTC® 10975™	-5.750	-7.734 to -3.766	Yes
MR Staph aureus vs. S. dysentriae ATCC® 13313™	-6.750	-8.734 to -4.766	Yes
MR Staph aureus vs. P. aureginosa ATCC® 27853™	3.500	1.516 to 5.484	Yes
MR Staph aureus vs. S. typhimurium ATCC® 14028™	3.250	1.266 to 5.234	Yes
MR Staph aureus vs. E. coli ATCC® 25922™	-0.7500	-2.734 to 1.234	No
MR Staph epidermidis vs. P. mirabilis NCTC® 10975™	6.750	4.766 to 8.734	Yes
MR Staph epidermidis vs. S. dysentriae ATCC® 13313™	5.750	3.766 to 7.734	Yes
MR Staph epidermidis vs. P. aureginosa ATCC® 27853™	16.00	14.02 to 17.98	Yes
MR Staph epidermidis vs. S. typhimurium ATCC® 14028™	15.75	13.77 to 17.73	Yes
MR Staph epidermidis vs. E. coli ATCC® 25922™	11.75	9.766 to 13.73	Yes
P. mirabilis NCTC® 10975™ vs. S. dysentriae ATCC® 13313™	-1.000	-2.984 to 0.9837	No
P. mirabilis NCTC® 10975™ vs. P. aureginosa ATCC® 27853™	9.250	7.266 to 11.23	Yes
P. mirabilis NCTC® 10975™ vs. S. typhimurium ATCC® 14028™	9.000	7.016 to 10.98	Yes
P. mirabilis NCTC® 10975™ vs. E. coli ATCC® 25922™	5.000	3.016 to 6.984	Yes
S. dysentriae ATCC® 13313™ vs. P. aureginosa ATCC® 27853™	10.25	8.266 to 12.23	Yes
S. dysentriae ATCC® 13313™ vs. S. typhimurium ATCC® 14028™	10.00	8.016 to 11.98	Yes
S. dysentriae ATCC® 13313™ vs. E. coli ATCC® 25922™	6.000	4.016 to 7.984	Yes
P. aureginosa ATCC® 27853™ vs. S. typhimurium ATCC® 14028™	-0.2500	-2.234 to 1.734	No
P. aureginosa ATCC® 27853™ vs. E. coli ATCC® 25922™	-4.250	-6.234 to -2.266	Yes
S. typhimurium ATCC® 14028™ vs. E. coli ATCC® 25922™	-4.000	-5.984 to -2.016	Yes

DMSO (10%)			
Staph aureus ATCC® 25923™ vs. MR Staph aureus	0.0	-1.984 to 1.984	No
Staph aureus ATCC® 25923™ vs. MR Staph epidermidis	0.0	-1.984 to 1.984	No
Staph aureus ATCC® 25923™ vs. P. mirabilis NCTC® 10975™	0.0	-1.984 to 1.984	No
Staph aureus ATCC® 25923™ vs. S. dysentriae ATCC® 13313™	0.0	-1.984 to 1.984	No
Staph aureus ATCC® 25923™ vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
Staph aureus ATCC® 25923™ vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
Staph aureus ATCC® 25923™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. MR Staph epidermidis	0.0	-1.984 to 1.984	No
MR Staph aureus vs. P. mirabilis NCTC® 10975™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. S. dysentriae ATCC® 13313™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
MR Staph aureus vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. P. mirabilis NCTC® 10975™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. S. dysentriae ATCC® 13313™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
MR Staph epidermidis vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
P. mirabilis NCTC® 10975™ vs. S. dysentriae ATCC® 13313™	0.0	-1.984 to 1.984	No
P. mirabilis NCTC® 10975™ vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
P. mirabilis NCTC® 10975™ vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
P. mirabilis NCTC® 10975™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No

S. dysentriae ATCC® 13313™ vs. P. aureginosa ATCC® 27853™	0.0	-1.984 to 1.984	No
S. dysentriae ATCC® 13313™ vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
S. dysentriae ATCC® 13313™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
P. aureginosa ATCC® 27853™ vs. S. typhimurium ATCC® 14028™	0.0	-1.984 to 1.984	No
P. aureginosa ATCC® 27853™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No
S. typhimurium ATCC® 14028™ vs. E. coli ATCC® 25922™	0.0	-1.984 to 1.984	No

Table 9 Table showing the p- values and the confidence intervals of the comparison for each gastroenteritis bacteria at different concentrations

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Staph aureus ATCC® 25923™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-9.500	-11.15 to -7.846	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	14.50	12.85 to 16.15	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-9.500	-11.15 to -7.846	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	14.50	12.85 to 16.15	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	24.00	22.35 to 25.65	Yes	****	< 0.0001

MR Staph aureus					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-13.75	-15.40 to -12.10	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-13.75	-15.40 to -12.10	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	13.75	12.10 to 15.40	Yes	****	< 0.0001
MR Staph epidermidis					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	1.000	-0.6537 to 2.654	No	ns	0.3617
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-11.50	-13.15 to -9.846	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	14.75	13.10 to 16.40	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-12.50	-14.15 to -10.85	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	13.75	12.10 to 15.40	Yes	****	< 0.0001

Gentamicin (10µg/mL) vs. DMSO (10%)	26.25	24.60 to 27.90	Yes	****	< 0.0001
P. mirabilis NCTC® 10975™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.7500	-0.9037 to 2.404	No	ns	0.6016
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-9.250	-10.90 to -7.596	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	10.25	8.596 to 11.90	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-10.00	-11.65 to -8.346	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	9.500	7.846 to 11.15	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	19.50	17.85 to 21.15	Yes	****	< 0.0001
S. dysentriae ATCC® 13313™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.5000	-1.154 to 2.154	No	ns	0.8378
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-8.750	-10.40 to -7.096	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	11.75	10.10 to 13.40	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-9.250	-10.90 to -7.596	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	11.25	9.596 to 12.90	Yes	****	< 0.0001

Gentamicin (10µg/mL) vs. DMSO (10%)	20.50	18.85 to 22.15	Yes	****	< 0.0001
P. aureginosa ATCC® 27853™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-10.25	-11.90 to -8.596	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-10.25	-11.90 to -8.596	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	10.25	8.596 to 11.90	Yes	****	< 0.0001
S. typhimurium ATCC® 14028™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-10.50	-12.15 to -8.846	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-10.50	-12.15 to -8.846	Yes	****	< 0.0001

Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	10.50	8.846 to 12.15	Yes	****	< 0.0001
E. coli ATCC® 25922™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-14.50	-16.15 to -12.85	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-14.50	-16.15 to -12.85	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.654 to 1.654	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	14.50	12.85 to 16.15	Yes	****	< 0.0001

b) Diethyl ether Extract

Table 10 Table showing the p- values and the confidence intervals of the comparison between bacteria that cause gastroenteritis

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significan
Extract (100 mg/mL)			
Staph aureusATCC® 25923™ vs. MR Staph aureus	12.75	10.69 to 14.81	Yes
Staph aureusATCC® 25923™ vs. MR Staph epidermidis	-1.000	-3.065 to 1.065	No
Staph aureusATCC® 25923™ vs. P. mirabilisNCTC® 10975™	-1.750	-3.815 to 0.3147	No
Staph aureusATCC® 25923™ vs. S. dysentriaeATCC® 13313™	2.500	0.4353 to 4.565	Yes
Staph aureusATCC® 25923™ vs. P. aureginosaATCC® 27853™	2.250	0.1853 to 4.315	Yes
Staph aureusATCC® 25923™ vs. S. typhimuriumATCC® 14028™	12.75	10.69 to 14.81	Yes
Staph aureusATCC® 25923™ vs. E. coliATCC® 25922™	12.75	10.69 to 14.81	Yes
MR Staph aureus vs. MR Staph epidermidis	-13.75	-15.81 to -11.69	Yes
MR Staph aureus vs. P. mirabilisNCTC® 10975™	-14.50	-16.56 to -12.44	Yes
MR Staph aureus vs. S. dysentriaeATCC® 13313™	-10.25	-12.31 to -8.185	Yes
MR Staph aureus vs. P. aureginosaATCC® 27853™	-10.50	-12.56 to -8.435	Yes
MR Staph aureus vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs. P. mirabilisNCTC® 10975™	-0.7500	-2.815 to 1.315	No
MR Staph epidermidis vs. S. dysentriaeATCC® 13313™	3.500	1.435 to 5.565	Yes
MR Staph epidermidis vs. P. aureginosaATCC® 27853™	3.250	1.185 to 5.315	Yes

MR Staph epidermidis vs. S. typhimuriumATCC® 14028™	13.75	11.69 to 15.81	Yes
MR Staph epidermidis vs. E. coliATCC® 25922™	13.75	11.69 to 15.81	Yes
P. mirabilisNCTC® 10975™ vs. S. dysentriaeATCC® 13313™	4.250	2.185 to 6.315	Yes
P. mirabilisNCTC® 10975™ vs. P. aureginosaATCC® 27853™	4.000	1.935 to 6.065	Yes
P. mirabilisNCTC® 10975™ vs. S. typhimuriumATCC® 14028™	14.50	12.44 to 16.56	Yes
P. mirabilisNCTC® 10975™ vs. E. coliATCC® 25922™	14.50	12.44 to 16.56	Yes
S. dysentriaeATCC® 13313™ vs. P. aureginosaATCC® 27853™	-0.2500	-2.315 to 1.815	No
S. dysentriaeATCC® 13313™ vs. S. typhimuriumATCC® 14028™	10.25	8.185 to 12.31	Yes
S. dysentriaeATCC® 13313™ vs. E. coliATCC® 25922™	10.25	8.185 to 12.31	Yes
P. aureginosaATCC® 27853™ vs. S. typhimuriumATCC® 14028™	10.50	8.435 to 12.56	Yes
P. aureginosaATCC® 27853™ vs. E. coliATCC® 25922™	10.50	8.435 to 12.56	Yes
S. typhimuriumATCC® 14028™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
Extract (50 mg/mL)			
Staph aureusATCC® 25923™ vs. MR Staph aureus	10.50	8.435 to 12.56	Yes
Staph aureusATCC® 25923™ vs. MR Staph epidermidis	-1.500	-3.565 to 0.5647	No
Staph aureusATCC® 25923™ vs. P. mirabilisNCTC® 10975™	-1.750	-3.815 to 0.3147	No
Staph aureusATCC® 25923™ vs. S. dysentriaeATCC® 13313™	1.500	-0.5647 to 3.565	No
Staph aureusATCC® 25923™ vs. P. aureginosaATCC® 27853™	2.250	0.1853 to 4.315	Yes
Staph aureusATCC® 25923™ vs. S. typhimuriumATCC® 14028™	10.50	8.435 to 12.56	Yes
Staph aureusATCC® 25923™ vs. E. coliATCC® 25922™	10.50	8.435 to 12.56	Yes
MR Staph aureus vs. MR Staph epidermidis	-12.00	-14.06 to -9.935	Yes
MR Staph aureus vs. P. mirabilisNCTC® 10975™	-12.25	-14.31 to -10.19	Yes

MR Staph aureus vs. S. dysentriaeATCC® 13313™	-9.000	-11.06 to -6.935	Yes
MR Staph aureus vs. P. aureginosaATCC® 27853™	-8.250	-10.31 to -6.185	Yes
MR Staph aureus vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs..P. mirabilisNCTC® 10975™	-0.2500	-2.315 to 1.815	No
MR Staph epidermidis vs. S. dysentriaeATCC® 13313™	3.000	0.9353 to 5.065	Yes
MR Staph epidermidis vs. P. aureginosaATCC® 27853™	3.750	1.685 to 5.815	Yes
MR Staph epidermidis vs. S. typhimuriumATCC® 14028™	12.00	9.935 to 14.06	Yes
MR Staph epidermidis vs. E. coliATCC® 25922™	12.00	9.935 to 14.06	Yes
P. mirabilisNCTC® 10975™ vs. S. dysentriaeATCC® 13313™	3.250	1.185 to 5.315	Yes
P. mirabilisNCTC® 10975™ vs. P. aureginosaATCC® 27853™	4.000	1.935 to 6.065	Yes
P. mirabilisNCTC® 10975™ vs. S. typhimuriumATCC® 14028™	12.25	10.19 to 14.31	Yes
P. mirabilisNCTC® 10975™ vs. E. coliATCC® 25922™	12.25	10.19 to 14.31	Yes
S. dysentriaeATCC® 13313™ vs. P. aureginosaATCC® 27853™	0.7500	-1.315 to 2.815	No
S. dysentriaeATCC® 13313™ vs. S. typhimuriumATCC® 14028™	9.000	6.935 to 11.06	Yes
S. dysentriaeATCC® 13313™ vs. E. coliATCC® 25922™	9.000	6.935 to 11.06	Yes
P. aureginosaATCC® 27853™ vs. S. typhimuriumATCC® 14028™	8.250	6.185 to 10.31	Yes
P. aureginosaATCC® 27853™ vs. E. coliATCC® 25922™	8.250	6.185 to 10.31	Yes
S. typhimuriumATCC® 14028™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
Gentamicin (10µg/mL)			
Staph aureusATCC® 25923™ vs. MR Staph aureus	10.25	8.185 to 12.31	Yes
Staph aureusATCC® 25923™ vs. MR Staph epidermidis	-2.250	-4.315 to -0.1853	Yes

Staph aureusATCC® 25923™ vs. P. mirabilisNCTC® 10975™	4.500	2.435 to 6.565	Yes
Staph aureusATCC® 25923™ vs. S. dysentriaeATCC® 13313™	3.500	1.435 to 5.565	Yes
Staph aureusATCC® 25923™ vs. P. aureginosaATCC® 27853™	13.75	11.69 to 15.81	Yes
Staph aureusATCC® 25923™ vs. S. typhimuriumATCC® 14028™	13.50	11.44 to 15.56	Yes
Staph aureusATCC® 25923™ vs. E. coliATCC® 25922™	9.500	7.435 to 11.56	Yes
MR Staph aureus vs. MR Staph epidermidis	-12.50	-14.56 to -10.44	Yes
MR Staph aureus vs. P. mirabilisNCTC® 10975™	-5.750	-7.815 to -3.685	Yes
MR Staph aureus vs. S. dysentriaeATCC® 13313™	-6.750	-8.815 to -4.685	Yes
MR Staph aureus vs. P. aureginosaATCC® 27853™	3.500	1.435 to 5.565	Yes
MR Staph aureus vs. S. typhimuriumATCC® 14028™	3.250	1.185 to 5.315	Yes
MR Staph aureus vs. E. coliATCC® 25922™	-0.7500	-2.815 to 1.315	No
MR Staph epidermidis vs. P. mirabilisNCTC® 10975™	6.750	4.685 to 8.815	Yes
MR Staph epidermidis vs. S. dysentriaeATCC® 13313™	5.750	3.685 to 7.815	Yes
MR Staph epidermidis vs. P. aureginosaATCC® 27853™	16.00	13.94 to 18.06	Yes
MR Staph epidermidis vs. S. typhimuriumATCC® 14028™	15.75	13.69 to 17.81	Yes
MR Staph epidermidis vs. E. coliATCC® 25922™	11.75	9.685 to 13.81	Yes
P. mirabilisNCTC® 10975™ vs. S. dysentriaeATCC® 13313™	-1.000	-3.065 to 1.065	No
P. mirabilisNCTC® 10975™ vs. P. aureginosaATCC® 27853™	9.250	7.185 to 11.31	Yes
P. mirabilisNCTC® 10975™ vs. S. typhimuriumATCC® 14028™	9.000	6.935 to 11.06	Yes
P. mirabilisNCTC® 10975™ vs. E. coliATCC® 25922™	5.000	2.935 to 7.065	Yes
S. dysentriaeATCC® 13313™ vs. P. aureginosaATCC® 27853™	10.25	8.185 to 12.31	Yes
S. dysentriaeATCC® 13313™ vs. S. typhimuriumATCC® 14028™	10.00	7.935 to 12.06	Yes
S. dysentriaeATCC® 13313™ vs. E. coliATCC® 25922™	6.000	3.935 to 8.065	Yes

P. aureginosaATCC® 27853™ vs. S. typhimuriumATCC® 14028™	-0.2500	-2.315 to 1.815	No
P. aureginosaATCC® 27853™ vs. E. coliATCC® 25922™	-4.250	-6.315 to -2.185	Yes
S. typhimuriumATCC® 14028™ vs. E. coliATCC® 25922™	-4.000	-6.065 to -1.935	Yes
DMSO (10%)			
Staph aureusATCC® 25923™ vs. MR Staph aureus	0.0	-2.065 to 2.065	No
Staph aureusATCC® 25923™ vs. MR Staph epidermidis	0.0	-2.065 to 2.065	No
Staph aureusATCC® 25923™ vs. P. mirabilisNCTC® 10975™	0.0	-2.065 to 2.065	No
Staph aureusATCC® 25923™ vs. S. dysentriaeATCC® 13313™	0.0	-2.065 to 2.065	No
Staph aureusATCC® 25923™ vs. P. aureginosaATCC® 27853™	0.0	-2.065 to 2.065	No
Staph aureusATCC® 25923™ vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
Staph aureusATCC® 25923™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. MR Staph epidermidis	0.0	-2.065 to 2.065	No
MR Staph aureus vs. P. mirabilisNCTC® 10975™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. S. dysentriaeATCC® 13313™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. P. aureginosaATCC® 27853™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
MR Staph aureus vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs. P. mirabilisNCTC® 10975™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs. S. dysentriaeATCC® 13313™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs. P. aureginosaATCC® 27853™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
MR Staph epidermidis vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No

P. mirabilisNCTC® 10975™ vs. S. dysentriaeATCC® 13313™	0.0	-2.065 to 2.065	No
P. mirabilisNCTC® 10975™ vs. P. aureginosaATCC® 27853™	0.0	-2.065 to 2.065	No
P. mirabilisNCTC® 10975™ vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
P. mirabilisNCTC® 10975™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
S. dysentriaeATCC® 13313™ vs. P. aureginosaATCC® 27853™	0.0	-2.065 to 2.065	No
S. dysentriaeATCC® 13313™ vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
S. dysentriaeATCC® 13313™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
P. aureginosaATCC® 27853™ vs. S. typhimuriumATCC® 14028™	0.0	-2.065 to 2.065	No
P. aureginosaATCC® 27853™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No
S. typhimuriumATCC® 14028™ vs. E. coliATCC® 25922™	0.0	-2.065 to 2.065	No

Table 11 Table showing the p- values and the confidence intervals of the comparison foreach gastroenteritis bacteria at different concentrations

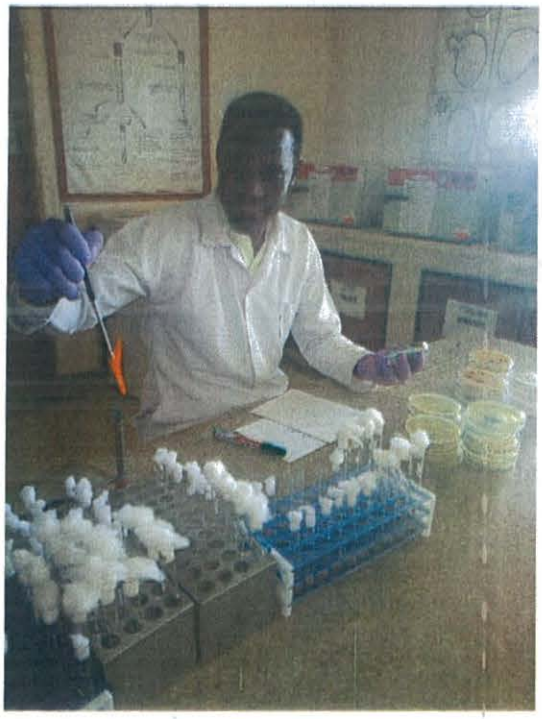
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Staph aureusATCC® 25923™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	2.250	0.6449 to 3.855	Yes	**	0.0038
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-11.25	-12.86 to -9.645	Yes	****	< 0.0001

Extract (100 mg/mL) vs. DMSO (10%)	12.75	11.14 to 14.36	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-13.50	-15.11 to -11.89	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	10.50	8.895 to 12.11	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	24.00	22.39 to 25.61	Yes	****	< 0.0001
MR Staph aureus					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-13.75	-15.36 to -12.14	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-13.75	-15.36 to -12.14	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	13.75	12.14 to 15.36	Yes	****	< 0.0001
MR Staph epidermidis					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	1.750	0.1449 to 3.355	Yes	*	0.0290
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-12.50	-14.11 to -10.89	Yes	****	< 0.0001

Extract (100 mg/mL) vs. DMSO (10%)	13.75	12.14 to 15.36	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-14.25	-15.86 to -12.64	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	12.00	10.39 to 13.61	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	26.25	24.64 to 27.86	Yes	****	< 0.0001
P. mirabilisNCTC® 10975™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	2.250	0.6449 to 3.855	Yes	**	0.0038
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-5.000	-6.605 to -3.395	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	14.50	12.89 to 16.11	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-7.250	-8.855 to -5.645	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	12.25	10.64 to 13.86	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	19.50	17.89 to 21.11	Yes	****	< 0.0001
S. dysentriaeATCC® 13313™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	1.250	-0.3551 to 2.855	No	ns	0.1668
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-10.25	-11.86 to -8.645	Yes	****	< 0.0001

Extract (100 mg/mL) vs. DMSO (10%)	10.25	8.645 to 11.86	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-11.50	-13.11 to -9.895	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	9.000	7.395 to 10.61	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	20.50	18.89 to 22.11	Yes	****	< 0.0001
P. aureginosaATCC® 27853™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	2.250	0.6449 to 3.855	Yes	**	0.0038
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	0.2500	-1.355 to 1.855	No	ns	0.9728
Extract (100 mg/mL) vs. DMSO (10%)	10.50	8.895 to 12.11	Yes	****	< 0.0001
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-2.000	-3.605 to -0.3949	Yes	*	0.0108
Extract (50 mg/mL) vs. DMSO (10%)	8.250	6.645 to 9.855	Yes	****	< 0.0001
Gentamicin (10µg/mL) vs. DMSO (10%)	10.25	8.645 to 11.86	Yes	****	< 0.0001
S. typhimuriumATCC® 14028™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-10.50	-12.11 to -8.895	Yes	****	< 0.0001

Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-10.50	-12.11 to -8.895	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	10.50	8.895 to 12.11	Yes	****	< 0.0001
E. coliATCC® 25922™					
Extract (100 mg/mL) vs. Extract (50 mg/mL)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Extract (100 mg/mL) vs. Gentamicin (10µg/mL)	-14.50	-16.11 to -12.89	Yes	****	< 0.0001
Extract (100 mg/mL) vs. DMSO (10%)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Extract (50 mg/mL) vs. Gentamicin (10µg/mL)	-14.50	-16.11 to -12.89	Yes	****	< 0.0001
Extract (50 mg/mL) vs. DMSO (10%)	0.0	-1.605 to 1.605	No	ns	> 0.9999
Gentamicin (10µg/mL) vs. DMSO (10%)	14.50	12.89 to 16.11	Yes	****	< 0.0001

Appendix III: Other experimental photo

Broth dilution method for determining MIC and MBC