ANTIMICROBIAL, CYTOTOXICITY, ACUTE ORAL TOXICITY EFFECTS AND PHYTOCHEMICAL COMPOSITION OF AQUEOUS AND METHANOLIC EXTRACTS OF *PHYSALIS PERUVIANA, BRIDELLIA MICRANTHA,* AND *CROTON MEGALOCARPUS*

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DECLARATION

I declare that this thesis is my original work and has not been presented in any other university for the award of any degree.

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DEDICATION

I dedicate this work to the Lord Jesus Christ and my dear family for granting me sufficient grace to complete it. Glory be to His holy name. Amen.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATC	Acute Toxic Classic method
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
NACOSTI	National Commission for Science, Technology, and Innovation
NCAPD	National Coordinating Agency for Population and Development
NO	Nitric oxide (Nitrogen oxide, Nitrogen monoxide)
OECD	Organization for Economic Co-operation and Development
PRA	Participatory rapid appraisal
RNA	Ribonucleic acid
ROS	Reactive oxygen species
UV	Ultraviolet light
WHO	World health organization

ABSTRACT

Microbial infections are the major causes of high morbidity and mortality in vulnerable persons globally. The emergence of antibiotic-resistant microbes has further complicated the management of pathogenic infections. The most considerable burden of microbial infections lies in the low- and middle-income countries, especially those in the African continent. The current antimicrobial drugs are inaccessible, unaffordable and cause undesirable effects. This has prompted the search for alternative and complementary therapies to cub microbial infections. Medicinal plants present a promising alternative source of potent antimicrobials due to their wide range of phytocompounds they contain. Medicinal plants have been used for a long time in traditional medicine to manage various diseases especially those of bacterial and fungal origin; however, their pharmacologic efficacy, toxicity, and safety profiles have not been fully validated scientifically. The World Health Organization (WHO) has recognized that over 80 % of the world population, especially in low-income nations, depend on traditional medicines for primary healthcare. As a result, the WHO has recommended the evaluation of herbal remedies as potential sources of pharmacologically active molecules for drug development. Considering the inefficiencies of synthetic antibiotics and the burden of microbial infections, this study was designed to investigate the antimicrobial, cytotoxicity, acute oral toxicity effects and phytochemical composition of the aqueous and methanolic extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus*, which are utilized in the Kenyan traditional medicine to treat microbial infections, on select microbial strains. The stem barks of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* were collected based on their traditional usage from Murang'a County, in Kenya. They were primarily identified by their local names and then authenticated by a taxonomist at the East Africa Herbaria at the National Museums of Kenya. Voucher specimens were prepared, and duplicates were deposited at the Department of Biological Sciences, Chiromo campus for future reference. The collected stem barks were naturally dried in the laboratory for two weeks, ground into powder form before extraction. The respective powders were extracted using water and methanol following standard methods.The antimicrobial activity of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* extracts on *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Candida albicans* were evaluated using the disk diffusion and Broth microdilution techniques described by the Clinical and Laboratory Standards Institute (CLSI). The bacterial and fungal strains were selected based on their availability and clinical significance. The brine shrimp lethality test was used to evaluate the cytotoxicity effects while the acute oral toxicity effects were evaluated according to the Organization for Economic Development and Cooperation (OECD; Document 425). Qualitative phytochemical screening was done following the standard phytochemical screening procedures. The aqueous extract of *P. peruviana* exhibited slight activity at concentrations of 50 µg/ml and 100 µg/ml on *S. typhimurium;* slight to moderate activity on *E. coli* and *S. aureus*; and moderate to high activity on *C. albicans,* demonstrating its antibacterial and antifungal effects. The methanolic bark extract of *P. peruviana* demonstrated slight to moderate antimicrobial effects against *E. coli*, *S. aureus* and *C. albicans;* however, a slight antimicrobial effect by this extract was observed on the *S. typhimurium*. The aqueous extract of *B. micrantha* produced slight activity on *E. coli* strain; slight to moderate activities on *S. typhimurium* strain, and moderate to high activities *S. aureus* bacterial strain and *C. albicans* fungal strain. The methanolic extract of *B. micrantha* showed moderate to high activities on *E. coli*, slight to moderate activities on *S. typhimurium*, slight to high activities on *S. aureus*, and slight to very high activities on *C. albicans* strains. The aqueous extract of *C. megalocarpus* demonstrated slight to moderate activities on *E. coli* and *S. aureus*, and moderate activities on *S. typhimurium* and *C. albicans* strains. Only the

aqueous *C. megalocarpus* was able to inhibit the growth of *S. typhimurium* at all the studied concentrations. The methanolic extract of *C. megalocarpus* exhibited slight to moderate activities on *S. typhimurium* and *C. albicans* strains; slight to high activities on *E. coli* strain, and slight to very high activities on *S. aureus* strain. Furthermore, the studied plant extracts exhibited low MIC values on selected microbes implying strong antibiotic activities. Both the aqueous and methanolic extracts of *B. micrantha* were toxic to the brine shrimp nauplii (LC_{50} 30µg/ml-100 µg/ml). All the other extracts were non-toxic to brine shrimp nauplii (LC_{50} >100 µg/ml). Notably, the aqueous extract of *P. peruviana* had an LC_{50} value of >1000 µg/ml denoting its remarkable safety. Acute oral toxicity studies showed that all the studied plant extracts were non-toxic at oral doses and therefore safe. The antimicrobial effects of the aqueous and methanolic extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* were attributed to the presence of phenols, flavonoids, among other antimicrobial-associated phytochemicals detected in this study. Furthermore, the cytotoxic effects of the aqueous and methanolic extracts of *B. micrantha* could be due to the presence of anthraquinones. The safety of the studied plant extracts was attributed to low concentrations or absence of toxicity associated phytochemical compounds. Therefore, it was concluded that the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* have antimicrobial effects on *E. coli, S. typhimurium, S. aureus* and *C. albicans* microbial strains. Also, aqueous and methanolic extracts of the studied plants do not cause acute oral toxicity effects in rat models and possess antimicrobial and safety associated phytochemicals. Additionally, extracts of *B. micrantha* are toxic to brine shrimp nauplii. Further studies aimed at isolating and characterizing antimicrobial molecules from the studied plant extracts are recommended. The specific modes of action and drug interactions of these extracts should be elucidated.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Microbial infections are among the major causes of morbidity and mortality in humans globally, especially in vulnerable groups (Elston *et al.*, 2015; World Health Organization, 2012, 2016). Furthermore, the increased rate of antimicrobial resistance to the available medications has caused major healthcare challenges (Frieri *et al.*, 2017; Morens *et al.*, 2004). The persistence of microbial infections during and after the treatment cycles has precipitated an overuse of antibiotics leading to other unprecedented outcomes (Cohen *et al.*, 2013).

Various antibiotic agents currently prescribed for various microbial infections have been associated with detrimental side effects which hinder their effective utilization (Caldwell and Cluff, 1974; Hao *et al.*, 2014; Lasemi *et al.*, 2016). Additionally, the presumably potent antimicrobial agents are arguably expensive and inaccessible to many (Dadgostar, 2019). Therefore, due to the inexorable challenges posed by microbial infections and the failures of conventional antimicrobial therapies, the need for alternative and complementary stratagems are warranted.

Medicinal plants have across ages formed a critical component of healthcare especially to persons of low income (Gathirwa *et al.*, 2011; James *et al.*, 2018; Kaminidevi *et al.*, 2015; Kareru *et al.*, 2007; WHO, 2005). The World Health Organization declared that over 80 % of the world population, especially in Asia and Sub-Saharan African states depend on herbals for health solutions (Qi, 2015; WHO, 2013).The enormous utilization and high confidence in traditional medicines are attributable to their alleged high efficacies, safety, acceptability, extensive usage, easy availability and accessibility (Anyanwu and Okoye, 2017; Farnsworth *et al.*, 1985; Rates, 2001). It is evidence that a battery of current potent medicines used in the

western medicine are derived from plants making herbals a potential source of safe drugs against maladies (Abreu *et al.*, 2012).

Despite the rich history of usage and claimed potency of medical plants, only a handful have been scientifically investigated for their bioactivities (Farnsworth *et al.*, 1985; Jouda, 2013; Kuglerova *et al.*, 2011). Moreover, concerning regarding the safety and toxicity of medicinal plants have been raised (George, 2011), thus hindering their integration into universal healthcare systems (Aydin *et al.*, 2016). These assertions have been fostered by the lack of traditional medicine practice policies and scanty research data supporting their healing claims as well as their assumed low toxicities. Consequently, it is important to empirically study and document the safety, toxicity and bioactivity of medicinal plants as they are viable sources of potent medicines.

In this study, the antimicrobial, cytotoxicity and acute oral toxicity of the aqueous and methanolic stem bark extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* were evaluated. In traditional medicine practice among the Agikuyu community of Murang'a County, these plants are used to manage typhoid among other disease associated with microbial infections (Maina, 2018). It is based on this ethnomedical background that these plants were selected for this study.

1.2 Statement of the problem

Microbial infections are among the leading causes of high morbidities and mortalities recorded in healthcare facilities globally (Keeling and Rohani, 2011; Selgelid, 2012). Their management has been faced by a myriad of bottlenecks due to resistance and tolerance by previously susceptible strains.The chemotherapeutic management of infectious diseases for prolonged period without eradicating the pathogen have contributed to the emergence of resistant microbes.

Furthermore, antibiotic therapy is associated with undesirable effects some of which are life-threatening (Lasemi *et al.,* 2016; Caldwell and Cluff, 1974). For instance, penicillins cause allergic reactions, dermatologic disorders, gastrointestinal complications, haemolytic anemia, bone marrow suppression, seizures among other adverse events (Lasemi*et al.,* 2016). Cephalosporins are associated with electrolyte imbalances, diarrhea, heamatologic abnormalities, vaginitis among others (Lasemi *et al.,* 2016).

Tetracyclines impair renal function, cause exfoliative dermatitis, pericarditis among other complications. On the other hand, macrolides are associated with anorexia, acute cholestatic hepatitis, ventricular arrhythmias and exacerbation of other pathologies including *torsade de pointes*. Other antibiotics like gentamycin are associated with nephrotoxicity, ototoxicity among a wide range of complications (Lasemi *et al.,* 2016). Additionally, accessibility to conventional antibiotics is challenging due to regulatory restrictions and high costs. This is a major challenge especially in the rural areas and in regions of low income.

Owing to the drawbacks and inefficiencies of conventional antimicrobial chemotherapy, alternative stratagems are required to curb infectious diseases at relatively cheaper costs than those involved in orthodox medicine, and with fewer or no toxic effects (Hao *et al.*, 2014). Medicinal plants on the other hand are a valuable source of antimicrobials owing to their long-term applications. Further, these plants are considered to be easily accessible, affordable and with fewer side effects compared to western medicine (WHO, 2005, 2008).

1.3 Justification of the study

Inspite of the extensive usage of medicinal plants to curb microbial infections, there are few focused empirical studies formulated to validate the claimed potencies (Ekor, 2014). Moreover, due to the lack of clear dosage regimens, formulation guidelines, marketing and

practice regulations in traditional medicine practice, toxicity and safety profiles of most medicinal plants have remained unknown (George, 2011).

Some of the plants used against microbial and associated diseases in traditional medicine are *P. peruviana, C. megalocarpus* and *B. micrantha.* Even though there is a rich history of ethnomedical applications of these plants in managing microbial infections, they have not been scientifically investigated and appraised for this bioactivity (Maina, 2018). Moreover, there is scanty research data on their cytotoxicity and safety despite their continued utilization.

Therefore, this study was designed to investigate the antimicrobial, cytotoxicity and acute oral toxicity effects of the aqueous and methanolic stem bark extracts to lay a framework towards their validation and as potential sources of safe and potent antimicrobials.

1.4 Study objectives

1.4.1 General objective

The main objective of this study was to investigate the antimicrobial, cytotoxicity and acute oral toxicity effects and qualitative phytochemical composition of the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus.*

1.4.2 Specific objectives

The following were the specific objectives of this study.

i. To evaluate the antimicrobial activity of the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* on selected microbial strains.
ii. To determine cytotoxic effects of the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* on brine shrimp nauplii.

iii. To investigate the acute oral toxicity effects of the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* in experimental rat models.

iv. To evaluate qualitative phytochemical composition of the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus*.

1.5 Research questions

This study was guided by the following research questions.

i. Do the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* have antimicrobial activity on selected strains?

ii. Do the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus*cause cytotoxic effects on brine shrimp nauplii?

iii. Do the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus*cause acute oral toxicity effects in experimental rats?

iv. Do the aqueous and methanolic barks extracts of *P. peruviana, B. micrantha* and *C. megalocarpus*possess antimicrobial associated phytochemicals?

1.6 Null hypotheses

This study adopted the null hypotheses as follows:

i. The aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* do not have antimicrobial activity on selected microbes.

ii. The aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* do not cause cytotoxicity in brine shrimp nauplii.

iii. The aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* do not cause acute oral toxicity in experimental rats.

iv. The aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* do not posses antimicrobial associated phytochemicals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbial infections

Among the most challenging ailments that are currently burdening healthcare systems are microbial infections (World Health Organization, 2012). Not only do pathogenic microbes cause diseases but also contribute to opportunist conditions which exacerbate health deterioration and complicate chemotherapeutic management (Selgelid, 2012). In the developing world, poor living conditions and over population coupled with poor hygiene have led to an increase in bacterial infections (Kuglerova *et al.*, 2011; Morens *et al.*, 2004). Indeed, research has indicated that bacterial strains that cause pneumonia, typhoid, tuberculosis, meningitis, urinary tract infections, diarrhea and cholera result in high morbidity and high mortality (Dadgostar, 2019; Jones *et al.*, 2008).

The utilization of antibiotic agents to manage microbial infections has been practiced for ages (Leekha *et al.*, 2011). Despite the marked success of antimicrobial chemotherapy, their wanton use in western medicine and for prophylaxis has led to a resurgence of resistant and tolerant strains (Frieri *et al.*, 2017; Yılmaz and Özcengiz, 2017). Furthermore, non-compliance to dosage regimens and improper storage of antimicrobial agents have contributed to the emergence of resistant microbial strains some of which were previously susceptible (Frieri *et al.*, 2017).

The development of bacterial biofilms has been recognized as a major trigger of resistance to antibiotics to over 1000 times (Douglas and Gitonga, 2016; Frieri *et al.*, 2017; Mwitari *et al.*, 2013). Additionally, the overuse of antibiotics in agriculture has greatly contributed to the current rise in antimicrobial resistant strains further negatively impacting health (Kaminidevi *et al.*, 2015).

Antimicrobial resistance poses a public health threat causing uncertainties in the future of healthcare (Dadgostar, 2019). This is witnessed in the prolonged hospital stays, increased management costs, unaffordable high-end antibiotics and therapeutic failures which culminate in unquantifiable strain. Additionally, antimicrobial resistance has been shown to impair the patient's immune system, which incapacitates the body's ability to counter pathogens. As a result, vulnerable subjects especially those under chemotherapy, surgery, dialysis and organ replacements suffer from various complications (Lasemi *et al.*, 2016).

Patients with underlying chronic conditions like diabetes mellitus, rheumatoid arthritis, cancer among others are seriously affected by antibiotic resistance (Morens *et al.*, 2004). Consequently, healthcare providers handling these classes of patients often resort to using last line of antibiotic drugs as a result of failures or ineffectiveness of lower antibiotics due to microbial persistence (Cohen *et al.*, 2013). These last line of antibiotics including carbapenems and polymyxins are expensive, not easily accessible and are associated with adverse side effects (Caldwell and Cluff, 1974).

A classic example of antimicrobial resistance involves methicillin resistant *Staphylococus aureus*, which is responsible for high annual mortality rates worldwide (Turner *et al.*, 2019). The prevalence of multi-drug resistant gram-negative bacterial strains have complicated the management of urinary tract infections and pneumonia (Frieri *et al.*, 2017; Li *et al.*, 2016). Furthermore, there has been an upsurge of antibiotic resistance to gonorrhea, tuberculosis and typhoid fever causing bacterial strains in the developing world thereby increasing healthcare costs adding to the economic strife in an already resource limited setup (Dadgostar, 2019; Frieri *et al.*, 2017).

2.2 Overview of the selected microbes used in this study

2.2.1 *Staphylococcus aureus*

This bacterial strain belongs to a group of facultative gram-positive bacterial strains of staphylococcaceae family and is found on the skin, mucous membranes and in the respiratory tract (Ondusko and Nolt, 2018). Morphologically, *S. aureus* occurs in grape-like clusters compromising of large, yellow colonies on agar media culture. This bacterial strain is non-motile, non-sporulating and reproduce asexually via binary fission (*Staphylococcus Aureus - StatPearls - NCBI Bookshelf*, n.d.).

In the clinical setup, *S. aureus* is responsible for various disease conditions including pneumonia, bacteremia, rheumatoid fever, meningitis, among others (Gnanamani *et al.*, 2017). In affected patients, especially those with major burns, septic wounds have been acknowledged as a chief cause of death. Moreover, *S. aureus* colonization on wounds may evoke systemic complications which add up to the detriment of the patient's health. *Staphylococcus aureus* is the leading cause of hospital-acquired illnesses which are transmitted via contaminated surfaces and aerosols (Gnanamani *et al.*, 2017; Tong *et al.*, 2015).

The emergence of methicillin resistant *S. aureus* strains, whose resistance to a wide range of antibiotics including β-lactams, quinolones and aminoglycosides among others, have been demonstrated, is a major heath concern (Al-Ayed *et al.*, 2016). The production of penicillin-binding protein 2a which is encoded by the *mec*A gene, over-synthesis of penicillinase and alteration of normal penicillin-binding protein domains, have been postulated as major mechanisms of antibacterial resistance in this strain (Turner *et al.*, 2019).

2.2.2 *Escherichia coli*

This is a rod-shaped anaerobic, facultative gram-negative bacterium which is non-sporulating (Lim *et al.*, 2010). *E. coli* is found in the lower intestinal tract of warm-blooded organisms where it either lives commensally or causes serious pathogenic effects. Clinically, it is known

to cause wound infections, watery diarrhea, sepsis, and food poisoning in infants and children with symptoms akin those of cholera (Erb *et al.*, 2007; Liu, 2019).

Escherichia coli infections are responsible for the high morbidity and mortality in children and the elderly especially in economically burdened setups which are often characterized by poor sanitation and hygiene (Allocati *et al.*, 2013; Kaper *et al.*, 2004; Poolman, 2016; Van Elsas *et al.*, 2011). Some clinical isolates of *E.coli* have demonstrated resistance to sulphonamides, chloramphenicol and the widely prescribed trimethoprim-sulphomethoxazole combined therapies (Erb *et al.*, 2007).

2.2.3 *Salmonella typhi*

Salmonella typhi is facultative gram-negative anaerobic bacterium of Enterobacteriaceae family (Jaroni, 2014; Saporito *et al.*, 2016) It is a flagellated, strain which reside in human intestines where it pathologically causes gastroenteritis (Dougan and Baker, 2014; Saporito *et al.*, 2016). It is transmitted through the consumption of water and food which are contaminated with faecal matter of infected patients (de Jong *et al.*, 2012; Trawinski *et al.*, 2020). Antimicrobial resistant *S. typhi* has emerged raising serious health concerns (Monack *et al.*, 2004; Parkhill *et al.*, 2001; Sanderson *et al.*, 2014).

2.2.4 *Candida albicans*

This is amorphologically dimorphic, diploid unicellular fungal strain widely known to cause oral thrush and genital infections (candidiasis) in humans (Wilson, 2019). Hyperglycemic state in patients suffering from diabetes mellitus has been shown to aggravate oral thrush (Silva *et al.*, 2012; Yang, 2003). Also, *C. albicans* is believed to be a key player among the opportunistic sexually transmitted infections in immunosuppressed and HIV/AIDS positive subjects (Barousse *et al.*, 2004; Donders *et al.*, 2018; McSorley, 2013; Wira *et al.*, 2011). *C. albicans* has demonstrated resistance against major antifungal drugs like flucytosine,

itraconazole and fluconazole making its management in affected patients a difficult endeavor (Sanguinetti *et al.*, 2015; Whaley *et al.*, 2017).

2.3 Herbal management of microbial infections

Throughout human history, medicinal plants have been a critical component of health (Farnsworth *et al.*, 1985). Most of the world population (over 80%) especially in low-income nations rely on herbal medicine for provision of medicines (WHO, 2005, 2013). In Kenya, herbal medicine utilization for healthcare needs has been explored and reported in various ethnomedical surveys (Amuka *et al.*, 2014; Kareru *et al.*, 2007; Maina, 2018; Onyancha *et al.*, 2019; Wambugu *et al.*, 2011). Among the uses of herbal medicines involved the fight against infectious diseases especially those caused by bacteria and fungi (Cheruiyot *et al.*, 2009; Chinsembu, 2016; Kareru *et al.*, 2007; Korir *et al.*, 2012; Maobe *et al.*, 2013; Mariita *et al.*, 2011; Mutembei *et al.*, 2018; Njoroge and Bussmann, 2006; Omwenga *et al.*, 2009).

Inaccessibility and high costs of conventional medicine have made many people especially in rural and remote areas to rely on herbal medicine as it is considered cheap and easily accessible (Kiringe, 2006; Leonti and Verpoorte, 2017; Pouliot, 2011). Furthermore, the perceived safety, efficacy and wide acceptability have also contributed to the reliance of traditional medicine in the management of infectious diseases (WHO, 2013).

In Kenya, various medicinal plants have been used for management of infectious diseases. For instance, the roots and leaf decoctions of *Osyris abyssinica* are used to treat dysentery and typhoid respectively (Kareru *et al.*, 2007). The bark decoction of *Cassine aethiopica* is used among the Mbeere people of Kenya as an antiseptic. On the other hand, *Rhus natalensis* roots are consumed to manage diarrhaea and influenza (Kareru *et al.*, 2007).

The root and bark decoctions of *Vitex strickeri*, *Comiphora Africana, Clerodendrum myricoides* are used to cure pneumonia (Korir *et al.*, 2012; Mutembei *et al.*, 2018). Additionally, various other medicinal plants including *Physalis peruviana, Croton*

*megalocarpus*and *Bridellia micrantha*have been extensively used to manage typhoid and pneumonia among other microbial-associated infections in traditional medicine(Kareru *et al.*, 2007; Maina, 2018).

2.4 Study plants

In this study, *Physalis peruviana*, *Bridellia micrantha* and *Croton megalocarpus* were selected based on their ethnomedical usage as antimicrobial drugs (Kareru *et al.*, 2007; Maina, 2018).

2.4.1 *Physalis peruviana*

Physalis peruviana is a small herb belonging to Solanaceae family which is widely distributed temperate and tropical regions (Lim and Lim, 2013). In French, it is called *amour en cage* ("love in a cage"). It is locally known as '*nathi*' by the Kikuyu of Murang'a County, Kenya (Maina, 2018). It has a round and smooth berry (fruit) which resemble a miniature yellow tomato measuring 1.25–2 cm in diameter (Puente *et al.*, 2011). A photograph of aerial parts of *P. peruviana* is shown in figure 2.1.

Traditionally, various parts are used against malaria, pneumonia and typhoid and fruits are taken as food (Kareru *et al.*, 2007; Maina, 2018; Mutembei *et al.*, 2018).

Previous studies have demonstrated anti-inflammatory, anti-hepatoma, antimicrobial activities in fruit and leaf extracts of *P. peruviana* (El-Kenawy *et al.*, 2015; Hassanien, 2011; Martínez *et al.*, 2010; Wu *et al.*, 2005).

Phytochemical analysis has revealed presence of ergosterol, campesterol, stigmasterol, lanosterol, total sterols, withanolides, carotenoids among other pharmacologically important phytochemicals (Ahmad *et al.*, 1999; El-Beltagi *et al.*, 2019; Fang *et al.*, 2012; Oliveira *et al.*, 2016).

Figure 2.1: A photograph showing aerial parts of *P. Peruviana* (*Photograph taken by Joseph Kathare)*

2.4.2 *Bridellia micrantha*

Bridellia micrantha is a medium to large deciduous tree which grows up to 20 m above the ground with spreading crown (Adeyemi *et al.*, 2008). It belongs to Euphorbiaceae family of herbs and trees which are characterized by succulent leafless branches; milky or watery latex; glands at the leaf base; and 3-lobed fruits. It is locally known as '*mukuigo*' by the Kikuyu of Murang'a County, Kenya (Kareru *et al.*, 2007; Maina, 2018). Figure 2.2 shows a photograph of *B. micrantha* plant.

The stem bark tinctures and decoctions of *Bridellia micrantha*are used to cure burns, soft tissue injuries, sexually transmitted infections, protozoa infections, gastrointestinal conditions, typhoid, pneumonia and dental diseases (Maina, 2018; Maroyi, 2017b). Leaf preparation is used to manage eye problems (Maroyi, 2017b).

Previous studies have indicated anti-ulcer activity against *H. pylori*-induced ulcers and antimicrobial activities against *S. typhi, S. enteritidis, S. flexneri, E. coli* and *M. tuberculosis* bacterial strains (Maroyi, 2017b). Furthermore, antidiabetic, hypolipidemic and antioxidant effects of extracts derived from *B. micrantha* have been reported (Adeyemi *et al.*, 2008; Anyanwu and Okoye, 2017).

Phytochemical investigations have revealed presence oftaraxerone, Friedelin, Taraxerol, Epifriedelinol, gallic acid, ellagic acid, anthocyanidin, delphiniridin and Benzene 1,3-bis(3-phenoxyphenoxy),2-pinen-4-one (Maroyi, 2017b).

Figure 2.2:Photograph of *B. micrantha* plant captured *in situ (Photo taken by Joseph M. Kathare)*

2.4.3 *Croton megalocarpus*

Croton megalocarpus is a deciduous tree belonging to Euphorbiaceae family which grow up to 35 m above the ground (Maroyi, 2017a). Its cylindrical bole measures up to 120cm wide and is unbranched up to 20 m high. This plant offers timber, firewood and medicine to local communities, which has led to its cultivation.It is locally known as ‘mukinduri’ by the Kikuyu of Murang’a County, Kenya (Maina, 2018).

Various parts of *C. megalocarpus* are ethnomedically used in the management of various ailments ranging from coughs, typhoid, pneumonia, wounds, joint pains, helminthic infections among other maladies (Kareru *et al.*, 2008; Maina, 2018; Maroyi, 2017a).

Previous studies have revealed presence of alkaloids, flavonoids, flavones, saponins, glycosides, terpenoids, sterols and tannins(Maroyi, 2017a). Moreover, clerodane diterpenoids including epoxy-chiromodine and chiromodine have been isolated from the stem bark of *C. megalocarpus*. Furthermore, lupeol, β-sitisterol, 3-β-O-acetoacetyllupeol, E-feruric, botulin among other compounds have been isolated (Maroyi, 2017a).

Various bioactivities of *C. megalocarpus* including, antifungal, antinociceptive, molluscicidal, anti-inflammatory, antioxidant, antibacterial among others have been (Gichui, 2016; Maroyi, 2017a; Ndunda, 2014).

Figure 2.3: Photograph showing aerial parts of *C. megalocarpus* plant*(Photo taken by Joseph Kathare)*

2.5 Medicinal plant as sources of potent antimicrobial agents

Medicinal plants have a great potential as sources of efficacious antimicrobials that can either inhibit or kill pathogens via multitarget approaches (Shakya, 2016). This can be attributable to the presence of various phytochemical amalgams which have been shown to confer wide spectrums of bioactivity (Abreu *et al.*, 2012; Phaniendra *et al.*, 2015). These phytocompounds are synthesized by plants to thwart pest and disease infestation, to promote growth and health, and to counter environmental stress (Harborne, 1998; Phillipson, 2001). It is therefore anticipated that consumption of plant-derived materials confers these benefits.

One of the plant phytochemicals comprise of saponins, which are polar compounds which are known to protect plants against pathogenic and herbivore attack (Sparg *et al.*, 2004; Velíšek,

2018). They are categorized as triterpenoids and steroidal glycosides depending on structural attachment of sugar moiety (Augustin *et al.*, 2011). They are prominently found in plants that are prone to insect and bacterial insults. Studies have demonstrated that saponins have antimicrobial, antimolluscicidal, antiparasitic, anti-inflammatory and antimutagenic properties (Arabski *et al.*, 2012; Kurmukov, 2013a).

Tannins and terpenoids are prominently present in barks, roots, fruits and leaves of medicinal plants, where they protect plants against microbial attack (Ashok and Upadhyaya, 2012; Chung *et al.*, 1998; Ky *et al.*, 2015). Research has revealed these phytochemicals possess antiseptic, antidiarrheal, diuretic, antimicrobial and antihemorrhoidal activities (Ky *et al.*, 2015; Scalbert, 1991; Serrano *et al.*, 2009). Furthermore, their antimicrobial activities have been shown to be effected through the disruption of microbial cell membranes and through the inhibition of critical enzymes involved in membrane integrity (Buzzini *et al.*, 2008; Kubmarawa *et al.*, 2007; Lipińska *et al.*, 2014; Ukoha *et al.*, 2011).

Alkaloids largely comprise of plant toxins and psychedelics indicated for various ailments including cancer and Alzheimer's disease (Aniszewski, 2015; Coqueiro and Verpoorte, 2019; Lu *et al.*, 2012). They have been shown to inhibit microbial growth by deterring prostaglandin and autocoid synthesis and release or their incorporation into microbial cell walls (Cushnie *et al.*, 2014; Debnath *et al.*, 2018; Wink, 2015).

Phenolics form the most versatile group of phytochemical metabolites with the broadest spectra of bioactivities (Naczk and Shahidi, 2006; Pereira *et al.*, 2009). Among phenolics, flavonoids, and phenols are major antioxidants which quench free radicals to maintain redox homeostasis (Kähkönen *et al.*, 2001; Mitchel Otieno *et al.*, 2016; Moriasi *et al.*, 2020a; Ozcan *et al.*, 2014). They also play important roles as signal transduction molecules and effectors which modulate cell growth and differentiation in living systems (Lee and Lee, 2010; Ozcan *et al.*, 2014). Based on their levels of oxidation, they can be divided into flavones, flavonols

and flavanones. Research has shown that they possess anti-inflammatory, hepatoprotective, antidiabetic and antibacterial activities (Das *et al.*, 2018; Antwi-Baffour, 2014; Tanase *et al.*, 2019). Furthermore, their cardioprotective, anticancer, neuroprotective and antiaging effects have been implicated (Aliyazcoglu *et al.*, 2013; Liaudanskas *et al.*, 2017; Martillanes *et al.*, 2017; Olas *et al.*, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant collection, identification, and processing

The stem barks of *Bridellia micrantha*, *Physalis peruviana*, and *Croton megalocarpus* were selected for this study based on their ethnomedical usage among the Agikuyu community of Murang'a County (Maina, 2018). They were submitted at the herbarium of National Museum of Kenya in Nairobi and botanically identified and authenticated by a taxonomist Mr. Mathias M. Mbale at National Museums of Kenya and assigned voucher specimen numbers as follows; *Bridelia micrantha* (NMK/01/2019), *Croton megalocarpus* (NMK/02/2019), *Physalis peruviana* (NMK/02/2019),where the voucher specimens were prepared and deposited. The plant materials were then collected and transported to the Department of Public Health, Pharmacology and Toxicology laboratories, at the College of Agriculture and Veterinary Sciences, Kabete Campus, University of Nairobi. The collected materials were then sorted and evenly spread to dry at room temperature (25 ^{o}C) for 2 weeks. They were then ground into a powder by an electric mill and stored in plastic containers awaiting extraction.

3.2. Extraction of the collected plant materials

The crude extracts of the studied plant materials were prepared according to the procedures described by Harborne (1998). The methanolic extracts of the studied plant materials were obtained by cold maceration method. Briefly, 250 g of respective plant powders were soaked in 1 litre of analytical-grade methanol in 2-litre conical flasks. The respective flasks containing the merc-menstruum mixtures were gently shaken and covered with aluminum foil.They were shaken once daily for two days, thereafter, the mixtures were decanted and filtered through Whatman filter papers (No.1). They were then concentrated under vacuum by

the help of a rotary evaporator. The resultant extracts were transferred into glass bottles and further dried in a hot-air oven at 35 ^{o}C for 5 days. They were weighed and their respective percentage yields determined.

For the aqueous extracts, about 50 g of respective plant powders were macerated in 500 ml of distilled water and heated for minutes at 58 ^{o}C. The mixtures were allowed to cool to room temperature and then filtered through the Whatman filter papers. The filtrates were transferred into freeze-drying flasks and fitted into a freeze-dryer, where they were lyophilized for 48 hrs. The dried extracts were weighed, and their respective percentage yields determined. All the extracts were stored in a refrigerator (4 ^{o}C) and only retrieved when required.

3.3 Study design

In this study a completely randomized study design was used from which experimental design were derived. Laboratory experimental designs were used to investigate the antimicrobial activity and toxicity effects of the aqueous and methanolic extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus.*

3.4 Investigation of the antimicrobial activities of the aqueous and methanolic bark extracts of the studied plants on selected microbes

In this study, *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (AT 10231) were obtained from the Department of Public Health, Pharmacology and Toxicology of the College of Agriculture and Veterinary Sciences, University of Nairobi, Kabete Campus. These strains were selected based on their clinical significance and availability. To investigate the effects of the studied plant extracts on the selected microbial strains, the disc diffusion and broth microdilution techniques described by the Clinical and Laboratory Standards Institute were followed (CLSI, 2014).

3.4.1 Preparation and standardization of microbial inoculum for experimentation

The fungal strain (*C. albicans*) was grown in Sabouraud dextrose agar (SDA; Oxoid) for 24 hrs according to the directions of the M100-S23 document of the CLSI (CLSI, 2014). Thereafter, sterile normal saline was used to standardize the inoculum so as to achieve a 0.5 McFarland standard at 530 nm using a Uv-V is spectrophotometer. Ranges of between 0.11 and 0.14 at OD_{530} were obtained. This was considered to be $1\text{-}5\times10^{6}$cfu/ml.

The bacterial strains (*E. coli, S. typhimurium* and *S. aureus*) were grown in Mueller-Hinton agar as per the CLSI guidelines for 24 hrs. Thereafter, the inocula were standardized to a turbidity equivalent to 0.5 McFarland scale of approximately $1\text{-}2\times10^{8}$ cfu/ml (CLSI, 2014).

3.4.2 The disc diffusion assay for antimicrobial susceptibility

In this assay, the CLSI guidelines (CLSI, 2014) were followed. Briefly, 1 g of each of the studied extracts were dissolved in 10 ml of 1 % DMSO (in sterile water) in a 15 ml centrifuge tube and thoroughly vortexed to make stock solutions of containing 100 µg/ml. The stocks were then serially diluted two-fold to give 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml and 3.125 µg/ml respectively.

Afterwards, 20 µl were aspirated and carefully impregnated on sterile Whatman discs of 6 mm diameter. The discs were gently pressed on the media containing 1 ml of the bacterial or fungal inocula to allow for proper drug-microbe contact. The assays were performed in triplicate with DMSO as negative control and streptomycin or ciprofloxacin or amphotericin B as positive controls. All the plates were incubated for 24 hrs at 37^{o}C, then diameters of zones of inhibition of microbial growth measured in millimeters.

3.4.3 The Broth microdilution technique for minimum inhibitory concentration (MIC) determination

In this determination, the modified CLSI method (CLSI, 2014), described by Golus *et al.* (2016) was adopted. Briefly, cultures were prepared and adjusted in Mueller-Hinton Broth media to 0.5 McFarland equivalent turbidity. Carefully, 10 µl of the previously prepared test extracts at a 10-fold concentration were transferred into Eppendorf tubes containing 90 µl of molten Mueller-Hinton agar in triplicate and gently vortexed. Microdilution was done in volumes of 100 µl in sterile 96-U-shaped multiwell plates in two-fold. In each of the micro-titre plates, the growth, sterility control and negative (1 % DMSO) controls were included for each of the tested microbial strains.

All the multiwell plates were allowed to settle at room temperature for the agar to solidify. Then, 2 µl of freshly prepared inoculate at concentration of 10^4cfu/spot were dispensed into the wells using a multichannel micropipette and allowed to interact at room temperature. The wells at the sides were added sterile water, and the plates were covered in zip-lock plastic bags and incubated at 35 ^oC for 18 hrs. The MIC was determined as the lowest concentration of the studied extracts which could completely inhibit microbial growth as per the CLSI recommendations (CLSI, 2014).

3.5 Evaluation of the effects of the studied plants extracts on brine shrimp nauplii

In this study, the brine shrimp lethality assay method described by Meyer *et al.* (1982) was used. Briefly, approximately 0.5 g of *Artemia salina* cysts (Sanders Great SaltLake, Brine Shrimp Company L.C., U.S.A.) were placed in an artificial sea containing 500 ml of brine water. They were incubated for two days to hatch into nauplii under continuous normal bulb illumination at 25 – 29 ^0C temperature and enough aeration.

Thereafter, ten nauplii were transferred using Pasteur pipettes into three sets of sample vials containing the either the studied plant extracts at concentrations of 0, 10, 100 and 1000µg/ml or podophyllotoxin in 5 ml brine solutionsin triplicate. The nauplii were then incubated for 24 hours and the number of survivors in each test vial were counted and documented. The percentage lethality was determined as a ratio of surviving nauplii in the test groups to those in the control (vehicle treated) group. LC_{50} values were derived from the line of best from a plot of percentage survival against concentration.

3.6 Evaluation of the Acute Oral Toxicity effects of the aqueous and methanolic bark extracts of the studied plant extracts

In this study, the guidelines posited by the Organization for Economic Co-operation and Development (OECD) in protocol document number 425 were adopted (OECD, 2008). Experimental female Winstar Rats weighing 150± 20 g were sourced from the Department of Public Health, Pharmacology and Toxicology animal breeding unit, acclimatized for 72 hrs before dosing (OECD, 2008). The studied plant extracts were reconstituted in normal saline solution to achieve the appropriate dose for administration according to the OECD guidelines (OECD, 2008).

On the experimentation day, the animals were fasted for 4 hrs and randomly assorted into groups of three rats.The experiment was initiated by administering a single dose of 175 mg/kg orally to the first group and normal saline (10 ml/Kg bw) to the control group.

Observations of wellness parameters (skin fur, eye colour, mucus membrane, salivation, lethargy, sleep, coma, convulsions, tremors and diarrhoea) were recorded at intervals of 30 min., 4 h., 24 h., 48 h., 1 week and 2 weeks for each individual rat. In the absence of observable signs of toxicity or mortality during the 14-day experimentation period, the next subsequent higher doses of 550 mg/kg and 2000 mg/kg respectively were administered into new groups of rats. All the experiments were done in triplicate (OECD, 2008). At the end of

the experiments, the experimental rats were euthanized and disposed off according to the set protocols.

3.7 Qualitative phytochemical composition of the aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus*

In this study, the standard protocols for qualitative phytochemical screening described by Harborne (1998) were followed. The phytochemicals that were evaluated include alkaloids, flavonoids, tannins, Saponins, Anthraquinonesand phenols.

3.7.1 Test for alkaloids

3.7.1.2 Dragendorff test

About 0.1 g of the aqueous and methanolic extracts of *P. peruviana, B, micrantha* and *C. megalocarpus* were extracted by boiling with 10 ml of 1% hydrochloric acid in independent test tubes. The mixtures were filtered and to about 2 ml of the filtrate, a few drops of the Dragendorff reagent were added. The formation of the red precipitates in the respective tubes indicate presence of alkaloids.

3.7.1.3 Mayer's test

To the 2ml remaining portion of the filtrate of the respective extracts in the Dragendorff test (section 3.6.1.1), a few drops of the Mayer's reagent were added along the sides of the respective tubes. The formation of the white creamy precipitates in the respective tubes indicated presence of alkaloids.

3.7.3 Test for flavonoids

To approximately 5 ml of ethanolic filtrates of the respective extracts, 2ml of 2% sodium hydroxide were added. The formation of an intense colour that decolorize on addition of a few drops of diluted hydrochloric acid indicate presence of flavonoids.

3.7.4 Test for tannins (ferric chloride test)

About 0.1 g of the aqueous and methanolic bark extracts of the studied plants were extracted by boiling with 20 ml of the distilled water. The mixtures were filtered through Whatman filter paper, and into 2 ml of the filtrate, a few drops of 5 % ferric chloride were added. The appearance of the dark green colour indicates a positive test for tannins.

3.7.5 Test for phenols

About 0.1 g each of the studied extracts were boiled with 10 ml of 70 % of ethanol for 5 minutes in water bath and then filtered while hot. The filtrates were cooled to room temperature and 2 ml of it be transferred into a clean test-tube then followed by dropwise addition of 5% ferric chloride solution. The appearance of green precipitates will indicate the presence of phenols.

3.7.6 Test for Glycosides

The aqueous and methanolic extracts (0.1 g) of the studied plants were re-extracted with 10 ml of chloroform. The mixtures were then be filtered, and the filtrates reduced by heating on a hot plate to dryness. Into the remaining filtrate after heating to dryness, 0.4 ml of the glacial acetic acid with trace amount of ferric chloride were added followed by 0.5 ml of the concentrated H_2SO_4 through the sides of respective test-tubes. The presence of blue colour in the acetic acid layer is a positive indication for cardiac glycosides' presence.

3.7.7 Test for saponins

About 0.5 g of the aqueous and methanolic extracts of the studied plants were dissolved in 5 ml of warm distilled water and vigorously shaken. The appearance of persistent frothing indicated presence of saponins.

3.7.8 Test for anthraquinones

Approximately 0.1 g of each of the studied extract were warmed 1 ml of chloroform in a water bath for 5 minutes. Afterwards, they were filtered through Whatman filter paper and allowed to cool to room temperature before adding equivalent volumes of 10 % ammonia. The mixtures were then shaken and the presence of pink coloration on the upper layer indicates presence of anthraquinones.

3.8 Data management and analysis

Quantitative data from antimicrobial and brine shrimp lethality experiments were tabulated on Excel spreadsheet (Microsoft 365) and exported to Minitab version 19.1 statistical software. Descriptive statistics were performed, and values were expressed as $\bar{x} \pm SEM$. One-Way ANOVA was used to determine differences among means followed by Tukey's *post hoc* test for pairwise comparisons and separations of means. Means that showed p values <0.05 were considered statistically significant. Acute oral toxicity results were treated according to the OECD (2008) guidelines. Qualitative data on wellness parameters in the acute oral toxicity and qualitative phytochemical screening studies were only tabulated. The obtained findings were presented in tables.

CHAPTER FOUR
RESULTS

4.1 Antimicrobial activities of the aqueous and methanolic extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* on selected microorganisms

4.1.1 Aqueous extracts

The obtained results showed that the zones of inhibition produced by the aqueous extract of *P. peruviana* on *E. coli* bacterial strain ranged from 6.50±0.50 mm at a concentration of 3.125 µg/ml to 9.00±0.58 mm at a concentration of 100 µg/ml (Table 4.1). Compared with the negative control, the zones of inhibition produced by this extract at all dose levels except at 3.125 µg/ml and 6.25 µg/ml were significantly higher ($p<0.05$; Table 4.1). However, the zone of inhibition caused by the positive control was significantly higher than the zones of inhibition recorded by all the other experimental treatments ($p<0.05$).

The *S. typhimurium* was not susceptible to the aqueous extract of *P. peruviana* at concentrations of 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml and 25 µg/ml respectively, and, the respective zones of inhibition were not significantly different from that of the negative control ($p>0.05$; Table 4.1). However, the zones of inhibition recorded by this extract at concentrations of 50 µg/ml and 100 µg/ml were significantly higher than those recorded at the other extract concentrations and the negative control. The standard antibiotic produced a significantly larger zone of inhibition than those of the rest of the treatments ($p<0.05$; Table 4.1).

No significant differences among average zones of inhibitions at concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml of *P. peruviana* were recorded against *S. aureus* bacterial strain. Similarly, the zones of inhibition recorded at extract concentrations of 3.125 µg/ml, 6.25 µg/ml and 12.5 µg/ml were not significantly different ($p>0.05$). However, the zones of inhibition recorded for the aqueous extract of *P. peruviana* at all the studied concentrations

were significantly larger than that recorded for the negative control ($p<0.05$). Overall, the reference antibiotic had the highest zone of inhibition that was significant at $\alpha_{0.05}$ (Table 4.1).

The effects of the aqueous extract of *P. peruviana* on *C. albicans* fungal strain were determined. In this study, no significant difference in zones of inhibition recorded at 3.125 µg/ml and 6.25 µg/ml were observed in *C. albicans* ($p>0.05$; Table 4.1). Likewise, the zones of inhibition recorded at extract concentrations of between 12.5 µg/ml and 50 µg/ml were not significantly different ($p>0.05$). However, at a concentration of 100 µg/ml, the obtained zone of inhibition was significantly higher than those recorded at all the other treatments ($p>0.05$). Notably, *C. albicans* was not susceptible to the reference drug and hence its zone of inhibition was the same as that of the negative control ($p>0.05$; Table 4.1).

Table 4.1: Antimicrobial effects of the aqueous bark extract of *Physalis peruviana* on selected microbial strains

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
3.125	6.50 ± 0.50^{ef}	6.00 ± 00^{d}	8.67 ± 1.33^{bc}	11.67 ± 0.88^{b}
6.25	7.50 ± 0.29^{de}	6.00 ± 00^{d}	9.00 ± 1.15^{bc}	11.67 ± 0.33^{b}
12.5	8.33 ± 0.88^{cd}	6.00 ± 00^{d}	9.00 ± 0.57^{bc}	12.67 ± 0.33^{ab}
25	8.83 ± 0.72^{bcd}	6.00 ± 00^{d}	10.17 ± 1.36^{b}	12.67 ± 0.67^{ab}
50	9.83 ± 0.17^{b}	7.33 ± 0.88^{c}	10.67 ± 1.86^{b}	12.83 ± 0.44^{ab}
100	9.00 ± 0.58^{bc}	8.67 ± 0.33^{b}	11.33 ± 0.33^{b}	14.00 ± 0.58^{a}
-ve Control	6.00 ± 0.00^{f}	6.00 ± 00^{d}	6.00 ± 00^{c}	6.00 ± 0.00^{c}
+ve Control	28.00 ± 0.00^{a}	27.00 ± 0.00^{a}	24.16 ± 0.16^{a}	6.00 ± 0.00^{c}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %).

The antimicrobial effects of the aqueous bark extracts of *Bridellia micrantha* were also investigated in this study. The results showed that at the lowest three concentrations tested, the mean zones of inhibition in *E. coli* bacterial strain were not significantly different ($p>0.05$; Table 4.2). Likewise, at the two upper concentrations (50 µg/ml and 100 µg/ml), the obtained zones of inhibition of *E. coli* were significantly similar ($p>0.05$). However, the

positive control antibiotic gave the largest zone of inhibition (26.67±0.33 mm) compared with the zones of all the other treatments in *E. coli* ($p<0.05$; Table 4.2).

The effects of the aqueous bark extract of *B. micrantha* on *S. typhimurium* were also investigated in this study, the results revealed no significant difference in zones of inhibition at extract concentrations of 6.25 µg/ml and 12.5 µg/ml and at concentrations of 50 µg/ml and 12.5 µg/ml ($p>0.05$; Table 4.2). Similarly, at a concentration of 3.125 µg/ml, the recorded zone of inhibition was not significantly from that of the negative control ($p>0.05$). However, the positive control drug showed a significantly larger zone of inhibition than the zones produced in all the other treatments ($p<0.05$; Table 4.2).

The effects of the aqueous bark extract of *B. micrantha* on *S. aureus* bacterial strain were determined. In this study, at all the extracts concentrations, the observed zones of inhibition were not significantly different ($p>0.05$; Table 4.2). However, the reference drug produced a significantly larger zone of inhibition compared with the zones produced by the studied extract at all concentrations and the negative control ($p<0.05$; Table 4.2).

The susceptibility of *C. albicans* fungal strain to the aqueous bark extract of *B. micrantha* was investigated in this study. The results showed no significant differences in zones of inhibition recorded at all the extract concentrations were observed ($p>0.05$; Table 4.2). Notably, *C. albicans* was not susceptible to the reference drug, hence, the zone of inhibition was like that of the negative control ($p>0.05$; Table 4.2).

Table 4.2: Antimicrobial effects of the aqueous bark extract of *Bridellia micrantha* on selected microbial strains

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
3.125	6.17 ± 0.17^{d}	6.67 ± 0.17^{d}	10.33 ± 1.20^{bc}	11.00 ± 0.58^{a}
6.25	6.83 ± 0.60^{d}	7.00 ± 0.00^{cd}	10.67 ± 1.45^{bc}	11.67 ± 0.33^{a}
12.5	8.50 ± 1.76^{cd}	7.00 ± 0.00^{cd}	11.33 ± 1.33^{bc}	12.17 ± 0.17^{a}
25	10.50 ± 1.04^{c}	7.83 ± 0.60^{c}	13.33 ± 1.67^{b}	12.67 ± 1.33^{a}
50	14.33 ± 0.88^{b}	10.00 ± 0.58^{b}	13.33 ± 1.67^{b}	12.67 ± 1.33^{a}
100	15.33 ± 1.67^{b}	10.00 ± 0.58^{b}	13.67 ± 1.20^{b}	13.00 ± 0.58^{a}
-ve	6.00 ± 0.00^{d}	6.00 ± 00^{d}	6.00 ± 00^{d}	6.00 ± 0.00^{b}
+ve	26.67 ± 0.33^{a}	27.00 ± 0.00^{a}	24.33 ± 0.33^{a}	6.00 ± 0.00^{b}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %).

The effects of the aqueous bark extract of *C. megalocarpus* on selected microbial strains were investigated. The results revealed no significant difference in zones of inhibition observed in *E. coli* at all the studied extract concentrations except at 3.125 µg/ml ($p>0.05$; Table 4.3). Similarly, the zone of inhibition observed at a concentration of 3.125 µg/ml of the studied extract was significantly like that of the negative control in *E. coli* ($p>0.05$). However, the positive control produced the largest zone of inhibition compared to the zones of inhibition observed at all the extract concentrations ($p<0.05$; Table 4.3).

The zones of inhibition produced by the aqueous bark extract of *C. megalocarpus* in *S. typhimurium* at concentrations of 3.125 µg/ml and 6.26 µg/ml, and, at concentrations of 25 µg/ml and 50 µg/ml were not significantly different ($p>0.05$; Table 4.3). However, the standard drug produced a significantly larger zone of inhibition in *S. typhimurium* than the zones of inhibition observed in all the extract concentrations and the negative control ($p<0.05$; Table 4.3).

Also, in this study, the antibacterial effects of the aqueous bark extract of *C. micrantha* on *S. aureus* were determined. The results indicated that at the zones of inhibition observed at

extract concentrations of 3.125 µg/ml and 6.25 µg/ml were not significantly different from that of the negative control in *S. aureus* bacteria ($p>0.05$; Table 4.3). The zones of inhibition produced by the aqueous extract of *C. micrantha* at concentrations of between 12.5 µg/ml and 100 µg/ml in *S. aureus* were not significantly different ($p>0.05$; Table 4.3). In this strain, the reference antibiotic showed a significantly larger zone of inhibition than the zones recorded in all the other setups of *S. aureus* strain ($p<0.05$; Table 4.3).

Furthermore, when the effects of the aqueous bark extract of *C. micrantha* on *C. albicans* were evaluated, the results indicated no significant differences in zones of inhibition observed at concentrations of 3.125 µg/ml and 6.35 µg/ml, 12.5 µg/ml to 50 µg/ml, and, between the control setups ($p>0.05$; table 4.3). However, *C. albicans* was more susceptible to 100 µg/ml of the aqueous bark extract of *C. micrantha* with a significantly larger zone than the zones observed in all the other setups ($p<0.05$; Table 4.3).

Table 4.3: Antimicrobial effects of the aqueous bark extract of *Croton megalocarpus* on selected microbial strains

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
3.125	6.33 ± 0.33^{c}	11.00 ± 0.00^{d}	6.17 ± 0.17^{c}	9.00 ± 0.00^{d}
6.25	7.00 ± 1.00^{bc}	11.00 ± 0.00^{d}	6.50 ± 0.29^{c}	9.67 ± 0.33^{cd}
12.5	8.67 ± 1.67^{bc}	11.33 ± 0.33^{cd}	7.16 ± 0.44^{bc}	10.33 ± 0.33^{bc}
25	8.83 ± 1.30^{bc}	11.83 ± 0.17^{c}	7.33 ± 0.33^{bc}	10.33 ± 0.60^{bc}
50	9.00 ± 1.00^{bc}	12.00 ± 0.00^{c}	8.33 ± 0.88^{bc}	10.66 ± 0.44^{b}
100	10.33 ± 2.03^{b}	13.00 ± 0.58^{b}	9.00 ± 1.00^{b}	12.17 ± 0.17^{a}
Negative	6.00 ± 0.00^{c}	6.00 ± 0.00^{e}	6.00 ± 0.00^{c}	6.00 ± 0.00^{e}
Positive	28.00 ± 1.15^{a}	27.00 ± 0.00^{a}	22.33 ± 1.76^{a}	6.00 ± 0.00^{e}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %).

4.1.2. Methanolic extracts

The results showed no significant differences in zones of inhibition of *E. coli* when the methanolic bark extract of *P. peruviana* was applied at concentrations of 50 µg/ml and 100 µg/ml ($p>0.05$; Table 4.4). Also, the zones of inhibition of *E. coli* by the methanolic bark extract of *P. peruviana* at concentrations of 6.25 µg/ml, 12.5 µg/ml and 25 mg/ ml were not significantly different ($p>0.05$; Table 4.4). Remarkably, the zone of inhibition produced by the positive control on *E. coli* was significantly larger than the zones in all the other incubations ($p<0.05$; Table 4.4).

The *S. typimurium* strain was not susceptible to the methanolic extract of *P. peruviana* up to the concentration of 50 µg/ml, as the zones of inhibition were like that of the negative control ($p>0.05$; Table 4.4). However, at 100 µg/ml of the methanolic extract of *P. peruviana*, the observed zone of inhibition was significantly larger than those observed at the rest of extract concentrations ($p<0.05$). The standard antibiotic recorded the largest zone of inhibition of *S. typhimurium* compared to all the other incubations ($p<0.05$; Table 4.4).

On *S. aureus*, the zones of inhibition produced by the methanolic extract of *P. peruviana* at concentrations of 6.25 µg/ml, 12.5 µg/ml and 25 µg/ml were not significantly different ($p>0.05$; Table 4.4). Likewise, at 50 µg/ml and 100 µg/ml extract concentrations, the zones of inhibition of *S. aureus* were not significantly different ($p>0.05$; Table 4.4). In this case, the standard antibiotic exhibited a significantly larger zone of inhibition compared with the zones in all the other extract concentrations ($p<0.05$; Table 4.4).

The effects of the methanolic bark extract of *P. peruviana* on *C. albicans* were also investigated (Table 4.4). In this study, no significant difference in zones of inhibition in *C. albicans* were observed among extract concentrations of 3.125 µg/ml, 6.25 µg/ml, and 12.5 µg/ml, and, between 25 µg/ml and 50 µg/ml($p>0.05$; Table 4.4). Notably, at the extract

concentration of 100 µg/ml, the observed zone of inhibition was significantly larger than those recorded in all the other extract concentrations and controls ($p<0.05$; Table 4.4).

Table 4.4: Antimicrobial effects of the methanolic bark extract of *Physalis peruviana* on selected microbial strains

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
3.125	6.90 ± 0.30^{cd}	6.00 ± 0.00^{c}	7.83 ± 1.17^{cd}	9.33 ± 0.33^{b}
6.25	8.33 ± 1.36^{bcd}	6.00 ± 0.00^{c}	8.67 ± 0.88^{bc}	9.66 ± 0.44^{b}
12.5	9.17 ± 0.44^{bcd}	6.00 ± 0.00^{c}	9.67 ± 0.88^{bc}	9.83 ± 0.60^{b}
25	10.17 ± 1.59^{bc}	6.00 ± 0.00^{c}	9.67 ± 0.88^{bc}	10.00 ± 0.00^{ab}
50	10.67 ± 0.333^{b}	6.00 ± 0.00^{c}	10.00 ± 0.00^{b}	10.00 ± 0.00^{ab}
100	11.83 ± 2.68^{b}	7.33 ± 0.33^{b}	10.67 ± 0.3^{b}	11.00 ± 0.58^{a}
-ve	6.00 ± 0.00^{d}	6.00 ± 0.00^{c}	6.00 ± 0.00^{d}	6.00 ± 0.00^{c}
+ve	30.00 ± 0.00^{a}	27.00 ± 0.00^{a}	23.67 ± 0.33^{a}	6.00 ± 0.00^{c}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %)

The effects of the methanolic bark extract of *B. micrantha* on selected microorganisms were also investigated in this study. The results showed that, upon application of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml of the methanolic bark extract of *B. micrantha*, the exhibited zones of inhibition were not significantly different ($p>0.05$; Table 4.5). However, at 100 µg/ml, the observed zone of inhibition was significantly larger compared to zones of inhibition in other extract concentrations and negative control ($p<0.05$; Table 4.5). In this setup, the positive control showed the highest zone of inhibition compared with all the other zones of inhibition ($p<0.05$; Table 4.5).

For *S. typhimurium*, the exhibited zones of inhibition by the methanolic extract of *B. micrantha*, ranged from 7.33±0.33 mm at 3.125 µg/ml to 11.33±0.67 mm at 100 µg/ml with significant differences ($p<0.05$; Table 4.5). No significant differences in zones of inhibition of *S. typhimurium* were observed in extract concentrations of 12.5 µg/ml, 25 µg/ml, and 50 µg/ml($p>0.05$; Table 4.5). Generally, a dose-dependent increase in zone of inhibition sizes

was observed with the increasing concentration of the extract with 100 µg/ml showing a significantly larger zone than those in lower concentrations ($p<0.05$; Table 4.5). In this setup, the positive control drug showed the largest zone of inhibition compared with all the other zones ($p<0.05$; Table 4.5).

Table 4.5: Antimicrobial effects of the methanolic bark extract of *Bridellia micrantha* on selected microbial strains

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
3.125	10.00 ± 1.53^{c}	7.33 ± 0.33^{e}	8.33 ± 0.88^{de}	6.67 ± 2.85^{d}
6.25	12.67 ± 2.85^{bc}	9.00 ± 0.58^{d}	9.00 ± 1.00^{d}	11.00 ± 0.00^{c}
12.5	12.67 ± 2.96^{bc}	9.33 ± 0.67^{cd}	10.67 ± 1.86^{cd}	12.00 ± 0.00^{b}
25	13.00 ± 2.00^{bc}	10.00 ± 0.58^{bcd}	12.67 ± 0.88^{bc}	12.33 ± 0.33^{b}
50	14.67 ± 0.33^{bc}	10.67 ± 0.88^{bc}	14.33 ± 1.20^{b}	13.17 ± 0.44^{b}
100	16.00 ± 1.53^{b}	11.33 ± 0.67^{b}	15.33 ± 0.33^{b}	19.00 ± 3.06^{a}
-ve	6.00 ± 0.00^{d}	6.00 ± 0.00^{e}	6.00 ± 0.00^{e}	6.00 ± 0.00^{d}
+ve	27.33 ± 1.45^{a}	27.00 ± 0.00^{a}	23.33 ± 0.67^{a}	6.00 ± 0.00^{d}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %)

In this study, the antimicrobial effects of the methanolic bark extract of *C. megalocarpus* were also evaluated. (Table 4.6). The results showed significant differences in zones of inhibition of *E. coli* by the methanolic bark extract of *C. megalocarpus*, and ranged from 7.00±0.58 mm at a concentration of 3.125 µg/ml to 15.00±1.00 mm at 100 µg/ml concentration ($p<0.05$; Table 4.6). It was observed that at the lowest tested concentration of this extract (3.125 µg/ml), the zone of inhibition produced was like that in the negative control ($p>0.05$). The positive control drug exhibited a significantly larger zone of inhibition than the zones of inhibition produced by the studied plant extract all concentrations in *E. coli* ($p<0.05$; Table 4.6).

The effects of the methanolic bark extract of *C. megalocarpus* on *S. typhimurium* bacterial strain were determined in this study. The results indicated no significant differences in zones

of inhibition of *S. typimurium* by the methanolic bark extract of *C. megalocarpus* at concentrations of 3.125 µg/ml and 6.25 µg/ml ($p>0.05$; Table 4.6). Similarly, no significant differences in zones of inhibition were observed between plates treated with this extract at concentrations of 50 µg/ml and 100 µg/ml, between 25 µg/ml and 50 µg/ml, and, between 6.25 µg/ml and 12.5 µg/ml($p>0.05$; Table 4.6). However, the standard antibiotic recorded a significantly larger zone of inhibition of *S. typhimurium* compared with the zones of inhibition observed in all the extract concentrations ($p<0.05$).

Upon subjecting *S. aureus* bacterial strain to the methanolic bark extracts of *C. megalocarpus* at various concentrations, the results revealed no significant differences in zones of inhibition among concentrations of 12.5 µg/ml, 25 µg/ml and 50 µg/ml($p>0.05$; Table 4.6). Generally, in this bacterial strain, a dose dependent increase in zones of inhibition was observed with significantly small zone at 3.125 µg/ml and significantly larger zone at 100 µg/ml ($p<0.05$; Table 4.6). However, the reference drug produced a significantly larger zone of inhibition compared with the zones produced in all the other setups ($p<0.05$; table 4.6).

The susceptibility of *C. albicans* to the methanolic bark extract of *C. megalocarpus* was also investigated in this study (Table 4.6). The results indicated that at concentrations of 3.125 µg/ml, 6.25 µg/ml, and 12.5 µg/ml of the methanolic bark extract of *C. megalocarpus*, the zones of inhibition produced were not significantly different ($p>0.05$; Table 4.6). Similarly, the zones of inhibition observed at concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml of the extract were significantly similar ($p>0.05$; Table 4.6). It was also noted that *C. albicans* was not susceptible to the positive control antibiotic, hence, the resultant zone of inhibition was like that of the negative control ($p>0.05$).

Table 4.6: Antimicrobial effects of the methanolic bark extract of *Croton megalocarpus* on selected microbial strains

Concentration (µg/ml)	Zone of inhibition (mm)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
3.125	7.00 ± 0.58^{fg}	7.00 ± 0.58^{ef}	11.00 ± 1.53^{d}	9.33 ± 0.33^{b}
6.25	9.00 ± 1.00^{ef}	7.67 ± 0.88^{de}	13.67 ± 1.86^{cd}	9.00 ± 0.00^{b}
12.5	10.83 ± 1.17^{de}	9.00 ± 0.58^{cd}	15.00 ± 0.00^{bc}	9.67 ± 0.33^{b}
25	$11.6670.33^{cd}$	9.33 ± 0.33^{c}	15.67 ± 0.33^{bc}	11.00 ± 0.50^{a}
50	14.00 ± 0.58^{bc}	10.17 ± 0.44^{bc}	16.33 ± 0.67^{bc}	11.33 ± 0.33^{a}
100	15.00 ± 1.00^{b}	11.00 ± 0.58^{b}	17.67 ± 0.88^{b}	12.00 ± 0.58^{a}
-ve control	6.00 ± 0.00^{g}	6.00 ± 0.00^{f}	6.00 ± 0.00^{e}	6.00 ± 0.00^{c}
+ve control	27.67 ± 0.88^{a}	27.00 ± 0.00^{a}	24.00 ± 1.00^{a}	6.00 ± 0.00^{c}

Values are expressed as $\tilde{x}\pm$SEM; means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test ($p<0.05$); Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); Negative control: DMSO (1.4 %)

4.1.3 Minimum inhibitory concentrations (MICs)

In this study, the lowest concentrations of the studied plant extracts and reference antibiotics, which completely inhibited microbial growth in the 48-hr incubation period at 37 ^{o}C, were considered as the minimum inhibitory concentrations (MICs).

The results revealed that the MICs of the aqueous bark extract of *P. peruviana* ranged from 6.25 µg/ml on *C. albicans* fungal strain to 100 µg/ml on *S. typhimurium* bacterial strain (Table 4.7). On *E. coli* and *S. aureus* bacterial strains, the MICs of *P. peruviana* were 50 µg/ml and 25 µg/ml respectively (Table 4.7). On the other hand, the MICs of the methanolic bark extract of *P. peruviana* were 100µg/ml on both *S. aureus* and *C. albicans* and 50µg/ml on *E. coli*. However, the MIC of the methanolic extract of *P. peruviana* on *S. typhimurium* was > 100 µg/ml (Table 4.7).

The MICs of the aqueous bark extract of *B. micrantha* were 50µg/ml and 100µg/ml for *E. coli* and *S. typhimurium* bacterial strains respectively and 12.5µg/ml for both *S. aureus* and *C. albicans* (Table 4.7). Similarly, the MICs of the methanolic bark extract of *B. micrantha* were 50 µg/ml for *E. coli*, 100 µg/ml for *S. typhimurium* and 12.5µg/ml for both *S. aureus* and *C. albicans* microbes (Table 4.7).

The MICs of the aqueous extract of *C. megalocarpus* were 100 µg/ml for both the *E. coli* and *S. aureus* bacterial strains and 25µg/ml on *S. typhimurium* and *C. albicans* microbial cultures (Table 4.7). On the other hand, the MICs of the methanolic bark extract of *C. megalocarpus* were 50 µg/ml for both *E. coli* and *S. aureus* and 100 µg/ml for both the *C. albicans* and *S. typhimurium* strains (Table 4.7).

Notably, the lowest MICs were recorded for aqueous bark extract of *B. micrantha* on *S. aureus* and *C. albicans* microbes (Table 4.7). Additionally, Ciprofloxacin (reference antibiotic) exhibited the lowest MICs on of 0.30µg/ml on *E. coli* and *S. typhimurium* bacterial cultures while Streptomycin had an MIC of 0.62µg/ml on *S. aureus* bacterial strain. Amphotericin B showed an MIC of > 100µg/ml on *C. albicans* fungus (Table 4.7).

Table 4.7: Minimum inhibitory concentrations of the aqueous and methanolic extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* on selected microbial strains

Plant extract	Minimum inhibitory concentration(µg/ml)			
	E. coli	***S. typhimurium***	***S. aureus***	***C. albicans***
P. peruviana (aq)	50	100	25	6.25
P. peruviana (met)	50	>100	100	100
B. micrantha (aq)	50	100	12.5	12.5
B. micrantha (met)	50	100	25	25
C. megalocarpus (aq)	100	25	100	25
C. megalocarpus (met)	50	100	50	100
+Ve control	0.30	0.30	0.62	>100

Positive control: For *E. coli* and *S. typhimurium* it was Ciprofloxacin (10 µg); For *S. aureus* it was Streptomycin (µg) and for *C. albicans* it was Amphotericin B (µg); aq: aqueous extract; met: Methanolic extract

4.2 Cytotoxic effects of the aqueous and methanolic extracts of the studied plants of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* on brine shrimp nauplii

The effects of the studied plant extracts on brine shrimp nauplii were also investigated in this study. The concentrations of the aqueous and methanolic extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* that could kill 50 % of the exposed brine shrimp nauplii

were determined and considered as mean lethal concentration (LC_{50}). The results are presented in table 4.8.

Generally, the LC_{50} values ranged from 10 µg/ml for the positive control (cyclophosphamide) to >1000µg/ml for the aqueous bark extract of *P. peruviana* (Table 4.8). The LC_{50} values obtained for both extracts of *C. megalocarpus* and the methanolic extract of *P. peruviana* were significantly higher than those recorded for both extracts of *B. micrantha* and the positive control drug ($p<0.05$;Table 4.8).

Table 4.8: Effects of the studied aqueous and methanolic plant extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* on brine shrimp nauplii

Plant extract	LC_{50}(µg/ml)
P. peruviana (aq)	> 1000
P. peruviana (met)	687.50 ± 5.94^{a}
C. megalocarpus (aq)	486.67 ± 3.15^{b}
C. megalocarpus (met)	458.33 ± 2.87^{c}
B. micrantha (aq)	67.35 ± 2.04^{d}
B. micrantha (met)	55.18 ± 1.27^{e}
+ control	10.00 ± 1.31^{f}

Values are presented as $\tilde{x}\pm$SEM; means with different lowercase alphabet superscript within the same column are significantly different by One-Way ANOVA followed by Tukey's test ($p<0.05$);aq: aqueous extract; met: Methanolic extract; Positive control; cyclophosphamide

4.3 Acute oral toxicity effects of the aqueous and methanolic extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* on rat models

In this study, the acute oral toxicity effects of the aqueous and methanolic stem bark extracts of the studied plants in laboratory rats were evaluated.Various wellness parameters were monitored throughout the 14-day experiment period and the findings recorded.

The results are presented in table 4.9. The results showed that, at all the orally administered doses (175 mg/Kg bw, 550 mg/Kg bw and 2000 mg/Kg bw) of the studied extracts, there were no observable signs of toxicity recorded in all the experimental rat models (Table 4.9)

since the wellness parameters were normal to the limit dose level of 2000 mg/Kg bw, the LD 50 values of all the studied plant extracts were considered to be >2000 mg/Kg bw according to the OECD/OCDE (2008) guidelines.

Table 4.9: Acute Oral Toxicity effects of the aqueous and methanolic bark extracts of the studied plants of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus* in experimental rats

Wellness parameter	Observation											
	30 minutes		4 hours		24 hours		48 hours		7 days		14 days	
	EGM	CGM	EGM	CGM	EGM	CGM	EGM	CGM	EGM	CGM	EGM	CGM
Skin and Fur appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Faecal matter consistency	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urination and urine appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Itching	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions and tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Aggression	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Grooming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Teeth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality/Death	None	None	None	None	None	None	None	None	None	None	None	None

EGM: Experimental group Rats (Administered with the studied plant extracts); CGM: Control group Rats (Administered with Normal saline only)

4.4 Qualitative phytochemical composition of aqueous and methanolic bark extracts of *Physalis peruviana, Bridellia micrantha* and *Croton megalocarpus*

The results showed presence of alkaloids, saponins, tannins, glycosides, flavonoids and phenols in the aqueous and methanolic extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* (Table 4.10). However, anthraquinones were absent in all the extracts except in the aqueous and methanolic extracts of *B. micrantha* (Table 4.10).

Table 4.10: Qualitative phytochemical composition of aqueous and methanolic bark extracts of *Physalis peruviana, Bridellia micrantha,* and *Croton megalocarpus*

Phytochemical	***P. peruviana* extracts**		***B. micrantha* extracts**		***C. megalocarpus***	
	Aqueous	**Methanolic**	**Aqueous**	**Methanolic**	**Aqueous**	**Methanolic**
Alkaloids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Anthraquinones	-	-	+	+	-	-
Phenols	+	+	+	+	+	+

+: Present; -: Absent

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

The resurgences of multi-drug resistant microbial strains have rendered the management and treatment of associated infections a challenging endeavor, leading to increased morbidity and mortality (Joray *et al.*, 2013). It is estimated that annually, over 2 million persons are diagnosed with mortal infections which are exacerbated by resistance, and, of the diagnosed cases, over twenty thousand patients succumb as a result of therapeutic failure (Frieri *et al.*, 2017; WHO, 2016). Globally, research has shown that antimicrobial resistance could cause over 10 million deaths by the year 2050 if not arrested early enough (WHO, 2016).

Due to the inadequacy of therapeutic tools to thwart microbial infections, there is an urgent need for the search of alternative and complementary strategies to curb these infections (Joray *et al.*, 2013; Yılmaz and Özcengiz, 2017). As a result, medicinal plants have proved to be a viable alternative with a high propensity for potent antimicrobials (Kaminidevi *et al.*, 2015; Muthii *et al.*, 2014).

Since antiquity, humans have utilized herbals and herbal-derived products to fight infections and promote their health (Mudzengi *et al.*, 2017; Turner *et al.*, 2019). Throughout the world, it has been estimated by the WHO that more that 80 % of the human population especially from low economy countries depend traditional medicine to meet their primary healthcare needs (WHO, 2013). The rich ethno history of their usage, their presumed efficacies and low toxicity profiles has accelerated their exploitation. Furthermore, the easy accessibility and affordability of traditional medicines as well as the insufficiencies of conventional medicine have elevated traditional medicine (Qi, 2015; WHO, 2005; 2013).

It is apparent that medicinal plants have contributed greatly to the conventional medicine as their products have offered lead molecules for drug development (Al-Ayed *et al.*, 2016; Friedman, 2017; Kaminidevi *et al.*, 2015). For instance, the potent drugs currently used to manage cancer, malaria among other conditions are derived from medicinal plants (Rates, 2001). Additionally, research has revealed that various plant extracts and plant-derived metabolites are very potent against non-resistant and resistant microbial strains (Atef *et al.*, 2019; Friedman, 2017; Frieri *et al.*, 2017).

Considering the fact that less than 20 % of the world medicinal plants have been scientifically explored for their therapeutic value, empiricalethnomedical exploration interest has been reinvented (Abreu *et al.*, 2012; Faron *et al.*, 2004). Therefore, medicinal plant bioprospecting especially in the Kenyan context, due to the rich indigenous flora is an impetus towards the search for affordable, accessible, efficacious and safe antimicrobial agents (Kareru *et al.*, 2007; Kokwaro, 2009).

Despite the prominent utilization of local herbals and herbal-derived products to manage microbial infections and associated maladies among the Kenyan traditional practioners, there is scanty empirical data to validate the claimed healing properties. As a result, the current study was designed to investigate the antimicrobial, cytotoxicity and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *P. peruviana*, *C. megalocarpus* and *B. micrantha*. Since these plants are used traditionally to fight microbial infections, their scientific exploration serves as a guide towards the discovery of lead compounds for antimicrobial chemotherapy (Amuka *et al.*, 2014; Maina, 2018).

To investigate the antimicrobial activities of the studied plant extracts of *Physalis peruviana*, *Bridellia micrantha* and *Croton megalocarpus* on the selected microbial strains, the most recommended antimicrobial susceptibility methodology described by the CLSI was employed

(CLSI, 2014). In this study, the standard disc diffusion and broth microdilution techniques were followed do determine the effects of the studied plant extracts on microbial growth. The zones of inhibition and the minimum inhibitory concentrations were considered indicators of antimicrobial activity.

Previous studies have shown that plant extracts exhibiting a zone of inhibition of above 6 mm on selected microbial strains have antimicrobial activity (Atef *et al.*, 2019; Jouda, 2013; Mwitari *et al.*, 2013; Nanasombat *et al.*, 2018). Plant extracts which show a zone of inhibition of between 6 mm and 9 mm are deemed to possess slight antimicrobial activity, those showing zones of between 9 mm and 12 mm have moderate activity while those exhibiting inhibition zones of 13-16 mm are considered to have high antimicrobial activity. Additionally, plant extracts which have inhibition zones ranging from 16-19 mm have very high antimicrobial activity while those exhibiting zones of inhibition with diameters of 20 mm or above on selected strains are considered to have remarkable antibiotic potency (Mwitari *et al.*, 2013; Nanasombat *et al.*, 2018; Saquib *et al.*, 2019).

In this study, the results revealed that the aqueous extracts of *P. peruviana* exhibited slight to moderate antimicrobial effects against *E. coli* and *S. aureus* bacterial strains in a dose dependent manner. On the other hand, this extract exhibited moderate to high activity on *C. albicans* demonstrating its antifungal effects. Against *S. typhimurium*, the aqueous extract of *P. peruviana* demonstrated slight activity at 50 µg/ml and 100 µg/ml (Mwitari *et al.*, 2013).

The methanolic bark extract of *P. peruviana* demonstrated slight to moderate antimicrobial effects against *E. coli*, *S. aureus* and *C. albicans* based on the criteria described earlier. However, a slight antimicrobial effect by this extract was observed on the *S. typhimurium* strain in this study. This implies that, these extracts have both antibacterial and antifungal properties with the antifungal effects being more pronounced. These results are consistent

with those earlier reported by Göztok and Zengin, (2015) on fruit extracts of *P. peruviana* and Higaki *et al.* (2016) who reported corroborating results on fruit fractions of the same plant.

On the other hand, the aqueous extract of *B. micrantha* indicated slight to high antimicrobial activities against *E. coli* strain based on the produced zones of inhibition sizes. Slight to moderate effects were observed against *S. typhimurium* strain while moderate to high effects were exhibited on *S. aureus* bacterial strain and *C. albicans* fungal strain (Mwitari *et al.*, 2013).

For the methanolic extract of *B. micrantha,* moderate to high antimicrobial activities were recorded against *E. coli* while slight to moderate effects were observed in *S. typhimurium* strain. Slight to high and slight to very high antimicrobial effects were noted in *S. aureus* and *C. albicans* respectively. Remarkably, at a concentration of 100 µg/ml of the methanolic extract of *B. micrantha* a zone of 19.00±3.06mm was recorded on *C. albicans,* indicating very high antifungal effects (Mwitari *et al.*, 2013). These findings corroborate those of Douglas and Gitonga, (2016).

Further, the aqueous extract of *C. megalocarpus* demonstrated slight to moderate antimicrobial activities against *E. coli* and *S. aureus* bacterial strains. On the other hand, moderate antimicrobial properties were exerted on *S. typhimurium* and *C. albicans* strains. Notably, of the three aqueous extracts studied, only *C. megalocarpus* exhibited antimicrobial effects on *S. typhimurium* at all the studied concentrations (Kariuki *et al.*, 2014; Matu and Van Staden, 2003).

Moreover, the antimicrobial activities of the methanolic extract of *C. megalocarpus* were graded as slight to moderate on *S. typhimurium* and *C. albicans* strains, slight to high activities for *E. coli* strain and slight to very high for the *S. aureus* strain, respectively. The

results are in agreement with an earlier report involving the methanolic, ethyl acetate and isobutanol extracts of this plant on selected microbial strains of human pathogenic bacteria (Obey *et al.*, 2016).

Moreover, research has shown that plant extracts which have MIC values that are less than 1µg/ml (1000 µg/ml) have antimicrobial activity with a potential of offering potent antibiotics (Anyanwu and Okoye, 2017; Ezeja *et al.*, 2012). In this study, the studied plant extracts exhibited low MIC values on selected microbes. Since the MIC values were much lower, it is anticipated that, the studied plant extracts can be strong antibiotics.

Medicinal plants are a host of various bioactive compounds with a broad spectrum of pharmacologic efficacies (Harborne, 1998). Research has shown that tannins, phenols, flavonoids, terpenoids among other phytocompounds are responsible for the antimicrobial activity of plants (Eldahshan and Singab, 2013; Kurmukov, 2013b; Molyneux *et al.*, 2007). Therefore, bioactivities reported in this study are attributable to these phytochemicals which work either solely or in synergy with others to cause the pharmacologic effects.

Since there were varied antimicrobial activities depending on the microbial strain, and the type of extract, it is thus, suggestive that, the phytochemicals which affect one strain may not affect the other one. Additionally, since various phytochemicals are solubilized and extracted with different solvents, the relative abundance of antimicrobial compounds may not be the same (Al-snafi, 2015; Kuglerova *et al.*, 2011). Moreover, even though water and methanol are both polar, their polarities are different, with water being more polar than methanol (Hamuel, 2012). Therefore, water concentrated very polar compounds than did methanol, thereby accounting for the differences in the antimicrobial activities of the studied plant extract.

Despite the long-standing utilization of herbals and their products for the management of various health conditions, serious concerns regarding their safety have been raised (George, 2011). Various factors that affect the therapeutic potency of herbal medicines are generally not adhered to. There is lack of standard procedures and regulations governing the preparation, labelling, marketing and dispensing of herbal medicines (Ekor, 2014). This has led to an emergence of unscrupulous practioners of herbal medicine thereby raising safety concerns. There are no dosage guidelines, clearly outlines contraindications, conventional drug- herbal drug interactions, and toxicity profiles of herbal preparations (Kuete *et al.*, 2015; Saad *et al.*, 2006). As a result, improper use of these medicines could cause life-threatening effects considering the insufficiency of scientific and clinical data.

Owing to the fact that the studied plants have for a long period been used in traditional medicine to manage infections among other diseases without clear scientific backup (Petrovska, 2012; Shakya, 2016), the current study sought to determine the cytotoxicity and acute oral toxicity effects of the aqueous and methanolic extracts of the studied plants.Assessment of safety is key to assure safe use of herbal medicines in traditional medicine and to offer scientific data which can guide further development.

The brine shrimp lethality assay technique is a rapid method widely used to screen cytotoxic effects of plant extracts and chemicals thought of therapeutic value (Meyer *et al.*, 1982). This technique was adopted to assess the cytotoxic/safety effects of the aqueous and methanolic stem bark extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* in exposed shrimp nauplii. In this method, the concentration of the plant extracts able to kill 50 % of nauplii following exposure is considered as the LC_{50}. Research has shown that plant extracts with LC_{50} values that are < 30µg/mlare very cytotoxic.Furthermore, plant extracts exhibiting LC_{50} values ranging from 30-100 µg/mlare toxic, while those having LC_{50} values that are over 100µg/ml are considered to be of low toxicity or safe (Gadir, 2012; Moshi *et al.*, 2010).

In view of the described criteria (Gadir, 2012; Moshi *et al.*, 2010), both the aqueous and methanolic extracts of *B. micrantha* were toxic as their LC_{50} values were between 30µg/ml and 100 µg/ml. All the other extracts proved to be non-toxic to brine shrimp nauplii and thus safe as their LC_{50} values were >100 µg/ml. Notably, the aqueous extract of *P. peruviana* was the safest among the tested extracts with LC_{50} value of >1000 µg/ml. These findings imply that both the aqueous and methanolic extracts of *C. megalocarpus* and *P. peruviana* are safe and can be used as alternative therapies for microbial infections. Moreover, caution should be exercised whenever extracts from *B. micrantha* are used to avert adverse events.

Since most of herbal medicines are administered orally, the acute oral toxicity effects of the studied plant extracts were investigated in rat models. In this study, the acute oral toxicity study top-down procedure described the OECD document number 425 was adopted (OECD, 2008). The results showed that all the studied plant extracts were non-toxic at oral doses and therefore safe. Considering these results, the studied plant extracts are safe for use in traditional medicine.

It is however notable that both the aqueous and methanolic extracts of *B. micrantha* were toxic to brine shrimp but non-toxic to experimental mice. This could imply that multicellular organisms have efficient machineries of handling drug agents as opposed to unicellular to lower organisms (Sahgal *et al.*, 2010). Additionally, the toxicity exerted by these extracts at the cellular level could be negligible so as to cause no observable signs of toxicity in experimental rat models (Naidu *et al.*, 2014; Sahgal *et al.*, 2010).

The medicinal value of plants is attributed to the secondary metabolites which they synthesize(Kurmukov, 2013b). The secondary metabolites (phytochemicals) are produced in response to stress which is associated with pathogenic attack, environmental stress and oxidative stress (Moriasi *et al.*, 2020b). Research has shown that each of these

phytocompounds are pharmacologically active with various effects in biological systems (Truong *et al.*, 2019).Of the phytoactive compounds present in medicinal plants, antioxidant amalgams posses the widest spectra of pharmacologic activities.

Flavonoids, phenols and tannins have been demonstrated to confer antioxidant and antimicrobial activities (Mutembei *et al.*, 2018). Besides, alkaloids and anthraquinones possess cytotoxic effects. From the findings of this study, it is therefore arguable that the antimicrobial effects of the aqueous and methanolic extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* are due to the presence of phenols, flavonoids among other antimicrobial-associated phytochemicals. Furthermore, the cytotoxic effects of the aqueous and methanolic extracts of *B. micrantha* could be due to the presence of anthraquinones (Bode and Dong, 2015).

The safety of the studied plant extracts reported in this study could be attributed to low concentration or absence of toxicity associated phytochemical compounds (Bode and Dong, 2015). Furthermore, the antimicrobial bioactive compounds anticipated to be present in the studied plant extracts in varied degrees do not cause observable signs of toxicity (Riaz *et al.*, 2018). These findings indicate that the studied plant extracts can be good alternative sources of safe antimicrobial compounds. Therefore, this study supports the traditional use of the studied plant extracts for the management of the claimed conditions.

5.2. Conclusions

Based on the obtained results, the following conclusions were drawn.

i. The aqueous and methanolic bark extracts of *P. peruviana, B. micrantha* and *C. megalocarpus* have antimicrobial varied effects on *E. coli, S. typhimurium, S. aureus* and *C. albicans* microbial strains.

ii. The aqueous and methanolic bark extracts of *P. peruviana* and *C. megalocarpus* are non-toxic, while those of *B. micrantha* are toxic, to brine shrimp nauplii.

iii. The aqueous and methanolic extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* do not cause any observable signs of toxicity in experimental rats.

iv. The aqueous and methanolic bark extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* possess antimicrobial associated phytochemicals

5.3. Recommendations from the study

From this study, the following recommendations were made:

i. The aqueous and methanolic bark extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* can be used as alternative and complimentary antimicrobial therapies against the studied strains.

ii. Both the aqueous and methanolic bark extracts of *B. micrantha* can be used as cytotoxic agents.

iii. The aqueous and methanolic bark extracts of *P. peruviana*, *B. micrantha* and *C. megalocarpus* are safe when orally administered.

5.4. Recommendations for further studies

Following this study, further investigations aimed at establishing the specific modes of action of the studied plant extracts are recommended. Moreover, drug interaction studies involving the studied plant extracts are encouraged. Further antimicrobial activity studies using other microbial strains, and, toxicological investigations of the studied plant extracts in other experimental models should be done. Also, isolation and characterization of specific antimicrobial phytocompounds from the studied plant extracts should be done.

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APPENDICES

Appendix 1: Institutional ethical approval letter

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2019/247

Dr. Joseph Murithi Kathare,
University of Nairobi
Dept. PHP & Toxicology
20/12/2019

Dear Dr. Kathare

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Antimicrobial activity of crude extracts of *Bridellia micrantha, Croton megalocarpus and Physalis peruviana* against *Salmonella typhi* and safety evaluation.

Dr. Joseph Kathare J56/87863/2016

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 9th December 2019. We have reviewed your application for ethical clearance for the study in Antimicrobial activity of crude extracts of B*ridellia micrantha, Croton megalocarpus and Physalis peruviana* against *Salmonella typhi* and safety evaluation.

The numbers of rats to be used in safety evaluation protocol and the laboratory *in vitro* assays meets minimum standards of the Faculty ethical regulation guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine

Appendix 2: Botanical Identification and authetication Certificate of the studied medicinal plants

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

4th February, 2019

REF: NMK/BOT/CTX/1/2

Joseph M. Kathare
Tel. 0721-421719
Nairobi

Dear Sir,

PLANT IDENTIFICATION

The plant specimens you brought to us for identification has been determined as follows:

01. *Bridelia micrantha* (Hochst.) Baill. (**Family:Euphorbiaceae**)
02. *Croton megalocarpus* Hutch.(**Family:Euphorbiaceae**)
03. *Physalis peruviana* L. (**Family: Solanaceae**)

Thank you for consulting the East African Herbarium for plant identification and confirmation.

Yours Sincerely,

NATIONAL MUSEUMS OF KENYA
04 FEB 2019
E.A
HERBARIUM NAIROBI

Mathias M. Mbaf

Botany Department

Appendix 3: Research findings Dissemination: Published Research Article 1

Pharmacogn J. 2021; 13(5): 1248-1256
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Antimicrobial Efficacy, Cytotoxicity, Acute Oral Toxicity, and Phytochemical Investigation of the Aqueous and Methanolic Stem Bark Extracts of *Bridellia micrantha* (Hochst.) Baill

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ABSTRACT

Introduction: Microbial infections cause high morbidity and mortality in humans globally. Antimicrobial resistance, emergence of new strains, high costs of antibiotics, inaccessibility-especially in remote areas, and adverse effects, impede successful eradications of pathogens, hence the need for novel strategies. *Bridellia micrantha* is used in traditional medicine to treat microbial infections; however, it has not been empirically validated. **Methods:** Antimicrobial activity of the aqueous and methanolic stem bark extracts of *Bridellia micrantha* was investigated using the disk diffusion and broth microdilution techniques, described by the Clinical Laboratory Standards Institute (CLSI) guidelines. The brine shrimp lethality assay technique was used to investigate the cytotoxic effects of the studied plant extracts in exposed nauplii. Acute oral toxicity effects of the studied plant extracts in Winstar rats were investigated following the up-and-down procedure described by the Organisation for Economic Development and Co-operation (OECD). Qualitative phytochemical screening was performed following standard procedures. **Results**: The aqueous and methanolic extract of *B. micrantha* indicated varied antimicrobial activities against *E. coli, S. typhimurium, S. aureus,* and *C. albicans,* with inhibition zones ranging from 6.00mm to 19.00mm. Furthermore, the studied plant extracts exhibited low MIC values (≤100 µg/ml) on selected microbes. Since the MIC values were much lower than 1000µg/ml (the cutoff for antimicrobial efficacy appraisal), it is anticipated that, the studied plant extracts can be strong antibiotics. The aqueous and methanolic stem bark extracts of *B. micrantha* were cytotoxic to brine shrimp nauplii, with LC_{50} values of 486.67±3.15 µg/ml and 458.33±2.87 µg/ml, respectively; however, these extracts did not elicit any observable signs of toxicity in rat models. Pharmacologically active phytochemicals, including flavonoids, alkaloids, saponins, tannins, phenols, and anthraquinones were detected in the two studied extracts. **Conclusions:** The aqueous and methanolic stem bark extracts of *B. micrantha* have appreciable antimicrobial activity against *E. coli, S. typhimurium, S. aureus* and *C. albicans*. Besides, the studied plant extracts are cytotoxic to brine shrimp nauplii; but they do not cause acute oral toxicity effects in rat models. Additionally, the studied plant extracts contain bioactive phytochemicals, with antimicrobial activity.

Key words: Brine shrimp lethality Assay, Minimum inhibitory concentration, Median lethal concentration (LC_{50}), median lethal dose (LD_{50}), Zone of inhibition.

INTRODUCTION

Microbial infections are among the major causes of morbidity and mortality in humans globally, especially in vulnerable groups [1–3]. Furthermore, the increased rate of antimicrobial resistance to the available medications has caused major healthcare challenges[4,5]. The persistence of microbial infections during and after the treatment cycles has precipitated an overuse of antibiotics leading to other unprecedented outcomes [6].

Owing to the drawbacks and inefficiencies of conventional antimicrobial chemotherapy, alternative stratagems are required to curb infectious diseases at relatively cheaper costs than those involved in orthodox medicine, and with fewer or no toxic effects[7]. Medicinal plants on the other hand are a valuable source of antimicrobials owing to their long-term applications. Further, these plants are considered to easily accessible, affordable and with fewer side effects compared to western medicine [8,9].

Bridellia micrantha (Hochst.) Baill is a medium to large deciduous tree which grows up to 20 m above the ground with spreading crown[10]. It belongs to Euphorbiaceae family of herbs and trees which are characterized by succulent leafless branches; milky or watery latex; glands at the leaf base; and 3-lobed fruits. It is locally known as '*mukuigo*' by the Kikuyu of Murang'a County, Kenya [11,12].

The stem bark tinctures and decoctions of *Bridellia micrantha* are used to cure burns, soft tissue injuries, sexually transmitted infections, protozoa infections, gastrointestinal conditions, typhoid, pneumonia and dental diseases [12,13]. Leaf preparation is used to manage eye problems [13].

Previous studies have indicated that *B. micrantha* has anti-ulcer activity against *H. pylori*-induced ulcers and antimicrobial activities against *S. typhi, S. enteritidis, S. flexneri, E. coli* and *M. tuberculosis* bacterial strains [13]. Furthermore, antidiabetic, hypolipidemic and antioxidant effects of extracts derived from *B. micrantha* have been reported [10,14]. Phytochemical investigations have revealed presence oftaraxerone, Friedelin, Taraxerol, Epifriedelinol, gallic acid, ellagic acid, anthocyanidin, delphiniridin and Benzene 1,3-bis(3-phenoxyphenoxy),2-pinen-4-one [13].

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Appendix 4: Dissemination of Research findings: Published Research Article 2

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Research Article

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Antimicrobial, Cytotoxicity, Acute Oral Toxicity, and Qualitative Phytochemical Screening of the Aqueous and Methanolic Stem-Bark Extracts of *Croton megalocarpus* Hutch. (Euphorbiaceae)

Joseph M Kathare, James M Mbaria, Joseph M Nguta, Gervason A Moriasi

ABSTRACT

Microbial infections are feared to cause over 10 million deaths by the year 2050, whereby 50% of the global burden squarely lies in less developed countries of Africa and Asian continents. The current drugs have suffered resistance by previously susceptible strains, are associated with severe side effects, among other therapeutic and economic drawbacks, hence the need for alternatives. Despite the widespread usage of medicinal plants by over 80% of the global population to treat common ailments, including microbial infections, only a few have been empirically validated. *Croton megalocarpus* is used to treat microbial-associated infections like pneumonia and typhoid among the Agikuyu community of Kenya. However, its healing claims and safety have not been evaluated empirically to date, hence this study. We investigated the antimicrobial, cytotoxicity, acute oral toxicity, and qualitative phytochemical composition of the aqueous and methanolic stem bark extracts of *C. megalocarpus*. The disk diffusion and broth microdilution techniques described by the Clinical Laboratory Standards Institute (CLSI) were adopted for antimicrobial assays. The acute oral toxicity effects of the studied plant extracts were evaluated according to the Organisation of Economic Co-operation and Development (OECD) guideline document number 425. The brine shrimp lethality assay technique was used to appraise the cytotoxic effects of the studied plant extracts. Qualitative phytochemical screening was performed following standard procedures. The results revealed that all the studied plant extracts had varied antimicrobial effects on selected microbial strains and showed MIC values of <1000 µg/ml indicating their antimicrobial potential. Moreover, the studied plant extracts had LC_{50} values of >100 µg/ml and >2000 mg/Kg bw in the brine shrimp lethality and acute oral toxicity assays, respectively, demonstrating their safety. Antimicrobial- associated phytocompounds were detected in the studied plant extracts suggesting they were responsible for the reported bioactivity. Further studies to establish the specific mode(s) of antimicrobial action, toxicological, and safety should be performed. Furthermore, antimicrobial investigations of the studied plant extracts on other clinically significant microbial strains and the isolation, characterization, and optimization of antimicrobials from the studied plant extracts should be done.

Keywords: Antimicrobial activity, *Croton megalocarpus*, Disk diffusion, Broth microdilution, Brine shrimp lethality assay, Acute oral toxicity.

INTRODUCTION

The rapid increase in antibiotic resistance by various pathogenic microbes, such as the multidrug-resistant *Staphylococcus aureus*, Enterobacteriaceae, and pseudomonads, is a major global health challenge [1, 3]. Research has indicated that resistant bacterial strains arise from inappropriate use of antibiotics, patient non-compliance to antimicrobial therapy, widespread usage of antibiotics in animal feeds as growth promoters, the transboundary transmission of resistant strains, among other factors [2]. This has complicated the treatment of various pathogenic infections with the current antimicrobial agents. Furthermore, the scarcity of novel, safe, affordable, and accessible antimicrobials, especially in the developing countries of the African and Asian continents, which account for over 50 % of all global infectious disease-associated deaths, warrants urgent intervention [4, 5]. Moreover, the presently used antimicrobials exhibit undesirable effects in patients, such as nephrotoxicity, hepatoxicity, gastrointestinal complications, among others [6].

Medicinal plants play an integral role in promoting the health and wellbeing of humans and animals [7, 9]. The World Health Organization (WHO) report indicates that over 80% of the global population, especially from the developing countries in the African and Asian continent, largely depend on traditional medicine to treat and manage common ailments [10, 11]. The reliance on traditional

Appendix 5: Research findings dissemination: Published Research Article 3

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Research Article

Antimicrobial, Cytotoxicity, Acute Oral Toxicity and Qualitative Phytochemical Screening of the Aqueous and Methanolic Extracts of *Physalis peruviana* L (*Solanaceae*)

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ABSTRACT

Pathogenic microbes are the major causes of morbidity and mortality globally, especially in children and in immunocompromised individuals. Despite the successes of antimicrobial therapy, various challenges, including antimicrobial resistance, therapeutic failure, deleterious side effects, high costs, and inaccessibility, hinder health and wellbeing, necessitating the need for alternative and complementary approaches. Medicinal plants have, for a long time, played an integral role in meeting the primary healthcare needs of over 80% of the global population, especially in low- and middle-income countries. However, despite the rich ethnomedical evidence of utilization there are insufficient empirical scientific data to validate and authenticate the therapeutic potential of medicinal plants. *Physalis peruviana* (*Solanaceae*) is used by the Agikuyu community of Kenya to treat malaria, pneumonia, typhoid, among other health conditions. Even though this plant has been used since antiquity to treat microbial-associated infections, there is no enough scientific proof of its pharmacologic efficacy against microbial infections. Moreover, the safety levels and toxicity profiles of herbal preparations of *P. Peruviana* are not adequately demystified scientifically. As a result, the current study investigated the antimicrobial, cytotoxicity, acute oral toxicity, and qualitative phytochemical composition of the aqueous and methanolic bark extracts of *P. Peruviana* and potential sources of alternative, efficacious, safe, and affordable antimicrobials. The disc diffusion and the Broth microdilution techniques were used to evaluate the antimicrobial activity of the studied plant extracts on selected microbial strains (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *and Candida albicans*). The brine shrimp lethality test was used to determine the cytotoxicity of the studied plant extracts. At the same time, the acute oral toxicity effects were investigated as per the guidelines of the Organization for Economic Co-operation and Development (OECD) outlined in document number 425. Qualitative phytochemical screening was performed using standard procedures. The aqueous bark extract of *P. Peruviana* exhibited slight antimicrobial activity against *S. typhimurium* and *E. coli*, slight to moderate activity against *S. aureus*, and moderate to high activity against *C. albicans*, in a concentration-dependent manner. Besides, the methanolic bark extract of *P. Peruviana* showed slight antimicrobial activity against *S. typhimurium* and slight to moderate activities against *E.coli*, *S. aureus*, and *C. albicans* microbial strains. Moreover, both of the studied plant extracts did not show any observable signs of acute oral toxicity effects in Winstar rats, and cytotoxicity in brine shrimp *Nauplii*. The studied plant extracts showed the presence of antimicrobial- associated phytochemicals. Further studies to establish specific mode(s) through which the studied plant extracts exert their antimicrobial activity should be done. Moreover, the antimicrobial effects of the studied plant extracts on other microbial strains of clinical significance should be evaluated. Additionally, extensive safety and toxicity evaluation of the studied plant extracts should be undertaken. Quantitative phytochemical evaluation, isolation, characterization and development of antimicrobial compounds from the studied plant extracts should also be done in future studies.

Keywords: Antimicrobial activity; *Physalis peruviana*; Disk diffusion; Broth Microdilution; Brine shrimp lethality assay; Acute oral toxicity

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