ANTIMICROBIAL ACTIVITY, BRINE SHRIMP CYTOTOXICITY AND PHYTOCHEMICAL COMPOSITION OF *CROTON DICHOGAMUS* PAX CRUDE ROOT EXTRACTS FROM KISUMU EAST SUBCOUNTY

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A thesis submitted in Partial fulfillment of the requirements for Masters of Science degree of the University of Nairobi (Pharmacology and Toxicology)

Department of public health, pharmacology and toxicology.

2021

DECLARATION

This thesis is my original work and it has not been presented for a degree in any other university. The journals, books, articles and other literature sources used for information have been duly acknowledged.

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DECLARATION OF ORIGINALITY

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DEDICATION

I devote this thesis to my lovely husband Douglas Mokembo, my sons Doron, Delan and Denzel who have greatly inspired me and to my parents, Henry Matara and Teresa Okondo.

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ACRONYMNS AND ABBREVIATIONS

ANOVA – Analysis of variance

CAM – Complementary and alternative medicine

CHD - coronary heart disease

CONC - Concentration

DMSO - Dimethyl sulfoxide

LC50 - Median lethal concentration

HSD - Honestly significant difference

MIC - Minimum inhibitory concentration

MBC - Minimum bactericidal concentration

MFC -Minimum fungicidal concentration

MHA - Mueller Hinton Agar

MHB - Mueller Hinton Broth

NSP - Non starch polysaccharides

URTI - Upper respiratory tract infections

SEM - Standard error of the mean

SPSS - Statistical package for social sciences

TRM -Traditional medicine

WHO - World health organization.

ZI – Zone of inhibition

ABSTRACT

The emergence of drug resistant strains of microbes has necessitated a hunt for more efficacious antimicrobials from the environment. As such, Greeneries (plants) have confirmed to be highly accumulated in potent medicinal values. In the current research the cytotoxicity, phytochemical constituents and antimicrobial activity of the crude root extracts of *Croton dichogamus* were evaluated. *Croton dichogamus is a* twig growing in Eastern Africa, where it is used as an anti-infective and tonic agent. The roots were obtained from collected from Kisumu East subcounty, then dried, milled and extracted using distilled water, acetone and 50% ethanol. The percentage yield of acetonic, aqueous and hydroethanolic extracts were 1.29%, 6.05% and 4.9% respectively. Microbroth dilution and agar well diffusion methods were used to estimate the antimicrobial activity of the extracts against five microorganisms. The cytotoxicity of the extracts was determine using brine shrimp bioassay. The fungal organism (C. *albicans*) together with the gram-positive bacteria (*S. aureus, B. cereus*) were considerably inhibited by the three root extracts while the gram-negative bacteria (E. *coli, P. aeruginosa*) were not inhibited by any of the three extracts at a concentration of 250mg/ml. Acetonic extract on *B. cereus* demonstrated the greatest antimicrobial activity giving a MIC of 10.42mg/ml and a zone of inhibition of 17.33±0.58 at a concentration of 250mg/ml. The LC_{50} values of acetonic, aqueous and hydroethanolic extracts (4.148µg/ml, 42.61µg/ml, 76.09µg/ml) were found to be below 100µg/ml which meant that all the extracts were highly cytotoxic. Saponins, anthracenes, flavonoids, tannins, phenols, terpenoids and polyuronides were the phytochemical components present. The current study reports that *C. dichogamus* had antimicrobial activity thus confirming the folklore claim. These results make a strong case for

isolation, and characterization of bioactive components responsible for the observed cytotoxicity, which could serve as leads to novel anticancer agents.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Complementary and alternative medicine (CAM) also known as folk medicine, Traditional medicine (TRM), ethno-medicine or native healing is a form of treatment that has been used to alleviate various illnesses since time immemorial (Petrovska 2012; Abdullahi, 2011). Folk medicine has been proven over years and has stood the test of time in diverse communities (Romero-Daza, 2002). From time immemorial, man has always been in an arduous pursue for sustainable ways of managing ailments. This practice has led to great inventions on the use of nature in alleviating complex maladies (Petrovska, 2012). The use of folk medicine has immensely risen with about 80% of the global populace depending on this approach as the main form of managing illnesses (Ekor, 2014). Many reasons have led to this system of treatment (medicinal plants) being accepted widely including its accessibility, availability, effectiveness and affordability (Patwardhan *et al.*, 2005). Even the World Health Organization (WHO) recognizes that folk medicine is a supreme medical management technique that assures good results to its users (WHO, 2009).

Despite the global demand and use of traditional medicine, there are safety concerns in that most of these products have not been scientifically confirmed to be effective and their safety has not been studied and documented (Ekor, 2014). There is no record of their effective dosages, potential adverse effects and food or drug interactions. As observed, mostly the safety of these herbal preparations is gambled with by use of incorrect labeling, inappropriate quality controls and inaccurate patient information (Raynor *et al.*, 2011).

Among all the traditional medications, herbal medicines have found the most use in the treatment of respiratory illnesses, ranging from infections, airway complications, allergic reactions among others. The traditional healers have a myriad of plants that are used to manage respiratory complications among them being *Croton dichogamus*. Mailu *et a*l., 2020, reported that *C. dichogamus* locally known as 'Rachar' a shrub growing along the lake basin was among the many herbs that were used to treat Asthma, pneumonia and coughs. The Eastern African communities namely Somalia, Tanzania, Madagascar, Kenya, Rwanda and Ethiopia have found it useful as a tonic to improve digestion (Hedberg *et al.*, 1983), to treat malaria, relive fever, it is also added to milk products as a dietary supplement (Aldhaher *et al*., 2017; Hedberg *et al*., 1983), in the treatment of polio-like symptoms, and chest pain (Kigen *et al.*, 2019) and also to manage stomach pains, cough, edema (Mutie *et al.*, 2020), to manage asthma (Mailu *et al.*, 2020) , arthritis, gonorrhea and in the treatment of respiratory ailments like tuberculosis, chest congestion and dry cough (Hedberg *et al.*, 1983).

Available studies have also indicated that *C. dichogamus* possess anti- mycobacterial and insecticidal and antiproliferative activities (Magadula, 2012 ; Tlankka *et al*., 2020). However, there is scanty information on its antimicrobial effects and its toxicity as well. The phytochemical composition of this plant is also not known. The treatment dosage and quality of the decoctions given by the herbalists are of great concern too.

1.2 Statement of Problem

Antimicrobial resistance has imperiled successful prophylaxis and eradication of the always rising range of diseases caused by fungi, parasites and bacteria (Tanwar *et al.*, 2014). An exponential advent and proliferation of drug resistant strains of microbes in the healthcare system has immensely rendered the antimicrobial missiles powerless, consequentially accelerating the frequency of therapeutic failure and death (Fankam *et al*.,2017). As such, scientists have been in an ceaseless search for new antimicrobial agents (Anand *et al.*, 2019). Fortunately, since historical times, plants have demonstrated that they are a reliable wellspring of potent bio actives (Dadson *et a*l.,2012). Research has shown that antimicrobials of plant sources have a great capability of resisting fungi, bacteria and viruses with negligible complications (Chandra *et al.*, 2017). Tests done on a number of extracts from plants and their secondary metabolites have also demonstrated that plants by themselves or their natural products might possess significant activity against a broad spectrum of human pathogens or they could exhibit synergist or potentiator effect on antimicrobial agents. (Abreu *et al*., 2012). Moreover, plant secondary derivatives have been found to possess resistance modifying effects (Rahma *et al*.,2007; Burnett-Boothroyd & McCarthy, 2011).

1.3 Justification of the study

For many years, the root of *Croton dichogamus* has being used in Kisumu East Subcounty to manage respiratory infections that are of bacterial origin (Mailu *et al.*, 2020). Despite of the traditional claim of the curative effect of the plant, there is a paucity of information in a manner that, there is no scientific validity on the efficacy and safety of the plant.

The current study was designed to fill this gap by the determination of the cytotoxicity and antimicrobial effect of the root extract of *C. dichogamus*.

Findings from the study were to ascertain the phytochemical constituents of *Croton dichogamus* and document how best it could be utilized in the management of diseases with minimal toxic effects to body cells.

1.4 Objectives

1.4.1 Overall objective

To evaluate the antimicrobial activity, brine shrimp cytotoxicity and phytochemical constituents of aqueous and organic extract of *Croton dichogamus.*

1.4.2 Specific objectives

1. To evaluate the antimicrobial activity of the organic and aqueous crude root extracts of *Croton dichogamus.*

2. To assay the cytotoxicity of the organic and aqueous root extracts of *C. dichogamus* in brine shrimp larvae.

3. To analyze the phytochemical composition of the aqueous and organic root extracts of *C. dichogamus.*

1.5 Hypothesis

The organic and aqueous root extracts of *C. dichogamus* have antimicrobial activity and are toxic to brine shrimp larvae.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional medicine

It involves the use of nature (animals, plants, fungi and shells) in the prevention and management of diseases (Abdullahi, 2011). The World Health Organization(WHO) describes folk medicine as "the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses," (WHO, 2000b). WHO further explains that traditional medicine (TRM) is a cultural heritage of medicinal practice that was in extensive use before the modern medicine (WHO, 2000a). There is overwhelming evidence that traditional medicine is widely accepted and used in treatment of diseases even with the existence of modern medicine (Abdullahi, 2011). Notwithstanding the considerable use of folk medicine, it is still an alternative method of treatment because it lacks the scientific proof of its safety and efficacy. This system also lacks a standard form of treatment since each society practices it according to the socio-cultural rules (Ekor, 2014 ; Abdullahi, 2011).

The use of TRM in Africa dates back to 4000 years when it was the only form of healing (Vedavathy, 2003). A recent report has indicated that since then the use of traditional medicine has been on the rise with approximately 80% of the population in Africa being dependent on TRM for survival against infections (Shewamene *et al*., 2017). In numerous African, communities, there are specified herbs in the management of diverse illnesses; microbial infections, correction of metabolic disorders, improving female and male sexuality, relieving pain and promoting the

general wellbeing of the body. The traditional medicine practitioners attempt to treat their patients wholistically by reconnecting their social-emotional equilibrium according to the community rules (Abdullahi, 2011). Thus, in the rural Africa, a great number of people have a complete trust in this system of treatment because it long existed and its results have been evidently optimum (Patwardhan *et al.*, 2005).

There is also an overwhelming evidence that the demand of traditional medicine is growing in Europe, Australia, Asia, and North America specifically in the management of chronic and terminal illness (cancers, rheumatic and neurological disorders, hypertension) that modern medicine cannot cure (Warren *et al*., 2013 ;Yuan *et al*., 2016).The United Kingdom is one of the countries that has set up public -sector hospitals for CAM medicine at London, Liverpool, Bristol and Glasgow (WHO, 2001). Moreover, the herbal preparations and complementary and alternative medications are now very popular and widely available in health food stores and pharmacies (WHO, 2001).

Modern pharmaceutical knowledge has borrowed so much from traditional forms of treatment, the first pharmacologically active constituent of morphine was separated from opium poppy plant (Hamilton & Baskett, 2000). Since then, many active compounds have been isolated from natural products, especially plants, with most of them substantiating their traditional uses (Hamilton and BAskett, 2000). Approximately 50% of the pharmaceutical products are derivatives of compounds isolated from plants and animals as active ingredients, with 80% of antimicrobial , cardiovascular, immunosuppressive and anticancer medications being of plant origin (Pan *et al.*, 2013).This has significantly increased the use vegetative greeneries in clinical practice especially as leads in the development of new drugs (Yuan *et al.*, 2016).

2.2 Herbal medicine

Herbal medications are prepared by subjecting medicinal plants through a process of extraction, fractionation, purification and concentration, then consuming them as per the condition to be treated (Firenzuoli and Gori, 2007). These herbal preparations may be in form of decoctions, concoctions, macerates, infusions, tinctures, ointments and dried powders (Gakuya, 2001).Lately, the use of herbal preparations has expanded widely with many people resorting to use these preparations in the management of a variety of maladies in many national health facilities. The herbal preparations have also been used globally in the treatment of acute and chronic illness.

2.3 Microbiology

Microbiology is the scientific investigation of disease-causing microbes such as viruses, fungi, protozoa, parasites and bacteria**.** Over the recent years, there has been a propagation and emergence of new strains of microbes that are highly resistant to available antimicrobial drugs (Fankam *et al*., 2017). The microbes often become intrinsically resistant to drugs by acquiring resistant genes from other organisms or by de novo mutation (Tenover, 2006). The acquired resistant genes will then enable the microorganism to release macromolecules that demolish the antibiotic by secreting an outflow of fluids that inhibit the entry of the drug into the intracellular target, by modifying the drug's target location or by providing a substitute pathway of metabolism that diverts the drug action (Tenover, 2006). To avoid this situation, wise use of antimicrobial agents is required by using the appropriate dose for the required duration. New antimicrobial agents which have different mechanisms of action need to be developed. The fast-resisting microorganisms are indicated in table 2.1 below with the current anti-infective regimen to manage their illnesses.

Table 2.1: Common Infectious agents and their regimen of treatment (Tamma *et al*., 2021)

Disease	Causative agent	Drugs
Atypical Pneumonae		
	Chlamydia pneumoniae	Azithromycin
	Legionella pneumophilia	Erythromycin
	Mycoplasma pneumoniae	Cefixime
Community acquired pneumonia		
	Streptococcus pneumonia	Ciprofloxacin
	Haemophiles influenza	Ceftriaxone
	Staphylococcus aureus	Levofloxacin
Upper Respiratory	*Streptococcus pneumoniae*	Ceftriaxone
Tract infections	*Hemophilus influenza*	Levofloxacin
		Azithromycin
		Erythromycin
		Cefixime
Sinusitis	*Hemophilus influenza*	Antihistamines
	Staphylococcus aureus	Azithromycin
		Amoxicillin
Urinary tract infection	*Staphylococcus saprophyticus*	Nitrofurantoin
	Pseudomonas aeruginosa	Azithromycin
	Escherichia coli	Erythromycin
		Amoxicillin/clavulanic
Sexually transmitted	*Chlamydia trachomatous*	Ceftriaxone
Infections	*Neisseria gonorrhea*	Doxycycline
	Treponema pallidum	Amikacin
	Hemophilus ducreyi	Benzyl penicillin

		Ceftazidime
		Azithromycin
Eye infections	*Neisseria gonorrhea*	Ciprofloxacin
	Staphylococcus aureus	Ofloxacin
	Chlamydia trachomatis	Levofloxacin
		Doxycycline
		Azithromycin
		Ceftriaxone
Skin infections	*Streptococcus pyogenes*	Clindamycin
	Staphylococcus aureus	Flucloxacillin
	Pseudomonas aeruginosa	Cephalexin
Gastritis	*Helicobacter pylori*	Clarithromycin
		Amoxicillin
		metronidazole
Food poisoning	*Salmonella typhi*	Hydration
	Escherichia coli	Metronidazole
	Bacillus cereus	Doxycycline
	Staphylococcus aureus	secnidazole
Bacterial meningitis	*Neisseria meningitidis*	Ceftazidime
	Hemophilus influenza	Cefixime
	Streptococcus pneumoniae	Ceftriaxone

2.4 In vitro bioassay methods

2.4.1 Brine shrimp lethality assay

Traditional forms of healing have utilized numerous plants whose safety and efficacy has never been tested and recorded. Traditional healers worldwide have assumed that the plant remedies and medicines are safe because of the period they have used them for treatment. Contrary to their claim, extensive studies have revealed that a number of these drugs are potentially mutagenic, toxic and carcinogenic (Elgorashi *et al.*, 2003). There is a need of using cytotoxic models to supply basic information regarding the plant to help select plant extracts which are devoid of carcinogenic properties for further research work.

Brine shrimp bioassay is a significant procedure for primary evaluation of the bioactivity of medicinal plants which correlates considerably with its cytotoxic and antitumor properties (McLaughlin *et al*., 1991; Okumu *et al*., 2020). Brine shrimp larvae are overly delicate and sensitive to toxins(Pelka *et al.*, 2000). Brine shrimp lethality test was first suggested by Meyer *et al.*, 1982 but was after sometime improved by McLaughlin et al, 1991. The technique involves the exposure of 48h old brine shrimp larvae (nauplii) to various concentrations of the plant extracts, then counting the number of dead and live nauplii. The ratio of the dead nauplii (immobile) and live nauplii (mobile) in comparison with controls (positive and negative) is used to approximate the cytotoxicity of the plant extract (Pelka *et al.*, 2000). The mortality rate is then calculated and the LC_{50} values are obtained.

This assay is simple, rapid, cost effective, accurate and convenient. It also requires a small amount of the plant extract (McLaughlin *et al.*, 1991). Since its discovery brine shrimp bioassay has proved useful in the detection of heavy metal, fungal toxins, cyanobacterial toxins , detection of pesticide, plant toxins and testing of the cytotoxicity of plant material and dental products

(McLaughlin *et al.*, 1991). The brine shrimp eggs are cheaply available in pet stores because they are used as food for fish. These eggs can remain viable for many years when kept in a dry place. The eggs hatch easily within 24-48 hours giving a big number of larvae than can be used to test may plant extracts (Pelka *et al.*, 2000). This method has been used for a number of year to assess the cytotoxicity of medicinal plants in Kenya, Tanzania, India, and Jordan (Rukenya, 2014).

2.4.2 Antimicrobial susceptibility testing

The antimicrobial activity of the plant extract is measured by the extent to which it inhibited the growth of microbial population. In testing the activity of the extracts various conditions need to be fulfilled, the plant extract has to come into contact with the microbial cell wall, the required conditions for microbial cell growth should be provided and the best criteria for determining the extent of growth should be selected (Hamburger and Hostettmann, 1991). The mostly used *in vitro* antimicrobial testing techniques include, dilution, diffusion, optical density and impedance methods (Rukenya, 2014). Dilution techniques have been found to give accurate and quantitative results which are not comparable with other methods (Manou *et al.*, 1998)**.**

2.5 Phytochemistry of medicinal plants

Phytochemical components are naturally occurring chemicals compounds and bio actives that are produced by plants to help them fight against bacteria, fungi, and viruses. The phytochemicals could be synthesized in the living cells of the plant through a series of complex and sometimes simple chemical reactions (Egamberdieva, 2017). All the plants as most of the autotropic organisms have two ways of producing and accumulating macronutrients of great nutritional and medicinal value; that is primary and secondary metabolism (Mendoza and Escamilla, 2018). Primary metabolites are the common compounds produced by all plants and are required for the functioning of the organism in which they are made. The primary metabolites may include amino

acids, sugars, lipids and nucleotides (Saxena *et al.*, 2013) On the other hand, secondary metabolites are the organic molecules that do not seem to have a straight forward benefit on the respiratory processes, photosynthetic process, nutrient absorption, active transport and the synthesis of carbohydrates, lipids and proteins (Mendoza and Escamilla, 2018). The secondary metabolites are the phytochemicals constituents of the plant and their biosynthesis is shown in figure 2.1 below.

Figure 2.1 A demonstration of the biosynthesis of plant secondary metabolites (Saxena *et al.*, 2013).

The phytonutrients function to shield the plant from infections and destruction from ecological dangers like Stress, UV rays, pathogenic hostilities, pollution and drought. They also give them the beautiful colors and aromatic scent and flavors they possess (Mendoza and Escamilla, 2018). In many recent studies it's been noted that phytochemicals have a very significant role in the maintaining the general well-being of animals and humans by protecting them from ailments (Saxena *et al.*, 2013). More than 4000 phytochemicals have been recorded most of which possess medicinal properties such as enzyme detoxification, antioxidant activity, hormone metabolism, stimulation of hormones, anticancer activity, modulation of hormones, decrease of platelet aggregation, disease preventing activities and immune boosting components *(Saxena et al*., 2013; Egamberdieva, 2017).

Various natural foods are an ample reservoir of potent phytonutrients like the nuts, whole grains, herbs, legumes, fruits, vegetables, spices and fungi. The phytochemicals are found accumulated in different ratios in various plant parts, like in the leaves, stems, roots, flowers and seeds (Saxena *et al*., 2013). These phytochemicals are the key determinants of the medicinal value of plants which are gauged by the positive physiological effects the cause in the human body systems (Egamberdieva, 2017). Each plant species synthesize an exceedingly diverse variety of phytochemical components which present an immense potential of the unearthing and developing new drugs. (Egamberdieva, 2017). Some of the phytochemicals produced by plants that have great medicinal benefits include alkaloids, terpenoids, tannins, saponins, flavonoids, steroids, and glycosides (Saxena *et al.*, 2013) as shown in table 2.2 below.

Table 2.2: Phytochemicals in plant with their biological functions(Muia *et al.*, 2020)

Medicinal value	**Phytochemical group**	**Biological function**
Antimicrobial	Phenols, alkaloids, Terpenoids	Inhibitors of microorganism and prevention of fungal infections
Antioxidants	Polyphenols, carotenoids, ascorbic acid, tocopherols, flavonoids	Inhibition of lip peroxidation, free radical quenching
Detoxifying agents	Flavones, carotenoids, coumarins, phenols, tocopherols, reductive acids, indoles, aromatic isothiocyanates, phytosterols, cyanates, retinoids	Inhibition of carcinogen actuation, inhibitors of tumorigenesis, inducers of drug inhibiting carcinogens
Anticancer	Flavonoids, polyphenols carotenoids, curcumins	Inhibitors of tumor, antimetastatic activity
Nonstarch polysaccharides (NSP)	Lignin, pectin, mucilage, gums, cellulose, hemicellulose	Delay in nutrient absorption water holding capacity, binding toxins
Neuropharmaceuticals	Alkaloids, terpenoids, biogenic amines	Cancer chemoprevention, antioxidants, treatment of nervous system disorders

2.6 Phytochemical classes

2.6.1 Phenolics

This is a class of phytochemicals that have at least one phenol group in their chemical structure.

They include flavonoids, polyphenols and phenolic acids. Phenolics have a hydroxy (OH) group

directly bonded to the aromatic hydrocarbon group. In all the plant kingdom phenolic phytochemicals have the greatest percentage of occurrence and they are popular because they exhibit a significant antioxidative property that guard the human body against free radical mediated disease processes like cancer (Saxena *et al.*, 2013). Recent research has demonstrated that phenolic phytochemicals have numerous biological activities such as anti-inflammatory, anticancer, prevention against osteoporosis, neurodegenerative disorders and diabetes mellitus (Egamberdieva, 2017).

2.6.2 Flavonoids

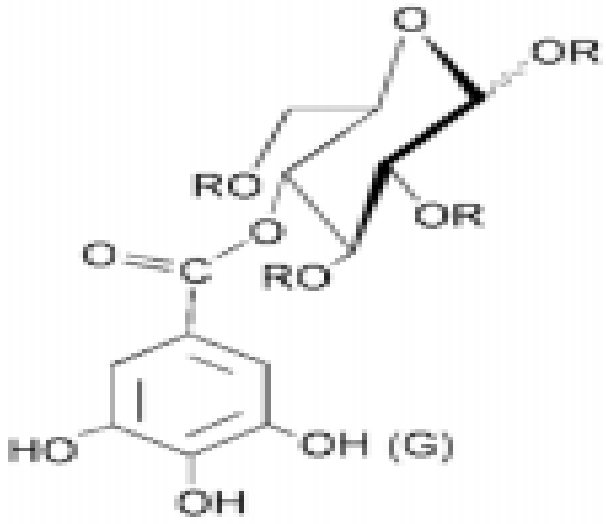
Flavonoids are the plant's secondary metabolites responsible for their flavor , color and antioxidant activity that protects them from unfavorable environmental conditions (Chaves *et al.*, 2020). They represent the largest group of phenolics that has been given much research precedence because of their rich source of biological and pharmacological activities like anti-inflammatory, cytotoxicity, estrogenic, anti-allergenic, enzyme inhibition, immunomodulatory, antimicrobial and anti-tumor properties. They also possess wide range of bioactive components that protect biological systems against deleterious effects like oxidative process and formation of free radicles (Mendoza and Escamilla, 2018).

Flavonoids could be polar or non-polar in nature. The polar flavonoids are extracted by a mixture of water and ethanol, while the less polar flavonoids like flavanols, flavones, aglycones , isoflavones and methylated flavones are well extracted by the use of organic solvents like acetone, hexane, dichloromethane, diethyl ether, ethyl acetate and chloroform (Chaves *et al.*, 2020).

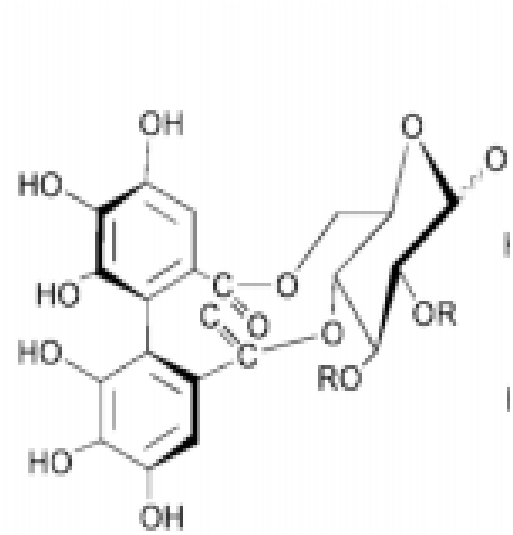
2.6.3 Tannins

They are high molecular weight phenolic compounds that are capable of forming complexes with proteins, alkaloids, carbohydrates and minerals (Chung *et al.*, 1998). Tannins can be divided into four categories according to their structural characteristics as ellagitannins, complex tannins, condensed tannins and gallotannins

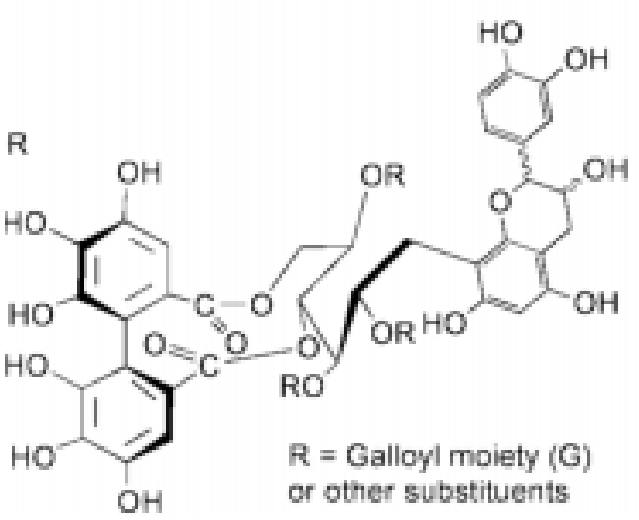
Gallo tannins are distinguished by the presence of galloyl units bound to different polyol-, catechin or triterpenoid components. Ellagitannins are those tannins in whose structure not less than 2 galloyl sections containing C-C are joined together. They also have the glycosidic bond linked to a catechin component. Complex tannins have at least a catechin component with a glycosidic bond linked to a gallo tannin or an ellagitannin unit. Condensed tannins compose of polymeric and oligomeric proanthocyanins that are formed by a bond of C-4 of one catechin with C-6 or C-8 of the next monomeric catechin. Tannins are useful sin the medical world as astringents, antidiarrheal anti-inflammatory, hemostatic, antiseptic, antioxidant and diuretic agents. They are also used in the treatment tumors that occur along the upper gastrointestinal tract (Saxena *et al*., 2013).



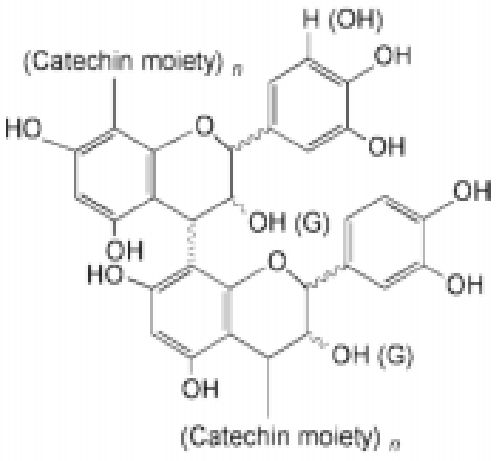
Gallo tannin



Ellagitannins



Complex tannin



Condensed tannin

Figure 2.1: Basic structures of classes of plant tannins(Chung *et al.*, 1998).

2.6.4 Alkaloids

They are basic, naturally occurring phytochemicals that have heterocyclic nitrogen atoms in their organic structure. Alkaloids are the most bitter substance in nature. They are sparingly soluble in water and well soluble in alcohol. Alkaloids could be classified according to the heterocyclic ring they contain as quinoline alkaloids like isoquinoline and quinine alkaloids such as heroine and codeine; pyridine alkaloids like piperine and coniine; Pyrrolidine alkaloids like hygrine; pyrrolidine -pyridine alkaloids like myosmine and nicotine and lastly pyridine -piperidine alkaloids like anabasine. (Saxena *et al*., 2013).

Alkaloids are potent antimicrobial agents and for this reason they are used in the pharmaceutical field for prophylaxis and treatment against microorganisms of bacterial and fungal origin. The chemicals produced by the alkaloids also perform an allelopathic function to ensure the plants' growth and survival against insects and herbivores. Most of these naturally occurring alkaloids have pharmacological properties such as antimalarial activity in quinine, anti-cancer activity in vincristine and vinblastine antihypertensive activity in indole alkaloids and antiarrhythmic activity in quinidine. Some alkaloids from opium poppy plant like codeine and morphine have been shown to have a stimulant effect and therefore they are used as analgesics. The anti- plasmodial activity in quinine has made it useful in the treatment of malaria a common tropical disease (Saxena *et al.*, 2013).

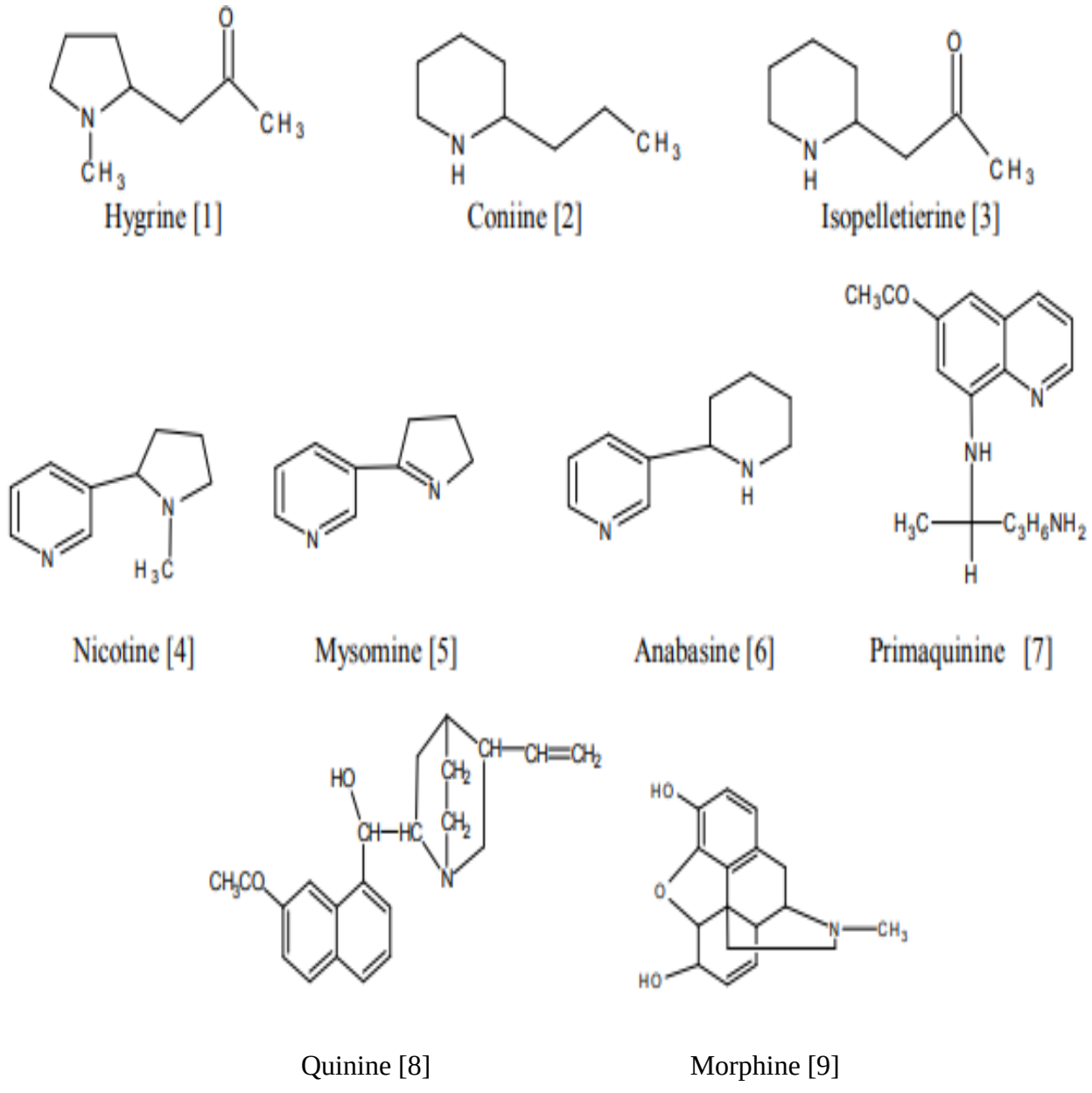


Figure 2.2: Chemical structures of naturally occurring alkaloids (Saxena et al, 2013).

2.6.5 Terpenes

Terpenoids or terpenes are a group of naturally occurring natural products that have been derived from five carbon isoprene units. Terpenoids and terpenes are the fundamental components of essential oils in plants. They are normally characterized with strong scent which serve to guard the plants against parasites and herbivores. They are either synthesized through the mevalonic acid pathway or glyceraldehyde phosphate pyruvic pathway (Mendoza and Escamilla, 2018). Terpenoids are categorized in regard to the number of isoprene components in them as shown in table 2.3 below.

Table 2.3: The classification of terpenes (Mendoza and Escamilla, 2018)

Isoprene units ***N***	**Carbon atom** ***N***	**Group**	**Example**
1	5	Hemi-terpenes	Isoprene
2	10	Mono- terpenes	thymol
3	15	Sesqui-terpenes	α-cadinene
4	20	di-terpenes	Taxol
6	30	Tri-terpenes	B-amyrin
8	40	Tetra-terpenes	B- carotene
9-30000	>40	Poly-terpenes	Rubber

Terpenoids are used widely because of their medicinal properties like antimalarial (artemisinin), antimicrobial, anti-ulcer, hepaticidal, antidiuretic (glycyrrhizin) and anticarcinogenic activity (Escamilla *et al*., 2018).

2.6.6 Saponins

Saponins are a class of secondary metabolites that have great differences in their chemical structures and form stable forms in aqueous solutions. Saponins are found in many higher plant parts like in the roots, tubers, leaves and seeds and blooms. Saponins are known as plant glycosides that contain a steroid, steroidal alkaloid or triterpene core structure that is also known as aglycone. A steroidal aglycone has 27 carbon atoms while a triterpenoid aglycone has 30 carbon atoms. Thus, the difference in saponins is a result of variations in the aglycone structure and the number of the sugar side chains they contain. Recent studies have indicated that saponins possess immunostimulant, hypocholesterolemia and anticarcinogenic properties. Saponins are also reported to significantly alter the feed intake, reproduction and growth of animals (Bachran *et al.*, 2008).

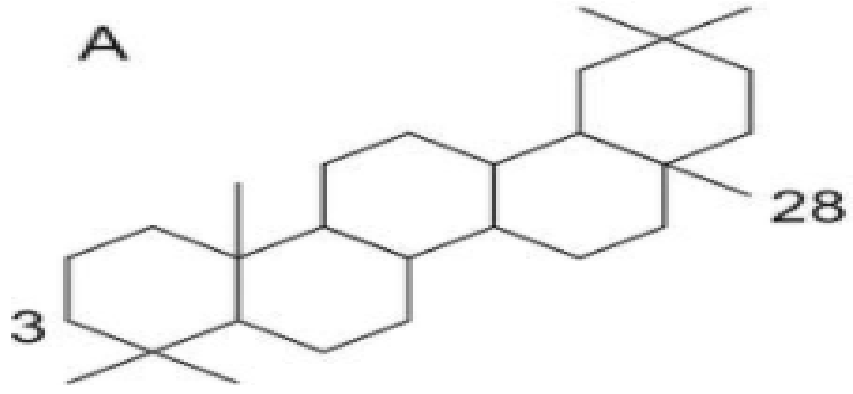
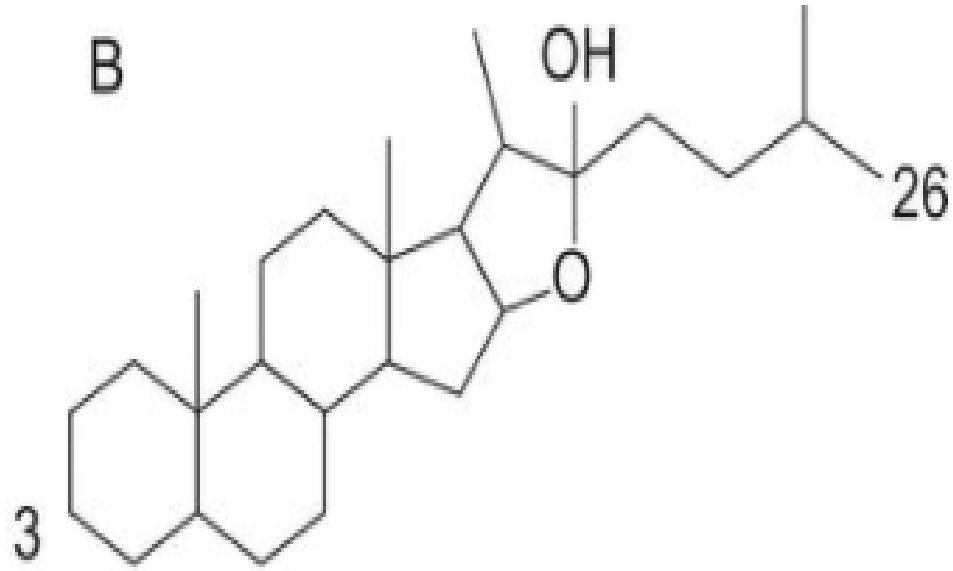


Figure 2.3: (A) Structure of a terpenoidal saponin (Bachran *et al.*, 2008).



(B) Structure of a steroidal saponin(Bachran *et al.*, 2008).

2.7 Literature on *C. dichogamus*

2.7.1 Morphology and geographical occurrences

C. dichogamus, a species of *Croton* genus is pyramidal twig or tree with symmetrical, frequent branching or sometimes straggling crown (African plant database, 2012). *C. dichogamus* grows in the wild and sometimes cultivated in tropical and subtropical regions (Fern, 2020). The twig, *C. dichogamus* displays a trailing wreath which has repeated branching. Its foliage is glossy, flat with fur underneath and the top side is normally brownish yellow in color (Fern, 2020,) Flowers of *C. dichogamus* are monoicous with each flower having 20 stamens that contain 6 sepals. There are no petals in this flower but the pistils contain five sepals, the portions are linear and are covered with fur, patterns are bipartite and the ovary is protected by a circular tabular scale (Aldhaher *et al*., 2016) . Fruits of *Croton dichogamus* are circular, divided into three parts and are a little bigger than a pea (Fern, 2020). The height of *Croton dichogamus* is approximately 7.5 meters but the

shrub is sometimes 2-5meters as seen in East tropical African countries like Kenya, Ethiopia, Uganda, Rwanda, Tanzania, and Mozambique (Fern, 2020). The habitat of *Croton dichogamus* includes bushlands, thickets, rocky grounds, dry forests, acacia woodlands, limestones, porous soils and lava where it many times form thick stands at an altitude from 550-1800 meters (Dadson, 2012; African plant database, 2012).

2.7.2 Traditional uses of *C. dichogamus*

The dried roots of *C. dichogamus* in Tanzania, are ground into fine powder then added to porridge for patients that have respiratory illnesses like tuberculosis because the twig is believed to relieve chest conditions (Hedberg *et al.*, 1983 ; Matara *et al.*, 2021). Fresh leaves of *C. dichogamus* are useful in the treatment of malaria, as a tonic and as a dietary adjuvant (Hedberg *et al.*, 1983) . Inhaling the smoke of the burning leaves of *C.dichogamus* is believed to act as an antipyretic to most patients (Hedberg *et al*., 1983 ; Mohagheghzadeh *et al.*, 2006). Eastern African communities in Tanzania like the Maasai and Batemi get rid of cardiovascular diseases induced by a high level of cholesterol by including *C. dichogamus* in all their soups and dairy products (Johns *et al.*, 1999). The agricultural and pastoral groups in Tanzania like the Mbulu have discovered that *C. dichogamus* is a reliable pesticide in preventing Bambara groundnuts from destruction by storage pests (Tlankka *et al*., 2020). The same community also uses stem barks of *C. dichogamus* as an antibiotic in the management of urinary tract infection (UTI) and tooth complications (Tlankka *et al*., 2020 ; Qwarse,2018).

In Kenya, the Samburu community use *C. dichogamus* as analgesic to manage stomach and chest pains (Aldhaher *et al*., 2017). The Loitoktok community use *C. dichogamus* in the treatment of

gonorrhea and arthritis (Muthee *et al.*, 2011). Along the lake region, the Luo community who call it 'Rachar' use the roots of *C. dichogamus* to relieve respiratory illnesses like asthma, pneumonia and persistent cough (Mailu *et al.,* 2020) . In Mutomo village, Kitui County, an infusion of the fresh leaves and stem barks of *C. dichogamus* are drunk to cure backache, malaria, stomach pains, chest pains, edema, fever and persistent coughs (Mutie *et al.,* 2020) . A concentrate of the fresh roots of *C. dichogamus* is used to treat reproductive disorders like infertility and impotence (Mutie *et al.,* 2020). Polio, chest pains and gonorrhea are managed in Narok county by drinking the juice from the fresh roots of *C. dichogamus* (Kigen *et al.*, 2019). Among the Marakwet group of people in Kenya, the boiled aerial parts together with the roots cure oral thrash, wheezing and abdominal cramps. In Ethiopia roots of *C.dichogamus* are used to make a paste for vaginal application to improve female reproductivity (Mutie *et al.*, 2020).

2.7.3 Bioactivity and bioactive principles

A number of secondary metabolites have been isolated from *Croton dichogamus* and identified using spectroscopic results (Aldhaher *et al.*, 2017).The roots, stems and leaves have been shown to contain flavonoids, tannins, saponins and terpenoids. (Johns *et al.,* 1999 ; Magadula, 2012) , while steroids, and flavonoids are also present in other plant parts (Aldhaher *et al.*, 2017; Johns *et al.*, 1999). The leaves have demonstrated a fair distribution of tannins, flavonoids and saponins while the stems contain a moderate composition of all the photochemical components.

Terpenes are the chief and most beneficial active ingredients found in *C. dichogamus*. The phytochemical screening of all the plant parts of *Croton dichogamus* indicated that terpenes had the highest percentage yield as compared to the other phytochemicals evaluated like tannins, alkaloids, flavonoids, phenols, steroids and saponins (Magadula, 2012). Studies have shown that *C.dichogamus* has approximately 20 groups of terpenes that display implausible biological

properties (Aldhaher *et al*., 2017 ; Aldhaher *et al*., 2016). Most of these terpene are from the class of halimanes, crotofolanes and neoclerodane moieties (Xu *et al*., 2018 ; Salatino *et al.*, 2007) . Nearly fifteen more terpenes which include 1 enantiomer, of a known sesquiterpenoids, 4 sesquiterpenoids, 2 Ent- halimane diterpenoids, 3 crotofolane diterpenoids, and 1 triterpenoid have also been isolated from *C. dichogamus* (Aldhaher *et al*., 2016).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study area

Fresh roots of *Croton dichogamus* were collected in October-November from the wild places in Kajulu, Kolwa and Manyatta wards of Kisumu East Sub County, Kisumu county, where the shrub is used in the treatment of Respiratory illnesses like Asthma and Pneumonia (Mailu *et al.*, 2020). Kisumu East subcounty covers an area of 135.90km^2 and is located at Longitudes 33˚20 East and 35˚20 East and latitudes 0˚ 50 south and 0˚ 20 South (Kisumu County Integrated Development plan, 2018). The administrative wards of Kisumu East Subcounty include Kolwa Central, Nyalenda A, Manyatta B, Kajulu East, Kajulu west and Kolwa East as shown in figure 3.1 below (Mailu *et al*.,2020).

The annual maximum temperature in Kisumu East Subcounty ranges between 25℃ to 33℃ while the annual minimum temperature ranges between 16˚C to 18˚C. The area receives an annual rainfall of 1300mm approximately which is evenly and adequately distributed with two peak fall in march-May and September-November (Kisumu County Integrated Development plan, 2018). The county is also endowed with a fresh water lake (Victoria) and seven permanent rivers which provide a potential for development of blue economy. The population of Kisumu East Subcounty is about 189 730 with fish farming, livestock farming and extensive sugar production being their major economic activity (Kisumu County Integrated Development plan, 2018). The root parts of *Croton dichogamus* were acquired from Kisumu County (Kisumu East subcounty). The laboratory work was done at the PHPT Department.

Kisumu East Subcounty.

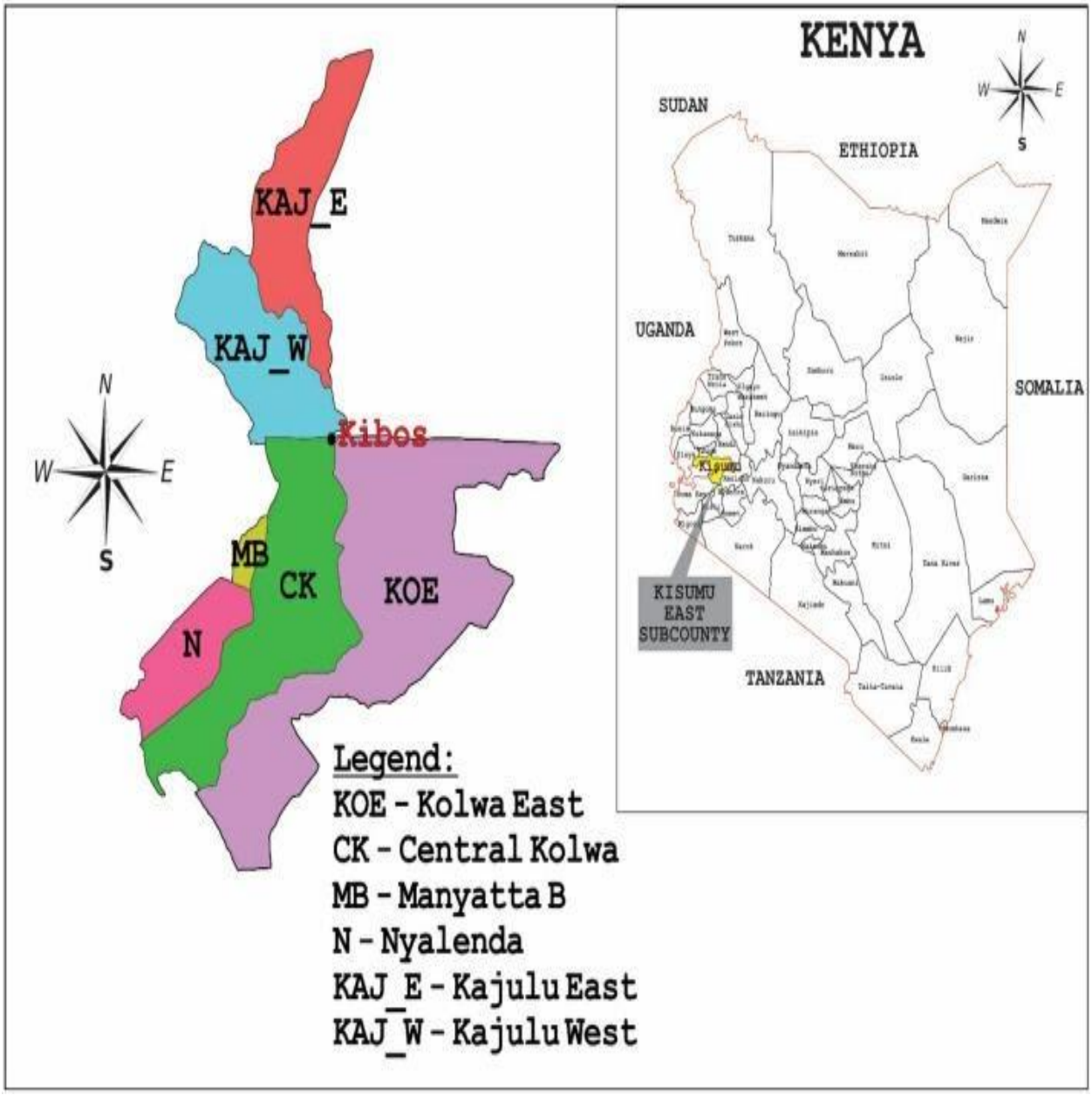


Figure 3.1: Showing Map of Kisumu East Subcounty and its wards (Mailu *et al*., 2020).

3.3 Collection and identification of plant material

Croton dichogamus (figure 3.2) fresh roots were sourced from Kisumu County (34˚54'59.99"E, 0˚ 14' 60.00"N), the area indicates in the map (figure3.1) in the month of October-November 2020, at the beginning of the study. The voucher specimen of *C. dichogamus* was deposited at the East African Herbarium located at the Kenyan National Museum grounds. The sample was identified as *Croton dichogamus* with NMK/BOT/CTX.1/2.as the reference number. The collected roots were then put in bags and taken to PHPT department, The University of Nairobi for further processing.

Figure 3.2: A picture of *Croton dichogamus* Pax taken by Dorine Matara

3.4 Preparation of plant extract

The roots of *C. dichogamus* were washed with running tap water and chopped with a knife into small pieces. The tiny root pieces were then air dried at normal temperature in a dust free well aerated room at the PHPT department for about 3 weeks. The dry roots were then milled into powder by an electric milling machine. The ground plant material was stored in sterilized, clean zip-lock, air-tight envelopes in portions of 500 grams, then kept in coldish, dry, ventilated place away from direct sunlight.

3.5 Aqueous Extract

Cold maceration method was used for extraction as described by Gakuya, 2001. 500gm of root powder of C. *dichogamus* was placed in an extraction jar and 2 liters of distilled water were added into the jar. This was followed by a thorough shaking of the combine for 72 hours in the morning and evening hours. After 3 days (72 hours) the macerate was filtered using a Whatman filter paper. The resulting filtrate was freeze dried for 24 hours. A light brown fine powder was formed that weighed 30.25gm. The aqueous extract which had a percentage yield of 6.05%w/w was kept safe in a bottle and refrigerated at 4˚C awaiting use.

3.6 Hydroethanolic (50% Ethanol) extract

A Mettler digital weighing balance was used to weigh about 500gm of powder of *Croton dichogamus*, which was placed into and extraction glass container. Exactly, 1 liter of distilled water was added to the jar followed by 1 liter of ethanol and allowed to soak for 72 hours with thorough quivering each morning and evening to increase the efficiency of extraction. The combine was then passed through a Whatman filter paper no.1. The resulting solution was then transferred into a conical flask and evaporated using a Rota evaporator whose operating temperature was 40˚C to

remove excess ethanol solvent. The resulting content was lyophilized to remove excess water and this produced a brownish extract weighing 24.50gm (Nguta *et al*.,2016). The extract was transferred into an amber colored bottle which was kept in the fridge at 4˚C.

3.7 Acetonic extract

Eight hundred grams of *C. dichogamus* was taken in an extraction jar and 2 liters of acetone were added gradually with thorough quivering to soak the powder uniformly. The solution was stirred continuously using a magnetic stirrer for three days. A number one Whatman filter paper was used to filter the combine. The filtrate was taken into a round bottomed flask and evaporated using a rotary evaporator set at 40˚C for about 5 hours. The resulting content was then placed into an amber glass container with a punctured foil. It was afterward kept on a warm sand bath for complete drying so result to a substance of constant consistency weighing 10.34 gm.

The acetone extract produced the lowest yield of extract that necessitated a second extraction. The two extracts gave 20.68gm of dry powder(Nguta *et al.,* 2016).

3.8 Ethical consideration

This research work was approved by the "Biosafety, Animal Care and Use Committee (BACUC) of the Faculty of Veterinary Medicine, University of Nairobi. A reference number **FVM BACC/2021/282** was assigned. A research permit was also issued from National Commissioner for Science and Technology Innovation (NACOSTI) with reference number of **913964.**

3.9 Antimicrobial determination

3.9.1 Organisms

Five microorganisms (4bacterial strains and a fungal organism) were acquired from the bacteriology Laboratory, in the PHPT department at the University of Nairobi for antimicrobial susceptibility testing. There details are mentioned in the table 3.1 below.

Table 3.3 Microorganisms used in the antimicrobial susceptibility test

Name of microbe	Organism group	Gram stain	Organism ref number
Candida albicans	fungus	-	ATCC 10223
Escherichia coli	bacteria	negative	ATCC 25922
Staphylococcus aureus	bacteria	positive	ATCC 25923
Pseudomonas aeruginosa	bacteria	negative	ATCC27853
Bacillus cereus	bacteria	positive	ATCC11778

3.9.2 Microbroth dilution technique

The microbroth dilution method as described by Muia *et al.*, 2020 was used. The bacterial organisms, *S. aureus, E. coli, B. cereus and P. aeruginosa* were cultured overnight in an incubator set at 37˚C on blood agar. The fungal organism *C. albicans* was also cultured overnight on nutrient agar at room temperature. Cultures were then suspended in 10ml sterile sodium chloride solution (physiological saline) which was kept at a concentration same as that of 0.5 MacFarland standards. This concentration was confirmed using MacFarland tubes as MacFarland1(NCCLS,1997). Stock solutions of each extract (hydroethanolic, acetonic and aqueous) including the positive controls (cephalexin and fluconazole) were prepped by dissolving 1000mg of the extract in 1ml DMSO (dimethyl sulfoxide) then adding 3ml of sterilized molten Mueller Hinton Broth (MHB). The stock

solutions had a strength of 250mg/ml. Five culture tubes of 2ml sterile MHB were then organized in duplicates. Serial dilutions which were two-fold were conducted from the stock solutions to make concentrations of 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml and 7.8mg/ml (Debalke *et al.*, 2018). About 0.1ml of each the five microorganisms were taken using a 1ml micropipette and inoculated into each tube of dilute plant extract and positive control. The tubes containing the fungal organism were placed at room temperature for 24 hours while the tubes containing the bacterial organisms were incubated at 37˚C for 24 hours. Observations were made from the tubes, where the lowest concentration of the plant extract that showed an inhibitory activity resulting to absence of growth (no turbidity) of microbes in the tube was indicated as the Minimum inhibitory concentration (MIC) value of the extract. A negative control tube was set by having plant extracts without microorganism. Positive control tubes had the microorganisms with the broad spectrum, commercially available drugs namely Cephalexin 500mg for the bacterial microorganisms and Fluconazole 200mg for the fungal microorganism. The Minimum bactericidal concentration (MBC) was determined by taking all the tubes that showed no visible bacterial growth (absence of turbidity) and culturing them aseptically in sterile molten Mueller Hinton Agar (MHA) using pour plate technique. Plates having bacterial organisms were incubated at 37˚C for 24 hours while plates with the fungal organism were placed at room temperature for 24 hours. MBC was recorded as the value with the least concentration that showed no bacterial growth. Normally MBC is defined as the least concentration where 99.9% or more of the previous inoculums are dead. This experiment was done in triplicates.

3.9.3 Agar well diffusion technique

Agar well diffusion technique as described in the Clinical Laboratory Standards Institute (CLSI) was used where sterile MHA was aseptically poured into petri dished and allowed to cool. The microorganisms (*Bacillus cereus, Escherichia coli, Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa)* were spread on MHA using a swab. A sterile cork borer was used to make hole of 7mm depth and 7mm diameter on the MHA. Using a Microtiter pipette, 0.1ml of the plant extracts of various concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml and 7.8mg/ml were added to the wells and left untouched on the bench for about an hour to ensure uniform disperse into agar. The bacterial strains were incubated for 24 hours at a temperature of 37˚C. The fungal organism was left at room temperature for 24 hours. The diameter of the zone of inhibition was measured in millimeters using a ruler. In this experiment, 1% DMSO was used as a negative control while cephalexin 500mg and Fluconazole 200mg were used as the positive controls for the bacterial and fungal organisms respectively. The experiment was conducted in triplicates and the negative and positive controls were also present.

3.10 Bioactivity of *C. dichogamus* using brine shrimp lethality assay

Standard viable eggs of brine shrimps were procured from a pet shop at Yaya center in Nairobi and preserved in a cool dry place in the PHPT department where the experiment was done. The batch number was X001M8M5IZ.

3.10.1 Hatching of the brine shrimp eggs

A marine salt solution was first prepared by taking 33gm of the marine salt in 1000ml conical flask. Water was then added to the marine salt gradually with constant stirring to hasten the dissolving of the marine salt. When all the marine salt was dissolve, more distilled water was added up to the mark of 1000ml.

A shallow rectangular plastic box with two chambers separated by a wall that had 1-2mm holes was used for hatching the brine shrimp eggs. This plastic box was filled with the marine salt solution. A rectangular hole of approximately 24cm was made on the lid of the smaller chamber to allow light to get in. Fifty milligrams(50mg) of brine shrimp eggs were taken and sprinkled in the dark chamber which had a lid covering it entirely (without a hole to illumine it). Dry yeast of about 6gm was sprinkled on the brine shrimp eggs in the dark chamber which served as food for the brine shrimps. This set up was placed under a light source of 40 watts electric bulb which was to illumine the smaller compartment that had a hole on the lid and attract the larvae (once hatched) to swim towards the lit compartment leaving the eggshells in the dingy compartment. About 48 hours were given for the hatching period (Gakuya, 2001).

3.10.2 Cytotoxicity bioassay

Exactly, 0.1gm of each extract (acetone, aqueous and hydroethanolic) and the positive control (vincristine sulphate) were taken into a volumetric flask, 1ml of DMSO was added to dissolve the extract then topped up to the 10ml mark using marine salt solution to make a stock solution (Okumu *et al.*, 2020). Thus, the strength of each stock solution was 10,000µg/ml. Vincristine sulphate was the positive control while dimethyl sulfoxide (DMSO) was the negative control in this experiment. Ten nauplii were taken from the hatching tray and placed into graduated tubes

using a Pasteur pipette. Three serial dilutions were prepared by transferring 500µl, 50µl and 5 µl of each plant extract stock solution to a set of five graduated tubes to make dilutions of 1000µg/ml, 100µg/ml and 10µg/ml respectively then the tubes were topped up to the 5ml mark using marine salt solution. For each dilution and the positive and negative controls, five graduated tubes were set. The tubes were kept at room temperature for 24 hours. Using a magnifying glass, the number of dead nauplii were counted and recorded against each concentration. For each dilution and control groups, percentage mortality was calculated. The results were analyzed using probit regression analysis and interpreted according to Meyer's criteria of cytotoxicity(Meyer *et al.*, 1982).

3.11 Phytochemical Screening.

The phytochemical composition of the root of *C. dichogamus* was identified qualitatively as demonstrated by (Trease and Evans, 2009 ; Usman *et al*, 2009 ; Visweswari *et al*, 2013).

3.11.1 Detection of Triterpenoids

About 0.5gm of the *C. dichogamus* root powder was soaked in 3ml of chloroform ($CHCL_3$). The combine was then filtered and few drops of concentrated (CONC) sulphuric acid (H2SO4) were put in the filtrate. Presence of a reddish-brown color is a positive confirmation of triterpenoids (Trease and Evans, 2009).

3.11.2 Detection of Flavonoid

About 0.5gm of the organic and aqueous crude extracts were placed in a test tube containing 5ml distilled water. The mixture was then boiled and filtered. Approximately, 3 drops of 10% ferric chloride solution were added to about 2ml of the filtrate. Appearance of a violet coloration was a positive indication of the presence of flavonoids (Trease and Evans, 2009).

3.11.3 Detection of saponins

Three milliliters of purified water were mixed with 0.5gm of the sample extracts and mixed well in a test tube. The mixture was then shaken vigorously for 2minutes. Appearance of foam which persists for 15 minutes was indicative of the presence of saponins (Trease and Evans, 2009).

3.11.4 Detection of phenols

About 2ml of distilled water was mixed with 0.3gm of the sample powder extract a test tube and warmed in water of a temperature of 40-50 ˚C for 3 minutes. To the solution, 3 drops of 5% ferric chloride were added. Occurrence of a bluish or green coloration was an indication of the presence of phenols (Usman *et al.,* 2009)**.**

3.11.5 Detection of tannins

Approximately, 0.5gm of the organic and aqueous extracts were taken and boiled in 20ml in purified water in a test tube. The solution was then filtered and 2ml of the filtrate was obtained in a test tube. To the filtrate, 3 drops of 10% ferric chloride were added. Appearance of a blackish blue color will indicate the presence of tannins (Visweswari *et al.*, 2013).

3.11.6 Detection of alkaloids

About 0.5gm of the plant extract was dissolved in 5ml of 1% hydrochloric acid then it was warmed for 5 minutes in a steam bath and filtered. About 3 drops of Dragendroff's reagent was added to 1ml of the filtrate. A reddish orange precipitation confirmed the presence of alkaloids (Trease and Evans, 2009).

3.11.7 Detection of polyuronides (Mucilage; Gums, Pectin)

Briefly, 2mls of the plant extract was mixed with 10ml of acetone. Development of a precipitate indicated the presence of polyuronides (Trease and Evans, 2009).

3.11.8 Detection of cyanogenetic glycosides

About 0.3gm of the sample was added to 2ml of chloroform. A picrate paper was then suspended inside the test tube. A fitting cork was used to close the test tube which was constantly boiled in a hot water bath for about 10 minutes. Conversion of the yellow picrate paper into red by the cyanide gas confirmed the presence of cyanogenetic glycosides (Trease and Evans, 2009).

3.11.9 Detection of Anthracenes

A portion of 0.3gm of the sample was obtained, followed by addition of 2ml of diethyl ether. Into this mixture 2ml of ammonia solution was added and allowed to react for 10 min. Appearance of a cherry red color confirmed the presence of anthracenes (Trease and Evans, 2009).

3.12 Statistical analysis

In this study, the experiments were conducted in triplicates. The data was analyzed using SPSS Version 23 for antimicrobial activity and SPSS version 20 for cytotoxicity assay. Using this software, mean, minimum, maximum and standard deviation values were obtained. Data was also subjected to inferential statistics where microbial growth inhibitions due to the various concentrations of crude extracts were compared among themselves to check for significant differences in the growth inhibitions. Since significant differences were detected from the preliminary ANOVA, Turkey HSD test using posthoc ANOVA was used to compare the means of two growth inhibitions so as to determine significance differences. A value of $P<0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Extract yield

The given quantities of *Croton dichogamus* was found to yield the amounts shown in table 4.1

Table 4.1: Yields of the *Croton dichogamus* root extracts.

Plant extract	weight of the powder (gm) in a solvent (liters)	Weight of the extract (gm)	% Yield %w/w
Aqueous extract	500gm in 2litres distilled water	30.25	6.05
Hydroethanolic extract	500gm in 1litres Ethanol & 1000litre distilled water	24.50	4.9
Acetonic Extract	800gm in 2litres acetone	10.34	1.29

The aqueous extract was a brown powder which was stable when kept at room temperature. The acetone extract was a sticky brown oily solid which melted when kept room temperature. Hydroethanolic extract gave a brown powder that was quite stable at room temperature. The aqueous extract gave the highest percentage yield while acetone gave the lowest yield.

4.2 Antimicrobial susceptibility testing of *Croton dichogamus*

4.2.1 Agar well diffusion method

4.2.1.1 Acetonic extract

The zone of inhibition of acetone extract of *C. dichogamus* against the five microbes is shown in table 4.2 and figure 4.1 below, where *B. cereus* recorded the highest zone of inhibition. The acetone extract was not active against the two gram negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) at a strength of 250mg/ml but the acetone extracts inhibited gram positive bacteria (*Staphylococcus aureus and Bacillus cereus*) and *Candida albicans* the fungal organism.

Table 4.2: Zone of inhibition (ZI) of acetone root extract of *Croton dichogamus*

CONC (mg/ml)	Zones of inhibition (mm)				
	Test organisms				
	B. cereus	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
250	17.33±0.33	12.33±0.88	0.00	0.00	15.00±0.58
125	16.33±0.88	11.33±0.33	0.00	0.00	13.67±0.33
62.5	13.67±0.88	9.67±0.33	0.00	0.00	10.67±0.33
31.25	5.67±2.8	2.67±2.67	0.00	0.00	5.33±2.67
15.6	0.00	0.00	0.00	0.00	0.00
7.81	0.00	0.00	0.00	0.00	0.00
CEPH /FLU (250mg/ml	29.67±0.33	28.88±0.33	27.67±0.67	29.67±1.67	29.67±0.33
DMSO	0.00	0.00	0.00	0.00	0.00

Key: Zones of inhibition were indicated as mean ± SEM of the triplicate experiments
0.00=meant that there was no observed inhibition
CEPH= cephalexin, the positive control for bacterial organisms
FLU =Fluconazole, the positive control for the fungal organism

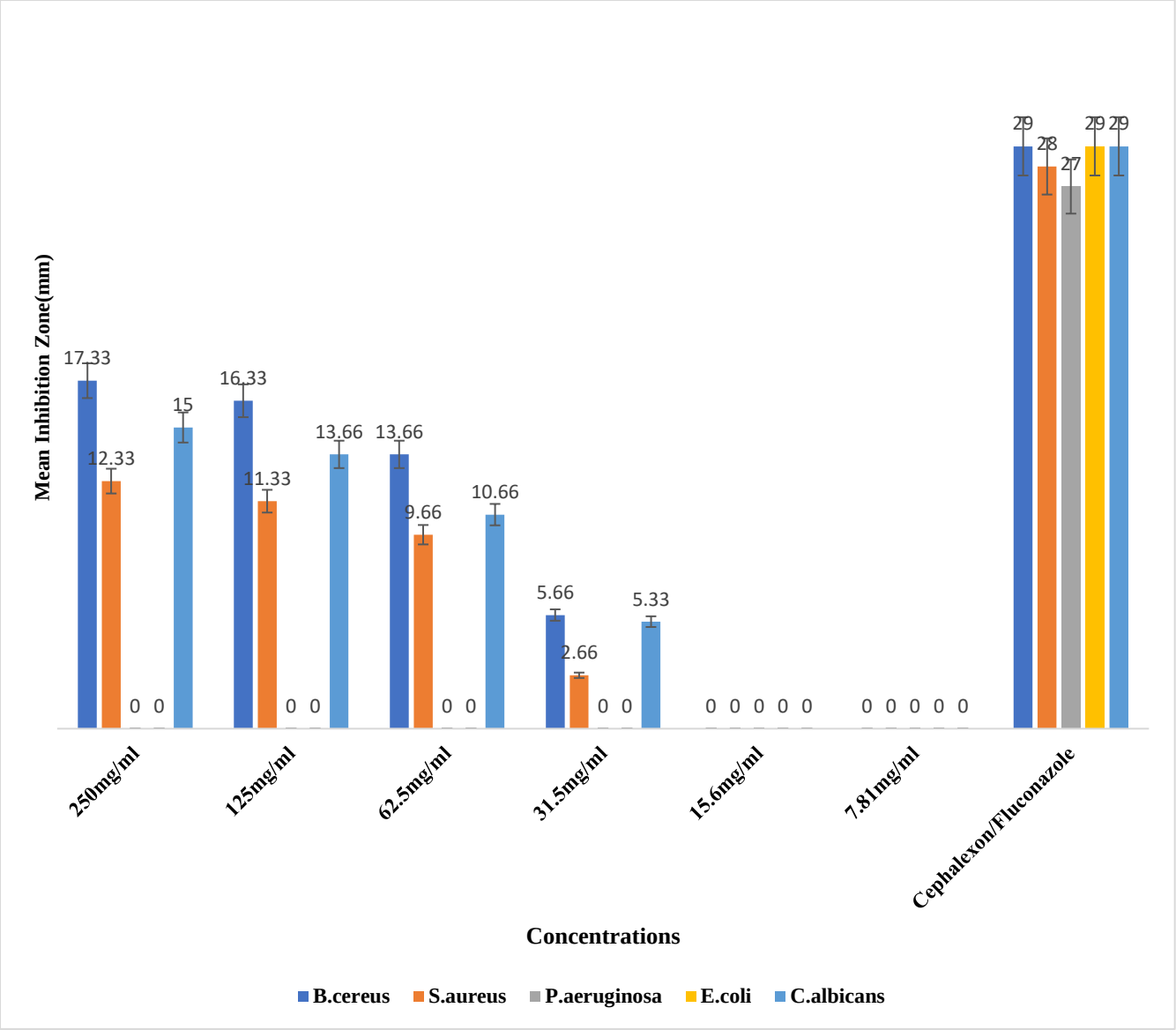


Figure 4.1: Growth inhibition of test microbes by varying concentrations of acetonic extract

From the data, there were notable statistically significant differences in the growth inhibition of the varying strengths of acetone extract against the human pathogens used. Measurable significance differences ($P < 0.05$) were observed between the growth inhibitions caused by the acetone extract and the reference drugs, conversely, there was no significance difference($p>0.05$) in the growth inhibitions caused by the three crude extracts**.** All concentrations did not inhibit *Escherichia coli and P. aeruginosa*, the diameter of zone of inhibition was zero for all concentrations thus all obtained p values were 1.00. Hence there were no significant differences between any two concentrations.

4.2.1.2 Aqueous extract

The zone of inhibition of aqueous extract of *C. dichogamus* against the five microbes is shown in table 4.3 and figure 4.2 below. The aqueous extract showed inhibition against *Candida albicans*, the fungal organism plus the gram-positive bacterial strains (*B. cereus*, *S. aureus*). However, the aqueous extract was not active against *Pseudomonas aeruginosa* and *Escherichia coli*, the gram-negative bacteria even at a strength of 250mg/ml.

Table 4.3: Diameter of the zones of inhibitions of the aqueous extract of *C. dichogamus*

CONC (mg/ml)	Diameter of zones of inhibition (mm) Test organisms				
	B. cereus	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
250	12.83±0.93	10.67±0.44	0.00	0.00	9.33±0.33
125	10.33±0.33	9.33±60	0.00	0.00	8.33±0.33
62.5	8.50±0.50	8.00±0.00	0.00	0.00	5.33±2.67
31.25	2.50±2.50	2.50±0.00	0.00	0.00	2.50±2.50
15.6	0.00	0.00	0.00	0.00	0.00
7.81	0.00	0.00	0.00	0.00	0.00
CEPH/FLU (250mg/ml)	29.67±0.33	28.33±2.19	27.33±0.33	28.67±2.19	29.00±0.00
DMSO	0.00	0.00	0.00	0.00	0.00

Key: Zones of inhibition were indicated as mean ± SEM of the three experiments
0.00= meant that there was no observed inhibition
CEPH= Cephalexin, the positive control for bacterial organisms
FLU =Fluconazole, the positive control for the fungal organism
CONC= Concentration
DMSO=Dimethyl sulfoxide

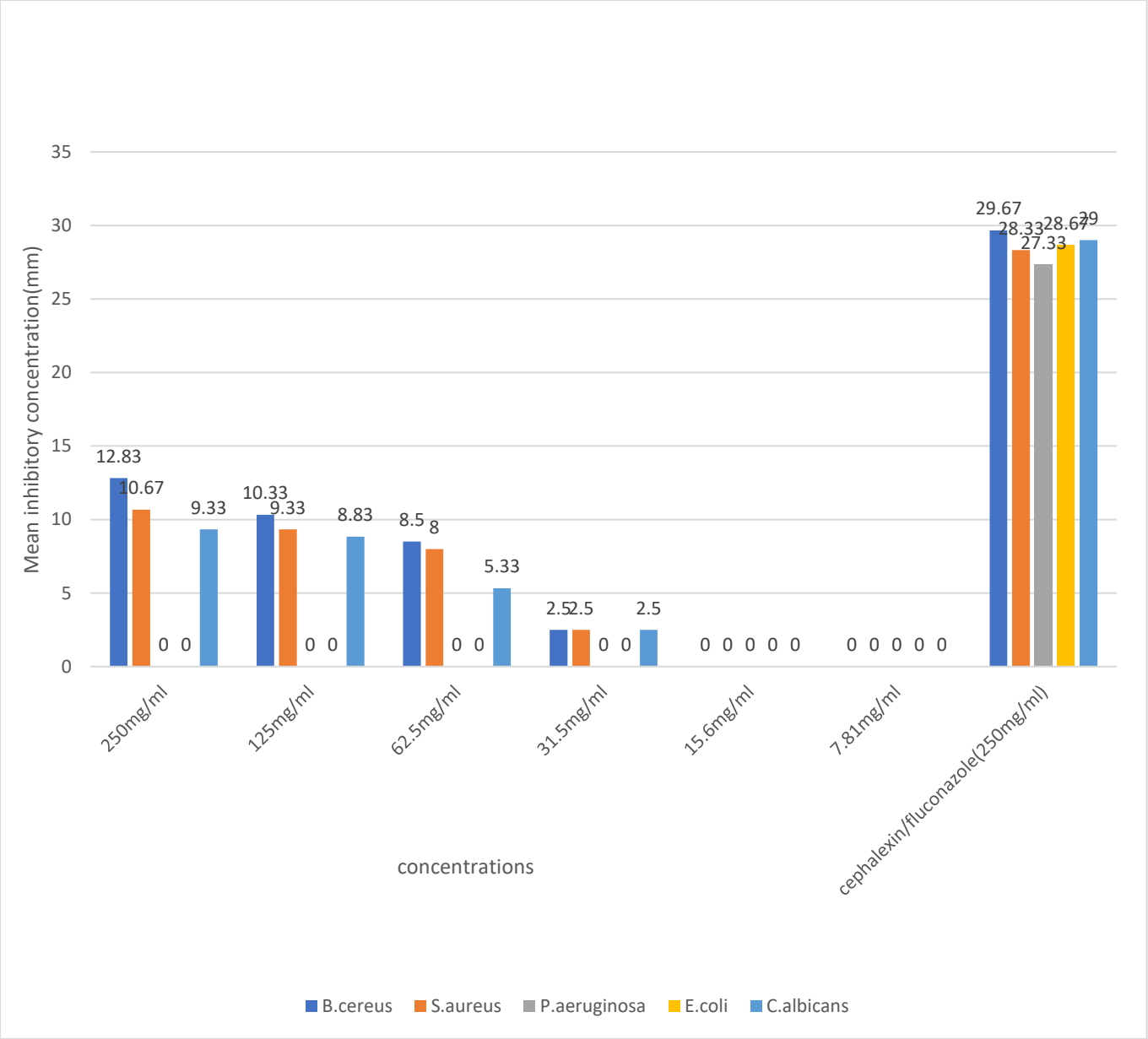


Figure 4. 2: Growth inhibition of test microbes by varying concentrations of aqueous extract

The various concentrations of aqueous extract had statistically significant differences in terms of growth inhibitions under each microbe investigated. There was statistically significant difference ($p>0.05$) between inhibitions caused by aqueous extract and the reference drugs however there was no significance difference in the growth inhibitions caused by the three crude extracts**.** All concentrations did not inhibit *E. coli* and *P. aeruginosa*, the diameter of zone of inhibition was zero for all concentrations thus all obtained p values were 1.00. Hence there was no significant differences between any two concentrations.

4.2.1.3 Hydroethanolic extract

The zone of inhibition of hydroethanolic extract of *C. dichogamus* against the five microbes is shown in table 4.4 and figure 4.3 below. *B. cereus* recorded the highest diameter of inhibition while the hydroethanolic extract was not active against *Pseudomonas aeruginosa* and *Escherichia coli*, the gram-negative bacteria, at a strength of 250mg/ml.

Table 4.4: Zone of inhibition (ZI) of hydroethanolic root extract of *Croton dichogamus*

Concentration mg/ml	Zone of inhibition (mm) Test organisms					
	B. cereus	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	
250	14.33±0.33	11.50±0.29	0.00	0.00	12.00±0.57	
125	12.33±0.33	10.33±0.33	0.00	0.00	10.33±0.33	
62.5	9.50±1.04	8.17±0.44	0.00	0.00	8.33±0.33	
31.25	5.33±2.68	0.00	0.00	0.00	5.17±2.59	
15.6	0.00	0.00	0.00	0.00	0.00	
7.81	0.00	0.00	0.00	0.00	0.00	
CEPH/FLU (250mg/ml)	30.67±0.33	30.33±1.45	28.33±0.88	26.67±0.88	29.33±0.67	
DMSO		0.00	0.00	0.00	0.00	0.00

Key: Zones of inhibition were indicated as mean ± SEM of the three experiments
0.00 =meant that there was no observed inhibition
CEPH= cephalexin, the positive control for bacterial organisms
FLU =Fluconazole, the positive control for the fungal organism

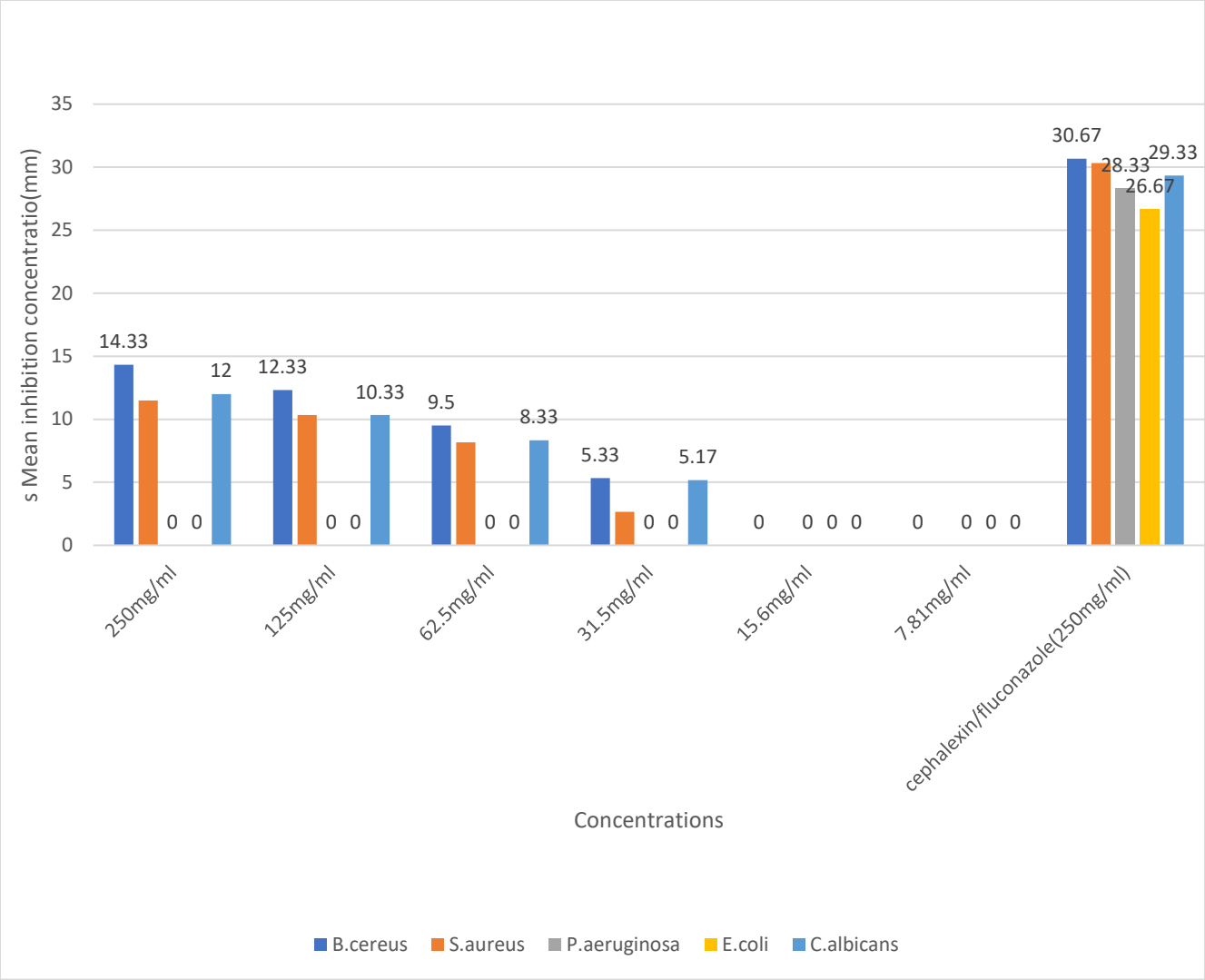


Figure 4. 3: Growth inhibition of test microbes by varying concentrations of hydroethanolic extract

The results showed statistically significant differences in terms of growth inhibitions among the various concentrations of hydroethanolic extract under each microbe investigated. Notable significance differences ($P<0.05$) were seen among the growth inhibitions caused by hydroethanolic extract and control drugs while no significant differences were seen in growth inhibitions caused by the three-crude extract. All concentrations did not inhibit *Escherichia coli* and *Pseudomonas aeruginosa* therefore their diameter of the zone of inhibition was zero for all concentrations thus all obtained p values were 1.00. Hence there was no significant differences between any two-concentration having *P. aeruginosa* and *E. coli.*

Table 4.5: summary of the zones of inhibition of *Croton dichogamus* crude root extracts against five microbes at various concentrations

Microorganisms	conc	Diameter of zones of inhibition (mm)				
	Mg/ml	Acetonic extract	50% Ethanol extract	Aqueous extract	Positive Control Cephalexin	Negative control DMSO
Bacillus cereus	250	17.33±0.33	14.33±0.33	12.83±0.93	29.67±2.48	0.00±0.00
	125	16.33±0.88	12.33±0.33	10.33±0.33		
	62.5	13.67±0.88	9.5±1.04	8.50±0.5		
	31.25	5.67±2.85	5.33±2.68	2.50±2.50		
	15.63	0.00±0.00	0.00±0.00	0.00±0.00		
	7.81	0.00±0.00	0.00±0.00	0.00±0.00		
Staphylococcus	250	12.33±0.88	11.5±0.29	10.67±0.44	28.67±0.58	0.00±0.00
Aureus	125	11.33±0.33	10.33±0.33	9.33±0.60		
	62.5	9.67±0.33	8.17±0.44	8.00±0.00		
	31.25	2.67±2.67	0.00±0.00	2.50±2.50		
	15.63	0.00±0.00	0.00±0.00	0.00±0.00		
	7.81	0.00±0.00	0.00±0.00	0.00±0.00		
P. aeruginosa	250	0.00±0.00	0.00±0.00	0.00±0.00	27.33±0.58	0.00±0.00
E. coli	250	0.00±0.00	0.00±0.00	0.00±0.00	28.66±4.163	0.00±0.00
Candida albicans					Fluconazole	
	250	15.00±0.58	12.00±0.58	9.33±0.33	29.00±2.52	0.00±0.00
	125	13.67±0.33	10.33±0.33	8.83±0.33		
	62.5	10.67±0.33	8.33±0.33	5.33±2.67		
	31.25	5.33±2.67	5.17±2.59	2.50±2.50		
	15.63	0.00±0.00	0.00±0.00	0.00±0.00		
	7.81	0.00±0.00	0.00±0.00	0.00±0.00		

Diameter of the zones of inhibition (ZI) were indicated as mean ± SEM of the three experiments 0.00 = no inhibition

4.2.2 Microbroth dilution technique

Table 4.2: The Minimum inhibitory concentration of the crude root extracts of *Croton dichogamus*

Extracts	Minimum inhibitory concentration (mg/ml) Test organisms				
	Bacillus cereus	*Staph aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
Acetone	10.42	13.02	>250	>250	31.25
Aqueous	28.65	31.25	>250	>250	67.71
Hydroethanolic	31.03	10.42	>250	>250	83.34
Quality control (DMSO)	-	-	-	-	-

Table 4.7 Minimum bactericidal concentration (MBC) of the root extracts of *C. dichogamus*

Extracts	Minimum Bactericidal concentration (Mg/ml) Test organisms				
	Bacillus cereus	*Staph aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*
Acetone	166.67	83.33	>250	>250	104.17
Aqueous	104.17	62.5	>250	>250	125
Hydroethanolic	83.33	125	>250	>250	166.67
Quality control (DMSO)	-	-	-	-	-

Table 4. 3: Average MICs & MBCs/MFCs(mg/ml) of Hydroethanolic, Acetonic and aqueous root extracts of *Croton dichogamus* against the five test organisms

Test microbe	Acetonic extract		Aqueous extract		50% ethanol extract	
	MIC	MFC/MBC	MIC	MFC/MBC	MIC	MFC/MBC
B. cereus	10.42	166.67	13.03	104.67	10.42	62.5
S. aureus	13.02	83.33	31.25	83.33	10.42	125
P. aeruginosa	>250	>250	>250	>250	>250	>250
E. coli	>250	>250	>250	>250	>250	>250
C. albicans	31.25	104.167	67.7133	83.33	83.33	166.67

Key: MFC= Minimum Fungicidal Concentration

MBC= Minimum Bactericidal Concentration

MIC= Minimum Inhibitory Concentration

The least minimum inhibitory concentration value was shown by the acetonic extract against *B. cereus* and the hydroethanolic extract against *B. cereus* and *S. aureus.*

4.3 Cytotoxicity studies

The results of brine shrimp bioassay are shown in figure 4.4, table 4.9 and table 4.10 below.

Table 4.9: Results on the cytotoxicity of *C. dichogamus* on brine shrimp larvae (*Artemia salina*)

Extract	Serial dilution(µg/ml)	Average no. of brine shrimp dead	Percentage mortality
Aqueous extract	10	2	16
	100	3	70
	1000	10	100
Hydroethanolic extract	10	0	0
	100	7	70
	1000	10	100
Acetonic extract	10	6	66
	100	8	86
	1000	10	100
Vincristine	10	0	6
	100	6	62
	1000	10	100

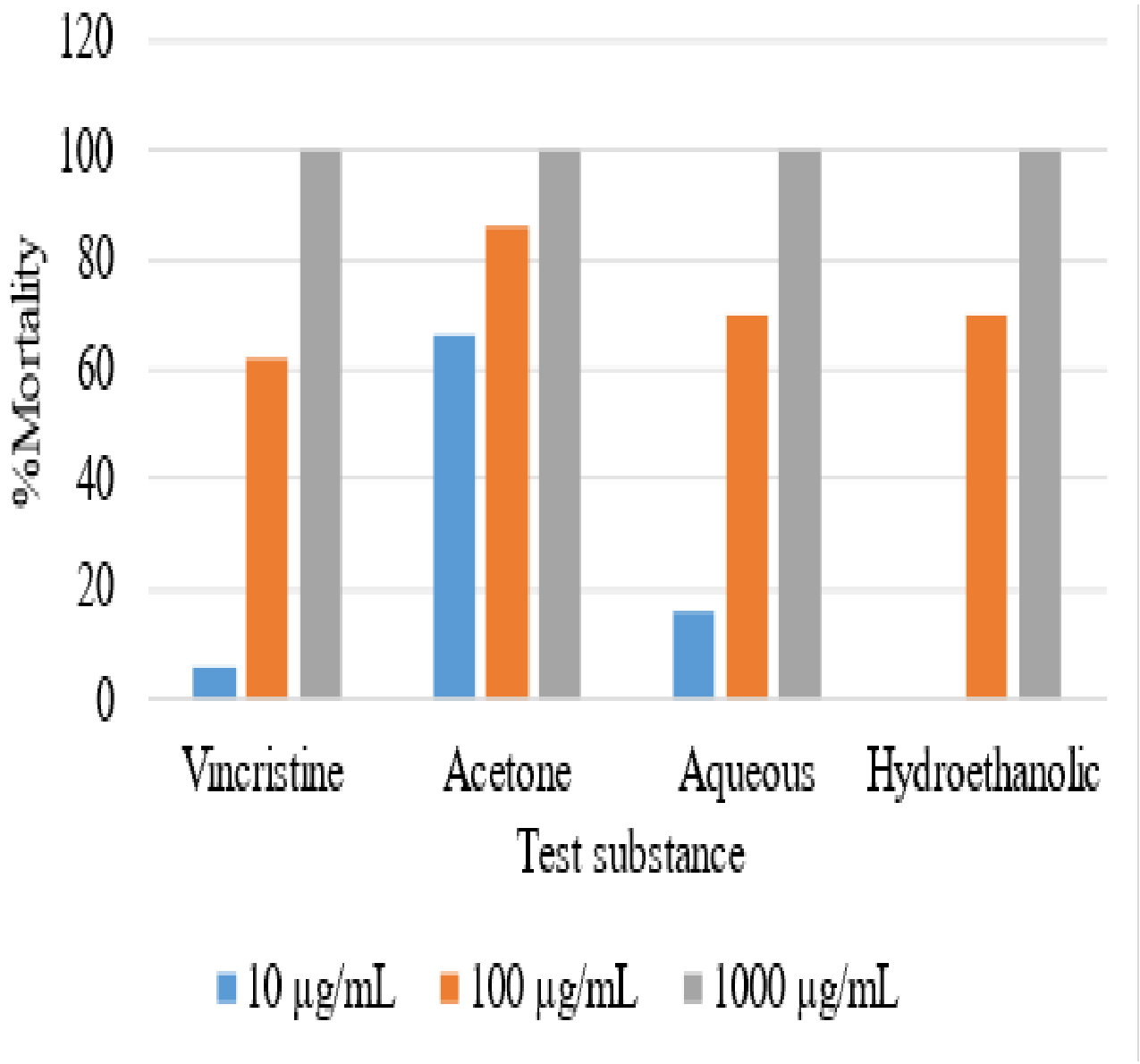


Figure 4.4: Comparison of brine shrimp mortality induced by vincristine (reference drug) together with hydroethanolic, aqueous and acetonic crude root extracts of *Croton dichogamus*

Table 4.10: The cytotoxicity profile of the hydroethanolic, acetone and aqueous crude root extracts of *Croton dichogamus* in comparison with Vincristine sulphate

Extract	Mean Mortality per concentration			Median lethal concentration	cytotoxicity	
	10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50} (95% CI)	Criteria for cytotoxicity (Meyer)	
Acetonic extract of *Croton dichogamus*	33	43	50	4.148(0.58-9.87)	High cytotoxicity	
Vincristine Sulphate Acetone	3	31	50	65.04(46.07-92.17)	High cytotoxicity	
Hydro-ethanol extract of *Croton dichogamus*	0	35	50	76.09(58.69-133.33)	High cytotoxicity	
Aqueous extract of *Croton dichogamus*	8	35	50	42.61(28.86-62.62)	High cytotoxicity	

According to the categorization criteria of cytotoxic compounds in the procedure given by Meyer *et al.*, 1982 and Nguta *et al.*, 2012 , LC_{50} values below 1000µg/ml were considered non-cytotoxic, values of LC_{50} between 500 to 1000µg/ml were considered weakly cytotoxic, values of LC_{50} between 100 to 500µg/ml were considered to be of moderate cytotoxicity and values of the range of 0-100µg/ml were termed as highly cytotoxic

Acetone extract demonstrated the most cytotoxicity (LC50 4.148µg/ml) then the water extract (42.61µg/ml) and lastly the hydroethanolic extract gave the lowest cytotoxic value of LC_{50} 76.09µg/ml. The three extracts recorded LC_{50} values below 100µg/ml. It was confirmed that the acetone extract of *C. dichogamus* was more lethal that Vincristine sulphate (reference drug) that had LC_{50} value of 65.04µg/ml.

4.4 Phytochemical Analysis

Table 4.4: Phytochemical results for the hydroethanolic, aqueous and acetone extracts of *C. dichogamus*

Detection of:	Water extract	50% Ethanol	Acetone
Saponins	+	+	+
Alkaloids	-	+	+
Flavonoids	+	+	+
Tannins	+	+	-
Terpenes	+	+	+
Phenols	+	+	+
Anthraquinones	+	+	+
Polyuronides	+	+	+
Cyanogenetic glycosides	-	-	-
Mucilage	+	+	+

Key: (-) Absent

(+) Present

CHAPTER FIVE

5.0 Discussion

Resistance of disease-causing microbes has gradually increased, posing a great worldwide threat on public health (WHO 2020). As such, development of more potent antimicrobials is crucial for the subsequent treatment of infectious ailments. Vegetative greeneries together with their natural products have demonstrated that they are an immeasurable reservoir of prospect antimicrobials because they contain a potential to combat a wide variety of human pathogens. The current study was designed to determine the antimicrobial effect of the root extracts of *C. dichogamus*; and also to evaluate the phytoconstituents and determine its safety in brine shrimp.

The aqueous extract recorded the highest percentage yield of 6.05%w/w while the organic extract (acetone) recorded the lowest percentage yield of 1.29%w/w. The percentage yield of the hydroethanolic extract was 4.9%w/w. Even though the acetonic extract was low in yield organic solvents have shown to contain the maximum load of phytochemicals (Usman et al, 2009). The mixing of the solvents of different polarities in extraction tend to solve the issue of low percentage yield observed in organic solvents(Usman et al, 2009).

Screening of various plant extracts against a wide range of infectious agents has proved that plant extracts possess an immense potential to fight against fungi, protozoa, bacteria, and viruses (Chandra *et al.*, 2017). In the current study, the three root extracts (acetone, aqueous and 50% ethanol) of *Croton dichogamus* demonstrated inhibition on the gram-positive strains of bacteria (*S. aureus, B. cereus*) as well as *Candida albicans*, the fungal microbe. However, these three extracts demonstrated no inhibitory effect on the gram-negative bacteria (*E. coli, P. aeruginosa*). The selectivity of action shown towards the gram positive and gram negative strains of bacteria

could be attributed to the impenetrable barricade of lipopolysaccharide present on the outermost membrane of the gram negative bacteria that prevent the entry of active compounds (Papo and Shai, 2005). This is a very different case with the gram-positive bacteria that liberally permit a close contact of the active compounds with the phospholipid bilayer of the cell membrane, thus enhancing ion absorptivity (Khanam *et al*., 2015). The presence of antibacterial property had also been confirmed by Magadula, 2012 who noted that the organic (ethanol) root extract of *Croton dichogamus* demonstrated inhibitory effect against two mycobacterium strains of *Mycobacterium madagascariense* and *Indicus pranii* with a Minimum inhibitory concentration of 1.25mg/ml.

The hydroethanolic extract against *S. aureus* and *B. cereus* plus the acetonic extract against *B. cereus* gave the highest MIC value. In comparison with the standard MIC range for antibiotic (0.015-0.107mg/ml) in clinical use, the results of the present research records a weak MIC range of 10.4-166.7mg/ml. (Dissanayake *et al.*, 2020). The results of this research recorded a significant difference between the inhibition caused by all the three extracts and the standard drugs, which therefore suggested that the reference drugs (Fluconazole and Cephalexin) had a strong antimicrobial activity than the extracts (hydroethanolic, acetone and aqueous) at the same concentration of 250mg/ml. There was a notable significant difference ($P<0.05$) in the mean zones of inhibition of the three extract and reference drugs (Fluconazole and Cephalexin). However, there was no significant difference ($P>0.05$) in the means zones of inhibition of the three extracts (aqueous, acetone and hydroethanolic), meaning that they had the same antimicrobial effect on the microbes tested. The presence of antimicrobial activity in *C. dichogamus* substantiated the folklore claim of its use to manage infectious ailments and could be considered as a prospective antimicrobial agent in the treatment of gram-positive bacterial strains.

Brine shrimp lethality assay is a convenient, rapid, inexpensive and reliable bench top procedure that is mostly used to determine the Median Lethal Concentration (LC_{50}) figure of extracts from plants using brine shrimp assay. The method of categorization of cytotoxins in brine shrimp assay employed by Meyer *et al.*, 1982 and Nguta *et al.*, 2012 was used in the present study. In this categorization, values of LC_{50}>1000µg/ml were considered nontoxic, values of LC_{50} between 500 to 1000µg/ml were considered weakly cytotoxic, values of LC_{50} between 100 to 500µg/ml were termed as moderate in cytotoxicity and lastly an LC_{50} values ranging between 0-100µg/ml were termed as highly cytotoxic. The data obtained from the present study showed that the three extracts were highly cytotoxic with acetonic extract (LC_{50} 4.148µg/ml) demonstrating a higher cytotoxicity than aqueous and hydroethanolic extracts which recorded values of LC50 42.61µg/ml and LC_{50} 76.09µg/ml respectively. The disparity in the cytotoxic activity in the three extracts could be ascribed to the difference in phytochemical components like terpenoids, tannins, saponins, phenols, flavonoids and alkaloids. (Nguta *et al.*, 2012). Since brine shrimp bioassay is employed to forecast the availability of any cytotoxic effect against cancer cell below 100µg/ml. the three extracts of *Croton dichogamus* are therefore imaginably cytotoxic having recorded LC_{50} values below 100µg/ml (McLaughlin et al, 1991).

Cytotoxic compounds exhibit their action by preventing the growth of cells at specific stages more especially the cells that display speedy growth. The chief mechanism of action of cytotoxic agents be by inducing apoptosis, detaining cell cycle or inhibiting angiogenesis. The overlapping of the confidence intervals (CI) noted between the reference drug, Vincristine sulphate (65.04(46.07-92.17)) and the plant extracts of hydro ethanol (76.09(58.69-133.33)) and aqueous (42.61(28.86-62.26)) indicated that there was no significant difference (P>0.05) in the lethality caused by vincristine sulphate and the two root extracts (hydroethanolic and aqueous) of *Croton dichogamus.*

There was however a significant difference ($P<0.05$) between the lethality observed in vincristine sulphate and the acetonic extract, in that the LC_{50} value of the acetone extract was quite low and was not overlapping with vincristine sulphate. The results suggested that acetone extract of *Croton dichogamus* was more lethal than vincristine sulphate (positive control).

Aldhaher *et al.*, 2017 reported that from the root extract of *Croton dichogamus* a cytotoxic compound called 10-epi-Maninsgin D was isolated, a substance which was found to be viable against CACO (human colorectal adenocarcinoma) cell line with notable prevention of cellular proliferation. Early on, another study done by Magadula, 2012 had indicated that the ethanolic root extract of *Croton dichogamus* was seen to be cytotoxic recording LC_{50} value of 40.70µg/ml. The above reports concurred with the current study that disclosed the presence of cytotoxic activity in the root extracts of *C. dichogamus*. In addition, a research has also discovered another compound (sesquiterpenoid) from the methanolic root extract of *C. dichogamus* called furocrotinsulolide which has recorded modest cytotoxic action against cancer cells at 30µm when investigated using CACO-2 cell line (Aldhaher *et al.*, 2016). Some of the compounds named above could be the reason why *C. dichogamus* exhibited significant cytotoxic activity in brine shrimp bioassay in the present research.

Plants being rich reservoirs of phytonutrients (Robards, 2003), their pharmacological and medicinal significance can be determined by the analysis of their photochemical constituents produced in their tissues as primary or secondary metabolites (Egamberdieva, 2017). In the same way medicinal usefulness of *C. dichogamus* could also be based on the presence of the active phytochemical components revealed in the study, its safety on body organs and its microbial responsiveness. A good ratio of the phytochemical compounds common to most plants like alkaloids, polyphenols, tannins, flavonoids, terpenoids and saponins (Egamberdieva, 2017) were

also found in *C. dichogamus*. This is quite a good indication that *C. dichogamus* can be of great medicinal value.

The present study confirmed the availability of polyuronides, phenols, saponins, tannins, flavonoids, anthracenes and terpenoids in the hydroethanolic, acetonic and aqueous crude root extracts of *Croton dichogamus.* The hydroethanolic and acetone extracts contained alkaloids while the aqueous extract recorded absence of alkaloids. The undesirable cyanogenetic glycosides were unavailable in all the three extracts (hydroethanolic, aqueous and acetone) of *C. dichogamus*. The hydroethanolic and aqueous extracts of *C. dichogamus* indicated the presence of tannins while they were absent in the acetone extract. These results were consistent with most recent studies that have reported the presence of terpenoids, alkaloids and flavonoids in most *Croton* species (Salatino *et al*., 2007; Xu *et al*., 2018). In another study, Johns *et al.*, 1990 reported the availability of the phytochemicals present in this study like saponins and phenols while Aldhaher *et al.*, 2016 indicated the presence of 20 more diterpenoids in the root extracts of *C. dichogamus*. Alkaloids, saponins, phenols and tannins were also present in the ethanolic root extract of *C. dichogamus* (Magadula, 2012). The disparity observed in the phytochemical composition in the above-mentioned studies with the current one could be attributed to geographical differences of the plant material.

Various reports have indicated that potent medicinal activities like antiproliferative (Aldhaher *et al.*, 2017), anti-inflammatory (Somteds *et al.*, 2019) and insecticidal (Tlankka *et al.*, 2020) properties that are found in *Croton dichogamus* could be ascribed to the elevated level of terpenoids (Matara *et al.*, 2021) which have also been reported in the present research. The use of *Croton dichogamus* as antibacterial agent in the treatment of respiratory infections antifungal and as tonic has been justified by the phytochemical components in it. The availability of terpenes in

the root extracts of *Croton dichogamus* explicate the usefulness of the plant in the treatment of respiratory disorders like chest pains, cough and asthma because terpenes are reported to soothe the respiratory mucous membrane that is irritated. Triterpenoids have antimicrobial effect, a reason why the plant is used to alleviate respiratory illnesses that are of bacterial genesis too (Magadula, 2012). In the pharmaceutical industry terpenoids like triterpenoids, diterpenoids, sesquiterpenoids have been used as insecticides, antibiotics and anthelmintics (Khanam *et al.*, 2015) .

The availability of saponins in the three root extracts of *C. dichogamus* is a reason why the plant is used in the treatment of coronary heart disease (CHD) and atherosclerosis. Saponins have been reported to have a hypocholesteremic activity on human beings and animals, their amphiphilic structure binds cholesterol thus forming insoluble complexes that are easily excreted in the bile. Saponins also obstruct the absorption of endogenous and exogenous cholesterol by interfering with its enterohepatic circulation , this leads to an increase in fecal matter and consequently lowering of serum cholesterol (Johns *et al.*, 1999).

Phenolic compounds in *C. dichogamus* pose as evidence on the usefulness of the plant in managing the inflammatory and bacterial illnesses that affect the respiratory tract like pneumonia, asthma pharyngitis, common cold and tuberculosis. Phenols have been shown to protect the body cells against oxidative conditions that inflame the tissues of the body (Rice-Evans *et al*., 1997).

The presence of alkaloids in *C.dichogamus* is an evidence that the plant can manage mild to moderate pain (Aldhaher *et al*., 2017). Alkaloid block the cyclooxygenase alleyway that subsequently block the inflammatory interleukins and cytokines that are the origin of pain. Other reports have also indicated that alkaloids can exhibit antispasmodic, antibacterial and antimalarial activities (Khanam *et al*., 2015).

Two extracts (hydroethanolic and aqueous) of *C. dichogamus* indicated the presence of tannins. Tannins have been reported to act as antioxidants owing the free radicle scavenging effect they exhibit. They have found use in the medical field as anti-tumor, antimicrobial and antiseptic properties (Khanam *et al*, 2015)

Flavonoids were present in the three extracts of *Croton dichogamus*. Studies have indicate that Flavonoids will bring down cases of upper respiratory tract infections (URTI) for the reason that flavonoids possess significant pharmacological activities on man such anti-cancer , antioxidant, anti-inflammatory, antiviral, antibacterial and anti-tumor (Khanam *et al*., 2015; Kaul *et al*., 1985). Further studies have reported that flavonoids have an ability to prevent inflammatory effects by decreasing the size of NF-Kb at the same time inhibiting the replication and metastasizing of two dangerous viral origins of URTIs (Kaul *et al*, 1985). Patients who have a high intake of flavonoids have a lower chance of getting cardiovascular, cancer and respiratory diseases (Khanam *et al*., 2015; Kaul *et al*., 1985). This fact substantiates the folklore use of *C. dichogamus* in managing respiratory infections. The presence of all the reported phytoconstituents play a role in the observed antimicrobial and cytotoxic properties. This is due to the fact that the occurrence and quantities of the phytochemical components in a plant determine its biological activity (Musila *et al.*, 2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

Acetonic, hydroethanolic and aqueous extracts of *Croton dichogamus* demonstrated a weak antibacterial action on the gram-positive strains of *Bacillus cereus and Staphylococcus aureus.* However, acetonic, hydroethanolic and aqueous extracts lacked antibacterial activity against the gram-negative bacteria; *Pseudomonas aeruginosa and Escherichia coli* even at the highest strength of 250mg/ml. Acetonic plant extract of *Croton dichogamus* showed the highest antibacterial effect among the three extracts tested.

The aqueous, hydroethanolic and acetonic extracts of *Croton dichogamus* exhibited a weak antifungal activity against *candida albicans* with the acetonic extract demonstrating the highest antifungal activity among the three extracts tested.

The hydroethanolic, acetonic and aqueous extracts of *C. dichogamus* demonstrated a very strong cytotoxicity by showing 100% mortality rate at 1000µg/ml.

The acetonic extract had the highest cytotoxicity against the brine shrimp larvae with LC_{50} of 4.148 and 100% mortality at 100µg/ml. The acetonic extract demonstrated a higher lethality when compared to the reference drug (vincristine sulphate).

The phytochemical screening of the hydroethanolic, acetone and aqueous extracts of *Croton dichogamus* indicated a presence of flavonoids, phenols, tannins, anthraquinones, alkaloids, saponins, terpenoids and polyuronides. This substantiated the traditional use of *C. dichogamus* as an antimicrobial, analgesic, hypocholesteremic and anti-inflammatory agent. The isolation, identification and characterization of these secondary metabolites may lead to new drug discovery and development opportunities.

6.2 RECOMMENDATIONS

A detailed quantitative analysis of the phytochemical composition of *C. dichogamus* plant parts (leaves, stem and roots) need to be done.

There is a need to determine acute, subacute and chronic toxicity *in vivo* to further clarify whether *Croton dichogamus* extracts are safe to be consumed and developed into the pharmaceutical pipeline.

Since the extracts demonstrated a value LC_{50} <100mg/ml, there is a need for isolation, identification and characterization of bioactive compounds responsible for the observed cytotoxicity. Further, in-depth studies must be done so as to fully understand the mechanism action underlying the potent activity

Since the plant is used for curative and insecticidal purposes, studies need to be done to determine the actual therapeutic dosage that is effective in both cases. Pharmacokinetic studies determining the dose range and frequency need to be done.

Other solvents or solvents mixtures need to be used in extraction in order to determine the solvent that gives the highest extraction yield. A solvent extraction toxicity profile is also needed.

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Appendices

Appendix 1: Plant identification

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

The East African Herbarium
P.O. Box 45166 00100 Nairobi, Kenya
Telephone: 3743513, 3742131/4 ext 2274
Fax: 3741424
E-Mail: botany@museums.or.ke

28th October, 2020

REF: NMK/BOT/CTX/1/2

Dorine Matara
University of Nairobi
Mobile: 0710784150

Dear Dorine,

PLANT IDENTIFICATION

The plant specimen that you deposited at the East African Herbarium for identification was determined as follows;

Croton dichogamus Pax (Family: Euphorbiaceae)

Thank you for visiting the EA Herbarium.

Yours faithfully,

Kennedy Matheka
Botany Department

NATIONAL MUSEUMS OF KENYA
28 OCT 2020
E.A
HERBARIUM NAIROBI

Appendix 2:Ethical Approval

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE BIOSAFETY, ANIMAL CARE AND USE COMMITTEE (FVM BACUC)
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY
P.O. Box 30197,

00100 Nairobi,

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

REF: FVM BACUC/2021/282
Dr. Dorine Matara Nyak,
University of Nairobi
Dept. of PHP& Toxicology,
20/01/2021

Dear Dorine,

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

Antimicrobial activity, Brine shrimp cytotoxicity and Phytochemical composition of *Croton dichogamus* Pax crude root extracts.

Dorine Matara J56/34184/2019

We refer to your MSc proposal submitted to our committee for review and your application letter dated November 30th 2020. We have reviewed your application for ethical clearance for the study.

The brine shrimp cytotoxicity protocol meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

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

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

Yours sincerely,

Kaluwa

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

Appendix 3: Research permit from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **913964**

Date of Issue: **22/April/2021**

RESEARCH LICENSE

This is to Certify that Dr., Dorine Nyak matara of University of Nairobi, has been licensed to conduct research in Kisumu on the topic: Antimicrobial activity, brine shrimp cytotoxicity and phytochemical composition of the Croton dichogamus Pax(Euphorbiaceae) crude root extracts for the period ending : 22/April/2022.

License No: **NACOSTI/P/21/10145**

913964

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix 4: Plant collection and preparation

The shrub, *C. dichogamus*

Fresh roots of *C. dichogamus*

Chopped pieces of *C. dichogamus* roots

C. dichogamus root powder

Appendix 5: Extraction process

Cold maceration followed by filtration

Rota evaporation

Freeze drying

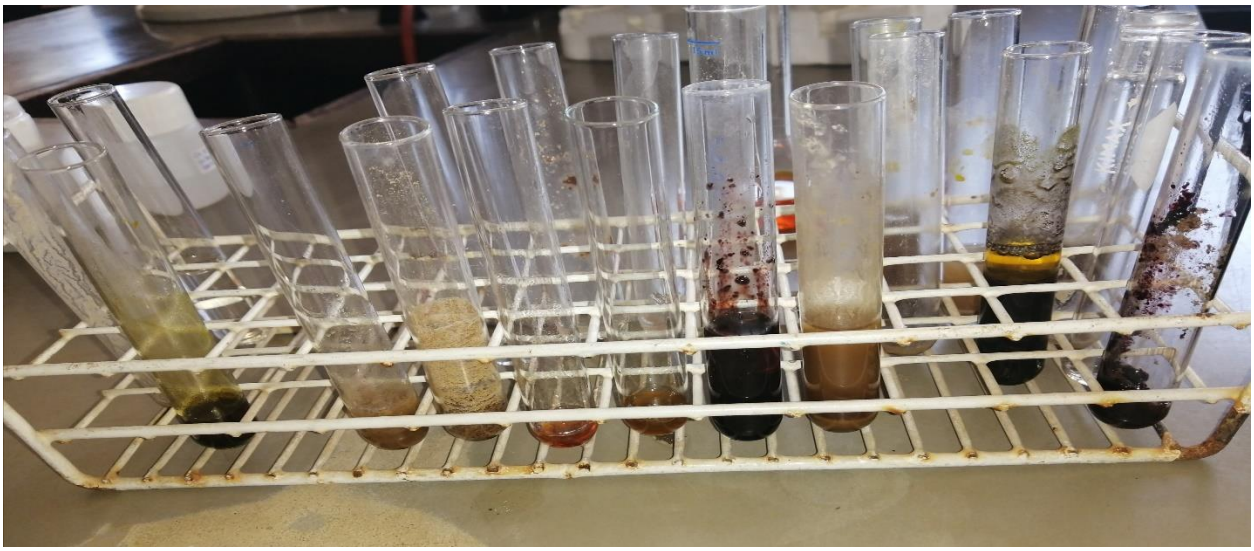
Appendix 6: Antimicrobial susceptibility testing

Appendix 7: Brine shrimp lethality Assay

Hatching of the brine shrimp eggs

Extract together with the nauplii

Appendix 8: Phytochemical analysis



Appendix 9: Raw data on Brine shrimp lethality assay

Sample	Tube number	Dose (µg/ml)	Number that died	Dose (µg/ml)	Number that died	Dose (µg/ml)	Number that died
Aqueous extract	1	10	2	100	10	1000	10
	2	10	0	100	6	1000	10
	3	10	1	100	10	1000	10
	4	10	2	100	4	1000	10
	5	10	3	100	5	1000	10
Acetone extract	1	10	8	100	10	1000	10
	2	10	6	100	10	1000	10
	3	10	6	100	8	1000	10
	4	10	5	100	5	1000	10
	5	10	8	100	10	1000	10
Hydroethanolic extract	1	10	0	100	8	1000	10
	2	10	0	100	6	1000	10
	3	10	0	100	8	1000	10
	4	10	0	100	8	1000	10
	5	10	0	100	5	1000	10
Vincristine	1		10				
	2		10				
	3		10				
	4		10				
	5		10				

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.88582136
R Square	0.784679481
Adjusted R Square	0.768116365
Standard Error	0.700602922
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	23.25381006	23.25381006	47.37511004	1.11348E-05
Residual	13	6.380977913	0.490844455		
Total	14	29.63478798			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.501546667	0.47860291	5.226768608	0.000163313	1.467587941	3.535505393	1.467587941	3.535505393
X Variable 1	1.52492	0.221550097	6.882957943	1.11348E-05	1.046290115	2.003549885	1.046290115	2.003549885

Appendix 10: Data on antimicrobial activity

Zone of inhibition of acetonic extract of C. dichogamus								
					95% Confidence Interval for Mean			
		N	**Mean**	**Std. Error**	Lower Bound	Upper Bound	Minimum	Maximum
Inhibition of B. cereus by acetone crude extracts	250mg/ml	3	**17.3333**	**.33333**	15.8991	18.7676	17.00	18.00
	125mg/ml	3	**16.3333**	**.88192**	12.5388	20.1279	15.00	18.00
	62.5mg/ml	3	**13.6667**	**.88192**	9.8721	17.4612	12.00	15.00
	31.5mg/ml	3	**5.6667**	**2.84800**	-6.5873	17.9206	.00	9.00
	15.6mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	7.81mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	**29.6667**	**.33333**	28.2324	31.1009	29.00	30.00
Inhibition of S. aureus by acetone crude extracts	250mg/ml	3	**12.3333**	**.88192**	8.5388	16.1279	11.00	14.00
	125mg/ml	3	**11.3333**	**.33333**	9.8991	12.7676	11.00	12.00
	62.5mg/ml	3	**9.6667**	**.33333**	8.2324	11.1009	9.00	10.00
	31.5mg/ml	3	**2.6667**	**2.66667**	-8.8071	14.1404	.00	8.00
	15.6mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	7.81mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	**28.3333**	**.33333**	26.8991	29.7676	28.00	29.00
Inhibition of P. aeruginosa by acetone crude extracts	250mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	125mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	62.5mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	31.5mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	15.6mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	7.81mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	**27.6667**	**.66667**	24.7982	30.5351	27.00	29.00
Inhibition of E. coli by acetone crude extracts	250mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	125mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	62.5mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	31.5mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	15.6mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	7.81mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	**29.6667**	**1.66667**	22.4956	36.8378	28.00	33.00
Inhibition of C. albicans by acetone crude extracts	250mg/ml	3	**15.0000**	**.57735**	12.5159	17.4841	14.00	16.00
	125mg/ml	3	**13.6667**	**.33333**	12.2324	15.1009	13.00	14.00
	62.5mg/ml	3	**10.6667**	**.33333**	9.2324	12.1009	10.00	11.00
	31.5mg/ml	3	**5.3333**	**2.66667**	-6.1404	16.8071	.00	8.00
	15.6mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	7.81mg/ml	3	**.0000**	**.00000**	.0000	.0000	.00	.00
	Fluconazole(250mg/ml)	3	**29.6667**	**.33333**	28.2324	31.1009	29.00	30.00

Zone of inhibition of hydroethanolic extract of C. dichogamus								
		N	Mean	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Inhibition of B. cereus by hydroethanolic crude extracts	250mg/ml	3	14.3333	.33333	12.8991	15.7676	14.00	15.00
	125mg/ml	3	12.3333	.33333	10.8991	13.7676	12.00	13.00
	62.5mg/ml	3	9.5000	1.04083	5.0217	13.9783	7.50	11.00
	31.5mg/ml	3	5.3333	2.68225	-6.2074	16.8741	.00	8.50
	15.6mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	7.81mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	30.6667	.33333	29.2324	32.1009	30.00	31.00
Inhibition of S. aureus by hydroethanolic crude extracts	250mg/ml	3	11.5000	.28868	10.2579	12.7421	11.00	12.00
	125mg/ml	3	10.3333	.33333	8.8991	11.7676	10.00	11.00
	62.5mg/ml	3	8.1667	.44096	6.2694	10.0640	7.50	9.00
	31.5mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	15.6mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	7.81mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	30.3333	1.45297	24.0817	36.5849	28.00	33.00
Inhibition of P. aeruginosa by hydroethanolic crude extracts	250mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	125mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	62.5mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	31.5mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	15.6mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	7.81mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	28.3333	.66667	25.4649	31.2018	27.00	29.00
Inhibition of E. coli by hydroethanolic crude extracts	250mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	125mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	62.5mg/ml	3	.0000	.00000	.0000	.0000	.00	.00

	31.5mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	15.6mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	7.81mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	Cephalexin(250mg/ml)	3	26.6667	.88192	22.8721	30.4612	25.00	28.00
Inhibition of C. albicans by hydroethanolic crude extracts	250mg/ml	3	12.0000	.57735	9.5159	14.4841	11.00	13.00
	125mg/ml	3	10.3333	.33333	8.8991	11.7676	10.00	11.00
	62.5mg/ml	3	8.3333	.33333	6.8991	9.7676	8.00	9.00
	31.5mg/ml	3	5.1667	2.58736	-5.9659	16.2992	.00	8.00
	15.6mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	7.81mg/ml	3	.0000	.00000	.0000	.0000	.00	.00
	Fluconazole(250mg/ml)	3	29.3333	.66667	26.4649	32.2018	28.00	30.00

Appendix 11: Published articles

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Research Article

Phytochemical Analysis and Investigation of the Antimicrobial and Cytotoxic Activities of *Croton dichogamus* Pax Crude Root Extracts

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Background. Increasing antimicrobial resistance has led to an arduous search for new potent drugs from nature. In this search, plants have proved to be rich reservoirs of efficacious medicinal components that manage ailments. The current study is designed to investigate the phytochemical composition, antimicrobial activity, and the cytotoxicity of the crude root extracts of *Croton dichogamus*, a shrub that is commonly used in the eastern Africa for the management of infectious diseases. *Methods*. The roots of *Croton dichogamus* were obtained, dried, ground, and extracted using three solvents (acetone, distilled water, and 50% ethanol). The antimicrobial activity was tested using agar well diffusion and microbroth dilution techniques against five human pathogens. The brine shrimp lethality assay was used to assess the toxic effect. *Results*. The phytochemical screening indicated the presence of terpenoids, flavonoids, tannins, phenols, polyuronides, saponins, and anthracenes. The brine shrimp lethality assay indicated that all the extracts were highly cytotoxic with LC_{50} values below 100 μg/ml. Acetonic extract had an LC_{50} value of 4.148 μg/ml, hydroethanolic extract had 76.09 μg/ml, and aqueous extract had 42.61 μg/ml. All extracts showed the antibacterial activity against Gram-positive bacteria (*B. cereus* and *S. aureus*) and a fungal organism, *C. albicans*. The extracts showed no antibacterial effect on the Gram-negative bacterial strains (*P. aeruginosa* and *E. coli*) at a concentration of 250 mg/ml. The highest antimicrobial activity was demonstrated by the acetonic extract on *B. cereus* which had an MIC of 10.42 mg/ml and a zone of inhibition of 17.33 ± 0.58 at a concentration of 250 mg/ml. *Conclusion*. In this research work, we report that *C. dichogamus* had the antimicrobial activity confirming the folklore claim. The results made a strong case for isolation of novel anticancer lead compounds.

1. Introduction

The emergence and propagation of antimicrobial resisting strains of microbes in clinical practice have extremely reduced the efficacy of antimicrobial weaponry, resulting to recurrence of therapeutic failure and mortality cases [1, 2]. Therefore, there is an urgent need for new effective antimicrobial agents [3]. Since classical times, human beings have been in a ceaseless search for ways of relieving such diseases, leading to great discoveries on the use of natural ways (like plants) of treating complex ailments [4]. Over the last three decades, the use of traditional medicine has immensely grown with approximately 80% of worldwide population relying on this system as the chief form of treatment [5]. The use of medicinal plants for reliving common ailments has been accepted widely because of their accessibility, availability, effectiveness, and affordability [6]. Various studies have demonstrated that antimicrobials of plant origin possess a huge potential to fight against bacterial, protozoal, viral, and fungal diseases with minimum

complications [7]. The screening performed on numerous plant extracts and their natural products has also shown that vegetative greeneries and their secondary metabolites have a notable activity against a wide range of microbes when used alone or they could also act as synergists or potentiators of other antimicrobial agents [8]. The World Health Organization (WHO) also acknowledges that traditional medicine is a prime healthcare system that gives good results to its users [9].

The plants that have been used extensively in traditional medicine are those of *Croton* genus (Euphorbiaceae family) that has 1300 species. Most of these species have displayed remarkable ability to manage a broad spectrum of diseases [6, 7]. *Croton dichogamus*, one of its species, is a medicinal shrub growing in Kenya, Tanzania, Somalia, Rwanda, Ethiopia, Mozambique, and Madagascar, where it often plays a significant role in traditional medicine [10].In Tanzania, the powdered roots of "Mhande," *C. dichogamus* are used by Sukuma to treat tuberculosis, while the smoked roots are used to relive chest pains and fevers [5]. In the Kenyan Lake Basin, among the Luo community, *C. dichogamus* (Rachar) is used to manage respiratory diseases such as asthma, pneumonia, and cough [9–11]. It is used in Somalia to treat gonorrhea [12], arthritis [5], stomach, and back pains [11, 12]. The Mbeere and Afan Oromo communities in Tanzania use dried leaves, roots, and stem barks of *C. dichogamus* as an antimalarial, antipyretic [5], and as a pesticide [13]. In Kitui County, the Kamba community prepares an infusion of the stem bark and leaves of *C. dichogamus*, locally known as "Mwalula," for drinking to alleviate back pains, malaria, stomachache, chest problems, fever, oedema, and cough [14]. In the same community, a root decoction is also drunk for the treatment of impotence and infertility [14].

Previous reports have indicated that ethanolic extract of *C. dichogamus* had an antibacterial activity against two *Mycobacterium* species, namely, *Mycobacterium indicus pranii* and *Mycobacterium madagascariense indicus*, giving a minimum inhibitory concentration (MIC) value of 1.25 mg/ml [15]. Studies have also shown that the essential oils from leaves of *C. dichogamus* had an antimalarial activity against *Anopheles gambiae* [16]. The antiproliferative activity [17], insecticidal activity [13], and hypocholesteremic activity [18] have also been reported.

Despite the extensive use of *C. dichogamus* for curative purposes and the traditional claim of its efficacy in the management of common ailments, the available literature is scanty. The pharmacological activity, antimicrobial property, and safety of *C. dichogamus* have never been investigated and documented. The motive of the current study was to evaluate the antimicrobial activity of acetonic, hydroethanolic, and aqueous root extracts of *C. dichogamus* against five human pathogens (*Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*) using agar well diffusion and microbroth dilution techniques. The phytochemical composition was evaluated, and the safety of the extracts was determined in a bench top assay using brine shrimp larvae (*Artemia salina*) [19].

2. Materials and Methods

2.1. Chemicals, Reagents, and Drugs. Absolute ethanol (Loba Chemic Pvt., India), acetone (Nice Chemicals Pvt., Ltd., India), dimethyl sulfoxide (Loba Chemic. Pvt., India), brine shrimp eggs (A.A Biotech Pvt., India), vincristine sulphate (Celon Laboratories Pvt., India), Mueller Hinton agar (HI-Media Laboratories Pvt., India), Mueller Hinton broth (HI-Media Laboratories Pvt., India), cephalexin (Medisel Ltd., Kenya), and fluconazole (Dawa Ltd., Kenya) were used in this study, and the chemical and reagents were of analytical grade.

2.2. Plant Collection and Authentication. Fresh roots of the shrub *C. dichogamus* were collected from Kisumu East Subcounty (0° 14′ 60.00″ N, 34° 54′ 59.99″ E), Nyanza province, in the month of November 2020 and transported to the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. The taxonomic identification was performed by Mr. Ken Matheka at the East African Herbarium located at the National Museum of Kenya. A voucher specimen of reference number of NMK/BOT/CTX.1/2 was deposited at the East African Herbarium.

2.3. Extraction of Plant Material. The fresh roots of *C. dichogamus* were washed, chopped into small pieces, air-dried, and ground into fine powder. The resulting plant powder was packed in sterile airtight ziplock bags and stored in a cool, dry shelf awaiting extraction.

Aqueous extract was prepared by cold maceration by adding 2000 ml of distilled water to 500 gm of the root powder. The mixture was then macerated for 72 hours with vigorous shaking in the morning and evening and then filtered. The filtrate was kept in a deep freezer for 24 h and then lyophilized to form a light brown powder which was stored in an amber bottle at −4°C in a refrigerator.

Hydroethanolic (50% ethanol) extraction was performed by taking 500 gm of the *C. dichogamus* root powder into an extraction jar and then adding 1000 ml of distilled water followed by 1000 ml of absolute ethanol. The mixture was macerated for 72 hours with vigorous shaking to increase the efficiency of extraction. The mixture was then filtered, and the filtrate was evaporated using a rotary evaporator set at 40°C to remove excess ethanol solvent. The resulting content was then freeze-dried to produce a light brown powder that was stored in the refrigerator.

The acetonic extract was prepared by taking 500 mg of *C. dichogamus* root powder into an extraction jar, adding 2000 ml of acetone gradually, and then shaken vigorously until a uniform consistency was obtained. The mixture was stirred continuously using a magnetic stirrer for 72 hours and then filtered. The filtrate was evaporated using a rotary evaporator whose operating temperature was set at 40°C for 4 hours. The resulting content was then placed into an amber colored bottle, covered with an aluminum foil, and then placed on a hot sand bath to get a consistent powder. The acetonic extraction was to be repeated to give enough yield that was required for the study.

2.4. Antimicrobial Studies

2.4.1. Test Microorganisms. A fungal microorganism and four bacterial strains given in Table 1 were obtained from the stock cultures from the bacteriology laboratory at the PHPT department.

2.4.2. Preparation of Cultures. The stock cultures were prepared according to the Clinical Laboratory Standards Institute (CLSI). A loopful of the pure cultures of each microbe was suspended in 10 ml sterile physiological saline to give a concentration equal to that of 0.5 MacFarland standards. According to Suffredini et al. [20], Gram-negative bacteria are never susceptible to plant extracts at a concentration lower than 200 mg/ml; thus, 250 mg/ml was a convenient dose for both Gram-positive and Gram-negative bacteria without the risk of nonspecific interaction. Stock solutions of 250 mg/ml were prepared by dissolving 1 g of the plant extracts (acetonic, aqueous, and hydroethanolic) in 1 ml of 1% dimethyl sulfoxide (DMSO); then, 3 ml of sterile molten Mueller Hilton was added to make 4 ml. Susceptibility studies were conducted according to the protocol described by Debalke et al. [21] with minor modifications. As described in the protocol [21], two-fold serial dilutions of 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml, and 7.8 mg/ml were made from the stock solution. The various dilutions made it easier to point out the exact concentration at which there was a complete bacterial inhibition. 1% DMSO was used as negative control while cephalexin, a broad-spectrum antibiotic, was used as a positive control for both Gram-positive and Gram-negative bacteria. Fluconazole was used as a positive control for the fungal microorganism.

2.4.3. Microbroth Dilution Technique. The microbroth method as described by Muia et al. [22] was used, where five culture tubes containing 2 ml sterile Mueller Hilton Broth were arranged. Two-fold serial dilutions were made from the stock solution. Using a micropipette, 0.1 ml of each microorganism was inoculated into every tube of diluted plant extract. The bacterial organisms were then incubated at 37°C for 24 hours, while the fungal organism was placed at room temperature for 24 hours. The observed lowest concentration of the plant extracts that retained its inhibitory effect resulting in no visible growth (absence of turbidity) of microorganism was recorded as the minimum inhibitory concentration (MIC) value of the extract. For the determination of MBC, all tubes that showed no visible bacterial growth were aseptically cultured in sterile molten agar using the pour plate method and incubated. The lowest concentration of the plant extract that shows no visible bacterial growth was noted as the MBC value.

2.4.4. Agar Well Diffusion Method. The agar well diffusion method was carried out as described by Clinical Laboratory Standards Institute (CLSI). Each test microorganism was spread on aseptically prepared nutrient agar by the use of a swab. Holes of 7 mm in diameter and 8 mm in depth were made using a sterile cork borer. 0.1 ml of the test extracts of varying concentrations were placed into the wells and allowed to stand on the bench for an hour for proper disperse into agar and incubated for 24 hours. The diameter of the zone of inhibition was measured in millimeters.

Table 1: Microbes used in the antimicrobial studies.

Name of microorganism	Microbe type	Gram stain	Strain type
Bacillus cereus	Bacteria	Positive	ATCC 11778
Staphylococcus aureus	Bacteria	Positive	ATCC 25923
Escherichia coli	Bacteria	Negative	ATCC 25922
Pseudomonas aeruginosa	Bacteria	Negative	ATCC27853
Candida albicans	Fungus	—	ATCC 102231

2.5. Brine Shrimp Lethality Studies

2.5.1. Hatching of the Brine Shrimp Eggs. Brine shrimp eggs were hatched according to the method described by Nguta et al. [23], where the brine shrimp eggs were incubated and hatched in a shallow rectangular plastic container containing marine salt solution which was made by dissolving 33 g of marine salt in a liter of distilled water. The plastic container had unequal chambers separated by a wall with 2 mm holes. Approximately 50 mg of viable brine shrimp eggs was sprinkled on the bigger chamber which was dark, while the smaller chamber was illumined by a 40 watts electric bulb. About 6 mg of dry yeast was sprinkled on the eggs to serve as food for the nauplii. Once hatched, the phototropic larvae swam from the dark chamber to the illuminated chamber leaving their egg shells behind. The hatching period was 48 hours [17, 19].

2.5.2. Cytotoxicity Bioassay. The stock solutions of aqueous, acetone 50% ethanol extracts, and vincristine sulphate (positive control) were prepared by taking 0.1 g of each sample and dissolving it in 1 ml DMSO and then topped up to the 10 ml mark with marine salt solution [19]. The concentration of the stock solution was 10,000 μg/ml. Dimethyl sulfoxide was used as a negative control, while vincristine sulphate was used as a positive control in the cytotoxicity bioassay. Ten nauplii (*Artemia salina*) were then transferred into graduated test tubes using a disposable pipette. Aliquots of 500 μl, 50 μl, and 5 μl representing the three concentrations of 1000 μg/ml, 100 μg/ml, and 10 μg/ml, respectively, of each sample were transferred into test vials. Five graduated tubes were set for each dose level per sample. Marine salt solution was added to all the test vials to make 5 ml volume. The tubes were kept at room temperature for 24 hours; then, the number of dead larvae was counted using a magnifying glass. The percentage mortality was determined for each dose level and controls. The median lethal concentrations (LC_{50}) were determined from the dead counts within 24 hours using probit regression analysis.

2.6. Phytochemical Studies. The crude root extracts of *C. dichogamus* were qualitatively screened for the presence of flavonoids, alkaloids, saponins, phenols, tannins, terpenoids, cyanogenetic glycosides, anthraquinones, polyuronides, and mucilage according to the procedures described by Visweswari et al. [24], Usman et al. [25], and Trease and Evans [26].

2.7. Statistical Analysis. All experiments were performed in triplicates; data were entered into Statistical Package for Social Sciences (SPSS), version 23, and the results are provided as mean ± SEM. One-way analysis of variance (ANOVA) and posthoc ANOVA using the Tukey HSD test was used to compare the differences in means among and between groups, respectively. Differences (among and between groups) were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Percentage Yield of Plant Material. The aqueous extract gave a percentage yield of 6.05% w/w, hydroethanolic extract gave 24.50% w/w, and acetone extract gave 1.29% w/w. The highest percentage yield was obtained from the aqueous extract, while the lowest yield was from the acetone extract.

3.2. Antimicrobial Activity. A varying antimicrobial activity was shown by the three extracts when investigated against the five microorganisms (Tables 2 and 3).

All the extracts were active against Gram-positive bacteria, *B. cereus* and *S. aureus*, while they were totally inactive against Gram-negative bacterial strains of *P. aeruginosa* and *E. coli* at a concentration of 250 mg/ml. The extracts were also active against the fungal organism, *Candida albicans*. Among the Gram-positive bacteria, the acetone extract of *C. dichogamus* exhibited the highest zone of inhibition on *B. cereus* (17.33 ± 0.58) at 250 mg/ml (Table 2) with an MIC value of 10.42 mg/ml (Table 3).

3.3. Brine Shrimp Cytotoxicity Assay. The results of the toxicity of the various extracts against brine shrimp larvae are shown in Figure 1 and Table 4. Acetonic extract had the highest toxicity (LC_{50} 4.148 μg/ml) followed by aqueous extract (LC_{50} 42.61 μg/ml). The hydroethanolic extract displayed the least toxicity (LC_{50} 76.09 μg/ml). All the extracts demonstrated LC_{50} values which were less than 100 μg/ml. The results obtained have shown that the acetonic extract was more toxic than the control drug (vincristine sulphate), which had a LC_{50} value of 65.04 μg/ml.

3.4. Phytochemical Composition. The phytoconstituents detected in the acetonic, hydroethanolic, and aqueous crude root extracts of *C. dichogamus* were flavonoids, saponins, phenols, terpenoids, anthracenes, and polyuronides, as given in Table 5.

According to the results, alkaloids were present in acetonic and hydroethanolic extracts but not in the aqueous extract. Tannins were present in aqueous and hydroethanolic extracts of *C. dichogamus* but were absent in the acetonic extract. Cyanogenetic glycosides were absent in all the three extracts.

4. Discussion

The propagation of drug resistance strains of microbes has posed a great challenge to global public health [1]. For this reason, the development of new therapeutic agents is critical in the future management of infectious diseases. Plants and their secondary metabolites have shown that they are a reliable resource of future antimicrobial agents as they have the ability to combat a wide range of human pathogens. The purpose of the current study was to investigate the antimicrobial activity and to qualitatively evaluate the phytoconstituents of the crude root extracts of *C. dichogamus*. The safety of the extracts was also determined in the brine shrimp cytotoxicity bioassay.

In this study, the highest yield was seen in the aqueous extract (6.05%) followed by hydroethanolic extract (4.9%). Acetonic extract gave a very low yield of 1.29% that necessitated a second extraction.

All the extracts of *C. dichogamus* showed activity against Gram-positive bacteria (*B. cereus* and *S. aureus*) and the fungal organism *C. albicans*, while all of the extracts showed no activity against Gram-negative bacteria (*P. aeruginosa* and *E.coli*). The selective activity of the extracts towards the bacteria strains could be due to the presence of an impermeable barrier of lipopolysaccharide on the outer membrane of Gram-negative bacteria that inhibit diffusion of active compounds [27]. On the other hand, Gram-positive bacteria freely allow the direct contact of active constituents with the phospholipid bilayer of the cell membrane leading to enhanced ion permeability [28]. The presence of the antibacterial property in *C. dichogamus* had also been confirmed in another study [15] that reported that the ethanolic root extract of *C. dichogamus* showed a weak antibacterial activity against two *Mycobacterium* species, namely, *Mycobacterium indicus pranii* and *Mycobacterium madagascariense indicus*, giving a minimum inhibitory concentration (MIC) value of 1.25 mg/ml.

The results of this study indicated that the MIC of the three extracts was quite weak (10.4–166.7 mg/ml) as compared to the MIC range of the commonly available antibiotics which is in a range of 0.015–0.107 mg/ml [29]. This study therefore indicated that all the extracts had a weaker antimicrobial activity even when compared to the standard drugs (cephalexin and fluconazole) at the same concentration of 250 mg/ml. It was established that there was no significant difference ($p > 0.05$) in the mean zones of inhibition of the acetone, aqueous, and hydroethanolic extracts. On the other hand, there was a significant difference ($p < 0.05$) between the mean zones of inhibition recorded for the three extracts and the positive controls (cephalexin and fluconazole) under various concentrations. The antimicrobial results of this study substantiate the traditional claim of the plant to treat ailments that are of bacterial origin

Table 2: Antimicrobial activity of *C. dichogamus* root extracts on various concentrations using the agar well diffusion technique.

Microorganisms	Concentration mg/ml	Zone of inhibition (MM)				
		Acetone extract	Hydroethanolic extract	Aqueous extract	Positive control	Negative control
					Cephalexin	
Bacillus cereus	250	17.33 ± 0.33	14.33 ± 0.33	12.83 ± 0.93	29.67 ± 2.48	0.00 ± 0.00
	125	16.33 ± 0.88	12.33 ± 0.33	10.33 ± 0.33		
	62.5	13.67 ± 0.88	9.5 ± 1.04	8.50 ± 0.5		
	31.25	5.67 ± 2.85	5.33 ± 2.68	2.50 ± 2.50		
	15.63	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
	7.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
Staphylococcus aureus	250	12.33 ± 0.88	11.5 ± 0.29	10.67 ± 0.44	28.67 ± 0.58	0.00 ± 0.00
	125	11.33 ± 0.33	10.33 ± 0.33	9.33 ± 0.60		
	62.5	9.67 ± 0.33	8.17 ± 0.44	8.00 ± 0.00		
	31.25	2.67 ± 2.67	0.00 ± 0.00	2.50 ± 2.50		
	15.63	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
	7.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
Pseudomonas aeruginosa	250	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27.33 ± 0.58	0.00 ± 0.00
Escherichia coli	250	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28.66 ± 4.16	0.00 ± 0.00
					Fluconazole	
Candida albicans	250	15.00 ± 0.58	12.00 ± 0.58	9.33 ± 0.33	29.00 ± 2.52	0.00 ± 0.00
	125	13.67 ± 0.33	10.33 ± 0.33	8.83 ± 0.33		
	62.5	10.67 ± 0.33	8.33 ± 0.33	5.33 ± 2.67		
	31.25	5.33 ± 2.67	5.17 ± 2.59	2.50 ± 2.50		
	15.63	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
	7.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		

Zones of inhibition were expressed as mean ± SEM of the triplicate experiments. 0.00 = no activity.

Table 3: Average MICs and MBCs for acetone, aqueous, and hydroethanolic crude extracts of *C. dichogamus* against the test microorganisms.

Test organism	Extracts					
	Acetone		Aqueous		Hydroethanolic	
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
B. cereus	10.42	166.67	13.03	104.67	10.41	62.5
S. aureus	13.02	83.33	31.25	83.33	10.41	125
P. aeruginosa	>250	>250	>250	>250	>250	>250
E. coli	>250	>250	>250	>250	>250	>250
C. albicans	31.25	104.167	67.7133	83.33	83.33	166.67

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration.

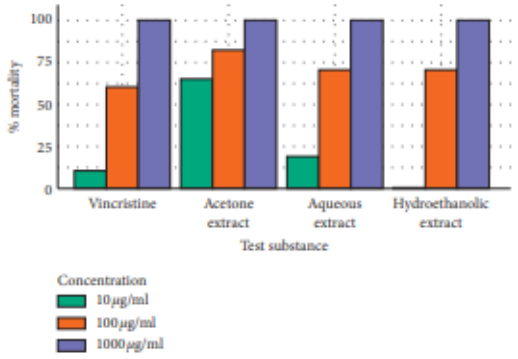

Figure 1: The comparison of the mortality induced by the crude root extracts (acetone, aqueous, and hydroethanolic) of *C. dichogamus* and vincristine sulphate.

Table 4: The toxicity profile of the acetonic, aqueous, and hydroethanolic root extracts of *C. dichogamus* as compared to vincristine sulphate.

Sample	Average mortality per test dose			Lethal concentration	Toxicity
	10 μg/mL	100 μg/mL	1000 μg/mL	LC_{50} (95% confidence interval)	Meyer's criteria
Vincristine sulphate	3	31	50	65.04 (46.07–92.17)	Highly cytotoxic
Acetone extract of *C. dichogamus*	33	43	50	4.148 (0.58–9.87)	Highly cytotoxic
Aqueous extract of *C. dichogamus*	8	35	50	42.61 (28.86–62.26)	Highly cytotoxic
Hydroethanolic extract of *C. dichogamus*	0	35	50	76.09 (58.69–133.33)	Highly cytotoxic

Table 5: Phytochemical analysis of the aqueous, hydroethanolic and acetonic extracts of *Croton dichogamus*.

Test	Aqueous extract	Hydroethanolic extract	Acetonic extract
Flavonoids	+	+	+
Alkaloids	−	+	+
Saponins	+	+	+
Phenols	+	+	+
Tannins	+	+	−
Terpenoids	+	+	+
Mucilage	+	+	+
Cyanogenetic glycosides	−	−	−
Anthraquinones	+	+	+
Polyuronides	+	+	+

Key (+) means presence of phytochemical and (−) means absence of phytochemical means.

like tuberculosis, pneumonia, and urinary tract infections [5, 30].

The brine shrimp bioassay is a rapid, reliable convenient, and inexpensive bench top procedure that determines the median lethal concentration values of plant extracts in a brine shrimp medium. The classification of toxicity in the brine shrimp bioassay described by Nguta et al. [23] and Meyer et al. [31] were used in the current study. In this classification, $LC_{50} > 1000\,\mu$g/ml were considered nontoxic, values of LC_{50} between 500 and 1000 μg/ml were considered weakly toxic, values of LC_{50} between 100 and 500 μg/ml were considered moderately toxic and values of LC_{50} between 0 and 100 μg/ml were considered to be strongly toxic. The results in the current study indicated that all the extracts were highly cytotoxic with acetonic extract (LC_{50} of 4.148 μg/ml) being more toxic than aqueous (LC_{50} of 42.61 μg/ml) and hydroethanolic (LC_{50} of 76.09 μg/ml) extracts. The difference in the cytotoxicity in the three extracts could be attributed to the phytochemical ratios of tannins, alkaloids, flavonoids, saponins, phenols, and terpenoids in them [23]. The brine shrimp lethality assay is normally used to predict the presence of the cytotoxic activity against cancer cells below 100 μg/ml; therefore, all the three extracts of *C. dichogamus* were potentially cytotoxic with LC 50 values below 100 μg/ml.

The cytotoxic agents work by interrupting the growth of cells at particular levels, especially those cells that exhibit a rapid growth. Their main mechanism of action may be due to arrest of cell cycle, induction of apoptosis, or inhibition of angiogenesis. In the current study, the overlap in confidence intervals of vincristine sulphate (65.04 (46.07–92.17)) and those of aqueous (42.61 (28.86–62.26)) and hydroethanolic extracts (76.09 (58.69–133.33)) suggests that there is no significant difference ($p > 0.05$) in the lethality induced by vincristine sulphate and that of aqueous and hydroethanolic extracts of *C. dichogamus*. However, there is a significance difference ($p < 0.05$) in the lethality of the acetonic extract and the control drug, since the LC_{50} value of the acetonic extract was too low with no overlap with the control drug. The acetonic extract was therefore more lethal than vincristine sulphate. The difference in the cytotoxicity in the three extracts could be attributed to the phytochemical ratios of tannins, alkaloids, flavonoids, phenols, and terpenoids in them [23].

The cytotoxicity report of this study resonates with that perfirmed by Magadula [15] who reported that ethanolic root extract of *C. dichogamus* gave the LC_{50} value of 40.70 μg/ml. The cytotoxic property of the root of *C. dichogamus* was confirmed by a study performed by Aldhaher et al. [17] who reported the presence of a cytotoxic compound (10-epi-Maninsgin D) in the root of *C. dichogamus* that was viable against CACO (human colorectal adenocarcinoma) cell line with significant inhibition of cellular proliferation. Another study performed later reported that a sesquiterpenoid known as furocrotinsulolide isolated from the methanolic root extract of *C. dichogamus* recorded a modest cytotoxic activity against cancer cells at 30 μm when tested on CACO-2 cell line [32]. These compounds could be responsible for the recorded cytotoxicity in the brine shrimp assay seen in the current study.

The phytochemical analysis in the current study confirmed the presence of saponins, phenols, polyuronides, tannins, triterpenoids, anthracenes, and flavonoids in aqueous, acetonic, and hydroethanolic root extracts of *C. dichogamus*. The alkaloids were present in acetonic and hydroethanolic extracts but not in the aqueous extract. The cyanogenetic glycosides were absent in the aqueous, hydroethanolic, and acetonic extracts. Tannins were present in aqueous and hydroethanolic extracts of *C. dichogamus* but were absent in the acetonic extract.

The presence of phytochemical constituents seen in this study like phenols and saponins was confirmed by a study performed by Johns et al. [18], while the presence of more than 20 diterpenoids was reported by Aldhaher et al. [17]. The presence of tannins, phenols, saponins, and alkaloids in reported in this study is inconsistent with the report given by Magadula [15] who indicated that those components were absent in the ethanolic root extract of *C. dichogamus*. This difference could be because of the geographical difference of the plant material.

In the current study, tannins were not detected in the acetonic crude root extract. However, for the aqueous and hydroethanolic root extracts, the amounts present were undetectable using GC-MS. The presence of tannins was also reported in another study performed by Johns et al. [18] who indicated that the methanolic root extract of *C. dichogamus* had a tannin content of 5.1 mg/g dry weight. Tannins are strong antioxidants because of the free radicle scavenging property in them. The fair content of tannins has been found useful in the medical field because tannins have antitumor, antimicrobial, and antiseptic properties [28]. This could be the source of the antimicrobial property in the extracts of *C. dichogamus*.

Studies have revealed that important pharmacological properties such as antiproliferative [17], anti-inflammatory [33], and insecticidal [13] activities which were present in *C. dichogamus* could be attributed to the high concentration of terpenoids [34] whose presence was also confirmed in the current study. Another study [32] also indicated that approximately 25 terpenoids were isolated from the root extracts of *C. dichogamus*. The presence of terpenoids in the extracts of *C. dichogamus* explains its use in the management of respiratory ailments such as cough, asthma, and chest pains and as terpenoids act to soothe the irritated mucous membrane lining the respiratory tract. Triterpenoids also exhibit antibacterial activities making the plant useful in treating respiratory infections that are of bacterial origin [15]. Reports have also established that in the pharmaceutical industry, terpenoids such as triterpenoids, sesquiterpenoids, and diterpenoids are used as anthelmintics, insecticides, and antibiotics [28].

The presence of phenolic compounds reported in this study was also confirmed in another study [18]. These compounds could be the reason why the plant is used to alleviate inflammatory conditions along the respiratory tract like asthma, pneumonia, pharyngitis, tuberculosis, and common cold. This is because phenolic compounds have been shown to protect the body cells against oxidative damage that cause inflammation in body tissues [35]. Kaul et al. [36] and Khanam et al. [28] reported that flavonoids give a reduced incidence of upper respiratory tract infections (URTI) because of their physiological effects on humans including antibacterial, antiviral, antiallergy, anti-inflammatory, antioxidant, and anticancer [23, 29]. In addition, reports have indicated that flavonoids reduce inflammation by reducing the size of NF-kB and stopping the replication and proliferation of two notorious viral sources of URTIs [36]. There is a positive correlation between increased consumption of flavonoids and reduced risk of respiratory infections, cardiovascular illness, and cancer illness [23, 29]. This supported the traditional use of *C. dichogamus* in the management of respiratory infections. Alkaloids inhibit the cyclooxygenase pathways which in turn inhibit inflammatory cytokines and interleukins that cause pain. Studies have also shown that alkaloids possess bacterial, antimalarial, and antispasmodic properties [28]. The presence of all the reported phytoconstituents plays a role in the observed antimicrobial and cytotoxic properties. This is due to the fact that the occurrence and quantities of the secondary metabolites determine the bioactivity of the plant [37].

This study is important because it served as a starting point in the discovery of new cytotoxic agents and the unveiling of the potent phytoconstituents in *C. dichogamus*. It also confirms the traditional claim of the presence of the antimicrobial activity in *C. dichogamus* and forms a basis for dose regulation of traditional preparations of *C. dichogamus* to avoid undesirable toxic effects.

5. Conclusions

The results of the current study confirmed that *C. dichogamus* possess a moderate antimicrobial activity and is highly toxic. The study also demonstrated that the roots of *C. dichogamus* are a good source of beneficial phytoconstituents. Despite the low yield, the acetonic extract demonstrated the highest antibacterial and antifungal activities against the tested microorganisms. The high cytotoxicity of *C. dichogamus* will limit its use as an antimicrobial agent. The previous statistical analyses performed on the antimicrobial and cytotoxic activities on different extracts at various concentrations were corroborated with the present findings and supported the traditional use of the plant as an antimicrobial agent. Thus, further study is required for dose adjustment among the communities that use the plant for curative purpose. Studies to determine the mechanism of action of this plant as an antimicrobial agent and cytotoxic agent are needed. Moreover, research is needed to isolate and identify the active phytoconstituents responsible for the cytotoxic activity in *C. dichogamus* for development of future anticancer drugs.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

JMN, DNM, and IOM designed the study, DNM funded the research, conducted the study, and prepared the first draft, and FMM and DNM analyzed the data. All the authors read, edited and approved the final manuscript.

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Botanical description, ethnomedicinal uses, phytochemistry and pharmacological effects of *Croton dichogamus* Pax (*Euphorbiaceae*)

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ABSTRACT

Croton dichogamus Pax (Euphorbiaceae) has been used widely in traditional ethnopharmacological practices against a wide number of ailments. The pharmacological activities, phytochemical composition and its safety aspects have been covered in a number of articles. The present review aims to provide a comprehensive literature overview regarding botanical description, phytochemical composition, local uses, pharmacology and toxicological effects of crude extracts, fractions and isolated compounds obtained using different solvent systems. The review was compiled through a thorough literature search from authentic resources using Google, Google Scholar, Medline, PubMed, Chemical abstracts, Web of Science, Scopus, Science Direct, peer reviewed articles, books and thesis. *Croton dichogamus* is an important ethnomedicinal plant used traditionally for the treatment of tuberculosis and other respiratory tract infections, stomach ache, fever, sexually transmitted diseases such as syphilis and gonorrhea, impotence, arthritis, tooth ache, infertility and malaria. Pharmacological and toxicological studies performed on the fresh plant parts and crude extracts prepared using different extraction solvents validates the ethnomedicinal utilization of *Croton dichogamus*. Studies performed validate the use of *Croton dichogamus* extracts in antimicrobial, antioxidative and antiproliferative therapy. Information on therapeutic validation in analgesia, hypertension, wound healing, gastrointestinal motility and diabetes mellitus is scanty. To further advance the local use of *Croton dichogamus* in the above-mentioned illnesses, there is an urgent need for further studies to validate the traditionally reported anecdotal efficacy and safety. Data on safety of various crude extracts of *Croton dichogamus* is also scanty. However, the available information on toxicology of *Croton dichogamus* suggests it is safe. The current review supports in part, the ethnomedicinal use of the medicinal plant. However, in-depth studies aimed at efficacy and safety evaluation, in addition to identification of compounds responsible for the reported activities is required. This information will support steps towards discovery of novel ligands with activity against illnesses reported above.

Keywords: *Croton dichogamus* Pax, Botanical description, Phytochemistry, Pharmacology, Toxicology, Traditional medicine.

INTRODUCTION

The medicinal value of plants has been exploited for disease alleviation since time immemorial [1]. The accessibility, availability, effectiveness, and affordability of medicinal remedies make traditional medicine a popular form of treatment [2].*Croton* (Euphorbiaceae) has 1300 species, most of them extensively used in traditional medicine, owing to their ability to treat a broad spectrum of diseases [3, 4]. Some species from this genus have important validated medicinal properties that can provide leads in drug design and development [5]. For instance, the leaves and stem bark extracts of *Croton cajucara* are used in form of pills for the treatment of diabetes, weight loss, high blood cholesterol and gastrointestinal disturbances; the methanolic root extracts of *Croton lobatus* have been reported to have activity against *Plasmodium falciparum* strains, sensitive to Chloroquine [4]. The dichloromethane leaf extract of *Croton zambesicus* showed *in vitro* cytotoxicity against human cervix carcinoma cells; anti-tumor activity was also shown by the red latex of *Croton lechleri* [4]. The methanolic leaf extracts of *C. lechleri* have also shown wound healing activity and an ability to heal gastric ulcers by reducing ulcer size and bacterial content of the ulcer [4]. Moreover, one of the active ingredients of a commercially available anti- ulcer agent Kelnac ™ is from the leaves of *Croton stellapilosus*, a Thai *Croton* species [5].

Croton is perceived to adapt to diverse climatic conditions and different soil types which could be the reason why its numerous species are found in most parts of the globe [6]. Due to its adaptive features, *Croton* genus has evolved at a very high rate, such that within the genus there are *Croton* subgenera like Geiseleria [7]. Its global availability and resilience to harsh climatic conditions has increased its

utilization as an anti-tumor, antidiabetic, antiviral, antispasmodic, antimicrobial, acetylcholinesterase inhibitor, anti-ulcer, anti-inflammatory and neuritic outgrowth-promoting agent [4].

C. dichogamus, one of the species of *Croton* has been reported to grow in Ethiopia, Kenya, Mozambique, Somalia, Rwanda, Madagascar and Tanzania, where it plays a key role in traditional medicine [8]. Phytochemical screening done on numerous crude extracts of the leaves, stem bark and leaves of *C. dichogamus* has reported the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids and numerous terpenoids [9, 5, 10]. The existence of a fair quantity of all these phytochemicals in *C. dichogamus* has qualified it for curative use in traditional medicine as a tonic to improve digestion [11], as an antimalarial, an antipyretic and as a nutritional supplement for milk [11, 12, 19], to treat polio-like symptoms, and chest pain [13], and also to alleviate back pains. It has also been used to alleviate stomachache, chest problems, oedema and cough [14], to manage asthma [15], arthritis and gonorrhea [16]. A study conducted in the mid-1900s in south Baringo district indicated that *C. dichogamus* is a highly nutritive shrub to both animals and humans containing 25.14% protein, 42.30% carbohydrate, 20.08% crude fiber, 1.08% calcium and 0.49% phosphorus [17]. Wild herbivores in Africa consume this plant as their main food especially the elephants, giraffes and buffaloes [18]. The current review therefore focuses on traditional uses, phytochemistry, biological activities and toxicologic effects of *C. dichogamus*.

Taxonomic tree

Domain - Eukaryota

Kingdom - Plantae

Phylum - Tracheophyta

Class - Magnoliopsida

Order - Malpighiales

Family - Euphorbiaceae

Genus - *Croton L.*

Specific epithet - dichogamus

Scientific Name - *Croton dichogamus* Pax

Morphology

C. dichogamus is a shrub with regular, repeated branching and a tapered or trailing wreath. The leaves are normally sleeky, tabular covered with silvery scales and the upper side is yellowish brown [8]. The flowers are monecious; each flower has twenty stamens that contain six sepals. This flower doesn't have any petals, its pistils have five sepals, divisions are sleeky and linear, designs are bipartite, the ovary is covered with round tabular scales [19]. *C. dichogamus* grows up to 7.5 meters tall or more but is usually only 2-5 meters. The fruit of *C. dichogamus* is spherical, three-parted, and slightly bigger than a pea [8].

The habitats of *C. dichogamus* may include acacia woodland, bushland, thicket, dry forest, on rocky ground, lava, limestone and porous soils and sometimes forming dense stands at elevations from 550-1800 meters [20].

Figure 1: Photo of *Croton dichogamus* Pax (Euphorbiaceae) plant. The picture was taken in Kisumu East, by Dorine Matara and authentication done at East African Herbarium.

Traditional uses

In Tanzania, the roots of *C. dichogamus* are milled then mixed with porridge for the treatment of tuberculosis because the shrub is believed to be efficient in the management of respiratory ailments [11]. The leaves are also used as tonic, antimalarial and as a nutritional supplement [11]. The smoke from the burnt leaves *of C. dichogamus* is inhaled by patients to provide relief from fever [11, 21]. The Batemi and Maasai of East Africa add C. *dichogamus* to milk and meat based soups to eliminate cardiovascular diseases caused by high levels of cholesterols [10]. The agro pastoral communities in Mbulu, Tanzania use *C. dichogamus* as a pesticide to control storage pests in groundnuts [22]. They also use it for the treatment of urinary tract infections and tooth-aches [22, 23].

Among the Samburu community in Kenya, C. *dichogamus* is used to alleviate chest pains, and stomach aches [9]. In Loitoktok district in Kenya, the plant is used to manage arthritis and gonorrhea [16]. In Nyanza province, the Luo community use *C.dichogamus* to nurse patients with asthma and other respiratory illnesses [15]. In a plant sanctuary called Mutomo, in Kitui County, *C. dichogamus* stem bark and leaves are made into an infusion that is drunk to alleviate back-pains, malaria, stomachache, chest problems, fever, oedema and cough [14]. A root decoction is also drunk for the treatment of impotence and infertility [14]. In Narok county, the roots of *C. dichogamus* are used to treat polio like symptoms, gonorrhea, and chest pain [13]. The Marakwet community in Kenya ingest the boiled roots, flowers and leaves of *C. dichogamus* to relieve abdominal pain, oral thrush and wheezing. The Ethiopians make a paste out of the roots of *C. dichogamus* for vaginal application to enhance female reproductivity [13].

Botanical description

Traditionally, *C. dichogamus* is referenced using various local names by different communities (Table 1).

Table 1: Local names associated with *C. dichogamus*

Community	Local name
Latin (scientific name)	*Croton dichogamus*
Swahili	Mhande
Dholuo	Rachar
Samburu	L-akirding'ai
Masaai	Enkitaru/Oloiborbenek
Batemi	Msiniya/Mgilahagi
Mbeere	Muthima
Kinyarwanda	Umuhuhe
Marakwet	Kelerwe
Maa	Olokidigai
Afaan Oromo	Maffuka
Dorobo	Localdongai
Girigiramu	Mbulu
Kamba	Mwalula

Phytochemistry

Secondary metabolites in *C. dichogamus* extracts

Phytochemical reports have shown the presence of a numerous metabolites which have been isolated from methanolic, n-hexane, dichloromethane and ethanolic crude extracts of *C. dichogamus* and identified by their spectroscopic data in comparison with available literature [9]. The methanolic root extract of *C.dichogamus* was reported to contain terpenoids, saponins, tannins, steroids, alkaloids and flavonoids [9, 10]. An ethanolic crude root extract from *C. dichogamus* indicated the presence of terpenoids and steroids [24]. The ethanolic leaf extract has been reported to contain flavonoids, saponins and tannins while the ethanolic stem extract has been reported to have a moderate distribution of terpenoids, saponins, tannins, steroids and alkaloids [24]. There is no phytochemical analysis performed on the fruits and flowers from *C. dichogamus*. The distribution of the compounds in various parts of C. *dichogamus* is represented below (Table 2).

Table 2: Secondary phytochemical metabolites identified from *Croton dichogamus*

Phytochemical component	Level of phytochemicals in different plant parts		
	Roots	Stems	Leaves
Terpenoids	+++	++	+
Saponins	++	++	++
Flavonoids	+	++	+++
steroids	+	+	+
Tannins	++	++	++
alkaloids	+	+	+

Key: Lowly present +, Moderately present ++, Highly present +++.

Quantitative phytochemical analysis and isolated compounds

Phytochemical analysis on the ethanolic root extract of *C.dichogamus* indicated that the terpenoids were higher in yield that the other compounds investigated, such as phenols, tannins, alkaloids, saponins and steroids [24]. The methanolic root extract of *C.dichogamus* was reported to be rich in terpenoids, saponins and tannins [10], while steroids, alkaloids and flavonoids were also present in minimal amounts [9, 10]. The ethanolic leaf extract was reported to contain a higher concentration of flavonoids, saponins and tannins while the ethanolic stem extract has been reported to have a moderate distribution of terpenoids, saponins, tannins, steroids and alkaloids [24]. Aldhaher *et al.*, [9] reported that a root extract of *C. dichogamus* contain 6.8 and 5.1 mg/g dry weight of total phenolic and tannin content respectively. Chapman *et al.*, [25], reported considerable amounts of saponins in froth assays and hemolytic assays of n-butanoic crude extract from C. *dichogamus* which were experimentally observed to be devoid of hemolytic crisis that is associated with saponins. Due to the existence of a higher concentration of terpenoids in *C. dichogamus* in comparison to the other compounds, studies have greatly focused on their quantities and curative effects [5, 9].

Phytochemical screening on n-hexane root extract of *C.dichogamus* has indicated that there are more than 20 types of terpenoids that possess incredible pharmacological activities [9, 5]. These diterpenoids belong to the clerodane, crotofolane, neoclerodane and halimane skeletal types [3, 4]. Crude n-hexane and methanolic extracts of *C. dichogamus* yielded 15 terpenoids; four sesquiterpenoids, one enantiomer of a known sesquiterpenoid, four Ent-clerodane diterpenoid, two Ent-halimane diterpenoids, three crotofolane diterpenoids and one triterpenoid as indicated below (Table 3).

Table 3: The main diterpenoids in the n-hexane crude root extract of C. *dichogamus* [8, 3]

Chemical name	Category of terpenoid	Molecular formular	Physical description	Percentage occurrence	Mass spectrum
4-patchoulene	sesquiterpenoids	C15H240	White oil	0.026	204.1878
Patchoulene-3-one	sesquiterpenoid	C15H22O	White oil	0.009	218.1671
Cadin1(6),2,4,7,9 penta-ene	sesquiterpenoid	C15H18O	White oil	0.036	198.1409
1(6),7,9-cadinatriene-4α.5β diol	sesquiterpenoid	C15H22O2	White oil	0.015	234.1620
1,3,5-cadinattriene-7R,10s-diol	Enantiomer of a sesquiterpenoid	C15H22O2-H	Yellow oil	0.008	233.1550
15,16-epoxy-13(16),14-ent-clerodadien-3-on	Ent-clerodane diterpenoid	C20H30O2	Yellow oil	0.038	302.2246
15,16-epoxy-4(18),13(16),14-entclerodatrien-3-ol	Ent-Clerodane diterpenoid	C20H30O2	White oil	0.008	302.2246

15,16-epoxy-3-keto-3(16),14-ent clerodadien-17,12S-olide.	Ent -clerodane diterpenoid	C20H26O4	Yellow oil	0.051	330.1831
15,16-epoxy-3,4 dihydroxy-3(16),14-ent-clerodadien-17,12S-olide	Ent -clerodane diterpenoid	C20H28O5	Yellow oil	0.077	371.1831
15,16-epoxy-5,13(16),14-ent-halimatriene-3-ol	Halimane diterpenoid	C20H30O2	Yellow oil	0.009	325.2138
15,16-epoxy-3-hydroxy-5(10),13(16),14-ent-halimatriene-17,12S-olide	Halimane diterpenoid	C20H26O4	White solid	0.016	330.1830
Crotohaumonoxide	Crotofolane diterpenoid	C22H26O2	Yellow oil	0.021	370.1780
Crotodichogamoin A	Crotofolane diterpenoid	C20 H22O4	Yellow gum	0.1	327.1590
Crotodichogamoin B	Crotofolane diterpenoid	C20H2202	Yellow oil	0.015	295.1695
Acetyl aleuritic acid	triterpenoid	C32H50O4	White crystal	0.017	497.3829

Crotodichogamoin B was shown to be an important biosynthetic intermediate of the crotofolane class (Crotohaumonoxide, Crotodichogamoin A and Crotodichogamoin B) [4]. A recent study has reported that a methanolic root extract of *C.dichogamus* yielded five other diterpenoids that are not included in the table above namely depressin, a Caspian diterpenoid, crothalimene A&B and crotocascarin A & B [9]. It was believed that the roots of *C.dichogamus* were the richest source of terpenoids, however an investigation on the ethanolic crude extract from the leaves of *C. dichogamus* has reported two other crotofolane diterpenoids which have not been observed in the methanolic and ethanolic root extracts, namely crotoxide A and crotoxide B [5].

Pharmacological activity

Antimicrobial activity

Antibacterial activity

Pharmacological studies have confirmed that *C. dichogamus* exhibit notable antibacterial properties [19]. The phytochemical components isolated from the plant have demonstrated mild to moderate activity against microbes of bacterial origin [4]. The ethanolic root extract of *C. dichogamus* were reported to have moderate anti-bacterial activity against two Mycobacterium species namely *Indicus pranii* and *Mycobacterium madagascariense indicus*, giving a Minimum Inhibitory Concentration (MIC) values of 1.25 mg/ml [24]. Methanolic root extracts of five other Croton species namely *Croton zambesicus, Croton megalobotrys, Croton macrostchyus, Croton sylvatica, Croton urucurana and Croton trillium* have been reported to inhibit both gram positive and gram negative bacteria especially *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Bacillus subtills* and *Pseudomonas aeruginosa* [6].

Pharmacological screening on a number of the organic extracts from *C. dichogamus* for anti-bacterial activity was consistent with studies done previously [27]. The ant-bacterial activity of the ethanolic root extract of C.dichogamus against *M. madagascariense indicus* and *Indicus pranii* was associated with a fair concentration of triterpenoids and alkaloids [24]. The antimicrobial property of *C. dichogamus* is linked to the very high concentration of terpenoids [24]. The methanolic root extracts which possessed the highest concentration of diterpenoids reported a considerably higher bacterial inhibition than the ethanolic extracts from stems and leaves [6].

Antimalarial activity

The essential oils obtained by steam distillation of the aerial parts of C. *dichogamus* [32], have demonstrated antimalarial or anti-plasmodial activity against *Anopheles gambie* and as a result, the extracts are extensively used against lake basin malaria [28].

Antifungal activity.

Even though the antifungal activity of *C. dichogamus* has never been investigated, some other *Croton* species in South America and Africa inhibited the growth of fungal organisms [4]. The methanolic root extract obtained from *Croton urucurana* hindered the growth of *Trichophyton mentagrophytes* and *Trichophyton tonsurans* [4]. The volatile oil of *Croton cajucara* also showed activity against *Candida albicans* [28].

The presence of catechins like gallocatechin and epigallocatechin in *Croton* species as demonstrated by phytochemical analysis has been noted to influence their antifungal activity [4]. However, pharmacological studies need to be staged to confirm the presence of the antifungal activity in *C. dichogamus*.

Antioxidant activity

There is an exciting free radical scavenging activity in Croton species [28]. Ferric thiocyanate (FTC) and DPPH (2,2- diphenyl-1-picryl-hydrazylhydrate) assays have confirmed the claim that *Croton* species have strong antioxidant activity [29, 4, 28]. Johns *et al.*, [30], indicated that the essential oils obtained from the leaves of *C. dichogamus* contain polyphenols like tannins and flavonoids which exerted significant antioxidant activity. Studies have also indicated that flavonoids in the methanolic stem extract of *C.dichogamus* increased production of antioxidative enzymes which promoted intracellular defense against free radicals [30, 28]. Ethanolic leaf extracts of *Croton cudatum* have shown strong hydrogen peroxide, superoxide and hydrochlorous acid free radical scavenging activity as well as antioxidative potential for chain breaking inhibition of lipid peroxidation. The antioxidation activity has been associated with the presence of flavonoids, cyanogenetic glycoside alkaloids and phenolic compounds [29].

Anti-proliferative activity

Compounds from *C. dichogamus* have shown a modest antitumor activity [9, 5]. For example 10-epi- Maninsgin D, a diterpenoid isolated from the n-hexane and methanolic crude root extracts of *C. dichogamus* when screened *in vitro* for cell viability against CACO (human colorectal adenocarcinoma) cell lines using red assay reduced CACO at 100mM showing significant inhibition of cellular proliferation [9]. The results from a study done later confirmed that another sesquiterpenoid known as furocrotinsulolide isolated from the methanolic root extract of *C. dichogamus* recorded a modest anticancer activity at 30µm when tested on CACO-2 cell line [5].

A fair composition of both diterpenoids and triterpenoids in *C. dichogamus* is believed to be the source of the antiproliferative activity [31]. *Croton cajucara and Croton mubago* which have a fair concentration of sesquiterpenoids in their ethanolic leaf extracts were reported to be active against melanoma(MALME-3M) and renal (UO-31) cell line [31].

Hypocholesteremic activity

Ethanolic stem extracts of *C. dichogamus* were seen to contain phytosterols like b-sitosterol that lower the serum cholesterol levels in humans and animals by preventing the endogenous and exogenous cholesterol absorption [32]. The methanolic stem extract of *C. dichogamus* was reported to contain saponins with an amphiphilic structure that bind with dietary cholesterol and with bile acids to prevent cholesterol absorption [10]. Hypolipidemic outcome was observed in assay with trans dehydrocrotonin from one *Croton* species, *C. cajucara* [4]. Pharmacological studies have also linked the hypocholesteremia activity to *Croton* species to the presence of clerodane diterpenoids, acetyl aleuritolic acid [4], saponins and polyphenols [32]. This has substantiated the folkloric use of *C.dichogamus* in the management of atherosclerosis and coronary heart disease [10]. Polyphenols such as flavonoids and tannins from the methanolic leaf and stem extract of *C.dichogamus* have also been reported to lower cholesterol levels through antioxidation [10].

Insecticidal activity

The crude powdered leaf extract of *C. dichogamus* was highly effective in protecting the Bambara groundnut seeds against damage by field pests and storage insects like *Callosobruchus maculatus* over a period of 180 days [22]. Moreover, the leaf powdered extract indicated great effectiveness against oviposition of bruchids such as *Callosobruchus maculatus* on the Bambara groundnut seeds with eggs on the surface with no toxic effects the herbivores [22].

The diterpene fraction from the methanolic leaf extract of *C. dichogamus* is the source of the insecticidal activity [4]. The diterpene moiety from *Croton linearis, Croton aromaticus and Croton californicus* demonstrated significant insecticidal activity [4].

Anti-inflammatory activity

Anti-inflammatory activity has been observed in most of the *Croton* species [3, 4]. Furocrotinsulide A, a compound isolated from the n-hexane leaf and stem extract of *C. dichogamus* and *Croton Poomae* demonstrated significant anti-inflammatory activity by inhibiting nitric oxide production better than standard drugs, indomethacin and dexamethasone [36, 19]. Bioassay -guided fractionation of the aerial parts of *Croton cilitoglandulifer* led to the isolation of tigliane diterpenoids which inhibited the enzyme cyclooxygenase-1(COX-1) and cyclooxygenase-2(COX-2) [34]. A tigane diterpenoid isolated from the branches and leaves of *Croton tiglium* displayed moderate inhibition of enzymes COX-1 and COX-2 [35] and crotonkinin A, a diterpenoid isolated from *Croton tonkinesis* also showed anti-inflammatory activity [4].

Recent studies have reported that the methanolic, n-hexane, ethanolic root and leaf extracts of *C.dichogamus* contained clerodane and sesquiterpenoid diterpenoids which exhibited significant anti-inflammatory activity [36].

Tonic activity

The word tonic refers to medicinal substances that are used to restore the general body health and function of various body organs. Studies have confirmed the folklore claim of *C. dichogamus* being effective as a tonic. Oliver [38], reported that aqueous leaf extracts from C. *dichogamus* contained uterotonic, aphrodisiac, muscle tonic, stomach tonic and muscle tonic activities. The crude extracts from the stem of *C. dichogamus* were also reported to have tonic effects [10].

Toxicology of *C. dichogamus*

C. dichogamus was tested for cytotoxicity using brine shrimp lethality assay. The ethanolic root extract, though toxic, was safer to Brine shrimp larvae with LC_{50} value of 40.70 µg/ml compared to cyclophosphamide, a standard anticancer drug that had LC_{50} value of 16.3µg/ml [24]. Maninsgnin D a compound isolated from the methanolic root extract of *C. dichogamus* was assayed for its cytotoxicity against HL-60, SMMCC-7721, A-549, MCF-7 and SW-480 human tumor cell lines by the MTT which gave an LC_{50} value of less than 40µm [37], indicating that the molecule is potentially toxic. This points towards a high lethality of *C. dichogamus* extracts, calling for dose adjustments in herbal formulations, and further in vivo studies to ascertain the safety of the medicinal plant.

CONCLUSION

A comprehensive literature overview has disclosed that *C. dichogamus* is an essential plant in ethnomedicine for the management of malaria, tuberculosis, asthma, cough, fever, impotence, stomach and tooth aches, chest and back pains, gonorrhea and syphilis. Organic and inorganic crude extracts from various plant parts of *C. dichogamus* have substantiated the folkloric use of the plant. Pharmacological studies have mainly focused on the assessment of the antimicrobial, antiproliferative, hypocholesteremic and pesticidal activity of the plant. A number of studies have evaluated the phytochemical composition of the plant parts using polar and non-polar solvents and various phytoconstituents have been isolated and identified. Despite the extensive use of *C. dichogamus* in traditional medicine, there is minimal scientific literature on its pharmacology and safety. There is a necessity of experimental evidence on antifungal, antipyretic, analgesic, antitussive and antispasmodic properties to validate the traditional use of *C. dichogamus*. There is scanty information appearing on the toxicological profile of *C. dichogamus*. This calls for further studies considering its wide utilization in folkloric medicine. Hence, there is an urgent need for further validation of anecdotal safety and efficacy of *C. dichogamus*, going forward.

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